International Seminar on Animal Industry 2012

FACULTY OF ANIMAL SCIENCE
BOGOR AGRICULTURAL UNIVERSITY

THE SECOND INTERNATIONAL SEMINAR ON ANIMAL INDUSTRY

"Empowering Local Resources for Sustainable Animal Production in Adapting to Climate Change"

5-6 July 2012
Jakarta Convention Center, Jakarta-Indonesia

PROCEEDING

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FOREWORD FROM CHAIRPERSON OF ORGANIZING COMMITTEE

Dear colleagues,

It is my great pleasure to welcome all of you to the Second International Seminar on Animal Industry 2012, and to Jakarta the capital city of Republic of Indonesia. This seminar is conducted by the Faculty of Animal Science - Bogor Agricultural University in collaboration with Animal Scientist's Association of Indonesia, Indolivestock 2012 Expo and Forum, Directorate General of Higher Education – Ministry of Education and Culture Republic of Indonesia, Directorate General of Livestock and Animal Health Services-Ministry of Agriculture, as well as Journal of Animal Science and Technology (Media Peternakan).

There will be 131 papers presented during the seminar consisted of 12 papers from invited speakers, and 119 papers from participants in which 72 papers will be presented orally and 47 papers will be presented as posters. The invited speakers come from several different countries including Australia, England, Japan, South Korea, South Africa, Sweden, Switzerland, United States of America, and of course Indonesia. The presenters for supporting papers come from several countries namely Malaysia, Thailand, Turkey, Iran, Irak and Pakistan, as well as from 22 different universities and research institutes in Indonesia.

This is a great opportunity for all of us to share knowledge and experience regarding the advanced development of animal science and technology in different part of the world especially related to the recent climate changes which may interferes animal production system. By closely collaborating and sharing information we will be able to overcome the problems better, faster and more comprehensive.

On behalf of the organizing committee, I would like to express my sincere thanks to Directorate General of Higher Education - Ministry of Education and culture for funding this seminar through Himpunan Profesi Grant, also to PT. Napindo Media Ashatama for partly funding the seminar and to Director General of Animal Livestock and Animal Health Services – Ministry of Agriculture for his support and collaboration. Thanks are also addressed to our sponsors namely PT. Nutreco, PT. Cheil Jedang, PT. Sinta Prima Feedmill, PT. Kaltim Prima Coal, CV. Swen IT. This seminar is also supported by some units of Bogor Agricultural University namely Department of Nutrition and Feed Technology, Department of Animal Production and Technology - Faculty of Animal Science, Graduate School, Diploma Program, and Graduate Business School.

Last but not least, I would like to thank the organizing committee who has been working very hard to make this seminar a successful event. For all participants, I

apologize for the inconveniences before, during, and after the seminar. I wish all of you will have a great time and a fruitful discussion. Thank you.

Jakarta, July 5th, 2012
Chairperson of Organizing Committee
Prof. Komang G. Wiryawan, Ph.D

FOREWORD FROM CHAIRPERSON OF ORGANIZING COMMITTEE

Ladies and Gentlemen,

Assalamualaikum warahmatullahi wabarakatuh

First of all, I would like to extend my warm welcome to all participants of the Second International Seminar on Animal Industry 2012 to Jakarta Convention Centre. Together with us in this seminar are delegates from various parts of the world: South Africa, Switzerland, Japan, Australia, UK, Sweden, South Korea, Pakistan, United States of America, Turkey, Iran, Irak, Thailand, and a part from the local delegates, our colleagues from various universities in Indonesia: from Sabang to Merauke, representatives from the government livestock service agencies, research centre as well as businessmen.

It is an honor for me, the Dean of Faculty of Animal Science, Bogor Agricultural University to be able to host such an important seminar. Let me begin by acknowledging the Napindo Media Tama Limited Corp. and Animal Scientist' Society of Indonesia for their collaboration in organizing this event. In this special occasion I would also like to express my appreciation to Dr. Ir. Suswono, MMA, the Indonesian Minister of Agriculture for his support and encouragement. We also extend our gratitude to Directorate General of Higher Education, Indonesian Ministry of Education and Culture as main sponsor of this seminar. My appreciation also goes to all invited speakers for their willingness to share their knowledge and vision with us. To the contributors and sponsors, I would like to express my great thanks. To all members of steering and organizing committee, I would like to express my deep appreciation for their effort to make this event successful.

Ladies and Gentlemen,

Global climate changing is a subject that is very intense we hear lately. It affects all sectors of our life including animal production system. The ability of our stakeholders to adapt to it will determine our survival. The emphasis of the seminar is on animal industry as this sector is seen as a leverage factor of the animal production system. The development of animal industry is vital in producing significant contribution of animal production system as a whole.

The objective of this seminar is primarily to present the development of science and technology innovations in animal industry, to disseminate the results of animal research on livestock production improvement, to broaden perspectives of stakeholders on potencies, prospects, and constrains on animal industry. Issue strategic with respect to animal breeding and genetic, feed and nutrition, animal

management and production, animal product's technology, socio-economic and policy, as well as animal disease and its prevention will also be discussed in depth.

Ladies and Gentlemen,

As we are all aware, the impact of globalization upon us is becoming manifest. To be able to join the mainstream, we have to improve our local competitiveness and uniqueness through optimalization of our local resources utilization. What needs to be strengthened may include persistency of culture identity since animal production systems in several countries are not only socio, technologic or economic aspects of the people. It is a culture of life.

Ladies and Gentlemen,

Over the next two days, I believe you will be discussing issues and matters regarding the empowering local resources for sustainable animal production in adapting to climate change. This seminar will include discussions based on more than 119 paper presentations that cover issues and topics encompassing animal breeding and genetic, feed and nutrition, animal management and production, animal product's technology, socio-economic and policy, as well as animal disease and its prevention. I believe you will find such topics interesting. Because the speakers are well known in their respective fields and will be able to provide you with the current state of the art of animal industry development in their region.

On this occasion, we will have the opportunity to work together to improve our contribution to animal industry development for the future. We have been fortunate enough to be given a great opportunity whereby we can learn from each other. I also hope that all of you will use this opportunity to strengthen the existing network. I am sure that all participants will greatly benefit from this seminar.

Let's get our act together for excellence and quality in research so that we can improve our contribution to the development of animal industry in the future.

Wabillahi taufiq wal hidayah
Wassalamualaikum warahmatullahi wabarakatuh

Jakarta, July 5th, 2012
Dr. Ir. Luki Abdullah, M.Sc.Agr.
Dean

SEMINAR PROGRAM

Thursday, 5 July 2012

Time	Ballroom	
	Event	Speaker
08.00 – 09.00	Registration	Secretariat
09.00 – 09.05	Opening Ceremony	MC
09.05 - 09.15	Report from Organizing Committee	Prof. Komang G. Wiryawan
09.15 - 09.30	Welcome Address from Rector of Bogor Agricultural University IPB	Prof. Hery Suhardiyanto
09.30-10.00	Opening and Keynote Speech by Minister of Agriculture of Republic Indonesia	Minister of Agriculture, Republic of Indonesia
10.00 – 10.10	Declaration of International Animal Science Student Association	
10.10 – 10.20	Sponsorship Appreciation	Committee
10.20 – 10.25	Photo Session	Photographer
10.25 – 10.40	Coffee Break	
	Moderator : Prof. Dr. Ir. Muladno, M.Sc	
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	Animal genetic potency for climate change adaptation	
11.05 – 11.30	Plenary 2 :	Prof. Jong K. Ha
	GM and non-GM rumen microorganisms in enhancing animal productivity	
11.30 – 11.55	Plenary 3 :	Dr. F. Leiber
	Mitigation of fermentation gasses through modification of nutrient metabolism	
11.55 – 12.20	Plenary 4 :	Prof. Fukuda
	Carrier protein in milk : basic and potential application	
12.20 – 14.00	Lunch and Poster Session	

Time	Room A Moderator : Prof. Cece Sumantri (Genetic, Breeding and Reproduction)	Room B Moderator: Dr. Ir. Idat G.P (Feed and Nutrition)	Room C Moderator: Dr. Ir. Asnath M. Fuah (Animal Manag. & Prod)
14.00 - 14.15	*M. A. Yaman*; Yurliasni., Zulfan & M. Daud* Improvement The Genetic Potential of Local Chicken by Combination of Crossbreeding, Selection Method,Cellular Analysis and Nutritional Requirements to Produce the Candidate of Local Layer	*A. Hayirli** Managerial and Nutritional Strategies to Minimize Lactational and Reproductive Losses in Heat-Distressed Dairy Cows.	*V.S. Lestari* & S.N.Sirajuddin* Factors Affecting to Biosecurity Adoption on Laying Hen Farmers
14.15 - 14.30	*M.I.A.Dagong, *, C. Sumantri, R.R. Noor, R. Herman & M. Yamin* Polymorphisms of Calpastatin (CAST) Gene and It's Association with Physical Meat Quality in the Local Sheep	*I.Badarina*, D. Evvyernie, T.Toharmat, E.N Herliyana, L.K. Darusman* Biodegradation of Coffee Husk Substrate During The Mycelial Growth of *Pleurotus ostreatus* and The Effect on *In Vitro* Digestibility	*M. Ma'sum* & A. Saleh* The Perception of The Slaughter Cattle's Farmers o Implementation of Artificial Insemination in Three Central Area of Slaughter Cattle in Indonesia
14.30 - 14.45	*A. Anggraeni*, H. Hasinah, S.A. Arta, B.Tiesnamurti, R. Misrianti, &E. Andreas* Genetic Variation of the IGF and OPN Genes in Holstein-Friesian Dairy Cattle of Historical and Non-Historical Twins	*D.Yulistiani** Ruminal Fungi Colonization of Stem Tissue of Untreated and Urea Treated Rice Straw Varieties	*A. Budiman, I. Hernaman*, & D. Latipudin* Utilization Of *Datura metel* Linn. to Decrease Transportation Stress on Sheep
14.45 - 15.00	*E. T. Margawati*, Paskah P. Agung & Muhamad Ridwan* Genetic Marker Approach for Confirming the Existing Twinning Trait in PO Cattle	*M. Amizi*, A., Yazid, M.E., A.Razak, M.N ,M. M. Ismail & M. A. Islam* Oil Palm Fronds (OPF) as Potential Affordable Source of Feeds for Ruminants for Small Holders	*S. Baba** Barrier to Adoption of Biogas Technology in South Sulawesi
15.00 - 15.15	*E.M. Sari*, R.R.Noor, C. Sumantri. Margawati.E.T* Carcass Traits Association with GH/AluI Gene Polymorphism in Indonesian Aceh Cattle	*Fatmawati* *In Vitro* Digestibility of Lampoyangan Grass (*Panicum Sarmentosum* Roxb) in Form of Hay and Silage	
COFFEE BREAK			

	Moderator : **M. Baihaqi, S.Pt.,M.Sc.** **(Animal Prod. Technology)**	**Moderator :** **Dr. Burhanudin Sundu** **(Feed and Nutrition)**	**Moderator :** **Dr. M. Amizi** **(Socio economic)**
15.30 - 15.45	*I.I. Arief*, R.R.A. Maheswari, T. Suryati & N.Kurniawati* Protein Quality of Fermented Beef by *Lactobacillus Plantarum* 1b1	*R.W.S. Ningrat &Khasrad* Effect of Waste Products on Ruminal Microbe Population and Rumen Charateristics *In Vitro*	*A.Phianmongkhol*, T.I. Wirjantoro, C. Chailungka, C. Prathum & A. Leotaragul* Public Perception in Thai Native Chicken (Pradu Hang-Dum Chiang Mai) via Food Contests
15.45 - 16.00	*H.R. Ansari-Renani*, J.P. Mueller, B. Rischkowsky, S.M. Seyed Momen, O. Alipour, M.Ehsani, S. Moradi* Cashmere Quality of Raeini Goats Kept by Nomads in Iran	*T. Rostini* & I. Zakir* Evaluation of Complete Ration Silage on Performance and Quality of Goat Meat	*M. M. Ismail* & M. Amizi* Trade Performance of Meat and Meat Preparation Sector in Malaysia : The Case of The Non-Ruminant Sector
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16.15 - 16.30	*Yamin, M.* &Rahayu, S* Wool Fibre of Local and Crossbred Sheep: Production, Processing Technique and Performance	*M. Taghavi-Nezhad, D. Alipour, P.Zamani,& S.Yadegari* The Effect of Essential Oils of Spearmint on the *In Vitro* Rumen Fermentation, Growth and Deaminative Activity of Amino Acid Fermenting Bacteria	*M. Ashfaq & S. Ali* Economic Losses Due to Delayed Conception of Dairy Animals Among Small Farmers in Pakistan
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16.30 - 16.45	*F.F. Munier* & H. Hartadi* Theobromine Content in Cocoa Pod Husk (*T. cacao*) Fermented by *Aspergillus* spp. in Different of Chop Sizes and Fermentation Times	*Hermon*,Suryahadi, K. G. Wiryawan, H .Soedarmadi* Effect of Energy and Protein Contents of Dietary on Local Beef Cattle Performance	*K. Fauziyah*, H. A. Sukria, Burhanuddin* A Model of Sustainable Ruminant Feed Industry in Jepara, Central Java

16.45 - 17.00	*A. Sofyan* & H. Herdian* Compariative Analysis of *In Vitro* Silage Digestibility Prepared by Different Drying Method	*Riyanto, J** Analysis of the Kinetics Fermentability, Degradability and Nutritive Value of Soybean Groats and Lemuru Fish Oil on *In Vitro* Rumen Gas and Methane Production	*Nuraini*, S. A Latif, A. Djulardi* Evaluation of Fermented Product by Monascus purpureus in Diet On Performance and Quality of Meat Broiler
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17.15 - 17.30		*Suparjo*, E.B.Laconi, K.G. Wiryawan,D.Mangunwidjaya* Evaluation of Nutrient Digestibility Of Goats Fed on Biofermented Cocoa Pods Using *Phanerochaete Chrysosporium* Supplemented By Mangan (Mn) and Calsium (Ca)	

Friday, 6 July 2012

Time	Ballroom	
	Event	Speaker
08.00 – 08.30	Registration	Sekretariat
08.30 - 08.35	Opening	MC
	Moderator : Dr. Ir. M. Yamin, M.Sc	
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	Prospect and challenges of animal agribusiness in Indonesia	
09.00 – 09.25	Plenary 6 :	Prof. Dr. L.C. Hoffmann
	Customer preference on meat commercial cut	
09.25 – 09.50	Plenary 7 :	Prof. E.R. Orskov, Ph.D.
	Improvement of local feed resources to increase nutrient efficiency to support sustainable animal production	
09.50 – 10.15	Coffee Break	
	Moderator : Prof. Dr. Ir. Dewi Apri Astuti, MS.	
10.15 – 10.40	Plenary 8:	Dr. Henk Enting
	Sustainability in Animal Production: Animal Nutrition Perspective	(Global Technical Manager Poultry)
10.40 – 11.05	Plenary 9 :	John Moran
	Planning Dairy Development in Tropical Asia	
11.05 – 11.30	Plenary 10 :	Prof. Max Shelton
	Development of tropical forages	
11.30 – 11.55	Lunch and Poster Session	
17.00 – 17.30	Closing Ceremony	

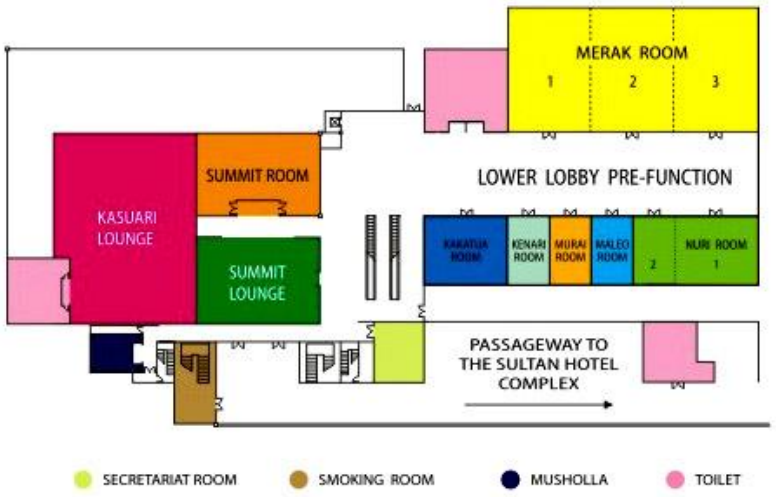
Time	**Room A** **Moderator :** **Dr. Despal** **(Feed and Nutrition)**	**Room B** **Moderator:** **Dr. Desianto** **(Feed and Nutrition)**	**Room C** **Moderator:** **Dr. Anneke Anggraini** (**Animal Manag. & Prod)**
14.00 - 14.15	*D.R. Lukiwati*, T. W. Agustini, B.A. Kristanto, Surahmanto* Production and Nutrient Uptake of Sweet Corn with Manure 'Plus' and Inorganic Fertilizer	*Suharti, S.*, N. Aizah, D. M. Suci, D.A. Astuti & E. Wina* *In Vitro* Fermentation and Bacterial Protein Synthesis in the Different Diets Supplemented with Lerak Extract plus Mineral (Ca, P, Mg, S)	*M. Younas** Effect of Climate Change (Heat Stress) on Livestock Production in Pakistan

14.15 - 14.30	*N. R. Kumalasari*, E. Bergmeier, & L. Abdullah* Diversity and Potency of Weed on Rice Field for Ruminant Feed in Java	*M. Sarwar*,M.Aasif Shahzad, M.Nisa* Effects of Feeding Different Level of Dietary Protein with or without Probiotics or Ionophores on Performance of Growing Kids	*M. Ulfah*, Mulatsih, S., N. M. Nurapriani, N. M.* The Characteristic of Farming System for The *Walik* Chicken in West Java, Indonesia
14.30 - 14.45	*Karti, P.D.M.H.K*, L. Abdullah, D.A. Astuti,., N. Kurniaty, & Nissa* Mineral Balance of *Brachiaria humidicola* Pasture which is Introduced with Legume Creeping in UP3J	*A.Sudarman*, K.G. Wiryawan, & A. Purnomoadi* Reducing Methane (CH_4) Emission of Sheep through Supplementation of Coconut Oil And/or Palm Oil into the Ration	*Rudi Afnan* & Martina Gerken* Long-Term Heat Stress in Relation with Productive and Reproductive Performances of Slow Growing Broiler Parents
14.45 - 15.00	*A.Pebriansyah*, Panca D.M. H. K. & A. T. Permana* Role of Arbuscula Mycorrhizal Fungi (AMF) in Overcoming Drought Stress Of Several Tropical Grasses (*Chloris Gayana, Paspalum Dilatatum,* and *Paspalum Notatum*)	*Y. S. Nur*, K. G. Wiryawan, R. Syarief , Nahrowi* Palm Press Fiber-Cr Organic Fermented As An Forage Alternative For Sheep	*Najamudin*, Amrozi, S. Agungpriyono, T. Laswardi, Yusuf* Anatomy and Morfometry of Male Reproductive Organ of Mouse Deer (*Tragulus javanicus*)
15.00 - 15.15	*L. Abdullah* *Indigofera zollingeriana* : A Promising Forage and Shrubby Legum Crop for Indonesia	*I. Prihantoro*, Y. Sari, L.Riyanti, Enggar, D.Evvyernie Amirroenas, T.Toharmat)* Nutritive Values of Forages Evaluated Using A Mixed Bacteria Isolated from The Rumen Liquor of Buffalo	*(Ulupi N*, I.I. Arief, B. Brahmantiyo & KE. Riwayati* Effectiveness of *Lactobacillus acidophilus* as Biocontrol to Prevent *Salmonella enteritidis* Infection on Laying Hens
COFEE BREAK			
	Moderator: **Prof. Toto Toharmat** **(Feed and Nutrition)**	**Room C** **Moderator :** **Prof. Iman Rahayu** **(Feed and Nutrition)**	**Moderator:** **Dr. Yantyati Widyastuti** **(Feed and Nutrition)**
15.30 - 15.45	*A.S. Tjakradidjaja*, K. G. Wiryawan, M. Afriyanti)* Fermentability and Digestibility *(In Vitro*) of Ration Containing Crude Curcin Extracted from *Jatropha curcas* L. Seed Meal by Microbes from The Rumen Fluid of Cattle and Buffalo	*Hafsah*, N.Marfuah, Sugiarto* Cholesterol Contents and Carcass Composition of Broiler Meats Fed by Different Level of Garlic Meal	*B. Sundu*, U. Hatta & H.B. Damry* Comparison of Mycotoxin Binders in the Aflatoxin B1-Contaminated Broiler Diets

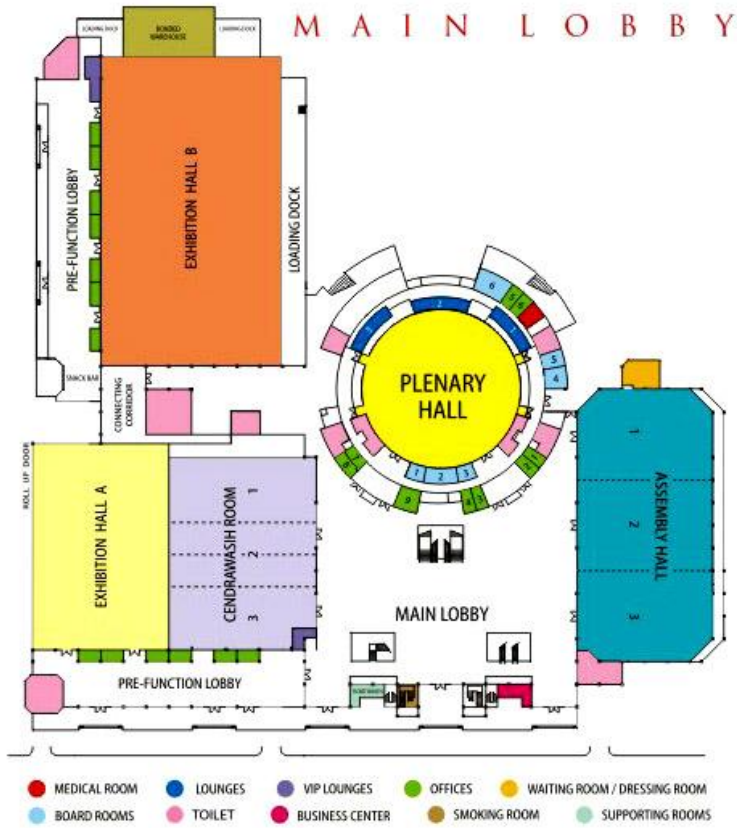
15.45 - 16.00	*Despal*, N.A. Qitri, K.B. Satoto & I.G. Permana* Comparison of portable and static types of silo on silage quality of total mixed ration contained ramie leaves (Boehmeria nivea, L. GAUD)	*D. Biyatmoko* & T. Rostini* The Supplementation Effect of Fish Oil, Corn Oil and Zinc in Fiber Ration on Cholesterol Profile, Omega-3 Omega -6 of Alabio Duck Egg	*Khalil** Improvement of Nutritive Values of Local Feedstuffs As Mineral Sources for Kampongs' Laying Hens
16.00 - 16.15	*D.A. Astuti*, S. Rahayu, B. Satoto, R. Priyanto, L. Khotidjah, T.Suryati and M. Baihaqi* Blood Metabolite Status Of Local Sheep Fed With *Indigofera Sp.*	*T. Budiati* , G. Rusul , W. N. Wan Abdullah , Y.M. Arip, R. Ahmad* The Bacteriological Quality of Chicken Offal and Spoiled Egg As Feed For Catfish and Tilapia Rearing in Penang, Malaysia	*S. Mawaddah*, W.Hermana, W.G.P iliang* The Content of Choles-terol, Fat, Vitamin A and E in the Meat, Liver, and Eggs in Japanese Quails Given Katuk Leaves Extract in the Diet
16.15 - 16.30	*Permana, I.G*. Suryahadi, & E. Qurimanasari* Greenhouses Gases Emissions from Dairy Cattle in Indonesia	*E. Sulistyowati*, T. Rostini, Suharlina, I. Martaguri, R. Muthia, A. Sudarwan, & K. G. Wiryawan* Production Performance of Broiler Chickens Fed Glucogenic and Lipogenic Diets	*T. Pasaribu, M. Prayuwidayati*, R, Palupi, K. G. Wiryawan, A. Sudarman, & R. Mutia* The Effects of Dietary Energy Sources on Immune Organs of Broiler Exposed Heat Stress
16.30 - 16.45	*Suryahadi*, A. S. Tjakradidjaja & D. Sunaryo)* Improving Production Performance of Peranakan Ongole Cows and Nutrient Digestibility of Rice Straw Based Diet with Supplementation and Complete Feed		

SEMINAR LAYOUT

LOWER LOBBY



MAIN LOBBY



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Invited Speaker

GM and Non-GM Rumen Microbes in Enhancing Animal Productivity

T. S. Park, J. K. Seo and Jong K. Ha

Department of Agricultural Biotechnology, Research Institute for Agriculture and Life Sciences, College of Agriculture and Life Sciences, Seoul National University, Daehak-dong, Kwanak-gu, Seoul 151-742, Korea

Abstract

Rumen microbes has been extensively used for manipulating the rumen ecology. Genetically modified or non genetic modified are both the type of application for enhancing the ruminal fermentation and improving the performance of ruminants. Construction of genetic modified rumen microbes has been succeeded with adding the various kinds of traits, such as improving fiber digestion, detoxification or reducing methane production in the rumen. This modification strategy mainly targeted into the predominant ruminal bacteria including Butyrivibrio fibrisolvens, Streptococcus bovis and Prevotella ruminicola. However, directly introducing the GM microbes to the rumen ecology is very limited. Recombinant Butyrivibrio fibrisolvens which was transformed with fluoroacetate dehalogenase gene can protect the host animal from plant toxin poisoning. Since the usage of GM microbes has restricted in most countries for its potential risks, many research have been focused on another approach with Non-GM microbes. There are many researches for checking the effect of diverse Non-GM rumen microbes for enhancing the CLA production, mitigation of methane emission, and the possibility as probiotics through in vitro and in vivo. The results show that this alternative use of Non-GM rumen microbes can be an intense candidate for improving the animal productivity through manipulation of rumen ecology. We discussed about how the GM and Non-GM rumen microbes have been used during the last decade and its potential aspects to contribute to the animal productivity.

Keywords: animal productivity, genetic modified, rumen microbes

Introduction

Manipulation of ruminal fermentation has been one of the preferred methods in improving the performances of ruminant animals and rumen microbes have been extensively used for manipulating rumen ecology. The contribution of rumen microbes in its ecology has been partially defined with predominant rumen

bacteria; *Butyrivibrio fibrisolvens*, *Prevotella ruminicola* and *Streprococcus bovis* which occupy a large population in the rumen and this fact allows them to be a proper target for genetic manipulation (Selinger *et al*., 1996; Ekinci *et al*, 2002). The leading purposes of genetic manipulation of predominant rumen microbes are increasing the fibrolytic activity, detoxification, limitation of protein degradation or improving microbial protein synthesis in the rumen (Forano and Flint., 2000). Although some successful evidences have been reported under in vitro conditions, efficacy of genetically modified rumen micobes has been marginal under in vivo conditions. Usage of non-GM rumen microbes can be an alternative way to avoid the potential risks of GM microbes. Some of recent areas of interest with non-GM rumen microbes are increasing the amount of CLA and mitigation of methane production. This paper will briefly discuss potentiality of GM and non-GM rumen microbes in enhancing animal productivity.

Genetically modified rumen microbes

The principal purpose which genetic modification has been intended during several decades is adding the beneficial traits (improvement of fiber digestion and amino acid synthesis, reduction of dietary protein degradation and alleviation of effect of toxic materials) (Gregg *et al*., 1994; Teather., 1985) into rumen microbes for their efficient fermentative action in the rumen. This modification strategy mainly targeted into the predominant ruminal bacteria including *B. fibrisolvens*, *S. bovis* and *P. ruminicola* (Forano and Flint., 2000). These efforts resulted in the construction of various kinds of GM microbes.

GM microbes often re-introduced into the rumen ecosystem for confirming the survival rate and capacity to be dominant in its environment but the period of survival was quite restricted, besides they usually do not maintain their recombinant traits (Kobayashi and Yamamoto., 2002; Krause *et al*., 2001). It is believed that foreign fibrolytic enzyme genes did not act properly in the rumen ecology. Inhibition by antibacterial-agent, bacteriocin, existed in the natural rumen ecology can be partly responsible for this. Despite non-significant impact on rumen fermentation and production with GM microbes, *B. fibrosolvens* transformed with fluoroacetate dehalogenase gene is regarded as one of positive examples (Gregg *et al*., 1998). Retaining the adequate population of recombinant bacteria to stabilize in the rumen is crucial factor for detoxification. In this experiment GM *B. fibrisolvens* maintained its population above 10^6 cells per ml and this was enough to detoxify the toxic molecule in the rumen ecology.

Despite successful expression of heterologous genes in the rumen microbes, the GM microbes have not been directly applied to the animal industry because of its potential risks. Possible gene transfer between rumen microbes and related environment or changes in the traits of rumen microbes are the most important

factors for examination of GMO's risks Forano and Flint., 2000). Though the risks of GMO are vague, the acceptability of GMO by the public who directly consume the animal products is usually low. Due to the difficulty of public agreement on certain evaluation criterion, assessment of GMO's risk is not yet fully constructed. Because the advantage of genetic modification is distinct and efficient use of this technology can be beneficial to animal industry, scientific standard is needed for the proper utilization of GMO.

Non-GM rumen microbes

Since the usage of GM microbes has restricted in most countries due to their potential risks, many research have been focused on another approach with Non-GM microbes. Microbes isolated from the rumen could be re-introduced into the digestive tract of ruminants hoping to enhance digestive functions.

Major bodies of research results are with rumen bacteria. Isolated rumen bacterium *Pseudobutyrivibrio xylanivorans* Strain Mz5T was evaluated for its possible role in the rumen ecology through in vitro studies (ČEPELJNIK*et al*., 2003). This strain produced butyrate as a major product and fiber degrading enzymes, and bacteriocin were also synthesized. Characteristics of this strain such as providing enengy to the colonocyte and reducing pathogenic bacteria may facilitate it as a possible candidate for probiotics. *Propionibacteria* is also the available nominee for the probiotics. According to the research by Stein *et al* (2006) increased milk production and the molar percentage of propionate were detected in dairy cows after the treatment of *Propionibacteria*. Young calves are main targets of probiotics because this stage is important for infants to develop their pre-gastric fermentative organs which are required for the efficient degradation of forage. Dosing probiotic mixture compound which consisted of five rumen microbes increased ADG, improved fibrolytic action and decreased incidence of diarrhea in young Holstein calves (Aldana *et al*., 2009). Since dairy cows need proper controlling for parturition and calving, probiotic supplementation is in a position to alter the rumen fermentation. *Megasphaera elsdenii* is well-known lactate-utilizing bacterium and Aikman *et al*. (2011) inoculated this bacteria into the rumen for the purpose of reducing rumen acidosis at the postcalving period. Though ruminal pH did not show significant change, dosing the bacterium resulted in lower acetate : propionate ratio and less fluctuation of ruminal pH and other positive effects on the animal performance such as milk production and energy balance. Similar positive response to the addition of rumen fungi has been reported previously. Rumen fungi secrete various enzymes to degrade fiber components, and some studies tried to use rumen fungi as probiotcs. *Piromyces* sp. isolated from goat improved digestibility and total VFA production in vitro, but the same culture did not show any significant effect for heifers (Samanta *et al*., 2008a,b)

Interests in CLA which has beneficial effect on humen health have been increased from the decades upward (Wahle *et al*., 2004) and extensive research efforts have been made to elucidate the mechanism of CLA formation in the rumen and to find ways of enhancing the process. However, only a few studies looked at possibility of using rumen microbes to enhance CLA production in the rumen. Rumen protozoa are able to synthesize CLA by the isomerase activity though they cannot convert the CLA to others including vaccenic acid and stearic acid (Or-Rashid et al., 2008). Among the classified rumen bacteria, *Butyrivibrio fibrisolvens* which is prevalent in the rumen isomerlizes linoleic acid at a higher rate than other ruminal microbes (Maia *et al*., 2007). *B. fibrisolvens* MDT-5 isolated from rumen of goat by Fukuda *et al* (2006) was used as CLA enhancer in the rumen. The strain was believed to have high linoleic acid isomerase activity and no CLA reductase activity. By analogy with this successful result of MDT-5 which show increment of CLA in pet animals, there is possibility of obtaining beneficial effects by using the same strain with domestic animals, although it is uncertain with ruminants which already have CLA-producing bacteria in their own environment.

Methane is major green house gas produced by diverse activities including agriculture industry. Among the agriculture aspect, ruminants take the great possession of methane production through their enteric fermentation. Therefore reducing methane emmision by ruminants is emphasized. Present approaches can be classified into largely two strategies. First approach is through improving the quality of nutrition supplied to the animals, so that more product may be produced per unit of methane. Secondly, through the modification of rumen fermentation using various methods actual production of methane can be reduced in the enteric fermentation (Iqbal *et al*., 2008). Homoacetogens are able to utilize H_2 coming from fermentation of feeds in the rumen. Removing hydrogen from the mechanism which was naturaly used by methanogens to produce methane is beneficial for animals, because if homoacetogen convert the CO_2 to acetate with hydrogen, the acetate generates energy for the animal and contributes to the animal productivity. Lopez *et al.* (1999) defined the competition ability of homoacetogen incubated with ruminal fluid including methanogen in vitro. Without any additives, homoacetogens did not reduce methane production, though the amount of methane was reduced after treatment with 2-bromoethanesulfonic acid. Paul *et al*. (2011) reported about novel sulphate-reducing bacterium(SRB), *Fusobacterium sp*, and characterized its available role in mitigating methane production in vitro. Sulphate reduction could be better options for removing hydrogen from the rumen ecology because these bacteria could compete with methanogens and homoacetogens for utilization of H_2. After incubation with novel isolated SRB, concentration of methane was reduced and the population of SRB also increased. Much more information is needed to apply the same technology to practice.

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Consumer Preferences in Meat

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Abstract

This paper addresses some of the requirements of modern consumers for fresh and processed meats. A focus is placed on the sensory quality attributes of meat tenderness, juiciness, flavour and odour and how these relate to the needs of the consumer. At the same time, cognizance is taken of the currentrequirements for healthy and nutritious food products, especially as pertaining to the lipid composition and the salt in the diet.Consumer desires for convenient and ready-to-eat meat products are discussed, as are some important issues surrounding process and/or production characteristics and their relation to animal welfare.

Keywords: meat, preference

Introduction

Animal producers around the world are currently faced with the common challenge of trying to feed the seven billion people on earth with an ever-diminishing supply of natural resources. Not only are there global decreases in the amount of suitable land available for farming, butthere are also a number of other universal hardships being faced, such as extreme weather patterns, degradation of land due to unsound farming practices and increases in zoonotic diseases. The only manner by which the production of meat protein can be increased is for animal producers to become more scientific in their production methodologies. This will almost certainly necessitate an increase in the intensification of animal production systems. Nonetheless, modern consumers are frequently expressing aversions to consuming meat that is derived from an intensive 'factory-like' production system.

The intensification of animal production invariably leads to theprovision of balanced feeds to the animals. This brings the needs of the animals into direct opposition with those of humans, where both would be competing for protein and energy resources such as those typically supplied by cereals and other crops. Additionally, a strong contender for these energy resources is the bio-fuel industry. This has caused many of the feedlot industries to use metabolic enhancers (Dikeman, 2007; Hansen, Frylinck& Strydom, 2012) so as to improve the food conversion

efficiency of the system. On the other hand, the use of antibiotics in feed are a common practice in the poultry industries, and to a lesser extent in the pork industries(Sofos, 2008). The use of stimulants and antibiotics, however, once again leads to mixed perceptions among consumers, primarily since different countries may permit or ban the use thereof.

Another major challenge faced by food producers is to ensure that highly perishable products reach the market in a'safe' manner, with a minimal decrease in quality. A large proportion of the meat production in the world is from regions that are situated far from the market, such as is the case in the South Americas. Such situationsdepend critically on the maintenance of the cold chain throughout transportation so as to minimise post-harvest waste. The transportation of meat across the globe also has implications in terms of the costs and the carbon footprint. The latter, in particular, has led to consumer movements in some first-world countries that promote the purchasing of"locally produced"foods sincethese are perceived to be more sustainable or environmentally-friendly.

Another major socio-economic challenge currently being faced relates to the fact that a large proportion of the world's people do not possess sufficient financial means to purchase high-quality protein sources, such as those produced from animals. In stark contrast, another portion of the world population may have a great surplus of financial resources and these individuals can often be highly critical in their food choices. In the latter case, extrinsic cues such as the carbon footprint and animal welfare issues associated with the production process become increasingly important purchasing drivers (Grunert, 2006) and the more affluent consumers are frequently willing to pay a price premium for their choices (Bennett, Anderson &Blaney, 2002). Although many producers have a strong social responsibility towards feeding the world's population, they are also faced with the reality of having to be economically viable. In other words, farmers will not produce food unless it is economically feasible for them to do so. According to the United Nations Food and Agricultural Organization (FAO), agricultural output will need to increase by 70% by 2050 in order to feed the world's population. Such a forecast will require the production of another billion tonnes of food grain and 200 million tonnes of livestock meat. The key to increasing food production from animals will rely on the improvement of productivity through selectively using genetic technologies to breed for increased animal production criteria (Gao, Zhang, Hu & Li, 2007; Allan & Smith, 2008). These breeding objectives should simultaneously also address welfare-friendly objectives, such as enhanced disease resistance (Thomas, Scollan& Moran, 2011). Other means of fulfilling the predicted requirementsmay include the breeding of animals that are appropriate or well-suited to their environments (Silanikove, 2000;Gregory, 2010; Craine, Elmore, Olsen &Tolleson, 2010; Bell, Charmley, Hunter & Archer, 2011).

Although all indications are that the price of meat as a protein source will continue to increase in the future, the demand for this commodity is also set to increase.

The increase in demand is strongly linked to the huge increase in buying power from China and India. The wealthier consumer, who can afford red meat, is frequently also a well-educated and travelled person who places value on the quality aspects of the meat (Martelli, 2009). However, this perceived quality is multi-dimensional and includes sensory quality, healthiness, convenience and process characteristics like animal welfare and production (frequently 'organic') systems (Grunert, 2006). The pressure on red meat sales due to the worldwide economic recession has caused a reappraisal of the factors which influence its appeal to consumers, which ultimately all comes back down to the quality of the meat. According to Wood, Enser, Fisher, Nute, Richardson &Sheard (1999), some of the factors determining meat quality include the absence of microbial hazards, the prevention of animal exploitation, the sensory appeal of the meat and the perceived healthiness, especially in relation to the amount and type of fat.

Sensory Quality

Irrespective of the purchasing ability of the consumer, each individual inevitably wants to have the best eating experience for their money. In the lower income groups, meat is eaten for its nutritional value, while in the higher income groups it is often consumed for the eating experience itself. In fact, the consumption of meat is seen as a sign of prosperity and wealth. Thus, as the wealth of a community increases, sodoes their meat consumption (Aaslyng, 2009). However, the type and amount of meat consumed is influenced byother factors, such as gender, age and marital status. Men eat more meat in general and a greater proportion of red meat than women (reviewed by Aaslyng, 2009). Additionally, older people typically eat more meat in general and a greater proportion of red meat than children. Families also tend to eat more meat than single people. Interestingly, Aaslyng (2009) notes that less-educated, adult men exhibit one of the highest levels of meat consumption, while young, well-educated women show one of the lowest levels of consumption.

It is self-evident that the traditional manner in which meat is prepared will influence the quality descriptors. A consumer eating a fresh steak will have different quality cues compared to one eating a traditionally dry/smoked or stewed meat product. Although a large amount of research has focused on the quality attributes of fresh meat (typically consumed as steaks), the desire for greater convenience in meal preparation has also resulted in an increase in the consumption of ready-to-eat meat products. The quality attributes of these would also differ and be more focused on perceived healthiness (fat levels, cholesterol, salt concentrations) and ease of preparation and consumption, with time-saving being of essence.

Ultimately, meat is consumed for pleasure. For fresh meats such as steaks, chops and roasts, three sensory attributes are of major importance for the hedonic value of the meat: tenderness, juiciness and flavour (both in the presence of fried flavour and

the absence of off-flavours) (Aaslyng, 2009). These three attributes have received a huge research focus in the past and will continue to receive attention in the future. The value of each characteristic also differs within each situation. For instance, when meat is very tender, then the value placed on juiciness and flavour becomes more important. However, tenderness is the most important of the three. It is now well established that no single factor influences these characteristics, but that it is rather a cumulative effect of a large number of factors that are extrinsic and intrinsic to the animal itself. The modern scientific animal producer will use a number of technologies (such as DNA markers for meat quality traits) and production systems (intensive feedlot that restrict movement thereby minimising the effect of exercise on muscle colour and toughness) to ensure that a fresh product is produced that meets the expected hedonic value deemed appropriate by the consumer. However, it is also known that a number of negative activities along the supply chain could negate these technologies.Of special interest to the modern discerning consumer is the welfare status of the animals in transit to the abattoir (see section 6).

The aforementioned quality characteristics are all applicable to cooked meat. Prior to cooking, the meat has to be purchased and the primary quality characteristic at this time is the visual appearance (colour of the meat). The colour of the meat is determined by numerous extrinsic and intrinsic factors such as the age of the animal, the environment in which the animal was raised (i.e. whether it was exposed to a high level of physical activity), the muscle type, the concentration of myoglobin pigments and then the chemical state of the myoglobin (Mancini, 2009; Mancini &Hunt, 2005). Other factors such as *ante mortem* stress also lead to abnormal colour developments, such as dark, firm and dry (DFD) or pale soft and exudative (PSE) meat. The former is typically associated with the meatfrom ruminants, whilst the latter is more frequent with that from monogastric animals. The stressor that causes these abnormal phenomenon are typically induced by human-animal interactions (Coleman & Hemsworth, 2012), but can also be caused by other factors such as extreme weather fluctuations (King, Wheeler, Shackelford &Koohmaraie, 2009).

After the meat has been purchased and cooked, the next important quality characteristic is the tenderness. This characteristic also plays a very important role in the consumer's willingness to repurchase the same product (Aaslyng, 2009). Although it is well recognised that the age of the animal plays an important role in determining the tenderness of meat (older animals have more heat stable collagen), most animals slaughtered in intensive production units are young adults, of which a large proportion are intact males. Age-induced toughness is thus generally of lessor importance. Other factors such as *ante mortem* stress also play an important role (King *et al*., 2009; Terlouw, Bourguet&Deiss, 2012) in terms of tenderness. When animals experience stress, they frequently try and move away from the stressor,which results in the metabolism of glycogen reserves prior to death. The entire aerobic/ anaerobic metabolism is influenced, causing the*post mortem* lactic acid production

(measured as pH) to deviate from the norm and resulting in either DFD or PSE. This in turnimpactson the activity of the proteolytic enzymes that are responsible for tenderising the meat (Devine *et al*., 2006). Numerous strategies may be applied in an attempt tonegate the decrease in tenderness, includingelectrical stimulation, carcass suspension, chilling regime, aging, use of external enzymes, mechanical, hydrodynamic shock, pressure and *pre-rigor* stretching (Thompson, 2002; Farouk, Wiklund &Rosenvold, 2009). Nonetheless, all of these methods are costly, and the quality of the end product still frequently differs considerably from that expected of the fresh meat product. Of special note in this regardare the *Bosindicus* breeds that are known to have higher levels of calpastatin, the inhibitor for the calpain enzymes, causing the meat from these breeds to be tougher (Shackelford, Koohmaraie, Miller, Crouse & Reagan, 1991; Strydom, 2006).

Juiciness is an important factor in the eating quality of meat, although its importance is determined by the specific meat product being consumed.For instance, juiciness is more important when consuming a steak than it is when consuming that meat which has been cut into small strips for a stew. Whereas *ante mortem* stress plays an important factor in determining the water-binding capacity of fresh muscle (by influencing the rate of decrease of muscle pH as well as the final pH), the main factor determining the juiciness of meat is the end-point temperature during the cooking (Aaslyng, 2009).Increased amounts of intermuscular fat also increases juiciness, especially when the meat is cooked at a high temperature. Aaslyng (2009) noted that the most important factor to increase the juiciness of meat is to educate the consumer on not over-cooking the meat. However, ethnic differences in food preparation also have to be taken into account when addressing this parameter.

The flavour of any meat is a combination of its taste and aroma, which are strongly influenced by additional factors such as mouthfeel and juiciness. Raw meat has hardly any aroma and only a blood-like flavour. During the application of heat, a complex series of thermally-induced reactions occur between the non-volatile components of the lean and fatty tissues (Elmore &Mottram, 2009). Over 1000 volatile compounds have been identified in meat. Elmore &Mottram (2009) reviewed the two main reactions that result in flavour development as meat is cooked. The first is the Maillard reaction, occurring between the reducing sugars and amino acids, and which is responsible for the typical meaty flavour and savoury, roast and boiled character. The second factor is lipid degradation that results in fatty aromas typically found in cooked meat. Inevitably, it is also the fat composition that is responsible for the flavour and aroma differences between species (Wasserman & Talley, 1968).

The diet has a strong influence on the fatty acid composition of animals, especially when considering monogastric animals. In ruminants, the rumen microorganisms in the digestive system have a major impact on the composition of fatty acids leaving the rumen for absorption in the small intestine (Jenkins, 1993; Doreau&Chilliard,

1997). Microbial enzymes derived from *Butyrivibriofibrisolvens*are responsible for the isomerisation and hydrolysis of dietary lipids and the conversion of unsaturated fatty acids (UFA) to various partially and fully saturated derivatives, including conjugated linoleic acid (CLA: C18:2 cis-9, t-11) (c), trans vaccenic acid (C18:1 t-11) (VA) and stearic acid (C18:0). Althoughlinoleic (C18:2 n−6) (LA) and linolenic (C18:3 n−3) (ALA) acids are the main UFA in the diet of ruminants, the processes occurring within the rumen ensure that the major fatty acid leaving the rumen is C18:0. The uptake of UFA into the small intestine by ruminants is similar to that in non-ruminant animals, but differs in the case of saturated fatty acids (SFA) (Bauchart, 1993). The intestinal absorption co-efficient of individual fatty acids is higher in ruminants than in non-ruminants, ranging from 80% for SFA to 92% for polyunsaturated fatty acids (PUFA) in conventional low fat diets. The higher absorption efficiency of SFA by ruminants has been attributed to the greater capacity of the bile salt and lysophospholipidmicellar system to solubilise fatty acids, as well as the acid conditions within the duodenum and jejunum (pH 3.0–6.0). The low pH is due to a low concentration of pancreatic hydrogen carbonate which reduces the conversion of SFA into insoluble calcium salts (which cannot be absorbed by the enterocytes). However, triacylglycerol resynthesis in ruminants takes place via the glycerol-6-phosphate pathway due to the virtual absence of 2-monoacylglycerol. The resynthesized lipid is carried as lipoproteins, chylomicrons and very low density lipoproteins (VLDLP) in the blood stream for uptake by the lipoprotein lipase enzyme and incorporation into the tissues. An important difference between non-ruminant and ruminant animals is that in the latter, the long chain PUFA, C20 and C22, are not incorporated to any great extent into triacylglycerols, but instead are incorporated into the membrane phospholipids and will be deposited in significant amounts in the intramuscular tissue (Enser, Hallett, Hewett, Fursey& Wood,1996; Offer, Marsden, Dixon, Speake& Thacker,1999).

Healthiness

The past number of years has been characterized by an increase in consumer interest in their nutrition and health, which has resulted in the development of health directives by governments for some food components, especially fats (Simopoulos, 2001).

Beef, Lamb and mutton contain high concentrations of SFA, so much so that their PUFA:SFA ratio is lower than the recommended minimum value of 0.45 for human diets. The excessiveconsumption of food with a high proportion of SFA is a major predisposing factor to the risk of coronary heart diseases (CHD), hypertension, stroke, diabetes and obesity in humans, which has led to a worldwide decline in red meat consumption (Webb, Casey & Van Niekerk,1994; Moloney, Mooney, Kerry & Troy, 2001). Although the relationship between dietary fat and the in-

cidence of diseases associated with the modern lifestyle are widely documented, especially CHD (Kritchevsky, 1998, 2000) and various cancers (Wood et al., 2003), this has also been challenged in the past few years (McAfee et al., 2010). As an example, in a meta-analysis and review of epidemiological cohort studies, no independent association could be found between the consumption of animal fat and breast cancer (Alexander, Morimoto, Mink & Lowe, 2010). The low PUFA:SFA ratio of ruminants is a consequence of the extensive biohydrogenation of ingested PUFA by the rumen microorganisms, leading to the formation of trans-MUFAs and SFA, which are then incorporated into the lipids in the muscle (Jenkins, 1993).

The degree of saturation of animal fats is influenced by its fatty acid composition (Webb & Casey, 1995). Accordingly, the quality of fat is determined by the fatty acid composition, which affects the palatability and shelf life. As mentioned, a shift in fatty acid composition can be induced by means of dietary manipulation, which will subsequently enhance the nutritional quality of red meat and fat quality. Dietary manipulation strategies are also available that minimise biohydrogenation of ingested PUFA in the rumen (Chikunya, Demirel, Enser, Wood, Wilkinson & Sinclair, 2004).

Another aspect that has been the focus of consumer attention as pertaining to red meat is the level of sodium (Na), due to its correlation with high blood pressure. Epidemiological studies indicate a positive association between excessive intake of Na, blood pressure and prevalence of hypertension (Appel et al., 2006). However, the Na levels in fresh meat are low. Rather, it is frequently the high levels of salt (NaCl) that are added to many processed meats consumed in the western diet that leads to an elevation in the Na levels. However, consumers are generally not always able to distinguish between the Na in fresh meat and that in processed meat.

Convenience

Within the current sophisticated world, the purchasing behaviour of the consumer has changed. Typically in an economically vibrant society, time isofpremium value and the modern consumer prefers the purchasing of a convenient product. This product should either be a ready-to-eat (RTE) one or it shouldbe packaged in such a manner that it requires minimal preparation time.The food industry has largely addressed this consumer desire by developing and producing a variety of RTE products which are now widely marketed in retail outlets across the globe. Since RTE products generally require minimal processing on the part of the consumer, the safety standards for these are normally stringent and are most often addressed by the implementation ofa strong HACCP (Hazard Analysis Critical Control Points) plan in most manufacturing facilities. In addition, a number of novel packaging strategies have been developed to extend the shelf-life of RTE products, such as modified atmospheric packaging (McMillin, 2008).

With food safety and transparency in mind, most countries have nowenactedfood labelling regulations that require that certain information be displayed on packaging, which includes (but is not limited to) a full ingredient list, nutritional composition data,recommended daily allowances (RDAs), as well as the declaration of certain common allergens.A great deal of research, however, has shown that consumers frequently experience difficulty in understanding much of the information presented on food labels (Shannon, 1993; Sadler, 1999; Kempden, 2011). When individuals do not understand labelling or information overload arises, they tend to avoid the presented information altogether (Kaswell&Padberg, 1992) and food labels become an ineffective information source and do not serve as successful purchasing drivers (Kempden, 2011).

New innovative mobile phone technology now allows consumers to selectively acquire additional information on certain characteristics of food products, and in so doing, assists with their interpretation of food labels. Using such applications, individuals are able to scan food product barcodes in store using their cellular phones and they can thereafter browse information relating to the nutritional composition of the product, the farm of origin, the carbon footprint and other pertinent content relating to animal treatment and environmental sustainability. Another technological trend geared towards convenience is shopping on-line, which permits consumers to choose which products they wish to purchase without going into the store.

Although there has undoubtedlybeen an increased preference for convenience foods, there has also been a recent consumer trend towards the purchasing of 'home-grown'or 'locally-produced' products, typically from weekend markets or farm-stalls. The driving ideology behind this trend is that'home-grown' and 'local' is best, with all attributes linked to the modern concepts of organically-produced, carbon foot print and so forth, which encompasses not only the production system but also the value chain as pertaining to transport and packaging. Underlying the purchasing of these products is a belief that they are healthy and safe to consume (Gellynck, Verbeke&Vermeire (2006), which may not always be the case, especially where there are no authorities to ensure that the necessary regulations are adhered to.

Process/production characteristics

The response to animal welfare is largely a citizen response based on extrinsic and intrinsic cues (Grunert, 2006). However, there are an increasing number of consumers who are willing to pay more for a product that is perceived to have been produced in an ethical manner, that includes accepted standards and norms as pertaining to animal welfare (Bennett *et al*., 2002; Napolitano, Girolami&Braghieri, 2010). Of course, the credibility of the authentication authority is of utmost importance as pertaining to the consumer's willingness to pay for the product being endorsed (Martelli, 2009; Van Loo *et al*., 2011). Animal scientists, food scientists and consumers all have different

ideas and perceptions onthe definitions ofmeat quality.There are unquestionably certain congruencies and divergences between producers and consumers (Sepúlveda, Maza&Pardos, 2011), indicating that the flow of information between these two ends of the value chain requires further development. To an animal scientist, quality would be linked to production performance, wherethe welfare aspects would be underwritten by the following five basic principles: adequate air, water, and feed; safe housing and sufficient space; appropriate complexity of the environment; regular supervision and effective health care; sensible handling. On the other hand, for a food scientist, quality would be linked to aspects measurable in the product, such as pH, colour, chemical composition as well as sensory characteristics. For the consumer, the following major dimensions have been identified which are considered as being relevant to the quality of animal products: sensory characteristics, including taste, odour, appearance, texture; healthiness, as animal-based foods are associated with their composition; intake of essential nutrients but these are also frequently deemed as potentially impairing human health (e.g. source of saturated fatty acids, vector of infections or pollutants); convenience, concerning the ease of preparation; and process characteristics, dealing with the way food products of animal origin are obtained, including farming systems, even though these aspects may have no effects on the other quality dimensions (Grunert, Beach-Larsen &Bredal, 2000). Each dimension aims to satisfy consumer purchase motives or values within the corresponding context (Grunert, 2006).

The animal producer is well aware that if they were to abide by the basic principles of animal welfare, the production performance of the animals would be improved. In dairy cattle, for instance, close human interaction with the animal will result in better milk yield (Hemsworth, Coleman, Barnett, Borg & Dowling, 2002). In young gilts, a positive experience with the stockperson will result in larger litter sizes (Hemsworth, Barnett, Coleman & Hansen, 1989), however, an important aspect in this regard would be the animal-stockperson interaction and the attitude of the latter to animals (Hemsworth, 2003).It is therefore in the best interest of the producer to ensure that the animal is comfortable and has all its needs addressed. It is also well known that the animal welfare, especially as relating to the *ante mortem* stress experienced by an animal, will result in a decline in meat quality (Mach, Bach, Velarde& Devant, 2008). However, in the developing world in particular, producers frequently have no inputs into the value chain of the animal leaving the farm *en route* to the abattoir, nor do they have any input on the activities associated during the offloading, lairage and ultimately stunning and killing of the animal.Hoffman and Lühl(2012), for instance,noted that there were numerous factors contributing to the stress (bruising) of cattle during theirtransportation in Namibia that were outside the control of the producer. Additionally, Hoffman and Fisher (2010) found that the condition of the roads influenced the stress experienced by pigs *en route* to the abattoir. Similarly, Huertas*et al*. (2010) also reported that the conditions of the

road influenced the level of bruising and thus the welfare of cattle transported to slaughterhouses in Uruguay. Alam*et al*. (2010) found that the treatment of cattle and spent water buffalos in Bangladesh at the point of sale and during the transport to the abattoir did not adhere to animal welfare guidelines. Most of the welfare malpractices noted was caused by others along the process/value chain and not the producer.It is of further interest to note that as from 1 January 2013, all countries exporting meat into the EU will have to meet the requirements of Council Regulation (EC) No 1099/2009 (2009), which includes requirements in the following areas: the layout, construction and equipment of slaughterhouses, handling and restraining of animals and stunning and slaughter (Cassidy, 2012).

In some cases, the modern marketplace itself places requirements on the producers that are actually detrimental to the animal's welfare, for example, the regulations found in most countriesrequiring that cattle be identified prior to being slaughtered. This practice results in the excessive handling of cattle during mustering in Namibia where the animals need to be hot branded for ownership identification (Hoffman &Lühl, 2012). In the review by Gregory (2008), it was concluded that the additional handling imposed by checking livestock passports needs to be reconsidered and that the use of remote animal identification methods may help solve animal welfare problems associated with the reading of ear tags. For the modern consumer, the idea of a wet market where live animals are kept and slaughtered in public is abhorrent, not only from the perceived inhumane treatment of the animals, but also from the beastiality response invoked when the animal is butchered in public. Gregory (2008) expands on the welfare issues related to wet markets such as excess handling and rudimentary care.

Conclusion

It is clear that as the profile of the modern consumer changes, their requirements for fresh meat and meat products are concurrently modified. Today, more emphasis is being placed on the ethical production of meat and it effect on the environment. Fortunately for the animal producer, ethical production and treatment for animals is positively correlated with good welfare practices. To meet the increasing global demand for animal protein, producers will need to become more scientific in their production systems – even when farming extensively. An area where there will be a rapid increase in the near future will be the genetic selection of animals to ensure that their performance meets the requirements of the consumer.

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Improving Local Feed Resource to Increase Nutrient Availability to Support Sustainable Agriculture

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Abstract

In temperate zones in so- called developed countries much of the agricultural land is used directly for animal feed e.g. grazing.Other crops are grown for winter feed e.g. grass for silage and hay, barley for grain and straw and also turnips and fodder beet. In so-called developing countries in subtropical and tropical zones the main product is human food and the by-products are used are animal feed. In some work it has been shown that it may be possible to improve the quantity and quality of by-products such as straw without affecting the quality and quantity of the primary products. More work is needed on this aspect as plant breeders need to be involved. Also methods of using other means to increase nutrient availability for animals have been discussed such as using duck and fish in rice fields , manure for biogas and composts e.g. total resource management.

Keywords: feed evaluation, by-products

Introduction

Animal Feed in so-called developed and developing countries

In so-called developed countries much of the agricultural land is used for producing feed for animals. In temperate zones animals graze in the summer and other parts of the land are used to provide feed for animals to be used in the winter where animals are kept indoors. The feed for winter maybe silage made from land producing silage from grass or hay from good quality grass. Some of the agricultural land can be used for fodderbeet and turnips for winter feed for animals. Some of the grain is used mainly for animals. The straw may be used as animal feed and bedding in winter and the barley grain used for concentrate feed for the farm animals. Wheat gain is used mainly for human food.

In the following I will concentrate more on animal feed from so-called developing countries in tropical and subtropical areas of Asia , including of course Indonesia. Here the main product from agricultural land is human food while the by-products are used for animal feed such as rice straw, wheat straw, rice bran, wheat bran, cassava leaves, skin of cassava roots etc.While farm animals mainly

cattle, sheep and goats may be grazing this would generally be on non agricultural land such as hills and mountains and under trees in Agroforestry systems . Most of the farmers are small farmers and the rural population is far greater than the urban population.

Improvement of local feed resources for animals

I was asked to speak about how to improve local feed resources and here of course we must speak about the main animal feed i.e. the by-products. Here I have some experience from years ago! We developed a very simple tool to measure the nutritive value of feed for ruminants namely the nylon bag technique (Ørskov and McDonald 1979.) This technique made it possible to very rapidly determine the nutritive value of feed for ruminants and one question that came to mind was whether it was possible to improve the nutritive value of straw without affecting the quality and quantity of grain. We tested 4 varieties of barley, the results of which are given in Table 1 (Reid *et al.,* 1988).

The straw varieties were given ad libitum to steers which also receiving 1.5 kg of concentrate daily. It was very clear that there were quite large differences in live weight gain varying from 106 to 400g/day. Also digestibility varied from 40.1 to 48.4% and feed intake varying from 3.4 to 5.2 kg per day

A student from India also tested 2 varieties of rice straw (Walli *et al* 1998). In the experiment described in Table 1 there were no differences in grain yield but on the whole, plant breeders have paid no attention to the results and not selected for straw quality putting all attention to grain yield and quality. In Indonesia rice straw is probably the most important source of roughage and plant breeders could pay more attention to whether the quality can be improved without affecting the quantity and quality of rice grains. There may well be other by-products as mentioned above which could be improved by selection without affecting the main product. As mentioned before,there are simple techniques such as using nylon bags or gas production which can be used to rapidly access the nutritive value of the feed. Plant

Table 1. Effect of variety of barley on straw utilization by steers also fed 1.5 kg of concentrate per day

Variety	Intake dry matter kg/d	Digestibility	Metabolisable Energy MJ/kg	Growth rate g/d
Gerbil	3.43	40.9	7.23	106
Igri	3.56	41.21	7.16	126
Golden Promise	4.43	45.2	7.35	198
Corgi	5.16	48.4	7.82	400
SE	0.18	1.2	-	40

breeders and animal nutrition experts need to work together to increase the value of the crop by-products.

Storage of by-products

During parts of the year there maybe an excess of by-products so storage is needed. Again there exist many methods which depend on type of feed, climate etc. Straw can be stored easily if dry and straw stacks made so that rain cannot penetrate them. For rice straw there is sometimes a problem as rice is grown in wet fields. It is also possible to store in wet form. If urea is relatively cheap it can be used to spray on the straws at about 4% of dry matter. Then the wet straw containing urea can be covered with plastic the urea will hydrolyze to ammonia which not only will preserve wet straw but also due to the alkali make it more digestible so animals can eat much more of it, maybe 50% more dry matter compared to untreated straw. Some by-products high in soluble carbohydrate maybe preserved as silage if the carbohydrate can ferment to give a low pH.

Type of animal and animal products

For many small farmers small ruminants e.g. goats and sheep are very important. They also serve as security or a type of bank. The feeds for small ruminants are often different from those needed by large ruminants. Goats in particular are very selective and very good at eating leaves from branches of trees which often form part of their food. Many trees particularly leguminous trees e.g. Leucaena, Caliandra, Glyricidia etc, have additional advantages in containing tannins which have anti-parasitic properties and help to reduce problems of gut nematodes and coccidia (Mui *et al* 2005). Milk from goats is generally very much in demand. In many areas goat kids are given milk from cattle as goat milk can be sold for considerably more than cattle milk.

If rice is the main product it is also possible to let ducks do the weeding of the field instead of using herbicides and pesticides. Ducks will excrete manure in the rice field which in turn can fertilize plankton which can be eaten by fish. In Vietnam (Minh *et al* 2006) showed that using this technique farmers income can be increased several fold and even rice yield is increased without the need for herbicides.

Another aspect which can increase income for small farmers is installation of small units for biogas production. These units are very important in Vietnam and India and are increasing in Indonesia.The manure from animals can be fermented to yield methane for cooking and the biogas slurry can be used as a fertiliser or in some areas with plenty of water it can go through fish ponds to feed the plankton for fish production.Thereafter that the water from the fish pond can be used for fertilizer.

Conclusion

There are several options available in so-called developing countries to improve the efficiency and quantity of nutrients for sustainable animal production. Since animal feed here consists mainly of crop by-products more work needs to be done to understand the possibilities of improving the quantity and quality and by-products without affecting quantity and quality of main products for human consumption. It is also necessary to know how best to preserve and improve the quality of by products externally for instance by using urea. Other options depend on soil, climate and socio-economic circumstances.

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Planning Dairy Development Programs in Tropical Asia

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Abstract

There has been a history of dairy development programs in tropical Asia that have either failed or required major revisions, for a variety of reasons. There have been obvious biological factors such as overestimating the level of performance of milking stock, or underestimating the required farm inputs to achieve targets for milk production and reproduction. All too often these programs suffer because of incorrect assumptions on the impact of the tropical environment on cow comfort, animal health, feed quality or cow appetites. However the most common reason is the lack of proper planning, both long and short term. This review discusses planning requirements for three scenarios, namely regional programs, "greenfield" sites and trouble shooting problems on existing farm developments.

Keywords: dairy development programs, on farm dairy programs, planning

Introduction

The last 20 years of dairy research, development and extension in many Western countries has produced quite sophisticated dairy production systems. Herd sizes have grown, efficient feeding systems have evolved and many farmers routinely monitor test results on their cows for milk production, composition and quality and for mastitis. They then use this information for making decisions on culling milking cows and for breeding genetically improved stock. High labour costs have led to much mechanisation, such as machine milking, forage conservation and feeding stock, while cows grazing at pasture are able harvest their own forages more efficiently than can farmers. Low population pressures, hence relatively cheap land, have allowed farms in Western countries to expand in both size and cow numbers.

Unfortunately this has not been the case for small holder dairy farmers in most Asian countries. Being in the tropics, feed quality suffers from high temperatures, humidities and often strongly seasonal rainfall patterns. Dairy cows are temperate

animals with thermo neutral (comfort) zones closer to 10°C than to 30°C. Furthermore, high humidities reduce feed intakes which exaggerate the adverse effects of high fibre forages on appetite. A good measure of heat stress, the Temperature Humidity Index, shows milking cows in the lowlands of the humid tropics to be in the "high stress" and "reduced performance" zones for much of most days throughout the year. Many dairy specialists correctly argue that potentially high performance dairy breeds, such as Friesians, may not necessarily be the best cattle genotype for tropical regions, except in highland areas or those with low humidities.

There are many socio-economic reasons why the efficiency of small holder dairy farming in Asia has not greatly improved over the last two decades. Granted, numbers of cows has greatly increased in most Asian countries, largely through government support for social welfare and rural development programs. The increased demand for milk (accentuated through school milk programs) and the concept of national food security are the driving forces behind most dairy development initiatives. However in terms of feed inputs per kg of milk produced or farm milk outputs, improvements have been slow. This is demonstrated by the inability of virtually all dairy industries in SE Asian to markedly improve their self-sufficiencies in milk over the last 10 years (Moran 2009) hence reduce their reliance on imported dairy products.

In addition to the above biological constraints, the other major problem to achieving national dairy development production targets has unfortunately been a common human failing, namely an inability to properly plan for such initiatives, in the short as well as the long term. This paper discusses this problem at three levels, firstly at a regional dairy program level, secondly a "greenfield" or new farm development level and thirdly, trouble shooting an existing dairy farm that is not performing, even to expectations. Many regional dairy development projects involve the construction of a series of medium to large scale dairy farms (say from 200 to 1000 milking cows) frequently on a "greenfield" site or one with little existing dairy infrastructure. The third level occurs all too often when poor planning has resulted in a new or existing farm that does not achieve realistic production and profit targets.

The importance of long term planning is paramount in any dairy development program. We often hear the comment "Failing to plan is planning to fail". Unfortunately this applies to much of the dairy development around tropical SE Asia.

Planning dairy programs

Regional dairy programs

A common problem with many regional dairy development programs is the desire to introduce the stock long before the infrastructure has been fully prepared to support them. Importing pregnant dairy heifers provides a small window of opportunity for their eventual calving and milk production, but all too often this window is too small to prepare for their change to a lactating cow, requiring optimum feeding

and herd management to settle into their new, often more hostile, environment.

Figure 1 lists ten steps that should be followed in any large scale regional dairy development program. It is essential to organise markets, milk processors, physical and social infrastructure before introducing stock. The actual cost of milk production cannot be determined until the stock are on site and their actual, rather than their predicted, levels of performance and required inputs can be quantified.

An additional step that overrides the success of all those in Figure 1 is a planned and ongoing supply of finances to ensure each step actually occurs "on time and on budget". This requires a long term commitment from financiers well before the program starts. This budget must incorporate realistic levels of cow performance based on local information or estimates and not those from other, generally temperate and hence less stressful, environments. The budget obviously needs to incorporate a cash flow as well as long term loan repayments and should not plan for any profits for several years into the programmed development.

A "greenfield" or new farm development site

Converting a greenfield site into a profitable and sustainable dairy farm also requires careful planning. The steps to take are similar to those in Figure 1 except that several of them would be taken for granted. For example, one would assume that there is an existing market and milk processors (Steps 1 and 2) or at least one that will definitely develop in time to utilise the raw milk from the new farm. Ensuring a sustainable feed supply (Step 3) and suitable staff (both managerial and general farm staff, Steps 4 and 5) are essential prior to introducing the stock.

Step 6, training the staff, could be taken for granted as that would have been ascertained prior to the project starting. The basic facilities must be constructed before the stock arrive. Of greatest importance, the assurance of sufficient and timely supply of finances is essential to ensure the project does not stall at any step along the way.

Probably the most important decision that needs to be made for a greenfield site is the proposed stocking capacity, that is the number of milking cow units to be maintained per ha of forage production area. One milking cow unit is one adult cow plus 20% of its replacement heifer, that is assuming a 20% replacement rate per annum. The farm should aim to supply as much of the annual forage requirements as possible, to give the farm management team more control over the supply and quality of such forages than if they have to be purchased off farm. The hardest part of this decision process is the assumption of annual growth rates of such forages. This has been discussed in detail by Moran (2005), who has concluded that to ensure all forages can be grown on farm, such target stocking capacities should range from 7 to 10 milking cow units per ha forage production area. Once this has been decided, then a more realistic calculation can be made of the total tonnages of forage that need to be purchased. The annual requirements for the other major dairy feed,

namely concentrates (either formulated or sourced as raw ingredients) also needs to be ascertained so that long term sources can be assured early in the project.

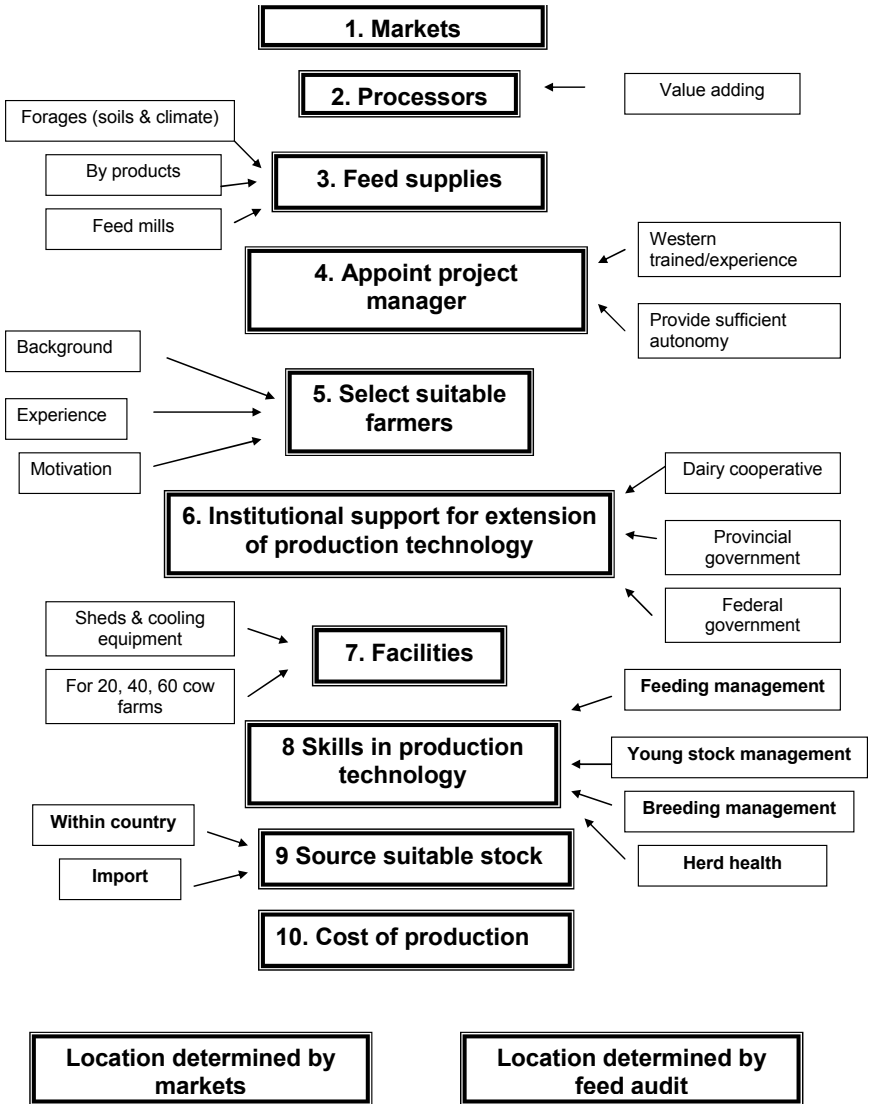

Figure 1. The ten steps to be followed in any regional dairy development program

Net cash flow means an estimate of farm expenses as well as farm income. The majority of cash is generated by the sale of milk. The shape of the lactation curve (Moran 2005) means that this can only be consistent from month to month with careful planning to source dairy stock at several times during the early years of the project, and not all at once. All too often such greenfield projects fail altogether or require major cash flow revision because all the stock were introduced at the same time.

Hitting the "white wall"

The above highlights a classic scenario where "new" farmers enjoy a rapidly increasing cash flow when all cows calf down over a short time frame. The farmer often then increases his cash input, sometimes into lower priority investments, neglecting the most important ones, such as maintaining a high quality (hence high intake) ration as cows approach mid and late lactation and ensuring optimum reproductive performance (using fertile bulls rather than depending entirely on artificial insemination and ensuring all field staff develop skills in heat detection). Persistency of milk production (as quantified by the average monthly decline in milk yield from peak) is one of the often neglected, key measures of success of a feeding program. It should be of the order of 8% rather than the all too common 12% or more (Moran 2005).

All too often herd milk production rapidly decrease as cows move into their less productive phases of their lactation phase, reduced cash flows follow and the farm's net income declines to such an extent that it's long term viability may be at risk. Such scenarios are rarely made public as national pride can be at stake, hence it is often repeated by new, inexperienced investors in dairy development.

Trouble shooting an existing dairy farm

This can cause the biggest problem because errors in design and construction of facilities, shortfalls in supplies of feeds, particularly forages, and inadequacies in managing the stock may have already introduced constraints on potential cow and farm performance. We will discuss this using a theoretical case study based on an actual situation.

A 150 milking cow free stall barn farm was established using pregnant grade Friesian heifers, all imported at the same time, in a hot humid environment in tropical SE Asia. Insufficient area was allocated to forage production and very few staff had had much experience with tropical dairy farming. Within its first 5 months of operation, milking stock were suffering from severe weight loss, stock (cows and calves) were dying, milk yields fell to average only 7 kg/d, cows were not cycling post-partum and there were increasing new cases of mastitis occurring every month.

Over the following 5 months the farm management, with consultant advice, were prepared to invest in a series of farm improvements which had dramatic beneficial effects of cow and farm performance. Milk yields and body condition increased and the cows started cycling. These farm improvements included:

- Developing more area for forage production
- Introducing a mixer wagon to allow for blending the ingredients and mechanical feed delivery
- Concentrating on ration formulation to balance energy, protein and other nutrient supplies

- Introducing a fermentable energy and a rumen degradable protein source
- Incorporating a small amount of rice straw in the diet to provide physically effective fibre
- Formulating lower cost rations to reduce feed costs
- Improving new born calf hygiene and colostrum feeding
- Routinely Californian Mastitis Testing cows followed by antibiotic treatment of subclinical mastitis cases and culling chronically infested cows
- Purchasing bulls for natural mating, rather than planning to practice artificial insemination
- Installing a water sprinkler system and cooling fans for better climate control
- Introducing recording systems, using both note books and computer software, to more closely monitor daily management practices
- Establishing a computer system to quantify milk income less feed costs and the proportion of feed consumed by non-productive stock each day
- Selling off bull calves and cull stock
- Initiating regular faecal and blood sampling and vaccination protocols for better disease management
- Using pregnancy diagnosis and record keeping for better reproductive management
- Importing pregnant stock with a range of expected calving dates
- Improving on farm biosecurity

Over a 12 week period, following these improved management practices:

- The number of cows dying decreased from 5 per week to zero
- Feed intakes increased from 8.4 to 15.0 kg DM/cow/day
- Average milk yields increased from 6.4 to 13.6 L/cow/day
- Body condition scores improved from 2.3 to 5.6 units (out of 8)
- The farm manager signed the consultant up for a further period
- The owner was seen more often with a smile on his face

Importing dairy stock from overseas

Very rarely, if at all, can dairy development programs rely on natural increases of heifers to populate the new regions. Calf mortalities are just too high in most SE Asian countries. For example, Moran (2011) reviewed the published data concluding that a range of 15 to 25% pre-weaning and early post-weaning mortality rates would be typical on many tropical dairy farms, in contrast to the 3 to 5% considered normal on well managed dairy farms in developed temperate countries. Such high mortality rates would also be indicative of large numbers of surviving calves that have suffered permanent health problems leading to reduced lifetime performance. Therefore the only way to source more dairy stock to improve farm and regional milk outputs,

assuming they can be fed adequately, is through an active program of importation. Australia and New Zealand seem to be the countries of choice, although Thailand also has an active dairy heifer export market especially to Malaysia and Vietnam.

When considering importations of dairy heifers, there are two major decisions to be made, namely what genotype is the most suitable and what age should they be on arrival. Unfortunately all too often, the first is considered a "given" by many decision makers who plan dairy development policies. That is, they must be "black and white"! If the dairy region is in the highlands (say above 800 to 1000 m above sea level) and or in a region without extremes in temperature and humidity, this is often the correct decision. However there seems little point in requesting Friesian heifers out of dams that have produced 5000 L milk per lactation, because it is highly unlikely that the imported heifers or their progeny will be managed well enough to achieve such milk yields, particularly if they are to be run on smallholder farms. In most cases, any dairy genotype imported from a developed country is likely to be of higher genetic merit that the typical milking cow in tropical Asia.

Jerseys or their crosses should be seriously considered in tropical dairy systems when climate constraints are apparent and/or when feeding and herd management is very much sub optimum. They are smaller, hence have lower maintenance requirements, have better climatic tolerance (due both to lower milk yields and physical characteristics such as sweat gland density and skin colour) and often better reproductive performance. In areas where premiums encourage farmers to produce milk with higher solids content, Jerseys also outdo Friesians. There are other dairy breeds that seem to perform better than Friesians in the torrid tropics such as Brown Swiss, or synthetic breeds such as Australian Friesian Sahiwals or the Girolanda (from Brazil), while the purebred Sahiwal (from Pakistan) justifies further consideration.

The other decision to make is whether to import pregnant heifers or yearling (virgin) heifers. Pregnant heifers are the most favoured because farmers get "two for the price of one", assuming the foetus is a dairy genotype. In addition as the heifer is pregnant (at least diagnosed as pregnant) she does not have to be mated soon on arrival at her new home where there is no guarantee that she will easily conceive. However with only a few months to adapt to her new environment, there is also no guarantee that that heifer will become a long term member of the milking herd once she calves down. All too often one hears stories of very high numbers (up to 30 or 40%) of imported heifers being culled and slaughtered after having only one calf. The most likely reason is that her poor feeding management post-calving and her higher genetic propensity to utilise body reserves to produce milk, have combined to result in anoestrus for many, many months post calving. Such animals have become very expensive dairy beef animals. In the long run, yearling heifers may be better economic propositions than pregnant heifers.

Lastly and of equal importance when importing dairy stock into tropical Asia, are issues of animal health. All countries, both importing and exporting countries

have disease management protocols. Such protocols must be strictly enforced and regularly reviewed, in case of new disease outbreaks in countries of origin. Foot and mouth diseases and brucellosis are the two most commonly talked about but there are others to consider. In one recent example, two diseases, namely bovine viral diarrhoea (BVD) and infectious bovine rhinotracheitis (IBR) were isolated in virtually every aborted foetuses arising from one importation. These can have long lasting adverse impacts on cow performance so require additional surveillance to ensure they do not enter the country with the consignment.

Conclusions

Dairy farming would have to be one of the most sophisticated forms of livestock production in the world and should only be undertaken after careful and logical planning, and with expectations of a long term investment before profits accrue. To be successful, such development programs must involve sourcing, or at least seeking support from, personnel with proven experience in both dairy farming practices and dairy farm business management. Much can be learnt from the litany of failed dairy development projects throughout tropical Asia so these mistakes will not be repeated, as unfortunately occurs all too often.

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Carrier Proteins in Milk: Basic and Potential Applications

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Introduction

Milk is an essential and perfect food for mammalian infants to promote their appropriate and healthy development during the lactation period. The biological role of milk is ensured by a broad spectrum of ingredients such as carbohydrates, lipids, proteins, minerals, and vitamins, which provide a rich nutritional source and confer passive immunity to newborns. Besides, some of milk components act as "messengers" to trigger innate behaviors (e.g., suckling behavior) (Schaal *et al*., 2003) and metabolic development (e.g., thermogenesis in adipose tissues) (Hondares *et al.*, 2010) in newborns. Among the milk components, several milk proteins play significant roles in carrying nutritional and bioactive substances. Caseins, the most abundant milk proteins, may be taken as carrier proteins in a broad sense, because many bioactive peptides are encrypted within its sequence and are released by the action of digestive enzymes in the gastrointestinal tract of infants (Meisel and Bockelmann, 1999), and moreover they can bind calcium ions. However, only carrier proteins that are capable of binding nutritional or bioactive substances in whey are focused here.

Whey is a clear and either almost no color or shallow yellow liquid which can be obtained by a removal of casein from skimmed milk, containing carbohydrates, peptides, proteins, minerals, and vitamins. It is well known that the major whey proteins are immunoglobulins, serum albumin, α-lactalbumin, and β-lactoglobulin (lacking in human and camel milk), however, recent proteomic research revealed that approximately 150 proteins are present in bovine whey including these major whey proteins (D'Amato *et al*., 2009). There are indeed many binding proteins in milk, including carbohydrate-, ion-, vitamin-, and lipid-binding proteins, some of which are supposed to be carrier proteins. Common abilities of the carrier proteins are in binding and solubilization of poorly water-soluble substances, which are widely diverse, and hence their physiological functions usually show very broad spectra.

In this paper, basic knowledge of biochemical properties and physiological functions of major carrier proteins so far known in milk (Table 1) are reviewed, and then their potential use for application is discussed.

α_1-Acid glycoprotein

α_1-Acid glycoprotein (AGP) is a heavily glycosylated 41 – 43 kDa protein with a p*I* of 2.8 –3.8 (Fournier *et al*., 2000). Molecular mass of protein moiety of bovine AGP is 23 kDa, therefore its carbohydrate content accounts for about 45% of the total weight of the whole molecule. AGP is expressed in hepatocytes and secreted into blood; its expression level increases several-fold in acute phase response to systemic reaction caused by tissue injury, inflammation, pathogenic infection, and trauma (Ceciliani *et al*., 2005). It is likely that several AGP isoforms in milk are not derived from blood plasma but from mammary epithelial cells and somatic cells (Gendler *et al*., 1982; Ceciliani *et al*., 2005). Extra-hepatic expression of AGP in protein and mRNA levels has been reported in a wide variety of cells and organs, supporting a hypothesis that an acute phase response may take place in extra-hepatic cell types and may be regulated by inflammatory mediators as observed in hepatocytes (Fournier *et al*., 2000).

The entire picture of physical functions of AGP remains unknown, however, it is undoubted that one of the most important biological functions of AGP is immunomodulation, and large part of this function is owing to the carbohydrate moiety. On the other hand, the protein part of AGP exhibits a common structural fold of lipocalin, a protein superfamily, of which members share a conserved β-barrel that consists of eight anti-parallel β-strands (Fig. 1). There is an internal cavity in the β-barrel structure of lipocalin, and various small hydrophobic molecules can be captured in there. Indeed, AGP has also an internal cavity as is known that AGP in blood plasma is capable of binding wide variety of compounds such as histamine (Chachaj *et al*, 1980), melatonin (Morin *et al*., 1997), serotonin (Schmid *et al*., 1973), and vanilloids (Szallasi*et al*., 1992). Furthermore, AGP can bind mainly neutral and basic drugs like tamoxifen (Schmid *et al*., 1973) and propanolol (Albani *et al*., 1984), via hydrophobic and electrostatic interactions, suggesting that AGP expression level in blood may affect pharmacological effects of exogenous drugs (Fournier *et al*., 2000).

AGP is secreted at a concentration of 162 ± 63.7 mg/L in bovine colostrum at 12 h postpartum, but it is no longer detectable at 3 d postpartum (Ceciliani *et al*., 2005). Secretion of AGP from neutrophils into milk was enhanced within 15 min by an exposure of Zymosan activated serum and phorbol 12-myristate 13-acetate, which mimics inflammatory activation (Rahman *et al*., 2008), indicating that at least neutrophils-derived AGP in milk is also involved with the anti-inflammatory activity. Natural ligands of AGP in milk are unknown.

Apolipoprotein

Apolipoproteins (apo-) are produced by hepatocytes and secreted into blood.

They form water-soluble macromolecules, lipoproteins including chylomicron, very low density lipoprotein (VLDL), intermediate density lipoprotein (IDL), low density lipoprotein (LDL), and high density lipoprotein (HDL), making complexes with lipids (triglycerides, cholesterol, and phospholipids) and one or more specific proteins (Mahley *et al*., 1984). They play crucial role in transporting and targeting lipids to the appropriate destination in the body. So far more than ten classes and subclasses are known: apo A (apo A-I, apo A-II, apo A-IV, and apo A-V), apo B (apo B48 and apo B100), apo C (apo C-I, apo C-II, apo C-III, and apo C-IV), apo D, apo E, apo H, apo J (clusterin), apo L, and apo M. Since a crystal structure of human apo E has first been solved to be an unusually elongated (65 Å) four-helix bundle, with the helices apparently stabilized by a tightly packed hydrophobic core that includes leucine zipper-type interactions and by numerous salt bridges on the mostly charged surface (Wilson *et al*., 1991), similar structures of human apo A-I (Ajees *et al*., 2006; Fig. 1), human apo C-I (Rozek *et al*., 1999), and human apo C-II (MacRaild *et al*., 2004) have been reported. Crystal structure of recombinant human apo M, which is a 25-kDa HDL-associated apolipoprotein, in complex with a fatty acid has recently been solved to be the typical lipocalin fold (Sevvana *et al*., 2009).

In bovine milk, apo A-I, apo A-II, apo A-IV, apo C-III, apo E, apo H, and apo J have been found by proteomic analysis (Yamada *et al*., 2002; Reinhardt and Lippolis, 2008; D'Amato *et al*., 2009; Senda *et al*., 2011; Table 1). Fong *et al*. (2007) have identified apo A-I and apo E to be minor components of the milk-fat-globule membrane (MFGM), which is surrounding lipid droplets budded off from the apical side of mammary epithelial cells. Besides liver, expression of several apolipoproteins has been observed in adrenals, brain, intestine, testis, and widely in other peripheral tissues (Elshourbagy *et al*., 1985; Powell *et al*., 1987; Boyles *et al*., 1990), but expression in mammary tissues is not evident, and hence apolipoproteins in milk are most likely derived from blood as observed in mice (Monks *et al*., 2001). Physiological functions of apolipoproteins in milk are supposed to be lipid solubilizer and transporter.

Lactoferrin

Lactoferrin (Lf) is a member of transferrin superfamily along with serum transferrin, ovotransferrin, and melanotransferrin, all of which function in iron transport and in inhibition of carbonic anhydrase (García-Montoya *et al*., 2012). Lf is produced by mucosal epithelial cells in various mammalian species and secreted into biological fluids including tears, saliva, vaginal fluids, semen, nasal and bronchial secretions, bile, gastrointestinal fluids, urine, milk, and colostrum (García-Montoya *et al*., 2012). Lf exhibits multiple physiological functions in relation to nutritional regulation and innate defense, such as regulation of iron absorption, immunomodulation, antioxidant and antimicrobial activities, and anti-carcinogenic and anti-inflammatory properties (García-Montoya *et al*., 2012).

Lf is approximately 80 kDa glycoprotein folded into two symmetrical lobes, N- and C-lobe, which are connected by a hinge region containing α-helix (Fig. 1). Each robe consists of two similar domains, domains I and II, which are composed by α-helices and β-strands. Each lobe can bind di- and trivalent cations with very high affinities, e.g., 10^{23} M^{-1} at pH 7.4 for Fe^{3+} (Aisen *et al*., 1978). Lf content in human milk is 1 to 3 mg/ml, of which only 3 to 5% are saturated by iron that causes their strong chelating ability and antimicrobial activity.

β-Lactoglobulin

β-Lactoglobulin (β-Lg), one of the major bovine whey proteins, is known to bind retinol, fatty acids, and a wide variety of hydrophobic compounds including serotonin (Taheri-Kafrani *et al*., 2011), however, its specific physical function is still unclear. Molecular weight of β-Lg is approximately 36 kDa, as being a dimer under physiological conditions, but it tends to dissociate into monomers at pH 2 to 3 (Sawyer and Kontopidis, 2000). β-Lg is also a member of the lipocalin superfamily. It is synthesized in mammary gland and secreted into milk at a concentration of 14.3 ± 4.6 g/L in the first milking sample, and then its concentration rapidly decreased over several days and reached a stable value of 2 to 4 g/L in the mature milk in case of cows (Levieux and Ollier, 1999). However, it is not detectable in human milk through the overall lactation period, supposed to be the major reason for cow's milk allergy in human infants. It should be pointed out that β-Lg is not universally distributed in mammalian milks, as it lacked in human and camel milks, and even exist, the ligand specificity is variable, e.g., horse and pig β-Lgs do not bind fatty acids (Pérez *et al*., 1993). Judging from these facts, a specific and significant physiological function of β-Lg seems to be implausible, although several possible roles including antimicrobial activity (Ouwehand *et al*., 1997), carrier of encrypted bioactive peptides (Mullally *et al*., 1997), and retinol transport (Pervaiz and Brew, 1985) have been suggested.

Odorant-binding protein

Odorant-binding protein (OBP), a member of the lipocalin superfamily, was first discovered as an abundant protein in bovine nasal mucosa in the early 1980's (Pelosi and Pisanelli, 1981). To date, among mammals, OBPs have been found in nasal circumstances of human, bovine, porcine, rabbit, elephant, dog, rat, mouse, and porcupine. OBP is capable of binding several odor molecules, such as a bell pepper odor, 2-isobutyl-3-methoxypyrazine, with sub-micromolar range of dissociation constants. OBP was initially assigned to solubilize hydrophobic odor molecules in aqueous mucosal layer prior to their reach to olfactory receptor, so-called "perireceptor event". Although intensive studies have been performed to date, the physiological function of OBP is still unclear.

Recent studies revealed the presence of OBP-like protein in bovine colostrum

and mature milk (D'Amato *et al*., 2009; Fukuda *et al*., 2009). This protein, bovine colostral OBP (bcOBP), showed 52% of sequence similarity against bovine nasal OBP. Secretion of bcOBP into milk was constant on the day of parturition until at least 10 d postpartum at a concentration of 181 ± 39 µg/L (Japaridze *et al*., 2012). Besides milk, bcOBP occurred in the nasal mucus, saliva, amniotic fluid, vaginal discharge, and blood plasma. Despite its low concentration, the distribution pattern and the finding that bcOBP harbored a characteristic sequence motif, CxxxC, which is conserved among insect and mammal pheromone binding proteins, suggest that bcOBP functions as a pheromone carrier (Japaridze *et al*., 2012). In sow milk, an odorant-binding protein was also found to be a potential pheromone carrier (Guiraudie-Capraz *et al*., 2005). Cross-reactivity of the monoclonal antibody against bcOBP was also found in the colostrum of farm animals belong to Cetartiodactyla, such as sheep, goat, camel, and pig (Japaridze *et al*., 2012).

Retinol-binding protein

Retinol-binding protein (RBP), a member of the lipocalin superfamily, is expressed in various cells and tissues. RBP binds retinoids including vitamin A and retinoic acid, which are essential for multiple physiological processes, ranging from vision to embryonic development, with high selectivity and affinity (Noy, 2000). RBP is also known to make a complex with transthyretin (see below). Among RBP subtypes, RBP4, which is synthesized in hepatocytes and adipocytes, is secreted into blood and transferred to various tissues *via* cardiovascular system and into milk. RBP4 is known to act as an adipokine with its increasing concentration in subjects with impaired glucose tolerance or type 2 diabetes (Cho *et al*., 2006). Elevated serum RBP4 causes insulin resistance, which seems to occur by an indirect inhibition of insulin signaling in adipose tissue through activation of pro-inflammatory cytokines in macrophages (Norseen *et al*., 2012).

During gestation period, vitamin A has a significant impact on the healthy development of lung of the fetus (Böhles, 1997). Despite that the nutritional importance of vitamin A is relevant, it is still unclear that RBP in milk can affects the nutritional status of the newborn throughout the breastfeeding period, because no clear relationship has been observed between plasma vitamin A level in mother and that in her neonate (Bhaskaram *et al*., 2000; Scaife *et al*., 2006).

Serum albumin

Serum albumin (SA) is an all-α protein whose dominative secondary structure is α-helix (Fig. 1). SA consists of three albumin domains, one of which contains five or six internal disulfide bonds. It belongs to albuminoid superfamily including α-fetoprotein and vitamin D-binding protein as member proteins. Molecular mass and p*I*

of mature bovine serum albumin (BSA) are 66 kDa and 4.7, respectively, containing 17 disulfide bridges and a free thiol group. BSA is supposed to be expressed in hepatocytes and secreted into milk *via* the bloodstream. SA can bind many molecules including monovalent and divalent ions, fatty acids, hormones, bilirubin, and water molecule. SA possibly plays significant roles in the maintenance of colloid osmotic pressure (Blunt *et al*., 1998) and in zinc transport (Smith *et al*., 1979) in blood, but its physiological function is controversial in milk, although thyroxine (Etling and Gehin-Fouque, 1984), zinc, and copper (Lönnerdal *et al*., 1982) have been found associated with SA in milk.

BSA is known as one of the major milk allergens that induce immediate allergic symptoms in allergic children (Goldman *et al*, 1963a; Goldman *et al*, 1963b). Conformational or sequential epitopes, which located on the loops connecting the α-helices, for the specific recognition of immunoglobulin E toward BSA are integrated by enormous number of the internal disulfide bonds (Restani *et al*., 2004). Although increasing digestibility of food allergens including BSA can decrease their allergenicity to some extent, it is still challenging to have hypoallergenic foods by technological treatments.

Transthyretin

Transthyretin (TTR), formerly prealbumin, is a homotetrameric transporter protein that can bind thyroxine, a thyroid hormone increasing the cell metabolism, and RBP-retinol complex resulting in stabilization of retinol-binding to RBP (Goodman and Leslie, 1972; Robbins, 1975). Molecular mass of the monomer is ranging from 14 to 16 kDa with glycosylation. Each monomer folds into a β-sandwich topology composed by two sets of four antiparallel β-sheets, which are connected by a short helical fragment (Blake *et al*., 1978). The dimeric structure is shown in Fig. 1. The tetramer is formed by assemble of the two dimers linked by hydrogen bonds and hydrophobic contacts. TTR is produced in the liver and excreted into blood (Felding and Fex, 1982). It is most probable that TTR is secreted into milk from the blood.

TTR binds and transport thyroxine accounting for 15 to 20% and up to 80% of the total thyroxine in the blood and the central nervous system, respectively (Hagen and Elliott, 1973). On the other hand, TTR concentration in serum negatively responds to the acute phase reaction (Dickson *et al*., 1982). Unusually low levels of TTR in patients with transthyretin-associated amyloidosis and a portion of at-risk individuals for this condition have been observed (Skinner *et al*., 1985), leading subsequent research effort for TTR to focus on the medical and clinical fields. Synthesize of TTR observed in the gastrointestinal mucosa of human fetus (Gray *et al*., 1985) may suggest a possible physiological function of TTR in milk to be a thyroxine transporter.

Vitamin D-binding protein

Vitamin D-binding protein (DBP), also known as Gc-globulin, binds more than 90% of lipophilic 25-hydroxyvitamin D, which is the principal circulating vitamin D metabolite in blood (Millen and Bodnar, 2008). DBP binds actin as well, indicating it may functions as a scavenger of monomeric actin released during cell lysis (Harper *et al*., 1987). DBP belongs to the albuminoid superfamily as same as SA. Molecular mass of bovine DBP is approximately 53 kDa with glycosylation. DBP was first discovered in bovine colostrum by Hollis & Draper (1979). Its expression level was evaluated to be 250 and 6 μg/mL in bovine colostrum and mature milk, respectively (Ena *et al*., 1992). DBP is synthesized in the liver and likely to be secreted into milk *via* the bloodstream (Hollis *et al*., 1981).

Possible physiological function of DBP in milk underlies normal bone formation in neonatal calves, in that supplemented vitamin D metabolites promote calcium absorption in the gastrointestinal tract (Mitchell and Lyles 1990; Cao *et al*., 2009). Normally little 25-hydroxyvitamin D passes into breast milk from the blood that causes vitamin D deficiency in the newborns (Kovacs, 2012). Vitamin D status in milk does not affect on calcium concentration of lactating mothers accustomed to a low calcium intake (Prentice *et al*., 1997), and moreover maternal adaptations during pregnancy and lactation appear to provide calcium to fetus and neonate without relying on vitamin D (Kovacs, 2012). Therefore, contribution of DBP to osteogenesis of neonates seems to be limited just in solubilization of 25-hydroxyvitamin D especially in the very short period of postpartum.

Possible Applications of Carrier Proteins in Milk

As described above, carrier proteins in milk exhibit a wide variety of biological functions. Safety of bovine milk is moreover guaranteed by a long eating experience, encouraging their application use in various areas including food industry, drug development, and clinical treatment. Several types of possible applications have to date been examined: (i) antibiotics, (ii) nutrient and drug carriers, (iii) immune bodies, and (iv) biomarkers.

Despite Lf promotes the growth of health-beneficial microorganisms such as *Lactobacillus* and Bifidobacteria (Sherman *et al*., 2004), it exhibits a broad spectrum of antimicrobial activity against Gram-positive and -negative bacteria, fungi, yeast, viruses, and even parasites. The iron binding ability of Lf confers antimicrobial activity, as being sequestering iron from the target pathogenic bacteria (Zarember *et al*., 2007; Valenti *et al*., 2011). Also Lf and its peptic peptide, lactoferricin, directly interact with cell surface of Gram-negative bacterial and can damage their outer membrane (Yamauchi *et al*., 1993). Indeed, many commercial uses of Lf and its derivatives have already existed on the basis of the fact that they exhibit broad bioactivities including antimicrobial, anticancer, anti-inflammatory, and immuno-

modulatory (García-Montoya *et al*., 2012). In terms of antimicrobial activity of Lf, for example, it offers applications in food preservation and safety with decreasing bacterial counts (Naidu, 2002).

Remarkable achievements have recently been reported in the development of nanoparticles, using milk carrier protein derivatives, which can deliver nutrients or drugs to the target sites efficiently. BSA-dextran conjugates prepared by Maillard reaction can bind ibuprofen through hydrophobic and electrostatic interactions, resulting in suppression of the precipitation of ibuprofen (Li and Yao, 2009). Nanoparticles of the BSA-dextran-ibuprofen complex can be formed by heat treatment. BSA nanoparticles coupled with cyclodextrin were indicated to be available for nasal delivery of anti-Alzheimer drug tacrine (Luppi *et al*., 2011). Octyl modified SA can form a core-shell structure in aqueous media by self-assembling due to core segregation and a combination of intermolecular forces (Gong *et al*., 2009), suggesting for intravenous water-insoluble drug delivery. Electrostatic nanocomplexes consisting of β-Lg and pectin can entrap and protect docosahexaenoic acid molecules with a very good colloidal stability, suggesting that they are useful tools for enrichment of acid non-fat clear drinks (Zimet and Livney, 2009). Lf conjugated poly (ethyleneglycol)-poly (lactide) nanoparticles was demonstrated to promote the uptake of intravenously administrated coumarin-6 into rat brain (Hu *et al*., 2009).

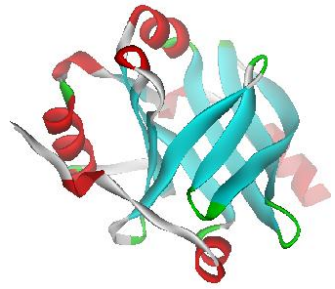
Taking advantage of the structural stability of β-barrel scaffold and the high structural plasticity of ligand binding site of lipocalin, site-directed random mutagenesis and selection *via* phage display against prescribed molecular targets were done, and by this means it was possible to confer antibody-like activities to lipocalin (Skerra, 2008). This artificial lipocalins with novel ligand specificities were termed as "anticalins", which have advantages in robustness, monovalency, flexibility, and broad affinities towards target molecules, compared with conventional antibodies. This approach is highly potential to provide many applications such as reagents for biochemical research and new drugs for medical therapy. Recently, a new anticalin against estradiol was successfully established using bilin-binding protein, a member of the lipocalin family, derived from *Pieris brassicae* (Liu *et al*., 2012). There have so far been reported on anticalins constructed by using the bilin-binding protein, human apo D (Vogt and Skerra, 2004; Eichinger *et al*., 2007), human neutrophil gelatinase-associated lipocalin (NGAL) (Goetz *et al*., 2002), and human tear lipocalin (Breustedt *et al*., 2005).

Several milk carrier proteins are highly expected as biomarkers for mastitis. Apolipoproteins are expressed in bovine milk with experimentally induced coliform mastitis (Boehmer *et al*., 2010), and furthermore, expression level of isoforms of the bovine prostaglandin D synthase also increase in bovine milk with mastitis (Baeker *et al*., 2002). Despite no direct clinical relevance has been elucidated, some proteins found in relation to human diseases are present in milk. Apo A-I localized in bronchial mucosa was found as a biomarker of chronic obstructive pulmonary disease

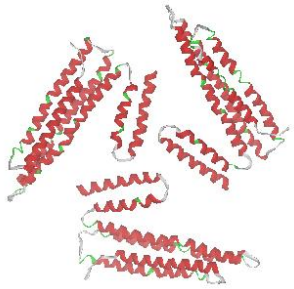
Table 1. Carrier proteins in bovine milk

Protein*	Molecular mass (kDa)	Oligomerization	Glycosylation	Ligands	Physiological functions
a1-Acid glycoprotein	23	monomer	yes	lipophilic compounds/steroids	immunomodulation/anti-inflammation
Apolipoprotein A-I	30	monomer	no	cholesterol	lipid transport/cholesterol and steroid metabolism
Apolipoprotein A-IV	43	monomer	no	fatty acids	lipid transport
Apolipoprotein C-III	11	monomer	no	fatty acids	lipid transport
Apolipoprotein E	36	monomer	no	fatty acids	lipid transport
Apolipoprotein J (Clusterin)	51	dimer/tetramer	yes	fatty acids	lipid transport/chaperone
Fatty acid-binding protein	15	monomer	no	lipophilic compounds/ fatty acids	lipid transport
b-Lactoglobulin	20	monomer/dimer	no	cholesterol/detergents/ fatty acids/ indoleamine/ vitamins	unknown
Lactoferrin	78	monomer/dimer/ trimer/tetramer	yes	iron	iron transport/antimicrobial activity
Lipopolysaccharide-binding protein	54	unknown	yes	lipopolysaccharide	antimicrobial activity
Odorant-binding protein-like (bcOBP)	20	dimer	no	unknown	pheromone transport?
Retinol-binding protein 4	23	monomer	no	retinol	vitamin transport
Secretoglobin, family 1D, member 2	11	dimer	yes	steroids?	unknown
Serotransferrin	78	monomer	yes	iron	iron transport
Serum albumin	70	monomer/dimer/ tetramer	no	Ca2+/Zn2+/Na+/K+/fatty acids/ hormones/ bilirubin/water	actin binding/ion transport/ regulation of colloidal osmotic pressure of blood
Similar to lipocalin	23	unknown	unknown	unknown	unknown
Transthyretin	16	tetramer	yes	thyroid hormone	hormone transport
Vitamin D-binding protein	53	unknown	yes	vitamin D	vitamin transport

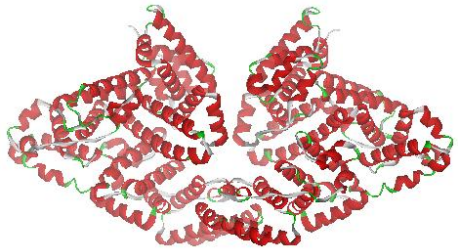
*Selected from D'Amato *et al*., (2009).



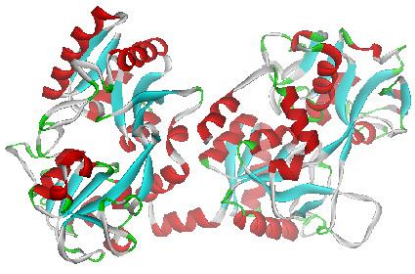
Human α_1-acid glycoprotein
(3KQ0)



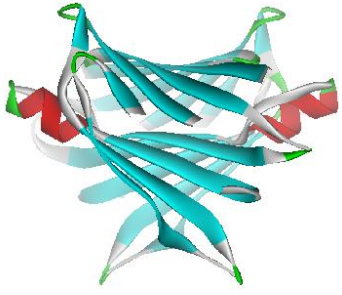
Human apolipoprotein A-I
(2A01)



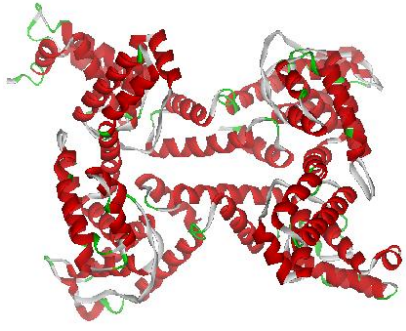
Bovine serum albumin
(3V03)



Bovine lactoferrin
(1BLF)



Human transthyretin
(3U2I)



Human vitamin D-binding protein
(1J78)

Figure 1. 3-D Structures of Milk Carrier Proteins

(Nicholas *et al*., 2010). Folate binding protein (FBP), which has been found in human and bovine milk (Metz *et al*., 1968; Ford *et al*., 1969), is a glycoprotein capable of binding folic acid and its analogues. FBP serves as biomarker and also mediates targeted therapies in cancer and inflammatory diseases, because it contains multiple *N*-glycosylation sites selectively expressed in tissues and body fluids (Mathias *et al*., 1996; Leamon *et al*., 1999; Smans *et al*., 1999; Jaiswal *et al*., 2012). NGAL, also a member of the lipocalin superfamily, is expressed in bovine mature milk with a concentration of 1 μg/mL (van Veen *et al*., 2006). NGAL is known to be a potential biomarker of acute renal injury after cardiac surgery (Mishra *et al*., 2005).

Conclusions

Carrier proteins in milk are essential for nutrition transfer, especially of poorly water-soluble compounds, from mother to her neonate. Their structural features are likely to be categorized into five: apo A-I type, albuminoid superfamily, lipocalin superfamily, transferrin superfamily, and transthyretin type, with the molecular mass ranging from 10 to 80 kDa. They bind ligands with a wide range of affinities mainly through hydrophobic and electrostatic interactions. Applications of milk carrier proteins examined to date are not primarily focused on solubilization and delivery of hydrophobic compounds, but on antimicrobial and immunological activities, owing to their important biological functions. Some challenges have been successful in this context, however, a lot of room remains to be elucidated. Potential of modified milk carrier proteins for nutrient and drug carriers and of natural milk carrier proteins for biomarkers of human and animal diseases are of particular interest in the future.

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Indonesia Farm Animal Genetic Resources in Adapting to Climate Change

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In developing countries, most people depend on agriculture. Crops and livestock are usually produced on the same farm with small plot of land. Grazing animals in open grassland also exist but its contribution in terms of animal products is relatively much less when compared to mixed crop animal farming system (Chantalakhana and Skunmun, 2002). Furthermore they stated that most farmers in developing countries can be classified as smallhorders, mostly live in rural areas and some in peri-urban. The role and contribution of animals in rural farms are far more complex than that in spesialized commercial farm since there exist so close and sophisticated relationships among farm family, animals, crops, social and agriculture.

Agriculture must become more productive if it is to feed a much larger world population. In responding to the daunting environmental challenges ahead, the combined effect of population growth, strong income growth and urbanization is expected to result in almost the doubling of demand for food, feed and fiber (FAO, 2009)

The Role of Livestocks

In 2050 world population is projected to rise to 9.1 billion from a current 6.7 billion, as results it require a 70-percent increase in farm production (FAO, 2009). Based on FAO (2008) report, the livestock sector has great potential to contribute to poverty reduction and the achievement of the Millennium Development Goals (MDG). Agricultural growth can be highly effective in reducing poverty as the largest share of the world's poor live in rural areas. Livestock provide food and income to the majority of the 1.2 billion people living on less than $1 per day. Demand for livestock products is growing fast in developing countries, faster than demand for staple crops, and will continue to do so in the foreseeable future. This demand growth can provide significant opportunities for many rural and peri-urban poor to increase returns from their livestock resources. However, effective and coherent pro-poor public policies, alleviating both institutional and technical constraints, are crucial to capitalise on the pro-poor opportunities offered by the livestock sector.

The FAO State of Food Agriculture (SOFA) 2010-2011 report stresses the importance of livestocks to the livelihoods of around one billion poor people. Livestock provides income, high-quality food, fuel, draught power, building material and fertilizer, thus contributing to food security and nutrition. For many small-scale

farmers, livestock also provides an important safety net in times of need. However, the FAO stressed the need for substantial investments and stronger institutions at global, regional, national and local levels, to ensure that continued growth of the livestock sector contributes to livelihoods, meets growing consumer demand and mitigates environmental and health concerns (FAO, 2010).

The livestock sector is one of the fastest growing parts of the agricultural economy. Livestock contributes 40 percent of the global value of agricultural production and supports the livelihoods and food security of almost one billion people. Globally, livestock contributes 15 percent of total food energy and 25 percent of dietary protein. Products from livestock provide essential micronutrients that are not easily obtained from other plant food products (FAO, 2010).

Rising incomes, population growth and urbanization are the driving forces behind a growing demand for meat products in developing countries—and they will continue to be important. To meet rising demand, global annual meat production is expected to expand from 228 currently to 463 million tons by 2050 with the cattle population estimated to grow from 1.5 billion to 2.6 billion and that of goats and sheep from 1.7 billion to 2.7 billion, according to FAO estimates (FAO, 2010).

Strong demand for animal food products offers significant opportunities for livestock to contribute to economic growth and poverty reduction. But many smallholders are facing several challenges in remaining competitive with larger, more intensive production systems (FAO, 2010).

Livestock can play an important role in both adapting to climate change and mitigating its effects on human welfare, FAO said (FAO 2010). To realize the sector's potential to contribute to climate change mitigation and adaptation based on enhanced capacities to monitor report and verify emissions from the livestock production new technologies will need to be developed.

Loss of Farm Animal Genetic Resources (FAnGR) Diversity

In general, Local breeds of domestic animals are much better suited to the conditions of biotic and abiotic stress than those improved breed. In addition, the local breed also reflect the cultural and historical identity of communities that have developed and continues as an integral part of life and traditions of various societies. A long with the advance of agriculture industrialized through, the indigenous breeds are disappearing in favor of modern high productivity but low ecological plasticity (Maxim *et al*., 2011).

In the last few decates, Farm Animal GenetIc (FAnGR) diversity has rapidly decline, mainly due to changing market demand and intensification of agriculture. Agriculture is shifting away from small production system to large commercial systems, and as a result, selection goals and production environment are now very similar throughout the world (Prentice and Anzar, 2010). According to the FAO,

approximately 20 percent of the world's breed of cattle, goat, pigs, horses and poultry are currently at risk of extinction, and at least one livestock breed become extinct per month over the past several years, resulting in its genetic characteristics being lost forever (Buerkle, 2007). So it is importance to conserve and maintain animal genetic resources to ensure the ability to respond to selection plateaus, consumer demand changes but more importantly biosecurity, environmental and food safety and potentially useful genes available in the gene pool (Pereira and Marques, 2008; Andrabi and Maxwell, 2007)

Genetic erosion of domestic animal diversity has placed 30% of the world breeds at risk of extinction often as a results of goverment policy / programs. Conservation and sustainable development of Animal Genetic Resources (AnGR) requires a broad focus that includes the many adaptive breeds that survive well in the low external input agriculture typical of developing countries (Drucker *at al*., 2001).

Major Indonesia FAnGR

The Bali breed is one of the four existing indigenous cattle breeds (Aceh, Pesisir, Madura and Bali) in Indonesia. The Sumban-Ongole and Javan-Ongole may also be considered local breeds. Although no official historical records exists, it is generally accepted that the Bali cattle is the domesticated direct descendant of the wild Banteng still surviving as an endangered species in three National Wild Reservation Parks (Ujung Kulon, Baluran and Blambangan) in Java (Martojo, 2012). Bali cattle still represents 27% of the total cattle population in Indonesia, and it is considered the pillar breed for small farmers (Purwantara *et al*., 2012) In oder to study the origin of of Indonesian cattle Muhamad *et al*. (2009) utilized the Y-chromosomes (Y), mitochondrial DNA (mt) and autosomal microsatellite alleles (µst). They concluded that Bali cattle is clearly separate from other breeds of catle. Noor *et al*. (2001a) using the isoelectric focussing method concluded that the Bali cattle has a unique β^{Bali} haemoglobin band that does not exist in *Bos taurus* cattle (Limmousin, Simmental and Brangus). Furthermore, Handiwirawan *et al*. (2003) found that The A and B alleles at INRA035 microsatelite locus are monomorphic and can be used for a spesific marker for Bali cattle. Allele A at locus HEL9 that has high frequency (92.90%) in Bali cattle and 100% in Banteng can also be used a supporting marker. All of the studies indicate that Bali cattle are native to Indonesia and have been domesticated in Indonesia.

In general, the productivity of native animals in the tropics is low, but their adaptability and survival in poor environments my be quite satisfactory. In many tropical countries, attempts to increase productivity have been made by importation of animals from temperate areas and crossbreeding with native animal. This importation and crossbreeding policy has been based on comparison of the high production of breeds from developed countries (primarily temperate) relative to

the production of native breed in less developed countries (primarily tropical), thus leading to unrealistic expectations of the potential for rapid improvement (Barker, 1995). However, while gains may be made for some traits in the crossbred progeny, their overall performance and that of subsequent generations may not be better than the native breed because of their inability to tolerate the adverse environmental conditions, for example poor nutrition and high temperature (Noor *et al*., 1992).

Pure breeding of Bali cattle can be found at Bali Island, Sumbawa Island, Flores Island and Bone district of South Sulawesi province. Indonesian government has allocated those islands as the main source of Bali cattle. Crossbreeding program can only be conducted outside areas. However due to indiscriminate crossbreeding, the pure Bali cattle at those areas have been contaminated with other breeds., i.e. Simmental, Limousine, Brangus, Charolais, indicating by high frequency of abnormal appearances, including abnormal color patterns and horn shapes (Handiwirawan *et al*., 2003)

The most recent study shows that the percentage of abnormal sperm on Bali cattle bulls is lower than those of Holstein, Limousine and Simmental bulls. In addition, the monomorphism for FSH sub beta, FSH receptor and GH exist in Bali cattle bulls and can be used as specific markers for the sperm quality. These three markers are closely related to the sperm quality (Ishak, 2012). This result clearly shows that native and domestic animal that experienced a long period of both natural and artificial selection can adopt the climate change. The Bali offers the advantage of a high resistance against most diseases, a remarkable ability to grow on low-quality fodder and a high fertility (McCool, 1991). The superiority of Bali cattle in extensive system and marginal conditions is also exhibit by most of native and domestic Indonesian FAnGR. List of Indonesian FAnGR that can be expected survive in the period of climate change is presented in Table 1.

Global Cimate Change

Reports have indicated that developing countries are more vulnerable to the effects of climate change due to their high reliance on natural resources, very limited capacity to adapt institutionally and financially, and high poverty level (Thornton *et al*., 2006). Drucker *et al* (2007) stated that the harsh effect of climate change give more impact in intensive livestock production system, such as pastoral communities whose livelihoods depend on climatic sensitive resources. In the face of climate challenges, adaptation of different livestock species to tropical conditions becomes crucial. FAO's Committee on Genetic Resources for Food and Agriculture noted that the management of animals under natural selection by pastoralists in marginal area plays an essential role in their adaptation and fitness in marginal environment (CGRFA, 2009).

In terms of global climate change, increasing resistance to pathogens and

Table 1. List of Indonesia Major Farm Animal Genetic Resources (FAnGR)

FAnGR	Breed	Investigators
Cattle	Aceh	Sari *et al*. (2010); Abdullah *et al*.(2012)
	Bali	Noor *et al*.(2001a); Sukmasari *et al*. (2002); Nijman *et al*. (2003); Mohamad *et al*. (2009); Handiwirawan *et al*. (2003); Handiwirawan and Subandriyo (2004); Purwantara *et al*. (2012); Martojo (2012); Ishak (2012)
	Katingan	Utomo *et al*. (2010); Utomo *et al*. (2011)
	Madura	Winaya (2010)
	Pesisir	Sarbaini (2004); Jakaria *et al*. (2007)
	PO	Hartati *et al*. (2010)
Buffalo	Spotted	Yulnawati *et al*. (2008)
	Swamp	Sumantri *et al*. (2010); Misrianti *et al*. (2010)
Horse	Manado	Takaendengan *et al*. (2011a); Takaendengan *et al*. (2011b)
Goat	PE	Zurriyati el al. (2011)
	Kacang	Batubara *et al*. (2011a); Batubara *et al*. (2011b)
	Marica	Batubara *et al*. (2011a); Batubara *et al*. (2011b)
	Samosir	Batubara *et al*. (2011a); Batubara *et al*. (2011b)
	Jawarandu	Batubara *et al*. (2011a); Batubara *et al*. (2011b)
	Muara	Batubara *et al*. (2011a); Batubara *et al*. (2011b)
	Bengali	Batubara *et al*. (2011a); Batubara *et al*. (2011b)
	Jeneponto	Rahardja (2007)
Sheep	Kisar	Salamena (2006)
	Fat Tailed	Noor *et al*. (2001b); Maskur and Arman (2010)
	Thin Tailed	Dagong *et al*. (2011)
	Garut	Inounu *et al*. (2008); Inounu *et al*. (2009); Dagong *et al*. (2011)
Chicken	Kampung	Sartika *et al*. (2004); Sartika *et al*. (2005); Zein and Sulandari (2008); Sartika *et al*. (2008); Sulandari *et al*. (2009); Mu'in *et al*. (2010); Nataamijaya (2008);
	Pelung	Iskandar (2004)
Duck	Alabio	Prasetyo and Susanti (2007); Rukmiasih *et al*. (2011); Matitaputty *et al*. (2011)
	Cihateup	Matitaputty *et al*. (2011); Rukmiasih *et al*. (2011);
	Tegal	Prasetyo and Susanti (2010)
	Mojosari	Prasetyo and Susanti (2007); Prasetyo and Susanti (2010)

pests to chemical, high freqency of epidemics growing, increasingly heavy pollution, reduction of genetic diversity may compromise the sustainable of livestock production. Local breeds adapted to local conditions, are often the most suited for providing environmental services, such as management of landscape, including the maintenance and promotion of a particular type of vegetation, crossing corridor preservation of habitats and wildlife. Local breeds may contribute to the prosperity of livestock farmers, even in the poor area, the ecological and cultural tourism (Maxim *et al*., 2011). Furthermore they emphasized that protecting local breeds can be done by identifying and promoting quality products. Many local races provide unique products of superior quality to those from commercial breeds.

The harsh effect of climate change is expected to have maximum impact on vulnerable pastoral community engaged in extensive livestock production system in dry lands. Osani and Bebe (2010) emphasized the importance of for the selection of animal in harsh environment. Analysis of progeny history records also provide inferences relating to the repeatability of kid survival and a measure of the environmental variance and can be used to predict future performance of dams (Falconer and MacKay, 1996). All these represent useful information for the selection of dams in harsh environment. Some action plan that could be applied include (i) the development of simple methods to characterize adaptive traits in marginal lands (Hoffmann, 2008); (ii) fostering participatory planning and the development of breeding goals and the design of breeding structures for community based adaptation to climate change (Osani and Bebe, 2010); (iii)understand herders perspective on how extensive livestock production system are tailored toward exploiting structural and environmental unpredictability (Kratly, 2008), and finally , this approach will contribute to the strengthening of livestock keepers' capacity and resilience (Hoffmann, 2008)

Conclusion

Global climate change represents a critical challenge to FAnGR in the 21st century. However, Indonesia has many native and domestic FAnGR that have adapted to harsh tropical climate and conditions for a long time. In order to survive the face of climate change, the FAnGR should be kept pure and utilized in sustainable way.

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Tropical Forages in Indonesia: Past experience and Future Opportunity

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Overview of Cattle Industry in Indonesia

Continuing economic growth in Indonesia is encouraging higher beef consumption with annual consumptioncurrently around 500,000 tonnes, or around 2kg/capita. This is low by comparison to many developed countries e.g. 33 kg/capitaare consumed annually in Australia.The Indonesian Government's objective is to raise consumption even further to 5kg (Dr John Ackerman,as reported in *Beef Central*).

Between 2004 and 2006, imported beef accounted for about 75% of total Indonesian demand (Beef Central).In response, the Indonesian government has initiated a program to boost domestic beef cattle production and productivity called: "PengembanganSwasembadaDagingSapi or Self Sufficiency in Beef Supply".Since smallholder production accounts for over 80% of beef production, it is vital that the supply capability of smallholdersby expanded.

The debate regarding the best way to achieve this target has focussed on lifting beef cattle numbers. The vice-minister for Agriculture was quoted as saying that from the "census for Beef Cattle and Buffaloes in 2011, the local beef cattle population is 14.8 million head. He said that "Indonesia would need 20 to 27 million head of cattle to support its own requirements. That's roughly the size of the beef herd in Australia" (Dr John Ackerman as reported in *Beef Central*).

However, the goal of lifting output is as much about increasing individual animal performance as it is about lifting overall cattle numbers. Expansion of the smallholder sectoris seriously hampered by poor nutrition which constrains both breeding herd productivity and growth rates of young animals to slaughter weight. Lifting productivity of both these parameters requires improved nutrition.

In our project "Improving smallholder cattle fattening systems based on forage tree legume diets in eastern Indonesia and northern Australia", the Australian Centre for International Agricultural Research (ACIAR) is focussing on increasing the productivity of cattle being fattened for sale by smallholders. By improving the nutritional quality of their diets using locally produced feeds, we aim to double the output of cattle fatteners. This has been identified as the best *way* to improve the livelihoods of the rural poor, who are otherwise largely involved in producing food for family consumption.

The Problem of Poor Nutrition

While the farm−gate price per kg live weight is similarfor Indonesian farmers and northern Australiangraziers, there are many additional problems in Indonesia viz. high calf mortality, lack of good quality bulls, long calving interval, poor growth rates and slaughter of productive cows.

Indonesian farmers produce an inferior product by all accepted indicators (rate of liveweight gain, carcass weight, age, dressing % (45% in Indonesia cf 54% in northern Australia) and carcass quality at slaughter). The lower productivity of Indonesian cattle can be related principally to inadequate nutrition, although other factors may also be influential. The key limitations are:

- Seasonal rainfall together with lack of land leading to scarcity of forage supply, especially in the dry season.
- Insufficient quantity and quality of forage fed and lack of understanding of the importance of good nutrition to achieve better animal performance.
- High calf mortality due to poor cow nutrition and management
- Stunted growth following weaning, when cattle are free grazed on native rangelands, reducing growth potential even when animals are returned to a higher quality diet.
- Other factors including: (a) Breed type: cattle in most of Eastern Indonesia have lower potential growth rates compared to tropically adapted cattle in northern Australia; (b) poor sanitation, housing and health management e.g. internal parasites; and (c) lack of sufficient water of appropriate quality

Of these limitations, the most important is poor animal nutrition under the traditional integrated crop/livestock feeding systems employed. Even in tethered fattening systems, cattle typically gain only 0.15 to 0.25 kg per day or 27− 46 kg in 6 months of intensive feeding prior to sale. In extensive free grazing systems,cattle lose weight during the dry season.

More and better quality forage is vital to improve all aspects of animal reproductive and growth performance. A faster rate of live weight gain (LWG) would mean faster turn off, higher liveweight and dressing percentage at slaughter, a higher quality product and therefore greater returns per kg of live weight.

The current minimum liveweight for slaughter of 250 kg is too small and contributes to the low dressing percentage (45%); it is the lowest of all beef cattle producing countries for which production data are collated. The average for tropical breeds in northern Australia is 54%. It is desirable, profitable and technically possible for Indonesian farmers to fatten to live weights of >300 kg.

Improved forage supply is central to achievement of each of these cattle development goals.

Need for Planted Forages and Importance of High Quality Diets

There has been little farmer interest in planted forages in Indonesia during the period 1960-1990) despite significant investments in forage research during that period. The reasons for lack of interest were:

(a) Low value for animal product, ready access to communal grazing, and lack of well-developed beef market leading to anon-commercial attitude to livestock,
(b) Since most cattle were free grazed, early work focused on improving communal, pastures, a concept that has generally proved unworkable due to the lack of grazing control. Some success was achieved with the introduction of highly grazing tolerant species e.g. *Stenotaphrumsecundatum* under coconuts (Shelton and Stur 1990), however gains were modest.

Interest in planted forages increased during the mid to late 90s due to changing circumstances, namely:

(a) Increasing value and demand for animal product due to increasein the wealth of Indonesia's population, which in turn has fostered the development of local markets,
(b) Less communal land available and thereforedeclining access to free forage,
(c) High labour requirement to herd free range animals.

Innovative smallholders began to tether their cattle and feed cut and carry forage collected from communal areas, and then eventually from forages deliberately planted on their own land. Initially, farmers were predominantly interested in using grasses which gave higher yields, more feed per unit area, and therefore lower labour costs. This bias towards feeding grasses remains today, for both farmers and Government personnel. During the two decade from 190 to 2010, there has been much good research work done; firstly, comparing the suitability and adaptability of various tropical grasses for cut and carry e.g. *Panicum maximum* cv. Simuang (purple pigeon grass), *Pennisetumpurpurteum* 'Napier', and the *Brachiaria* Hybrid 'Mulatto'; and secondly, developing successful participatory approaches to achieving uptake of planted forages by smallholder farmers (Stur *et al.* 2007). There were a number of forage projects in Indonesia, mostly supported by International aid funds e.g. 'The Forages for Smallholders Project' (1995-2005) supported byAusAID / CIAT funds, and 'Pastures for Plantation Crops' (1988-1995) supported by ACIAR.

As farmers became more commercially oriented, they needed to improve reproductive performance, calf and weaner management, and growth rates, in order to speed up turn-off. These improvements are principally achieved by feeding higher quality diets. Tropical grasses alone are insufficient as they are low in protein and digestibility, especially as they mature.The response of farmers was to supplement with feed concentrates e.g. rice bran, pig and poultry concentrates; while this achieved the objective of better animal performance, supplementation with concentrates was expensive and reduced profits.

The alternative was to make greater use of tropical legume forages which are naturally high in protein and generally of higher digestibility than tropical grasses; an approach that is gaining acceptance but still not widely practiced or understood.

The stylos(*Stylosanthes guianensis, S. hamata, S. scabra*) were the initial legumes of choice by Government agencies due to their quick growth on most soils including acidic, infertile sandy soils.However, they are not as high quality as some other legumes due to their higher proportion of fibrous stem when mature, and they are often short term. For these reasons, there has been limited use of herbaceous legumes, although another ACIAR project (SMAR/2006/003LPS: Integrating forage legumes into the maize cropping systems of West Timor)is having success with the integration of herbaceous legumes with maize plantings.

In the meantime, farmers of their own volition began to make greater use of forage tree legumes (FTL) (Gutteridge and Shelton 1994;Shelton *et al.* 2000), most notably leucaena (*Leucaena leucocephala*), sesbania (*Sesbania grandiflora*), gliricidia (*Gliricidia sepium*)and calliandra (*Calliandra calythyrsus*). But other species were also used e.g. *Acacia leucophloea*in Timor.

Role of Forage Tree Legumes

It became apparent that forage tree legumes were more appropriate source of leguminous forage for smallholders for several reasons(Gutteridge and Shelton 1994):

- They are deep rooted and have the ability to provide forage through the dry season when it is most needed.
- They are true perennials and do not require to be replanted on a regular basis.
- They are readily harvested and transported requiring less time for harvesting even than grass.
- They supply multi-purpose products for households e.g. apart from forage, they are a source of timber, fuelwood and even human food.
- There are several species of forage tree legumes, while introduced,grow naturally in Indonesia in a wide range of differing environments.

With best possible nutrition involving substantial use of expensive purchased concentrates, the potential growth rate of Bali cattle is about 0.76 kg/d or 139 kg in 6 months (Mastika, 2003). With improved nutrition based on 30−40% of high−quality forage tree legumes (FTL) in the diet, Bali cattle are capable of 0.4−0.5 kg/d or 73−91 kg in 6 months (Mastika, 2003).

Quigley and Poppi (2009) reported "that the potential growth rate of 6 to 12 month old Bali calves can be lifted from 0.1 - 0.2 kg/d, under prevailing feeding scenarios, to over 0.4 kg/d, closer to the potential liveweight gain of approximately 0.65 kg/d (Figures 1 and 3). Simple feeding strategies available to farmers include the provision of feeds high in protein, such as *Leucaena leucocephala*, *Sesbania*

grandiflora or copra meal and rice bran. At low levels of inclusion (10 g DM/kg W/d) these protein supplements resulted in financially beneficial increases in live weight gain".

To achieve these levels, increased access to, and improved knowledge of, the nutritional value of FTL is required. This approach has potential to not only increase slaughter weights, but also reduce the slaughter of productive females.

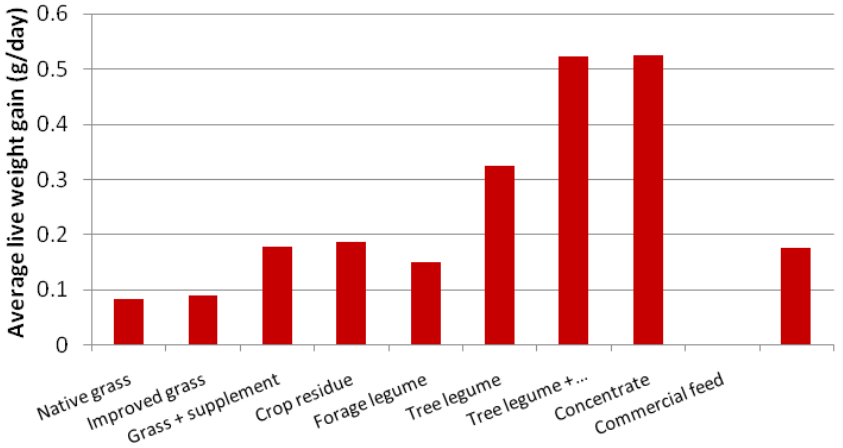

Figure 1. Average daily gain of weaned early weaned Bali cattle (60-110 kg live) fed a range of diets in JawaTimur, Nusa Tenggara Barat, Nusa Tenggara Timur and Sulawesi Tengah (Quigley and Poppi 2005).

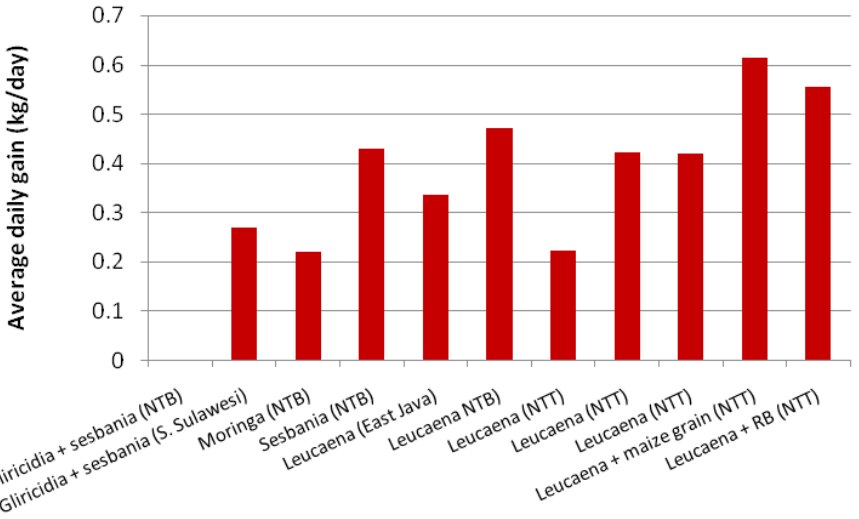

Figure 2. Average daily gain of weaned early weaned Bali cattle (60-110 kg live) fed a range of tree legume diets in JawaTimur, Nusa Tenggara Barat, Nusa Tenggara Timur and Sulawesi Tengah (Quigley and Poppi 2005).

The Key Tree Legume Species

As mentioned, there are several species of FTL being used as a source of forage in Indonesia; however this paper will focus on two of the highest quality species, namely, *Leucaena leucocephala* and *Sesbania grandiflora*.

Leucaena in Indonesia

The tropical leguminous shrub *Leucaena leucocephala* (leucaena) is widespread in Indonesia where it occurs as an invader of disturbed areas where soil and climate parameters are conducive to its growth. Where these areas overlap with ruminant production, some farmers have evolved feeding systems utilizing freshly harvested leucaena as a much needed source of protein in cattle, goat and buffalo diets. Leucaena is especially widespread in the seasonally dry Provinces of NTB and NTT and is often the sole feed especially in the dry season. There is immense potential to increase the use of this valuable underutilized forage.

Leucaena was promoted in the semi-arid islands of Nusa Tenggara Timur province in eastern Indonesia in the 1930s to 1960s by government institutions and NGOs to reduce degradation and increase production and promote sustainable agricultural systems (Piggin and Nulik 2005). They reported that "the Amarasi system in Timor is based around the use of leucaenaas forage for tethered cattle and goats. The Sikka system in Flores was developed in the 1960s and involves contour rows of leucaena to prevent erosion and create indirect terraces where corn, peanuts, and mungbeans are grown and mulched with leucaena clippings from the hedgerows".

"These systems each now cover about 50,000 hectares or 70% and 30% of the Amarasi and Sikka areas respectively, contributing substantially to farm production, wood supply, and stabilization of the resource base. Given that villager families farm about 2 ha, this suggests that some 25,000 farm families may be growing leucaena in both Amarasi and Sikka" (Piggin and Nulik 2005).

While the above examples of use of leucaena by smallholders are well documented, recent investigations have shown that the feeding of leucaena to livestock is more widespread than previously thought. For instance, we visited Balinese villages in western Sumbawa (Jati Sari), villages on the southern coast of Sumba, and villages north of Sengiti on the eastern coast of Lombok, that have for extended periods, based their livestock feeding around the use of both naturally occurring and planted leucaena. The Balinese farmers in Jati Sari informed us that leucaena feeding was common practice in Bali before they migrated to Sumbawa approximately 30 years ago. On arrival, they initially found jobs as labourers in the shrimp fisheries, but eventually were able to buy land and use their previous experience to become cattle fatteners.

We have no doubt that similar practices exist in many other areas of those Provinces, and probably in other Provinces of Indonesia.

The potential for making use of existing leucaena areasfor livestock feeding, or extending the area of leucaena by new plantings, is enormous; there are large areas of uplifted coral landscapes, in both NTB and NTT that are eminently suitable for the growth of leucaena. Indeed our early results from plantings in the village of Kuanheum and Batulesavillages in Timor and in Pametinganga hamlet in Kambataana village, Pandawaisubdristrict in Sumba, demonstrate this conclusion.

The origin of the naturally occurring variety is not known and may be one of the original giant K-series provenances distributed from the University of Hawaii. Our recent work in NTT has demonstrated the superiority of cv. Tarramba,an Australian released variety (Gutteridge and Shelton 1996), compared to the local variety; it is higher in palatability, yield, and psyllid tolerance. Tarramba leucaena is currently being distributed widely in NTT and now NTB as part of the ACIAR project referred to above. It is imperative that local seed production of this variety is promoted to ensure its widespread distribution and use.

Leucaena toxicity. Whilst leucaena can support excellent animal growth rates, it possesses an anti-nutritive property - the toxic non-protein amino acid mimosine. However, mimosine is readily degraded by plant and rumen microbe enzymes to 3-hydroxy-4(1H)-pyridone (3,4-DHP) (Hegarty *et al.* 1964) and then, if the bacteria *Synergistes jonesii* is present, to harmless by-products which are likely to have nutritional value (Jones and Megarrity 1986). In the absence of the specific gram negative bacteria *S. jonesii*, undegraded DHP is absorbed into the blood stream and is then excreted in urine. While clinical symptoms include enlarged thyroid glands, subclinical toxicity is more common and presents as reduced appetite and reduced animal productivity. Subclinical 3,4-DHP toxicity can retard animal growth by 30-50% (Jones and Winter 1982; Quirk *et al.* 1988). The most practical method for checking the protection status (presence of bacteria capable of degrading DHP) of ruminants is analysis of urine for presence of DHP (Graham 2010). The method is inexpensive and confirms not only the presence of bacteria but also their effectiveness in degrading DHP when ruminants are consuming high leucaena diets.

As part of The University of Queensland's ongoing study into leucaena toxicity, the Australian Centre for International Agricultural Research (ACIAR) has funded a survey of the Indonesian provinces of Nusa Tenggara Barat (NTB) and Nusa Tenggara Timur (NTT). The aim was to determine the local protection status of ruminants to leucaena toxicity in twenty villages chosen from the islands of Lombok, Sumbawa, Sumba and Timor.

Preliminary urine test results showed large variability, not only between islands, but also between animals in the same village and between villages on the same island.

On every island, there were some animals excreting high levels of toxin while other animals consuming large amounts of leucaena were excreting no toxin in

their urine. These latter animals are of particular interest for further studies, as they possess ruminal bacteria capable of complete degradation of mimosine and DHP. Interestingly, there were also animals excreting high amounts of urinary DHP toxin in neighbouring villages, in some cases less than 1 km apart. Cattle of Balinese villagers in Sumbawa and cattle in Timor with well-established leucaena feeding practices, were excreting low amounts of toxin, and showed protection against leucaena toxicity.

A surprising finding was that goats and buffalos within Sumba, an island previously thought to be unprotected due to its isolation from other islands, showed low or no toxin excreted while on high leucaena diets.

The findings of this survey are encouraging and will aid research into improving current inoculation practices to prevent toxicity wherever leucaena feeding is practised.

Sesbania in Indonesia

Sesbania (*Sesbania grandiflora*) is a multi-function tree. Its main use In southern Lombok, the major region for goat and cattle production, is as the main (and sometimes the only) component of ruminant diets (Dahlanuddin*et al.* 2005). It was originally introduced to Lombok in the 1970s as part of a national program, aimed initially at improving soil fertility and replanting barren areas.

Dahlanuddin*et al.* (2005) reported that "Sesbania has the highest nutritive value and is the most widely used of all tree legumes available for livestock feeding in Lombok. It is planted in single rows along the bunds of rice paddies. The leaves are cut and fed fresh in a cut-and carry system; the branches are dried for firewood. Farmers harvest only the side branches of the tree to avoid tree mortality, and to make the trunk straight for pole timber when cut at around 3 years of age. Southern Lombok has limited forest resources, making sesbania the main source of firewood and timber for both housing and animal pens".

Dahlanuddin*et al.* (2005) reported that "sesbania is planted on approximately 25% of rice field bunds on Lombok, mostly on the southern part of the island (which is the main rice region in Lombok). Mature seeds that drop naturally during the dry season provide sufficient seedlings in the early wet season for transplanting onto the bunds. Each farmer plants an average of 520 plants, 40-60cm apart. From an estimate of the total length of the bunds over Lombok island, circa 65K small farmers plant sesbania".

The fact sheet on the tropical forages web site (www.tropicalforages.info) indicates that"an annual dry matter yield of 27 kg of green leaf/tree was achieved by harvesting side branches. When fed to ruminant animals, it may comprise up to 70% of total forageallowance during the dry season. Anecdotal reports of high liveweight gains in cattle are common. In India, milk yield was increased by 8% (9.2-9.9 l/day) when cattle were fed 5 kg fresh leaf/day". However, the plant contains canavanine,

the nutritional implications of which are unknown. It also Contains low quantities of condensed tannins and the seeds are poisonous to fish.

The system has potential for expansion to other areas of Lombok and Indonesia with similar agroecological conditions.Sesbania planting is a valuable and sustainable technology that fits well into smallholder farms. It allows farmers to produce high quality forage. However, better agronomic information is required on planting configuration, management for optimal forage and wood production, and genetic diversity.

Empowering Local Resources for Sustainable Animal Production Due to Climate Change

The theme of this conference is Empowering Local Resources for Sustainable Animal Production Due to Climate Change. Forage tree legumes contribute to this goal in many ways:

Contribution to sustainability

1. They can be crucial contributors of forage to the productivity of household ruminants, lifting the economic returns to rural families, and improving the welfare of both farmers and their animals.
2. They are long-lived perennials that stabilize often degraded environments protecting them from erosion and degradation.
3. Being legumes they contribute N to the integrated crop-livestock systems so common in Indonesia.
4. They provide much needed forage for livestock reducing grazing pressure on communal lands.

Contribution to climate change

1. The improvement in quality of ruminant diets by the addition of forage tree legume foliage reduces methane emissions. “CSIRO research shows that northern (Australian) cattle fed on a diet of predominantly leucaena, a legume tree, emit less methane than cattle grazing on tropical grasses” (Charmley 2011).
2. Substantial amounts of C are sequestered in the woody frame and timber of FTL. Research being conducted at the University of Queensland indicate that leucaena-grass pastures sequester C at depth in the soil profile (below 60 cm) and can store 370 – 400 t ha-1 C compared with 270 t ha-1 C in grass only (buffel grass) pastures; however, the amounts are highly dependent on management practices, soil type and climate (Kathryn Conrad, personal communication).

Conclusions

The Indonesian Government has made a priority to lift cattle productivity and move towards self-sufficiency in beef production. Smallholders are crucial to this strategy as they supply a high percentage of output. The best way to achieve this goal is to improve the amount and quality of forages used by farmers to feed their cattle; this will have a direct positive effect on reproductive efficiency of females (by lifting calving percentages and reducing calf mortality) and the rate of growth of young bulls post weaning and therefore turn off from smallholder cattle fattening enterprises.

Forage tree legumes can play an important role as they are commonly present in rural environments, they are perennial and supply dry season forage, and many farmers already use them in their feeding programs.The key tree legume species are*Leucaena leucocephala* and *Sesbania grandiflora* but other species such as *Gliricidia sepium* are also important. FTL have the additional advantage of promoting sustainability of animal production due to their longevity and have the potential to reduce greenhouse gases via their C sequestration capability and to reduce methane emissions from ruminants consuming higher quality diets.

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I. Breeding and Genetics

Improvement the Genetic Potential of Local Chicken By Combination of Crossbreeding, Selection Method, Cellular Analysis and Nutritional Adjustment to Produce the Candidate of Local Layer

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Abstract

The present research has been conducted in five stages: genetic selection, crossbreeding, cellular analysis, evaluating the nutritional requirements and egg production. This research used 10 adult male chickens from crossing between female of Kampung chicken with male of laying chicken and 60 female Arab (silver and golden). Crossing carried out by artificial insemination and all parameters measured on offspring. The research design used was randomized factorial (2x4) and data was processed statistically using analysis of variance. To be able to show the maximum genetic potential then any growth stage of chicken during the selection process were examined by nutrient requirements mainly protein and energy balance using feed conversion and feed efficiency parameters. The treatments of energy: protein balance were; A (3000 Kcal:21%), B(2900 Kcal:20%), C(2800 Kcal:19%) and D(2700 Kcal:18%). Analysis of cellular parameters (protein, RNA and mRNA) in muscle tissue was performed on F3 derivatives of Kamaras to indentify as laying hens and also evaluated the ability of production.At the end of period, other parameters were measured: egg production, egg quality (Haugh Unit and grade of eggs, egg shape and eggshell color). Research results showed that crossbreeding and selection based on the performance of the exterior on local chicken produced two type of local layer hen that meet the criteria of commercial laying hens: medium and heavy type of Kamaras chicken. The cellular analysis results showed that chicken Kamaras has characteristics similar to the laying hens where the pectoral muscle growth was very slow. Egg production of both types of Kamaras chicken much higher than pure kampung chicken ranged between 144-170 egg/year. Egg quality analysis resulted that the Kamaras chicken (both medium and heavy type) produced eggs with high quality especially in terms of Haugh unit values (> 70), egg weight, eggshell thickness, grade (A) and quality of egg yolk (reddish yellow) better than Kampung chicken or Arab chicken. However, energy and protein content in diet significantly affected the egg production and egg quality in both type of Kamaras chicken. In conclusion, the egg productivity and egg quality of Kamaras chicken as a the result of selection and crossbreeding program were better than other types of

local chickens. In fact, Kamaras could be developed as a candidate of local laying chicken for future.

Keywords: cellular analysis, crossbreeding, local chicken, nutritional requirements, selection method

Introduction

Breeding program of local animal will be able to support economic stability and food security programs which are more valuable than the import of animal from abroad. This program will stimulate the Indonesian people to produce its own products and have alternative jobs for community. One of the great potential of local animal in Indonesia is local chicken (ayam kampung) which could be developed by genetic improvement to reduce the dominance of imported chicken (broiler or layer). This effort will support the genetic development of local chickens as a commercial layer chicken that is more productive and profitable for business activity. These objectives could be achieved by a sustainable strategic approach including genetic selection and nutritional adjustment (M. Aman Yaman *et al.,* 2000a: 2000b: 2010) and up-grading technology through genetic approach and breeding technology (May, 1971: Brillard, 2003).

The breeding technology developed for local chicken selection should have advantages in terms of output, applicative and more economical (Ansah *et al.,* 1985: Bennewitz *et al*., 2007). The previous studies of local chicken have categorized two types of local chicken from Aceh Province resulted by genetic selection using exterior parameters, namely (a) the type of potentially meat chicken and (b) the type that can be oriented as laying hens. The previous results also showed that the average local chicken eggs only produced between 70 to 90 eggs /year. Until now, the conventional program of genetic selection for local chicken have not been able to raise the potential of local chickens as productive laying hens (M. Aman Yaman *et al.,* 2008: 2009). In order to solve such problems, the application of genetic programs for selecting local chicken needs to be done through a combination of a more comprehensive, more effective and applicable method using crossbreeding program.

In principle, to stimulate the gene expression of local chicken as layer requires the supply of nutrients according to their requirements since the starter, grower, pre-laying and laying period (M. Aman Yaman *et al.,* 2002: Wattiaux, 2006). Development of methods of selection, crossbreeding and nutritional approaches in an effort to generate a new strain of local chickens for egg production will be highly appropriate for a local chicken. It is possible to perform crossbreeding program using artificial insemination to increase the expression of genetic potential (Szwaczkowski

et al., 2000: Sapp *et al.,* 2004). The present research will focus on producing a layer chickens resulted by crossbreeding between local chicken, Arab chicken and Hy-Line Brown layer. The combination of breeding and nutritional approach could be considered to produce a new strain of local chicken which genetically able to produce egg higher than the original local chicken. The characteristic of egg produce by this chicken also must be qualified for market standard as a commercial chicken egg.

Materials and Methods

Ten adult male selected chickens (crossing between female of Kampung chicken with male of laying commercial chicken) and sixty female Arab chicken were used to evaluate the quality of offspring as a candidate of layer chicken. Crossbreeding was carried out by artificial insemination and all parameters were performed on offsprings (F1 – F3). The research design used was randomized factorial (2x4) and data was processed statistically using analysis of variance. Evaluation of genetic potential was performed in any growth stage of chicken during the selection process. The effect of nutritional adjustment on selection program was examined by treatment of protein and energy balance to evaluate feed conversion parameter.

The treatment of energy and protein balance were A (3000 Kcal:21%), B(2900 Kcal:20%), C(2800 Kcal:19%) and D(2700 Kcal:18%). At 90 days of age, analysis of cellular parameters (protein, RNA and mRNA) in muscle tissue was performed on breast muscle sample of offspring (F3) to indentify selected chicken as a candidate of laying chicken. At the end of period, other parameters were measured: egg production, egg weight, haugh unit (HU), egg grade, egg shape and eggshell color.

Sampling of hatching egg resulted by crossbreeding to produce F1-F3 offspring were selected by the following criteria ; eggshell color is reddish-white, oval shape, egg weight from 35 to45 gram and fertile. Sample of breast muscle and liver tissue were collected from chicken to evaluate the response of chicken type and nutritional treatments on cellular parameters for selection program carried out by the method of dislocation neck (cervical spine fracture) and chemical analysis. All sample tissue was preserved by immersing into liquid nitrogen and stored at -80° C until analysis. Protein, RNA and mRNA contents were analyzed by chemical treatment and measured by spectrophotometer (M. Aman Yaman *et al.,* 2000).

Results and Discussion

The present result showed that crossbreeding between local chicken, layer chicken and Arab chicken produced two types (heavy and medium types) of productive laying hen (called as Kamaras chicken) with a specific performance and difference in egg productivity (Table 1). Both of Kamaras chickens have a similar body shape but difference in body weight during mature sex and dominant color.

Heavy type chicken has a black dominant color and gray dotted, while medium type has a black dominant color and white/golden dotted. At the age of mature sex, body weight was 1580 gram (heavy type) and 1420 gram (medium type). The average of egg production of both types of Kamaras chicken ranged between 171 to 177 eggs /year. There was no difference in egg quality of both types of chicken. However, the FCR of medium was lower (2.6 kg) compared to the heavy type chicken (2.9 kg) during 90 days of rearing period. The results also show that through crossbreeding program and combination of selection methods produced a derivative type of local chicken which has an ability to produce eggs was higher than the origin of local chicken but still has a similar characteristic in egg quality.

Table 1. Parameter of Kamaras chicken (female line) resulted by cross breeding program as a candidate of local productive laying hens

Specification	Heavy type	Medium type
Body shape	Layer	Layer
Dominant color	Black and gray dotted	Black and white dotted
Body weight of *pra-laying* (gram)	1580	1420
First laying age (week)	19	18
Clutch (egg/week)	5	4
Mean of egg production (egg/year)	177	171
Egg weight (gram)	35-47	32 - 43
Haugh unit	74	72
Egg shell color	Creamy white	Creamy white brown
Hatchability (%)	93	91
Feed conversion rate (FCR) for 90 days	2.9	2.6

Combination selection program and mating strategy to explore the potential of genetic during breeding process will largely affect the derivative type of chicken (M. Aman Yaman *et al.,* 2008 and 2009). On poultry breeding program, the dominant characteristic that appears in exterior parameter followed by analysis of cellular parameter will be used as a very useful parameters to produce a new type of chicken in accordance with the breeding purpose (Brah, 2005; Bennewitz *et al.,* 2007) including breeding program to stimulate the increase in egg production and egg quality of local chicken. The combination of selection program on a local chicken through cellular and exterior parameters influenced the appearance of the chicken peformance and egg production (Wattiaux, 2006) . The present result approved that crossbreeding followed by a strike selection method using local chicken, layer

chicken and female Arab produced a new type of chicken which can replace the role of local chickens as laying hens in the future.

Analysis of cellular parameters (Table 2) in the two types of chicken Kamaras showed that protein, RNA and mRNA contents in the pectoral muscle has equal to those in commercial laying hens. These indications show that the chicken Kamaras could be categorized as slow-growing type of chicken similar to the characteristics of laying hens. Cellular content in breast muscles of poultry could be used as a reference for functional selection of laying hens or broilers, in which the concentration of protein, RNA and mRNA of chicken breast muscle layer is lower than broilers due to the different types of fiber muscle (Kita *et al.,* 2002 and M. Aman Yaman, 2010). The results of this study approved that the chicken Kamaras have a very strong character as laying hens that can be developed into a productive laying hens.

Table 2. The concentration Protein, RNA and mRNA contents on breast muscle of Kamaras chicken (F3) resulted by crossbreeding between male selected chicken and female Arab chicken

Parameter	Medium type	Heavy type
Chicken age (days)	90	90
Body weight (gram)	857	993
Breast muscle weight (gram)	17.15	18.49
Protein (mg)	3023	3155
RNA (mg)	12.93	13.24
mRNA (*ug*)	3276	3340

It was also observed that protein and energy balance in the feed significantly affected on egg production rate and egg quality (HU value) in both type of Kamaras chicken. However, there was no influence of chicken type (heavy and medium types) on egg production of Kamaras chicken. It was known that adjustment of protein and energy balance increased the number of eggs and egg quality. The egg production of Kamaras chicken fed on diet containing 19% of crude protein and 2800 Kcal of energy significantly increased than other nutritional treatments (Table 3). It was also informed that the requirement of protein and energy balance was different between Kamaras chicken and commercial layer chicken. In general, commercial laying hens require 17% of protein and 2900 Kcal of energy in ration. It was due to the difference in genetic characteristic as the result of crossbreeding between 3 types of chicken. The present result also showed that breed and chicken type, genetically affected the egg production but the nutritional balance in particular; protein, energy and minerals is a important factor affecting the production and egg quality of laying hens (Kino, 1993; Bennewitz *et al.,* 2007). The two types of Kamaras chicken

produced between 147-170 eggs/year higher than origin chicken (70-90 eggs / year). The influence of genetic characteristic from the Arab and layer chickens as male and female line during crossbreeding program caused an increase in egg production of Kamaras chicken.

Table 3. The effect of chicken type and nutritional treatment on number and egg quality of Kamaras chicken (F3) resulted by crossbreeding between male selected chicken and female Arab chicken

Type of offspring	Nutritional treatments	Number of egg/year	HU	Egg weight (g)	Egg grade	Egg yolk color	Eggshell thickness (mm)
Heavy	A	157[b]	68[b]	33	A	Reddish yellow	0.33
	B	162[c]	71[b]	35	A	Reddish yellow	0.34
	C	167[c]	73[b]	36	AA	Reddish yellow	0.37
	D	142[a]	64[a]	32	B	yellow	0.32
Medium	A	156[b]	67[a]	34	A	Reddish yellow	0.32
	B	164[c]	70[b]	34	A	Reddish yellow	0.35
	C	170[c]	73[b]	36	AA	Reddish yellow	0.36
	D	147[a]	65[a]	32	B	yellow	0.32

Different superscript in the same line means significantly different ($P<0.05$)

The differences in protein and energy balance in ration also effected egg weight, shell thickness, albumen height, yolk color of egg. It was also observed that the value of HU and grade eggs on both types of chicken Kamaras was significantly affected by protein and energy balance in ration. The egg weight of Kamaras chicken fed on ration contained 19% of crude protein and 2800 kcal of energy was higher and it was also followed by an increase in egg components, egg grade and HU. The results are consistent with several previous studies that nutritional adjustment resulted in increased egg weight and albumen quality that have an impact on increasing the Haugh Unit of egg (M. Aman Yaman *et al.,* 2008:2009 and 2010).

Conclusions

Cross breeding program between selected local chicken, Arab chicken and Layer Hy Line Brown has produced a potential local laying chicken called as Kamaras chicken. The third offspring of crossbreeding use 3 type chickens generated two types of local laying chicken (medium and heavy types) with the high egg production than the origin local chicken. It was also known that the egg production and egg quality of Kamaras chicken could be stimulated by fed on ration contain 19% of protein and 2800 Kcal of metabolizable energy (ME).

In conclusion, egg production and egg quality of Kamaras chicken from cross breeding, genetic selection and nutritional adjusment was better than their origins so it could potentially be developed as a local chicken laying.

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Physical Meat Characteristics of Local Thin Tail Sheep based on Calpastatin (CAST) Genotype variation

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Abstract

Calpastatin (CAST) is an indigenous inhibitor of calpain that involved in regulation of protein turn over and growth. The objective of this research was to identify genetic polymorphisms in the part of intron 5 - entire exon 6 of CAST gene in local sheep and their association with meat quality and muscle composition. A PCR-SSCP method was carried out to identify genetic variation of CAST gene. In total 401 heads of sheep from 8 subpopulations were investigated, three groups of samples were thin tail sheep from Sukabumi, Jonggol and Kissar. The rest samples were Priangan sheep from Margawati and Wanaraja and fat tail sheep from Donggala, Sumbawa and Rote islands. Twenty one heads from Jonggol were used for meat and muscle identification. SSCP analysis revealed that three different SSCP patterns corresponded to three different alleles in the CAST locus (CAST-1, 2 and 3 allele) with six different genotypes. Genetic variation between local sheep populations were calculated based on genotypic and allelic frequencies. Most populations studied were polymorphic, with genotype frequencies of CAST-11, CAST-12, CAST-22, CAST-13, CAST-23, and CAST-33 were 29.7%, 38.2%, 24.2%, 2.5%, 4.5% and 0.7% respectively. CAST-1 and 2 alleles were most commonly found in all populations with total frequency 95.8%, while rare allele was CAST-3 (4.2%) and only found in thin tail population. Based on sequence analysis identified a non-synonymous amino acid variation in exon 6 induced Gln/Leu substitusion. There was no association between CAST alleles and genotypes with meat quality.

Key words: calpastatin, local sheep, meat quality, PCR-SSCP

Introduction

Local sheep is one of the genetic resource potential to be developed. This is due to their several advantages which are prolific, good adaptability to the harsh

environments, disease resistance, shorter production cycles and relatively requires small capital. In addition, in some densely populated areas like Java, sheep are able to substitute some beef that have to be imported each year. Based on 2008 data, special needs of sheep in West Java alone reach the range of 3.343.365 heads (Ditjennak 2009). The amount of the request indicates that the prospects for sheep farming is still wide open.

In relation to sheep development efforts, some weaknesses of the local sheep is that their slaughter weights and daily body weight gain are low vaired between 54 - 174 g/head/day (Yamin *et al.* 2009), in addition, the carcass quality and meat is also highly variable and do not meet international market standards. In order to solve the problem the genetic quality improvement efforts are needed to increase productivity, carcass and meat quality so the impact on lamb production will increase the contribution of lamb to total meat production in the country that currently only around 5% (Ditjennak 2009).

Molecular biotechnology advances allow the selection can be done at the DNA level through the use of marker genes that have an association with the highly ecomomic traits. One of marker genes associated with body weight in local sheep was calpastatin (Sumantri *et al*. 2008). Numerous other studies have also shown a relationship of calpastatin gene with carcass quality (Schenkel *et al*. 2006), and meat quality, especially tenderness (Casas *et al*. 2006; Curi *et al*. 2009).

Calpastatin (CAST) is a member of the calpain-calpastatin system involving three molecules of the enzyme μ-calpain, m-calpain and calpastatin that serves as both calpain inhibitor. This system plays a important role in diverse physiological processes such as regulation of protein turn over and growth (Goll *et al*. 1992), and myoblast migration (Dedieu *et al*. 2003), therefore CAST is believed as a good candidate gene for growth, carcass and meat quality.

Information on meat quality based on various calpastatin gene genotype in the local sheep currently is not yet available, so the information is needed in order to describe the effect of this gene on meat quality.

Materials and Methods

Sample and Genotyping

This study used 21 heads of Thin Tail Sheep (TTS) from UP3J Jonggol that reared intensively. Sheep then grouped based on their genotype variation based on Dagong et. al. (2011) methods. A pair of PCR primer, forward: 5'-GTTATGAATTGCTTTCTACTC-3' and reverse: 5'-ATACGATTGAGAGACTTCAC-3' was designed to amplify part of intron 5 and whole exon 6 of CAST gene, as described by Zhou et al. (2007). PCR amplification was carried out in 25 μl reaction containing 50-100 ng genomic DNA, 0.25 μM of each primer, 200 μM dNTPs (Fermentas), 4.0 μM Mg^{2+}, 0.5 U of Toptaq DNA polymerase (Qiagen, Hilden, Germany), and 1x

the reaction buffer. The condition of thermal cycling consisted of pradenaturation at 95 °C for 5 min, followed by 35 cycles of denaturation 95 °C for 30 s, annealing 56 °C for 45 s, and extension 72 °C for 45 s. The final extension step was at 72 °C for 5 min. Amplification was carried out in a thermal cycler (Mastercycler Personal 22331, Eppendorf, Germany). The PCR amplicon were checked on 1.5% agarose gels in 0.5 x TBE buffer containing 10% of ethidium bromide at 100 volt for 45 min and visualized by UV transiluminator. A SSCP procedure was used to identify variation in the amplicon of CAST locus. The sheep that have been known to represent the CAST genotype then slaughter each genotype to identify their physical meat charactreristics.

Physical Meat Characteristics

Meat quality was measured based on the physical parameters which include: pH measurement by pH meter and measured after aging for 24 hours. Meat tenderness was shown by the enormous strength (kg/cm^2) required to cut the meat cores indicated by the needle cutter Warner Bratzler Shear Force (WBSF) that moves over the scale with a measurement sensitivity of 0.1 kg/cm^2. Water Holding Capacity was measured with a planimeter by finding out the amount of water (mg H_2O). Cooking loss was measured by substracting the initial weight with the weight after sample cooked at 80 °C for 1 hour.

Association of CAST gene polymorphims with physical meat quality was analyzed by t-test with the following statistical equation:

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sigma \sqrt{\frac{1}{n_1} + \frac{1}{n_2}}} \qquad \sigma = \sqrt{\frac{\sum_{i=1}^{n}(\bar{X}_i - \bar{X}_1)^2 + \sum_{i=1}^{n}(\bar{X}_i - \bar{X}_2)^2}{n_1 + n_2 - 2}}$$

Where :

$\bar{X}_1$ and $\bar{X}_2$ = Mean value in genotype 1 and genotype 2
n_1 and n_2 = Number of sample in genotype 1 and genotype 2
σ = Total varians

Physical meat quality data corrected in advance using the following statistical equation:

$$X_i \ correction = \frac{\bar{X} standard}{\bar{X} \ observed} x \ X_i \ observed$$

Where :
X_i correction = value of physical meat characteristics after being corrected by sex and age

$\bar{X}$ standard = mean of physical meat characteristics of standard population

$\bar{X}$ observed = mean of physical meat characteristics of observed population

X_i observed = value of physical meat characteristics before being corrected by sex and age

Results and Discussion

Differences of physical meat characteristic

Means value of physical meat quality from different CAST genotypes on the local sheep are shown in Table 1. There was no significant difference (P>0.05) either in tenderness, water holding capacity, cooking loss and pH from three different CAST genotyes (CAST-11, CAST-12 and CAST-22) in local sheep. Similar results with a previous study by Zhou *et al*. (2008), who reported that all allelic variation (CAST-1, 2 dan 3) or variations of genotypes were identified in the CAST locus did not significantly affect the lamb tenderness.

Tenderness value of research results in the range 2 - 3 in a tender category, but no differences among the three genotypes. In contrast to some previous studies that identified a significant association between CAST variation with beef tenderness. In cattle, CAST gene variations have been used commercially as genetic markers. Two markers are currently available commercially were *GeneSTAR Tenderness* and *Igenity TenderGENE*. *GeneSTAR* using SNP G/A in 3'UTR region (Barendse 2002),

Tabel 1. Mean value of physical meat characteristics from various CAST genotypes of local Thin Tail Sheep

Physical meat characteristics	Genotypes					
	CAST-11 (n=4)	CV(%)	CAST-12 (n=10)	CV(%)	CAST-22 (n=7)	CV(%)
Tenderness (Kg/ cm^2)	3.16±0.72	22.78	2.98±0.79	26.51	2.49±0.71	28.81
Cooking loss (%)	49.54±3.24	6.54	43.76±3.61	8.25	46.32±6.53	14.10
Water Holding Capacity (WHC) (MgH_2O)	117.31±13.70	11.68	99.46±19.83	19.94	103.73±19.27	18.58
Persentage of WHC (% MgH_2O)	39.10±4.56	11.66	33.15±6.60	19.91	34.57±6.42	18.57
pH_{ult} (24 h)	5.57±0.07	1.26	5.75±0.31	5.39	5.80±0.32	5.54

Note: CV = Coefficient of variation (standard deviation/mean x 100%)

while *Igenity TenderGENE* using SNP G/C in intron 5 region (Van Eenennaam *et al*. 2007).

Differences in CAST gene effect sheep meat tenderness and beef probably caused by the fact that sheep meat is more tender due to the rate of myofibrils proteolysis of sheep meat is faster than beef (Koohmaraie *et al*. 1991), therefore, the differences in sheep meat tenderness has smaller effect on meat tenderness.

Conclusion

There was no difference between physical meat quality with CAST genotype variation in local sheep.

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Genetic Variation of the IGF1 and OPN Genes in Holstein-Friesian Dairy Cattle of Historical and Non-Historical Twins

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Abstract

The application of DNA molecular techniques can be used to determine the mutation cases at DNA fragments associated with fertility traits in cattle. This study was aimed to identify genetic variants of Insulin Like Growth Factor 1 (IGF-1) and Osteopontin (OPN) genes to be considered as candidate genes controlling fertility traits in HF cattle of historical twins (27 heads) and non historical twins (15 heads) from West Java, Indonesia. Historically twinning cattle was defined as either the cows ever calved twins (more) or their offspring (female and males). Investigation of genetic variants was done by applying PCR-RFLP method by using restriction enzyme of SnaB1 for The IGF-1 gene and Bsr1 for the OPN gene. Amplification produced DNA products of 249 bp (IGF-1 intron-1) and 290 bp (OPN intron-4). Genotyping on the IGF-1 gene locus Snab1 produced only one DNA fragment, meaning all the cows having the BB genotype (249 bp). Monomorphic of the IGF-1 gene was probably due to no mutation (C/T) at the nucleotide as a cutting site. Instead, it was discovered genetic variants of the OPN gene locus Bsr1, resulting three DNA banding patterns, these genotypes were successively CC (200 and 90 bp), CT (290, 200, 90 bp) and TT (290 bp). The proportion of CC, CT and TT genotypes in historical twinning cattle (30%: 56%: 14%) differed to those of non historical twinning one (20%: 40%: 40%). Former cattle had the amount of CC and CT genotypes higher than the latter uterine milk uterus fertility for embryo growth in HF dairy cows observed.

Keywords: Hostein Friesian, IGF-1 gene, OPN gene, PCR-RFLP, twinning

Introduction

Productivity of dairy cows is strongly determined by the level of their fertility. The potency of a cow to give twin births needs to be studied to get information on how far this trait can be inherited. The inheritance of twin births in cattle had a similar pattern to quantitative traits, it was controlled by many genes and interacted with environment (Lien *et al*., 2000). DNA molecular techniques focusing on genomic analysis can be used to examine the insidences of mutation of the sequences of DNA fragments related to the changes in breeding values or performances of valuable traits.

Genetic polymorphisms of two fertility genes in cattle, including the IGF1 and OPN genes, using the method of restriction fragment length polymorphisms (RFLP) or others have been studied. The IGF1 gene in cattle is located in the chromosome 5 (BTA5), of which containing QTL regions of controlling twin (multiple) births. So this gene was possible to be used as a candidate gene to increase the genetic potency of cows to calve twin or multiple (Lien *et al*., 2000). The IGF1 gene played an important role in regulating folliculogenesis and possibly be involved also in regulating multiple ovulations in cows (Kim *et al*., 2009). Therefore, the IGF1 gene could be used as a positional candidate gene and intron 2 IGF1 gene was highly significant (P= 0.003) associated with twinning traits in cattle (Kim *et al*., 2009).

Osteopontin (OPN) gene in cattle is located in the chromosome 6 (BTA6), closely located to the QTL genes of milk production (Leonard *et al*., 2005). The bovine OPN gene consisted of 6 exons with the size of about 7 kb from genomic DNAs (Gen Bank accession number: NW_255516) and encoded a 278-AA protein (Kerr *et al*., 1991). Regulation and immediately functional implication of the conditions involving the OPN gene gave temporary and partial possibilities as the result of joining activities in developing and ensuring the maintenance of a pregnancy (Johnson *et al*., 2003). The objective of this study was to determine genetic polymorphisms of the IGF1 and OPN genes in HF cattle with historical twin and non-historical twin raised by small dairy farmers in Lembang District, West Java Province.

Materials and Methods

Blood and DNA Samples

A total blood sample number of 42 heads of HF dairy cattle consisting 27 heads of historical twin birth cattle and 15 heads of non-historical twin birth ones as the control. Genomis DNA was extracted from fresh blood sampes by using standard phenol-chloroform protocol (Sambrook and Russel, 2001).

Amplification and Polymorphism Identification

Amplification of IGF-1 and OPN gene fragments was done by using poly-

merase chain reaction (PCR) methods. Reagents were used for amplification of both fragments are 2 µl of DNA sample, 25 pmol of each primers (Table 1), 200 µM of dNTPs mixture, 1 mM of $MgCl_2$, and 0.5 unit of DreamTaq™ DNA polymerase with its buffer (Fermentas) in 25 µl of total solutions. Amplification process was running within GeneAmp® PCR system 9700 (Applied Biosystems™) with the condition of pradenaturation at 95°C for 5 minutes, 35 cycles consisting of denaturation at 95°C for 30 second, primer annealing at 60°C for 45 second and extension at 72°C for 1 minute, and the final extension at 72°C for 5 minutes.

Polymorphism identification both in IGF-1 and OPN gene fragments were detected by restricted fragment length polymorphism (RFLP) methods. The restricted enzyme which used for IGF-1 gen fragment was SnaBI (New England Biolabs) and for OPN was BsrI with following manufacture's instructions. The product of RFLP methods were visualized on 2% agarose gel (w/v) which stained by EtBr (ethidium bromide). Allele identification was followed Siadkowska *et al*. (2006) for IGF-1 gene and Leonard *et al*. (2005) for OPN gene.

Table 1. Primers information were used

Gene	Sequence (5'-3')	PCR Product	Restriction enzyme	Reference
IGF-1	F: ATT ACA AAG CTG CCT GCC CC	249 bp	SnaBI	Siadkowska *et al.*, 2006
	R: ACC TTA CCC GTA TGA AAG GAA			
OPN	F: GCA AAT CAG AAG TGT GAT AGA C	290 bp	BsrI	Leonard *et al*., 2005
	R: CCA AGC CAA ACG TAT GAG TT			

Statistical Analysis

Genotype frequency represents the ratio of a genotype to total population. Allele frequency is a ratio of an allele to the overall allele at a locus in the population. Mathemathics model genotype and allele frequency (Nei and Kumar, 2000) is represented as follows:

$$X_{ii} = \frac{n_{ii}}{N} \; x \; 100\%$$

$$X_i = \frac{(2n_{ii} + \sum_{i \neq j} n_{ij})}{2N}$$

Note :

χii = iith genotype frequency
nii = number sample of ii genotype
nij = number sample of ij genotype
N = total sample
χi = ith allele frequency

Results and Discussion

Amplification and Genotyping of the IGF1 Gene Fragment

Amplification of IGF-1 gene fragment was successful to amplified the 249 bp fragment were located in intron 1 of IGF-1 gene. Genetic polymorphism of the IGF-1 gene was detected by PCR-RFLP method by Siadkowska *et al*. (2006) through the examination of the presence of a C/T base transition at the 472 nucleotide position in non-codign region of *Bos taurus* IGF-1 gene. The substitution of C to T produced a new SnaBI (IGF-1|SnaBI) restriction site. The animals were genotyped by follow Siadkowska *et al*. (2006). Animal with homozigot TT was indicated by the presence of two fragments i.e. 223 and 26 bp, while the genotype homozigot CC was indicated by absent of SnaBI restriction site and showed only one fragment i.e. 249 bp. The heterozigot CT was indicated by the presence of three fragments i.e. 249, 223 and 26 bp.

The RFPL analysis samples show that the genotype of HF cattle was homozigot CC. The allele was found are allele C. This result caused the frequency of the CC genotype obtained are 100%, regardless of the CT and TT genotype are 0%. This result was contrast to those of some previous studies by detecting the presence of genetic polymorphisms in the bovine IGF-1 gene. Polymorphism short tandem repeat (STR) in the 5'flanking region of intron 3 on IGF1 gene was identified by Kirkpatrick (2001). Single strand conformation polymorphism (SSCP) in the 5' flanking region of intron 1 on IGF-1 gene was also identified as a transition of T/C known as RFLP|SnaB1 (Ge *et al*., 2001). Two polymorphisms of the IGF1 gene, the insertion/dilesi TTTG (InDel) in intron 4 and RFLP|DpnI in intron 5 were found in Norway cattle (Lien *et al*., 2000).

Amplification and Genotyping of the OPN Gene Fragment

Amplification of the intron 4 OPN gene which located at the chromose 6 (BTA6) investigated in HF cattle resulted in a fragment length of 290 bp. The amplification product was then restricted by BsrI enzyme to detect the presence of point mutation in the intron 4 OPN gene. Genetic polymorphism of the OPN gene in this study followed the methods of Leonard *et al*. (2005) which examined the transition C/T in the 5' non-code area in the intron 4 of *Bos taurus* OPN gene .

The substitution of C to T produced a new BsrI (OPN|BsrI) restriction site. Animal with homozigot CC was indicated by the presence of two fragments i.e. 200 and 90 bp, while the genotype homozigot TT was indicated by absent of BsrI restriction site and showed onlyone fragment i.e. 290 bp. The heterozigot CT was indicated by the presence of three fragments i.e. 290, 200 and 90 bp.The analysis of RFLP on the OPN|SbrI within HF cattles with historical twin from Pangalengan district show that there was found three genotypes, namely the CC genotype, CT, and the TT genotype.

Table 2. Genotype and allele frequency of the OPN gene in HF historical twin and control

Population	Cattle (Head)	Genotype Frequency (%)			Allele Frequency (%)	
		CC	CT	TT	C	T
Pangalengan	Non-twin (0)	-	-	-		-
	Sub total (17)	24 (4)	53 (9)	24 (4)	50	50
	Twin (10)	40 (4)	60 (6)	0 (0)	70	30
Lembang	Non-twin (15)	20 (3)	40 (6)	40 (6)	40	60
	Sub total (25)	28 (7)	48 (12)	24 (6)	52	48
Total	Twin (27)	30 (8)	56 (15)	14 (4)	57	43
	Non-twin (15)	20 (3)	40 (6)	40 (6)	40	60
	Total (42)	26 (11)	50 (21)	24 (10)	51	49

Note: (….) was blood samples

The frequencies of the occurrences of CC, CT and TT genotypes of the OPN gene of HF historical twin cattles from Pangalengan ditrict were 24, 52 and 24% repectivelly. For HF cattles from Lembang district were found some interesting things. For non-historical twinning cattles as the controls were identified three genotypes, namely CC, CT and TT genotypes, with the frequencies of the occurences of the respective genotypes were succesively 20, 40 and 40%y. For historical twin animals in this location were found none animal having the TT genotype (0%), so those historical twin cattle had only two genotypes of CC (40%) and CT (60%) respectively.

Conclusion

Genotyping on the intron 1 region of IGF-1 gene in the BTA5 in HF cattles of both historical twin and non-historical twin resulted in no genetic polymorphism (monomorphic) as the DNA fragment representing solely the CC genotype. This is as indication of the C/T substitution in the intron1 IGF1 gene might be disappearence, so this gene was unable to be functioned as a candidate gene in studying twinning traits in HF cattles.

Genotyping on the intron 4 of OPN gene in HF cattles with historical twins and non-historical twins resulted in three genetic variance, providing CC, CT, and TT genotypes, but their frequencis was varied. This result proved that the C/T transition in the non-code area on intron 4 of OPN gene could be used as an early indicator as a candidate gene to study its control on milk uterus secretion to mediate twinning birth in in HF cattle.

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Genetic Marker Approach for Confirming the Existing Twinning Trait in PO Cattle*

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Abstract

Up to present, Indonesia still depends on importing of the beef cattle from overseas both for slaughtering and feedlot needs. The Ongole ascendant (PO) cattle is one of Indonesian beef cattle representatives that has adapted very long in Indonesia and has a potential source of meat production. Almost 1% PO cows in central Java delivered twin calves both by AI and nature fertilization. This study was designed to confirm the existence of genetic marker associating with twinning trait in PO cattle. Amount of 35 DNA samples were collected from PO cows with twin birth experiences and twin calves. Amount of 12 DNA control samples was also involved. Three pairs of primers for microsatellite genetic markers of BMS-1216; BM-321and RM103 were used for amplification of all DNA samples. PCR products were visualized through 8% ND PAGE. The result showed that all fragments with twinning experience were on right sizes of BMS1216 (143-165bp); BM-321 (108-126bp) and RM 103 (114-144bp) of the Bovine chromosome 5. Even though negative samples delivered not clear bands but there were still in the right sizes of applied microsatellites. Microsatellite marker is only an early clue that obtained fragments might be bearing the putative locus associating with twin trait. Therefore in the coming study it needs to confirm the existence of candidate gene(s) associating with twinning traits by using SNP (Single Nucleotide Polymorphism) markers.

Keywords: genetic marker, twinning trait, PO cattle, confirmation

Introduction

Traditional genetic selection in livestock has been conducting up to present to select important traits which have economic values. This manner has contributed many advantages in livestock development this present. However, this traditional selection manner has been considered as not an effective way in breeding program since this selection is time consuming and involves many persons to measures the performance of desired traits.

In the past 20 years ago, there was a great changing in livestock breeding from quantitative genetics shifting to molecular genetics with emphasized in the identification of quantitative trait loci (QTL) and marker-assisted selection (MAS). In that past, advances in molecular genetics have lead to either the identification of multiple genes or genetic markers or single gene associated with specific traits (Dekkers, 2004). In addition, those genetic markers could identify QTL or genomic regions that affect quantitative traits.

Genetic selection in cattle for some difficult traits or the trait with low heritability, such as reproduction, it has traditionally had very little success (Allan *et al*., 2009). The advances in DNA technologies, genetic selection of traits with low heritability could improve reproductive efficiency more rapidly in cattle. One important trait of reproductive traits is twinning birth in industry of cattle producers. This twinning trait is shown very small percentage in cattle since cattle is a uniporous cattle with a single delivered calf in each birth. As reported byKomisarek and Dorynek (2002), the accidence of twinning birth is smaller (about 1%) in beef cattle and more than 4% in dairy cattle then increased in more age in cows.

The QTL study associated with twinning trait was reported in chromosome 5 of bovine by using genome-wide linkage analysis(Lien *et al*., 2000).Microsatellite markers have been used in initial genome mapping (De Atley *et al*., 2008) and also used to identify the location of Quantitative Trait Location (QTL). Even though today it most efforts involved SNP (Single Nucleotide Polymorphism) marker in genome mapping. Microsatellites have been identified in both coding and non-coding regions of the genome and have been utilized to detect QTL (Sellner, *et al*., 2007).

Our observation of twinning birth in Ongole ascendant (PO) at one district of Grobogan regency in central Java, it was often occurred and delivered every year. Owners or stakeholders of twinning birth have fertilized their cows by using either bull (naturally) or AI (Artificial Insemination), (unreported data).Based on that information, this study was designed to confirm those cows whether the twinning trait affected genetically or by chance. All DNA samples of cows delivering twin birth were used for confirming the candidate gene (s) associated with twinning birth in PO cattle by application of microsatellite markers.

Materials and Methods

DNA Genome Samples

Samples of DNA were collected from fresh blood of 35 cows delivering twinning birth at some districts under Grobogan regency of Central Java Province in 2011. As 12 DNA control samples (PO without twin background) were also included. A modified method of high salt (Montgomery and Sise, 1990) was used to extract the DNA samples.

PCR Optimization and Amplification

In prior to DNA amplification, it was performed optimization of PCR in order to get suitable annealing temperatures of three pairs of microsatellite primers using a PCR gradient (Techne, UK). The work of PCR was conducted based on the suitable annealing temperatures. Three pairs of primers for microsatellite genetic markers were BMS-1216 (Accession No. G18633); BM-321 (Accession No. G18515) and RM103 (Accession No.U10391) drawn from NCBI, see Table 1. Those primers were used for amplification of all DNA samples. The PCR reagent consisted of 2 µl DNA template; 4 µl of 10 ρm primer; 2.5 µl of buffer for Taq polymerase; 2 µl of 25mM $MgCl_2$, 2 µl of 2.5 mM dNTP, 0.3 µl Taq Polymerase (1 unit per 1 µl) , and added dd-H_2O up to 25 µl of total volume. A program of PCR was performed as following procedure: 2 min at 94 ºC as initial denaturation, followed by 30 cycles of 30 sec at 94 ºC, 45 sec at 54/59 ºC, and 30 sec at 72 ºC, with a subsequent 5 min final extension at 72 ºC.

Table 1. Nucleotides of the Used Primers

Primer	F/R	Primer Sequens	Acc No.	Annealing (°C)
BMS1216	F	GCCTGCATGTGTCTGTGG	G18678	59
	R	TCTGTGTCGGAATACCCTCC		
RM103	F	TCTGTGCACTTTACATTTAACAGA	U10391	54
	R	GTGGTCTATTGAACTTTTGTTCAGA		
BM321	F	AAGGGTCAGACAAAACTTAGCA	G18515	54
	R	ATCCTTGCCCTAATTCTCATTC		

Sources: Ihara *et al.* (2004).

Visualization of Targeted Fragment

PCR products were visualized through 8% ND-PAGE (Non-Denaturing Poly-Acrylamide Gel Electrophoresis). Targeted fragments were detected by confirming the emerged bands with the 100bpDNA ladder. The right sizes of band performance

indicated the candidate locus of the candidate of desired gene encoding the twin birth trait.

Results and Discussion

Samples of DNA collected for this study were drawn from all cows delivering twin calves and some of twin calves. Total of DNA samples was 35 and stored at -20°C. Optimization of PCR for confirming the closely suitable annealing temperatures was conducted to all three primer pairs of chosen microsatellite genetic markers by using a gradient PCR. The obtained annealing temperatures were 54 °C (RM 103; BM 321) and 59 °C (BMS 1216).

Based on those annealing temperatures, each of DNA samples (35) were amplified with the mentioned above of PCR program and PCR reaction using 3 pairs of genetic microsatellite primers. Therefore, it was found 105 fragments bearing candidate locus that expected containing genes encoding twinning trait in PO cattle, see Figure 1.

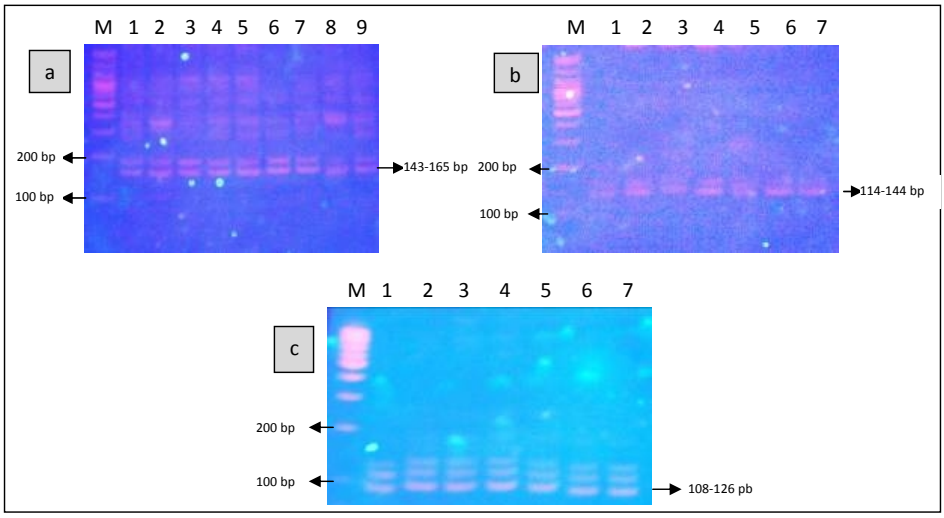

Figure 1. Amplification results on 8% ND-PAGE using primers of BMS 1216(a), RM 103 (b), BM 321 (c). M: 100bp DNALadder; No. 1 to 7; 1 to 9: DNAsamples of PO cattle.

Figure 1 showed representative fragments of the PO DNA twin samples. The results of DNA amplification showed that all fragments or bands were on right sizes of BMS1216 (143-165bp); BM-321 (108-126bp) and RM 103 (114-144bp) on the Bovine chromosome 5. This finding indicates that the all DNA samples might bear the putative locus of twinning trait that encoded by twinning gene(s). The whole fragments showed at the same pattern of monomorphyc double bands.

Therefore, allele frequency of the three microsatellite markers is a hundred percent of monomorphyc. In this study, there was not found specific allele(s) for twinning trait since the twinning trait is influence by multi genes (Komisarek and Dorynek, 2002). Twinning trait therefore could be associated with either high ovulation rate (Meuwissen *et al.*, 2002; Allan *et al*., 2009) or higher milk production (Fricke and Wiltbank, 1999; Wiltbank *et al*., 2000; Weller *et.al*., 2008;) or could be associated with growth trait.

As comparation, negative samples of PO DNA or without twinning birth experience were also amplified with those three microsatellites. However the result seemed to be a weak approval of either bearing or without bearing the putative locus of twinning traits (Figure 2). Compared to the twinning sample, 12 DNA samples without twinning background did not show a significant difference between twinning and negative (control) samples.

This finding is still a rough report as an initial clue that the whole DNA samples derived from all cows and calves showed as early proof for twinning birth traits. This finding might be supported by the source of collected DNA samples were derived from cows with twinning history in their live and twin calves.

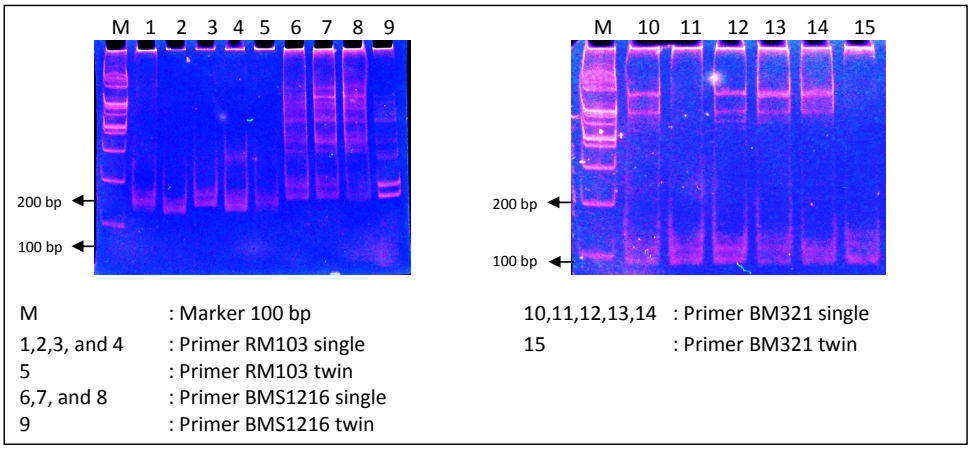

Figure 2. Negative (control) samples amplification results on 8% ND-PAGE using primers of BMS1216, RM103, and BM321.

Identified locus in this study was still a wider location of desired gene of twinning birth. The incidence of twinning birth is associated with the high of ovulation rates in cattle (Allan *et al*., 2009). Genomic scans for ovulation rates have been studied in selection of cattle population to increase the incidence of twinning rate (Kappes *et al*., 2000).Twinning trait is multiple genes that can be traced by implementation of molecular genetics of microsatellite. Advances in molecular genetics could identify multiple genes or genetic markers associated with genes that affect traits of interest in livestock, including genes for single-gene traits and QTL or ge-

nomic regions that affect quantitative traits (Dekkers, 2004). In addition, this molecular genetics has advantage in enhancing the selection response of particular traits that are difficult to improve by conventional selection such as traits with a lower heritability or traits for which the measurement of phenotype is difficult, expensive and only possible late in life, or not possible on selection candidates.

Therefore, this result needs to be further studied with emphasizes on using SNP markers. Nowadays, most efforts involve a Single Nucleotide Polymorphism (SNP) marker. The SNP marker could detect mutation of by either insertion of deletion of single nucleotide.

Conclusion

Based on the applying of three microsatellite markers in the confirming of twinning traits in PO cattle it showed that all DNA samples emerged the right size of targeted fragments in the same pattern of monomorphyc bands. This is an early indication that the obtained fragments might be bearing putative locus encoding twinning trait. Further study in Single Nucleotide Polymorphism (SNP) might be necessary to confirm the existence of the twin gene(s) in PO cattle.

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Carcass Traits Association with GH/AluI Gene Polymorphism in Indonesian Aceh Cattle

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Abstract

This study was conducted in order to identify polymorphism of growth hormone gene in the exon five and to determine the association of GH/AluI polymorphism with carcass quality in Aceh cattle. A total of 42 DNA genome samples were extracted from two Aceh cattle population, i.e., Banda Aceh (12), Aceh Besar (30), and the PCR-RFLP was used to amplify 404 bp of GH gene. The result showed that the LL genotype was the only genotype found in Aceh cattle population, and the alele frequency of alel L is 1. This finding indicated that there was not evidence of polymorphism of GH/AluI in Aceh cattle, and there was not correlation of GH/AluI gene with carcass quality of Aceh cattle. It could be affected by small number of sampling size. However, this study suggests that GH gene could be possible used as genetic marker.

Keywords: Aceh cattle, GH gene, PCR-RFLP, Polymorphism

Introduction

The enhancement of beef cattle's productivity in Indonesia will be more appropriate if it is done through a selection which is not only based on the phenotype, but also combined with direct selection on the level of DNA which codes the phenotype in which the quality needs to be improved. The bovine genome map made based on markers on genome DNA uses molecular technique such as RFLP, microsatellite, minisatellite, PCR-RFLP, and PCR-SSCP make it possible to identify gene locus which are responsible for trait variations having economic values.

GH gene as a genetic marker is frequently used in researches because growth hormone gene (GH) is one of the genes which influence growth (Di Stasio *et al*.

2005). Casas *et al.* (2004) reported that QTL for growth traits, carcass composition and beef quality was spread on chromosome 1, 2, 3, 16, 17, 19, 20, 21 and 26. GH gene has a great role in beef cattle's performance (Breier. 1999), hence it is very interesting to identify GH gene polymorphism on Aceh cattle.

Materials and Methods

This research included field and laboratory activities. Field activity was conducted in Banda Aceh and Aceh Besar slaughterhouses. The DNA extraction and characterization of GH/Alu I gene diversity was conducted in Animal Molecular Genetics Laboratory, Faculty of Animal Science, Bogor Agriculture University, whereas the examination of carcass and meat quality was carried out in Ruminansia Besar Laboratory, Department of Animal Production and Technology. This activity was done in October 2009 - November 2010.

Blood Sample of Aceh Cattle

This research used Aceh cattle's meat (muscle) in which this cattle is local ones originating from Aceh province. Twelve (12) samples were taken in RPH Banda Aceh, and thirty (30) samples from RPH Antassalam, Aceh Besar (Table 1). The sample used was *longisimus dorsi* muscle on the $12^{th} - 13^{th}$ rib. The cattle slaughtered were bred traditionally, in which during daytime, they were herded, and put in stalls at nights. The slaughter was done traditionally.

Table 1 The number of meat sample used for GH/AluI gene analysis

Population	Number
RPH Banda Aceh	12
RPH Aceh Besar	30
Total	42

Genome DNA Extraction (Meat)

DNA extraction was done from the meat. The extraction procedure followed phenol-chloroform method (Sambrook *et al*. 1989).

GH Gene Amplification

The amplification of GH gene fragment was done using PCR (*polymerase chain reaction*) method. The PCR machine used was *thermal cycler* (*Ependorf 5332*). The arrangement of primers used can be seen in Table 2.

Table 2 Primers GH/AluI (Gen Bank M57764.1, Gordon *et al*., 1983)

Locus	Temperature *annealing*	Product PCR	Sequences of Primers
GH/AluI	59 ºC	404 bp	F:5'-TAGGGGAGGGTGGAAAATGGA-3'
			R:5'-GACACCTACTCAGACAATGCG-3'

Genotype Determination Using PCR-RFLP

The genotype determination of each individual was done using *restriction fragment length polymorphism* (RFLP) which was visualized on agarose gel 1.5% with the buffer of 0,5x TBE (tris borat EDTA), functioned on 100 V for 40 minutes, and colored with ethidium bromide on *UV trans illuminator. Alu*I was utilized as a cutter enzyme for the target gene fragment.

Data Analysis

Gene Frequency. The genotype frequency was the ratio of the number of a certain genotype towards population number. Allele frequency was the ratio of a certain allele towards the whole allele in a locus in a certain population. The frequency of each allele in each locus was counted based on Nei dan Kumar (2000) formula:

$$X_i = (2n_{ii} + \sum n_{ij}) / (2N)$$

Results and Discussions

GH Gene Amplification Results

The amplification of growth hormone (GH) gene fragment done on Aceh cattle showed primers forward on the position of intron 4 and primers reverse on the position of flaking region 3 (Figure 1). The GH gene fragment amplification was conducted using thermal cycler (*Ependorf 5332*) machine on the temperature of annealing 63 ºC.

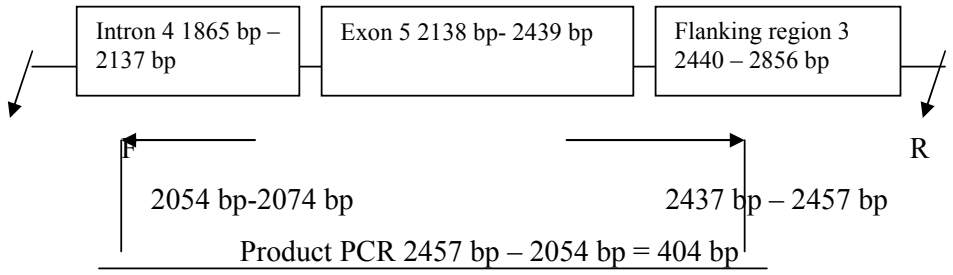

Figure 1. Primers forward position, primers reverse and PCR GH gene product

The result of gene fragment amplification visualized on agarose gel 1.5% is presented on Figure 2. The length of GH gene fragment amplification is 404 bp.

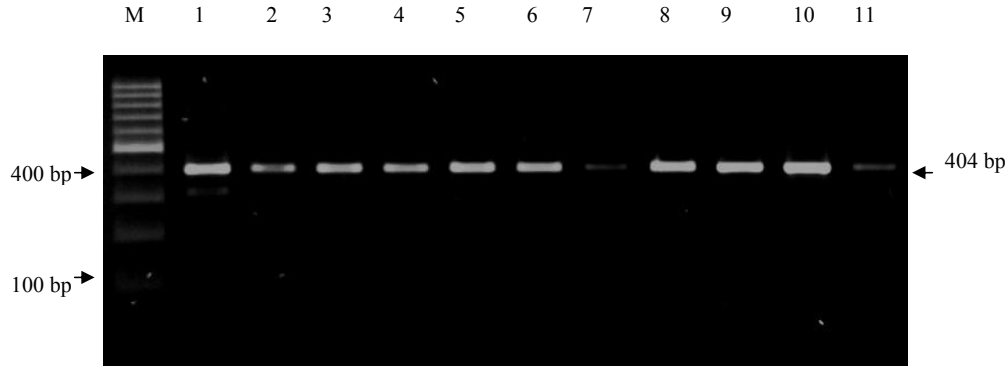

Figure 2. Visualization of GH gene fragment amplification result on agarose gel 1.5% (M: marker 100 bp, 1 -11: research sample)

The amplification results conducted by Gordon *et al.* (1983), Yao *et al.* (1996), Ge *et al.* (2003) and Zakizadeh *et al.* (2006) with the same primers showed that annealing primers GH/AluI gene fragment on 59 ºC for 80 seconds, 65 ºC for 30 second, and 57 ºC for 60 seconds resulted in good PCR product. The annealing temperature used in this research was 63 ºC for 45 second to obtain optimal PCR product so that it can be read clearly.

Identification of GH gene variants using PCR-RFLP

The determination of GH gene genotype in this research was carried out using PCR-RFLP with AluI as the cutter enzyme. AluI enzyme recognized AG|CT cutting site. Based on the sequence of GH gene DNA fragment being amplified, two AluI cutting sites were obtained; they are fragments with the length of 87, 132, and 185 which are knows as leucyne allele (L) (Picture 3).

The cutting using AluI enzyme on AluI GH gene fragment as much as 404 bp only resulted in one kind of fragment: a fragment which can be cut (two bars) known as LL genotype, whereas the fragment which cannot be cut (one bar) known as VV genotype and combined fragment (three bars) knowns as LV genotype cannot be found in this research (Figure 3).

The visualization result using agarose 1.5% shows that GH/AluI locus on the Aceh cattle sample population being observed is uniform. The genotype found on Aceh cattle in this research is LL genotype. Based on the analysis, the LL genotype frequency was one (1). This made the LV and VV genotype frequency zero (0). Based on Nei dan Kumar (2000) equition, allele L frequency is 1 and V allele frequency is 0. This result is likely caused by the very limited research samples.

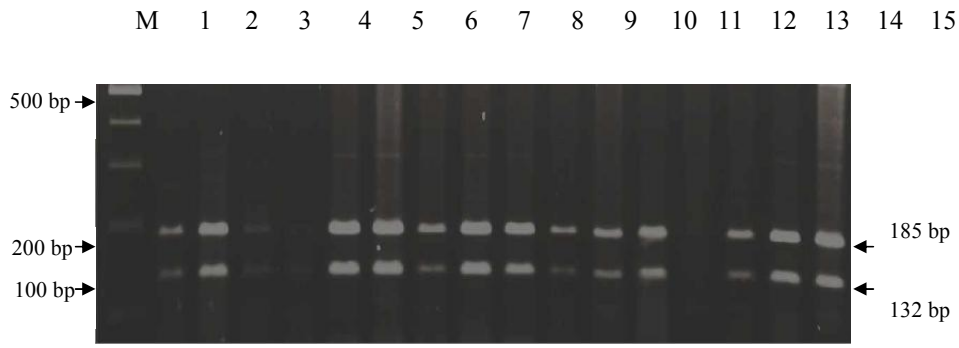

Figure 3. Visualization of GH|*Alu*I gene fragment PCR-RFLP on aragose gel 1.5% (M: Marker 100 bp, 1-16: research sample)

Analysis Results on Aceh Cattle' Carcass and Meat Quality

The value of parameter average of Aceh cattle carcass and meat quality can be seen on Table 3. The pH value of the meat in this research has the average of 5.46, which shows the pH range of normal meat 5.4 – 5.8. The meat color is in category I (score 1 – 5) according to SNI (Indonesian National Standard, 2008). The degree of tenderness 4 -5 is considered moderate, not too tender and not too tough.

Table 3 The results of Aceh cattle's meat and carcass quality

Parameter	n	Average ± standard deviation	Indonesian National Standard
Weight (kg)	42	301.88 ± 125.59	-
Eye muscle area (cm^2)	42	34.19 ± 3.51	-
pH	42	5.46 ± 0.39	5.4-5.8
Tenderness (kg/cm^2)	42	4.79 ± 1.74	4-5
Cooking loss (%)	42	35.38 ± 6.24	-
DMA (%)	42	29.94 ± 4.55	-
Meat color	42	3.8 ± 1.81	1-5

The research results identify that Aceh cattle's carcass/meat has finer meat fiber and its color is red. The quality and meat structure greatly depend on types of meat and location. Marbling is also very influenced by the breeding system and food given.

Conclusions

Based on this research results, it can be identified that the use of GH/AluI only resulted in LL genotype and monomorphic, so that it cannot be used as a marker to

associate with the carcass quality on Aceh cattle. This phenomenon is likely due to the limited number of samples and the existence of natural selection towards LV and VV genotype as the consequence of Aceh cattle adaptation's to the local environment. Thus, a further research is still necessary, by using more samples and if diversity is found, sequencing needs to be done so that it results in more accurate research results.

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Identification of Holstein-Friesian Lactating Cows as Good Replacement Stocks under Small-Scale Dairy Farming in a Highland of West Java, Indonesia

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Abstract

Good replacement stock has an essential role to improve animal productivity and to ensure the sustainability of domestic small-scale dairy cattle farming. This study was done in HF dairy farmers as the members of two Milk Collection Units (of 23 MCUs) at North Lembang Milk Cooperation Unit (NLMCU). Research was located in the selected dairy cattle area of having dense cow population, high milk production and prospective region in a mountainous field of Lembang Subdistrict, West Java. Farmers as samples were dominated by small-scale ownerships by commonly applying a low input system. A recorder was contracted to record daily milk yield (morning and evening) of individual lactating cows in a rotation manner for one record per cow per month during five months of lactation from June to October in 2010. The total number of lactating cows recorded in the accordance of the recorder capability was 180 heads. The observed cows varied in the status of early lactation (1-4) and lactation period (1-5). Averages of test day milk from the 1st to 5th months (TD1-TD5) were successively 17.2 ± 4.2, 16.0 ± 3.9, 14.8 ± 3.4, 13.4 ± 2.6, and 13.0 ± 2.3 l/d. Cumulative milk production for 5 months of lactation (CMP 5mo.) was calculated as the sum of the multiplication of each standardized TD (TD2-TD6) x 30 (number of days each month). The resulted showed the average of the estimated CMP 5mo. was 2296 ± 469 lt. (1200-3780 lt.). Cows with CMP 5mo. above 2296 lt. could be considered as good replacement stocks. As the results of this evaluation was still early, data of completed milk yield and related lactation traits should be continually collected to get more precisely information in identifying qualified replacement stocks to ensuring the improvement of dairy cattle milk yield and productivity.

Key words: dairy cattle, milk yield, replacement stock, small dairy farmer

Introduction

National dairy cattle population currently was around 407,767 hds (DGLS, 2010). The highest population was in East Java Province by 141,199 hds (34.6%), followed by Central Java by 190,562 hds (34.4%), and West Java by 120,768 hds (29%). Java is well known as the Island with the densest population and the highest intensified-land use. This condition made the dairy businesses were mostly carried out under an integrated crop-livestock system, of which the sources of forages as animal feeding directly influenced by the availability of agricultural and industrial crops by-products (Devendra, 1999; Anggraeni and Sofyan, 2008).

Small-scale dairy farmers commonly faced many constraints such having limited land and capital beside of unwell keeping in breeding and farming practices (Kusmaningsih *et al*., 2008). Cattle were commonly kept in a freestall housing system from where the supplies of feeding and input production closely related to the outside. This caused of most of small dairy farmers more likely taking the decision of maintaining only lactation cows compared to heifers and calves (female). Lactating cow was considered directly generate income through the saling on their milk yield every day, so they could pay daily cost of input production.

Producing replacement stoks (RSs), especially young lactating cows to replace cows having a low productivity and to renew old cows, was therefore an essential aspect in the effort to increase domestic fresh milk production. Provision of female cattles as RSs with a high genetic potency to express milk yield in the high agro-ecosystem in central dairy area in Java then should get attention. The aim of this research was to identify young lactating cows to be considered as qualified RSs by producing milk 30% above the average in small dairy farmers.

Materials and Methods

This field research was carried out in a highland of the central dairy region in the North Lembang Milk Cooperation Unit (MCU), Lembang District, West Java. Research location was selected for the reason of having high density of dairy cows and a low rate of animal mutation. Dairy farmings studied were represented by small-scale dairy cattle farmings as the members of the North Lembang MCU. HF lactating cows observed came from two Milk Collecting Groups (MCGs) of Gunung Putri and Nagrak from the total of the existing 23 MCGs. These two MCGs were selected as having the densest population and reaching high milk yield compared to those of other MCGs. HF lactating cows studied were identifed by purposive sampling.

Test day milk yields (morning and afternoon) or milk TDs were recorded for individual cow per month during five months of lactation observed. Data were recorded rotationally by a contracted recorder, from July to November, 2010. Re-

corded cows were selected in more similar status of lactation, by considering the biological aspects of early months or stages of lactation and lactation periods. The total number of cows recorded for their milk TDs in one month depended on the recorder's capability. Previous analysis showed that recorded milk TDs data were distributed within early lactations at 1-5 months and lactation periods of 1-5 times. Their effects on milk TDs were investigated.

Based on the previous analysis showed months of lactation significantly affected on milk TDs ($P<0.01$), so its effects were standardized to the ranges of 2-6 months (TD2-TD6) of lactation. This was done by formerly estimating milk TD curve of individually lactating cow with regarding to develope an appropriate regression equation (linear or quadratic) in developing suitable correction factors. Partial cumulative milk production for 5 months (CMP 5mo.) of each cow was calculated as the sum of the multiplication of each standardized TD (TD2-TD6) x 30 (number of days in one month). Cows having CMP 5 mo of 30% above the average were then identified as qualified RSs.

Results and Discussion

General Research Condition

Research Location. North Lembang MCU was well known for the potency of their HF cows in producing high milk in central dairy area in Lembang District, West Java. This MCU supervised a large number of small dairy farmers as the members. Most working area of this MCU were located high land at the altitude of around 1,200-1,257 m asl. Climate had the temperature in a range of 15.6 - 16.8 ^{o}C during rainy season and 30.5 - 32.7 ^{o}C for dry season. Rainfall was around 1,800-2,500 mm per year. Ensminger (1971) stated *Bos taurus* dairy cows could produce milk optimally at the ranges of temperature of 10-15.6 °C and humidity of 50-70%. Cows still produced more milk at the temperature up to 21.1 ^{o}C.

Dairy Farmers. Dairy cattles were commonly raised at a small-scale by dairy farmers. The North Lembang MCU had 22 working area, 23 Milk Collecting Groups (MCGs), and 603 Milk Collecting Units (MCUs). MCUs were built to facilitate the collecting fresh milk from the farmers. The two selected Gunung Putri and Nagrak MCGs were identified with their HF lactating cows produced milk yield around 12.7 lt/d and 15.9 lt/d respectively.

Management. Animals were very commonly kept in pens with their location separated from the houses, either behind or beside of farmers' houses. The size of housing of mature cow was around 1.5 m x 2.0 m. Roofing was made by any materials such as tiles, zinc, asbestos, and their combination. Wall mostly opened through

outside. Floor used a variety of materials such as cement, wood, rubber and their combination. Rubber was used as the base of floor for facilitating farmers more easy in cleaning pens and keeping animals not easily slipped. Water resources were from local water drinking company or well and used for drinking and bathing animals as well as cleaning pens.

Feeding Animal. Feeding animals highly depended on agricultural and industrial crops by products. Farmers fed animals by mixturing concentrate. Some additional feeding as protein and energy sources were used, such as bran, soybean, cassava pulp, and beer. Feeding forages to animals varied widely. Generally, farners gave wild, elephant and King grasses. Forages were cut from their own garden or community land or from the sourrounding. Some farmers rent grassing land from Local Government Forestry. Farmers had the difficulty to obtain forages during the dry season, so the effort of finding forages could be expanded farther even in other districts.

Milk Production

Total HF lactating cows recorded on their milk TDs were 184 hds. By plotting milk TDs into five months of lactation resulted general pattern of these milk TDs continually decreasing by progressing lactation. Decreasing trends occured for all lactation periods (1-5). The averages of test day milk yields (TD1-TD5) were succesively 15.2, 14.8, 13.8, 12.5, and 12.2 lt at the first lactation; and 17.4 , 16.1, 14.9, 13.4, and 13.0 lt at the fifth lactation.

By distributing milk TDs into different early months of lactation (1-5) showed that those milk TDs were expanded from the first up to the eight months of lactation (Table 2). HF lactating cows studied seemingly showed of having milk TDs curves close to the normal milking curve. By progressing months of lactation, milk TDs continually decreased. The averages of milk TDs (TD1-TD8) for all lactation

Table 1. Daily milk yield of HF lactating cows by lactation period

Lactation period	Number of animal (hds)	Test Day Milk Yield (Litre)				
		TD1	TD2	TD3	TD4	TD5
1	34	15.2 ±3.0	14.8±3.2	13.8±2.9	12.5±2.3	12.2±2.3
2	39	17.3±4.4	16.6±4.3	14.9±3.2	13.6±3.0	13.2±2.4
3	35	18.0±4.8	16.4±4.3	15.0±3.9	13.6±2.8	13.2±2.6
4	24	18.0±5.1	16.5±3.9	15.6±3.6	13.8±2.5	13.4±2.1
5	22	17.4±4.1	16.1±3.9	14.9±3.5	13.4±2.3	13.0±2.0
Average	184	17.2±4.2	16.0±3.9	14.8±3.4	13.4±2.6	13.0±2.3

Table 2. Daily milk yield of HF lactating cows based on early months of latation (milk TD1-TD8)

Month of lactation	1st Month	2nd Month	3rd Month	4th Month	5th Month	6th Month	7th Month	8th Month
Average	18.2	17.5	16.6	15.5	14.3	14.0	13.1	12.3
SD	4.52	4.37	3.51	3.58	3.09	2.88	2.80	2.64
Record	55	86	120	154	154	99	68	34

periods were succesively 18.2, 17.5, 16.6, 15.5, 14.3, 14.0, 13.1, and 12.3 lt.

Milk TDs of HF lactating cows in this study were higher compared to those of HF cows maintained in Colimber MCG as another MCG in the North Lembang MCU (Sukandar, 2010). HF cows were reported in producing milk TDs (TD1-TD10) succesively 15.4±4.3, 18.5±3.8, 18.0±5.0, 16.6±7.7, 16.2±4.4, 14.5±2.8, 14.1±3.7, 12.3±2.0, 11.9±6.9, and 10.6±4.0 lt/d.

TD milk yileds of HF cows in this study were also lower than those daily milk yields of Holstein heifers kept in Brazil in 1988-1991, by reporting milk TD1-TD10 succesively 21.8, 23.6, 23.3, 22.1, 20.9, 19.7, 18.3, 17.3, 16.0, and 15.0 lt/d. (Machado *et al*., 1999). The differences possibly caused by some kactors such as animals milking ability, management and environment.

Standardization of Milk Production

As previously escribed, the excisting differences of the months (stages) of lactation and lactation periods of observed HF lactating cows required the examining their effects on recorded milk TDs. Differences in the early months of lactation certainly made a major influence on daily milk yileds among lactating cows studied. The significantly effect of different months of lactation on milk TDs (TD1-TD5),

Table 3. Daily milk yields of HF lactating cows standardized into TD2-TD6 months of lactation

Lactasion Period	Σ Cow (Hds)	Standardized Test Day Milk Yield (Litre)					Average (Litre)
		TD 2	TD 3	TD 4	TD 5	TD 6	
1	34	16.3±0.7	15.2±0.7	14.0±0.6	13.0±0.5	12.3±0.4	14.2±0.6
2	39	17.9±0.7	17.0±0.6	15.3±0.5	14.0±0.5	13.2±0.4	15.5±0.5
3	35	19.1±0.7	17.0±0.7	15.2±0.6	14.0±0.5	13.2±0.4	15.7±0.6
4	24	18.5±0.8	16.8±0.8	15.8±0.7	14.4±0.6	13.5±0.5	15.8±0.7
5	22	18.4±0.8	17.0±0.8	15.5±0.7	14.3±0.6	13.1±0.5	15.7±0.7
Significancy		ns	ns	ns	ns	ns	-

Description: The effects of lactation period on individual standardized daily milk yields (TD2-TD6) were not significant (P>0.05).

that was identified previously, were then standardized to 2-6 months of lactation. Standardization of milk TDs was more frequently developed by a quadratic equation. The averages of daily milk yields as the result of standardization into TD2-TD6 was presented (Tabel 3).

Further examination of the influence of different lactation periods (1-5) on the standardized milk TDs (TD2-TD6) proved that not one of this effect was significant (P>0.05). This implied that standardization was not required to the lactation periods.

Cumulative Milk Production

Cumulative milk production of five months of lactation after standardized to the months of lactation of TD2-TD6 was abbreviated by CMP 5 mo. CMP 5 mo. for each lactating cow from the total HF lactating cows of 154 hds were evaluated. The estimated CMP 5 mo. of individual HF cows were then be ranked, starting from the highest to the lowest ones. The average of the estimatef CMP 5 mo. of the HF lactating cows were by 2,296 ± 469 lt with a range between 1,200-3,780 lt. By considering the qualified RSs as those HF lactating cows having CMP 5 mo. of 30% above the average, therefore those qualified RSs should reach CMP 5 mo. above 2,985 lt. These qualified RSs were identified for 10 hds, succesively for the HF cows No. 4218, A00046, 56, 3707, A.03000, 8354, 2965, 101 138, 31 424, 36 173, 102 361, and 102 096. The first rank was for the cow of No. 3780 with CMP 5 mo of 4,218 lt., while the lowest was for the cow of No. 096 with CMP 5 mo. by 3,015 lt.

HF cows possesing CMP 5 mo. more than 2,296 liters could be considered as qualified RSs. However, to ensure more cows available be used as good RSs in regarding to replace the cows of less productive and the old ages, then HF lactating cow with CMP 5 mo. exceeding the average of 2,296 ± 469 could be possible to be considered as good RSs. By including the cows with CMP 5 mo. above the average as good RSs could guarantee more good RSs were available for small dairy farmers. These RCs could considrerably produce optimal milk under small-scale dairy farmings. From this research results, a following-up action was required for collecting more data in daily milk yields and related traits to indentifed good RSs better. Lacating cows that could be identified accurately as good RSs became a key factor of ensuring sustainable of small-scale dairy farmings in our country.

Conclusion

- The number of HF lactating cows identified as qualified RSs for having CMP 5 mo) 30% above the average denden closely to the average TD milk yields of the cows in the targetted location.
- HF lactating cows classified as qualified RSs in this research location (Gunung Putri and Nagrak MCGs) if their estimated CMP 5 mo. exceeded 2,985 lt.

- Qualified HF RSs in this research were identified for 10 animals, from whict at the first rank was for the cow No. 3780 (CMP 5 mo. = 4,218 lt.) and at the tenth rank was for the cows No. 102 096 (CMP 5 mo. = 3,015).

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Qualitative Traits of *Walik* Chickens, The Rare Indigenous Chicken, in West Java, Indonesia

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Abstract

The Walik chicken is one of the rare indigenous chickens in Indonesia owned frizzling feathers. Since the external genetic information of Walik chickens is very limited, therefore, the study on the qualitative traits of such rare indigenous chicken is necessary to support their comprehensive repertoire that would be useful for their preservation efforts and potency development. Thirty six Walik chickens (15 cocks, 21 hens), and 42 Walik chickens (16 cocks, 26 hens) from Sumedang and Bogor District, respectively, were used in this study. The variety on base color of feather, color of the plumage, flick feather, feather pattern, shank color, and comb types of the chickens were identified based on Hutt (1949), and Somes (1988). The frequency of autosomal genes, sex-linked genes, and feather pattern were quantified based on Nishida et al. (1980), and Stanfield (1982). The Walik chickens from Sumedang and Bogor District population have shown predominantly similarities on the plumage color (i), the wild feather pattern (e+), single comb (p), and white/yellow shank (Idid). However, the Walik chickens from Bogor District population were dominated by the strip feather/B (52%), and the silvered-flick feather/S (54%). The Walik chickens from Sumedang District population were dominated by the plain feather/b (54%), and golden flick feathers/s (84%). The low frequency occurence of some qualitative traits could be useful for selection in order to conserved the rare traits. Further studies on the quantitative traits, and the molecular analysis need to be done to complete a set of characterization of the Walik chickens.

Key words: qualitative traits, walik chicken

Introduction

The *Walik* chicken is chategorized as one of the rare indigenous chickens in Indonesia based on their small population number, very limited data of their morphological features and biological parameters, and very limited data on their

current utilization (Sartika & Iskandar, 2007). The *Walik* chicken own a frizzling type of feather (Sartika & Iskandar, 2007), and has various names based on the geographic region of sampling, such as *Walik* or *Rintit* chickens in West Java. The monogenic traits based on pigmentation differences, and comb types are one of approach that can be used to describe the genetic variations in chickens. The qualitative traits of the chickens also have important economic, cultural, and religious function, therefore the specific characteristics must be carefully identified and considered in developing breeding programs. In term of *Walik* chicken in Indonesia, till now, the data on their qualitative traits is very limited. Therefore, the objective of this study was to identify the qualitative traits of the *Walik* chicken populations cared under traditional farming system in West Java to provide base line data that would be useful for their conservation efforts and potency development.

Materials and Methods

The study areas were selected based on purposive sampling method. The initial survey to identify the individual households kept the *Walik* chickens was done by interviewing the head of villages, and the oldest people in a society who know well the people in the study areas as described on the snow ball methods. Thirty six *Walik* chickens (15 cocks, 21 hens) from 4 villages (Padanaan, Palasah, Ujungjaya dan Keboncau) in Sumedang District, and 42 (16 cocks, 26 hens) *Walik* chickens from 9 locations (*Kampung* Cangkrang, *Desa* Cikarawang, *Kampung* Carang pulang, *Desa* Situgede, *Desa* Babakan Lebak, *Desa* Babakan Lio, *Desa* Cibeureum Dramaga, *Desa* Neglasari, and *Desa* Kahuripan) in Bogor District, West Java, Indonesia were used in this study. The variety on base color of feather, color of the plumage, flick feather, feather pattern, shank color, and comb types of the chickens were identified based on Hutt (1949), and Somes (1988). The frequency of autosomal genes (plumage color and comb types), sex-linked genes (variety on base color of feather, flick feather, and shank color), and feather pattern were quantified based on Nishida *et al*., (1980), and Stanfield (1982).

Results and Discussion

The frequency distribution pattern of the qualitative traits in Walik chickens is presented on Table 1. The *Walik* chickens, either from Sumedang and Bogor District have shown predominantly similarities on the plumage color (i), the wild feather pattern (e^+), single comb (p), and white/yellow shank (Idid). However, the *Walik* chickens from Bogor District population were dominated by the strip feather/B (52%), and the silvered-flick feather/S (54%). Whereas the Walik chickens from Sumedang District population were dominated by the plain feather/b (54%), and golden flick feathers/s (84%).

The coloured plumage of brown, black, and mixture were predominantly observed among *Walik* chickens to white colour either in Bogor or Sumedang District population (Table 1). Pigmentation differences, which are attributable to melanin, produce a variety of plumage colours in the chickens. The presence and level of melanin pigments such as trichochrome is related to feather colour and is considered to be indicative of genetic differences among certain plumage colours (Smyth, 1991). There are 2 kinds of melanine, namely eumelanine and pheomelanine. Eumelanine forms the black and blue colour of feather, whereas the pheomelanin forms the red-brown, salmon and dark yellow (Brumbraugh and Moore, 1968). The strip base color of feather present if the distribution of melanine on seconday feather

Table 2. Frequency Distribution of Qualitative Traits in Walik Chickens found in Sumedang and Bogor District, West Java, Indonesia

Qualitative Traits	Gene Frequency	
	Sumedang (n=36)	Bogor (n=42)
Plumage Color		
qI	0	0.01
qi	1.00	0.99
Variety on base color of feather		
qZB	0.47	0.52
qZb	0.54	0.48
Flick feather		
qZS	0.16	0.54
qZs	0.84	0.47
Feather pattern		
qE	0.20	0.10
qe+	0.43	0.51
qe	0.37	0.38
Comb type		
qP	0.24	0.17
qp	0.76	0.83
Shank color		
qZId	0.5	0.79
qZid	0.5	0.21

qI= white, qi= colored-plumage, qZB= strip; qZb= plain, qZS= silver, qZs= golden, qE= black, qe+ = wild, qe= columbian, qP= pea, qp= single, qZId= white/yellow; qZid= black/green.

is blocked. The variety of base color of feather is the sex linked gene that will be found as Z^BW and Z^BZ^B or Z^BZ^b, respectively in male and female (Hutt, 1949).

The relatively low frequency of the white plumage colour (Tabel 1) can be attributed to the fact that white chickens (especially cocks) are important components in traditional religious of the community, therefore they are readily to be sold. Large variation in plumage colour on the indiginous chicken population is indicative of unconscious selection effort. Ensminger (1992) stated that plumage color and pattern, skin color, shank, and comb type are inherited by single pairs of genes that able to influence the preference of the consumers. However, till know there was a very limited data that the variation in plumage colour of the indiginous chickens in Indonesia is mainly due to the lack of conscious selection or breeding programs towards choice of colour.

Our finding also showed that the single comb/p predominates with a frequency of 76%, and 83% for *Walik* chickens in Sumedang and Bogor District population, respectively, to pea comb (24%, and 17%). The higher frequency of single to pea comb indicated that Walik chickens are mainly recessive for comb type. If the heterozygote genotipe has any relative advantages, improvement of the stock would be slow to medium since only 17% and 24 % posessed the pea comb that is generally regarded as the dominant comb type.

The higher frequency of white/yellow shank color (79%) to black/green color (21%) of *Walik* chickens in Bogor District population is in line with the report of Sartika & Sofjan (2007) who found the white/yellow skin was dominant in the indiginous chickens in Indonesia. The melanine on epidermis relates to the black color of chicken shank, whereas the lipochrome on epidermis and melanine on dermis relate to the green color of chicken shank (Jull, 1951). It has been generally assumed that the red junglefowl is the sole wild ancestor of the domestic chicken (Crawford, 1990, Fumihito *et al*. 1994, Romanov & Weigend, 2001, Sulandari & Zein, 2008). However, Eriksson *et al*., (2008) demonstrates that though the white skin allele originates from the red junglefowl (Gallus gallus), the yellow skin allele originates from a different species, most likely the closely related to grey junglefowl (Gallus sonneratii). Therefore, the molecular characteristics is also important to be observed to complete a set of identification and characterization of the indiginous chickens in Indonesia, mainly the *Walik* chickens. The rearing of *Walik* chickens either in Sumedang and Bogor Districts is also an integral part of the smallholder farming system, where they kept by the rural poor to fulfill multiple function. This could also become a sources of variation of the qualitative traits of chickens since the presence or absence of the carotenoid pigments, primarily xanthophylls, in the feed is also responsible for the diversity in skin colour of chickens (Eriksson *et al.* 2008).

Indigenous chickens of the tropics are important reservoirs of useful genes and posses a number of adaptive traits (Horst, 1989). However, diverse human needs

in the form of selective breeding for distinct phenotypes also contributed to the diversity of the present day chicken populatios maintaned in different parts of the tropics (Dessie *et al*., 2011). From our findings, we predict that the *Walik* chicken have a high similarity with the *Kampong* chicken which naturally have e^+ gene, as described by Nishida *et al*. (1980). We also predict that the *Walik* hens from Sumedang Districts, which were dominated by the plain feather (54%), have shown similarities with the Single Rhode Island Red that mainly was developed as the meat producing chickens. The foreign gene from Barred Plymouth Rock was also identified based on the strip feather of Walik chicken from Bogor District population. Historically, the Barred Plymouth Rock are developed as egg producing chickens. Weigend and Romanov (2001) stated that the identification and characterization of the chicken genetic resources generally requires information on their population, adaptation to a specific environment, possession of traits of current and future value and socioecultural importance, which are crucial inputs to decisions on conservation and utilization. Therefore we recommend that the further studies on the quantitative traits, in term of egg and meat production need to be done to predict the utilization potencies of *Walik* chicken.

Conclusions

The *Walik* chickens from Sumedang and Bogor District population have shown predominantly similarities on the plumage color (i), the wild feather pattern (e^+), single comb (p), and white/yellow shank (Idid). However, the *Walik* chickens from Bogor District population were dominated by the strip feather/B (52%), and the silvered-flick feather/S (54%). Whereas the Walik chickens from Sumedang District population were dominated by the plain feather/b (54%), and golden flick feathers/s (84%). The low frequency occurence of some qualitative traits could be useful for selection in order to conserved the rare traits. Further studies on the quantitative traits, and the molecular analysis need to be done to complete a set of identification and characterization of the *Walik* chickens.

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The Classification of Body Measurement on Syrian Hamster (*Mesocricetus auratus*) Based on Factor Analysis and Principal Component Analysis

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Abstract

Mesocritus (M.) auratus (Syrian hamster) is not only used a laboratory animal especially in medical research, but also is looked after as a pet animal. Genetic information can be obtained by studying body morphometry of Syrian hamster, and quantitative characteristics can be useful for conservation and breeding development. Body size characteristics of Syrian hamster is becoming the focus of this study. It is, therefore, this study is conducted to classify Syrian hamster into large, medium and small body sizes on the basis of factor analysis (FA) and principal component analysis (PCA). Hotelling T2-statistic showed differences in linear measurement of body surface between male and female Syrian hamsters (P<0.01). Results of FA showed that variable affecting the body size of male M. auratus in this study was body length; however, it did not influence the body size of female significantly. Body length in male hamsters, therefore, can be used as a reference in a selection program based on body size measurements that are not directly related to body weight. Body length is also found as discriminatory variable for body size, and the chest width was the discriminatory variable for body shape for both male and female. The distribution of colors that occur in each body size class indicated that M. auratus still has a variety in coat colors. Results of clustered diagram show that the crowd in body shape of male is greatly different from that of female Syrian hamsters. Classification the male and female Syrian hamster can be performed based on the value of first factor scores (SF-1) for practical purposes, as well as the body size. Both male and female Syrian hamster can be classified into large, medium and small groups, and each hamster individual data have SF-1 score and it own body score. It is concluded that classification of body size in M. auratus based on factor analysis and principal component analysis showed the same results.

Keywords: body measurement, classification, factor analysis, Mesocricetus auratus, principal component analysis

Introduction

Mesocricetus (*M*.) *auratus* (Syrian hamster) was the first species which was used as a laboratory animal at a university in Jerrusalem in 1930 (Hafez, 1970). Sanders and Sandadura (1992) reported that *M. auratus* was the appropriate laboratory animal for studying the effect of boiled coffee on hypercholesterolemia. Bornhop *et al*. (2003) also used *M. auratus* in observing Luminescent Lanthanide Chelate Contrast Agents for detecting early - stage malignant lesions in Syrian hamster cheek pouch. Most of *M. auratus* had been used as laboratory animals in medical research, nowadays, the animals are becoming popular as pet animal, and it had been used as pet animals in England since 1880 (Grzimek, 1975).

Genetic information can be obtained by studying body morphometry of *M. auratus*. Characteristics in body size and shape of *M. auratus* is determined through Principal Component Analysis (PCA); Factor Analysis (FA) is used to determine factors for classifying body size on the basis of relations between body size varibles which are independent each other (Gaspersz, 1992). The last analysis can be viewed as an expansion analysis of PCA. The original variables can be used as a factor if total variability was approximately 80 - 90% (Gaspersz, 1992).

Quantitative characteristics can be useful for conservation and breeding development. Body size of *M. auratus* is included in medium size (Henwood, 2007), but the hobbyist like hamster with small size; as a result, body sizes of *M. auratus* is becoming the focus of this study. Therefore, this study is conducted to classify *M. auratus* into large, medium and small body sizes. Classification through FA and PCA (Gaspersz, 1992) was carried out on the basis of linear sizes of body surfaces which include body length (X_1), chest width (X_2), pelvic width (X_3) and wrist circumference of front arm or front leg (X_4).

Materials and Methods

The experiment was conducted in the Laboratory of Animal Genetics, Department of Animal Production and Technology, Faculty of Animal Science, Bogor Agricultural University, Bogor. Syrian hamsters used in this experiment were 58 heads (24 males and 34 females) at the mature body age. Other materials used were vernier caliper and thread for measuring linear variables of body surface. Hotelling T^2-statistic was used to compare linear measurements of body surface between males and females with the formula suggested by Gaspersz (1992). If the results reject H_0 hypothesis, the two average values of linear variables of body for were different and the test were continued with FA (Factor Analysis) and PCA (Principal Component Analysis). The FA used was calculated on the basis of formula suggested by Gaspersz (1992) as follows : $X_p = c_{p1} F_1 + c_{p2} F_2 + \ldots + c_{pm} F_m + \epsilon_p$ with description: X_p = random variables; c_{ij} = parameters reflecting the importance of

j^{th} factor in composition from i^{th} respons; in FA was known as weighting (loading) from i^{th} respons at the same factor of j^{th}; F_j= the same factor of j^{th}; ϵ_p= error from i^{th} respons, in FA was known as i^{th} specific factor which was random; i= 1, 2, 3,4 (p= 4); j= 1, 2, 3, 4 (m= 4); p= numbers of variables measured. Factor score (SF) was calculated using formula of Gaspersz (1992) as follows: $SF= C' K^{-1} (X_j - X)$; j= 1, 2, . . , n; with description: SF= matrix for factor score (descendant from covariance); C'= matrix for weighting factor (descendant from covarian); K^{-1}= invers from covarian matrix K; X_j= vector for observation of j^{th} individu; X= vector average value from variable X; n= sample size. Classification based on body measurements (small, medium and big or large) was conducted using formula recommended by Gaspersz (1992) which were: large group if SF > SF + sSFs, medium group if SF – sSF < SF < SF + sSF, small group if SF < SF – sSF, with description: SF= factor score, SF= average of factor score, sSF= standard deviation of factor score. Principle Component Analysis (PCA) by Gaspersz(1992), equation model for body parts were: $Yp= a_1pX_1 + a_2pX_2 + a_3pX_3 + a_4pX_4$; with description: Yp= p^{th} principle component (p= 1, 2, 3, 4); X_1= body length, X_2= chest girth, X_3= pelvic width, X_4= wrist-circumference. Classification on the basis of body linear size that was observed (small, medium and large) was conducted using Garpersz (1992) formula large group: if $y_{h1} > y_1 + s_{y1}$; medium group: if $y_1 - s_{y1} < y_{h1} < y_1 + s_{y1}$ and small group if $y_{h1} < y_1 - s_{y1}$. Data was processed using statistical software of Minitab.

Results and Discussion

Table 1 demonstrates descriptive statistics for body linear size variables of Syrian hamster. T^2-Hotteling statistic showed that there were differences in body linear sizes between male and female Syrian hamster ($P<0.01$). These differences were also observed by Fox *et al*. (1984). The highest body linear size variables influencing body sizes of male and female hamsters was obtained on the basis of communality at Factor Analysis.

Tabel 1. Average and standard deviation for body linear sizes of male and female Syrian hamster (*Mesocricetus auratus*) (cm)

Sex	Body length	Chest girth	Pelvic width	Wrist circumference
♂	9.45±0.70 (7.41%)[1] (n=24)[2]	1.87±0.15 (8.02%)[1] (n=24)[2]	1.12±0.09 (8.04%)[1] (n=24)[2]	1.54±0.14(9.1%)[1] (n=24)[2]
♀	9.22±0.55 (5.97%)[1] (n=34)[2]	1.88±0.22(11.7%)[1] (n=34)[2]	1.10±0.12 (10.91%)[1] (n=34)[2]	1.69±0.09 (5.33%)[1] (n=34)[2]

[1] (%)= coefficient of variance; [2] n= sample numbers.

Table 2 showed the communality (proportion of variability of each variable origin) in body linear size variables of Syrian hamster. The results demonstrated that the body length (X_1) was expressed as a factor F1 of body size in males. Variable affecting the body size of male Syrian hamster in this study was body length (X_1). This variable had a value of 0.70 which was the primary factor affecting the body size of male significantly. According to Pontoh (2007), a weighting factor value closing to +1 or -1 can be used as primary factor. Body length of male Syrian hamster showed a positive relationship between the closeness of the body length and the F1-factor. The higher the body length then the body size will be greater in male. The results demonstrated that the body length (X_1) was expressed as a factor F1 of body size in females. Weighting factor of body length (X_1) on the F1 factor had the greatest value, ie. -0.55. According to Hair, Jr. *et al.* (1998), the weighting factor was greater than ± 0.50, but was not greater than ± 0.55; so the statistical value of the weighting factor has no effect. The results stated that body length was the main factor determining body - size in males, but not in females.

Body length in male, therefore, can be used as a reference in a selection program based on body size measurements that are not directly related to body weight. Linear size of the body in Syrian hamster observed was associated with the condition of the body skeleton.

Table 2. Factor weighting (correlation between original variable with factor), Eigen value (λ), total variance (%), cumulative variance (%) and body size communality score of male & female Syrian hamster (*Mesocricetus auratus*)

Variables measured (Male)	Factor				Communality score
	F1	F2	F3	F4	
Body length (X1)	0.70	-0.01	-0.00	-0.01	0.49
Chest girth (X2)	-0.01	-0.13	0.08	-0.01	0.02
Pelvic width (X3)	0.04	-0.05	-0.03	0.06	0.01
Wrist circumference (X4)	-0.05	-0.09	-0.10	-0.03	0.02
Eigen value (λ)	0.50	0.03	0.01	0.00	0.54
Total variance (%)	91.1	5	3	0.9	100
Variables measured (Female)	F1	F2	F3	F4	
Body length (X1)	-0.55	-0.04	0.01	-0.01	0.31
Chest girth (X2)	0.15	-0.16	0.04	-0.01	0.05
Pelvic width (X3)	0.01	-0.08	-0.09	0.00	0.01
Wrist circumference (X4)	-0.02	-0.01	0.00	0.08	0.01
Eigen value (λ)	0.33	0.03	0.01	0.01	0.38
Total variance (%)	87	8.6	2.5	1.9	100

F1= 1st factor, F2= 2nd factor, F3= 3rd factor, F4= 4th factor

Table 3. Body size and shape equations with the total diversity and Eigen values in the Syrian hamsters (*Mesocricetus auratus*) male and female

Sex	Equation	Total Diversity	Eigen Value
Male	Size = $X_1 - 0.02X_2 + 0.06X_3 - 0.07X_4$	91.1%	0.50
	Shape = $0.04X_1 + 0.80X_2 + 0.28X_3 + 0.53X_4$	5.0%	0.03
Female	Size = $0.96X_1 - 0.26X_2 - 0.01X_3 + 0.04X_4$	87.0%	0.33
	Shape = $0.24X_1 + 0.86X_2 + 0.44X_3 + 0.06X_4$	8.6%	0.03

X_1= body length, X_2= chest width, X_3= pelvic width, X_4= wrist circumference

Discriminatory variable for body sizes of male and female Syrian hamster were body length (X_1) based on the highest Eigen value on body size equation (Table 3). Everitt and Dunn (1998) stated that the first principal component represents the overall size of the body must explain the total variability between 50%-95%. The first principal component is the body size. Discriminatory variable for body shape of male and female Syrian hamster were chest width (X_2). Everitt and Dunn (1998) stated that the second principal component represents the overall body shape, must explain the total diversity of at least 1%.

Classification the male Syrian hamster can be performed based on the value of first factor scores (SF-1) in Table 2; can be divided into large group with SF-1 $\geq$ 1.01 (five heads), medium group with SF-1 -1.01 to + 1.01 (13 heads) and small group with SF-1 $\leq$ -1.01 (six heads). Classification the female Syrian hamster can be performed based on the value of first factor scores (SF-1) that can be divided into large group with SF-1 $\geq$ 1.06 (five heads), medium group with SF-1 -0.94 to + 1.06 (13 heads) and small group with SF-1 $\leq$ -0.94 (six heads).

Classification based on the score of body size based on Table 3 demonstrates that male with a score value $\geq$ 10.03 (five heads) is for large group, a score value 8.63 to 10.03 (13 heads) is for medium group and a score value $\leq$ 8.63 (six heads) is for small group. The classification for the females results in the score value > 9.02 (five heads) for the large group, the score value : 7.88 to 9.02 (23 heads) for the medium group and the score value $\leq$ 7.88 (six heads) for the small group. Distribution of Agouti Non Golden (ANG), Agouti Golden (AG), and Non Agouti (NA) in each group of Syrian hamster indicated that the coat color was not related to body size.

Conclusion

Variable that affects the body size of male M. auratus in this study was body length; however, it did not influence the body size of female significantly. Body length was found as well as discriminatory variable for body size of male and female. Discriminatory variable for body shape of male and female M. auratus were

chest width. The distribution of colors that occur in each body size class indicated that M. auratus still has a variety in coat colors. Classification of body size in M. auratus based on factor analysis and principal component analysis showed the same results.

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Phenotypic Characteristics of *Legund* Chickens in West Java, Indonesia

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Introduction

The *Legund* chicken is the rare Indonesian native chicken that is naturally devoid of feathers on its neck due to the autosomal incomplete dominant naked neck (Na) gene. This gene is not only responsible for defeathering the neck region, but also restricts the feathering areas around the body by 20-30% in the heterozygous (Nana) and up to 40% in the homozygous (NaNa) genotype. Due to the association with the reduction in feather coverage, improved heat tolerance and better adaptability, survivability, and productibility under heat stress conditions, the Na can be used as a marker gene that is economically important in modern breeding system. However, the characteristic of this chicken has been poorly characterized to date in Indonesia due to their small population number, very limited data of their phenotypic characteristics, and biological parameters, and very limited data on their current utilization. Therefore, the purposes of this study were to observe the phenotypic characteristics, the influence of imported breed and the purity level of native gene of the *Legund* chicken populations cared under traditional farming system in West Java. The research findings will be very important to provide base line data that would be useful for the desaining their conservation program and improvement of utility development.

Material and Methods

Thirty one (3 cocks and 28 hens), and 32 (8 cocks and 24 hens) *Legund* chickens respectively from Subang, and Bogor District populations, West Java, Indonesia were observed in this study. The purposive sampling method was used to select the study areas. The identification of individual households owned the *Walik* chickens was done by using the snow ball method. The phenotypic characteristics such as variety on base color of feather, color of the plumage, flick feather, feather pattern, shank color, and comb types of the chickens were identified based on Hutt (1949), and Somes (1988). The frequency of autosomal genes (feather color and comb types), sex-linked genes (feather feature, feather shine, and shank color), and feather pattern were quantified based on Nishida *et al*., (1980), and Stanfield (1982). Prediction of

introgression rate of imported breeds (*Rhode Island Red*, *White Leghorn* and *Barred Plymouth Rock*), and purity level of native gene were calculated based on Nishida *et al*. (1980).

Results and Discussion

The control of gene constitution on phenotypic characteristic of *Legund* chicken either on Subang or Bogor District population was ii E+ B_ ss Id_ P. However, we didn't find a single comb (pp) of *Legund* chickens on Subang District populations (Table 1).

Table 1. Frequency Distribution of Phenotype Characteristics in *Legund* Chickens found in Subang and Bogor District Populations, West Java, Indonesia

Phenotypic Characteristics	Loci	Genotypes (Phenotypes)	Genes	Gene Frequency	
				Subang	Bogor
Feather color	I > i	I – (White)	qI	0.02	0.03
		ii (Colored)	qi	0.98	0.97
Feather pattern	E > e^+ > e	E_ (Black)	qE	0.03	0.10
		e^+ (Wild)	qe^+	0.97	0.90
		ee (Colombian)	qe	0.00	0.00
Feather feature	B > b	B_ (Barred)	qB	0.96	0.89
	(Sex linkage)	Bb (No barred)	qb	0.04	0.11
Feather shine	S > s	S_ (Silver)	qS	0.37	0.21
	(Sex linkage)	ss (Gold)	qs	0.63	0.79
Shank color	Id > id	Id (White / Yellow)	qId	0.65	0.52
	(Sex linkage)	id (Black / Grey)	qid	0.35	0.48
Comb type	P > p	P_ (Pea)	qP	1.00	0.53
		pp (Single)	qp	0.00	0.47

The feather colour of *Legund* chickens was expressed as brownish, red, black, white, and barred or show a mixture of colours which are found to be highly phenotypic variability. This variation may result in increasing of their survival under local conditions. The relatively low frequency of the white plumage colour (Tabel 1)) can be attributed to the fact that white chickens (especially cocks) are important components in traditional religious of the community, therefore they are readily to be sold. In term of genotipe, we had difficulty to recognize the heterozigous and homozigous genotipe of *Legund* chickens in the field as the owners applied a very poor breeding record. However the frequency distribution of feather was

dominated by the barred feather, either on Subang District or Bogor District, 0.96 and 0.89, respectively (Table 2). Horst (1988) stated that in homozygous condition, naked neck chickens have a completely bare neck whereas in the heterozygous condition they have a bare neck with a tuft of feathers. Since Na is a major 'marker' gene identified by qualitative criteria (visual, biochemical or serological) that may show association with quantitative traits, either because of pleiotropy (Johnson and Rendel, 1968), or because of linkage with other genes (Crawford, 1990), therefore the further investigation of sex linkage genes on that chickens should be done based on a complete breeding record.

Table 2. The Comparison of Introgression Rate of Imported Breeds (Q) Rhode Island Red (SR), White Leghorn (WL) and Barred Plymouth Rock (BR) to *Legund* Chickens in Subang and Bogor District Population

Population	Introgression Rate			$Q^{SR} + Q^{WL} + Q^{BR}$	Purity Level of Native Gene (%)
	Q^{SR}	Q^{WL}	Q^{BR}		
Subang	-0.31	0.02	0.94	0.65	35
Bogor	-0.37	0.03	0.86	0.52	48

We also predict that the the gene of Barred Plymouth Rock mainly influenced the *Legund* chickens in both population. The introgression rate of Barred Plymouth Rock (Q^{BR}) to the Legund chickens populations was highest (0.94) followed by White leghorn (Q^{WL}) and Rhode Island Red (Q^{SR}) (Table 2). This finding is congruent with the Legund chickens that is predominated by the barred feather (B_) (Table 1), as a special character of Barred Plymouth Rock. We then predicts that the *Legund* chickens are primarily developed by the farmers as meat producers, event in the field the farmers utilize this chickens for dual purposes of meat and egg producers. Further study on the morphological features and productivities of the chickens should be done to predict their production potency.

The purity level of native gene in *Legund* chicken at Subang District population was lower (35%) than that of Bogor District population (48%). Nishida *et al*. (1980) found that the purity level of native gene in Indonesian indigenous chicken, mainly Kampong chicken was 28-55% (46% for West Java). The differences of breeding practices to improve chicken productivities applied by the farmers may result on this differences. Recent findings show that Na gene result a better heat tolerance and better adaptability, survivability, and productibility under heat stress conditions. The gene is clearly expressed under unfavourable conditions such as higher temperatures (Cahaner *et al*., 1993), smaller diurnal or seasonal fluctuations and under poor management conditions (Islam & Nishibori 2009). Therefore the existency of such gene must be maintained and can be used as a marker gene that is economically important in modern breeding system. The breeding program that also

maintain Na gene must be set up to support the the conservation and utilization of *Legund* chickens in Indonesia.

Conclusions

The control of gene constitution on phenotypic characteristic of *Legund* chicken either on Subang or Bogor District population was ii E+ B_ ss Id_ P. The introgression rate of Barred Plymouth Rock (Q^{BR}) to the Legund chickens populations was highest (0.94) followed by White leghorn (Q^{WL}) and Rhode Island Red (Q^{SR}). The purity level of native gene in *Legund* chicken at Subang District population was lower (35%) than that of Bogor District population (48%). Maintaining the Na gene is needed to support the conservation efforts and improvement of utilization of *Legund* chickens in Indonesia.

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Morphometric Performances of Thin Tail Sheep with Differences *Calpastatin* (Cast-1) Genotipees

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Abstract

Calpastatin (CAST) is an indigenous inhibitor of calpain that involved in regulation of protein turn over and growth. The objective of this research was to compare the morfometric performances of thin tail sheep with difference of CAST genotipees. PCR-RFLP method was carried out to identify genetic variation of CAST gene. Based on the identification, variation of CAST gene that found were MM and MN with the single Calpain genotype variation, TT. Nine thin tail sheeps from Jonggol were used for this research. The sheeps clustered based on the variation CAST gene, 5 sheeps were MM genotype and 4 sheeps were MN genotype. Variation of CAST gene gave significantly differences in morfometric performances. Sheeps with MM genotype have longer body length, heart girth, wither depth, and rump width than sheep with MN genotype. Sheeps with MM genotype had longer Ossa vertebrae cervicales Ossa vertebrae thoracicaeOssa vertebrae lumbales, Os vertebrae sacrales and Os scapulae.

Keywords: calpastatin, thin tail sheep, morfometric performances

Introduction

Thin tail sheep is one of the Indonesian native sheep that have potential to be developed. Although the adult animal's body weight is relatively small compared with sheep tail fat, but thin tail sheep are well adaptable to the limited availability of food and high temperature and child sheep mortality are relatively low (Subandriyo, 2003).

Progress in molecular biology allow livestock selection efforts can be done at the gene level, ie by looking for the diversity of genes that control livestock productivity. One marker genes associated with weight gain in the local sheep genes is gene that controlling regulation of calpain and calpastatin synthesis (Sumantri *et*

al., 2008). According to Camau *et al*. (2007), calpain and calpastatin are included in the calpain system. Calpain system is enzymes that contribute in meat tenderness by proteolytic post-slaughter. Calpain system has three members of the protein that is μ-calpain, m-calpain and calpastatin. Activity of μ-calpain and m-calpain is affected by Ca^{2+} ions. The functions calpain enzyme on live animal is to degrade proteins in the myofibril myofibrillar structure formation (Scanes, 2003).

Calpastatin is an enzyme to inhibit protein degradation muscle cells by the enzyme μ-calpain and m-calpain. Increased calpastatin activity can increase muscle mass (hypertrophy) accompanied by a decrease in meat tenderness. Calpastatin associate with myostatin regulate muscle growth rate, so the diversity of calpastatin gene is expected to affect the local sheep growth properties, and therefore variations in calpain system genes in sheep will not only influence the rate of postmortem meat tenderness but also expected to influence muscle growth.

The objective of this research was to study the comparative morphometry performance of thin tail sheep in the different variations of calpastatin genotypes.

Materials and Methods

This research used thin tail sheep from the Jonggol Animal Science Teaching and Research Unit (JASTRU). Based on the preliminary research for the detection of calpain and calpastatin gene diversity obtained calpastatin gene variations, that should have 3 variations of the genotypes MM, MN and NN, obtained only 2 variations genotipee, MM and MN, and NN gene variation was not found. M denotes the normal calpastatin allele, whereas N indicates that mutation calpastatin allele. Samples taken from sheep that had calpastatin genotypes MM and MN with the same calpain genotype (TT). Sheep with MM calpstatin genotype obtained of 5 samples, while for sheep with MN genotype obtained 4 samples. Sheep selected were male with a thin tail sheep ready for slaughter age is in the range of 1 to 1.5 years (I1).

The study was conducted in April to August 2011 at the Outdoor Laboratory of Small Ruminants, Animal Production and Technology Department, Faculty of Animal Science, Bogor Agricultural University.

Measurements on live animals were body weight and morphometrics performances. The morphometrics performances were measured body frame conformation. Body frame conformation Measurement used tuberosity and the processus that clearly visible in live sheep. This measurement was carried out to study the pattern of development and growth of both overall and per body part of sheep. Parameters observed in measurements of morphometry were:

a. Primary Morphometrics
   - Body length
   - Body height
   - Wither depth
   - Wither widhth

- Hips leight
- Hearth girth
- Rump width

b. Part of *Columna vertebralis*
   - *Ossa vertebrae cervicales*
   - *Ossa vertebrae thoracicae*
   - *Ossa vertebrae lumbales*
   - *Os vertebrae sacrales*

c. Extrimity Length
   - *Os scapula*
   - *Os humerus*
   - *Ossa radius-ulna*
   - *Ossa metacarpalia*
   - *Os femoris*
   - *Ossa tibia-fibula*
   - *Ossa metatarsalia*

The data obtained were analyzed using Student's t test, two-tailed hypothesis to compare differences of calpastatin gene variations between MM and MN. Mathematical model according to Steel and Torrie (1991) was:

$$t = \frac{(Xa - Xb) - (\mu a - \mu b)}{Sxa - xb}$$

explanation :

t : T value to be compared with the t table to determine the acceptance of the hypothesis

$(Xa - Xb)$: The average difference in sample a and b

$(\mu a - \mu b)$: Difference in the average of population a and b

$Sxa - xb$: Standard error value

Results and Discussion

Morphometric measurements used to determine rate of livestock growth. Morphometric performance of sheep with differences of calpastatin genotypes are presented in Table 1. Body weight, daily body weight gain, height, chest height and width of the hips did not show significant differences. Body length of thin tail sheep MM genotype significantly longer than MN genotype. The main components that affect body length are the joints of the spine (vertebrae Columna). Columna vertebre arranged from *Ossa vertebrae cervicales, Ossa vertebrae cervicales, Ossa vertebrae lumbales* and *Os vertebrae sacrales*. Based on the measurement, all part of *Columna vertebralis* of MM genotype thin tail sheep significantly longer than the MN genotype, so muscle formed on a commercial cuts of neck, rack and loin will be longer when compared with the MN genotype.

Hearth girth and wither depth are parameters that indicate the dimensions of rib cage (rib cage). Based on the measurements, MM genotype of thin tail sheep had significantly larger hearth girth and longer wither depth than the MN genotype. It showed that MM genotype of the thin tail sheep had rib cage dimensions larger

than the MN genotype. MM genotype of Thin tail sheep had a longer *Os scapulae* compared with MN genotype. Os scapula sizes would give a influence of shoulder percentage.

Tabel 1. Morphometric performance of sheep with differences of calpastatin genotypes

Parameters	Calpastatin Genotypes			
	MM (n=5)	CV (%)	MN (n=4)	CV (%)
Primary Morphometrics				
Body weight (kg)	20.56 ± 2,27	12.77	19.12 ± 2.09	10.93
Body length (cm)	54.13 ± 2.83[a]	5.22	51.28 ± 2.22[b]	4.32
Body height (cm)	56.46 ± 2.54	4.50	55.81 ± 1.67	2.99
Hips height (cm)	57.69 ± 1.95	3.38	57.30 ± 2.28	3.98
Heart girth (cm)	63.17 ± 2.76[A]	4.36	59.86 ± 2.30[B]	3.84
Wither depth (cm)	26.18 ± 1.17[a]	4.49	25.04 ± 1.19[b]	4.74
Wither width (cm)	14.64 ± 0.38	2.57	14.59 ± 0.47	3.22
Ramp width (cm)	13.03 ± 0.73[A]	5.62	11.83 ± 0.58[B]	4.91
Part of *Columna vertebralis*				
Ossa vertebrae cervicales (cm)	11.59 ± 0.64[a]	5.48	11.01 ± 0.49[b]	4.47
Ossa vertebrae cervicale (cm)	18.48 ± 0.96[a]	5.22	17.51 ± 0.75[b]	4.28
Ossa vertebrae lumbales (cm)	11.32 ± 0.59[a]	5.20	10.73 ± 0.45[b]	4.16
Os vertebrae sacrales (cm)	7.99 ± 0.41[a]	5.18	7.58 ± 0.34[b]	4.46
Part of Extrimity				
Os scapula (cm)	21.72 ± 0.96[A]	4.40	20.64 ± 0.84[B]	4.07
Os humerus (cm)	15.82 ± 0.70	4.40	15.64 ± 0.47	2.99
Ossa radius-ulna(cm)	14.98 ± 0.67	4.45	14.82 ± 0.44	2.96
Ossa metacarpalia (cm)	10.05 ± 0.44	4.39	9.93 ± 0.30	3.05
Os femoris (cm)	13.09 ± 0.38	2.92	12.94 ± 0.39	2.99
Ossa tibia-fibula (cm)	20.74 ± 0.93	4.49	20.50 ± 0.64	3.11
Ossa metatarsalia (cm)	15.93 ± 0.54	3.37	15.92 ± 0.47	2.97

Different superscript letters on the same lines suggested a significant difference between treatments (P <0.05), superscript capital letter stating the difference highly significant (P <0.01), n = number of samples (tail), KK= coefficient of variance (standard deviation / average x 100%).

Hips Width indicate the distance between the pubis (O*s pubis*) to the hip band (*Ossa membri pelvini*). This parameter indicate the distance between the feet, which is the stifle area muscle deposition. Hips Width of

MM genotype of thin tail sheep were larger than that of MN genotype. Based on the overall data, thin tail sheep with MM genotype had larger morphometri performance than MN genotype. It suggested that calpastatin had potential to influence the growth of bones, especially in body axis. It was closely related to the function of calcium as bone formation and calpain enzyme whose activity is influenced by the concentration of ions Ca^{2+}.

Conclusions

Thin tail sheep with MM genotype had larger morofometri performance than MN genotype. It suggested that calpastatin had potential to influence the growth of bones, especially in body axis.

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II. FEED AND NUTRITION
Sub Theme: Agrostology

Production and Nutrient Uptake of Sweet Corn Treated with Manure 'plus' and Inorganic Fertilizer

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Abstract

Most of the land in Indonesia used for food-crops production belongs to acid soil characterized by low phosphorus (P), and low pH. Rock phosphate (RP) and scallop shell meal as phosphorus source, combined with manure maybe a promising technique to overcome those problems. A field experiment was conducted on an acid soil (low pH and low available Bray II extractable P) to evaluate the effect of manure, manure 'plus' (RP and scallop shell meal, combined with manure), and inorganic fertilizer on corn production and nutrient uptake of stover. A completely randomized block design with 3 replicates was used. The treatments were manure (T1), manure 'plus' (T2), RP+ZA (T3), SP+urea (T4), manure+RP+ZA (T5), manure+SP+urea (T6), manure 'plus'+RP+ZA (T7), manure 'plus'+SP+urea (T8). Dosage of manure was 1 ton/ha. Dosage of N, and P was 200 kg N/ha, and 150 kg P2O5/ha, respectively. All plot received basal fertilization of KCl (150 kg K2O/ha). Zea mays saccharata was cut at ground level on 70 days after planting and analyzed for dry matter yield, nitrogen and phosphorus uptake. Result showed that corn production and nutrient uptake, significantly influenced by the treatment. Manure 'plus' (T2) resulted nutrient uptake of stover significantly higher compared to manure (T1). Manure 'plus' combined with inorganic fertilizers (T7, T8) resulted in significantly higher of corn production and nutrient uptake compared to T1 and T2, and tend to be higher compared to the other treatments. Therefore, manure 'plus' (RP+scallop shell meal+manure) could improve the quality of manure and increased corn production and nutrient uptake in acid soil.

Key words: manure, nitrogen, phosphorus, scallop shell meal, Zea mays saccharata

Introduction

Crop-livestock system (CLS) has been used by farmers in Indonesia. The characteristic of CLS is crops yield for food, stover for ruminant feed and manure can be used as organic fertilizer. Farmyard manure is traditionally widely used for improving soil fertility, but since it is relatively low in phosphorus in relation to nitrogen and potassium, it is often supplemented with a phosphatic fertilizer or with a mixed fertilizer with a high phosphate component (Ann, 1993). Superphosphate fertilizer (SP) has been widely used to improve food crop production on non-productive lands in Indonesia. However, the high cost of SP is now focusing attention on rock phosphate (RP) fertilizer and scallop shell meal as P sources. In addition, Lukiwati (2002) showed that RP and SP fertilizer increased maize grain yield, dry matter (DM) stover yield of maize stover over those of the control that did not receive P fertilizer. Finely ground RP is an apatite mineral not readily soluble in water, and when added to acid condition, the solubilization of RP is increased. Therefore, and expensive P source (SP) can be replaced by RP when the application of the latter is combined with amonium sulphate fertilizer (Lukiwati *et al*., 2001) or decomposition process of organic materials (Sumida and Yamamoto, 1997). Combination of manure 'plus' and inorganic fertilizer as being an alternative strategy for more rational and sustainable agriculture. The objective of the research was to investigate the influence of manure and manure 'plus' combined with inorganic fertilizer (N, P) on sweet corn yield and nutrient uptake of stover in the acid soil.

Materials and Methods

A field experiment of completely randomized block design with three block as replicates was conducted in Semarang-Central Java Indonesia, during 70 days on acid soil with the following pH values 5.15 (block I), 5.30 (block II), and 5.11 (block III). Available P (Bray-I) was 2.17 (block I), 1.48 (block II), and 1.18 ppm (block III). Soil nitrogen content (%N) were 0.132 (block I), 0.164 (block II), and 0.142 (block III). Thus the soil was acid, low in both of P and N content. The experiment was conducted on 350 m^2 divided into 24 plots. Each plot size was 3m x 3m or 9 m^2. The treatments consisted of P fertilizers from two sources; RP-27 and SP-18. Nitrogen fertilizers from two sources; urea and amonium suplphate. Level of both P and N fertilizers was at 66 kg P/ha and 100 kg N/ha, respectively. Level of manure and manure 'plus' at 1 ton/ha. Manure made from cattle dung and urine mixed with waste of forage of straw waste, while it combined with P and scallop shell meal as P source was called manure 'plus'. A basal application fertilizer was KCl at 125 K/ha, was applied to each plot. The treatments of fertilization were T1 (manure), T2 (manure 'plus'), T3 (RP + AS), T4 (SP + urea), T5 (manure + RP + ZA), T6 (manure + SP + urea), T7 (manure 'plus' + AS), T8 (manure 'plus' + urea). Sweet corn seed

was dibbled into small holes, spaced 100 x 50 cm. Each plot contained 30 plants from 15 holes planting. The sweet corn was harvested at 70 days after planting. After harvesting of sweet corn, the stover was cut close to the ground and measured for DM yield, nitrogen and phoshorus uptake. The analyses of variance for sweet corn yield, DM yield, N and P uptake of stover were made using the general linear model procedue of SAS. Significant differences among the treatments were calculated using DMRT.

Results and Discussion

Result showed that sweet corn yield, DM yield, N and P uptake of sweet corn stover (Table 1) were significantly ($P<0.05$) influenced by the treatments. Table 1 showed that manure 'plus' (T2) resulted sweet corn yield and DM yield non-significantly different compared to manure (T1). The application of manure 'plus'combined with inorganic fertilizer (T7 and T8) resulted in significantly different of sweet corn yield and DM yield of stover compared to manure and manure 'plus' only (T1 and T2). Inorganic fertilizer (T3 and T4) resulted in non-significant difference on sweet corn yield compared to T1 and T2. Dry matter production with SP+urea (T4) showed significantly higher compared to T1 and T2. Dry matter production of T3 showed a significant difference compared to T1 and tend to higher compared to T2. Manure + SP + urea (T6) resulted in significantly higher of corn production compared to T1 and T2, while T5 significantly different compared to T1 and tend to higher compared to T2. However, the application of manure combined with inorganic fertilizer (T5 and T6) resulted in significant different of DM yield of stover compared with T1, T2, T3 and T4. Application of both manure and manure 'plus' resulted in similar sweet corn yield, and DM yield of stover compared in the same combinations of inorganic fertilizers, i.e. T5 vs T7, and T6 vs T8. Combination of N and P fertilizers from difference sources resulted in similar sweet corn yield, and DM yield of stover, i.e. T3 vs T4.

Application of manure and manure 'plus' combined with inorganic fertilizers T5, T6 and T7, T8 resulted in N and P uptake of sweet corn stover non-significantly different. Application of T5, T6 and T7, T8 resulted N and P uptake tend to be higher compared with inorganic fertilizer only (T3, T4). Combination between organic and inorganic fertilizer resulted nutrient uptake tended to be higher compared to inorganic only. The application of manure and manure 'plus' combined with inorganic fertilizers (T7 and T8) resulted in significantly different of phosphorus and nitrogen uptake of sweet corn stover compared to manure and manure 'plus' only (T1 and T2). Inorganic fertilizer (T3 and T4) resulted in significantly different on N and P uptake of sweet corn stover compared to T1 and non-significantly different compared to T2.

Table 1. Production of sweet corn and dry matter, nitrogen and phosphorus uptake of stover with manure 'plus' and inorganic fertilizer

Treatments	Sweet corn (kg/m^2)	Dry matter	N uptake (g/m^2)	P uptake
Manure (T1)	0.63^{c}	139.90^{e}	2.01^{d}	0.72^{c}
Manure 'plus' (T2)	0.81^{bc}	161.43^{de}	3.07^{c}	1.01^{b}
RP + AS (T3)	1.02^{abc}	170.49^{cd}	3.17^{c}	0.88^{b}
SP + urea (T4)	1.05^{abc}	199.96^{bc}	3.36^{bc}	0.91^{b}
M + RP + AS (T5)	1.15^{ab}	222.74^{ab}	3.84^{ab}	1.14^{ab}
M+SP+urea (T6)	1.31^{a}	248.79^{ab}	4.11^{ab}	1.42^{a}
M'plus'+ AS (T7)	1.36^{a}	269.87^{ab}	4.82^{ab}	1.39^{a}
M'plus'+ urea (T8)	1.51^{a}	279.49^{a}	5.38^{a}	1.21^{ab}

Different superscript in the same column means significantly different ($P<0.05$).

Inorganic fertilizers (T3 and T4) resulted in higher on sweet corn yield, DM production, N and P uptake of stover compared to organic fertilizers (T1 and T2). Soil fertility is more limited without inorganic fertilizers (ntrogen and phosphorus), and only using manure or manure 'plus' (Min *et al*., 2002). Nutrient content (P, N) of manure 'plus' (T2) was increased, because made from manure with RP and scallop shell meal added. The agronomic effectiveness of RP can be enhanced through acid condition (Bationo and Kumar, 2002; Lukiwati, 2002). Sumida and Yamamoto (1997) showed that decomposition process of organic materials could released organic acids, and decreased pH and redox potential might have increased the availability of plant nutrients which high solubility in acid condition. Therefore, manure 'plus' (T2) resulted in higher of sweet corn yield, DM production, and nutrient uptake compared to manure (T1) only. Combination between manure and manure 'plus' with N and P fertilizers increased the availbility of soil nutrient i.e N and P for sweet corn plant (Lukiwati *et al*., 2010). Combination between manure or manure 'plus' with inorganic fertilizers (N and P) could improve nutrient balance for sweet corn plant. Application of both manure or manure 'plus' resulted in similar sweet corn yield, DM yield and nutrient uptake of stover compared in the same combination of inorganic fertilizers. The dosage of both organic and inorganic fertilizers were not different, respectively. Combination of N and P fertilizers from different sources resulted in similar of sweet corn yield, and DM yield of stover. The dosage of those fertilizers were not different, respectively. Therefore, sweet corn yield, DM and nutrient uptake response to those fertilization were not different as well. The same result reported by Toth *et al*. (2006). N and P – based manure applications did not differ in ability to supply nutrients for crop growth. Lukiwati *et al*. (2001) reported that P fertilizers (SP, RP) in combination with N fertilizers (AS,

urea) resulted in similar DM yield and crude protein content of *Setaria splendida.* According to Nassir (2001), reactive RP when it was directly applied at initial rates of between 80-360 kg P_2O_5/ ha, not only increased yields of corn, but resulted in similar yields than SP and also increased soil pH. Phosphorus fertilizers could increase the plant growth, especially if the P nutrient is a major limiting factor to the plant production (Lukiwati, 2002). Combination between manure or manure 'plus' with inorganic fertilizer could improve nutrient balance for sweet corn plant.

Conclusions

Manure 'plus' resulted in higher of sweet corn yield, dry matter yield and nutrient uptake of stover compared to manure only. Organic fertilzers (manure and manure 'plus') combined with inorganic fertilizes resulted in higher of sweet corn yield, DM yield and nutrient uptake of stover compared to application both of organic' or inorganic fertilizers separatedly. Therefore, organic fertilzer could increase sweet corn yield, DM yield, and nutrient uptake in acid soil, if it is combined with inorganic fertilizer.

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Indigofera zollingeriana: A Promising Forage and Shrubby Legum Crop for Indonesia

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Abstract

Indigofera zollingeriana is a shrubby legume that has been studied since 2009 at Department of Animal Science and Technology, Bogor Agricultural University. Observation of agronomical parameters was conducted to investigate forage regrowth patern, herbage production and cutting management. Some soil ecological parameters were also observed to recognize rhizo effect on chemical and biological soil properties. Evaluation of nutrinional parameters were conducted as well to investigate utilization of nutritional value of the legume. Agronomical study revealed I. zollingeriana is a branchy regrowing plant that showed appropriate harvesting time at 60 days, easy to grow with tea growing structure model, and produced about 6-10 ton DM ha^{-1} harvest^{-1}. Cultivation of I. zollingeriana significantly increased 20% of soil organic carbon, P-solubilizing bacteria colony, stablized soil-P and -N consentration and soil pH. Meanwhile investigation of nutritional aspects revealed highly nutritional value indicated by high protein content and digestibility; good utilizable fiber, high dry matter digestibility, low tannin and saponin content. According to these results, I. zollingeriana is able to be developed as one of promising shrubby legume to improve livestock production in Indonesia due to nutritional, agronomical and ecological reasons.

Keywords: Indigofera zollingeriana, legume crop, nutrition value, forage production

Introduction

Significant problem in ruminant animal production system is low nutrition intake that cause low animal productivity. About 70% of forages used by farmers originates from local grasses that has been known low in protein content (7-9%) and high crude fiber, and most of them low in digestibility. To improve feed quality and nutrition intake, farmers use concentrate as additional menu in ruminant ration. However use of concentrate has led regionally to increase of feed raw materials

price and uncertainty in quality. Farmers have difficulties in controlling feed qulity due to not only variation of feed material quality but also its low availability in the market. This, in turn leads to reduction of animal production, particularly dairy production. Importation of some feed materials like soybean meal has in some cases solved the problem, however farmers are not able to access the materials as the consequence of price and availability. Use of other agroindustrial waste becomes more trading issue rather then technical aspect. But still farmer do not have big chance to access high and stable quality of raw feed materials.

Recommendation to use more concentrate portion ruminant animals more problem raise in controlling feed availability and quality. Seeking local raw feed materials originating from high quality forages such as legumes become an alternative to suplement even substitute concentrate feed raw materials. The recent success with *Leucaena* as a new high quality forage species for northern Australia has prompted a search for other legume tree and shrub species suitable for introduction to other grazing system (Blunt and Jones, 1977; Clem *et al*., 1993). It is a good example that may inspire farmer in Indonesia.

Use of legume particularly tree or shrub legume species may reduce feed cost and contributes to environment. Legume is able to improve soil fertility through biological nitrogen fixation (Danso *et al*., 1992) and other symbiotic solubilizing bacteria. Many studies about legume-michorrizal fungi symbiosis revealed benefits for P eficiency. One of promising legume we have studied is *I. zollingeriana*. The scientific name of this legume crops has determined by our colleague at Indonesian Institute of Science (LIPI). This shrub legume may be as one of promising forage crop, which confirms nutritionally high quality (Abdullah, 2010; Abdullah and Suharina, 2010; Tarigan *et al*., 2010)) and farmers begin widely establish the legume crop in Indonesia to improve goats milk production.

This paper aims to describe the role of *I. zollingeriana* as a source of high quality forage, and its role on soil ecology.

Material and Methods

Agronomy study about *I. zollingeriana* has been conducted since year 2009 at research station of Bogor Agricultural University to investigate effect of foliar fertilizer on forage production improvement and the role of the crops in maintaining soil fertility (Abdulah, 2010; Abdullah and Suharlina, 2010). Some chemical and biological soil properties were observed to investigate the effect of Indigofera's rhizosphere on N, P and P-solubelizing bacteria (Suharlina, 2010). Compiled data based on *in vitro* test in investigation of nutritional value of Indigofera leaf in dairy goat rument (Jovintry, 2011) was extracted for this paper. Besised, *in vivo* experiment involving *I. zollingeriana* as substituting feedstuff to commercial concentrate was carried. In form of pellet, its leaf was fed to 2 groups of dairy goats consisting of

third period of lactating saanen and second period of lactating etawah crossbred (Apdini, 2011). Data are extracted and used for discussion of the paper.

Results and Discussion

Persistent Forage Production

Forage production is an important parameter indicating the potential of dry matter available to ruminants. Observation of *I. zollingeriana* forage biomass production for 9 times of defoliation carried out with interval of 60 days showed that dry forage production ranged between 6-10 tons /ha/crop. Variations occur due to the season and intensity of branching formation and intensive leaf development. This leads to overlapping leaves, so that the effectiveness of photosynthesis is reduced. Although forage production fluctuated, but it still showed higher average yields than those of other legume species such as *Gliricidia* and *Calliandra*. *I. zollingeriana* forage productivity is affected by defoliation intensity. As discussed earlier, defoliation with a height of 1 m showed a more persistent production (Figure 1.)

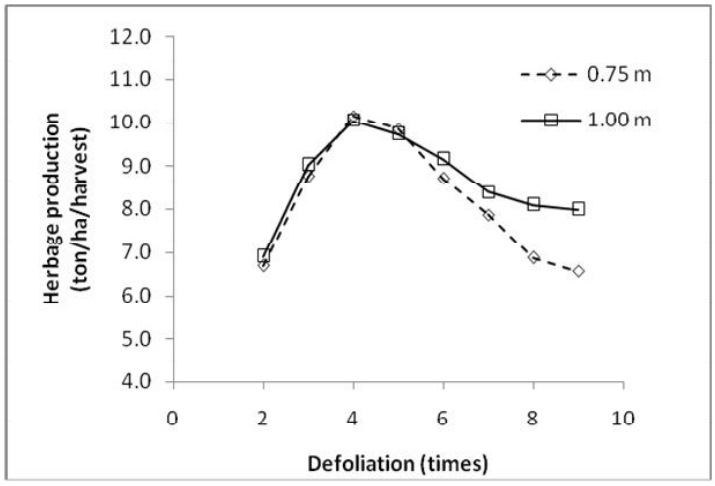

Figure 1. *I. zollingeriana* forage production during the period of observation with defoliation for 9 times (defoliation intensity 60 days).

High Quality Forage Source

Indigofera has both desirable agronomic characteristics and high nutritive value to be useful as forages. The nutrition value of a feed is determined by its ability to provide the nutrients required by an animal for its maintenance, growth and reproduction. Farmers have recognised for decades that some Indigofera species offer considerable potential nutrition (Table 1.) for improving the productivity of goat. Nutrition value of Indigofera as shown in Tabel 1 varied depended on soil

Tabel 1. Nutrition value of Indigofera zollingeriana recorded during agronomic study fertilizer application and nutritional (*in vitro*) study

Parameters	Value	
	Without fertilizer	With fertilizer
Crude protein (%)	27.68±0.75	31.31±1.04*
Tannin (%)	0.36±0.05	0.61±0.01
Saponin (%)	0.41±0.02	1.17±0.07
NDF (%)	38.30±1.72	51.05±1.55
ADF (%)	28.62±0.82	45.29±1.10
Ca (%)	1.16±0.02	1.78±0.06
P (%)	0.26±0.01	0.31±0.01
K (%)	1.31±0.11	1.42±0.08
Mg (%)	0.46±0.02	0.51±0.04
IVDMD (%)	75.44±2.02	85.50±4.24
IVOMD (%)	72.06±2.81	80.65±3.16

Source: Abdullah, 2010; Jovintry, 2011, * Suharlina, 2010 using supramin as N source.

fertility and application of foliar fertilizer (Abdullah, 2010). Trials carried out at Livestock Research Center in Medan showed improved daily gain of local goat and local x Boer cross bred during feeding program with *Indigofera* (Tarigan, 2009).

Forage quality of Indigofera changed depended on defoliation management. Interval defoliation every 60 days showed a best quantity and quality forage (Abdullah and Suharlina, 2010) than 90 days (Tarigan, 2009). The intensity of defoliation was investigated as high as 1m to 1.5 m above ground, which produced highest herbage production and nutritive value. *In vivo* experiment using pelleted Indigofera's leaf for both two groups of lactating Etawah crossbred and Saanen revealed high persistence of milk production prior a month of dry periods. Ration with commercial concentrate produced lower milk (379 ml/head) than ration containing pelleted Indigofera's leaf (762 ml/head) a month prior a month of dry periods (Apdini, 2011). Use of Indigofera leaf in lactating does reduced feed cost for about US$ 0.39 dolar/L of milk (Abdullah *et al*., 2012).

Ecologically Beneficial

Legumes produce most biologically fixed nitrogen and are therefore crucial to maintaining the N-balance in nature. A very high yielding leguminous crops can add up to 500 kg of nitrogen to the soil per hectare per year was estimated (NAS 1979). Study on the ecological function of *I. zollingeriana* revealed the legume could be expected to maintain soil fertility. This is evident from previous studies, that *I.*

Tabel 2. Effect of *Indigofera zollingeriana* on soil nutrient properties and P-solubilizing bacteria population

	C-organic (%)	N total (%)	P available (%)	P-solubilizing bacteria*
Before Indigofera cultivation	0.97±0.17	0.16±0.01	1.48±0.18	8.66±2.81
After Indigofera cultivation	1.27±0.06	0.13±0.03	1.48±0.04	37.53±6.8

*Cell per collony/100g; data were extracted from Suharlina, 2010.

zollingeriana plants can maintain the content of N and available P in soil, and improved soil organic C content and P-solubilizing bacteria population (Table 2).

Conclusions

I. zollingeriana is a shrubby legume species that plays an important role in improving the quality of feed and maintain soil quality. The results provide information that these plants can be recommended as a source of high quality forage for ruminants.

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Potential of Weeds for Ruminant Feed on Rice Fields in Java

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Abstract

Weeds compete with rice for sunlight, nutrients and water, and may reduce the yield. However, in traditional rice farming systems farmers utilize many kinds of weeds for food, forage or household purposes. Rice weeds are also utilized to feed ruminants.The aims of this research were to explore the weed diversity of rice field in various regions and rice farming systems in Java. Field researches were conducted in November 2011 – January 2012. Designated research areas: West Java: Cianjur (upland) and Karawang (lowland); Central of Java: Karanganyar (upland) and Brebes (lowland); East Java: Malang (upland) and Gresik (lowland). Locations of plots have been selected according the principles of stratified random sampling. Weed assemblages were sampled in fields of intensive and extensive farming systems as well as in fallowed areas before weeding time in two seasons. The result showed that approximately 295 species of weeds growth in rice field and terrace area. Dominant weed species present at the terrace in experimental site were Cynodondactylon, Eleusineindica, and Fimbristylismiliaceae. Potency of weed for ruminant feed gains approximately 903,5 g/m2 at terrace area.

Keywords: potency, ruminant feed, weeds diversity

Introduction

Increasing the level of rice self-sufficiency of Indonesia led to an increase in the intensity of rice (*Oryza sativa* L.) cropping in many areas since the 1970s. In Indonesia, rice cultivation covers a total of about11.5 million hectares throughout the archipelago, on Java alone around 5.4 million hectares (National Development Planning Board, 2003).About 70% of the area comprise irrigated rice fields, which have been estimated to be 100 times more productive than upland rain-fed rice farming upland rice (Fairhurst and Dobermann, 2002).

One consequence of cropping intensification in irrigated systems is a shift and an increase in weeds populations. Weeds compete with rice for sunlight, nutrients (Nyarko and Datta, 1993) and water, and causes loss of yield by about 10-50% (Chin

et al, 2000). Weed management therefore poses a significant challenge to Indonesian farming systems. Herbicide treatments change the weed community composition interrelated to crop productivity (Ulber 2010). A new approach to biodiversity friendly management is therefore to enhance the benefits derived from desirable weed species with high value for the farmer and the agroecosystem. The use of weed species is possibly the most efficient management to overcome problems of pollution through herbicide usage, slow breakdown or burning. Weed plants and vegetation vary enormously in morphology, phytomass and species composition depending on habitat conditions and land-use (Soerjani *et al*., 1987). Due to their chemical and physical characteristics weeds may or may not be grazed by ruminants. The feeding value of rice field weeds is little-known in scientific literature but the local farmers have considerable knowledge which should be combined. The main objectives of the present study were ‑to assess characteristics of farming systems in Java and to evaluate the potential of rice weed biomass as ruminant feed.

Materials and Methods

The research was done in October 2011 until March 2012 in lowland (0-100 m) and upland areas (>400 m)of rice field in Java, i.e. Karawang (33 – 53 masl), Brebes (26 – 44 masl), Gresik (14 – 41 masl), Cianjur (527 – 856 masl), Karanganyar(403 – 714 masl) and Malang (526 – 684 masl). In each of the sites, suitable landscape sections of ca. 5 km x 5 km size were selected and 15 plots located therein.The weeds were sampled in cultivated areas, fallowed areas and on rice field terraces.

Fresh biomass was measured from weed sources in each plot by placing squareframes sized 30 cm x 30 cm. The weeds were sampled on three plots on each rice field and terrace in each district. Weed samples were collected in each plot, cut and weighed as fresh biomass. The samples were dried at 60°C for 48 h and weighed as dry biomass.

Results and Discussion

Overview

The study revealed that rice farming systems in Indonesia are dependent on elevation. In upland areas continuous water supply throughout the years facilitated weed control and reduced herbicide application. Herbicides were commonly applied in lowland areas. The proportion of farmers who used herbicides on their farms was 60% or more in the lowland areas while less than 15% of the farmers applied herbicides in the upland areas (Table 1). Weed biomass source sites in upland areas were mostly on terraces as a result of continuous control in rice field area while in the lowlands the most important weed biomass sources were fallows. Manual weeding was common practice to reduce weeds in almost every rice field area. Weeding is

Table 1. Characteristics of rice farming systems

Parameters	District					
	West Java		Central of Java		East Java	
	Cianjur	Karawang	Karang-anyar	Brebes	Malang	Gresik
Herbicide application (%)	13.33	60	0	66.67	6.67	66.67
Manual weeding (%)	71.5	100	100	100	100	93.3
Time of fallow (weeks)	3-4	6-10	3-5	3-8	3-4	10-14
Weed biomass source	Terrace	Fallowed area	Terrace	Fallowed area and terrace	Terrace	Fallowed area and terrace

usually done two times, specifically 20 and 40 days after transplanting of rice plants, with the exception of Gresik.

In Gresik, direct seeding was practiced due to a shortage of labor and the higher costs of rice transplanting. Manual weeding in Gresik would commonly be done simultaneously with rice plant thinning to reduce labor costs. Labor for manual weeding is becoming increasingly scarce. Therefore the application of herbicides in the first tillage increases to eradicate all weeds that emerged in the time of fallow. In addition, poor maintenance of the irrigation infrastructure reduces water availability for rice production in the dry season and increases surface water sand flooding in rice field areas in the rainy season.

Weed Biomass

Weed biomass is the most important indicator of feed availability for ruminant in rice field area. There was a significant difference in weed biomass among rice field areas, with upland areas (Cianjur, Karanganyar and Malang) producing higher biomass values than lowland areas (Karawang, Brebes, Gresik) (Table 2). There were no significant differences between the locations of eastern, central, or western Java, respectively. Fresh weed biomass in the first rice growing season (early rainy season) yielded 891-2369 g/m^2(Table 2). Weed biomass varies enormously between regions and even between small farms in the same village due to differences of environmental factors and farming management. The results showed that high values of fresh weed biomass had the potential to supply ruminant feed in Java although variability in water regime affected the populations and growth of weed species.

Fresh weed biomass in Javanese rice fields differed from that found byRoder *et al*. (1998) who measured 220-990 g/m^2/year of fresh weed biomass over the rice growing season in northern Laos. There are many factors affecting the weed biomass

Table 2. Weed biomass in rice field areas

Parameters	District					
	West Java		Central of Java		East Java	
	Cianjur	Karawang	Karang-anyar	Brebes	Malang	Gresik
Characteristic of region	Upland	Lowland	Upland	Lowland	Upland	Lowland
Fresh weight (g)	2369.0[a]	900.1[b]	1912.2[a]	922.3[b]	2130.7[a]	890.7[b]
Dry weight (g)	284.3[a]	140.1[b]	245.8[a]	151.3[b]	258.3[a]	127.5[b]
Dry matter (%)	12.0[d]	15.6[a]	12.9[c]	16.4[a]	12.1[d]	14.3[b]

Different superscript in the same line means significantly different ($P<0.05$)

between countries, such as seasonal and climatic variation as well as different farming management systems (Machado, 2005). Yakup (2007) reported that weed infestation in rice farms increased along with elevation. Common weeds in the upland rice field areas in West Java are *Myriophyllum aquaticum* and *Sagittaria guayanenesis* while in lowland areas *Leersia hexandra, Sacciolepis interrupta* and *Ipomea aquatic* are common (Yakup, 2007).

The combination of herbicide application and manual weeding in the lowland rice field areas of Karawang, Brebes and Gresik resulted in a significantly higher degree of weed suppression. Variability of surface water condition affects the growth ofrice weed communities (Juraimi *et al.,* 2011). Hence, weed biomass in upland areasis concentrated on terraced sites since weeds have been suppressed on about 70% of the rice field areas through ponding surface waters. In all lowland areas, rice weeds were found to grow mainly in times of fallow and the values were not significantly different from each other.

There were biologically small but statistically significant differences in the comparison of dry matter percentage in each location. In the lowland areas higher dry matter values were found than in the upland area. Cutting times and plant structural composition seems to affect production yields (Yolcu *et al.,* 2006).

Conclusions

Selecting weed species suitable for ruminant feed supply and production in a particular region (based on agro-pastoral farming) is very important to sustain animal production both economically and ecologically. Rice weeds have the potential to yield substantial amounts of biomass in fallowed areas and terraces. Rice weed biomass in upland areas is currently higher than in lowland areas due to the combination of herbicide application and manual weeding in the lowlands.

Xinqing *et al.* (2006) suggested four principles for selecting appropriate species as feed, i.e. biogeographical matching of species, ecological matching, production yield and forage quality.It is essential, therefore, to further study the phytogeographical, ecological, biological and functional traits of the Javanese wild plants of rice fields.

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Mineral Balance of *Brachiaria humidicola* Pasture which is Introduced with Creeping Legumes Creeping at UP3J

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Abstract

The purpose of this research was to study mineral balance of Brachiaria humidicola pasture which was introduced with creeping legumes. Plants materials used consisted of B humidicola, three kinds of legumes, i.e. Pueraria javanica, Centrosema pubescens and Calopogonium mucunoides, NPK fertilizer, manure and Soil Potential Microorganisms were obtained from Agrostology Laboratory, Faculty of Animal Science, Bogor Agricultural University. The experiment used block randomized design with eight treatments and four replications. AThe treatments were P_1: Control (Pasture concisted of Brachiaria humidicola), P_2: B. humidicola introduced with Pueraria javanica, Centrosema pubescens and Calopogonium mucunoides with NPK fertilizer of NPK), P_3: B. humidicola with P.javanica, P_4: B. humidicola with C. Pubescens, P_5: B. humidicola with C. mucunoides, P_6: B. humidicola with P. javanica and C. Pubescens, P_7: B. humidicola with P. javanica and C. mucunoides, P_8: B. humidicola with P. javanica, C. pubescens and C. Mucunoides. P_3 until P_8 used NPK half doses, manure and soil potential microorganisms. Parameters observed were level and intake of N, P, K, Ca, Mg from forage, blood serum, and sheep feces, dry matter consumption. The data were analyzed with analysis of variance and the differences between treatments were analyzed with Duncan Multiple Range Test (DMRT). Consumption of N in each treatment was not sufficient for the needs of N from a sheep everyday, except P_3, P_4 and P_7. Consumption of P in all treatments was not sufficient. Consumption of K and Mg for all treatments were sufficient. Consumption of Ca in each treatment was not sufficient, except P_4. The mineral imbalance of P and Ca occurred in all treatments except P2, N for P_1, and P_6, Mg for P_5 and P_8. The mineral balance occured on K. To solve the problem of mineral imbalance can be done with the addition of organic or inorganic fertilizers in pasture.

Keywords: pasture, legume and grass, mineral balance

Introduction

Excellent quality forages could result the best production of ruminants. Ruminants can consume a wide variety of forages plants. Feeding of legume forage will increase the protein intake needed by ruminants. High mineral uptake such as N, P, Ca from pasture can increase the quality of forage (Karti, 2010). The lack of mineral content from the forage in Pastures can result in mineral deficiency, especially for pregnant sheep because the minerals are also used for the development of the fetus. *Brachiaria humidicola* has an average protein content of 6.6% and 55% TDN (Vendramini *et al*, 2008), while the TDN requirement for pregnant sheep was 86% and 14.16% protein (NRC, 1985) at the age of 4 months pregnancy with a body weight 20-30 kg. Based on these requirement, the addition of feed in the pasture is necessary. One of them is by introducing creeping legume. The purpose of this research was to study mineral balance of *B. humidicola* pasture which is introduced with creeping legume.

Materials and Methods

The materials used consisted of pasture of *B. humidicola,* three *kinds of* creeping legumes, i.e. *Pueraria javanica*, *Centrosema pubescens* and *Calopogonium mucunoides,* NPK fertilizer, manure and Soil Potential Microorganism obtained from Agrostology Laboratory, Faculty of Animal Science, Bogor Agricultural University. The experiment used block randomized design with eight treatments and four replications. The treatments were P_1: Control (*Pasture of Brachiaria humidicola*), P_2: *B. humidicola* introduced with *Pueraria javanica*, *Centrosema pubescens* and *Calopogonium mucunoides* and fertilized with NPK), P_3: *B. humidicola* with *P.javanica,* P_4: *B. humidicola* with *C. Pubescens,* P_5: *B. humidicola* with *C. mucunoides,* P_6: *B. humidicola* with *P. javanica* and *C. Pubescens,* P_7: *B. humidicola* with *P. javanica* and *C. mucunoides,* P_8: *B. humidicola* with *P. javanica*, *C. pubescens* and *C. Mucunoides.* P_3 until P_8 used NPK half doses, manure and soil potential microorganisms. Parameters observed were level and intake of N, P, K, Ca, Mg from forage, blood serum, and sheep feces, dry matter consumption. The data were analyzed with analysis of variance and the differences between treatments were analyzed with Duncan Multiple Range Test (DMRT).

Results and Discussion

Consumption of dry matter in the treatment of P_2-P_8 is higher when compared with controls. The highest dry matter intake at P4 treatment. Introduction of legumes can increase dry matter intake. Consumption of dry matter of P_2-P_8 higher than the control due to the introduction of legume. Intake of N, P, K, Ca was higher in $P_{2-}P_8$ compared with controls. Intake of N, P, Ca was highest in P_4, K intake in P_3,

Table 1. Mineral consumption of N, P, K, Ca and Mg, which is absorbed (in serum) and the waste (the feces) on *B. humidicola* Pasture which is introduced with legume creeping

Treatments	DM Consumption (g/ekor/hari)	Mineral Intake (g)					Serum (g)					Feses (g)				
		N	P	K	Ca	Mg	N	P	K	Ca	Mg	N	P	K	Ca	Mg
1	592.08	6.28	0.59	5.74	1.48	2.49	0.02	0.02	0.29	0.14	0.01	6.75	1.12	2.19	7.87	1.36
2	753.75	7.91	1.28	14.32	2.19	1.96	0.02	0.02	0.41	0.16	0.02	7.24	1.06	2.11	9.95	1.36
3	1001.71	16.83	1.20	18.33	4.31	3.01	0.04	0.03	0.59	0.22	0.03	12.52	1.50	2.50	17.33	2.20
4	1121.14	22.09	1.46	15.58	7.18	2.58	0.03	0.02	0.41	0.24	0.03	11.10	1.79	2.69	19.28	2.35
5	884.57	12.12	1.06	15.83	3.63	1.95	0.03	0.03	0.38	0.21	0.02	11.15	1.50	5.13	14.95	2.03
6	985.30	12.81	0.99	10.44	3.84	2.96	0.04	0.03	0.35	0.22	0.03	12.91	1.68	1.87	23.06	2.27
7	1004.07	15.36	1.10	15.56	5.42	3.01	0.03	0.02	0.44	0.21	0.03	11.45	1.81	1.71	17.47	2.31
8	958.09	13.7	0.77	14.08	5.27	2.01	0.03	0.03	0.30	0.21	0.02	10.92	1.25	1.92	16.19	2.11

P_1: Control (*Pasture of Brachiaria humidicola*), P_2: *B. humidicola* introduced with *Pueraria javanica*, *Centrosema pubescens* and *Calopogonium mucunoides* and fertilized with NPK), P_3: *B. humidicola* with *P.javanica,* P_4: *B. humidicola* with *C. Pubescens,* P_5: *B. humidicola* with *C. mucunoides,* P_6: *B. humidicola* with *P. javanica* and *C. Pubescens,* P_7: *B. humidicola* with *P. javanica* and *C. mucunoides,* P_8: *B. humidicola* with *P. javanica*, *C. pubescens* and *C. Mucunoides.*

Mg intake in P_3 and P_7. Mg intake was lowest in P_5. Introduction of legumes can increase the intake of N,P, K, Ca and Mg. Uptake of N, K, Ca, Mg were higher in P_3-P_8 compared with P_2 and control. N uptake was highest in P_4 and P_6, P uptake in P_3, P_5, P_6 and P_8, K uptake was highest in P_3, P_4 uptake on Ca and Mg uptake in P_3, P_4, P_6, P_7. Introduction of legumes can increase the uptake of N, P, K, Ca, Mg. Mineral retention of N, P, and Mg were higher in treatment P_3-P_8. Mineral retention of N, P, K, Ca and the highest in P_6, P_7, P_5, P_6 and P_4. Introduction of legumes can increase the retention of mineral N, P, K, Ca and Mg.

Table 2. Mineral balance (without urine) in *B. humidicola* pasture which is introduced with legume creeping

Treatments	Mineral Balance(g)				
	N	P	K	Ca	Mg
P1	-0.49	-0.55	3.26	-6.54	1.11
P2	0.65	0.21	11.81	-7.92	0.59
P3	4.27	-0.33	15.23	-13.25	0.78
P4	10.95	-0.36	12.48	-12.34	0.2
P5	0.95	-0.47	10.33	-11.53	-0.11
P6	-0.14	-0.72	8.22	-19.44	0.66
P7	3.88	-0.73	13.41	-12.26	0.68
P8	2.75	-0.51	11.86	-11.13	-0.12

P_1: Control (*Pasture of Brachiaria humidicola*), P_2: *B. humidicola* introduced with *Pueraria javanica*, *Centrosema pubescens* and *Calopogonium mucunoides* and fertilized with NPK), P_3: *B. humidicola* with *P. javanica,* P_4: *B. humidicola* with *C. Pubescens,* P_5: *B. humidicola* with *C. mucunoides,* P_6: *B. humidicola* with *P. javanica* and *C. Pubescens,* P_7: *B. humidicola* with *P. javanica* and *C. mucunoides,* P_8: *B. humidicola* with *P. javanica*, *C. pubescens* and *C. Mucunoides.*

Nitrogen balance in P_1 and P_6 showed a negative value means there is imbalance of nitrogen, and indicating that soil in this treatment is deficiency with nitrogen. Magnesium balance in P_5 and P_8 showed a negative value means there is imbalance of magnesium, and indicating that soil in this treatment is deficiency with magnesium. Phosphorus imbalance in all treatments except P_2. P_2 is the treatment is given with NPK fertilizer with a higher P levels compared with other treatments. The mineral imbalance occurred on Ca, but mineral balance occurred on K. Mineral balance can be improved by the introduction of legume and fertilizer P and Ca by using organic or an organic fertilizers in pasture. If not done can cause mineral deficiencies of Ca and P, and resulting in decreased growth.

Conclusions

Consumption of N in each treatment was not sufficient for sheep requirement, except P_3, P_4 and P_7. Consumption of P in al treatments were not sufficient. Consumption of K and Mg for all treatments were sufficient. Consumption of Ca in each treatment was not sufficient, except P_4. Introduction of legumes can increase consumption of dry matter, intake, uptake and retention of mineral N, P, K Ca and Mg

The mineral imbalance of P and Ca occurred in all treatments except P_2, N for P_1 and P_6, Mg for P_5 and P_8. The mineral balance occured on K. To solve the problem of mineral imbalance can be done with addition of organic or anorganic fertilizers in pasture.

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Role of Arbuscula Mycorrhizal Fungi (AMF) in Overcoming Drought Stress of Several Tropical Grasses

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Abstract

Grasses productivity is affected by soil water availability. Arbuscular Mycorrhizal Fungi (AMF) was inoculated to support plant to overcome drought stress during its growth.The aim of this study was to understand the role of Arbuscular Mycorrhizal Fungi (AMF) to support growth and the production of grasses in drought stress condition. Three species of tropical grasses: Chloris gayana, Paspalum notatum, /and Paspalum dilatatum were used. The research used completely randomized design with 4 treatments consisting of M0S0= without mycorrhizal and daily watering, M0S1= without mycorrhizal and without watering; M1S0 = with mycorrhiza and daily watering; M1S1= with mycorrhizal and without watering. and 5 replications. The four treatments research were as follows; Each type of grasses were obsereved in a separate study. The result showed that mycorrhizal played significant role in improving growth and root dry weight biomass of Chloris gayanain drought condition. Paspalum notatum is the most adaptive grass in the drought condition. Chloris gayana has the growth and a better production than Paspalum dilatatum.

Keywords: Arbuscular Mycorrhizal Fungi (AMF), Chloris gayana, drought stress, forage, Paspalum dilatatum, Paspalum notatum

Introduction

The livestock sector development can not be separated from the provision of high quality forage and sustainable. More than 60% of feed consumed by ruminants is forage, either in fresh or in dried form. Green fodder can be grasses, legumes or other types of leaves. The types of forage can be administered alone or mixed with grass or legume be given only in the form of grass.

The forage is one source of forage for grazing and appropriate use as forage cut because of its ability to regrow after the cutting or grazing. Tropical grasses

such as *Chlorisgayana, Paspalumdilatatum* and *Paspalumnotatum*is are specific tolerant grasses species (Nahak, 2011). They have good adaptability to defoliation and regrouwth. However some of tropical grasses species do not adaptive to drought condition, which mainly showed suffer in growth and produce low herbage and its qualityAvailability of feed grass that is still lacking is mainly influenced by soil conditions, climate and water availability.

Water is needed for plant growth in optimum level. Availability of water in the soil is mainly determined by the intensity and distribution of rainfall, which in turn affects ground water pool and nutrient supply. Water shortage is one of the main problems for the growth and development of a plant. Shortage of water internally at the plant resulted in a decrease direct and magnification of cell division. In the vegetative growth stage, the water used by plants for cell division and enlargement are indicatedby the increase in plant height, diameter magnification, multiplication leaf, and root growth (Sasli, 2004).

At this time in Indonesia forage crops can be developed on the dry soil conditions. Haryadi and Yahya (1988) describes drought stress in plants can be caused by two things: (1) lack of water in the root zone, (2) the rate of evapotranspiration is higher than the rate of water absorption by roots of plants that need high water on the leaves. Plants experiencing drought stress are stunted, due to the availability of water in plants and soil affect soil nutrient uptake by plant roots.

One of alternative that can be applied to several types of plants cultivated in water stress is overcome by the use Arbuskula Mycorrhizal Fungi (AMF) on the plant. The symbiosis between AMF and its host plant is a symbiotic mutualism (mutually beneficial). This symbiosis involves the provision of photosyntate by the host for the fungus and host plant otherwise acquire nutrients and water from the ground taken by its hyfa. This association did not lead to infection of the roots of disease

Karti (2005) explains that the role of mycorrhizal addition to improving nutritional status of plants, may also increase drought resistance. Rungkat (2009) explains that the mycorrizal plants usually grow better than plants that do not mycorrizal. Mycorrhizae have a role for growth and crop production, namely: a) increase the absorption of nutrients, b) protect host plants from the damaging effects caused by drought stress, c) be able to adapt quickly to the contaminated soil, d) can protect plants from root pathogens e) can improve the productivity of the soil and stabilize soil structure of the soil. In the tropical grass plants on the growth of mycorrhizal influence is also quite good. Objective of the present study was to know the effects of drought and the addition of mycorrhizal fungi on growth and productivity arbuskula some tropical grasses *Chloris gayana, Paspalum dilatatum* and *Paspalum notatum.*

Materials and Methods

The location of research was conducted at the Faculty of Animal Sciences in Greenhouse, Agrostologi Laboratory, Laboratory of Dairy Cattle Nutrition, Faculty of Animal Science, Bogor Agricultural University. Implementation of the recearch began in July 2010 to July 2011.

Materials used in this study were: Pols *Chlorisgayana, Paspalumnotatum, Paspalumdilatatum*, Latosol soil, and NPK and KCl fertilizer, and FMA obtained from the Forest and Environmental Biotechnology Research Center of Biological Resources LPPM IPB. Equipment used were scales, pots, watering tools, pieces of stone, plastic, shovel soil, brown envelopes, scissors, solatip, ruler and oven.

Data were statistically analized using completely randomized design with 4 treatments consisting of M0S0= without mycorrhizal and daily watering, M0S1= without mycorrhizal and without watering; M1S0= with mycorrhiza and daily watering; M1S1= with mycorrhizal and without watering, and 5 replications. The four treatments research were as follows;. Each type of grasses were obsereved in a separate study. The followed by the Duncan's Multiple Range Test to determine signigicant differences.

Results and Discussion

Effect of Treatment of Groundwater Levels

The moisture of soil content that describes the amount of available water is absorbed by plant roots for growth to an extent where the water becomes available and the plants have withered. Average percentage of soil moisture content in rhizosphere of *Paspalum notatum, Paspalum dilatatum,* and *Chloris gayana* at the time of harvest, are shown in Table 1. The data were collected at the condition of permanent wilting point (M1S1 and M0S1). Based on analysis of variance the treatments significantly influenced ($P<0.01$) soil water content of the rhizosphere of *Paspalum notatum, Paspalum dilatatum,* and *Chloris gayana.*

The highest value of soil moisture content on the treatment indicated by the treatment of M0S0 and M1S0 on *Chloris gayana* (36.0% and 39.9%), *Paspalum notatum* (66.9% and 65.3%), and *Paspalum dilatatum*(38,1% and 42.4%). In addition, it is shown that the treatment of M1S1 and M1S0 treated has less water availability compared to those of M0S1 and M0S0. This indicated effective role of mycorrhizae in grass crops in term of water absorption.

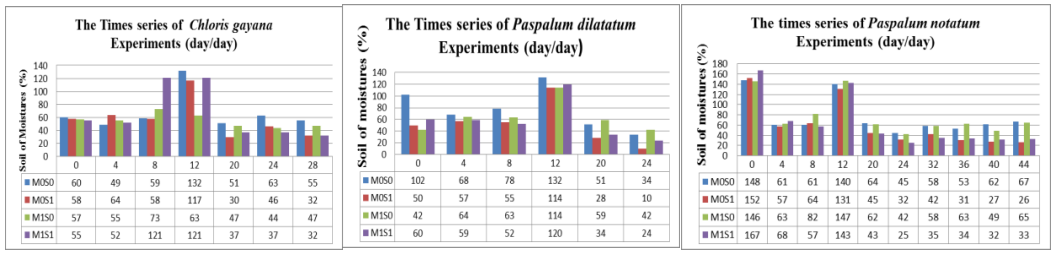
In *Chloris Gayana*, M0S0 treatment resulted in highest value of soil water content on day 12th, whereas M1S0 provide the highest value on day 16 to day 20, this suggests an increment effect of mycorrhizal soil water content after a few days on infected plants (Figure 1a). In *Paspalum dilatatum* soil water content was affected by the treatment on day 12th, whereas the lowest treatment occurred on

Table 2. Mineral balance (without urine) in *B. humidicola* pasture which is introduced with legume creeping

Treatment	Percentage soil moisters (%)		
	Chloris gayana	*Paspalum notatum*	*Paspalum dilatatum*
M0S0	36.0±8.0^{A}	66.9±2.5^{A}	38.1±15.2^{A}
M0S1	27.9±5.0^{B}	26.2±1.1^{C}	10.4±4.3^{C}
M1S0	39.9±8.1^{A}	65.3±3.8^{A}	42.4±8.0^{A}
M1S1	27.2±10.5^{B}	32.5±4.7^{B}	27.7±6.6^{B}

Description:Different letters in the same column indicate significantly different effect (P<0.01). M0S0: without mycorrhizal and watered every day; M0S1: without mycorrhiza land flushing; M1S0: the mycorrhizae and watered every day; M1S1: with the mycorrhizal and not watered.
Different plant conducted a separate study.

day 24th at M0S0, M0S1, M1S0, and M1S1, it indicated that the response levels the highest water *Paspalum dilatatum* plants occurred on day 12th and the lowest soil water content levels near the permanent wilting point (Figure 1b).



(a) (b) (c)

Figure 1. Effect of soil moisture treatment of plants *Chloris gayana*: M0S0= no mycorrhiza and watered every day; M0S1= without mycorrhizal and not watered; M1S0= with mycorrhizal and watered every day; M1S1= with mycorrhizal and not watered

Conclusions

The role of mycorrhiza was effective in maintain soil water content in both *Paspalum notatum* and *Paspalum dilatatum*, but it was not effective in *Chlorisgayana.*

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Mineral Concentration of Forage Grasses at Different Salinity Levels of Soil

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Abstract

Climatic change increase the sea level that causes soil salinization. High salt concentration in soils inhibits crop growth and production The lower limit of saturation extract electrical conductivity of saline soil is conventionally set at 4 dS m^{-1}. The research was conducted to evaluate mineral concentration of five forage grasses (Panicum maximum, Setaria sphacelata, Euchlaena mexicana, Brachiaria brizantha, and Cynodon plectostachyus) at non saline soil (EC = 0,5 dS m^{-1}) and saline soil (EC= 11 dS m^{-1}). The experiment design in this research using split plot with forage grasses as main plots and different salinity level of soil (non saline and saline) as sub plots. Sodium concentration of herbage increased significantly ($P<0.05$) at saline soil. Herbage nitrogen concentration was not different between non saline and saline soil, except for B. brizantha. Forage grasses had similar concentration of phosphorous at different salinity level of soil, except for S. sphacelata. Potassium concentration of P. maximum S. sphacelata, E. mexicana, B. brizantha, and C. plectostachyus herbage was significantly lower ($P<0.05$) at saline soil. In conclusion, high salt concentration at saline soil reduced potassium uptake. Sodium uptake was higher at saline soil than non saline soil.

Keywords: mineral concentration, saline soil, non saline soil, forage

Introduction

The effect of global warming is climatic change that increase the sea level. It was reported that the increasing of sea level was 3 mm/year. Increasing sea level causes soil salinization along the coast area of island. The sea water that contain high concentration of sodium will be intrusion to the soil along the coast. The high temperature with low rainfall will cause sodium move toward the top soil that affect the plant growth. Abrol (1988) reported the salt affected areas in Indonesia was approximately 13.2 million ha.

Three main groups of salt affected areas are saline soil, saline-sodic soil and

sodic soil. Saline soil contains sufficient neutral soluble salts that adversely affect the growth of most crop plants. The soluble salts are mainly sodium chloride and sodium sulfate with EC (electrical conductivity) more than 4 dS/m and ESP (exchange sodium percentage) less than 15. Saline-sodic soil has EC more than 4 dS/m and ESP more than 15. Sodic soil has EC less than 4 dS/m and ESP more than 15 (Majerus, 1996). In relation to crop growth, Abrol (1988) classify soil salinity into five classes. There are non saline soil, slightly saline soil, moderately saline soil, strongly saline soil and very strongly saline soil. Saline soil has EC between 0–2 dS/m, salinity effects are negligible. Slightly saline soil has EC between 2–4 dS/m, yields of sensitive crops may be restricted. Moderately saline soil has EC between 4–8 dS/m, yields of many crops are restricted. Strongly saline soil has EC between 8–16 dS/m, only tolerance crops yield satifactorily. Only a few crops yield satisfactorily at EC more than 16 dS/m or very strongly saline soil.

The effect of soluble salt at soil to plant growth is very complex. Salinity will cause ionic stress, osmotic stress and secondary stress. Accumulation of sodium (Na) and chloride (Cl^-) at leaves harm the plant growth. The high osmotic pressure hampers the plant water uptake, resulting the physiological drought. Excessive sodium ions at the root surface may disrupt plant potassium uptake that is vital for the maintenance of cell turgor, membrane potential and the activity of many enzimes (Xiong and Zhu, 2002). Wang *et al*. (2002) reported the total chlorophyll of elephant grass decrease from 181 at control to 125 at soil with EC 10 dS/m. Increasing NaCl at liquid media from 0 to 100 mM significantly decrease leaf area, chlorophyll content and photosintetic rate of *Leucaena leucocephala* and *Centrosema pubescens* (Kusmiyati *et al.,* 2009a). Salinity also affects nutrient uptake. Kusmiyati *et al.* (2009b) reported increasing NaCl concentration from 0 mM to 300 mM at liquid media significantly decreased nitrogen (N), phosphorous (P) and potassium (K) uptake at shoot and root of elephant grass and king grass.

The experiment was designed to evaluate herbage mineral concentration (nitrogen, phosphorous, potassium and sodium) of *Panicum maximum, Setaria sphacelata, Euchlaena mexicana, Brachiaria brizantha, and Cynodon plectostachyus* at saline soil compare with non saline soil. The obtained results can contribute to a better knowledge of understanding the physiological effect of salinity to develop a tolerant plant.

Materials and Methods

The experiment was conducted at greenhouse in Animal Agriculture Faculty, Diponegoro University, Tembalang Campuss – Semarang. Split plot design with completely random design was used to arrange the experiment. The main plot was forage grasses (R1= *Panicum maximum,* R2= *Setaria sphacelata,* R3= *Euchlaena mexicana,* R4= *Brachiaria brizantha, and* R5= *Cynodon plectostachyus*). The sub-

plot was soil salinity level (T1= non saline soil, T2= saline soil). There were three replications.

Pols of each forage grass were planted at pot that contain 10 kg of soil. The first cut was one month after planting to make uniform planting material. Fertilizer dosage are use 60 kg N/ha/cutting, 150 kg P_2O_5/ha and 100 kg K_2O/ha. Grasses were cut 8 weeks after the first cut. Herbage was cut 5 cm above the surface of the soil. Shoot material were weighed and dried in open air for one week. Dried tissues were re-weighed and ground to pass through a 1 mm screen for subsequent tisssue analysis.

Forage N contents were measured by Kjedahl method (AOAC, 1975). Phosphorous was analyzed by spectrophotometer method (Sulaeman *et al*., 2005). While potassium and sodium content were measured by flamefotometry (Sulaeman *et al*., 2005). The mineral concentration (N, P, K, and Na) were calculated in term of g/kg dry matter (DM). The results were analyzed using analysis of variance, then followed by LSD test to compare the different mineral concentration between non saline and saline soil at each forage grass (Steel and Torrie, 1980).

Results and Discussion

Non saline soil was latosol soil that was taken from Tembalang sub district, Semarang city, Central Java. While saline soil was taken from Kaliori sub-district, Rembang–Central Java. Saline soil was classify as alluvial type. Electrical conductivity of saline soil was 11.1±0.35 dS/m with pH 8.3±0.11. According to Abrol (1988), the electrical conductivity of saline sail is classified as strongly saline soil. Non saline soil had EC 0.5±0.01 dS/m and pH 6.81±0.01.

Analysis of variance showed that nitrogen of herbage were significantly different between forage grasses ($P<0.05$) (Table 1). Herbage nitrogen concentration was not different between non saline and saline soil, except for *B. brizantha* (Tabel 1).

Table 1. Nitrogen concentration of grasses at different levels of soil salinity

Grasses	Non saline soil	Saline soil	Mean
P. maximum	18.99±0.38^{a}	19.96±0.81^{a}	19.48±1.70^{a}
S. sphacelata	18.87±0.74^{a}	19.00±1.21^{a}	18.94±0.90^{a}
E. mexicana	18.55±0.84^{a}	19.31±0.96^{a}	18.93±0.93^{a}
B. brizantha	19.42±1.20^{a}	14.46±1.58^{b}	16.94±3.32^{b}
C. plectostachyus	15.62±0.29^{a}	15.21±0.54^{a}	15.42±0.46^{c}
Mean	18.29±1.94^{a}	17.59±2.82^{a}	

Means followed by a different letter at the same row or column were significantly different at the 0.05 probability level according to LSD test. Number followed by a different letter at the same species of grass was significantly different at the 0.05 probability level according to LSD test.

Nitrogen was absorbed by plant in the form of nitrate (NO_3^-) and ammonium (NH_4^+). Nitrate move to the root surface mainly by mass flow. Mass flow reference to the movement of water together with dissolved electrolytes (ions) through the soil (Tisdale and Nelson, 1975). The reduction of herbage N concentration at saline soil compared with non saline soil of *B. brizantha* and *C. Plectostachyus* were 25% and 2% respectively, while there were no reduction of N concentration at *P. maximum, S. Sphacelata* and *E. Mexicana*

Forage grasses had similar concentration of phosphorous at different salinity level of soil, except for *S. sphacelata* (Table 2). Reduction of phosphorous concentration was ranged from 4% to 10%. Plants absorb most of their phosphorous as the primary orthophosphate ion (H2PO-). Phosphorous moves from soil to roots by ion diffusion process. Plant absorb P by contact exchange (Tisdale and Nelson, 1975). Nitrogen and phosphorus uptake of forage grasses was not different between non saline and saline soil. At saline soil, forage grasses still can absorb nitrogen and phosphorous because the water was available. At this experiment, water at saline soil was maintained at field capacity.

Table 2. Phosphorous concentration of grasses at different levels of soil salinity

Grasses	Non saline soil	Saline soil	Mean
P. maximum	1.79±0.29^{a}	1.50±0.29^{a}	1.66^{bc}
S. sphacelata	1.99±0.05^{a}	1.78±0.06^{b}	1.89^{abc}
E. mexicana	2.46±0.48^{a}	2.27±1.07^{a}	2.36^{a}
B. brizantha	2.21±0.04^{a}	2.01±0.80^{a}	2.11^{ab}
C. plectostachyus	1.44±0.36^{a}	1.38±0.21^{a}	1.41^{c}
Mean	1.98^{a}	1.79^{a}	

Means followed by a different letter at the same row or column were significantly different at the 0.05 probability level according to LSD test. Number followed by a different letter at the same species of grass was significantly different at the 0.05 probability level according to LSD test.

Potassium concentration of *P. maximum S. sphacelata, E. mexicana, B. brizantha, and C. plectostachyrus* herbage was significantly lower at saline soil ($P<0.05$) (Table 3). Potassium concentration reduction of *P. maximum, S. Sphacelata, E. Mexicana B. brizantha* and *C. plectostachyus* are 48%, 74%, 55%, 55%, and 34%.

Sodium concentration of five grasses herbage increased significantly at saline soil ($P<0.05$) (Table 4). Saline soil has high concentration of NaCl. High salinity in growth media cause excessive sodium at the root surface. Sodium at high concentration has a strong inhibitory effect on potassium uptake by root (Xiong and Zhu, 2002). Sodium and chloride shoot concentration increased and K decreased as the external NaCl concentration increased (Teakle *et al*., 2006). This condition will

Table 3. Potassium concentration of grasses at different levels of soil salinity

Grasses	Non saline soil	Saline soil	Mean
P. maximum	21.19±2.77[a]	10.95±0.91[b]	15.42[b]
S. sphacelata	38.59±4.48[a]	10.01±3.33[b]	24.42[a]
E. mexicana	20.04±3.85[a]	8.90±1.04[b]	14.47[b]
B. brizantha	13.31±0.32[a]	5.97±1.80[b]	9.64[c]
C. plectostachyus	21.56±2.96[a]	14.03±2.62[b]	17.80[b]
Mean	22.94[a]	9.86[b]	

Means followed by a different letter at the same row or column were significantly different at the 0.05 probability level according to LSD test. Number followed by a different letter at the same species of grass was significantly different at the 0.05 probability level according to LSD test.

Table 4. Sodium concentration of grasses at different levels of soil salinity

Grasses	Non saline soil	Saline Soil	Mean
P. maximum	1.15±0.35[b]	6.77±1.65[a]	3.96[c]
S. sphacelata	1.16±0.35[b]	8.86±1.89[a]	5.01[b]
E. mexicana	0.99±0.03[b]	9.09±0.75[a]	5.04[b]
B. brizantha	1.19±0.39[b]	11.63±0.81[a]	6.41[a]
C. plectostachyus	1.15±0.36[b]	10.57±1.34[a]	5.86[ab]
Mean	1.13[b]	9.38[a]	

Means followed by a different letter at the same row or column were significantly different at the 0.05 probability level according to LSD test. Number followed by a different letter at the same species of grass was significantly different at the 0.05 probability level according to LSD test.

cause nutrient imbalances and deficiencies. Paksoy *et al.* (2010) reported K application to plant growth media significantly increased mineral content of okra seedling under saline condition. Tolerant plants had more K uptake than the susceptible ones. Potassium had an important role in salt tolerance (Rubio *et al.* 2004). Salinity will cause ionic stress, osmotic stress and secondary stress. Accumulation of sodium (Na^+) and chloride (Cl^-) at leaves harm the plant growth. Excessive sodium ions at the root surface may disrupt plant potassium uptake that is vital for the maintenance of cell turgor, membrane potential and the activity of many enzimes (Xiong and Zhu, 2002).

The percentage enhancement of sodium concentration were ranged from 488% to 877% at saline soil compared with non saline soil. Sodium concentration at *P. maximum was* 488%, while *B. Brizantha was* 877% at saline soil compared with non saline soil. Among the five grasses that were tested at this experiment, *P. maximum*

showed the most tolerant plant. *P. maximum* was capable to suppress the uptake of sodium, also suppressed the reduction of potassium uptake.

Conclusion

It could be concluded that nitrogen concentration in herbage of *Panicum maximum, Setaria sphacelata, Euchlaena mexicana* and *Cynodon plectostachyus* was not different between non saline and saline soil. Herbage phosphorous concentration of *Panicum maximum, Euchlaena mexicana, Brachiaria brizantha, and Cynodon plectostachyus* was similar at non saline and saline soil. High salt concentration at saline soil reduced potassium uptake and increased sodium uptake of *Panicum maximum, Setaria sphacelata, Euchlaena mexicana, Brachiaria brizantha, and Cynodon plectostachyus*. Among the five grasses that were tested at this experiment, *P. maximum* showed the most tolerant plant.

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II. FEED AND NUTRITION
Sub Theme: Feed Technology

Theobromine Content in Cocoa Pod Husk (*Theobroma cacao*) Fermented by *Aspergillus spp*. in Different of Chop Sizes and Fermentation Times

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Abstract

Theobromine is one of alkaloid in cocoa pod husk (CPH). Fresh CPH has high theobromine content reach 0.40% (400.000 ppm) which is fresh CPH portion in ruminant ration more than 30% to be affected of health. This research aim was to know the change of theobromine content of CPH with different chop sizes were fermented in the different time of incubation by Aspergillus spp. Three species of Aspergillus spp. were used in this research i.e. A. niger, A. oryzae and A. ficuum BPT. There were 4 chop sizes i.e.; irrigular size (A_1), 1x5 cm (A_2), 3x5 cm (A_3), and 5x5 cm (A_4). The incubation times were; 0, 5, 7, and 9 days, with three replications. The solely parameter was theobromine content which were prepared according to AOAC (1990) and analyze using HPLC (Chen et al., 2008). The result of research showed that CPH fermented using A. niger, the lowest of theobromine content for chop size A_2 and incubated 9 days was 38.088 ppm. CPH fermented using A. oryzae had the lowest of theobromine content in A_2 and 9 days only 36.215 ppm. CPH fermented with A. ficuum had the lowest of theobromine content in A_2 and 9 days reach 42.914 ppm. It was concluded that the best of CPH fermented for reducing of theobromine content for 1x5 cm (A_2) chop size with 9 days fermentation, fermented by A. oryzae.

Keywords: theobromine, cocoa pod husk, Aspergillus spp., chop sizes, incubation times

Introduction

Cocoa pod husk (CPH) utilization is expected to be capable of feed fulfilling the ruminant animal requirement yearly, so the animal will not be lacking of nutrients for its life and production. This condition has reason because cocoa trees have fruit in

a long year which is CPH abundant available in harvest season. Utilization of fresh CPH as feed supplement has limiting factor which one alkaloid is theobromine. CPH has high of theobromine content or 3,7-dimethylxanthine (Mahyuddin and Bakrie, 1993; Aregheore, 1999). The high concentration theobromine affected the health of animal (Golding, 1985). The theobromine content of CPH about 0.17-0.20% (Wong and Hassan, 1988), while in cocoa beans reached 1.5-3.0%, and in dry cocoa beans reached 1.8% (Willson, 1999).

Decreasing of theobromine content can be done with fermentation method using microbe agent such as fungi. Some researchers used *A. niger* as decomposer of CPH in solid fermentation process (Yamaoka-Yano and Mazzafera, 1999; Huq, 2006) or *A. oryzae* (Lubis *et al*., 2002). They found that CPH theobromine content were reduced due to the role of enzyme activity that secreted by *Aspergillus spp*.

Based on those results, in this research we used *A. niger, A. oryzae* and *A. ficuum* in solid fermentation with CPH as a substrate. The aim of this research was to decrease theobromine content of CPH in Laboratory scale that subsequently the fermented CPH would used for alternative forage, and also we studied about storage time of fermented CPH as feed.

Materials and Methods

This experiment was fermented CPH using three of *Aspergillus spp*.i.e. *A. niger, A. oryzae* and *A. ficuum,* in four types of chop sizes i.e. irregular size (A_1) as control, 1 x 5 cm (A_2), 3 x 5 cm (A_3), and 5 x 5 cm (A_4) and four kinds of incubation times i.e. 0, 5, 7 and 9 days. CPH was air dried for 8 hours after chopping, while powder fungi were used as much as 1% and then added urea 1% of CPH weight. Urea dissolved with sterile water in ratio 1 : 10 parts. Fresh CPH used 1 kg every treatment which this was spread in plastic box (the first coating). CPH sprayed with urea and inoculated of *Aspergillus spp*., the next with same procedure was done for the second and third coatings.

After completion of fermentation process, CPH was removed and to be air-dried for 6 hours. The weighing of CPH and to be continued with samples drying using oven in temperature of 55 ^{o}C for 4 days. About 100 g of fermented CPH was dried using freeze dryer for 4 days. Dried CPH was ground with the mortal which was surrounded by ice to maintain optimal temperature and theobromine content, and then followed by analysis of theobromine content of CPH. Analysis of theobromine content was recommended by EFSA (2008) using High-Performance Liquid Chromatography (HPLC).

Results and Discussion

Theobromine as alkaloid compound cause bitter in cocoa beans and CPH. Al-

kaloid is organic component from plant which has contain of nitrogen, commonly as alkalis and it has biology activity. The alkaloid in plant has function for attact of pest (Urich, 1994). Fermentasi treatment can decrease CPH theobromine content. CPH non fermented has higher of theobromine content (Table 1).

Table 1. Theobromine content of cocoa pod husk fermented by *A. niger* (ppm)

Incubation times (days)	Treatment of chop sizes				Average
	A1	A2	A3	A4	
0	157.560	134.410	171.554	186.193	162.429
5	76.008	71.093	78.857	81.778	76.934
7	38.545	38.103	46.680	52.697	44.006
9	38.477	38.088	43.837	45.727	37.507
Average	77.648	70.424	85.232	91.599	

Note: A_1= irregular size, A_2= 1 x 5 cm, A_3= 3 x 5 cm, A_4= 5 x 5 cm.

The finding results showed that fermentation process affected the decreasing of CPH theobromine content. In *A. niger* fermentation treatment, there was an effect on CPH theobromine content due to differences of chop sizes and incubation times. Reducing of CPH chop size was parallel to reducing of theobromine content. The A_2 and A_4 chop sizes showed average of the lowest and the highest of theobromine content, respectively. On the other side, increasing of incubation time was inversed with decreasing of average of theobromine content. The lowest average of theobromine content was achieved through fermentation at 9 days of incubation (reduced 76.91%). From this experiment, the lowest of theobromine content (38.088 ppm) was achieved in A_2 chop size when incubated during 9 days.

Fermentation using *A. oryzae* in treatment of chop sizes and incibation times showed effect to decreasing of CPH theobromine content that was presented in Table 2. Reducing of CPH chop size had correlation with reducing of theobromine content. The A_2 chop size showed average of the lowest theobromine content, while A_4 was the highest theobromine content. The longer incubation time supported reducing theobromine content. The lowest average of theobromine content was occur at 9 days fermentation (reduced 23.81%). From this experiment, the lowest of theobromine content (36.215 ppm) was achieved in A_2 chop size when incubated during 9 days.

The finding results showed that fermentation CPH using *A. ficuum* affected the decreasing of theobromine content (Table 3). Decreasing of CPH chop size had relation with decreasing of theobromine content. The A_2 chop size showed average of the lowest for theobromine content. On the other side, increasing of incubation time was inversed with decreasing of average of theobromine content. The lowest

Table 2. CPH theobromine content KBK nonfermented and fermented with *A. niger* (ppm)

Incubation times (days)	Chop sizes				Average
	A1	A2	A3	A4	
0	181.891	191.058	195.084	197.083	191.279
5	62.904	64.337	67.120	75.050	67.353
7	53.275	53.926	53.600	58.360	54.790
9	46.016	36.215	46.880	53.096	45.552
Average	86.022	86.384	90.671	95.897	

Note: A_1= irregular size, A_2= 1 x 5 cm, A_3= 3 x 5 cm, A_4= 5 x 5 cm.

Table 3. CPH theobromine content KBK nonfermented and fermented with *A. ficuum* (ppm)

Incubation times (days)	Chop sizes				Average
	A1	A2	A3	A4	
0	175.409	156.430	214.776	223.609	192.556
5	68.566	69.884	81.920	91.207	77.894
7	62.146	66.348	75.749	88.566	73.202
9	45.052	42.914	58.360	80.048	56.593
Average	87.793	83.894	107.701	120.858	

Note: A_1= irregular size, A_2= 1 x 5 cm, A_3= 3 x 5 cm, A_4= 5 x 5 cm.

average of theobromine content was achieved through fermentation at 9 days of incubation (decreased 28.09%). From this experiment, the lowest of theobromine content (36.512 ppm) was achieved in A_2 chop size when incubated during 9 days.

The average for theobromine content in CPH non fermentation (0 day) was 192.556 ppm (192.556 mg/kg), kg). This value was lower from reported by Haryati and Harjosuwito (1984); Wong and Osman (1988) about 0.17-0.20% (1,700-2,000 mg/kg), but EFSA (2008) reported higher reach 0.40% (4,000 mg/kg). Different of theobromine content for non fermented CPH was caused by different treatment of chop size. This research, CPH was chopped and air dried during 8 hours which was decrease water content and followed by decreasing of theobromine content. The cocoa bean had theobromine content higher reached 1.5-3.0% (15,000-30,000 mg/kg) (Harrison, 2001), while dry beans had theobromine content lower only 1.8% (18,000 mg/kg) (Willson, 1999). The CPH theobromine content was the lowest among 3 varieties of *Aspergillus spp*. after fermentation process that was *A. niger* in A_2 with incubation of 9 days (45.052 ppm).

Reducing of CPH theobromine content caused by decomposing activity of *Aspergillus spp.* which was during fermentation process to result heat (increasing

of substrate temperature). Beside that occured hydrolysis in CPH substrate. In this step, theobromine dissolve in cell liquid and came out together with evaporation process. Rohan (1963) and Alamsyah (1991) were reported that theobromine content reduce during fermentation process because soluble in cell liquid and diffusion in cocoa bean nib. Diffusion process will stop if occur balance of theobromine content in cocoa bean. Decreasing of CPH theobromine content was relatively low between *A. niger* compared *A. ficuum*. This case had relatition with ability *A. niger* to utilize theobromine as energy source. According to Asano et al. (1993) and Hakil et al. (1999) that *A. niger* had ability to utilize theobromine as energy source through methylxanthines degradation.

Conclusions

The chop size of 1x5 cm and incubation times of 9 days affected decreasing of CPH theobromine content. The best of CPH fermented for reducing of theobromine content for chop size 1x5 cm (A_2) with 9 days fermentation, fermented by *A. oryzae*.

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Differences in Drying Method of King Grass (*Pennisetum hybrid*) Silage Samples Prepared for *in Vitro* Digestibility Analysis

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Abstract

In vitro digestibility of silage was influenced sample preparation methods because silage contained volatile compounds had potentially losses during preparation. An experiment had been conducted to evaluate different methods for preparing of a silage sample which was used in vitro digestibility studies. King grass (Pennisetum hybrid) silage were sampled at 21 d incubation for in vitro gas production analysis. Sample preparation was conducted by oven drying at 60°C while freeze drying method at -20°C and both of them conducted during 20 hours. The variables measured were in vitro dry matter and organic matter digestibility (IVDMD and IVOMD), gas production, volatile fatty acids (VFA) production. The experiment was arranged on completely randomized design with t-test analysis. Results showed that either IVDMD or IVOMD from silage dried by oven and freeze drying methods were similar. Production of volatile fatty acid (VFA) and acetate: propionate ratio (C2:C3) had also no differences between silage prepared by oven and freeze drying methods. However, total gas production from silage during 48 hours incubation affected by drying methods significantly (P<0.05). Gas production of freeze dried silage (Y) could be predicted by gas production from oven dried silage (X) as followed the equation was Y=1.0846X+0.7947 (R2=0.997). It was concluded that oven drying method could be used for the sample preparing method of silage at the in vitro digestibility analysis.

Key words: freeze drying, gas production, in vitro digestibility, oven drying, silage

Introduction

Silage is defined as forage that is preserved in the controlled fermentation (McDonald *et al*., 1991). It is a complex routine preparation to determine chemical constitution and characterisation of silage products. Due to silage is a product of

microbial fermentation activity which produce volatile and unstable chemical compound such as ammonia, volatile fatty acids, and lactic acid (Rêgo *et al.* 2010). To overcome the problems, many researchers often use a freeze drying technique for a better sample preparation in future analysis (Grabber, 2009). However, there are limitations applying this method including relatively expensive equipment, more complex sample preparation, etc. On the other hand, there are some relatively simple preparation methods to proceed, such as oven drying (Kamarloiy and Yansari, 2008) or freeze drying (Calabrò *et al.*. 2005, Grabber, 2009).

It is necessary to investigateeffects of substrate preparation (i.e. freeze versus oven dried) on *in vitro* digestibility of king grass (*Pennisetum hybrid*) on the basis of *in vitro* gas production technique. This is because sample preparation method produced highly significant influence on *in vitro* degradation test. Calabrò *et al*. (2005) indicated that there was significant interaction between sample method preparation and fermentability parameters. Total gas production is known to give a good estimation of digestibility (Beuvink and Kogut, 1993). This method is also able to predict fractions of rumen fermentable organic matter, crude protein and starch escaping rumen degradation as well as *in vitro* organic matter digestibility (DeBoever *et al*., 2005).

Therefore, the objective of this study was to investigate the effect of sample preparation method on king grass silage digestibility based on determination of *in vitro* dry matter and organic matter digestibility, total gas and volatile fatty acids production using gas production technique.

Materials and methods

Silage was made from king grass (*Pennisetum hybrid*). Grass was wilted for 24 hours to increase dry matter (DM) content. Feed materials were chopped with shredded size 1-3 cm. Inoculants 1% (v/w) was added into grasses and water was also added to adjust moisture content up to 75%. After mixing homogenously, all ingredients were packed in plastic bag (5 kg/pack) and incubated for 21 days. Samples were prepared by two methods of drying. The first group was dried by oven-drying (60°C) for 20 h, and the other one was dried by freeze-drying method using a freeze dryer Leybold-Heraeus GT Lyovac type-2 (Peterswan Ltd., Edinburgh) at -20°C for 20 h. Samples were then ground with a mortar and sieved by a filter (1.0 mm screening).

Evaluation of silage digestibility, volatile fatty acids (VFA) and ammonia (NH_3) productions were measured by the total gas production using Menke *et al*. (1979) that was modified by Jayanegara *et al*. (2009). *In vitro* DM and organic matter (OM) digestibility (IVDMD or IVOMD) were measured according to Blümmel *et al*. (1997) method. *In vitro* digestibility was determined by calculating degradation percentage of DM or OM after incubation for 24 h.

Ground silage samples (380 mg, DM 86.4%) were placed into the syringe to the pre-incubation for 24 h at 39°C. Rumen fluid (10 ml) and buffer solution (20 ml) were inserted into syringe with saturated CO_2. Composition of buffer solution per 100 ml rumen fluid (Menke *et al*., 1979) consisted of macrominerals (23.7 ml), micro-minerals (0.012 ml), bicarbonate buffer solution (23.7 ml), resazurin 4% (0.122 ml), reducing solution (4.96 ml) and distilled water (47.5 ml). Rumen fluid was taken from fistulated beef cattle (Ongole crossbred) which had been conditioned to feeding standard (feed composition consisted of 60% forage and 40% concentrate). Gas production kinetics was calculated based on exponential equation (Ørskov and McDonald,1979). The estimated value of a, b, c were calculated by fitting curve method using Neway Software (Rowett Research Institute, Aberdeen, UK) installed at Microsoft Office Excel 2007® that was developed by Chen (1997). VFA was analysed using gas chromatography method (Friggens *et al*., 1998) and NH_3 analysis using spectrophotometric method (Broderick and Kang, 1980).

Evaluation of *in vitro* digestibility and fermentability were arranged on factorial completely randomized design with 2 factors of treatments. Each treatment consisted of 3 replications with 2 sub samples. Pangola grass (*Digitaria decumbens*) was used as standard sample and each syringes containing silage, standard samples and blank were randomly allocated in the incubator. Incubation was carried out for 48 h and gas production was observed at 0, 1, 2, 4, 6, 8, 12, 18, 24, 36 and 48 h after incubation. Data of IVDMD and IVOMD, gas production, VFA total, acetate (C_2), propionate (C_3), butyrate (C_4), and NH_3 were analyzed with analysis of variance (ANOVA) and if among the treatments showed significant differences ($P<0.05$) followed by t-test (Gomez and Gomez, 2007).

Results and discussions

Effect of drying methods on *in vitro* IVDMD and IVOMD were shown at Table 1. IVOMD and IVDMD of silages prepared by freeze drying method were 5-6% higher than those of silages prepared by oven drying method. However, drying methods had no significant influences on those variables. *In vitro* digestibility of silage could be influenced by many factors which were sample preparation methods and chemical component of feedstuff.

Fresh or freeze-dried silages tended to be more fermentable than oven-dried silage (Calabrò *et al*., 2005, Grabber *et al*., 2009), the nutritive value, especially protein and crude fiber in silage, was closely related to *in vitro* degradability (DeBoever *et al*., 2005). In many cases, overheating affected solubility of nutrients. Proteins were binding to NDF content in silage if drying temperature was more than 70°C (Cone *et al*., 1996). Because of sample was dried at low temperature (60°C), there was no significant alteration of total nutrient solubility indicated by a similarity in digestibility of oven-dried sample to that of freeze-dried sample. Calabrò *et*

Table 1. *In vitro* digestibilities, gas production parameters, production of volatile fatty acid (VFA) and amonia (NH_3) from silage prepared by oven and freeze drying

Variable		Drying Methods	
		Oven (60 °C)	Freeze Dry (-20 °C)
In vitro digestibility			
IVDMD[1]	(%)	43.74±3.18	46.29±8.62
IVOMD[2]	(%)	50.61±3.01	53.83±10.55
Gas production[3]			
a	(ml/h)	-0.450±0.401	-0.518±0.479
b	(ml/h)	48.32±2.623	50.746±4.675
a+b	(ml/h)	47.87±2.517	50.227±4.580
c	(ml/h)*	0.032±0.006b	0.025±0.003a
Total VFA	(mM)	161.11±63.74	162.99 ± 79.10
Acetate (C_2)	(mM)	114.11±47.86	109.79 ± 54.89
Propionate (C_3)	(mM)	34.89±11.93	39.25±17.61
Butyrate (C_4)	(mM)	12.11±5.31	13.96 ±7.94
Ratio C_2:C_3		3.20±0.63	2.75±0.39
NH_3	(mg/100 ml)	32.13±3.68	32.08±4.68

[1]IVDMD: *in vitro* Dry Matter Digestibility, [2]IVOMD: *in vitro* Organic Matter Digestibility, [3]Gas Production from Soluble Fraction (a) and Potential Soluble Fraction (b), and Rate of Gas Production (c), * Mean with different superscript at same row showed significant difference (P<0.05).

al. (2005) reported that there were no differences in OM degradability (707 vs 708 g/kg) from silage dried by freeze- and oven- drying (65°C). VFA and ammonia were readily vapor by increasing temperature in drying chamber. Unstable chemical compound (VFA and NH_3) could be exhausted after silo was opened and silage was dried (Rêgo *et al.,* 2010). To maintain volatile or unstable compounds, in silage could be conserved by freeze drying (Grabber, 2009). Differences of drying methods were significantly influence kinetics of gas production during 48 h incubation. Gas production was higher for freeze-dried sample than that of oven-dried sample (Figure 1A). This result indicated that gas production from silage was higher when it was prepared by freeze-drying than by oven-drying.

Gas production obtained from freeze-dried silage (Y) could be estimated by that from oven-dried silage (X) following the equation: $Y=1.085X+0.795$ (R^2= 0.998) (Figure 1B). This equation showed a significant analysis based on consistent increases in gas production from both silages prepared by different methods. Oven-dried silages could be used as estimation of gas production produced by freeze-dried silages. Deinum and Maassen (1994) stated that forage dried at at 70 ^oC produced

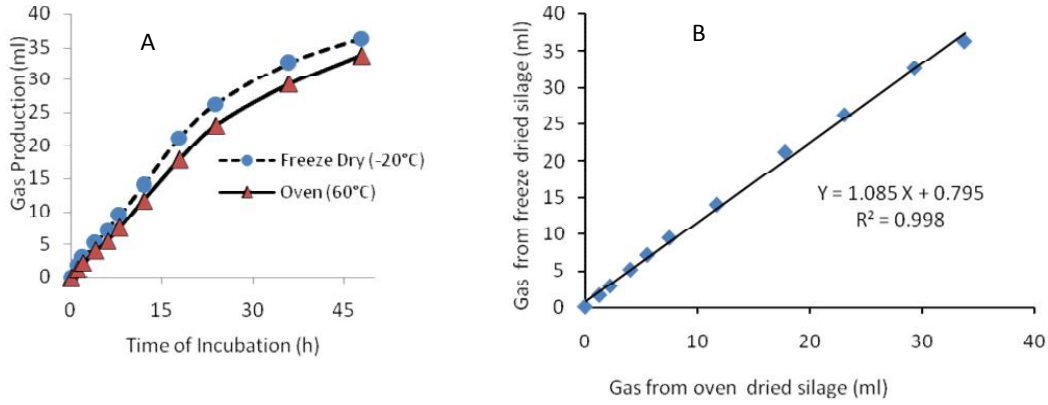

Figure 1. Gas production kinetics (A) and relationship of gas production (B) from silage prepared by freeze and oven drying

less nutrient destruction, but drying up to 105 °C caused more protein bound to NDF fraction and increased other compound losses. This was due to Maillard reaction to occur between amino acid (protein) and glucose that strengthened chemical linkage in those fractions and less fermentable indicated by lowering gas production.

Variables of soluble (a) and insoluble fraction (b) showed non-significant differences between sample prepared by both drying methods. However, gas production rate (c) data was able to show nutrient loses produced by oven-drying method. Fresh silage (200 mg) produced gas (45.6 ml) higher than dried silage (32.4 ml) at 24 h incubation (Calabrò *et al*., 2005). Drying at low temperature was able to keep volatile compound in silage, and this type of silage could be similar to that of fresh silage. Metabolites in silage were dominated by organic acids such as lactic, acetic, propionic, and butyric acids, N-ammonia (McDonald *et al*., 1991).

There were no significant differences between oven- and freeze- drying for silage preparing method on total VFA, acetate, propionate, butyrate and ammonia. Average productions of acetate, propionate and butyrate were 112.0, 37.1, and 13.1 mM, respectively, with average ratio of acetate (C_2) and propionate (C_3) was 2.98 and ammonia production was around 32.0 mg/100 ml. Calabrò *et al*. (2005) reported that freeze- and oven- drying produced different effects on corn silage, but were not statistically significant. The results showed that total VFA (90.90 vs 82.94 mmol/g OM), acetate (65,60 vs 54.56 mmol/g OM), propionate (23.4 vs 22.26 mmol/g OM), butyrate (1.99 vs 3.03 mmol/g OM). Moreover, those differences value showed that not significant differences. Based on fermentability variables, there were similarity in responses between the two methods. Volatile compound produced by microbes during ensilage was not significantly lost when silage sample was prepared by oven drying (60 °C, 20 h). A higher drying temperature caused many losses of compound in fresh forage decrease in digestion rate, conversely a lower drying temperature (50-70°C) caused less several loses (Deinum and Maasen, 1994).

Conclusion

There were no differences between oven-dried (60 °C, 20 h) and freeze-dried (-20 °C, 20 h) samples on in vitro digestibility and fermentability. Gas production of freeze-dried silage (Y) could be predicted by that from oven-dried silage (X) using the following equation: $Y=1.085X+0.795$ ($R^2=0.998$). It was concluded that oven-drying (60 °C, 20 h) method can be used for preparing silage samples in *in vitro* digestibility analysis.

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Effect of Prebiotic on Broiler Performance: A Meta-Analysis

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Abstract

A meta-analysis of results from broiler chicken trials was conducted from several countries between 2003-2012 to demonstrate the effects of dietary prebiotic versus basal diets on feed consumption, weight gain, body weight gain and feed conversion of broiler chicken. A number of thirty seven references (peer reviewed papers) was collected to analyse by using statistical meta-analysis. Criteria for selecting studies were : 1) pen trial, 2) prebiotic dosage, 3) negative and/or positive control, 4) antibiotic inclusion, 5) replication, 6) result and discussion. Results were averaged "by treatment" using paired T-test to compare the use of dietary prebiotic on broiler performance. Compared with negative control, the final body weight and body weight gain of broiler chicken with the inclusion of prebiotic give significan effects (P<0.05) and was found to be 2.22 % and 2.83 % greater than unsupplemented. However, dietary prebiotic slight improve (P>0.05) 1.974 % feed intake and 1.153 % feed conversion. Comparisons between this present of meta-analysis and those done previously on another prebiotic trial suggest that dietary prebiotic may improve broiler performance.

Keywords: broiler, chicken, meta-analysis, prebiotic

Introduction

A prebiotic compound is defined as a nondigestible food ingredient that can be utilized by intestinal microflora, which beneficially affects the host (Gibson and Roberfroid, 1995). Oligosaccharides as prebiotics are carbohydrates composed of short chain of monosaccharides. Some are thought to enhance the growth of beneficial organisms in the gut and others are thought to function by competing with pathogenic bacteria for attachment sites in the lumen (Kim et al., 2011). Oligosaccharides are carbohidrates that yield 2 to 10 monosaccharides upon hydrolysis (Benites et al., 2008).

However, certain oligosaccharides such as fructo-oligosaccharides, galacto-oligosaccharides and mannan-oligosaccharides due to their unique chemical structures, are resistant to digestive enzymes in the upper digestive tract of a host enter in the hindgut, fermented by the intestinal microflora and transit unchanged into the large intestine (Rehman *et al*., 2007). Prebiotics have indirect favorable effects on the host immunity. They stimulate the growth of lactic acid-producing bacteria. These bacteria can influence the immune system by producing immunostimulating compounds (Houshmand *et al*., 2012). Mannan-oligosaccharides (MOS) are present in the cell wall of yeast and have been shown to alter microbial populations in livestock (Biggs *et al*., 2007). MOS have unique functions as a competitive binding site, the bacteria bind to it and are carried out of the gut rather than binding to the intestine (Griggs and Jacob, 2005). Inulin, a prebiotic polyfructan, extracted from chicory (*Chicorium intybus*) that contains β (2 → 1) glycosidic bond and resistant to host-derived digestive enzymes (Rehman et al., 2007). But, the major prebiotics of interest as poultry feed additives are fructo-oligosaccharide and mannan-oligosaccharide (Shanmugasundaram and Selvaraj, 2012).

However, there were inconsistency and inconclusive data of the application of prebiotics on broiler performance (Griggs and Jacob, 2005). An alternative to find out this problem is the meta-analysis approach that allows integrating different variables and establishing systematic responses adjusted to the diversity of available experimental publications (Andrette *et al.*, 2012). Therefore, this study was performed using meta-analysis to investigate the relationship of several prebiotics on broiler chickens performance.

Materials and Methods

Literature Preparation

Indexed publications with in vivo experimental results on broiler feed supplemented with prebiotics were selected. The search strategy to select the publications was to consult different online data sources with key words in English. The main criteria for selecting publications were a) diets with prebiotics comparing without prebiotics, b) research with no challenge treatment, c) effects of prebiotics on broiler chicken performance. After the selection of the publications, the performance variables such as feed intake, body weight, body weight gain and feed conversion were tabulated to permit the descriprive analysis of studies included in the database.

Description of the Database

The database composed from 37 articles publised (peer reviewed journal) between 2003 and 2012. The most frequent preiodicals in the database were Journal of Poultry Science (32.43 % of the papers), British Poultry Science (29.73 %), Journal of Animal Physiology and Animal Nutrition (10.81 %), South African Journal of

Animal Science (8,11 %), Journal of Applied Poultry Research (5.41 %), International Journal of Poultry Science (5.41 %), Australian-Asian Journal of Animal Science (5.41 %), Brazilian Journal of Poultry Science (5.41 %) and Journal of Animal and Veterinary Advances (5.41 %). Most of the experiments were conducted in European (43.24 % of the papers), Australian (24.32 % of the papers) and American (18.92 % of the papers) institutions.

The studies included in the database total 13,928 broilers, with an average of 376 broilers per paper. The genetics were described in 86.49 % of the papers (40.54 % Cobb and 35.14 % Ross). Most (59.46 %) of the papers used male broilers, 13.51 % used mixed lots and 27.03 % did not describe broiler sex in the study. The facilities used included floor (32.43 %) and cages (51.35 %) and 16.22 % of the authors did not present the installation type. Average temperature in experimental facilities was 28.18 ^{o}C (ranging from 18 - 35 ^{o}C). No information about stress caused by environmental conditions (heat, cold or humidity) or sanitary challenge was presented in database papers. Vaccination (for Mareks, Coccivac, ND, IB or IBD) was described in 7 studies under analysis. However, only data from animals vaccinated in the pretrial period were considered for analysis. The other papers did not describe the procedures for animal vaccination. Corn and soybean meal were the main ingredients (70.27 %), where wheat based diets (18.92 %) also used for broiler diets.

Statistical analysis

The meta analysis considered only the prebiotic that was evaluated in two or more than two independent studies. The effect size was calculated for each study : effect = mean of the group fed prebiotic – mean of control group/mean of control group (Faria-Filho *et al.*, 2006). Statistical analysis was performed using 47 pairs data on literatures for each of parameters and conducting paired t-tests and probability of statistical significance.

Results and Discussion

Fourty seven experimental data from thirty seven articles (2003-2012) was collected and divided into 4 prebiotics, FOS, GOS, MOS and Inulin. Some of articles which conducting research using chito-oligosaccharides, *Aspergillus* prebiotics, isomalto-oligosaccharides, stachyose did not used as meta-analysis data because insufficient articles that report the effect of those prebiotics on broiler chicken.

No significant differences was seen of meta-analysis of the use of prebiotics on feed intake of broiler chickens compairing with control diet. Table 1 showed that average feed intake was 2634.46 g/birds in the basal diets versus 2686.48 g/birds in the basal diets and prebiotic addition. The use of prebiotic give 1.974% improvement compared with basal diet without any supplementation of prebiotics but did not give any significant effect (P>0.05) on feed intake of broiler chickens. It was reported

Table 1. Meta-analysis of the effect of prebiotics on feed intake of broiler chicken

Statistical Analysis	Feed Intake		Body Weight		BWG		FCR	
	Without	With	Without	With	Without	With	Without	With
Overall average	2634.46	2686.47	2035.78	2080.93	891.94	917.15	1.69	1.67
Difference (%)		1.974		2.22		2.83		1.153
t-value (Paired test)		P>0.05		P<0.05		P<0.05		P>0.05

that dietary prebiotic from FOS or MOS did not improve feed intake of broiler chickens (Kim *et al*., 2011; Swiatkiewicz *et al*., 2011). The use of prebiotics may reduce feed intake of broiler chickens (Biggs et al., 2007) while others have reported no significant effects (Yang *et al*., 2008[a]; Abdel-Raheem *et al*., 2011).

The use of prebiotics give significantly effect (P<0.05) on body weight by 2.22% improvement, compared to basal diets without any prebiotics. Table 1 showed that body weight of broiler chicken fed prebiotics was 2080.93 g/birds, higher than broiler chicken fed basal diets (2035.78 g/birds). It was reported that the use of prebiotics may improve body weight of broiler chickens (Ortiz *et al*., 2009; Houshmand *et al*., 2011). In contrast, however, another research reported that prebiotics added in broiler feed did not improve body weight (Rehman *et al*., 2008).

In the present meta-analysis, body weight gain was greater (P<0.05) for the broiler chicken diets with prebiotic addition. From Table 1, it showed that body weight gain of broiler chickens fed prebiotics was 917.15 g and 2.83% higher than broiler chickens fed basal diet without any prebiotics addition (891.94 g). Kim *et al*. (2011) observed that body weight gain were improved in broilers fed diets supplemented with 0.25% FOS and 0.025% MOS compairing with basal diet. This result also similar with Xu *et al*. (2003); Abdel-Raheem *et al*. (2011) and Yang *et al*. (2008[b]).

Nonsignificant (P>0.05) changes of feed conversion ratio of broiler chickens using prebiotic diets were 1.153% or 0.02 point compared to basal diets without any prebiotics. Table 1 showed that feed conversion ratio of broiler chickens fed prebiotics was 1.67 compared with basal diets (1.69). Kim *et al*. (2011) observed that dietary FOS or MOS did not improve feed conversion of broiler chickens. Another result reported that the use of MOS have no effects on FCR (Yang *et al*., 2007; Houshmand *et al*., 2012).

From the meta-analysis data indicated that there were inconsistency results of the use of prebiotics on broiler performance. In one hand, it was reported that the use of prebiotics could improve broiler performance, but in the other hand, addition of prebiotics on broiler diets did not have any significant effect on broiler

performance. This variability on the effectiveness of prebiotics may be due to the effect of different factors: type and inclusion level of prebiotics, type of diet, animal characters and degree of hygiene in husbandry conditions (Verdonk *et al*., 2005; Biggs *et al*., 2007).

The lack of beneficial effects of the prebiotic also related to stress factors because of temperature, humidity and stocking density (Houshmand *et al*., 2012). Environmental challenge and disease also the main factor of the effect of prebiotic. Yang *et al*. (2008[a]) reported that the use of MOS could improved the broiler performance of the *E. coli* challenged birds more than FOS did by the end of the experiment, whereas, within the unchallenged birds, FOS largely increased the BWG of birds compared to MOS.

Conclusion

The meta-analysis performed in this study allowed us to address and systematically quantify the effects of prebiotics on broiler performances. Finally, it can be concluded that the use of prebiotics could improve body weight and body weight gain but not feed intake and feed conversion ratio. Also, it can be continued to analysis the use of MOS as feed additive on broiler performance due to sufficiency and consistency of the data.

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A Model of Sustainable Ruminant Feed Industry in Jepara, Central Java

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Abstract

Feed availability and quality are among the main problem in animal production in Indonesia, whereas agricultural and agroindustrial by products are very potential feedstuffs. On the other hand, there are lack of accurate data and information of local feedstuffs quantity and availability. The aims of this research are to identify local feedstuff availability, to evaluate its carrying capacity, and to develop model of feed industry in Jepara . It is expected that the present research may provide a basic data and information for feed industry development in Indonesia. Survey method was used in this study, primary and secondary data were descriptively analyzed. Location of this research was determined with purposive sampling method and number of respondents with randomized sampling. The results showed that the production of available local feedstuffs were: soybean straw was 98% of total production, cassava leaf was 98%, rice bran was 100%, and fermented soybean (tempe) by product was 100%. The optimal carrying capacity of Jepara based on the real production was 27480.79 AU. Model of sustainable ruminant feed industry was designed based on integrated farming that consist of feedmill, agroindustrial industry, feed raw material industry, organic fertilizer and bioenergy industry. Recomended feedmill capacity in Jepara is 577.20 tons/month to produce concentrate for 9620 beef cattle. Based on this study, Pakis aji, Mlonggo, and Bangsri are recommended for feedmill establishment.

Keywords: Jepara, model of feed industry, ruminant

Introduction

Feed availibility and quality are among of the main problem in animal production in Indonesia. Whereas agricultural and agroindustrial by products are very potential feedstuffs. On the other hand, there are lack of accurate data and information of local feedstuffs quantity and availability. The competitive market very supported feedmill development (Sukria dan Krisnan, 2009). Based on characteristic of animal farming, ruminant depends on biological prosess and natural resources so goverment have to concern with local potential feedstuff. The aims of this research was to identify local feedstuff availability, to evaluate its carrying capacity, and to develop model of feed industry in Jepara. It is expected that the present research may provide a basic data and information for feed industry development in Indonesia.

Materials and Methods

Survey method was used in this study, primary and secondary data were descriptively analyzed. Location of this research was determined with purposive sampling method and number of respondents were decided with randomized sampling. Potential, effective, and real production of agricultural by product was calculated based on Dry Matter (DM), Crude Protein (CP), Total Digestible Nutrient (TDN). The potential production (ton DM) was calculated with formula: wide of crop area (ha) x DM productivity (ton/ ha) and production of agroindustrial by product was calculated with formula: total of by product (ton) x DM (%) (Tabrany, 2006). The effective production was calculated with formula: the potential production x proper use factor. The proper use factor of rice straw was 70%, corn straw was 75%, peanut straw was 60%, soybean straw was 60%, sweet potato leaf was 80%, cassava leaf was 30%, and sugar cane sprout leaf was 80% (Reksohadiprojo, 1984). The real production was calculated with formula: the effective production x feedstuff used factor of Jepara. Carrying capacity was calculated by dividing production (ton/year) based on DM, CP, and TDN with nutrient consumption for 1 animal unit (AU) (ton/ year) (Syamsu, 2006) with assumption that 1 AU consumes 9,59 kg/day DM, 1,151 kg/day CP, and 5,71 kg/day TDN (NRC, 1988). Analysis of model of sustainable ruminant feed industry consist of population and nutrient consumption analysis, feedstuff availibility evaluation, and feedstuff carrying capacity analysis.

Results and Discussion

Production of agricultural by product in Jepara consisted of available and unavailable product. The available production became feed use factor of agricultural by product, feed use factor of rice straw was 70%, corn straw was 91%, peanut straw was 95%, soybean straw was 98%, sweet potato leaf was 90%, cassava leaf

was 98%, and sugar cane sprout leaf was 95%. Because of the production based on CP (%DM) was the lowest production, so it became a limiting factor in production of agricultural by product in Jepara. Production of agricultural by product in Jepara was showed in Table 1.

The result showed that production of agricultural by products in Jepara were available among January with March and May with October. Lack of feedstuff happened at April, November, and December. The real production was available and exploited agricultural by product. Real production in Jepara was 37,23% of effective production, while 62,77% of production was exploited for another industries. Production of agroindustrial by product in Jepara (ton CP/year) was 3.440,08 consist of rice bran was 3.401,54, soybean cake (tahu) by product was 14,48 and fermented soybean (tempe) by product was 24,05.

Carrying capacity Jepara was capability of Jepara to provide ruminant feedstuff in form of agricultural and agroindustrial by product that accomodate for a number of ruminant in Jepara. The result of carrying capacity showed that based on potential production, Jepara could accommodate 40.156,46 AU, based on effective production 36.207,88 AU, and based on real production 27.480,79 AU. Carrying capacity based on real production was optimum population that could be accomoded by Jepara based on available and exploited feedstuff. Based on real production, Jepara excessed about 8.781,21 AU.

Nalumsari, Batealit, and Mayong Sub-district were potential agricultural by product center in Jepara. Developing Feedstuff industry could support this potency, it managed agricultural by product as crude fiber (CF) source, while, alternative feedmill location were Pakis Aji, Mlonggo, and Bangsri Sub-district.

Tabel 1. Production Potential of Agricultural by Product Based on CP (%DM) Jepara (tons/year)

Feedstuffs	Production CP (%DM)		
	Potential Production	Effective Production	Real Production
Rice Straw	7,540.44	5,278.31	3,694.81
Corn Straw	1,683.70	1,262.78	1,149.13
Soybean Straw	6.23	3.74	3.66
Peanut Straw	3,377.82	2,026.69	1,925.36
Sweet Potato Leaf	68.43	54.75	49.27
Cassava Leaf	2,374.11	712.23	697.99
Sugar Cane Sprout	731.16	584.93	584.79
Total	15,781.89	9,923.42	3,694.81

In general, Jepara has potential for developing beef cattle and buffalo (Tabrany, 2006) so model of feedmill industry was recommended to produce concentrates feed for beef cattle. Jepara had 9.620 beef cattles, they were dominated Ongole hybrid with weight 200 kg, a beef cattle consumed 3% of weight DM and 1% of weight concentrate (Tillman *et al*, 1991) so concentrate consumption was needed by beef cattles in Jepara was 19,24 ton/day or 577,2 tons/month.

The sustainable feed industry was developing of sustainable feed industry; it was an effort to manage resources conservation trough technology development and based on institution in order to provide available feed in Jepara. Developing of sustainable feed industry based on efficiency and zero waste principle. Model of sustainable feed industry based on integrated farming in Jepara was showed in Figure 1.

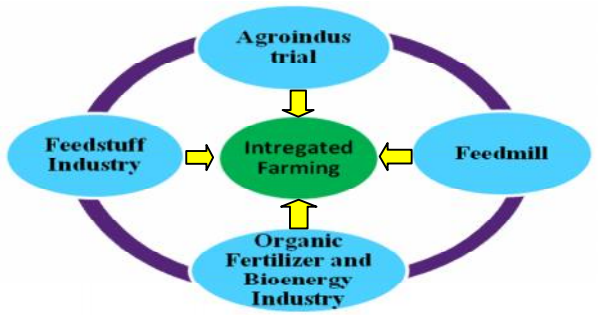

Figure 1. Model of Sutainable Ruminant Feed Industry Based on Intregated Farming in Jepara

Figure 1 showed that intregated farming consisted of:

1. Feedstuff industry that managed and processed agricultural by product. Jepara had real production of agricultural by product 8.105,01 tons/year and carrying capacity 19.292,36 AU so it provided feed for 5.772 AU beef cattles.
2. Agricultural industry that managed and processed agricultural product from local farmer, whereas its main product and by product became feedstuff for feedmill.
3. Feedmill that produced concentrates 577,2 tons/month for 9.620 beef cattles.. Alternative feedmill location were Kecamatan Pakis Aji, Mlonggo, and Bangsri Sub District.
4. Organic fertilizer and bioenergy industry that managed feces became organic fertilizer and bioenergy. 9.620 beef cattles produced 192.400 kg/day solid feces and 86.580 wet feces with assumption that a beef catle produced 20 kg/day solid feces and 9 kg/day wet feces. If 3 beef cattles provided energy for a family, so Jepara could provide energy for 3.206 families.

Conclusions

The most available agricultural by product in Jepara were soybean straw was 98%, cassava leaf was 98%, rice bran was 100%, and fermented soybean (tempe) by produc was 100%. Carrying capacity of Jepara based on potential production was 40.156,46 AU, effective production was 36.207,88 AU, and real production was 27.480,79 AU. Model of sustainable feed industry based on integrated farming consisted of feedstuff industry, agricultural industry, feedmill, organic fertilizer and bioenergy industry. Feedmill was recommended to produce concentrates for 9.620 beef cattles that need 577, 20 ton/month. Recommended alternative feedmill location were Pakis Aji, Mlonggo, and Bangsri Sub-district.

Acknowledgement

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The Effect of Effective Microorganisms-4 (Em 4) Addition on the Physical Quality of Sugar Cane Shoots Silage

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Abstract

This research aimed to find out the physical quality of silage sugarcane shoots with the addition of Effective microorganism (EM-4). This research was conducted in the Laboratory of animal science and in the Laboratory of Chemistry and Microbiology the Faculty of Agriculture Sriwijaya University. Shoots of sugar cane were cut into pieces as long as pieces 2-3 cm and dried up to obtain water levels 60-70%,then the addition of EM4 was done according to dose treatment and stored for 21 days. Physical observation, temperature and the amount of lactic acid bacteria were analyzed descriptively while the measurement of the degree of acidity and percentage fungi of used Completely Randomized Design with 5 treatments and 3 replications. The treatments were: T0= shoots of sugar cane without treatment (control), T1= shoots of sugar cane+4%EM4 (v/w), T2= shoots of sugar cane+6%EM4 (v/w), T3= shoots of sugar cane+ 8%EM4 (v/w), T4= shoots of sugar cane+10%EM4 (v /w). The results showed that the temperature of silage sugar cane ranges from 27.5-29oC.The ever increasing doses of EM4 showed a yellowish green color. The flavor was sour and fresh fragrant, the increase in the amount of lactic acid bacteria, significantly affect (P<0.05) pH (4.3-5.2) and the percentage of fungi (0.52-2.29%). The conclusion of this research is with increasing the dose of the effective microorganism 4 treatment up to 10 % can retain physical qualities of shoots of sugar cane shoots silage.

Key words: effective microorganism-4, physical quality, silage, sugar cane shoot

Introduction

Cane is chief source of sugar confection replace rain large tropical countries and subtropical . Plantation in pt nusantara (PTPN) vii in love sweet south sumatra cane crop production in 2010 reached 69.23 ton/hectares from the acreage of the sugar cane plantations 12.715 and produce 14% shoots of sugar cane from produc-

tion (Koeshartowo, 2011). Shoots of sugar cane is waste not much used by producers sugar potentially as providing fodder potential. Besides, plant cane ordinary harvested season so that can be used as an alternative a grass substitute who in the season persediaannya greatly reduced. According to Muchtar *et al*. (1983) declaring that shoots of sugar cane as a grass substitute elephant not give negative influence against cattle cut and dairy.

Shoots of sugar cane can be given in the form of fresh or dried. But weakness of shoots of sugar cane was to have the nature of quick-drying, wilt and yellow in time relatively quickly, therefore necessary preservation as silage. Prayitno (2010) reported that ingredients nutrients shoots of sugar cane processed better than given fresh. Sumasih et al. (2009) declaring that silage is the storage and fermenting forage fresh in anaerobic condition with the help of lactic acid bacteria. Composition nutritional value in silage will experience change namely carbohydrates should abate, but protein rough on silage good will not undergoing many changes.

Effective microorganism 4 (EM-4) is a mixture of various microorganisms which can be utilized as a source of inoculum in improving feed quality. Utilization of the EM-4 as a source of mikrobia in sugar cane shoots in silage laboratoris can improve nutrition and expected to play a role in overcoming the constraints of lack of feed dry all season.

Materials and Methods

This research was carried out at the farm as well as Enclosure Experiment Laboratory of Nutrition and Chemical Laboratory and Microbiology Agricultural Faculty of Agriculture University of Sriwijaya for 4 months.

The materials used in this research is derived from the shoots of sugar cane sugar cane plantations love sweetly district of suspects ogan, ilir effective microorganism-4 (em-4) derived from palembang cinde market and chemicals and equipment for analysis .

The making of silage shoots of sugar cane

The shoots sugar cane first cleaned then will be cut with 2-3 cm and dikering anginkan to the water level 60-70%. Each sample was em-4 in accordance with the dose of the treatment. Later included in into the plastic bags to dense and bound to happen anaerobic condition and deposited for 21 days. After the fermentation of the 21 days finished, the sample measure temperature it is, and then opened and was taken immediately samples analyzed.

Research design and data analysis

The design that was used in this study is a randomized design complete (RAL) consisting of 5 and 3 times a recycling treatment:

T0 = Shoots sugar cane without treatment (control)
T1 = Shoots sugar cane + 4% EM-4 (v/w)
T2 = Shoots sugar cane + 6% EM-4 (v/w)
T3 = Shoots sugar cane + 8% EM-4 (v/w)
T4 = Shoots sugar cane + 10% EM-4 (v/w)

Data is processed and analyzed with the fingerprint of the deskriftif variety in accordance with the draft is used, If the treatment effect is real then conducted tests in Multiple Areas of Advanced Multiple Range Test Duncan (DMRT) (Steel and Torrie, 1991).

Variable

The variables observed ware the physical characteristics including temperature, color, aroma and texture of a percentage of the fungi (Yusmadi, 2008), pH (AOAC, 1990), and the amount of lactic acid bacteria (Fardiaz, 1987).

Results and Discussion

Characteristics of Silage

The temperature of the sugar cane shoots during the process of silage ensilase ranges from 27.5-29 ^{o}C, temperature is a characteristic of a good silage. This is in accordance with statement Okine *et al*. (2005), which reported good quality silage is produced at temperatures between 25 to 37 ^{o}C. The difference in temperature is due to the existence of the activity of micro-organisms that produce carbon and heat production due to the process of respiration in the initial phase (phase aerobic) ensilase so that the formation of carbon dioxide (CO_2), water (H_2O) and heat (Coblentz, 2003).

Research results show that sugarcane shoots aromatic treatment T0, T1, T2, and T3 is an aromatic acid, whereas treatment T4 was flavorful fragrant fermentation. The difference in this fragrance guess because the higher the level the use of EM-4 to 10% dose (T4), leading to an increased number of lactic acid bacteria (Table 1), in which the bacteria that produce fragrant aroma of fermenting. Smells sour on treatment of T0, T1, T2, and T3 is allegedly the end product of fermentation lactic acid not only alone, but also produces butyric and acetic acid, alcohol. Abdelhadi *et al*. (2005), stating the characteristics of good silage is aromatic acids and fragrant fermentation (Abdelhadi *et al*., 2005).

Color observation silage sugarcane shoots on each treatment having the same color from fresh green before ensilase to green yellowish. The addition of the EM-4 to 10% of the dose of the accelerating phase of anaerobic, as more and more adding EM-4 to dose 10% then the more the amount of lactic acid bacteria too which helps speed up anaerobic phase. Coblentz (2003) States that the increase in temperature can affect the structure of silage for example color change silage becomes dark.

Tabel 1. The physical characteristics, pH and total of lactic acid bacteria silage shoots of sugar cane

Treatment	Tempera-ture	Colour	Aromatic	Texture	pH	% fungi	∑ LAB
T0	29	Green yellowish	Acid	rather delicate	5.2±0.1^{c}	2.29±0.25^{b}	3.4 x 10^{8}
T1	28	Green yellowish	Acid	rather delicate	4.6±0.1^{b}	2.24±0.20^{b}	4.1 x 10^{8}
T2	28	Green yellowish	Acid	rather delicate	4.5±0.0^{b}	2.16±0.44^{b}	4.6 x 10^{8}
T3	28	Green yellowish	Acid	rather delicate	4.5±0.0^{b}	1.49±0.87^{b}	4.9 x 10^{8}
T4	27.5	Green yellowish	Fragrant	refined	4.3±0.0^{a}	0.52±0.41^{a}	5.2 x 10^{8}

Note: different supercript shows significant (P<0.05).

The research results of texture for silage sugarcane shoots silky-textured show that until rather delicate. The addition of the EM-4 at a dose of 10% (T4) can fine-tune the texture of sugar cane, silage at the helm had expected the EM-4 can be rough fibers and degrades the Flex bond lignoselullosa, so that the texture of silage sugarcane shoots becomes refined. This is in accordance with statement Darmawan (2010), which reported the use of EM-4 in fermentation can lower the levels of crude fiber. Good silage will see a refined texture (Ratnakomala *et al*., 2006; Ridla *et al*., 2007).

Statistical results show the addition of the EM-4 at the helm of different silage sugarcane real (P>0.05) against the percentage of fungi. T4 treatment has the lowest percentage of fungi (0.52%) while the highest on treatment T0 (2.29%). The addition of the EM-4 to 10% of the dose of the accelerating phase of anaerobic, because the higher the addition of EM-4 to 10% of the dose the higher the amount of lactic acid bacteria too which helps speed up anaerobic phase so that the fungus cannot grow with fertile, while in treatment T0 (control) is allegedly a longer phase of aerobnya because it is not added to the EM-4 so slow lactic acid bacteria evolved as a result of fungi can utilize aerobic phase to grow and thrive. Fungal growth on this research only at the top of the course, because dense less than perfect, so the process of respiration continued as a result formed CO_2, H_2O and heat. Water that is formed, causing difficult going anaerobic conditions, so that fungi grow and develop. This fungus will produce mikotoksin/toxins that can interfere with the health of cattle (Coblentz, 2003).

pH Silage Shoots of Sugar Cane

The results showed the addition of diverse EM-4 silase in the sugar cane

different real (P>0.05) in degrees acid (pH). The result showed that the continued degrees acid (pH) silase this research is varied in sugar cane criteria from good to bad. The quality of a good silase found in treatment T4 (4.3) and the quality of poor for treatment T0 (5.2). Macaulay (2004) said that can silase the quality of being inducted into four categories, which is well (pH 3.2-4.2), good (pH 4.2-4.5), bad (pH 4.5-4.8), and very bad (> 4.8). The addition of the EM-4 pH affect the quality of silage, because the higher the awarding of EM-4 to 10% dose, then the greater the decrease in pH. A decrease in the pH of the silage is influenced by lactic acid bacteria during the process of ensilase. With the large number of lactic acid bacteria contained in the silage sugarcane leaf would be more effective to facilitate the process of ensilase and will continue to progress until the pH is low enough to inhibit the growth of mikoorganisme predominantly adverse (Lopez, 2000).

Total Lactic Acid Bacteria

Lactic acid bacteria are a group of bacteria that are capable of converting carbohydrates (glucose) into lactic acid. Bakterisidal effects of lactic acid are associated with a decrease in the pH of the environment being 3 to 4.5 so that the growth of other bacteria including pembusuk bacteria will be hampered. The largest amount of lactic acid bacteria in treatment of T4 (5.2 x 108 CFU/gr), while the lowest treatment T0 (3.4 x 106 CFU/gr). The difference in the amount of lactic acid bacteria is affected by pH of silage. the pH will determine which microorganisms are active in making silage. Lactic acid bacteria shows optimal activity at pH= 4.3 (Woolford, 1984). Treatment with additional t4 EM-4 to 10% placeman phænogamous during the lowest ensilase. With the number of lactic acid bacteria contained in silage shoots of sugar cane of inhibiting the growth will mainly mikoorganisme is harmful. Chen and Weinberg (2008), declare silage good dominated by working lactic acid bacteria and produces lactic acid on the contrary, the process of fermentation silage a less well cause clostridia develops characterized by high levels of butyric acid (elferink *et al*., 2000).

Conclusions

Based on the research inconclusive that increases in dosages effective microorganism- 4 treatment of 10% dosages can improve the physical quality of of silage shoots of sugar cane.

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Chemical and Physical Quality of Sago (*Metroxylon sago* Rottb.) Waste Based Wafer Complete Ration for Aceh Beef Cattle

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Abstract

Sago (Metroxylon sago Rottb.) waste up till now had not been exploited in optimal and only partly small are applied as component of feed, especially for ruminant. The potential of sago as animal feed is quite large and will increase in value when processed into wafer complete ration. This experiment was conducted to study chemical and physical quality of sago waste based wafer complete ration which were preserved in various different periods. Determination of chemical and physical variables of wafer complete ration were color, texture, smell, water content, specific density, water activity, and storage capacity. Data were analyzed by analysis to completely randomized factorial design with two factors (A: the levels of sago waste used in wafer complete ration 10.20, 30, and 40%, B: storage time of 2, 4, 6, and 8 weeks). The results indicated that the wafer complete ration with various different formulations did not affect specific density and water activity. A significant different (P<0.05) was observed in water content with the highest water value in ration containing 40% sago waste. The period of preservation effect was significant (P<0.05) on water content, density and specific density, but did not influence water activity texture and smell. The preserved wafer complete ration for six weeks were still in good condition, but after eight weeks the wafer started to change in physical texture. It is concluded that sago waste based wafer complete ration has high quality in terms of physical and has storage capacity to keep in good condition for six weeks.

Keywords: complete ration, sago waste, wafer

Introduction

Sago (*Metroxylon sago* Rottb.) waste, up till now, had not been exploited in optimal and only partly small are applied as component of feed, especially for ruminant. The potential of sago as animal feed is quite large and will increase in value when processed into wafer complete ration. Wafer complete ration is a feed

physically formed to a compact and concise which is expected to ease in handling and transportation, has complete nutritional content, and use relatively simple technology so easy to apply (Trisyulianti *et al*., 2003). Wafer complete ration is feed processing technology, especially in the dry season. Basic research and applications, in particular regarding the potential of improving the quality of sago waste as feed, it until now still not widely applied. Therefore, studies on the utilization of sago waste are continuously still very necessary. Most research has so far directed sago waste in the utilization of sago waste as raw material for biofuels and fungi or bacteria growth substrate for the production of extracellular enzymes (Akmar & Kennedy, 2001). However, the use of information sago waste as raw material for the manufacture of a complete wafer as a livestock feed ration is still very limited.

Observing this, a study needs to be done on the potential of sago waste as raw material for the manufacture of a complete wafer; this is an effort to ration the supply and implementation of strategies that feed technological innovation-oriented economy that is capable of providing complementary feed at any time. This study aims to determine the quality and physical properties of wafer-based complete rations of sago waste residue made up of different formulation and different storage time.

Materials and Methods

Experimental equipment

Equipment used in this study were felt wafer hidrolic machine (temperature of 150 ^{o}C, pressure 200-300 kg/cm^2 for 15-20 minutes), machine mixer, hammer mill, mixing container, AW meter, and termohigrometer.

Complete rations formulation

Complete ration used in this study were concentrates containing raw materials, such as coconut cake, rice bran, sago, molasses, vitamins, minerals and sago waste. Ration treatments consisted of : P1= ration containing 10% sago waste, P2= ration containing 40% sago waste, P3= ration containing 30% sago waste, P4= ration containing 40% sago waste. Complete ration wafer formulations were prepared using the method of trial and error. Ration of a complete wafer composition was shown in Table 1.

Preparing wafer complete rations

Wafer complete ration was prepared as follows : (a) all concentrated sources of raw material was dried by the sun, (b) all raw materials for concentrate were milled using a hammer mill to mash size, (c) treated feed materials (sago waste) was mixed with molasses as an adhesive material (5%) until blended, after being mixed with the concentrate to be a complete ration, mixing was done manually, (d) complete

ration was incorporated into rectangular molds measuring 25 cm x 25 cm x 5 cm. After hot compression was performed at a temperature of 150 ^{o}C with a pressure of 200-300 kg/cm^{2} for 15-20 minutes, cooling was done by placing sheets of wafer in the open air for 24 hours until the moisture content and the weight was constant, then the results were put in sacks.

Table 1. Composition of wafer complete ration

Feed Material	Treatment			
	P1	P2	P3	P4
Sago waste (%)	10	20	30	40
Rice bran (%)	33.5	30	25	20
Coconut cake (%)	25	23.5	23.5	23.5
Sago (%)	25	20	15	10
Molasses (%)	5	5	5	5
Vitamin (%)	0.5	0.5	0.5	0.5
Minerals (%)	1	1	1	1
	100	100	100	100

P1= ration containing 10% sago waste, P2= ration containing 40% sago waste, P3= ration containing 30% sago waste, P4= ration containing 40% sago waste.

Wafer tester

Wafer testing are: (a) wafer that has been made to cut the size of 5 cm x 5 cm x 2 cm samples were then taken for the proximate analysis (dry material, ash, crude protein, crude fiber, crude fat, Beta-N, and TDN), testing the physical properties (water content, density and water activity). The wafer was then stored for 2, 4, 6, and 8 weeks. Treated wafer that would be stored, was placed in a sackt to determine the difference.

Statistical analysis

The data obtained from the results of the study were analyzed using completely randomized factorial design with two factors (A: ration, B: storage time) with 3 replications; if significantly different occurred, the data will be tested further with Orthogonal Contrast Test (Steel and Torrie, 1995).

Observed variables

Observed variables in determining the quality and physical properties of the wafer was done by analyzing the nutrients of complete ration wafer (proximate analysis), wafer content (AOAC, 1984), density wafer (Trisyulianti *et al*., 2003),

water activity (Syarief and Halid, 1993) , texture and storage capacity (2, 4, 6, and 8 weeks).

Results and Discussion

Chemical composition of wafer complete rations

Chemical composition of wafer complete rations and sago waste (dry matter, ash, crude protein, crude fiber, crude fat, Beta-N, and TDN) of four treatments was shown in Table 2 and Table 3.

Table 2. Chemical composition of wafer complete rations

	Treatment			
	P1	P2	P3	P4
Dry matter (%)	87.03	86.02	86.08	85.09
Ash (% DM)	5.44	5.53	5.67	6.03
Crude protein (% DM)	14.92	15.13	14.33	13.53
Crude fiber (% DM)	12.73	11.93	11.23	11.53
Crude fat (% DM)	5.74	6.51	7.06	7.61
Beta-N (% DM)	58.36	59.56	60.26	58.06
TDN (% DM)	71.17	71.93	70.48	69.03

Results of analysis: Nutritional Laboratory, Department of Animal Husbandry, Unsyiah (2011). P1= ration containing 10% sago waste, P2= ration containing 40% sago waste, P3= ration containing 30% sago waste, P4= ration containing 40% sago waste.

Table 3. Chemical composition of sago waste

Dry matter (%)	Ash (% DM)	Crude fat (% DM)	Protein (% DM)	Crude fiber (% DM)	Carbohydrate (% DM)
85.29	4.30	0.16	4.99	33.33	50.61

Results of analysis: Nutritional Laboratory, Department of Animal Husbandry, Unsyiah (2011)

Water levels

Wafer complete rations with a kind of different composition of sago waste significant affected (P<0,05) moisture content. The water content of the composition of the residue on the wafer with real sago 40% was higher when compared to other wafer sago waste. Wafer complete rations with a composition of dreg sago 10% had fewer cavities causing evaporation that occured over the resistor, while the wafer with a composition of 40% had the sago waste cavities that were more numerous

and large causing evaporation was running fast. Moisture contents of each treated wafer was indicated in Table 4.

Table 4. Water content value of wafer complete rations

Treatment	Storage time			
	2 weeks	4 weeks	6 weeks	8 weeks
P1	13.44±0.12[a]	14.00±0.34	14.07±0.87	14.30±0.77
P2	14.34±0.20	13.59±0.55[b]	14.90±0.61	15.80±0.61
P3	14.44±0.15	14.35±0.45[b]	13.89±0.15	14.56±0.67
P4	13.00±0.56[a]	14.45±0.67	13.56±0.54	14.12±0.65

The value of flats with different superscripts in the same column showed a significantly different (P <0.05). P1= ration containing 10% sago waste, P2= ration containing 40% sago waste, P3= ration containing 30% sago waste, P4= ration containing 40% sago waste.

Storage time of wafer complete rations affected significantly (P<0.05) water content. Average value of the highest water levels in storage was for 2 weeks, because the wafers absorb water from the environment. Average value for six weeks was not stable, this was caused by humidity and temperature values that changed frequently. The interaction between factor A (wafer complete ration) and factor B (storage time) were not significantly different to water content although P2 treatment had the highest water content which was equal to 15.80±0.61. Storage conditions were likely to increase the water content. This occurred due to the influence of humidity, and ambient temperature during the storage periods. The activity of microorganisms can be on tap on the water content of 8-12%, so that the feed material was not easy to mold and rot (Verma *et al*.1996).

Wafer density

Density of the wafer determined the dimensional stability and physical appearance of a complete wafer feed (Jayusmar *et al*., 2002). Wafer density was a measure of the cohesiveness of the sheet and the particle size depended on the density of materials used and the amount of pressure exerted during the four sheets of wafer manufacturing process. Wafers that have a high density of the feed would provide a solid and hard texture; so it would be easily in both the storage and handling of shocks during transportation and was expected to last longer in storage (Trisyulianti *et al.,* 2003). Ration of a complete wafer density value was indicated in Table 5.

Wafer complete rations with different composition of sago waste had no effect on density. Week-long storage of two bonds between the particles of the material was still strong. Wafer density decreased at week 4 to week 8. The interaction between factor A (wafer complete rations) and factor B (storage time) did not

significantly affect the density of the wafer feed. The lowest density value obtained was in treatment P3 which was 0.45±0.04 g/cm^3, while the highest 0.78±0.04 g/cm^3. Density values were not stable due to high relative humidity caused the liquid condensed on the surface of the material; so that the surface material became wet, and was very conducive to microbial growth and damage.

Table 5. Wafer complete rations density with various storage times (g/cm^3)

Treatment	Storage times			
	2 weeks	4 weeks	6 weeks	8 weeks
P1	0.50±0.01 ^{b}	0.56±0.05 ^{b}	0.48±0.02 ^{a}	0.52±0.00 ^{b}
P2	0.78±0.04 ^{c}	0.65±0.06 ^{c}	0.58±0.05 ^{b}	0.50±0.08 ^{a}
P3	0.66±0.05 ^{c}	0.45±0.04 ^{a}	0.49±0.06 ^{b}	0.49±0.09 ^{b}
P4	0.78±0.33 ^{c}	0.52±0.06 ^{a}	0.51±0.01 ^{a}	0.61±0.00 ^{b}

The value of flats witth different supperscripts in the same column showed a significantly different at (P<0.05). P1= ration containing 10% sago waste, P2= ration containing 40% sago waste, P3= ration containing 30% sago waste, P4= ration containing 40% sago waste.

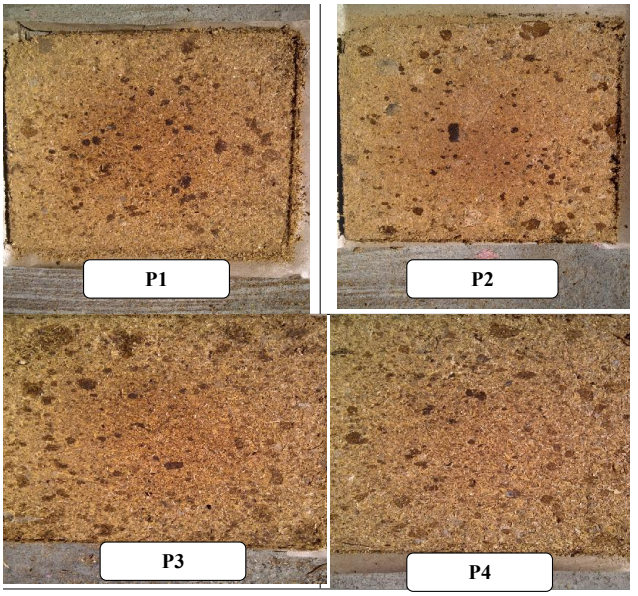

Figure 1. Wafer complete rations density with various storage times. P1= ration containing 10% sago waste, P2= ration containing 40% sago waste, P3= ration containing 30% sago waste, P4= ration containing 40% sago waste.

Water activities

Wafers with a variety of different composition of sago waste did not significantly affect the activity of water (Table 6). Storage time did not significantly affect the activity of water. Water activity at the beginning of week 2 to 8 was still high. Water activity was the amount of free water used for growing microorganisms (Syarief and Halid, 1993). Storage up to 4 weeks did not change the color to black. The interaction between factor A and factor B showed no significantly different results. The lowest water activity obtained at P1 was equal to 0.60±0.05. This was because the humidity was low, the liquid surface of the material would evaporate a lot; so that microbial growth was hampered by dehydration and a dark surface material.

Tabel 6. Wafer complete rations of water activity with various storage times

Treatment	Storage time			
	2 weeks	4 weeks	6 weeks	8 weeks
P1	0.60±0.05	0.75±0.08	0.79±0.00	0.72±0.00
P2	0.72±0.02	0.73±0.03	0.79±0.00	0.75±0.00
P3	0.69±0.09	0.70±0.06	0.80±0.08	0.77±0.07
P4	0.70±0.06	0.74±0.07	0.82±0.07	0.80±0.01

The value of flats wits different supperscripts in the same column showed a significantly different at ($P<0.05$). P1= ration containing 10% sago waste, P2= ration containing 40% sago waste, P3= ration containing 30% sago waste, P4= ration containing 40% sago waste.

Conclusion

Wafer complete rations with different composition of sago waste does not affect the specific gravity, density of water activity, but affected water levels with the highest value found in the wafer with a composition of 40% sago waste. Storage for 8 weeks old greatly increases the water content, lower specific gravity and density, but does not affect the activity of water. The wafers stored up to 6 weeks is still in a good condition, but at 8 weeks of storage, the surface of the wafer start to rancid black.

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Quality of Vegetable Waste Silages Treated with Various Carbohydrate Sources

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Abstract

The aim of this research was to evaluate the quality of vegetable waste silages, using rice bran, onggok (cassava flour waste) and pollard as carbohydrate sources. Vegetable waste was collected from local traditional market, consisted of corn husk, chinese cabbage dan cabbage. Research was held in Randomized Block Design consisted of six treatments with 3 replications. Treatments were (T1) vegetable waste+rice bran, (T2) vegetable waste+rice bran+rice straw, (T3) vegetable waste+onggok, (T4) vegetable waste+onggok+rice straw, (T5) vegetable waste+pollard, (T6) vegetable waste+pollard+rice straw. Lactobacillus plantarum 1A-2 was used as innoculant. The quality of silages was evaluated by measuring pH, temperature, population of lactic acid bacteria and lactic acid production. Nutrient characteristic was determined by proximate and fiber analysis. Results showed that pH of silages were not affected by treatments, but silage treated with rice bran, with or without rice straw addition, had higher temperature compared with others (29oC or 28,3oC). The highest population of lactic acid bacteria (1.9 x 107cfu/ml) was found in silage using rice straw and onggok (T4), but highest lactic acid production (0,91) was measured in silage using rice straw and rice bran (T2). In general, the use of rice bran as carbohydrate sources gave highest lactic acid production followed by pollard and onggok. Different carbohydrate source gave different nutrients characteristic. Silage with highest protein content was measured in silage with pollard as carbohydrate source, followed with rice bran and onggok.

Keywords: Lactobacillus plantarum 1A-2, onggok, pollard rice bran, vegetable waste silages

Introduction

Forage is major feed for ruminant, but increasingly difficult to obtain due to competing land uses with human interests. Therefore, it is necessary to find new resources that can substitutes the forage. Vegetables waste is a potential feed source

for ruminant, to overcome lack of grass, especially in dry season. Vegetable waste is part of the vegetables that are not consumed by humans, usually already discarded by traders, so has no economic value. Traditional farmers already give vegetable waste to their cattle, but its high water content made it easily decayed and cannot be stored for long period. This limitation increased the cost and difficulties in handling because farmers have to take vegetable waste from traditional market everyday. Treatment is necessary to improve the quality and the nutritional value of vegetable waste as feed.

Silage production is a method of moist forage preservation which is widely used all over the world (Saele, 2002). It is based on natural fermentation when lactic acid bacteria (LAB) ferment water soluble carbohydrates to organic acids, mainly lactic acid, under anaerobic conditions. As a result, the pH decreases, inhibiting detrimental anaerobes, and so the moist forage is preserved (Merry and Davies, 1999). A combination of anaerobic condition and acidity protects the forage from the proliferation of deleterious bacteria and fungi, and it also increases the palatability of the forage due to lactic acid production (Yang *et al*., 2001, Weinberg *et al*., 2003, Filya, 2003).

In terms of storage, silage is more durable because of spoilage bacteria do not resistant to low pH, so its availability and quality of feed can be assured. Silage can also be used as probiotics and organic acid sources for livestocks as an alternative to antibiotics. In order to improve the ensiling process and to obtain a high-quality fermented product, various chemical and biological additives have been developed and used during silage fermentation. The biological additives are advantageous because they are safe and easy to use, non-corrosive to machinery, do not pollute the environment and are regarded as natural products (Filya *et al*., 2000; Weinberg and Muck, 1996). Seale *et al.* (1986) found that sugar is a limiting factor in producing good-quality fermented products. Sugar mainly serves as a carbon source for microorganisms. Molasses, lactose and a mixture of cereal grains and malt, dextrose, corn or tapioca flour have been used as additives (Zahar *et al*., 2002).

A successful ensiling process requires a minimum concentration of fermentable sugars (3-5% in DM). However, the majority of carbohydrates in plants are in the form of fibrous polymers that make up the cell wall and are not fermented by lactic acid bacteria (LAB). In order to obtain the necessary level of fermentable water-soluble carbohydrates (WSC) for the lactic fermentation in crops which are low in WSC, the use of carbohydrate sources has been suggested.

Research on the ensiling of vegetable waste is still limited, and the effects of different additives may vary from one to another, resulting variety of resulting silages. Therefore, the objective of this experiment was to evaluate the quality of vegetable waste silages, using rice bran, onggok (*cassava flour waste*) and pollard as carbohydrate sources.

Materials and Methods

Vegetable waste was collected from local traditional market in Bogor, consisted of corn husk, chinese cabbage and cabbage. After chopping, vegetable waste was air dried to decrease water content. It was sprayed with *Lactobacillus plantarum* 1A-2 as an innoculum and divided into equal portions for the application of treatments. All innocula were diluted with distilled water, so that they were applied at the same rate (10 ml of solution/kg of vegetable waste).

Experiment was held in a randomized block design consisted of six treatments with 3 replications. Treatments were (T1) vegetable waste + rice bran, (T2) vegetable waste + rice bran + rice straw, (T3) vegetable waste + onggok, (T4) vegetable waste + onggok + rice straw, (T5) vegetable waste + pollard, (T6) vegetable waste + pollard + rice straw.

Each treatment was packed into ±15 L plastic drum as silos in triplicate and sealed. Silos were stored at room temperature. After 45 days ensiled, each silo were sampled for chemical and microbial analyses. The DM content was determined by oven drying for 48 h at 60 ^{o}C. After drying, samples were ground through a 1-mm screen miller, and stored in glass bottle at room temperature for chemical (proximate and fiber) analysis. The quality of silages was evaluated by measuring pH, temperature, population of lactic acid bacteria and lactic acid production. Nutrient characteristic was determined by proximate and fiber analysis at Laboratory of Feed Technology, IPB.

Another portion of original sample was diluted with autoclaved distilled water and blended in high-speed blender for 30 s. The diluted samples were enumerated for LAB on pour-plates using MRS agar (DeMan, Rogosa, and Sharpe) with TPC (Total Plate Count) method, cultivated at 30 °C for 48 hours (modification from Cappucino and Sherman, 1983). Colonies were counted from plates of appropriate dilutions containing a minimum of 30 colonies. pH was immediately measured from the remainder of diluted sample.

Results and Discussion

Quality of silage can be observed from the physical characteristics of the resulting silage. After 45 days of ensiling, vegetable waste silage in this study exhibiting a yellowish green color, fresh aroma and not slimy. There was only a few fungal contamination visually observed on the surface of the silage due to aerobic conditions. Those physical characteristic indicated a successful fermentation process. Use of rice bran, onggok (cassava byproduct), and pollard as source of carbohydrates in each treatment resulted in only a little color differences of silages. Silage using onggok had a lighter color while silage using pollard and rice bran had a darker color. Physical appearance of vegetable waste silages from each treatment can be seen in

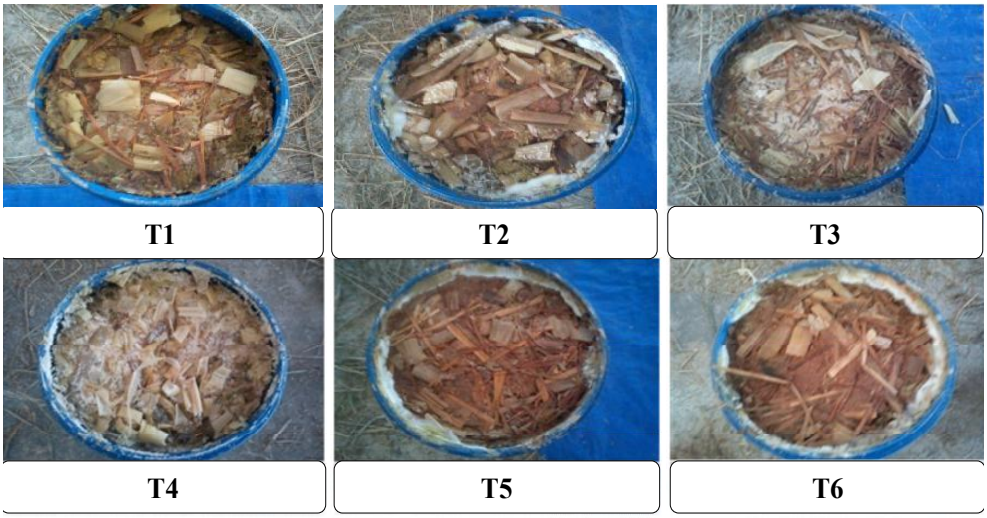

Figure 1. Physical appearance of vegetable waste silages. T1= vegetable waste + rice bran; T2= vegetable waste + rice bran + rice straw; T3= vegetable waste + onggok; T4= vegetable waste+ onggok + rice straw; T5= vegetable waste + pollard; T6= vegetable waste + pollard + rice straw.

Other variables for evaluating silage quality is the chemical (temperature, pH and lactic acid production) and microbiological (LAB population) characteristics of silage presented in Table 1. Temperature of silage at the end of fermentation varied between treatments. The highest temperature was recorded (29 ^{o}C) on silage with rice bran addition (T2), which was significantly higher compared to others.

Value of pH, lactic acid production, and population LAB were not signifantly affected by carbohydrate sources addition. Vegetable waste silages treated with ong-

Table 1. Chemical and microbiological characteristics of vegetable waste silages

	Treatments					
	T1	T2	T3	T4	T5	T6
Temperature (^{o}C)	28.33±0.01^{b}	29.00±0.00^{b}	27.00±0.00^{a}	27.33±0.57^{a}	27.33±0.57^{a}	27.00±0.00^{a}
pH	3.91±0.01	3.94±0.04	3.42±0.46	3.55±0.42	3.91±0.09	3.72±0.19
Lactic acid (%)	0.41±0.10	0.30±0.05	0.23±0.01	0.29±0.11	0.30±0.06	0.36±0.11
LAB (cfu/g)	7.10x10^{8}± 4.98x10^{8}	10.40x10^{9}± 4.10x10^{8}	6.97x10^{8}± 3.59x10^{8}	16.5x10^{9}± 8.97x10^{8}	6.71x10^{8}± 2.08x10^{8}	5.48x10^{8}± 3.06x10^{8}

Note: LAB= Lactic Acid Bacteria; T1= vegetable waste + rice bran; T2= vegetable waste + rice bran + rice straw; T3= vegetable waste + onggok; T4= vegetable waste + onggok + rice straw; T5= vegetable waste + pollard; T6= vegetable waste + pollard + rice straw.

gok gave the lowest pH value (3.42 and 3.55), while the highest pH value was obtained from silages treated with rice bran (3.91 and 3.94). This condition may relate to the acidity of onggok which was lower than rice bran and pollard. A pH range of 3.7-4.2 was generally considered to be beneficial for crop preservation (Kung and Shaver, 2001), but according to Bates *et al*. (1996), pH ranged between 3 and 4 was still considered adequate.

High quality silage is likely to be achieved when lactic acid is the predominant acid produced, as it is the most efficient fermentation acid, and reduces silage pH (McDonald *et al*., 2002), thus lactic acid production was correlated with pH value. Higher lactic acid production would result in lower pH value, but in this experiment, some silages with low lactic acid production also had low pH value. Lactic acid production in this study was ranged between 0.23–0.41%, with no significant differences among treatments. Silages treated with onggok (T3) had the lowest lactic acid production compared to other carbohydrate sources. According to Coblentz (2003), onggok have low WSC content (3%), while rice bran and pollard have higher WSC content (5% and 12%). Low WSC content means low nutrient sources served for LAB to produce lactic acid.

Lactobacillus plantarum 1A-2 added as inoculants during silage making to increase population of lactic acid bacteria, in order to stimulate lactic acid fermentation, accelerate the decrease in pH, and thus improve silage preservation of homo - fermentative lactic acid bacterial strains. These bacteria produce large amounts of lactic acid in the silage in a short time and stabilize it with minimal losses (Filya, 2003). In general, different carbohydrate sources had not influenced population of lactic acid bacteria significantly. The highest population of LAB was observed in silages with onggok addition (T4) (16.5 x 10^9 cfu/g), while pollard addition (T6) produced silages with the lowest population of LAB (5.48 x 10 cfu/g ^{8}).

Table 2. Nutrient composition of vegetable waste silages

Nutrient (%)	Treatments					
	T1	T2	T3	T4	T5	T6
DM	86.57	88.44	88.54	90.75	91.64	87.21
Ash	26.46	15.37	2.52	7.59	5.84	9.61
CP	7.51	9.40	3.56	4.59	14.52	13.45
CF	25.79	28.15	14.26	19.76	10.99	16.41
EE	1.70	0.30	0.12	1.01	0.19	1.15
NFE	25.11	35.22	68.08	57.80	60.10	46.59

Note: DM= Dry Matter; CP= Crude Protein; CF= Crude Fiber; EE= Ether Extract; NFE= Non Fiber Extract; T1= vegetable waste + rice bran; T2= vegetable waste + rice bran + rice straw; T3= vegetable waste + onggok; T4= vegetable waste + onggok + rice straw; T5= vegetable waste + pollard; T6= vegetable waste + pollard + rice straw.

Ensiling has been a preferential method in maintaining the energy content of forages, ensuring a good nutritional value when used as feed (Vervaeren *et al*., 2010). Table 2 shows nutrient characteristics of vegetable waste silages after 45 days of ensiling. Use of pollard as carbohydrate source gave the highest CP content (14.52%) than other treatments, while the use of onggok provided the lowest CP content of vegetable waste silages (3.56%). This result is correlated with CP content of carbohydrate sources added in silage production. Pollard has CP content around 15%, while rice bran and onggok have only CP content around 11% and 2% (Furqaanida, 2004). Sapienza and Bolsen (1993) stated that CP content of qood quality silage ranged between 10.50%-15.20%. Above the optimum range will result in poor silage quality and cannot be stored for a long time due to biochemical reactions between amino acids and sugars causing the Maillard reaction which produced brown silage.

The contents of CF and EE in vegetable waste treated with rice bran were the highest in all silages (28.15% and 1.70) as predicted, because rice bran had the highest CF and EE content compared with other carbohydrate sources used in this experiment. Silage with EE content more than 2% will be easily contaminated and classified as bad quality of silage (Sapienza and Bolsen, 2003). The highest EE in this experiment was 1.70% indicating a good quality of silage.

Fiber compositions of silages were not affected significantly by treatments (Table 3). The use of rice straw as silage material increased the value of NDF, compared with silages without rice straw (T2 vs T1, T4 vs T3, T7 vs T6), and also increased lignin content of silages except for T2. Neutral detergent fiber (NDF) is related to the filling effects of feeds in the rumen. Pollard addition (T5 and T6) resulted in the lowest NDF, ADF, cellulose and lignin contents in silage, but exhibiting the highest level of hemicelluloses (33.09%). Use of onggok as carbohydrate source resulting in the highest cellulose content (49.55%) and lowest hemicelluloses (4.18%).

Table 3. Fiber composition of vegetable waste silages

Fiber (%)	Treatments					
	T1	T2	T3	T4	T5	T6
NDF	58.77	72.69	58.28	76.44	52.96	56.33
ADF	51.59	49.65	33.37	72.26	19.87	32.43
Hemicellulose	7.18	23.04	24.91	4.18	33.09	23.90
Cellulose	33.44	30.41	23.71	49.55	16.70	22.38
Lignin	10.77	10.24	9.03	18.14	2.67	5.27

Note: NDF= Neutral Detergent Fiber; ADF= Acid Detergent Fiber; T1= vegetable waste + rice bran; T2= vegetable waste + rice bran + rice straw; T3= vegetable waste + onggok; T4= vegetable waste + onggok + rice straw; T5= vegetable waste + pollard; T6= vegetable waste + pollard + rice straw.

Conclusions

Physical characteristic of vegetable waste silages was good, with a yellowish green colour and good aroma, indicating that fermentation process was successful. Use of rice bran, onggok (cassava byproduct), and pollard as carbohydrate sources for ensiling vegetable waste did not show significant differences in chemical and microbiological characteristics, nutrient and fiber compositions of resulted silages. It can be concluded that all carbohydrate sources used in this experiment can be use as silage additive resulting in a good vegetable waste silage.

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II. FEED AND NUTRITION
Sub Theme: Poultry

Evaluation of Fermented Rice Bran-Tofu Waste by *Monascus purpureus* in the Diet on Performance and Quality of Meat Broiler

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Abstract

An experiment was conducted with 80 unsexed broilers of the Arbor Acress strain to evaluate utilization of fermented product by Monascus purpureus in broiler diet on performance and carcass quality. This study involved a completely randomized design (CRD) with 5 treatments (0, 5, 10, 15, and 20% of fermented product by Monascus purpureus in diets) and 4 replicates per treatment. Diets were isonitrogenous (22% crude protein) and isocaloric (3000 kcal/kg diet). Measured variables were performances (feed consumption, weight gain, feed conversion), quality of meat carcass (fat and cholesterol). Data were analyzed by analysis of variance for CRD. Increasing fermented product by Monascus purpureus levels in the diets increased feed consumption, weight gain but decreased (P<0.01) feed conversion, meat carcass (fat and cholesterol). In conclusion, up to 20% of fermented product by Monascus purpureus could be included for the broiler diet to increased performances and decreased 33.88% cholesterol of meat broiler.

Keywords: feed conversation, fermentation, meat cholesterol, Monascus purpureus

Introduction

Product fermented high carotenoids (β carotene and monacolin) based on by-product could be used as alternative poultry diet, subtituted conventional feed stuffs still import. In Indonesia some of corn, soybean meal and fish meal are still imported from abroad. It resulted in a high cost of diets for poultry. The utilization of waste materials from agricultural or industrial wastes (by-products) is often applied to overcome the problem of feed shortage in poultry industry. Feed diversification in the poultry diet is one of many attempts to reduce the cost of feed in the poultry industry.

Another advantage using fermented product high monacolin was reduced egg and meat cholesterol, so egg and meat safe to eat for anyone including people with

hypercholesterolemia. Results of the research before, reported that utilization fermented products by *Monascus purpureus* (high monacolin) substituted corn 40.06% and decreased cholesterol of meat 35.09 respectively (Nuraini, 2012). Eisenbrand (2005) reported that used of 2.4 g/day product fermented with *Monascus purpureus* containing 10 mg monacolin (lovastatin) for 12 weeks decreased total cholesterol, LDL cholesterol, triglycerides and increased HDL cholesterol blood serum of rats. According Endogrul (2004), monacolin or lovastatin is secondary metabolites produced by *M. purpureus* as hypocholesterolemia agent.

Substrate for fermented product high carotenoid can used solid substrate based on agro waste/waste agricultural products such as waste of (sago, cassava and tofu) are widely available in the area of West Sumatra (Nuraini *et al.,* 2009). Wastes are potentially large to be used as animal feed, because high availability, while still containing nutrient content and not compete with human needs. The success of a solid media fermentation of carotenoid fungi is depend on the given optimum conditions such as: substrate composition, substrate thickness, inoculum dose and duration of incubation. Result of the research before reported that the optimum conditions of *M. purpureus* to produce rich monacolin and to increase the nutrient content of fermented products were composition of substrate contain of a mixture of 80% rice bran and 20% tofu waste, the thickness of the substrate 1-2cm, inoculum dose 10% and long incubation 8 days. Nutrient content of fermented product by *M. purpureus* increased if compare with before fermentation. Protein content increased from 14,85% to 20,22 %, monacolin increased from 0 mg/kg to 400,71 mg/kg. So that this experiment want to study the effect of feeding fermented product by *M. purpureus* (high carotenoid monacolin) in the diet on performance and quality of meat broiler.

Materials and Methods

One hundred (100) 4 days old CP 707 broiler chicks were study in this experiment. The chicks were individually weighed and randomly selected and allocated to each of the fife different level of fermented product by *M. purpureus.* Product fermented contain 60% rice bran with 40% tofu waste then added aquades (water content 70%), stirring evenly, sterilized the material 30 minute after boiling water, then allowed to reach room temperature. Inoculated with 10% inoculum *M. purpureus* and incubated for 8 days (Nuraini *et al.,* 2009). After the fermentation products are harvested, dried by using sunlight. Own diets were formulated from ingredients such as corn, soybean meal, fish meal, rice bran, product fermented by *M. purpureus*, coconut oil and $CaCO_3$. The broilers were given a diet with 22% crude protein and 3000 ME kcal/kg feed. Composition of ration and their nutrient content are presented in Table 1 and 2.

Table 1. Composition of ration treatments (%)

Feedstuff	Treatments (%)				
	A	B	C	D	E
Corn	52.50	50.50	48.50	46.50	44.50
Rice Bran	9.75	7.50	5.25	3.00	0.50
Soybean Meal	12.00	11.00	10.00	9.00	8.00
Fish Meal	23.00	23.00	23.00	23.00	23.00
Coconut Oil	2.25	2.50	2.75	3.00	3.50
RBTWF	0.00	5.00	10.00	15.00	20.00
Topmix	0.50	0.50	0.50	0.50	0.50
Amount	100	100	100	100	100

Note: RBTWF= Rice Bran-Tofu Waste Fermented.

Table 2. Ingredient content and energy metabolism of ration treatments

Ingredient	Treatments (%)				
	A	B	C	D	E
CrudeProtein (%)	22.04	22.04	22.05	22.6	22.03
Ether Extract (%)	4.90	5.14	5.37	5.61	6.09
Crude Fiber (%)	4.87	5.23	5.59	5.95	6.26
Calsium (%)	1.04	1.02	1.00	0.98	0.96
Phosphor (%)	0.59	0.58	0.57	0.56	0.55
ME (Ccal/kg)	3,003.30	3,003.75	3,004.20	3,004.65	3,022.50

Note: ME= Metabolism Energy.

The experimental design used was Completely Randomized Design (CRD) with 5 treatments were: 0%, 5%, 10%, 15%, and 20% of fermented product in the diet and 4 replications. The variable observed were feed intake (g/bird), weight gain (g/bird), feed conversion, meat cholesterol(mg/100g), meat fat (%). Data obtained was subjected to analysis of variance. Where significant differences occurred, the means will be separated using Duncan multiple range test (DMRT).

Results and Discussion

Feed Consumption, Body Weight Gain and Feed Conversion: The effect of feeding fermented product by *M. purpureus* on performance and quality of meat broilers are presented in Table 3. Increasing product fermented by *M. purpureus* in

Table 3. Broiler performance fed fermented rice bran-tofu waste by *Monascus purpureus*

Parameter	Diet				
	0% PF	5% PF	10% PF	15% PF	20% PF
Feed Consumption (g/bird)	1911.45^{c}± 19.58	1993.50^{b}± 19.02	2052.30^{b}± 19.46	2091.60^{ab}± 19.13	2115.40^{a}± 19.00
Weight Gain (g/bird)	782.42^{c}± 12.38	858.72^{bc}± 11.40	893.69^{b}± 12.35	959.70^{ab}± 12.29	986.99^{a}± 11.30
Feed Conversion	2.45^{d}±0.03	2.32^{c}±0.02	2.29^{b}±0.03	2.19^{ab}±0.02	2.14^{a}±0.02
Meat Cholesterol (mg/100g)	114.04^{a}± 10.11	105.42^{b}± 11.23	93.14^{c}±10.34	88.12^{d}± 11.67	75.40^{e}± 10.34
Meat Fat (%)	12.42^{a}±1.23	11.22^{a}±1.26	10.19^{ab}±1.24	9.90^{b}±1.30	8.79^{b}±1.26

Note: Means in the same row with different superscript differ significantly (P<0.05); PF= product fermented by *Monascus purpureus*.

the broiler diet were significantly (P<0.05) affected feed consumption, weight gain, feed conversion meat cholesterol and meat fat.

Feed consumption of broiler highest at the treatment using 20% product fermented by *M. purpureus*, it showed that the fermented product by *M. purpureus* preferred (palatable) up to 20% in the diet, eventhough with reduction of corn and soybean meal in each of these treatments. This is caused by fermentation with *M. purpureus* produced a distinctive flavor that is preferred bird (palatable). In accordance with the opinion of Murugesan *et al.* (2005), fermentation products have a preferred flavor and has a few vitamins (B1, B2, and B12) that are preferred when compared to original material.

The effect of feeding fermented product by *M. purpureus* on weight gain, the present data demonstrated that treatment fed PF 20% and PF 15% (986.99g/bird and 959.70g/bird, respectively) higher than (P<0.05) as compared to birds fed PF 10%, 5%, and PF 0% (893.69g/bird,858.72g/bird and 782.42g/bird, respectively). High weight gain of broiler, it mean high feed nutrient in the diet which use to produce meat, so it can increase weight gain. According Gunawardana *et al*. (2008), weight gain is influenced by feed intake, especially protein intake. High weight gain indicate that the product fermented by *M. purpureus* until level 20% in broiler diets that reduce the use of corn and soybean meal (40.34% and 35.67%, respectively) was still preferred (palatable) by livestock. In addition, the high weight gain in treatment E (20% PF) compared to treatment A (0% PF) due to the product fermented by *M. purpureus* produced unsaturated fatty acid were oleic acid (omega 9), linoleic acid (omega 6) and linolenic acid (omega 3) (Lin *et al.,* 2005). According Grobas and Mateos (1999), 1.5% to 2% linoleic acid is needed for birds during growth or the production phase of the first egg laying period. Linoleic acid deficiency in the diet can reduce egg production.

The low feed conversion ratio at treatment E than in treatment A is caused by feed intake and weight gain also differed significantly (P<0.05). According Varkoohi *et al.* (2010), feed conversion ratio is the ratio between feed intake in producing a number of meat. Feed conversion can be used as a picture of the production coefficient, the smaller value mean more efficient use of feed to produce meat.

Meat Cholesterol and Meat Fat

The data of meat cholesterol and meat fat showed that the treatment fed PF 20% had lower mean value (P<0.05) of meat cholesterol and meat fat than the treatment PF 15%, PF 10%,PF 5% and PF 0%. Low cholesterol of meat broiler in treatment E compared to other treatments, associated with the use of fermented product rich monacolin. Increasing fermented product by *M. purpureus* in the diet caused the higher content of carotenoids monocolin. Monacolin is hyphocholesterolemia agent. According to Erdogrul and Azirak (2004) that red yeast rice (fermentation by *M. purpureus*) produced monacolin that can inhibit the action of the enzyme-CoA reductase Hydroksimetyl Glutaryl (HMG Co-A reductase) that play a role in the formation of mevalonat in the synthesis of cholesterol so that cholesterol is not formed. The results of this study showed that fermented product by *M. purpureus* until level 20% decreased meat cholesterol 33.88%.

Conclusion

Increasing fermented product by *Monascus purpureus* in the diet can improved the performance and meat quality of broiler. Feeding fermented product by *M. purpureus* up to 20% in broiler diet obtained 986.99 g/bird body weight, feed conversion ratio 2.14 can reduced meat cholesterol 33.88%.

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Cholesterol Contents and Carcass Yields of Broiler Meats Fed Different Level of Garlic Meal

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Abstracts

Garlic is one spice herbal that can be used to reduce levels of fat and cholesterol in the human blood. This materials can also be applied to reduce fat and cholesterol content of meat products through the manipulation of animal diets. The study objective was to identify the cholesterol content of meats and carcass yield of broiler fed different level of garlic meals. The experiment was used 100 day old chicks (DOC) of broiler. It was designed by Completely Randomized Design with 5 treatments and 4 replicates. Differences every treatment were tested with Duncan's multiple range test. Experimental diets used were: control diet (R0); inclusion of 1.5% garlic meal (R1); inclusion of 3% garlic meal (R2); inclusion of 4.5% garlic meal (R3); and inclusion of 6% garlic meal (R4). Feed and water were offered ad libitum, birds were slaughtered at 42 days old. The result showed that feed treatments had significant effect (P<0.05) on cholesterol content of meat with the value as 123.20mg/100g (R0); 123.00mg/100g (R1); 102.95mg/100g (R2); 73.29mg/100g (R3); and 58.69mg/100g (R4). The inclusion of 6% of garlic meal in the diets reduced cholesterol contents from 123.2 mg/100g (R0) to 58.69mg/100g (R4), or reducing to be 47.26%. While the carcass yields (carcass persentage, components, and abdominal fat), actually an increased but not to significant effects. Increasing of garlic meal in the broiler ration reduced the cholesterol content of the broiler meats.

Keywords: broiler, carcass, garlic meals, meat cholesterol

Introduction

High quality meat associated with the feed quality and maintenance management. Quality of the feed affects the quality of meat produced. The broiler chicken as well known to be more efficient in converting feed to animal product. The nature of the broiler meat of which is a high fat and water contents. High water content in

meat is one of the factors that support the development of fungi or micro-organisms that can reduce the meat quality (Ketaren, 1989). In addition broiler meat are also known to contain high levels of fat and high cholesterol, thereby reducing the level of palatability for people suffering from heart disease and cholesterol. To overcome this constraint the manipulation of feed needed to improve the quality of meat produced, one of the local feed ingredients that can be added to improve the quality of animal meat is garlic. The inclusion of garlic in chicken diets may reduce the fat and cholesterol of the produced.

Garlic (*Allium sativum*) is widely distributed and used in all parts of the world as a spice and herbal remedy for the prevention and treatment of a variety of diseases, ranging from infections to heart diseases. In the past two decades, particular attention has been focused on the cholesterol-lowering activity of garlic. Garlic that had metabolic effects may decrease blood glucose, blood cholesterol and triacylglycerol (Horie *et al*., 1991). Wibowo (1989) stated that garlic may accelerate growth and increase the weight gain. Bordia *et al*. (1975) reported that the essential oils of onion and garlic can prevent fat-induced hyperlipemia. A marked reduction of serum cholesterol levels (53% and 34%) were observed in rats fed a diet supplemented with 2 or 3% garlic powder. Similar effects of garlic were found in rats fed diets containing either cholesterol or lard. Plasma and liver cholesterol as well as total liver lipids were reduced by about 30% by garlic supplementation, whereas plasma triacylglycerols were reduced only in the group fed lard (Chashnidl et.al., 1982). Depressed hepatic cholesterol levels in chickens fed 2% garlic for 14 d were observed by Sklan *et al*. (1992). Various garlic extracts exhibited hypocholesterolemic effects on chickens, mainly through the inhibition of the key enzymes in cholesterol and lipid synthesis (Qureshi *et al*., 1983). Konjufca *et al*. (1997) found that the essential oils of garlic can reduce cholesterol content of meat broiler. The objective of this study was to identify the cholesterol content of meats and carcass compostion of broiler fed different level of garlic meals

Materials and Methods

Chicken and Housing

A total of 100 one-day-old chicks (DOC)broiler mixed sex (males and females) strain Arbor Arcres CP 707 was used. Chickens were kept in 20 plots with the size for each plot 1 x 1 x 0.5 m and placed 5 chicks. Each plot was equipped with a feed and drinking water. The treatments were randomly distributed among the plots. During the experiment all of the chicken were given ad-libitum feeding and drinking water. The chicken was weight individually at the begining of the experiment and at six day interval thereafter.

The chickens were reared up to 41 days old and were weight individually.

At 42 days old the chickens were deprived of feed for 8 hours and all the chicken were wight individually prior to slaughter. After slaughter, feather were removed by dipping the chicken in to the warm water (approx.60-70 ^{o}C). Carcass yield was weight of dead chicken without feather, head, neck, legs, and digestive organs. The chickens were cut into the parts according to standar procedure of dissection (Jensen, 1989). Variable determined were carcass weight, carcass components, abdominal fat, and cholesterol content of breast meat. The meat samples for cholesterol analysis was taken from the breast meat as much as 100g of each treatment. Cholesterol analyzes performed by the method of Lieberman-Burchrad (Tranggono *et al.*, 1989).

Diets and Treatments

The control diets was performed to be met the nutrient requirement with protein and metabolyzable energy with 21% and 3000 kcal respectively. The diet was fed as crumbles form and was specially prepare for this research. The composition and analyzed value of the diets in shown in Table 1. The diets used in this experiment were not separated between starter and grower diets according to NRC (1994) recomended. The chickens were fed similar feed composition througout the experiment. Feed ingredients used and the composition of treatment rations are listed in Table 1.

Table 1. Diets Composition and Nutrient Content of the Feed Treatmens

Diets	Treatments				
	R0	R1	R2	R3	R4
Yellow corn	52	51	50	49.5	49
Ricebran	7	6.5	6	7	6.5
Coconut meals	10	10	10	11.5	11.5
Soybean grain	15	15	15	13.5	12
Fish meal	15	15	15	13.0	14
Topmix	1	1	1	1	1
Garlic meal	0	1.5	3.0	4.5	6.0
Nutrient Contents*):					
Crude protein (%)	21.01	21.02	21.02	20.02	20.01
Crude fat (%)	1.08	1.07	1.71	1.05	1.04
ME (kcal/kg)	3,009	3,022	3,034	3,007	3,004
Crude fiber (%)	4.57	4.51	4.45	4.70	4.67
Ca (%)	0.33	0.33	0.33	0.29	0.31
P (%)	0.09	0.09	0.09	0.09	0.08

*) Based of feeds analysis in the Nutrition Laboratory Animal Science Department, Argicultural Faculty Tadulako University, Palu (2010)

The dietary treatment are as follows: R_0= control diets without inclusion garlic meals; R_1= diets with the inclusion garlic meals 1.5%; R_2= diets with the inclusion garlic meals 3.0%; R_3= diets with the inclusion garlic meals 4.5%; R_4= diets with the inclusion garlic meals 6.0%.

Design Experiment and Statistical Analysis

The experiment included 5 dietary treatments, and the experimental design is Completely Randomized Design with 5 treatment and 4 replicates. Data from the experiments were analyzed by using analysis of variance according to the type design was used, if there is a difference between the treatments followed by Duncan Multiple Range Test (Steel and Torrie, 1991).

Results and Discussion

Cholesterol Contents

Results of cholesterol contents that influenced by inclusion of garlic meal in the diets as shown in Figure 1.

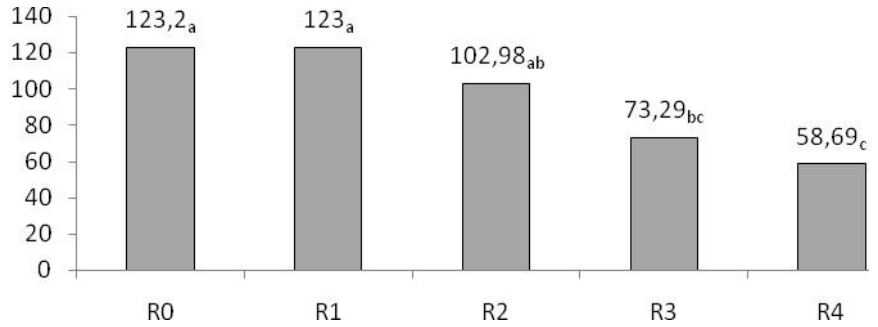

Figure 1. Mean of cholesterol content of meats of each treatment in mg/100g

Results of variance analysis showed that garlic treatment had significant effect ($P<0.05$) on meat cholesterol. It was found a decrease of cholesterol content of the meats by increasing of dietary garlic meals in the diets. The treatment that inclusion of 4.5% and 6% of garlic meal in the diets had cholesterol contents significantly lower compared to control diets. This is possible because the component in garlic-containing sulfur called *allicin* reduced the development of cholesterol in the meat. Keusgen (2002) found that garlic contains *allicin*, a component that acts as an antibacterial. *Allicin* from fresh garlic extract has a wide-ranging antibacterial activity for both gram negative and gram positive. *Allicin* is not formed on the intact plant garlic, because garlic contains *aliin* and enzymes intact alinase. When garlic is chopped or crushed then reacts with the enzyme alinase to be form *alicin*. The use of garlic in the form of pasta 3.8% in the diets can reduce cholesterol 18% and 23%,

respectively in broiler and layer chickens in an age of 12 weeks had been carried out for 4 weeks (Qureshi *et al*., 1983). Thiosulfanat compounds in garlic are formed due to enzyme activity *alinase* of *aliin* (an amino acid containing sulfur), through it can be influenced the *alicin* content and resulted lower cholesterol levels in the blood (Wahyuono 1999). Mamonto (1992) stated that there is a relationship between blood cholesterol and meats cholesterol, decreased of blood cholesterol will be followed by a decrease in cholesterol of meat. This statement was applied by Jaya (1997) that resulted the addition of 1% garlic in broiler feed can reduce about 17.10 mg/dl (8.97%) and blood cholesterol levels around 13.02 mg/dl (7.06%) cholesterol levels of meat. Another experiment was done by Eckner *et al* (1993) that found the inclusion of garlic extract in the chicken diets could be reduced the triglyceride, total cholesterol, and phospholipids in the blood. Garlic extract was also reported have a fibrinolytic effect, increase the mobility of cholesterol and triglycerides (Schneider, 1985), and as antiaterogenik which prevents the occurrence of atherosclerosis by preventing oxidation of LDL (Heinle and Betz, 1994). Effects of hipocholestrolemia in the garlic probably caused by the presence of alicin as a bioactive compound (Borek, 2001).

Carcass Yields

Results of carcass yields that consist of carcass percentage, abdominal fat, and carcass component as shown in Table 2. The results of variance analysis showed that the treatment provided no significant effect on carcass composition, abdominal fat and carcass components. This is probably caused by the content and quality of diets consumed relatively the same nutrients content, resulting relative the same of body weight and ultimately result in similar carcass component as well. Jull (1979) states that the percentage of broiler carcass in the range of 65%-75% based on live weight,

Table 2. The mean of carcass, abdominal fat, and carcass component for each treatment

Carcass yields (%)	Treatments					P-value
	R0	R1	R3	R3	R4	
Carcass	66.76	66.82	67.26	68.88	69.29	0.28
Abdominal fat	2.21	1.51	1.24	1.14	0.99	3.01
Carcass components:						
Breastmeat	38.30	39.67	38.51	38.84	37.15	0.89
Drumstick	15.57	14.48	14.95	14.96	14.77	0.83
Thigh	13.89	13.96	13.61	13.45	14.18	0.40
Back	21.36	20.78	21.36	21.17	22.31	0.60
Wings	10.88	11.11	11.56	11.58	11.59	1.99

while the results of this study is in the range from 65.55 to 70.15%, slightly lower. Budiansyah (2003) reported that carcass components relative to body weight gain equal to the percentage of carcasses that would produce no different.

Although there is no significant difference among the treatments effect on carcass yield, but the higher used of garlic powder in the ration resulted a higher percentage of carcasses. Increase the percentage of carcass due to the increase in body weight, the higher body weight resulted the greater percentage of carcass as well (Siregar *et al.*, 1980). This indicated that garlic had fungtion to become a growth promotor. Substances that play role as a growth promoter in garlic is *scordinin*. Physiological effects of *scordinin* was tested by Wibowo (1989) on mice, that indicated that it increase growth and body weight. In contrast, the results of abdominal fat content showed reduced with the increasing of garlic meals in the diets.

Conclusion

There were reducing of cholesterol contents of broiler meats by inclusion of garlic meal in the diets. The use of garlic meal up to a level of 6% in the ration of broiler was reduced significantly cholesterol contents of meats from 123.2 mg/100g to 58.69 mg/100g, or reducing 47.26%. While the carcass yields (carcass persentage, components, and abdominal fat), actually an increased but not to significant effects. Increasing of garlic meal in the broiler ration reduced the cholesterol content of the broiler meats.

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The Supplementation Effect of Fish Oil, Corn Oil, and Zinc in Fiber Ration on Cholesterol Profile, Omega-3 and Omega-6 of Alabio Duck Egg

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Abstract

As a complete nutrient product, the egg of Alabio duck contains high cholesterol, therefore it needs some attention. This research was conducted to reduce this cholesterol level and at the same time to improve its deposit of omega -3 and omega-6 in the egg, by providing fiber ration containing fish oil, corn oil, and zinc (Zn). The experimental design being used was factorial of 2x5x2 with 100 ducks. The first factor was fiber (F) and the second one was oil and Zn (O). Results showed that the treatments were effective enough in reducing cholesterol and improving the omega -3 and omega- 6 of the egg. The best product was shown by treatment of F1O4 containing 6 % fiber (F1) with 2% fish oil + 4% corn oil + Zinc (O4), either in single or interaction, in decreasing cholesterol and LDL cholesterol at the same time improving HDL cholesterol in plasma, omega -3 (linolenate), and omega -6 (linoleate) of the Alabio duct egg.

Keywords: Alabio duck, Cholesterol, HDL, LDL, Omega-3 and- 6

Introduction

The egg of Alabio (*Anas plathyryncho,* Borneo*)* duck has been a big choice of consumers as it has high nutrient contents, such as 13.1% protein, 14.5% fat, 0.5 % carbohydrate, 1 % ash, and 19.9 g/100 g calory (Biyatmoko (2007[a]). This duck egg contributes as much as 54.14% out ot total egg production with 67.32 % productivity in South Kalimantan (Biyatmoko, 2007[b]). However, since egg contains high cholesterol, it becomes a health concern (Froning *et al*., 1990). Cholesterol is part of egg yolk (5.2%), 65.5 % triglyceride, and 28.3 % phospholipide (Sirait, 1986). Amrullah (2004) reported that cholesterol in an egg ranged from 198 to 208 mg/egg, even riched 270 mg/egg (Cotteril *et. al*.1977).

One of the efforts of reducing cholesterol is thru *gastrointestinal* system, by binding pancreatic bile mechanism. Supplementation of fish oil and plant oil could lessen egg cholesterol as a result of hipolipidimic effect, and improve omega-3 and-6 (Supriyatna, 1999). Ration rich of omega-3 comes from fish oil, while, omega-6 rom corn oil. Taneja *et al.,*(1995) described that addition of fish oil and corn oil without buffer supplementation could give negative impact on other nutrient absorption. Zinc as a buffer, has several roles, such as in cellular activity, synthesis and metabolism of protein (Lloyd, 1978). The absence of buffer would cause lost of appetite leading to anorexia, slow growth and production, as well as misabsorbsion of nutrient, including lipid.

The objective of this research was to decrease cholesterol and improve omega-3 and omega-6 in Alabio duck egg thru the inclusion of fiber ration containing fish oil, corn oil, and zinc.

Materials and Methods

This research was carried out for three months utilising 100 Alabio ducks. Experimental design being used was randomised block in factorial of 2 x 5 and 2 replication with five ducks for each replication. Diets were formulated as iso calory-protein based on production stage, containing 18% CP (crude protein) and EM (energy metabolic) of 2750 kcal/kg. Factors implemented were:

First Factor, levels of fiber (F), were F1= 6% and F2= 8%.

Second Factor, levels of oil (O), were

O1 = 6% fish oil + Zn
O2 = 4% fish oil + 2 % corn oil + Zn
O3 = 3% fish oil + 3 % corn oil + Zn
O4 = 2% fish oil + 4 % corn oil + Zn
O5 = 6% corn oil + Zn

Variables evaluated were cholesterol, HDL-plasma cholesterol, LDL-plasma cholesterol, omega-3, and omega-6. Data were analysed as Anova, if there is found a significant difference, followed by DMRT.

Results and Discussion

Cholesterol contents (Table 1) of the egg were not affected ($p>0.05$) by interaction of fiber and oil levels. However, fiber level or oil diet treatment significantly ($p<0.01$) affected egg cholesterol. Fiber of 6% (F1) and 2% fish oil + 4% corn oil+ Zn (O4) were considered as the best treatment. Supplementation of fish oil and corn oil which are rich MUFA and PUFA were able to decrease the cholesterol as they have hypolipidemic effect, decreasing the capacity transfer of LDL and increasing plasma HDL thus lowering the egg cholesterol (Abbey *et. al.,*1990).

The same pattern happened in high density lipoprotein (HDL), the individual factors showed significant differences ($p<0.05$) (Table 2). The 6% fiber diet (F1) was the best in improving plasma HDL (215.890 mg.dl^{-1}), while the 2 % fish oil + 4 % corn oil + Zn (O4) increased the highest HDL (248.575 mg.dl^{-1}). Fish oil plays some roles of reducing transfer capacity of LDL cholesterol and plasma lesitin (cholesterol acyltransferase), as well as stimulating thromboxane and improving the ratio of HDL_2HDL_3 (Abbey *et.al.,* 1990).

The plasma low density lipoprotein (LDL) cholesterol showed significant respond ($p<0.05$) on the interaction of fiber and oil supplementation in lowering the LDL (Table 3). The best combination was found in F1O4 with the lowest LDL (13.58 mg.dl^{-1}). Mechanism of cholesterol reduction by fish oil was effective in reducing plasma LDL, playing a role as antiagregasion and reducing plasma triglyceride (Eritsland *et. al*., 1994 and Sinclair, 1996) as a result of the oxidation of EPA dan DHA (Jandacek *et. al*.,1991). Besides, this supplementation altered the structure of LDL and LDL receptor in the hepatic cells to be active to uptake the LDL cholesterol, thus lowering the plasma LDL cholesterol in the egg (Schectman *et al*.,1996).

The omega-3 (Table 4) was also affected significantly ($p<0.01$) by the interaction of fiber and oil. The best combination was contributed by F1O4 (6% fiber+ 2 % fish

Table 1. Averages of Alabio Duck Egg Cholesterol (mg.g^{-1}) with Fiber and Oil Diets

Fiber levels (F) (%)	Oil and Zn (O)					Averages
	O1	O2	O3	O4	O5	
F1	11.768	7.434	7.428	6.333	7.296	8.051^{A}
F2	12.253	7.560	7.673	7.003	7.362	8.370^{B}
Averages	12.010^{c}	7.497^{b}	7.550^{b}	6.668^{aa}	7.329^{b}	8.210

Note Different superscripts within coloumns were different very significantly ($p<0.01$) and within rows ($p<0.05$).

Table 2. Averages of Alabio Duck Egg HDL Cholesterol (mg.dl^{-1}) with Fiber and Oil Diets

Level of fiber (F1)	Oil and Zn (O)					Averages
	O1	O2	O3	O4	O5	
F1	195.000	195.860	213.220	253.820	221.550	215.890^{A}
F2	196.880	195.270	195.010	243.330	216.660	209.430^{B}
Averages	195.940^{a}	195.565^{a}	204.115^{b}	248.575^{c}	219.105^{c}	212.660

Note Different superscripts within coloumns were different very significantly ($p<0.01$) and within rows ($p<0.05$).

Table 3. Averages of Alabio Duck Egg LDL Cholesterol (mg.dl^{-1}) with Fiber and Oil Diets

Level of fiber (F)	Oil and Zn (O)					Averages
	O1	O2	O3	O4	O5	
F1	57.098^{e}	32.449^{d}	15.36^{b}	13.58^{a}	15.51^{b}	26.799
F2	16.828^{c}	32.555^{d}	38.745^{e}	15.223^{b}	16.5^{c}	23.970
Averages	36.963	32.502	27.052	14.401	16.005	25.384

Note: Different superscripts within the same rows and coloumns were different significantly ($p<0.05$)

Table 4. Averages of Alabio Duck Egg Omega-3 (mg.100 g^{-1}) with Fiber and Oil Diets

Level of Fiber (F)	Oil and Zn (O)					Averages
	O1	O2	O3	O4	O5	
F1	192.5^{c}	179.3^{ab}	177.8^{ab}	236.2^{e}	170.6^{ab}	191.28
F2	169.3^{ab}	168.3^{ab}	166.3^{a}	202.5^{d}	181.4^{bc}	177.56
Averages	180.90	173.80	172.05	219.35	176.0	184.42

Note: Different superscripts within the same rows and coloumns were different significantly ($p<0.05$)

Table 5. Averages of Alabio Duck Egg Omega-6 (mg.100 g^{-1}) with Fiber and Oil Diets

Level of Fiber (F)	Oil and Zn (O)					Averages
	M1	M2	M3	M4	M5	
F1	1773.1^{c}	1604.6^{b}	2072^{d}	2632^{e}	2669^{e}	2150.14
F2	1750.1^{b}	1332.8^{a}	1644^{b}	1756^{b}	1763.1^{b}	1649.13
Averages	1761.60	1468.70	1858.00	2194.00	2216.05	1899.63

Note: Different superscripts within the same rows and coloumns were different significantly ($p<0.05$)

oil + 4 % corn oil + Zn (236.2 mg.100 g^{-1}). This high level of omega-3 was due to the fact that corn oil contain α linolenic dan linoleic (Kreutler, 1980). There were two reasons for the lowering effect; first, the high fiber content (8%) in F2O1 and F2O2, dan second, the level of zinc (29 ppm) as limited factor.

The best combination increased the omega-6 was given by F1O5 (6% fiber + 6% corn oil + Zn (O4) that was 2669 mg.100 g^{-1} (Table 5). The increase of omega- 6 in an egg would be linearly correlated to the level of omega-6 PUFA being consumed from the diet. Scaife *et al.,* (1994) stated that omega- 6 in diet is absorbed and

deposited without any changes. However, its deposit in the egg was found high only in the corn oil with a certain proportion.

Conclusion

The inclusion of fiber ration with fish oil, corn oil, and zinc was effective in reducing cholesterol, at the same time increasing the omega-$_3$ and omega-6 of Alabio duck egg. The best recommendation was given to the ration containing 6% fiber and 2% fish oil + 4% corn oil + Zinc, as its interaction or as individual in lowering cholesterol and LDL and increasing the plasma HDL cholesterol as well as omega-3 (linolenic) and omega-6 (linoleic) in Alabio duck egg.

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The Bacteriological Quality of Chicken Offal and Spoiled Egg as Feed for Catfish and Tilapia Rearing in Penang, Malaysia

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Abstract

Chicken offal, spoiled egg and commercial fish feed were used as feed in cultured ponds of catfish and tilapia in and around Penang, Malaysia. The total aerobic bacteria, coliform and fecal coliform was investigated on catfish (Clarias gariepinus) , tilapia (Tilapia mossambica) and its water. A total of 48 samples (12 intestine of catfish, 12 intestine of tilapia, 24 water samples) were taken from 8 pond in and around Penang, Malaysia. All fish samples fed by chicken offal or spoiled egg were heavily contaminated by total aerobic bacteria (5 to 7.26 log10 cfu/gr), coliform (2800 to 11000 MPN/gr) and fecal coliform (3 to 430 MPN/gr). Meanwhile, fish fed by commercial pellet were less contaminated by total aerobic bacteria (5.33 to 6.58 log10 cfu/gr), coliform (7 to 11000 MPN/gr) and fecal coliform (3 to 110 MPN/gr). Chicken offal and spoiled egg polluted the water in aquaculture system more than commercial fish feed. The highest l evel of total aerobic bacteria, coliform, fecal coliform in pond water were 7.21 log10 cfu/ml, 11000 MPN/ml and 2800 MPN.ml. The bacteria load of chicken offal and spoiled egg might contaminate catfish, tilapia and its water. These will be a concern for quality and safety of catfish and tilapia in term of nutrition and human health.

Key words: chicken offal, spoiled egg, catfish, tilapia, bacteriological quality

Introduction

In Malaysia, catfish and tilapia were the important aquaculture products and marketed for domestic consumption (FAO, 2012). The prices of those fishes were more consistent compared to marine fish. Thus, the demand of catfish and tilapia

tended to increase in the last decade. However, some problems have been met. Those were the limited land, the rising of production costs, lack of skilled labor, the threat of diseases, the high food safety and quality requirements. These issues made the aquaculture development to be more difficult (FAO, 2012). The rising of production cost can be reduced by replacing the commercial pellet feed with cheaper feed such as food waste from restaurant, chicken offal, spoiled egg or others.

In Malaysia, chicken offal and spoiled egg have been found to be used as feed for catfish and tilapia. However, the study about the effect of those feed for microbiological aspect in catfish and tilapia has not yet been done. To filling this gap, the present study will investigate microbiological aspect in catfish and tilapia which fed by different type of feed. The objective of this study was to investigate the bacteriological quality of chicken offal and spoiled egg as feed for catfish and tilapia rearing in Penang, Malaysia. The bacteriological aspects studies were total aerobic count, *coliform* count and *fecalcoliform* count in the intestines of fish and water of ponds. Fapohunda et al. (1994) reported that the verification of the microbiological quality, such as *coliform* and *fecalcoliform*, could explain whether the harvest or production presents a health hazard or not to human. *Coliform* and *fecalcoliform* were reported to be the most heavily contaminate the intestinal tract of fish (Salle, 1964).

Materials and Methods

Catfish and tilapia were obtained from 8 ponds located in Penang -Malaysia in November 2008 – September 2009. Sampling was done 3 times visiting for each ponds. During each visit 5-6 live catfish and tilapia were purchased from ponds, placed in sterile plastic bags and transported in polystyrene box to the laboratory. On arrival at the laboratory, the intestines of fish samples were pooled and analyzed for Aerobic Plate Count (APC), *coliform* and *fecal coliform*. Water samples were also obtained from the ponds where both catfish and tilapia were reared. This sampled and kept in sterile jar at 4 ^{o}C during transportation to the laboratory. The ponds were studied based on the type of feed given to the fish. The feed types were chicken offal for catfish feed, spoiled egg for tilapia feed and commercial fish pellet feed for both. The chicken offal was steamed and grinded before feed to the fish. The spoiled egg was mixed with other ingredients and was formed to pellet.

The intestines of catfish and tilapia were taken aseptically and chopped by using sterile knife. Twenty five grams of intestines was mixed with 225 ml of 1.5% Pepton Water (Oxoid) and homogenized by using stomacher (Interscience) for 120 sec. The dilution was prepared by pipeting 1 ml of aliquot and mixed with 9 ml of 1.5% Pepton Water (Oxoid). The dilution was done from 10^{-1} until 10^{-6}. About 100 ml of aliquot was spread on Plate Count Agar (Merck) and incubated at 37^{o}C for 24-48 hours. Total number of colonies were counted and calculated as BAM Manual

Protocol (Maturin *et al*., 2001). Total aerobic count was expressed as log cfu/g.

Coliform and *fecalcoliform*count were determined by using MPN method (Feng *et al*., 2002). One ml of 10^{-1} until 10^{-5} dilution of intestines and water samples were transferred into three tubes of Lauryl Sulfate Tryptose (LST) Broth (Oxoid) and incubated at 37°C for 24h. Approximately, 10 µl of broth from positive tubes were transferred into 10 ml of Brilliant Green Lactose Bile (BGLB) broth (Merck) and incubated at 37°C for 24 hours. Turbid tubes with gas were considered as positive and coliform count were expressed as MPN/g or MPN/ml. *Fecalcoliform* count was determined by transferring 10 µl of BLGB broth from positive tubes into three tube of EC broth (Merck) and was incubated at 44-45 °C for 24 hours. Tubes showing gas and turbidity were considered positive for the presence of *fecalcoliform* and these were expressed as MPN/g or MPN/ml. *E. coli* cultures (Food Microbiology Laboratory, School of Industrial Technology, USM) were used as control.

Statistical Analysis

The difference in APC, *coliform* and *fecal coliform* ponds was determined by using one-way ANOVA, SPSS software for Windows Version 13.

Results and Discussion

In present study the microbiological quality of catfish and tilapia fed by using chicken offal, spoiled egg and commercial feed were evaluated. Our results showed that total aerobic count on intestinal of catfish and tilapia fed bychicken offal and tilapia fed by spoiled egg were observed relatively higher compared to catfish and tilapia fed by commercial fish feed. The aerobic plate count ranged from 5.00 to 7.26 $\log_{10}$ CFU/gcatfish fed with chicken offal, 5.97 to 6.69 $\log_{10}$ CFU/gcatfish fed with commercial feed, 5.33 to 6.58 $\log_{10}$ CFU/g tilapia fed spoiled egg, 5.11 to 6.11 $\log_{10}$ CFU/g tilapia fed commercial fish feed. Later on, the fish which were not eviscerated properly, the bacteria might spread and contaminate through the apparatus of the fish intestinal such as intestinal wall and intestinal cavity. The proteolytic enzymes originated from intestines and/or the inside of intestinal canal might act for the spoilage process (Andreji *et al*., 2006).

In water samples, chicken offal and spoiled egg showed to be relatively higher on total aerobic count compared to commercial fish feed. There were no significant differences between type of feed on catfish, tilapia and water ($P>0.05$). The aerobic plate count in water samples ranged from 6.00 to 7.21 $\log_{10}$ CFU/g for water obtained from the pond use chicken offal, 6.00 to 6.9 $\log_{10}$ CFU/gfor pond use commercial feed, 5.32 to 6.58 $\log_{10}$ CFU/g for water obtained from the pond use spoiled egg, 5.05 to 6.18 $\log_{10}$ CFU/g for pond use commercial feed.

Chicken offal was more contaminate the water in pond compare to spoiled egg. Other study reported that chicken loaded by aerobic bacteria (Cohen *et al*.,

2007). Spoiled egg was formed as pellet to feed tilapia. The pellet form could fed tilapia efficiently and reduced the remained feed in water. Thus, the contamination in water was observed less than chicken offal which was not formed in pellet. The form of pellet in fish feed can significantly reduce pollution caused by fish feeding and improve both the feed efficiency as well as fish health (Agriculture Fisheries and Conservation Technology, 2004).

The detected values of *coliform* in intestinal of catfish and tilapiafed by using chicken offal and spoiled egg showed to be relatively higher compared to commercial fish feed. These were observed also in water samples. Thus, chicken offal and spoiled egg might be the source of coliform. There were significant different between intestines of catfish fed chicken offal and catfish fed commercial fish feed ($P<0.01$). These were observed in tilapia and water samples also.

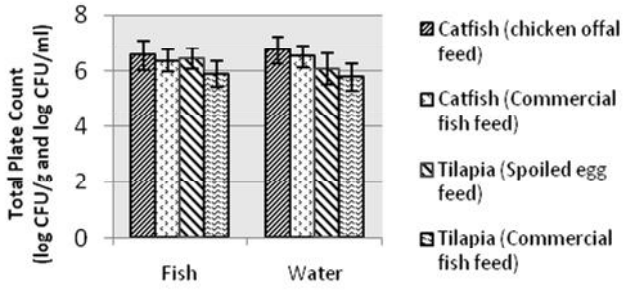

Figure 1. Distribution of Aerobic Plate Count in catfish, tilapia and water obtained from pond in Penang

Coliform count was 1100 MPN/g in the intestines of catfish fed with chicken offal, from 240 to 750 MPN/g in catfish fed with chicken offal, from 2800 to 11000 log MPN/g tilapia fed spoiled egg, from 7 to 11000 MPN/g tilapia fed commercial fish feed. Other study found that chicken and spoiled egg loaded by *coliform* (Cohen *et al*., 2007; Theron*et al*., 2003). The composition of the intestinal flora is related in varying degree to the level of contamination of water and food in the environment (Geldreich *et al*., 1966).

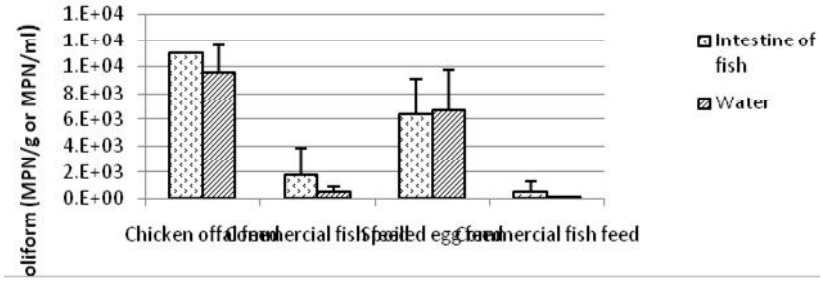

Figure 2. *Coliform* count in catfish, tilapia and water obtained from pond in Penang

Coliform count ranged from 2100 to 11000 log CFU/g in water samples from pond use chicken offal, 110 to 1100 log CFU/g from catfish pond fed with chicken offal, 40 to 1100 log CFU/g from tilapia pond fed spoiled egg, 70 to 110 log CFU/g from tilapia pond fed commercial fish feed. Chicken offal and spoiled egg used as feed might cause the increase of *coliform* in water. Chickenand spoiled egg which contained with *coliform* (Cohen*et al*., 2007; Theron *et al*., 2003) can transmite to the water (Pearson *et al*., 1987).

Intestines of catfish were highly contaminated with *fecalcoliform* (Figure 3) with the mean level of 20 MPN/g, followed by integstines of tilapia with the mean level of 3.67 MPN/g. Other studies found that *fecalcoliform* was observed in catfish and tilapia from the pond (Saber *et al*, 2004; Leung *et al*., 1992).

The mean for *fecalcoliform* count in catfish fed with chicken offal (20 MPN/gr) was relatively higher compare to *fecalcoliform* in catfish fed with commercial fish feed (9 MPN/g). In water samples from those sites, the significant difference was observed. The mean of *fecalcoliform* in pond water (fed with chicken offal) was 48 MPN/g which was higher than those fed with commercial fish feed (8.3 MPN/g). Chicken and egg could be the sources for the presence of *fecalcoliform* (Schwaiger *et al*., 2008; Cohen *et al*., 2007).

In tilapia, *fecalcoliform* were no significant different between tilapia fed by using spoiled egg and commercial fish feed. This was also observed in water samples. The mean values were 3.67 MPN/g (tilapia fed spoiled egg), 3 MPN/g (tilapia fed commercial feed), 3 MPN/g (water of pond use spoiled egg) and 3 MPN/g (water of pond use commercial feed).

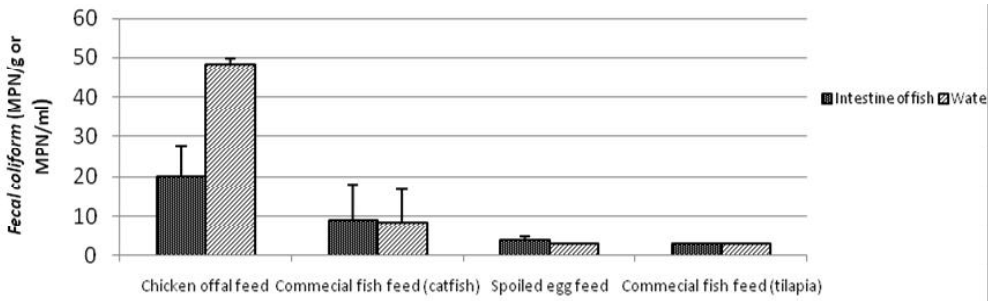

Figure 3. *Fecalcoliform* count in catfish, tilapia and water obtained from pond in Penang

Conclusions

The results of this study showed that using chicken offal and spoiled egg as feed to catfish and tilapia cause heavily contamination by total aerobic bacteria (5 to 7.26 log cfu/g), *coliform* (2800 to 11000 MPN/g) and *fecalcoliform* (3 to 43 MPN/g). These were relatively higher compared to fish fed by commercial pellet which had total aerobic bacteria (5.33 to 6.69 log cfu/g), *coliform* (7 to 11000 MPN/g) and

fecalcoliform (3 to 11 MPN/g). Chicken offal and spoiled egg polluted the water in aquaculture system more than commercial fish feed.

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Production Performance of Broiler Chickens Fed Glucogenic and Lipogenic Diets to Overcome Environment Temperature

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Abstract

Two types of diet, glucogenic and lipogenic, were equally tested to 24 broiler chicken in completely randomized design for four weeks to evaluate their effects on body weight, feed intake, feed conversion ratio (FCR), carcass, and whole carcass weight. Diets were designed to contain 1.05% and 7.0% of palm oil, as glucogenic and lipogenic ones, respectively. Both diets were formulated as isoprotein (22%) and isocaloric (3050 kcal/kg) to overcome the environment temperature of 25.8-30.4^{0}C, around the poultry housing at campus. The birds were weighed at the beginning, weekly, and at the end of the diet application. The difference of these body weights were calculated as body weight gain (BWG). Feed intakes were accumulated weekly to the end. The FCR was calculated as the ratio of feed intake to body weight gain. Carcass and whole carcass were then compared to their body weight gain for each diet. Results showed that even though there were no significant differences ($p>0.05$), however, BWG in glucogenic diet chicken were 2.3% higher than that of in lipogenic diet birds. While, the feed intake in lipogenic diet chicken were significantly higher ($p<0.05$) than that of in glucogenic diet birds. Accordingly, the FCR was also significantly higher ($p<0.05$) in lipogenic diet of broiler chicken. None of carcass variables was significantly different, however, the glucogenic diet birds were 10.6% heavier than that of the lipogenic diet birds. Having higher in BWG, carcass weights, and carcass ratios, yet, lower in Feed intake and FCR, it can be concluded that glucogenic diet fed to broiler chickens was more efficient in overcoming the environment temperature, therefore the production performance was better than that of lipogenic diet.

Key words: glucogenic, lipogenic, production performance, broiler chicken

Introduction

Glucogenic and lipogenic diets refer to the level of fat source being included in the diet, the higher fat content, it is as lipogenic diet. This, even was clearly differenciated from one another by the inclusion of 0 and 2.5% of fat from palm oil in Glucogenic and lipogenic diets, respectively (vanKnegsel et, 2007). Feeding both diets or even a combination or mixed diet would come up with certain consequences relating to the stage of the physiological condition of the animal. Glucogenic source for birds is usually derived from amino acid, containing high protein diet, as well as it was out of D-glucose. While, lipogenic source could be from glucose and triglycerol. Therefore, glucose, amino acid, and glycerol are the keys for lipid metabolim in non ruminants (Larson, 1985).

Heat stress environment may lead to production and physiological impairment. Designing proper diet is a key to maintain these performances. High density diets made of different source or different level of certain ingredient, such as palm oil may be expected to keep production performance. Therefore, it was crucial to evaluate the effects of the glucogenic and lipogenic diets on the performance of broiler chicken under the environment temperature, 28-31 ^{o}C.

Materials and Method

Animals and Diets

Twenty four broiler chicken were allocated into two different diets in completely randomized design. As the purpose of this small study was to evaluate between glucogenic and lipogenic therefore there was no control diet. There were three replications and four birds for each replication. Birds being used were at one week day old at the beginning of the treatment for four weeks.

All ingredients for both diets were the same, with the exception of the level of each feedstuff. These diets were composed with the same level of crude protein (CP), that was 22% (isoprotein) and the same level of metabolizable energy (ME), that was 3050 kcal/kg (isocaloric). The fat content derived from palm oil were 1.05 and 7.0% for glucogenic and lipogenic diets, respectively. Environment temperatures were recorded at am, noon, and pm using thermometer.

Data Collection and Statistical Analysis

Collecting data were conducted weekly for feed intakes and body weights. Body weight gain (BWG) was calculated by the difference at the week- four and week-one. Feed intakes (FI) were accumulated by weeks all the way to the end. Feed conversion ratio (FCR) was calculated as ratio of feed consumed to body weight. Carcass and whole carcass (abdominal organs included) weights were as-

Table 1. Diet composition for broiler chicken

Composition	Glucogenic	Lipogenic
Corn Gluten meal, %	4.96	13.16
Corn grain, %	60	44.17
Soy bean meal, %	25	24.73
Fishmeal , %	7.91	10
Palm Oil, %	1.05	7.0
Calcium Carbonate, %	1.08	0.94
Nutrients:		
Dry Matter, %	85.12	78.49
EE, %	3.48	3.61
CP, %	22	22
ME, Kcal/kg	3,050	3,050
Ca, %	0.69	0.68
P, %	0.47	0.45

sessed at the end of the experiment. Carcass ratios were calculated by comparing toward body weight.

Data of body weight gain, feed intake, FCR, carcass weights were presented as the means with standard deviation and were analyzed using a Paired t- test (Myers, 1986).

Results and Discussion

Nutrient intakes

Looking at the diet composition and nutrient content (Table 1), it is known that both diets have relatively the same ether extract contents, 3.48% and 3.61%, respectively were for glucogenic (1.5% palm oil) and lipogenic (7.0% palm oil) diets. These ether extract levels were lower than that of in ration containing lower corn gluten meal (3%) and about the same amount of soybean meal (24.50%), was 5.92% (Sugiharto *et al*., 2010).

The data revealed that there were slight differences, even though not significant ($p>0.05$) in nutrient intakes between both diets in broiler chicken (Table 2). As in dry matter intake of glucogenic diet bird was a 10.29 g higher (0.6%), its crude protein and metabolizable energy were quantitatively higher as well, even though they contained the isoprotein and isoenergy. However, the ether extract intake of the lipogenic diet bird was slightly higher (3.1%) for as much as 1.82 g. This intake suggested that the lipogenic diet comprising 7% fat in the concentrate resulted in

Table 2. Nutrient intakes of glucogenic and lipogenic diets in broiler chicken

Nutrient Intakes	Glucogenic	Lipogenic
Dry Matter, g	1,690.41±37.63	1,680.12±58.17
Ether Extract, g	58.83±1.31	60.65±2.1
Crude Protein, g	371.89±8.28	369.63±12.80
Metabolizable Energy, kcal	5,155±114.76	5,124. 37±177.42

such amount of ether extract content that was high enough to make a small difference in its intake compared to that of in glucogenic diet birds.

Production performance and Environment Temperature

The results (Table 3) showed that body weight gain in glucogenic diet broiler was a little bit heavier for 29.6 g or 2.28% than that of the lipogenic diet. However, the feed intake was greater significantly ($p<0.05$) for as much as 157.63 g, equaled to 7.94% in lipogenic diet chicken. However, in terms of bulkiness, the dry matter intake in glucogenic diet was quantitatively higher (10 g) in Table 2. On the other hand, glucogenic diet was significantly more efficient ($p<0.05$) in converting into body weight gain. This number (1.54) is about in the same range as in the FCR of fasted and unfasted birds, were 1.49-1.54, respectively (Sugiharto *et al*., 2010). This FCR is also close to the FCR of chicken kept in different density, were about 1.55-1.66 (Sunarti *et al*., 2010). This suggested that the energy availability in glucogenic diet is about at the right level as it is in both fasted and unfasted birds to cover the energy requirement in fulfilling these birds' body weights. It seemed that the lower level of palm oil content in glucogenic diet was more efficient for the broiler kept during this environment temperature (25.8-30.4 ^{o}C; average of 27.9 ^{o}C). While, in 34 ^{o}C, broiler fed 8% palm oil or 8% soybean oil had FCR of 1.89-2.19 (Zulkifli *et al*, 2007).

Table 3. Body weight gain, feed intake, and FCR of broiler chicken fed glucogenic and lipogenic diets

Variable	Glucogenic	Lipogenic
Pre-treatment body weight, g	195.03±7.0	174.50±15.39
Post-treatment body weight, g	1488.89±20.44	1438.75±96.34
Body weight gain, g	1293.85±27.4	1264.25±111.72
Feed Intake, g	1985.92±43.49^{a}	2140.55±10.96^{b}
FCR	1.54±0.0^{a}	1.73±0.11^{b}

Note: Significant differences ($p<0.05$) between treatments.

Inspite of having been the isoenergy diets, with the higher ether extract (EE) content (3.61%) in lipogenic diet, making its energy availability might be a bit slower to be converted into body weight. Therefore, this might be the cause that the birds with this diet consumed more feed, consequently, with the lower body weight, made the FCR is higher in lipogenic diet. These data were in coherent relationship when considering the higher body weight gain, lower feed intake, therefore smaller feed conversion ratio was found in glucogenic diet birds.

Carcass weights and ratios

Carcass weight of birds fed glucogenic diet was slightly different by 111.8 g or 11.87% higher quantitatively, than that of lipogenic diet birds (Table 4). Whole carcass was also quantitatively higher by 127 g or 11.86% in glucogenic diet birds. In fact, both weights of carcass belonged to the birds with glucogenic diets were heavier than that of in lipogenic diet. In addition, the ratios of body weight gain to carcass or whole carcass were higher (0.82 and 0.93) in this glucogenic diet birds, compared to carcass ratio in lipogenic diet broiler. The glucogenic carcass were higher than that of in broiler chicken (around 0.66) with Ca-PFA as reported by Dewi *et al*. (2011).

This suggested that the higher the readiable starch such as in corn grain (60%) in glucogenic diet, the easier it would be metabolized and converted into body weight and carcass in broiler chicken with lower feed intake in the average environment temperature of 27.9 ^{o}C. However, these carcass weights were lower than that of broiler chicken (1242.39–1425.44 g) kept in the average environment temperature of 28.39 ^{o}C as reported by Sunarti *et al*. (2010).

Table 4. Carcass weight of broiler chicken fed glucogenic and lipogenic diets

Variables	Glucogenic	Lipogenic
Carcass, g	1,054±155.40	942.2±140.10
Whole carcass, g	1,198.3±145.43	1,071.3±124.80
Carcass:BWG	0.82±0.03	0.75±0.10
Whole carcass:BWG	0.93±0.11	0.85±0.09

Conclusion

Regarding to feed intake, body weight, feed conversion ratio, carcass weight, and carcass ratio to body weight, it is obvious that the glucogenic diet containing 1.5% palm oil and 60% corn grain is efficient and could be applied for broiler chicken under environment temperature.

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Comparison of Mycotoxin Binders in The Aflatoxin B_1-Contaminated Broiler Diets

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Abstract

Mycotoxin contaminations in poultry feed have been the second major stumbling block in feed industry after increasing price of conventional feedstuff. Among over 300 mycotoxins identified, aflatoxins B1 have been a main concern for animal nutrionist, particularly in tropical regions. The use of mycotoxin binders to replace fungicide or mold killer has been long practiced in poultry diet. An experiment was conducted to determine the effect of mannan-polysaccharides extracted from copra meal in an attempt to bind mycotoxin in broiler diets. Three hundred broiler chicks were used and were allocated to treatment diets in brooder cages from days 1 to 14. On days 15, eighty birds were transferred into the individual metabolism cages for faecal collection and digestibility studies. The rest of the birds were then allocated into floor cages for the bird performance parameters. The treatments diets were: (1) control diet, (2) control diet + 0.10% bentonite, (3) control diet + 0.02% yeast mannan, (4) control diet + 0.02% copra mannan (CM) and (5) control diet + 0.05% CM. The diets were mixed with or without aflatoxin B1. The feeds and water were offered ad-libitum. Total faecal collection was done for three consecutive days for digestibility measurements. A completely randomized factorial design was used to test the effect of 5 diets in the presence of two levels of aflatoxin B1 on bird performance by using 4 replicate cages. Coefficient of feed digestibility was significantly improved due to diet treatments where contaminating the feed with 0.3 ppm aflatoxins B1 impaired feed digestibility. There was an interaction between diet treatments and level of aflatoxins B1 in coefficient of feed digestibility ($P<0.05$). A decreased digestibility was only found in contaminated diet when the diet was not supplemented with mycotoxin binders. The use of 0.05% CM improved feed efficiency. In conclusion, 0.05% CM in the diet can be effectively in broiler diets.

Key words: aflatoxin B1, broiler, copra mannan, mycotoxin binder

Introduction

Mycotoxin contaminations in poultry feed have been the second major stumbling block in feed industry after increasing price of conventional feedstuff. A survey conducted by FAO indicated that 25% of world's grain was contaminated by mycotoxins (Devegowda and Murthy, 2005). Among over 300 mycotoxins identified (Yiannikouris and Jouany, 2002), aflatoxins B_1 have been a main concern for animal nutritionist, particularly in tropical regions. The use of mycotoxin binders to replace fungicide or mold killer has been long practiced in poultry diet.

In the market, the use of clay as a mycotoxin binder has been long used, such as bentonite and zeolite. However, Chestnut (1992) reported the weaknesses of these mycotoxin binders when used in animal feed. These mycotoxin binders have been reported to be effective for aflatoxins only and also bind other essential nutrients that are useful for animal growth. Use of mannan polysaccharide from yeast to bind mycotoxins has been practiced for more than three decades. Hashmi et al., (2006) found that mannan from Saccharomyces cerevisiae could reduce mortality rate from 47,5 % to 30% in poultry fed diet containing 300 ppb aflatoxin. Copra manan extracted from coconut may have the same property as found in yeast mannan. Accordingly, a study was conducted to determine the efficacy of copra mannan as mycotoxin binder in poultry diet.

Material and Methods

Mannan extraction

The method of Kusakabe and Takashi (1988) was used to extract beta mannan from copra meal that was purchased locally. About 16 liters of 24% NaOH were added to 2 kg of copra meal in a 25 liters stainless steel bucket. The mixture of copra meal with NaOH was occasionally stirred for 24 hours at room temperature. The slurry was then filtered through a porous cloth bag. The filtrate was neutralized with 12 N H_2SO_4 until the pH of the solution was about 5.5. The resultants precipitate (copra mannan) being collected by centrifugation, was dialysed against tap water to remove salts. The leftover residue that was identified as copra mannan by Kusakabe and Takashi (1988) was quantified and expressed as a g/kg copra meal.

Animals and Feed

An experiment was conducted at poultry house at University of Tadulako, Palu, Indonesia for 6 weeks. Three hundred unsexed broiler chicks were used in this experiment. The birds were placed in brooder cages based on the treatment diets for 14 days before transferring them into floor pens. On days 15, eighty birds were transferred into the individual metabolism cages for faecal collection and digestibility studies. The rest of the birds were then allocated into floor cages for the bird

Table 1. Composition of the experimental control diet (g/kg)

Ingredients	Starter diet	Grower diet
Full fat soybean meal	249.8	189.7
Maize	602.0	621.0
Fish meal	130.0	130.0
Palm oil	2.7	21.2
Dicalcium phosphate	10.3	25.9
Salt	0.7	6.6
Methionine	1.0	2.5
Lysine	1.5	1.1
Vitamine and Mineral Mixture	2.0	2.0
Calculated composition:		
Crude protein	229.5	210.0
Crude fibre	28.5	25.4
AME (MJ/kg)	13.39	13.40
Lysine	12.3	11.1
Methionine + cystiene	9.0	9.2

Table 2. Experimental diets

Diets	Aflatoxins	Replications
Control diet (D1)	-	4
	+	4
Control diet + 0.02% copra mannan (D2)	-	4
	+	4
Control diet + 0.05% copra mannan (D3)	-	4
	+	4
Control diet + 0.02% yeast mannan (D4)	-	4
	+	4
Control diet + 0.1% Bentonite (D5)	-	4
	+	4

performance parameters. The treatments diets were: (1) control diet, (2) control diet + 0.10% bentonite, (3) control diet + 0.02% yeast mannan, (4) control diet + 0.02% copra mannan (CM) and (5) control diet + 0.05% CM. The diets were mixed with or without aflatoxin B_1. The feeds and water were offered *ad-libitum*. Total faecal collection was done for three consecutive days for digestibility measurements.

A completely randomized factorial design was used to test the effect of 5 diets in the presence of two levels of aflatoxin B_1 on bird performance by using 4 replicate cages.

Statistical analysis

A completely randomized factorial design was used with five different diets, two level of aflatoxins and four replicate cages per treatment. Seven to eight birds were placed in each cage Data were analyzed by analysis of variance. Differences among treatments were tested for significance by using Multiple Range Duncan Test (Steel and Torrie, 1980).

Results and Discussions

Data of the effect of aflatoxin, mycotoxin binders and interaction of aflatoxin and mycotoxin binders on broiler performance are shown in Table 3, 4, and 5.

Table 3. Effects of aflatoxin B1 on broiler performance

Treatments	BWG (g)	FCR	DMD (%)	Faecal moisture (%)
Without aflatoxin	2,201	1.72	79.0[a]	68.8[b]
0.3 ppm aflatoxin B1	2,177	1.74	78.1[b]	70.7[a]
SEM	23.5	0.03	0.4	0.3

Note: Values with the different superscript within a column are significantly different (P<0.05). BWG: body weight gain; FCR: feed convertion rasio; DMD: dry matter digestibility.

Table 4. Effects of mycotoxin binders on broiler performance

Treatments	BWG (g)	FCR	DMD (%)	Faecal moisture (%)
Control (Con)	2,129	1.79[a]	77.4[b]	73.0[a]
Con. + 0.02% copra mannan	2,168	1.75[ab]	78.5[ab]	68.8[b]
Con. + 0.05% copra mannan	2,235	1.68[b]	78.9[a]	68.2[b]
Con.+ 0.02 % yeast mannan	2,246	1.68[b]	78.9[a]	68.9[b]
Con. + 0.1% bentonite	2,165	1.78[a]	78.9[a]	69.9[b]
SEM	29.8	0.02	0.6	0.5

Note: Values with the different superscript within a column are significantly different (P<0.05). BWG: body weight gain; FCR: feed convertion rasio; DMD: dry matter digestibility.

Table 5. Effects of interaction between aflatoxin and mycotoxin binder on broiler performance

Parameters	Treatments										SEM
	D1		D2		D3		D4		D5		
	+	-	+	-	+	-	+	-	+	-	
BWG (g)	2,141	2,117	2,223	2,113	2,201	2,269	2,267	2,226	2,172	2,158	15.1
FCR	1.79	1.79	1.71	1.78	1.70	1.66	1.66	1.71	1.75	1.81	0.01
DMD (%)	78.9[a]	75.8[b]	79.0[a]	77.9[a]	79.0[a]	78.9[a]	79.0[a]	78.8[a]	79.0[a]	78.9[a]	0.3
Faecal-moisture (%)	70.1[a]	75.9[b]	69.6[a]	68.1[a]	66.8[a]	69.6[a]	67.9[a]	69.8[a]	69.7[a]	70.0[a]	0.2

Note: Values with the different superscript within a column are significantly different (P<0.05). BWG: body weight gain; FCR: feed convertion rasio; DMD: dry matter digestibility.

Although it has been believed that contamination of the diet with aflatoxin could deteriorate feed quality and thus bird performance, addition of 0.3 ppm aflatoxin B_1 in broiler diets did not impair broiler performance in the present study. This might be due to a low concentration of aflatoxin given to the birds or a shorter challenged time (42 days). According to Devegowda and Murthy (2005), a decreased body weight gain was observed when the birds were fed a diet containing 2.5 ppm aflatoxin. This concentration is far above the concentration of aflatoxin offered to the birds in this present study. To asses such speculation, an experiment with a longer time of experiment and a higher concentration of aflatoxin is needed.

The more promising figure found in this present study is that there is an improvement in the feed efficiency of birds fed diets supplemented with mycotoxin binder. This finding clearly supports previous reports of Murthy and Devegowda (1995). Among mycotoxin binders used in this current study, the use of either 0.05% copra mannan or yeast mannan could significantly improve feed efficiency. This improvement was not found when the birds were offered diet with only 0.02% copra mannan. This may indicate that 0.02% copra mannan was not effective enough to bind more aflatoxin in the diets supplemented with 0.3 ppm aflatoxin. The use of 0.1% bentonite also did not improve feed efficiency.

Interaction between mycotoxin binders and aflatoxin treatments was found in dry matter digestibility and faecal moisture. A decrease in dry matter digestibility and faecal moisture only took place when aflatoxin-contaminated diet was not supplemented with mycotoxin binders. In conclusion, the use of 0.5% copra mannan can be used as effectively as other commercial mycotoxin binders. Supplementation of the diet with mycotoxin binders decreased moisture content of the faeces.

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Improvement of Nutritive Values of Local Feedstuffs as Mineral Sources for Kampong Laying Hens

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Abstract

Three mineral feeds composed mainly of three locally available materials (limestone originated from Bukit Kamang, freshwater oyster shell and bone ash) were investigated to improve their nutritive values as mineral sources for diet of Kampong laying hens. The first formula was enriched with micro minerals (P1). The second was enriched with micro minerals and supplemented with vitamin (P2). The third was supplemented with both micro minerals and vitamin and amino acid DL-methionin (P3). The minerals were mixed at the level of 6% into basal diet. Two other diets were used as controls. The first was basal diet mixed with 6% of mixture limestone, oyster shell and bone ash (P4), while the second was basal diet mixed with a commercial mineral feed (P5). The five experimental diets were then fed to 150 Kampong laying hens. The hens were divided into 3 groups based on body weight: heavy, medium and light. Each group was subdivided into 5 subgroups in accordance with number of treatments, so that each treatment consisted of 3 replicates containing of 10 hens. Parameter measured included: feed intake, egg production, FCR, eggshell quality, mineral retention and mineral composition of tibia bone. The results showed that laying performances of Kampong hens were significantly improved, when local mineral feeds were fortified with micro minerals, vitamin and amino acid. The hens fed with diet supplemented with local mineral feed and enriched with micro minerals, vitamin and amino acid showed no significantly different performances with that of supplemented with commercial mineral feed.

Keywords: Kampong laying hens, limestone and freshwater oyster shell, mineral feed

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Introduction

Center for intensive rearing of Kampong laying hens in West Sumatra are located in three sub districts: Talawi (Sawahlunto city), Suliki (Lima Puluh Kota district) and Tanjung Emas (Tanah Datar district). Farmers kept the hens of about 1000

– 2000 birds each in individual cages made from wood and bamboos. The eggs produced in term of size and shell strength were found poorer than that of egg from free-scavenging Kampong hens (Anwar and Khalil, 2005).

Poultry under intensive production systems are particularly susceptible to minerals, vitamin and amino acid deficiencies. Laying hens need 3-4% Ca in the diet during production period mainly for eggshell formation (Scholtyssek, 1987). Calcium metabolism and shell formation is dependent upon enzyme systems and various trace minerals are required as co-enzymes. There are six critical micro minerals that are frequently deficient in diet for laying hens, i.e.: cupper (Cu), selenium (Se), iodine (I), iron (Fe), manganese (Mn) and zinc (Zn) (NRC, 1994). Other micro nutrients that are related with mineralization process and frequently deficient in diet for laying hens are vitamin D and B12 and sulfur-containing amino acid. Vitamin D is essential for normal shell calcification (Plaimast and Kijparkorn, 2010) and vitamin B_{12} is an integral part of different enzyme systems (McDowell, 1989), while methionine is generally the first liming amino acid in corn-soybean diet and adequate sulfur amino acids must be present in the diet for maximum egg size (Miles *et al*., 1986, Abd El-Maksoud *et al*., 2011).

Inclusion of mineral-vitamin-amino acid in a premix in formulated diet has become indispensable practice because feed ingredients do not contain all essential minerals, vitamins and amino acid. Such premix might be produced from locally available mineral feedstuffs by fortifying with essential micro minerals, vitamin and amino acid.The province of West Sumatra abounds with mineral feed sources in the form of: limestone, fresh water oyster shell and bone meal. One of the most intensively exploited limestone hill deposit is named Bukit Kamang, located at Kamang Mudik villages, Kamang Magek sub district, Agam district. The meal products not only contains high calcium (Ca) of about 38-40% but are also rich on micro minerals of iron (Fe), manganese (Mn) and selenium (Se) (Khalil and Anwar, 2007). Fresh water oyster (*Corbicula sp*) was abundantly found in fresh water bodies in West Sumatera. The shell parts which are used as feed in coarse ground form contain about 26-30% calcium (Khalil, 2003). Bone meal is produced by small-scale home industries and contained of relatively high Ca and P of about 20.8 % and 12.5%, respectively (Anwar and Khalil, 2005).

The present research aimed to study the effect of supplementation of local mineral formula containing Bukit Kamangs' limestone, fresh water oyster shell and bone meal with micro minerals of Zn, Cu and I and vitamin (D and B12) and sulfur amino acid (methionine) on the laying performances of Kampong hens.

Materials and Methods

Four mineral feeds were formulated which composed mainly of three locally available materials: Bukit Kamangs' limestone, fresh water oyster shell and bone

meal. The first formula, called as local mineral, composed only of Bukit Kamangs' limestone, fresh water oyster shell and bone meal. The second was the local mineral fortified with micro minerals of Zn, Cu and I. The third was the local mineral fortified with both micro minerals and vitamins of D3 and B12. The fourth was the local formula enriched with micro minerals, vitamins and amino acid of methionine. The nutrient compositions of the formulas were justified to the standard requirements for laying hens recommended by Weinreich *et al*. (1994).

Each mineral formula was mixed in the level of 6% with basal diet. Basal diets were prepared by using three main component of commercial layer concentrate, corn and rice bran in the level of 20, 42 and 32 %, respectively. Another diet was mixed with a commercial mineral premix (MINERAL B12 produced by EKA FARMA, Semarang) and considered as control; so that there were in total five experimental diets as treatments:

Treatment 1 (P1): Basal diet + local mineral
Treatment 2 (P2): Basal diet + local mineral + micro minerals
Treatment 3 (P3): Basal diet + local mineral + micro minerals + vitamins
Treatment 4 (P4): Basal diet + local mineral + micro minerals + vitamins + amino acid.
Treatment 5 (P5): Basal diet + commercial mineral feed.

The nutrient and energy compositions were justified to the standard requirements of Kampong laying hens during production period recommended by Mulyono (1999).

The experimental diets were offered to 120 Kampong laying hens for 24 weeks. The hens were divided into three groups based on body weight: light (1150-1349 g/bird), medium (1350-1499 g/bird) and heavy (1500-1800 g/bird). Each group which composed of 40 birds was then subdivided into 5 subgroups in accordance with the number of treatments, so that each experimental unit consisted of 8 birds. Parameters measured included: body weight, feed intake, feed conversion ratio (FCR), hen-day egg production, number and weight of egg production, eggshell quality (weight and percentage of eggshell), mineral retention and weight and mineral composition of tibia bone. All data were subjected to statistical analysis using variance analysis in a completely block design with 5 treatments and 3 blocks as replicates. Duncan's Multiple Range (DMRT) was applied to separate means. Differences were considered significant at $P<0.05$ (Steel and Torrie, 1981).

Results and Discussion

Laying Performances

Results in Table 1 show that total feed intake for 24 weeks ranged 17,655 -18,109 g/bird, while daily feed intake ranged 105 g–108 g/bird. These data did not differ significantly ($P>0.05$). This might be occurred because mineral, vitamin

Table 1. Feed intake, feed conversion ratio and egg production of Kampong laying hens fed diets containing different mineral formula sources for 24 weeks

	Experimental diets with mineral formula sources:				
	P1	P2	P3	P4	P5
Initial body weight, g/bird	1,423.3	1,400.0	1,373.3	1,443.3	1,393.3
Final body weight, g/bird	1,650.0	1,816.7	1,640.0	1,683.3	1,653.3
Total feed intake, g/bird	1,7685.1	18,109.0	17,869.3	17,654.6	17,931.6
Daily feed intake, g/bird	105.3	107.8	106.4	105.1	106.7
Egg production, eggs/bird	47.8^{c}	60.2^{b}	60.7^{b}	70.8^{ab}	74.4^{a}
Egg production, g/bird	2,167.8^{c}	2,551.2^{b}	2,660.8^{b}	2,846.2^{ab}	3,294.4^{a}
Hen-day egg production, %	28.0^{c}	35.9^{b}	36.1^{b}	42.1^{ab}	44.3^{a}
Feed conversion ratio	8.16^{c}	7.09^{b}	6.71^{b}	6.20^{ab}	5.44^{a}

Note: a, b, c, d – values in the rows with different superscript differ significantly (P<0.05).

and amino acid did not have profound influence on body weight and feed intake of birds

Laying performances in terms egg production and feed conversion ratios were significantly (P<0.05) affected by the treatments. Kampong hens fed diet containing local mineral (P1) showed the lowest egg production in term of number, total weight and hen-day egg production and the poorest feed utilization efficiency. Supplementation of local mineral with micro minerals, vitamins and amino acid (P2, P3 and P4) improved laying performances (P<0.05). Egg production increased about 16.1 egg/bird, from 47.7 (P1) to 63.9 egg/bird (P2, P3 and P4), total egg mass 518.3 g/bird (from 2167.8 to 2686.1 g/bird), hen-day egg production 10% (from 28.0 to 38.0%), while feed conversion ratios decreased of about 1.49 (from 8.16 to 6.67). Previous study with commercial laying hens showed that supplementation of local mineral with micro minerals Zn, Cu and I improved laying performances (Khalil, 2010). The beneficial effects of Cu, Zn and I supplementation on laying performances of commercial layers were reported by Swiatkiewicz and Koreleski (2008), El-Husseiny *et al*. (2009) and Cepuliene *et a*l. (2008).

Although the results were not significantly difference, supplementation of local mineral with mixture of micro minerals, vitamins and amino acid gave positive effect on laying performances. As presented in Table 3, Kampong hens fed with diets containing local mineral and fortified with micro minerals and vitamins (P3) or mixture of micro minerals, vitamins and amino acid (P4) showed higher egg production and lower feed conversion ratio than those of fed with diet containing mineral formula fortified with only micro minerals (P2). When local mineral were enriched completely with mixture of micro minerals, vitamins and amino acid (P4), laying performance were found not significantly different with those of commercial

premix (P5). Kampong hens fed on the diets containing local mineral and fortified with micro minerals, vitamins and amino acid (P4) (average: 71 eggs/bird; 2846 g/bird; 42%, respectively) showed not significantly different in feed utilization efficiency and egg production in term of number, total weight and hen-day egg production with those fed with diet containing commercial premix (P5) (74 egg/bird; 3294 g/bird; 44%).

Egg Weight and Eggshell Quality

The results of egg weight, quality of eggshell and mineral retention are presented in Table 2. Average egg weight ranged 38.7 to 40.5 g/egg. There were not significant effect (P>0.05) of supplementation of local mineral with micro minerals, vitamins and amino acid. Lack of influence of micro mineral and vitamin supplementation on egg weight were also reported by some researcher. Mabe *et al.* (2003) reported that the addition of 60, 60 and 10 mg/kg of Zn, Mn and Cu, respectively, to basal diet did not significantly influence egg weight in commercial laying hens. Abdallah *et al* (1994) suggested that remove supplemental iron or some other kinds of minerals (Cu, Zn or Mn) from laying hens diets did not affect the egg weight of hens. Kato *et al*. (2003) reported that supplementation of vitamin B_{12} at 10µg/kg in a corn soy based diet in Lohmann laying hens during the second cycle of production gave no significant effect on egg mass.

The data presented in Table 2 also show that fortifying local mineral with micro minerals, vitamins and amino acid did not significantly (P>0.05) influence shell weight, shell thickness, shell per cent and mineral and ash content of shell. These results were confirmed by Holoubek *et al*. (2002) who reported that the addition

Table 2. Average egg weight, shell weight, per cent of shell weight and shell thickness of Kampong laying hens fed diets containing different mineral formula sources for 24 weeks

Parameter	Experimental diets with mineral formula sources				
	P1	P2	P3	P4	P5
Egg weight, g/egg	40.5	39.7	42.0	38.7	39.7
	(4.2)	*(0.8)*	*(4.1)*	*(1.1)*	*(2.4)*
Eggshell weight, g/egg	3.8	4.5	3.7	3.5	3.8
	(0.4)	*(1.8)*	*(0.1)*	*(0.3)*	*(0.2)*
Per cent egg shell, %	8.7	8.6	8.6	8.5	8.8
	(0.5)	*(0.2)*	*(0.5)*	*(0.6)*	*(0.6)*
Eggshell thickness, mm	0.48	0.50	0.49	0.49	0.50
	-0.01	*(0.00)*	*(0.01)*	*(0.01)*	*(0.00)*

Value in italic parentheses: standard deviation (SD) (±).

of micro minerals of Cu and Fe to feed mixture has not significant impact on the quality of egg shell of commercial layers.

It is concluded that the nutritive values of local mineral formula composed of Bukit Kamangs' limestone, fresh water oyster shell and bone meal were improved by fortifying with micro minerals, vitamins D and B12 and methionine. Laying performances of Kampong hens fed diet containing local mineral and fortified with micro minerals, vitamins and methionine were found not significantly different with those fed diet supplemented with commercial mineral feed.

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The Content of Cholesterol, Fat, Vitamin A and E in the Meat, Liver, and Eggs in Japanese Quails Given Katuk Leaves Extract in the Diet

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Abstract

Katuk leaves extract (Sauropusandrogynus L. Merr) is a medicinal plant that has been studied because it has many benefits. Katuk leaves in animal feed can be used in the form of meal and extract. The advantages of Katuk Leaves Extract (KLE) in the diet are easy to store, need in a relatively small amount and low fiber content. This experiment was conducted using one hundred fifty six female Japanese quails starting at 4 weeks old and raised uptill 10 weeks of age. The parameters observed were the content of cholesterol, fat, vitamin A and E in the meat, liver, and eggs. The treatments were control diet (R0); control diet with 0.15% KLE (R1) and control diet with 0.30% KLE (R2). The quails were divided into 3 treatment groups with 4 replications (13 quails in each replicate). The data of quail performances were analyzed by a completely randomized designed and further with Duncan's test if there were any significant differences in the treatments. The data of the cholesterol, fat, vitamin A and E inthe meat, liver, and eggs were analyzed descriptively. The advantages of giving KLE in quails' diet were decreased the content of cholesterol, fat in the liver and eggs, and increasedthe vitamin A and Econtentin the meat and eggs.

Keywords: cholesterol, katuk (Sauropusandrogynus L. Merr), quails, vitamin A, vitamin E

Introduction

The level of cholesterol intake higher than the level of need along with the lifestyle of the people who tend to consume lots of fatty foods.Food intake with high cholesterol continuously causesthe increasing levels of cholesterol in the blood. Excess cholesterol (hypercholesterolemia) causes harmful health effects. Prevention

of high cholesterol in body such as by eating animal food products that low in fat and cholesterol. Katuk plants *(Sauropus androgynus L. Merr)* is an alternative plant that can decrease the cholesterol egg yolk and layer carcasses (Ibrahim, 2004). The purpose of extraction process is to take some or all substance in the plant. Subekti (2007) reported there were active compounds that contribute of decreasing cholesterol in egg yolks, liver and carcass treatment probably caused by phytosterol particularly stigmasterol high. Katuk leaves contain phytosterols 2.43 g/100 g and 466 mg/100 g dry fresh able to decrease serum of cholesterol, eggs, carcasses, and liver in quail (Subekti, 2007). Phytosterols which covers sterols and stanols of plantsare found in plants as fats. Katuk leaves also contain vitamins A and E. The content of vitamin A which is quite high at 4337.34 µg/g can provide yolk color better. The fuction of vitamin E such as for avian reproduction, improve performance and strengthen the status imunoglobin.

Materials and Methods

The experiment was conducted from June to September 2009. This research used quail (*Coturnix coturnix japonica*) 8-week-old female as many as 208 animals, were placed in battery cages and divided into 3 treatments with 4 replicates (13 quails in each replicate). The Treatment diets were control diet (R0); R0 + 0.15% KLE (R1); R0 + 0.30% KLE (R2).The first prosess of extraction was preparation of making Katuk Leaves Meal (KLM) showed in Figure 1 and Figure 2 presentedthe prosess of Katuk Leaves Extract (KLE). The preparation of KLEused 70% ethanol solvent.The analysis method for cholesterol content in meat, liver and eggs based on the Liebermann-Burchad (Kleiner and Dotti, 1962). The analysis method for fat content in meat, liver and eggs made by sochlet method.Vitamin A and E content in meat, liver and quail eggs were analyzed by HPLC method (High Performance Liquid Chromatography).The experimental design used a completely randomized design.

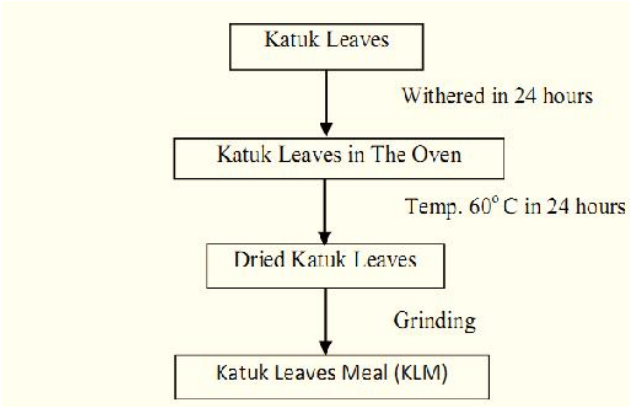

Figure 1. The scheme of Katuk Leaves Meal Prosess (KLM)

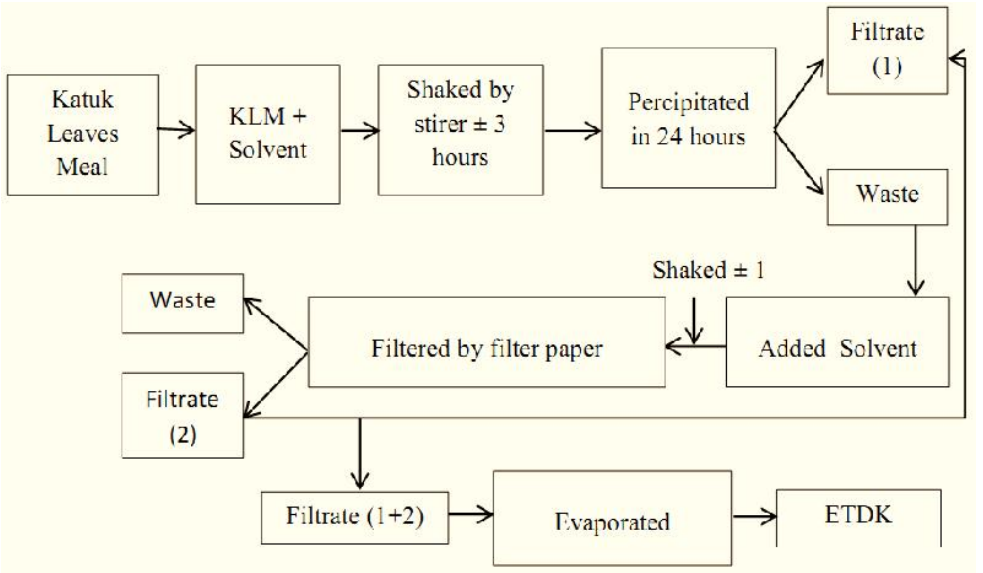

Figure 2. The scheme of Katuk Leaves Extract Prosess (KLE)

The nutrient content of control diet and katuk leaves extract presented in Table 1, the nutrient content of treatment based on calculations presented in Table 2.

Table 1. The Nutrient Content of The Control Diet and Katuk Leaves Extract (As fed)

Nutrient (%)	Material	
	Control Diet*	KLE**
Dry Matter (%)	87.59	70.35
Ash (%)	10.45	5.65
CP (%)	18.21	19
Fiber (%)	9.58	0.21
Fat (%)	5.61	2.40
Beta-N (%)	43.74	43.21
Ca (%)	3.58	0.05
P (%)	1.25	0.03
Bruto Energy (Kal/g)	3980	3122

* Based on Analysis ITP Laboratory, IPB 2010
** Based on Analysis Fish Nutrition Laboratory, FPIK, IPB 2011

Table 2. The Nutrient Content of Treatment Based on Calculations

Nutrien	Diet		
	Control Diet	0.15% ETDK	0.30% ETDK
Dry Matter (%)	87.59	87.56	87.54
Ash (%)	10.45	10.44	10.44
CP (%)	18.21	18.21	18.21
Fiber (%)	9.58	9.57	9.55
Fat (%)	5.61	5.61	5.60
Beta-N (%)	43.74	43.74	43.74
Ca (%)	3.58	3.57	3.57
P (%)	1.25	1.25	1.25
Bruto Energy (Kal/g)	3980	3978	3977

Results and Discussion

Analysis of the cholesterol content (Figure 3) in meat, liver and eggs were conducted when the quails at 14 weeks old. The content of cholesterol in the liver and eggs decreased by increasing levels of KLE in feed. The giving of KLE 0.15% in feedcould decreasethe cholesterol2.92% of the control, while increasing the level of 0.30% KLEcouldlower 22.81% of control.The decreationof cholesterol content in eggs by giving KLE 0.15% as many as 13.86% of control, by increasing the level 0.30% KLE cholesterol content decreased below R1 (0.15% KLE) of 19.86% of control.

The percentage decrease in the cholesterol content in meat, liver and eggs respectively 8.3%, 22.55%, 35.58% from the quail that gave feedcontrol diet. The lowering in cholesterol content with giving KLEwas influenced by the active compound (phytosterols) that contained in the katukleaves.While the role of phytosterols, especially stigmasterol can lower cholesterol in egg yolks, liver, and quail carcass treatment (Subekti, 2007). The levels of cholesterol in liver appeared significantly higher than the meat and eggs, this is because the liver is where cholesterol synthesis in addition to the main gut, skin, testis, and aorta. In addition it is the highest organ role in cholesterol synthesis.

Figure 4 shows the fat content of meat, liver and eggs after 14-week-old quail. The same trend of decrease in cholesterol in the liver and eggs also occurs in fat. Fat content in the liver by giving 0.15% KLEcould decrease 3.21% of control, and by giving 0.30% KLE9.62% fat could be decreased. It also occured in the egg, which fell 6.93% and 16.02% of control by giving KLE respectively 0.15% and 0.30% in the feed. The decreationof fat in liver content, meat and eggs due to lower

cholesterol, because cholesterol is a lipid that has a similar molecular shape fat, or cholesterol were included special types of lipids called steroids.

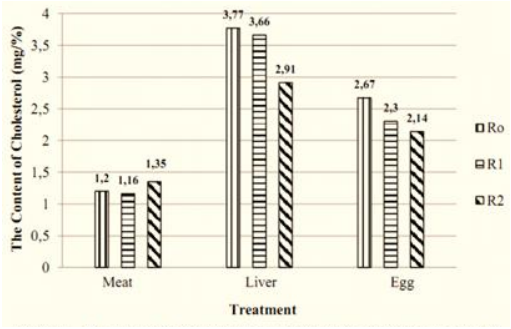

Figure 3. Cholesterol Content in Meat, Liver, and Egg Quail

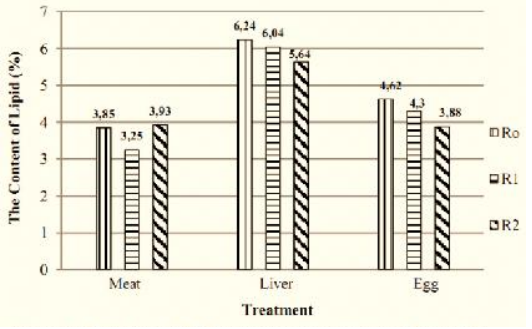

Figure 4. Lipid Content in Meat, Liver, and Egg Quail

The giving 0.15% and 0.30% KLE in the ration of the liver and eggs tend to be lower than the group without giving KLE (R0), it is presumably because the active compounds contained in extracts of katuk leaves is extracted perfectly. The increasing content of vitamin A on meat (Figure 5) with 0.15% and 0.30% KLE in feed as many as 2.44% and 16.81%.The increasingvitamin A of 0,30% KLE(R2) two times larger than the0,15% KLE(R1) because the content of vitamin A is deposited largein meat. Figure 5 shows that the vitamin A content of the highest in the eggs deposited, it is possible because the animals that produce livestock products, if given the treatment it is shown the greatest influence on the resulting product.The same trend is shown in an increase in vitamin E (Figure 6). The content of vitamin E in the liver by giving 0.15% and 0.30% KLEtend to be lower than the quails didn't feed KLE (R0) occured also in the study Subekti (2003).

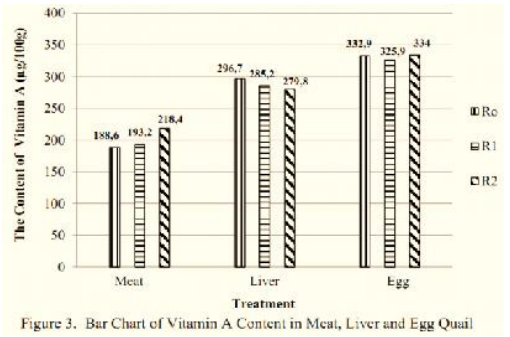

Figure 5. Vitamin A Content in Meat, Liver, and Egg Quail

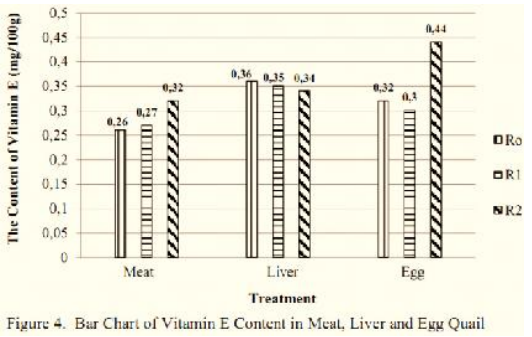

Figure 6. Vitamin E Content in Meat, Liver, and Egg Quail

Conclusions

The advantages of giving KLE in quails' diet were decreased the content of cholesterol, fat in the liver and eggs, and increased the vitamin A and Econtentin the meat and eggs.

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The Effects of Dietary Energy Sources on Immune Organs of Broilers Exposed to Heat Stress

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Abstract

The aim of this experiment was to study the effect of palm oil addition as energy source in the ration on the percentage of limfoid gland, thymus gland and bursa fabricius of broilers exposed to heat stress. The research was conducted in Experimental Station, Faculty of Animal Science, Bogor Agricultural University, in November to December 2011. The experiment was conducted in a Completely Randomized Design (CRD) consisting of two treatment rations with three replications. The treatments were as follow: R1 = ration with 22% crude protein and 3050 kcal Metabolism Energy containing 1% palm oil; R2= ration with 22% crude protein and 3050 kcal Metabolism Energy containing 7% palm oil. The parameter observed were limfoid gland percentage, thymus gland percentage and bursa fabriosis percentage. The result of the experiment showed that the addition of palm oil in the ration until 7% DM basis in broiler'ration did not significantly affect the lymphoid organ percentage, thymus gland percentage, and the bursa fabriosis percentage in broilers exposed to heat stress. It could be concluded that addition of palm oil as energy sources in the ration gives similar effect on broilers exposed to heat stess.

Keywords: broiler, heat stress, lymphoid organ, thymus gland, and bursa fabriosis

Introduction

Global climate change has significant effects on agriculture production, including poultry production. Heat stress is one of big concern in poultry industry, especially in tropical and sub-tropical countries. It has been well known that heat stress could reduce the productivity and increase the mortality of the chicken, that will in turn affects the performance of poultry industry. High environmental temperature could hamper the average daily gain of the chicken, as results of lower appetite and feed intake.

Broiler chicken is known as a homoeothermic animal. Body temperature regulation of this animal was directly affected by environmental temperature. Therefore, as part of its thermoregulation, broiler chicken should control feed intake to meet the need for maintenance, production, and heat production (Furlan & Macari, 2002). Objective of this experiment was to study the effect of palm oil addition as energy source in the ration on the percentage of limfoid gland, thymus gland and bursa fabricius of broilers exposed to heat stress.

Materials and Methods

The experiment was conducted in Experimental Station, Faculty of Animal Science, Bogor Agricultural University, from November to December 2011. Twenty four of 1 week old chickens were used in this experiment. The broiler chickens were then divided into two groups and the treatment ration arrangement was presented in Table 1.

Table 1. Dietary treatment formulation

Ingredients	R1 (%)	R2 (%)
Corn Gluten meal	4.96	13.16
Corn grain	60.00	44.17
Soybean meal	25.00	24.73
Fish meal	7.91	10.00
Oil	1.05	7.00
Calcium Carbonate	1.08	0.94
Total	100	100
Metabolism Energy (kcal/kg)	3050	3050
Dry Matter, %	85.12	78.49
Crude Protein, %	22.00	22.00
Ca, %	0.687	0.682
P, %	0.465	0.454

The experiment was conducted in a Completely Randomized Design consisting of 2 treatment rations with 3 replications. Each experimental unit consists of 4 broiler chickens. The treatments were as follow: R1 = ration with 22% crude protein and 3050 kcal Metabolism Energy containing 1% palm oil; R2= ration with 22% crude protein and 3050 kcal Metabolism Energy containing 7% palm oil. The parameter observed were limfoid gland percentage, thymus gland percentage and bursa fabricius percentage. All data collected were subject to analysis of variance, followed by Duncan's Multiple Range Test (Steel and Torrie, 1995).

Results and Discussions

Average daily environmental temperature and humidity observed at afternoon during the experiment was 30.38 °C and 80.35% respectively. Whereas, comforts zone poultry production is 25 – 28 °C for temperature and 60% - 70% for humidity. This high environmental temperature condition caused the broiler chickens exposed to heat stress during the experiment. This situation also supported by low average daily feed intake as well as high water intake.

Data on the percentage of thymus gland, percentage of lymph, and percentage of bursa fabricius at the end of the experiment were presented on Table 2. It could be seen from the table that long term heat stress caused broiler chicken difficult to achieve the standard body weight, because significant amount of energy in the diet will be used for heat production and heat dissipation. Heat stress also has deteriorated effects on physiological organs such as lymph, thymus gland, and bursa fabriosis.

Table 2. Percentage of thymus gland, lymph, and bursa fabricius at the end of the experiment

Dietary treatment	Lymph (%)	Thymus gland (%)	Bursa fabricius (%)
R1 (carbohydrate as energy sources)	0.22	0.71	0.14
R1 (palm oil as energy sources)	0.21	0.78	0.11

The difference of energy sources did not significantly affect ($P>0.05$) the percentage of thymus gland, percentage of lymph, and percentage of bursa fabricius at the end of the experiment. No significantly different in these parameters probably because of no difference in energy content of the dietary treatments during the experiment. Tizard (1998) stated that bursa fabricius is lymphoid organs that function to support growth and differentiation of cells of antibody system. Moreover, Riddell (2004) stated that bursa fabricius which is located in dorsal part of cloacae produce Cell B which is then synthesized the plasma cell and antibody. Therefore, this organ is very important in body immune system.

Conclusion

Difference energy sources in the diet of broilers exposed to heat stress did not significantly affect the lymphoid organ percentage, thymus gland percentage, and the bursa fabricius percentage.

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Zink Suplementation on Complete Tea Waste Ration (*Camelia sinensis*) to Evaluate Performance Reproduction of Young Rabbit Does

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Abstract

Tea waste (Camelia sinensis) has high protein and crude fiber resources and it has been reported that 30% in the ration could give good performance in growing rabbit. Problem with the waste is lag of Zn absorption due to lignin content so it will effect to the reproduction. The objective of this study was to evaluate the effect of level Zn supplementation on ration containing 30% tea waste on reproduction performance of young rabbit does. Twenty young rabbit does were assigned to four pellet complete rations and synchronization of estrus with such a dose of PGF2α. The treatments were control with Comercial ration ; R1 was ration with 30% tea waste ; R2 was R1 plus 50 ppm Zn and R3 was R1 plus 100 ppm Zn. Parameters observed were pregnant and lactation feed intake (g/head/d), frequency of first matting to pregnant, the pregnant presentation, length of pregnant (day), litter size, birth weight, weaning weight, milk production and mortality pre weaning. Completely Randomized Design was used with four treatments and five replications. Result showed that the treatments were not significant affected yet to the feed intake and all performance reproduction of young rabbit does. It was concluded that 30% of tea waste plus Zn could be used as source of protein and mineral for rabbit does reproduction ration where the result condition same with control ration.

Keywords: rabbit does, tea waste, Zn supplementation

Introduction

Tea waste as a byproduct of manufacture soft drinks industry has potential to use as source of feed, because of the availability and nutritional value. Some studies has been reported that tea waste can be used in rabbit ration up to more than 30% without a negative impact on performance (Khotijah *et al*., 2004). However, the effect of the ration has not been evaluated for the reproductive performance of rabbit. A good reproduction performance by using cheap ration is one of the breeders goal.

Green tea waste has high protein and fiber content. As a roughage they has 29.01% lignin content (Istirahayu, 1993). According to James (1990), fibers with high lignin content could bind some minerals as a result of carboxyl, hydroxyl and methoxyl – lignin bound, so that interfering the mineral absorption which is very important for the reproduction pathway. One of the essensial minerals for the reproduction metabolism is Zn. The functions of Zn are has affect to the growth, reproduction, bone and blood formation, metabolism of nucleic acids, proteins and carbohydrates. Zinc acts as essential components or enzymes activators and called metalloenzim (Scott *et al*., 1982). Zinc plays a role in the process of cell development which needed by somatic cells during pregnancy and weaning. The mineral also involves in the process of protein synthesis for the milk production. It is reported that Zn deficiency during pregnant resulted in birth defects and fetal death (Lutwak-Mann and McIntosh, 1971). Zinc plays a critical role in the repair and maintenance of the uterine lining following calving, speeding the return to normal reproductive function and estrus. Zinc deficiency occurred during the standard dosing period of guideline rabbit developmental toxicity studies may be associated with a modest increase in resorption rate and a transient inhibition of embryonic growth (Pitt *et al*., 1997). So that the presence of Zn in the ration is very important to note. Tea waste utilization in such a mount of reproductive ration requires additional essencial mineral such Zn. This study was aimed to evaluate the effect of level Zn supplementation in ration containing 30% tea waste on reproduction performance of young rabbit does.

Materials and Methods

Animals and diets

A total of 20 young rabbits does of New Zealand White cross breed were used. The rabbits were housed in individual cages in the same room, receiving ration and water ad libitum. Treatments were made up of commercial ration and basal diets varying in supplemental zinc contents, provided as zinc sulfate (ZnSO4) as described in Table 1.

Experimental design

The experimental design was Completely Randomized Design, with four treatments and five replications. The treatments were control with Comercial ration; R1 was ration with 30% tea waste; R2 was R1 plus 50 ppm Zn and R3 was R1 plus 100 ppm Zn. Parameters observed were pregnant and lactation feed intake (g/head/day), frequency of first matting to pregnant, the pregnant presentation, length of pregnant (day), litter size, birth weight, weaning weight, milk production and mortality pre weaning.

Table 1. Ration formulation and their chemical composition as DM basis

Feedstuff	Treatment Ration			
	K	R1	R2	R3
Tea waste (%)	C	30	30	30
Soybean meal (%)	O	20	20	20
Yellow corn (%)	M	26	26	26
Wheat bran (%)	E	17.5	17.5	17.5
Molases (%)	R	5	5	5
Palm Oil (%)	C	0.5	0.5	0.5
$CaCO_{3\ (\%)}$	I	1	1	1
ZnSO4(ppm)	A L	0	50	100
Dry matter (%)	86.45	88.31	88.31	88.31
Ash (%)	7.40	6.15	6.15	6.15
Crude protein (%)	16.59	21.92	21.92	21.92
Crude fiber (%)	9.75	10.58	10.58	10.58
NFE (%)	46.00	44.39	44.39	44.39
Ca (%)	0.82	1.33	1.33	1.33
P (%)	0.35	0.46	0.46	0.46
Zn (ppm)	70.00	50.00	100.00	150.00
NDF (%)	38.67	55.73	55.73	55.73
ADF (%)	16.77	22.2	22.2	22.20
Gross energy (kkal/kg)	4164	3879	3879	3879

Synchronization of estrus and Mating

The animal were treated by synchronization of estrus with PGF2 alpha hormone injections and then mated naturally. The checking of pregnancy were done in 12-14 days after injection. The parameter observed were collected during two months evaluation.

Results and Discussion

The means of dry matter intake of different physiological status were shown in Table 2. Daily dry matter feed intake in all physiological status were notsignificant different in all treatments. The average of dry matter intake ranged from 84.14 to 121 g/head/d and result showed that tea waste supplemented Zn tended to increase

compared to the commercial ration. Does rabbits supplemented with 50- 100 ppm zinc, as well as non-supplemented animals.

Table 2. Dry matter Intake in Different Physiological Status

Physiological Phases	Treatments			
	K	R1	R2	R3
	----------g/head/day----------			
Pre pregnant	85.0±18.32	105,55±14.00	93.560±27.57	104.22±22.61
Pregnant	84.14±21.88	90.47±5.05	95.02±21.14	94.11±14.35
Lactation	94.07±34.41	110.70±25.29	103.60±39.86	121.01±36.84

Performance Reproduction

The frequency of first mating to be pregnant ranged from 1 – 1.33 time. Length of pregnancy ranged from 31.33 to 32.5 days. The data showed as the normal range according to Smith and Mangkoewidjojo (1988). The birth weight from does treated by 30% tea waste with 0-100 ppm Zn supplementation (R2) did not significantly different compared to control diet (commercial) (Table 3). The total number of kits born, weaned and total body weight of kits were similar among the treatments.

Table 3. Performance Reproduction of Young Rabbits Does

Parameters	Treatments			
	K	R0	R1	R2
Frequency of first matting to pregnant	1.00	1.00	1.33	1.00
P Pregnant presentation	100.00	100.00	66.67	100.00
Length of pregnant (day)	32.00	32.50	31.33	31.33
Litter size	5.70	6.75	7.00	7.00
B Birth weight (g/head)	51,58	33,80	41,97	39,43
Weaning weight (g/head)	220.04	205.38	149.28	136.95
Milk production (g/does/day)	62.23	61.72	70.60	67.60
Pre weaning Mortality (%)	5.00	62.50	42.5	34.39

Litter size were not significantly different in all treatments, but there was a tendency to increase litter size with increasing Zn in the ration. Presence of Zn

could maintain the cell division, so that pre natal disability and mortality can be reduced (Lutwak-Mann and McIntoch, 1971).

The treatment did not give significantly different effects on the mother's milk production. There was an increasing of 13.45% in the waste tea ration and 8.63% milk production of comercial ration. Suplementasi Zn had affected to the milk production and growth (Bayu, 2004); the adequacy of Zn vary depending on physiological conditions, such as the amount of Zn that must be absorbed to replace the endogenous expenditure, network formation, growth and milk secretion (Reviana, 2004).

Pre-weaning mortality of kit affected by birth weight, environmental conditions, feed and does milk production and mothering ability (Junus, 1982). The highest kit mortality were in R0 and the lowest in the treatment of the commercial ration. There was a reduction in kit mortality on ration which treatment with Zn supplementation, where in Zn suplementation the mortality reduce from 42.9% to 37%. This value was higher than Lebas *et al.* (1984) reported, where pre-weaning mortality on Europe Rabbit farm was 20%.

Conclusions

Result showed that the treatments were not affected to the feed intake and all performance reproduction parameters of young rabbit does. It was concluded that 30% of tea waste plus Zn could be used as source of protein and essensial mineral source for rabbit does reproduction ration without significant different compared to comersial ration.

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Lipid Deterioration of Layer Diet That Contains Lemuru Fish Oil (*Sardinella longiceps*) and Turmeric (*Curcuma domestica*) as Antioxidant During Storage Period

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Abstract

A research aimed to to evaluate lipid deterioration of layer diet which contains lemuru fish oil (Sardinella longiceps) and turmeric (Curcuma domestica) as antioxidants during storage period. The experimental design used was completely randomized design with 5 x 4 factorial and 2 repetitions. The factors were diet (P) ; P0 : 3% LFO (Sardinella longiceps) in diet, P1 : 3% LFO (Sardinella longiceps) + 0,3% turmeric (Curcuma domestica) in diet, P2 : 3% LFO(Sardinella longiceps) + 0,6% turmeric (Curcuma domestica) in diet, P3 : 3% LFO(Sardinella longiceps) + 0,9% turmeric (Curcuma domestica) in diet, P4 : Used of ration 3% LFO (Sardinella longiceps) + 0,02% BHT. Another factor is storage period (Q) ; Q0 : 0 weeks, Q1 : 2 weeks, Q2 : 4 weeks, Q3 : 6 weeks. The measured were moisture content, extract ether content, free fatty acid and peroxide numbers. The use of antioxidant had significant effect (P<0,01) to decrease free fatty acid, peroxide number and extract ether content. Moreover, storage period had significant effects (P<0,01) in increasing moisture content, free fatty acid, formation peroxide numbers; in contrast, it decreased extract ether content. It is worth noting that the diet at the fourth week of storage could still be used. Similarly, the use of different levels of antioxidant and different storage period had significant effects (P<0,01) on moisture content, free fatty acid and formation of peroxide number. In conclusion that the use of use of 0,9% ; P3 : (3% LFO(Sardinella longiceps)+0,9% turmeric (Curcuma domestica) in diet, turmeric and BHT were able to decrease free fatty acid, and peroxide number formation.

Keywords: antioxidant, free fatty acid, peroxide number and moisture content, storage period

Introduction

Poultry productivity is mostly affected by quantity and quality of diet; which contains highly nutritious components. Therefore, it is crucial to maintain diet qual-

ity in certain storage period as rancidity and nutritive value decrease may occur. Fish oil supplementation is commonly used to fulfil energy requirement in poultry (Saerang, 2003; Fenita *et al* 2005 ; Santoso *et al*, 2010.). Lemuru fish oil *(Sardinella longiceps*) is one of feed supplement, which is a waste product of Lemuru fish oil processing industry. Lemuru fish oil is rich of unsaturated fatty acid and omega-3 (25.17%). Research has been proven that feeding lemuru fish oil is economical as diet supplement (Fenita 2002; Sudibya 1998; Sastrodiharjo *et al* 1998; Fenita *et al* 2005; Fenita *et al* 2010) and stated that Lemuru fish oil supplementation up to 3% significantly increase egg production and has a better feed conversion ratio. A negative effect of Lemuru fish oil supplementation in diet is a short storage period as it may experience deterioration. Diet deterioration such as rancidity and decreasing nutritive value are due to prooxidant which proceeds oxidation process; therefore, a rancidity inhibitor is require to minimize nutrition deterioration (Winarno, 2004) and Fenita *et al* (2005; 2010). There are two types of antioxidants; synthetic and natural antioxidant. Butylated Hydroxytoluena is an effective synthetic antioxidant; however its toxicity contributes negative side effects. In contrast, turmeric (*Curcuma domestica*) is an alternative natural antioxidant that contains antimicrobial agents. Turmeric, a herbal plant, is widely used in Indoesian society as food preservative. According to Suwandi dan Hidayat (1995) antioxidant activity of turmeric is much greater than other herbal plants (curcumin, desmetoxy curcumin, dan bisdesmetoxy curcumin). Sengngeng (1996) mentioned that the use of turmeric of 0,6% as antioxidant as well as natural anti-toxin in broiler chicken as it significantly maintain peroxide number, decreases crude fat and crude aflatoxin. Aim of this research was to evaluate lipid deterioration of layer diet diet which contains lemuru fish oil (S*ardinella longiceps*) and turmeric (C*urcuma domestica)* as antioxidant during storage period.

Materials and Methods

The experiment design used was completely randomized design with 5 x 4 factorial and 2 repetitions. The factors were diet (P); P0: 3% LFO (S*ardinella longiceps*) in diet, P1: 3% LFO(S*ardinella longiceps*) + 0.3% turmeric (C*urcuma domestica)* in diet, P2: 3% LFO(S*ardinella longiceps*) + 0.6% turmeric (C*urcuma domestica)* in diet, P3: 3% LFO(S*ardinella longiceps*) + 0.9% turmeric (C*urcuma domestica)*in diet, P4: Used of ration 3% LFO (S*ardinella longiceps*) + 0.02% BHT. Another factor is storage period (Q); Q0: 0 weeks, Q1: 2 weeks, Q2: 4 weeks, Q3: 6 weeks. The data were analyzed by using analysis of variance (ANOVA), any significant results would be tested by using Duncan Multiple Range Test (Stell and Torrie, 1999). The variables observed were moisture content, extract ether content, free fatty acid and peroxide numbers. Lemuru fish oil is supplied by PT. Bali Mayu Desa Nagara/Nagari. Bali. Feed formulation . The formulation is referred to Rasyaf (1994) and Fenita (2010) with ± 17% crude protein and ± 2750kcal/kg of diet.

Results and Discussion

Results showed that diet which contains lemuru fish oil (S*ardinella longiceps*) and turmeric (C*urcuma domestica)* as antioxidant during storage period had a significant effect on moisture content (P<0,01). In general, a higher amount of turmeric meal resulted in higher moisture content. The higher moisture content may due to moisture content of the turmeric meal. Sumardi (1992) revealed that of 100 grams diet intake, 11.40 grams is moisture content that is contributed by turmeric meal. A DMRT test found a significant result of moisture content (P<0.01). The highest moisture content was at the 6th week of storage (Q3 11.09%); however, the lowest moisture content was at 0 week (Q0 10.37%). Fenita *et al* (2005) stated that the moisture content is probably influenced by storage room temperature and humidity. Furthermore, Syamsu (2003) mentioned that storage period affects moisture content of diet. Antioxidant level and storage period had significant interraction with moisture content (P<0.01). BHT treatment (P4) and six weeks storage period showed the highest moisture content 11.26%; however, the lowest moisture content was at the storage of 0 week and 10% BHT treatment.

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Crude fat content, There was insignificant different between P3 and P0; however, P0 was significantly different from P1, P2 dan P4. Generally, a higher turmeric level resulted in a higher decrease fat content of diet. Lemuru fish oil supplementation in P1, P2, P3 is able to minimize the fat content decrease. According to Sejati (2002) a lower concentration of oxidizeable materials is able to inhibit oxidation; in contrast, at a higher concentration antioxidant materials can be prooxidative. Storage period highly significantly decreased crude fat content (P<0.01). The decrease in crude fat content is also determined by storage period; the longer storage period the more deterioration diet occurs. Ketaren (1986) mentioned that fat rancidity is caused by

Table 1. Average moisture content (%), crude fat content (%), free fatty acid and peroxide number acid

Storage period (Week)	Level of antioxidant					Average	F
	P0	P1	P2	P3	P4		
				Moisture content (%)			
0 (Q0)	10.21^{ab}	10.33^{bcd}	10.48^{cd}	10.71^{f}	10.10^{a}	10.37^{A}	
2 (Q1)	10.33^{bcd}	10.51^{dc}	10.51^{dc}	10.83^{fgh}	10.29^{bc}	10.49^{B}	
4 (Q2)	10.70^{f}	10.85^{fgh}	10.67^{cf}	11.01^{hi}	10.78^{fg}	10.80^{C}	
6 (Q3)	10.97^{hi}	11.15^{ij}	10.96^{ghi}	11.12^{ij}	11.26^{i}	11.09^{D}	
Average	10.55^{A}	10.71^{B}	10.65^{AB}	10.92^{C}	10.61^{AB}		
Interaction							**
				Crude Fat Content (%)			
0 (Q0)	5.28	5.27	5.24	5.04	5.27	5.22^{c}	
2 (Q1)	5.13	5.21	5.22	4.96	5.16	5.14^{bc}	
4 (Q2)	5.00	5.14	5.18	4.90	5.05	5.05^{b}	
6 (Q3)	4.44	4.45	5.01	4.39	4.73	4.60^{a}	
Average	4.96^{ab}	5.02^{bc}	5.16^{c}	4.82^{a}	5.05^{bc}		
Interraction							ns
				Free fatty acid			
32.99^{a}	32.38^{a}	32.44^{a}	31.86^{a}	32.24^{a}	32.38^{A}		
41.94^{c}	39.00^{b}	34.58^{a}	33.47^{a}	33.47^{a}	36.49^{B}		
51.51^{fg}	49.17^{ef}	46.62^{dc}	45.97^{a}	48.66^{c}	48.39^{C}		
62.22^{j}	56.44^{i}	53.05^{gh}	52.98^{gh}	54.75^{hf}	55.89^{D}		
47.17^{C}	44.25^{B}	41.67^{A}	41.07^{A}	42.28^{A}			
Average							**
				Peroxide number (mg O/100 g sampel)			
0 (Q0)	2.15^{a}	2.10^{a}	2.06^{a}	2.03^{a}	2.02^{a}	2.07^{A}	
2 (Q1)	2.66^{b}	2.71^{b}	2.61^{b}	2.51^{b}	2.55^{b}	2.61^{B}	
4 (Q2)	3.85^{e}	3.81^{de}	3.62^{d}	3.66^{de}	3.14^{c}	3.62^{C}	
6 (Q3)	4.30^{g}	4.17^{fg}	4.18^{fg}	4.08^{f}	4.19^{fg}	4.18^{D}	
Average	3.24^{C}	3.20^{BC}	3.12^{BC}	3.07^{AB}	2.98^{A}		
Interaction							**

P0: 3% LFO (S*ardinella longiceps*) in diet, P1: 3% LFO(S*ardinella longiceps*) + 0,3% turmeric (C*urcuma domestica)* in diet, P2: 3% LFO(S*ardinella longiceps*) + 0,6% turmeric (C*urcuma domestica)* in diet, P3: Used of ration 3% LFO(S*ardinella longiceps*) + 0,9% turmeric (C*urcuma domestica)*, P4: Used of ration 3% LFO (S*ardinella longiceps*) + 0.02% BHT. Another factor is storage period (Q); Q0: 0 weeks, Q1: 2 weeks, Q2: 4 weeks, Q3: 6 weeks. Bars with different letters indicate the group mean is significantly different ($P<0.01$).

several factors: (1) Odor absorption by fat (2) Enzymatic action in fat content tissue materials (3) Microbial action (4) Oxygen oxidation. Furthermore, Winarno (2004)

stated that fat deterioration might be caused by tainting, hydrolization and oxygen. It is found that there is no correleation between antioxidant level and storage period (P>0.05).

Free fatty acid, utilization of turmeric *(Curcuma domestica)* antioxidant and BHT significantly decreased (P<0.01) free fatty acid; however, storage period very significantly increased free fatty acid (P<0.01). Effects of antioxidant utilization and storage period on free fatty acid during the experiment are shown on table 2. Utilization of turmeric antioxidant showed that free fatty acid of P3 treatment group was highly significant (P<0.01) compared to P0 and P1. Length of storage mearurement on different storage periods showed that at the storage period of 0 week had the lowest free fatty acid content (32.38%); in contrast, the highest free fatty acid content was at the measurement of week 6th (55,89%). Ketaren mentioned that free fatty acid is formed as fat hydrolisa and oxidation process. Moisture content of diet was increasing as time storage was prolonged (Table 5) which stimulated fat hydrolization of diet so that the free fatty acid would be increasing. A rapid increase of free fatty acid indicates fat deterioration and a decrease in fat content of stored diet.

The storage up to 4 weeks had free fatty acid of 48.39%; however, at the 6 weeks of storage, the free fatty acid of 55.89%. A higher percentage of free fatty acid in diet is an indication that the diet cannot be given to animals as mentioned by Anggorodi (1985) that diet cannot be given to the animals if the free fatty acid content is more than 50%. There was a significant interaction between antioxidant and storage period (P<0.01) on free fatty acid. The lowest free fatty acid was on P3 (0 week storage); whereas, the highest free fatty acid was on P6. In general, antibiotic utilization on diet and length of storage are contributing factors to increase free fatty acid content on diet.

Peroxide number

Results showed that turmeric *(Curcuma domestica)* and BHT (P treatment groups) and storage period (Q treatment groups) were highly significantly affect peroxide number (P<0.01) as shown at Table 2. Different level of antioxidant used had a very significant effect on peroxide number (P<0.01). A rapid increase of fat deterioration was due to an increase in storage period which resulted in an increase in peroxide number. Peroxide formation was stimulated by the present of oxygen and light.which accelerates oxidation process and increases peroxide number; which are followed by hydroperoxide formation as fat. After that, fatty acid is broken down in form of aldehyde, keton and free fatty acid (Ketaren, 1986 ; Fenita, 2010). There are a very significant correlation (P<0.01) between oxidant level on diet and storage period on peroxide formation. The lowest peroxide number was at P4 at 0 week of storage period (2,02 mg O/100 g sample). However, the highest peroxide formation was at P0 and 6 weeks storage period (4,30 mg O/100 g sample). In general, antioxidant on diet and storage period significantly affects peroxide formation. The

less turmeric (Curcuma domestica) on diet with a longer storage period would result in an increase in peroxide number.

Conclusion

In conclusion, 0.9% turmeric *(Curcuma domestica)* and BHT have an equal ability as antioxidant to minimize peroxide formation. BHT as antioxidant is more capable to maintain fat content than 0.9% turmeric *(Curcuma domestica)*.

A prolonged of the storage period may increase moisture content, peroxide formation and to decrease crude fat level. Diet at 4 week of storage period can be given to the animals; however, there is an increase in moisture content, over 50% free fatty acid, peroxide formation and a decrease in fat level.

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Effects of Dietary Supplementation of Natural Feed Additive on Leucocyte Profile and Lymfoid Organ of Broiler

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Abstract

Herbal, probiotic and prebiotic as natural feed additive have potential to replace antibiotic in poultry diet. This experiment was conducted to study the effect supplementation of herbal, probiotic, prebiotic and synbiotic in the diet on leucocyte profile and lymfoid organ of broiler. The experiment used 180 day- old Ross broiler chicks (unsexed) which were reared for 5 weeks. This experiment used completely randomized design with 6 dietary treatments and 3 replications. The dietary treatments were : 1. Basal diet as negative control (without feed additive), 2. Basal diet + antibiotic (bambermycin 0.05%) as positive control, 3.Basal diet + probiotic (EM4, 1cc/l), 4. Basal diet + prebotic (fermacto, 0.2%), 5. Basal diet + herbal mixed (Curcuma longa, Curcuma xanthorriza and Zingiber officinale, 1.5%), 6. Basal diet + synbiotic (EM4 , 1cc/l and Fermacto, 0.2%). At the end of feeding trial (5 weeks of age), 6 birds each treatment were sacrificed to measure the lymfoid organ (sleen and bursal fabricius). Blood sample were collected for heterofil and lymfosit measurement. There were no significant difference on spleen, heterofil (H) and lymfosit (L) due to dietary treatments. However, bursal fabricius of birds fed antibiotic, probiotic and synbiotic diets significantly ($p<0.05$) lower than birds fed negative control diet. Birds fed probiotic diet had lowest mortality as compared to other treatments. In conclusion, probiotic supplementation gives better health performance than other natural feed additive.

Key words: broiler, herbal, lymfoid organ, prebiotic, probiotic

Introduction

Feed additives have become essential components of feeds especially for monogastric animals. The use antibiotics as growth promoters has been banned in many countries due to public concern about their residues in animal products and the development of antibiotics resistance bacteria (Lee *et al*., 2004). This condition lead the nutritionist to investigate natural products as alternative to replace antibiotics.

Probiotic, prebiotic and herbal can be used as alternatives to replace antibiotic as growth promoter in poultry diet. Probiotics are living microorganism, not absorbed in the digestive tract, no tissue residue, no mutation of other mocroorganism and they improved growth and feed efficiency (Lopez, 2000). It was reported that probiotics benefit to host animal by improving immunity, preventing harmful microorganism, providing digestive enzymes and stimulating syntesis B groups vitamin (Rolfe, 2000; Gunai *et al*., 2006; Asli *et al*., 2007). Prebiotic have been defined as non digestible feed ingredient as substrates for growth beneficial bacteria already existing in caecum and colon. Several studies have shown that addtion of prebiotics to poultry diet improved the performace and immune response through improving gut microflora (Piray *et al*., 2007, El- Husseiny *et al*., 2008). Synbiotic was combination of prebiotic and probiotic. The aplication of probiotics and prebiotics in broiler diet improved the performace (Aftahi *et al*., 2006; Kermanshahi and Rostami, 2006).

Herbs and spices can be use as alternatives to AGPs (antibiotic growth promoters) in poultry diet due to their anti microbial properties, antioxidant activity and digestion aid including stimulation of endogenous enzym activity. Among the herbs, *Curcuma longa* (turmeric), *Curcuma xanthorrhiza* (javanese turmeric) and *Zingiber officinale* (ginger) has been used for centuries as medicinal plant in Indonesia. The main bioactive compound from *Curcuma longa* and *Curcuma xanthorrhiza* is curcumin. Curcumin have wide spectrum of biological actions including anti inflammatory, antioxidant, anticarcinogenic, antimutagenic, anticoagulant, antifertilty, antidiabetes, antibacterial, antifungal, antiprotozoa, antiviral, antivibrotic, antiulcer, hypotensive and hypocholesteremic activity as reviewed recently (Chattopadhyay *et al*., 2004). *Curcuma xanthorrhiza* also had essential oil known as xanthorrhizol. This compound have some biological action including antibacteria, antifungal (Rukayadi, 2011), antioxidant, antiplatelet effect, immnomodulatory and cardiovascular protective properties (Jantan, 2011). *Zingiber officinale* or ginger contain zingiberen and zingerol as mayor componen that can stimulate digestive enzyme. The bioactive of ginger can reduce phatogenic bacteria and improve the appetite. Herawati (2010) reported that feeding red ginger in the diet improved broiler performance and feed efficiency.

Today, the farmer can choose one of some natural products for improve poultry productivity and health performance. Therefore, this study was conducted to evaluate the effects of dietary probiotic, prebiotic and herbal on leucocyte profile and lymfoid organ of broiler.

Materials and Methods

Animals and Diets

This experiment was conducted at Laboratory of Poultry Nutrition, Faculty of Animal Science, Bogor Agricultural University. The experiment used 180 day-

old *Ross* broiler chicks (unsexed) which were reared for 5 weeks. The chicks were reared on deep litter system in open side house with standard management conditions. The dietary treatments were : 1. Basal diet as negative control (without feed additive), 2. Basal diet + antibiotic (bambermycin 0.05%) as positive control, 3.Basal diet + probiotic (EM4, 1cc/l), 4. Basal diet + prebotic (fermacto, 0.2%), 5. Basal diet + herbal mixed (*Curcuma longa*, *Curcuma xanthorriza* and *Zingiber officinale*, 1.5%), 6. Basal diet + synbiotic (EM4 , 1cc/l and Fermacto, 0.2%). *Curcuma longa*, *Curcuma xanthorrhiza* and *Zingiber officinale* powder were purchased from local market. These herbal were mixed (ratio 1:1:1) before incorporated at level 1.5% to the experimental diet. Level of herbal in this experiment base on our previous research. Antibiotic, probiotic and prebiotic used in this experiment were commercial product. All level of these feed additive according to company recomendation. Basal diet was formulated to met broiler requirement according to NRC (1994) recommendation. The ingredient and nutrient composition are presented in Table 1. Proximate analysis of basal diet was conducted according to AOAC (1984). At the end of feeding trial (5 weeks of age), 6 birds each treatment were sacrificed to measure the lymfoid organ (spleen and bursal fabricius). Blood sample were collected for heterophil and lymphocyte measurement.

Table 1. Composition of basal diet, *as fed*

Ingredients	%
Yellow corn	46.0
Rice bran	12.2
Soybean meal	27.0
Fish meal	10.0
Vegetable oil	3.5
CaC03	0.8
Premix[1]	0.5
Total	100.0
Nutrients analysis	
Gross Energy (kcal/kg)	4,455
Crude Protein (%)	23.15
Crude Fiber (%)	5.28
Ca (%)	0.97
P total (%)	0.58

Note: [1]Each kg premix contain : Vit.A 9000 IU, Vit.D 2000 IU, Vit.E 12 IU, Vit.B1 0.5 mg, Vit. B6 1 mg, Niacin 15 mg, Panthotenic acid 12.5 mg, antioksidan 100 mg.

Statistical analysis

This experiment used completely randomized design with six dietary treatments and three replications (10 birds/replication). All data were subjected to analysis of variance according to Steel and Torrie (1995). Significant treatment means were futher tested using Duncan's multiple range test (Duncan, 1955).

Results and Discussion

The effects dietary supplementation of natural feed additive on leucocyte profile and lymfoid organs of broiler are presented in Table 2.

There was no significant different on spleen, heterophil and lymphocyte percentage due to dietary treatments. Spleen is cite for lymphocyte production. Supplemented natural feed additive did not affect the spleen organ, it indicated that lymphocyte production did not affected by the treatments. Lymphocytes are important immune cells that play critical role in maintaining immune function. Results in this experiment showed that hen fed natural feed additive had a good immun function as compared to that of antibiotic.

Bursa fabricius of hen fed herbal diet significantly ($p<0.05$) higher than those of other treatments. Herbal used in this experiment contain some bioactive compound from ginger, turmeric and javanese turmeric. These bioactive compound might be cause bursal fabricius bigger than other natural feed additive. Our results showed that natural feed additive can replace antibiotic in poultry diet. Among natural feed additive, probiotic was better than prebiotic, synbiotic and herbal, it has the lowest mortality rate.

Table 2. Effects of natural feed additive on spleen,bursal fabricius, heterofil, lymfosit, and mortality of broiler at 5 weeks of age

Treatment	Spleen (%)	B. Fabricius (%)	Heterophil (%)	Lymphocyte (%)	Mortality (bird)
Basal diet	0.17±0.06	0.12±0.02^{b}	34.67±19.60	64.33±18.04	6
Antibiotic	0.18±0.05	0.09±0.01^{c}	22.33± 4.04	77.00± 5.00	5
Herbal	0.14±0.01	0.36±0.01^{a}	20.67±15.04	78.67±14.57	8
Probiotic	0.17±0.04	0.08±0.01^{c}	51.33±24.83	48.33±25.38	3
Prebiotic	0.24±0.09	0.11±0.03^{b}	21.00± 8.19	78.00± 7.94	8
Synbiotic	0.20±0.07	0.09±0.01^{c}	36.67± 5.69	62.67± 5.13	4

Note: $^{a-c}$ Means in the same coloum with different superscript are significantly different ($p<0.05$)

Conclusion

In conclusion, probiotic, prebiotic, synbiotic and herbal could replace antibiotic as antibiotic growth promoter in poultry diet. Among natural feed additive, probiotic was better in decreasing mortality.

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Effects of Dietary Supplementation of Herbal Mixed on Ammonia and Protein Content of Laying Hen Manure

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Abstract

Poultry manure can be valuable resource as a fertilizer, it can also a potential to air pollution. This experiment was conducted to study the effect supplementation of herbal mixed (Naturbro®) on ammonia and protein content of laying hen manure. Thirty six Hy-line pullets (19 weeks of age) were randomly assigned to cage which were reared for 6 weeks. This experiment used completely randomized design with 4 dietary treatments and 3 replications. Birds were fed basal diet as control or basal diet supplemented with 0.5, 1.0, 1.5% Naturbro®, respectively. Feed and water were provided ad libitum. At the end of feeding trial, manure was collected for 24 h and immediately frozen for ammonia and protein analysis. The results indicated that all supplemented treatments significantly ($p<0.05$) reduced ammonia production in manure as compared to the control diet. However, the effect of herbal mixed on protein content was not consistent, only birds fed 0.5% herbal mixed significantly ($p<0.05$) reduced protein content in manure. In conclusion, herbal mixed at level 0.5% was effective to reduced ammonia and protein content in laying hen manure.

Key words: ammonia, herbal mixed, laying hen, manure, protein

Introduction

Poultry industry must be environmentally sound to ensure its long-term sustainable growth. Poultry manure can be valuable resource as fertilizer but also can be a pollution to the environment especially to air pollution. Ammonia (NH_3) is one of mayor environment concern due to its emmision led to atmospheric acid deposition and impaired poultry productivity. Many studies reported that high level NH_3 on the farm could reduce feed effeciency, growth rate and egg production, damage the respiratory tract and impaire immune responses (Miles *et al*., 2004; Reece *et al*.,1980; Deaton *et al*., 1984; Nagaraja *et al*., 1984). Reducing NH_3-volatilization is very importan to maintain human and animal health and clean environment.

Many experiments had demonstrated that dietary manipulation to be useful tool to reduce ammonia. Kim and Patterson (2004) reported that zinc supplementation in broiler diet reduced nitrogen loss in poultry manure without detrimental effect on grorwth performance. Reducing protein content in poultry diet with addition amino acid synthetic had benefit effect to reduce nitrogen in poultry manure, thus potential for lowering NH_3 emmision to environment (Namroud *et al*., 2008; Ferguson *et al*., 1998). Feeding probiotic and prebiotic (oligofructose) in poultry diet reduce volatile ammonia and urease activity in broiler excreta (Yeo and Kim, 1997; Yusrizal and Chen, 2003).

Indonesia has many herbal that potential to be use as feed additive, but research related to ammonia in poultry manure is still limited. The objectives of this study were to determine whether supplementation of herbal mixed in the diet could be used to reduce ammonia and protein in laying hen manure without adversely affecting bird production.

Materials and Methods

This experiment was conducted at Laboratory of Poultry Nutrition, Faculty of Animal Science, Bogor Agricultural University. Thirty six *Hy-line* pullet (19 weeks of age) were randomly assigned to individual cage which were reared for 6 weeks.

Table 1. Composition of basal diet, *as fed*

Ingredients	%
Yellow corn	49.0
Rice bran	14.0
Pollard	8.0
Soybean meal	17.7
Fish meal	4.0
Palm oil	3.0
CaC03	3.7
DCP	0.6
Total	100.0
Nutrients analysis	
Gross Energy (kcal/kg)	3,456
Crude Protein (%)	16.60
Crude Fiber (%)	4.82
Ca (%)	4.82
P total (%)	0.88

Birds were fed basal diet as control or basal diet supplemented with 0.5%, 1.0%, 1.5% Naturbro® (comercial herbal mixed), respectively. Basal diet was formulated to met layer requirement according to NRC (1994) recommendation. The ingredient and nutrient composition are presented in Table 1. Proximate analysis of basal diet was conducted according to AOAC (1984). Feed and water were provided *ad libitum*. At the end of feeding trial, manure was collected for 24 h and immediately frozen for ammonia and protein analysis.

Statistical analysis

This experiment used completely randomized design with four dietary treatments and three replications (3 birds of each replication). All data were subjected to analysis of variance according to Steel and Torrie (1995). Significant treatment means were futher tested using Duncan's multiple range test (Duncan, 1955).

Results and Discussion

The effects of supplementation of herbal mixed on ammonia, water content and crude protein of laying hen manure are shown in Table 2.

Ammonia content in manure of hen fed herbal diet reduced significantly ($P<0.05$) as compared to that of control diet. Ammonia content in laying hen manure reduced 46.17%-56.36% due to the herbal supplementation 0.5%–1.5% in the diet. This finding could be due to the action of some component in herbal mixed. NaturBro® contains *Curcumae rhizoma*, *Sesbania folium* leaves, *Melaleuceae fructus* and *Caryophylli folium* leaves. Each herbal in NatureBro® content antibacterial properties, beside other function for improving metabolism in poultry. *Curcuma rhizoma* had curcumin as antibacteria, *Sesbania folium* leaves had saponin as antibacteria and *Caryophylli folium* leaves had essential oil as antibacteria. All of these antibacterial properties in NatureBro® inhibit ureolitic bacteria activity to produce urease enzyme, therefore ammonia production reduced significantly ($P<0.05$). This

Table 2. Effects of suplementation of mixed herbal on ammonia, water, and crude protein content of laying hen manure

Treatments	NH3 (mM)	Water content (%)	Protein (%DM)
Control	108.82 ± 16.31^{a}	87.94±3.72	33.64 ± 0.65^{A}
Herbal (0.5%)	57.29 ± 43.70^{b}	85.77±2.18	20.95 ± 4.98^{B}
Herbal (1.0%)	47.49 ± 11.65^{b}	86.73±2.07	32.77 ± 1.42^{A}
Herbal (1.5%)	58.58 ± 22.66^{b}	87.43±1.95	33.02 ± 3.32^{A}

Note:Superscript in the same coloum in capital letter are significantly different ($p<0.01$), while superscript in small letter are significantly different ($p<0.05$)

finding was in agreement with Yeo and Kim (1997) who reported that supplementation probiotic decreased urease activity in small intestine of broiler.

There was no significant different in water content of laying hen manure due to dietary treatments. Water content was one of many factors that influence ammonia production, but our results showed that no relation between ammonia production and water content. This finding was in agreement with Kitai and Arakawa (1979) who reported that no relationship between water content and ammonia production in manure due to antibiotic treatment in broiler diet.

Protein content in manure of hen fed 0.5% herbal diet was reduced significantly ($P<0.05$) as compared to other treatments. Herbal mixed at level 0.5% in laying hen diet improved protein digestibility, therfore protein retention was increased and protein in manure was decreased significantly ($P<0.05$). Reduction protein content in manure will reduce ammonia production directly, because nitrogen that will be converted to ammonia already decreased.

Conclusion

It was concluded that suplementation 0.5% herbal mixed in the diet was effective to reduced ammonia production and protein content in laying hen manure.

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The Effect of Feeding Fermented *Jatropha curcas* Meal on Percentage of Carcass and Giblets of Kampong Chickens

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Abstract

Jatropha curcas meal (JCM) is potential as poultry feed due to its rich in nutrients content (24.71% protein). Fermentation of JCM using Rhizopus oligosporus decreased the phorbolester, but the crude fiber and phytic acid were still high. The objective of this experiment was to evaluate the effect of feeding fermented JCM using Rhizopus oligosporus supplemented with cellulase and phytase on percentace of carcass and giblets of kampong chickens. The experiment used 40 birds of 200 kampong chickens which were reared from day old chicks up to 10 weeks of age. This experiment used completely randomized design with 5 treatment diets and 4 replications, each replication used 10 birds. The diets were: T0 (control diet, without JCM), T1 (the diet contained 5% untreated JCM), T2 (the diet contained 5% fermented JCM and cellulase 200 ml/ton), T3 (the diet contained 5% fermented JCM and 1000 FTU phytase), and T4 (the diet contained 5% fermented JCM and cellulase 200 ml/ton and 1000 FTU phytase). The parameters observed were percentage of carcass, heart, liver, spleen, kidney, gizzard and pancreas of 6 and 10 weeks old of the birds. The results showed that feeding untreated or fermented JCM 5% did not influence the percentage of carcass and giblets of 6 weeks old as well as of 10 weeks old kampong chickens, except for the gizzard of 10 weeks old. The gizzard of the T3 was higher ($p<0.05$) than that of the control. It was concluded that feeding untreated as well as fermented Jatropha curcas 5% was safe for the kampong chickens.

Keywords: carcass, fermentation, giblets, Jatropha curcas meal, kampong chicken

Introduction

Jatropha curcas meal (JCM) is potential as poultry feed due to its rich in nutrients content. JCM with shell contains 24.71% protein (Sumiati *et al*., 2008),

the seed kernels contains 31-34.5% protein (Martinez –Herrera *et al*., 2006). The gross energy of kernels ranged from 31.1 to 31.6 MJ/kg DM, and the levels of amino acids, except lysine, were higher than that of the FAO/WHO reference protein for a five year old child on a dry matter basis (Martinez –Herrera *et al*., 2006). The availability of this rich nutrients is limited by toxins and antinutrients contained in the meal. These toxic and antinutrients include curcin(lectin), tannin, trypsin inhibitors, phytate, saponin and phorbolesters (Francis *et al*., 2006). Apart from these, phorbolesters that are present at high levels in the kernels have been identified as the main toxic agent responsible for toxicity (Makkar *et al*., 1997). Untreated *Jatropha curcas* meal was toxic to rats, mice and ruminants (Becker and Makkar, 1998) as well as to poultry (Sumiati *et al*., 2007). Its need to detoxify the JCM in order to fully ultilize of the meal. Sumiati *et al*. (2007) conducted various treatments (physical, combination of chemical + physical, and biological) to detoxify Indonesian *Jatropha curcas* meal as poultry feed. Fermentation using *Rhizopus oligosporus* was the best method to detoxify the toxins and thus increasing the nutrititive value of the *Jatropha curcas* meal for poultry. However, the crude fiber and phytic acid of the meal were still high. Poultry can not digest fiber, especially cellulose, even the fiber could interfere other nutrients contained in the feed. Sing (2008) reported that phytic acid is an anti-nutritional constituen of plant derived feeds. As a reactive anion, it forms a wide variety of insoluble salts with mineral including phosphorus, calcium, zinc, magnesium and copper. Therefor, the objective of this experiment was to evaluate the effect of feeding fermented JCM using *Rhizopus oligosporus* supplemented with cellulase and phytase on percentace of carcass and giblets of kampong chickens.

Materials and Methods

The experiment used 40 birds of 200 kampong chickens which were reared from day old chicks up to 10 weeks of age. The JCM was fermented using *Rhizopus oligosporus*. This experiment used completely randomized design with 5 treatment diets and 4 replications, each replication used 10 birds. The diets were: T0 (control diet, without JCM), T1 (the diet contained 5% untreated JCM), T2 (the diet contained 5% fermented JCM and cellulase 200 ml/ton), T3 (the diet contained 5% fermented JCM and 1000 FTU phytase), and T4 (the diet contained 5% fermented JCM and cellulase 200 ml/ton and 1000 FTU phytase). The composition of experimental diets is presented on Table 1.

The parameters observed were weight percentage of carcass, heart, liver, spleen, kidney, gizzard and pancreas of 6 and 10 weeks old of the birds. The data were analysed using ANOVA (analyses of variance) according to Steel and Torrie (1995).

Table 1. The composition of the experimental diets

Ingredient	Treatments (%)				
	T0	T1	T2	T3	T4
Yellow corn	51.23	53.21	53.21	53.21	53.21
Rice bran	20.50	15.00	14.50	14.50	14.50
Soybean meal	17.00	16.50	16.50	16.50	16.50
Untreated *J. curcas* meal	0.00	5.00	0.00	0.00	0.00
Fermented *J. curcas* meal	0.00	0.00	5.00	5.00	5.00
MBM	7.50	7.00	7.00	7.00	7.00
Palm oil	3.00	2.50	3.00	3.00	3.00
Salt	0.10	0.10	0.10	0.10	0.10
Vit-min mix	0.50	0.50	0.50	0.50	0.50
Dl-methionine	0.173	0.187	0.187	0.187	0.187
Total	100	100	100	100	100
Cellulase, ml/ton			200	0	200
Phytase, FTU/kg[1)]			0	1000	1000
Calculated nutrients[2)]					
ME, kcal/kg	2,855.64	2,862.71	2,865.11	2,865.11	2,865.11
CP, %	18.23	18.39	18.26	18.26	18.20
EE, %	5.60	5.15	5.43	5.43	5.40
CF, %	3.81	4.77	5.65	5.65	5.65
Ca, %	0.91	0.91	0.91	0.91	0.91
nPP, %	0.61	0.56	0.56	0.56	0.56
Na, %	0.14	0.13	0.13	0.13	0.13
Lysine, %	0.83	0.83	0.82	0.82	0.82
Methionine, %	0.36	0.37	0.37	0.37	0.37
Meth + cystine, %	0.62	0.62	0.62	0.62	0.62

[1)] DSM Nutrition Product
[2)] Nutrient compositions based on Leeson and Summers calculation (2005)

Results and Discussion

Fermentation JCM using *Rhizopus oligosporus* decreased phorbolesters, trypsin inhibitors, phytic acid, and saponin. Feeding untreated or fermented JCM 5% in the diets did not influence the percentage of carcass and giblets of 6 weeks old (Table 2) as well as of 10 weeks old (Table 3) kampong chickens, except for the gizzard of 10 weeks old. The gizzard of the T3 was higher ($p<0.05$) than that of the

control. The increasing of gizzard was due to higher crude fiber in the diet of T3, i.e. 5.65% compared to the control diet, i.e. 3.86%. Feeding 5% of untreated as well as fermented JCM were safe for the liver, heart, kidney, pancreas, and other giblets of the kampong chickens. It indicated that the phorbolesters contained in the diets was low, and it indicated that the JCM used in this experiment was from *J.curcas* seed contained low phorbolesters. Makkar *et al*. (1998) reported that there were different varieties of J.*curcas*, non-toxic and toxic varieties. The toxic varieties contained phorbolesters up to 2.7 mg/g kernel and non-toxic ones just contained up to 0.11 mg/g kernel. Sumiati *et al*. (2010) reported that untreated JCM used in this experi-

Table 2. Percentage of giblets of kampong chickens at 6 weeks of age

Giblets	Diet treatments (% of live weight)				
	T0	T1	T2	T3	T4
Heart (%)	0.58±0.09	0.50±0.08	0.51±0.04	0.63±0.08	0.60±0.15
Liver (%)	2.75±0.12	2.77±0.51	2.71±0.36	2.85±0.30	2.81±0.16
Spleen (%)	0.28±0.05	0.27±0.07	0.28±0.10	0.34±0.02	0.27±0.08
Kidney (%)	0.73±0.13^{AB}	0.55±0.20^{A}	0.78±0.12^{AB}	0.97±0.17^{B}	0.84±0.26^{AB}
Gizzard (%)	4.04±0.34	4.23±0.49	4.88±0.31	4.00±0.67	4.41±0.40
Pancreas (%)	0.32±0.08	0.34±0.07	0.34±0.08	0.23±0.02	0.36±0.03

Mean values within the same row with different superscripts are significantly different (P<0.01).
T0= control diet, without JCM, T1= the diet contained 5% untreated JCM, T2= the diet contained 5% fermented JCM and cellulase 200 ml/ton, T3= the diet contained 5% fermented JCM and 1000 FTU phytase, T4= the diet contained 5% fermented JCM and cellulase 200 ml/ton and 1000 FTU phytase.

Table 3. Percentage of giblets of kampong chickens at 10 weeks of age

Giblets	Diet treatments (% of live weight)				
	T0	T1	T2	T3	T4
Heart (%)	0.46±0.14	0.50±0.11	0.50±0.04	0.48±0.06	0.50±0.05
Liver (%)	1.95±0.43	2.28±0.26	2.05±0.30	2.01±0.16	2.18±0.24
Spleen (%)	0.30±0.06	0.32±0.12	0.22±0.05	0.23±0.05	0.40±0.22
kidney (%)	0.75±0.33	0.94±0.22	0.74±0.10	0.62±0.21	0.74±0.08
Gizzard (%)	3.20±0.09^{a}	3.94±0.12^{ab}	3.60±0.13^{ab}	4.06±0.95^{b}	3.40±0.62^{ab}
Pancreas (%)	0.33±0.21	0.35±0.04	0.26±0.04	0.32±0.03	0.32±0.11

Mean values within the same row with different superscripts are significantly different (P<0.01).
T0= control diet, without JCM, T1= the diet contained 5% untreated JCM, T2= the diet contained 5% fermented JCM and cellulase 200 ml/ton, T3= the diet contained 5% fermented JCM and 1000 FTU phytase, T4= the diet contained 5% fermented JCM and cellulase 200 ml/ton and 1000 FTU phytase.

ment contained 24,33 µg/g phorbolesters and the fermented meal contained 15.28 µg/g phorbolesters.

Feeding high phorbolesters *Jatropha curcas* meal at the level of 5% in the diet to the broilers caused 100% mortality at the age of 22 days and it damaged the liver as well as kidney (Sumiati *et al*., 2007)

Conclusions

It was concluded that feeding untreated as well as fermented jatropha curcas meal 5% in the diet was safe for the kampong chickens.

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Dietary Supplementation of *Andrographis Paniculata* Nees Meal on Performance and Serum Cholesterol of Laying Hen

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Abstract

Andrographis paniculata (sambiloto) is well known as medicinal plant and use as supplement in the poultry ration. The objective of this research was to evaluate the effect sambiloto meal as feed additive in the diet on performance and serum cholesterol in laying hen. Thirty six of laying hens were used in this experiment. The basal diet contained 16 % crude protein and 2850 kkal/kg metabolizable energy. Completely randomized design was used in this experiment with four treatments and three replications (3birds/replication). The treatment were basal diet as control or basal diet + 0.3 g, 0.6 g, 0.9 g sambiloto meal/ kg live weight of laying hen, respectively. The parameters observed were feed consumption, hen day production, egg weight, feed conversion, mortality, triglyceride, HDL, LDL, and total cholesterol in serum. The results showed that the diet with 0.6 g sambiloto meal/kg live weight of laying hen significant (P<0.05) increased of feed consumtion and hen day production but was not influence on egg weight and feed conversion ratio. The sambiloto meal significantly (P<0.05) reduced total serum cholesterol and LDL on level 0.9 g sambiloto meal/ kg live weight of laying hen.

Keywords: Andrographis paniculata, cholesterol, laying hen, performance, sambiloto

Introduction

Andrographis paniculata is known as medicinal plant and use as supplement in the poultry ration. *A. paniculata* has many kind of names,such as Sambiloto, Ki Oray, Ki Peurat Bidara Sadilata, Sambilata, Takila, Ampadu and Pepaitan (Hanan, 1996). The *A. paniculata* is available and spread enough throughout the Indonesia. The active compound of *A. paniculata* are andrografid and neoandrografolide (Santa, 1996). Andrografolide have the effect as imunostimulan and antibacteria (Puri ***et al,*** 1993). The concentration of andrografolie was varies depending on the area for example in Bogor, Sukabumi and Sukaharjo are 1.92 %; 1.96% and 2,1%

respectively. The leaf extract of *A. paniculata* contain a noticeable amount of total phenol (5.96 mg/g) which play a major role in controlling antioxidant (Prakash S. *et al,* 2011). A mixture *of Andrographis* and mengkudu (*Morinda citrifolia*) extract given through drinking water produced body weight gain and feed efficiency better than the control (Zainuddin, 2003).

Materials and Methods

Animals and Housing

Thirty six laying hens 33 weeks of age with average 1401.4 ± 103.3 g of body weight were used in this experiment. The experiment were conducted for 7 weeks and all chicken were reared in layer cages.

Experimental Diet

Basal diet contained 16 % crude protein and 2850 kkal/kg metabolisable energy. All chickens were fed and water drinking *ad libitum*. Basal diet was supplemented with *A. paniculata* meal: 0.3 g, 0,6, 0,9 g/kg body weight. The nutrient composition of *A. paniculata* meal was presented in Table 1.

Andrographis paniculata Meal

A. paniculata leaf were collected from local area. The leaves of A.*paniculata* was sun-dryer, powdered and kept ready for experimental used.

Design of Experiment

Completely randomized design was used in this experiment divided into four treatments and three replications. The treatments were basal diet + 0.3 g *A.paniculata* meal/kg BW, basal diet + 0.6 g *A. paniculata*/kg BW, and basal diet + + 0.9 g *A. paniculata* meal/kg BW. Parameters observed were feed consumption, hen day production, egg weight, feed conversion rasio (FCR), triglyceride, HDL, LDL, and cholesterol total in serum. The feed ingredient and nutrient composition is presented in Table 2.

Table 1. The nutrient composition of *A. paniculata* leaves meal

Nutrient	Composition (%)
Moisture	88.01
Crude protein	11.03
Crude fiber	22.75
Ether extract	3.04

Table 2. Ingredients and chemical composition of the the layer ration in the experiment

Feed Ingredient	Total (%)
Yellow corn	56.75
Rice bran	11.4
Pollard	2
Fish meal	6
Soybean meal	14.5
Coconut oil	1.5
DCP	0.25
CaCO3	7.6
Chemical composition :	
Metabolisable energy (kkal/kg)	2900
Crude protein (%)	16
Calsium (%)	3.3
Phospor non Phytat (%)	0.34
Lysin (%)	0.89
Methionin (%)	0.33

Collection and Analysis of Data

The collected data were analysed using analysis variance procedure and Duncan's Multiple Test was used to compare differences between treatmen means. The data on daily feed consumption, egg production and egg weight and feed conversion ratio were calculated. Serum cholesterol triglyceride, HDL, LDL were collected at laying hen 40 weeks of age

Results and Discussion

The feed consumption, hen day production, egg weight and FCR are shown in Table 3. The results showed that hen fed 0.6 g *A. paniculata* meal per kg BW significantly ($P<0.05$) increased in hen day production as compared to other treatments. But hen fed 0.3 and 0.9 g per kg BW had lower feed consumption and hen day production than control. This was due to increase the feed consumption but FCR did not significant. The andrografolide active substance caused growth regulator (increased appetite), which can increase the consumption of nutrients for egg formation. *A. paniculata* had the effect of imunostimulan and antibacteria (Puri *et al*., 1993) was caused performance at hen fed 0.6 g *A.paniculata* meal highest. Ulfah (2006) also reported that mechanism of active substance medicinal plant was affects as appetite stumulating substance, digestion enhancers, bacterial steering agents,

Table 3. Mean values of feed consumption, egg production, egg weight, and FCR for layer

Parameters	Treatment groups			
	P1	P2	P3	P4
Feed consumption g/bird/day	73.91±32.8[b]	69.82±56.08[a]	89.81±38.80[a]	70.19±74.64[b]
Hen day production %	37.3±4.73[b]	42.47±9.47[b]	62.38±2.32[a]	43.09±1.71[b]
Egg weight g/egg	53.45±2.50	50.23±3.38	48.96±5.84	46.58±7.21
Feed conversion ratio	3.54±0.45	3.57±0.49	3.72±1.39	3.51±0.20

P1= basal diet, P2= basal diet + 0.3 g/ kg BW, P3= basal diet + 0.6 g/ kg BW, P4= basal diet + + 0.9 g/ kg BW.

Table 4. The effect of *A. paniculata* meal in laying diet on triglyceride, HDL, LDL and cholesterol total serum

Variable	Treatment			
	P1	P2	P3	P4
Triglyceride mg/100 ml	124.11±4.69	109.20±11.72	135.20±8.51	124.80±16.74
HDL mg/100 ml	52.17±2.11	52.64±7.49	53.84±3.65	43.62±4.38
LDL mg/100 ml	79.06±5.46[bc]	84.19±11.62[c]	68.6±4.62[ab]	65.79±3.02[a]
Total cholesterol mg/100 ml	168.89±7.82[b]	183.4±17.25[c]	160.08±7.10[ab]	143.87±4.29[a]

P1= basal diet, P2= basal diet + 0.3 g *A. paniculata* meal/kg BW, P3= basal diet + 0.6 g *A. paniculata* /kg BW, P4= basal diet + + 0.9 g *A. paniculata*/kg BW.

metabolic modifiers, odour neutralizing component on regulating of performance health condition of animals. Mathiavanan *et al*. (2006) reported that *A. paniculata* was use 2 g/kg was gave positif responce on performnace of broiler chicken.

The hen fed 0.9 g *A. paniculata* meal per kg BW significantly ($P<0.05$) decreased serum cholesterol total and LDL as compared than other treatments (Table 4). It showed that andrografolide play a role in lowering fat absorption. Nugroho (2001) reported that mice fed extract of A. *paniculata* at 160 mg/100 g body weight for 8 weeks decreased of cholesterol total but increased of HDL. But the result of this experiment showed that of serum LDL and HDL were decreased. Therefore, *A. paniculata* meal can used to prevent atherosclerosis at level 0.9 g/kg BW.

Conclusion

Dietary supplementation of *A. paniculata* meal at 0.6 g per kg BW was effective to increased egg production. *A. paniculata* meal at 0.9 g per kg BW in ration was effective to decreased serum total cholesterol and LDL.

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Effect of Mannanases-predigested Palm Kernel Meal in the Diets on Nutrient Digestibilities and Broiler Performance

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Abstract

Two studies were conducted to determine the efficacy of using mannanase treated and untreated palm kernel meal (PKM) with different commercial mannanases in various broiler diets. Forty five birds and 160 birds were used for digestibility and performance studies respectively. Three different diets (PKM with no enzyme and PKM pretreated with mannanase A or B) were offered to the birds for digestibility study. In contrast, 7 different diets containing 0% PKM as control (T1), 10% PKM (T2), 10% mannanase A-treated PKM (T3), 10% mannanase B-treated (T4) were offered for performance study. The pre-treated PKM was heated in the oven at 90oC for 15 minutes to inactivate the enzyme prior to its mixing with the diets. The study was conducted for 42 days and the birds were fed their respective diets ad libitum. Faecal discharge was collected for three consecutive days for faecal coefficient of digestibility and ileal digesta was taken from Mackel's diverticulum to 1 cm before caeca for ileal amino acid digestibility. Data indicated that pre-digested PKM with both mannanases increased digestibility of crude fibre and AME of PKM (P<0,05). However, an increased ileal protein digestibility and a decreased moisture content of faeces were only found in birds fed the mannanase A treated PKM. Addition of 10% PKM in the broiler diet negatively affected body weights of birds when compared with the birds fed the control diet (T1). Predigestion of PKM with mannanase A improved feed intake and body weight gain significantly. In conclusion, Predigested PKM with mannanase could be effectively used to improve the nutritive value of PKM.

Key words: mannanase, palm kernel meal, predigestion and broiler

Introduction

Palm kernel meal (PKM) as an agricultural by-product is produced abundantly in Indonesia. Since 2008, Indonesia has been the world's biggest producer of palm

kernel meal (FAO, 2008), being about 2.04 million tonnes/year. Although palm kernel meal appears to have favourable nutrients for growing chickens, nutrient qualities are poor due to high fibre content, particularly mannan, and low digestibility (Sundu *et al*., 2008). Accordingly, the use of this by-product in poultry diet is limited.

Treatment of low quality feedstuffs with exogenous enzymes has been a focus of research in animal feed industry. Conventional use of an enzyme through its direct application onto the diet may inactivate the enzyme when the enzyme containing diet was pelleted due to its heat exposure (Sundu *et al*., 2006). Instead of using conventional method of enzyme application onto the diet, predigestion was used to cope with the problem of enzyme damage due to pelleting. Predigestion is an enzymatic treatment taking place in a chamber. Setting up the temperature and moisture during treatment process have been an important procedure to be considered. Two studies were conducted to determine the effect of predigestion using mannanase in palm kernel meal based diets.

Materials and Methods

Animals and Diets

Forty five birds and 160 birds were used for digestibility (experiment 1) and performance studies (experiment 2) respectively. Three different diets (PKM with no enzyme and PKM pretreated with mannanase A with the activity of 800 U/g or mannanase B with the activity of 1100 U/g) were offered to the birds for digestibility study. Due to commercial reason, the brands of these two enzyme products were confidential. Palm kernel meal and pre-digested palm kernel meal being used in these two studies were kindly provided by Wilmar Company. Prior to mixing with other feed ingredients and feed additives, the pre-digested palm kernel meal were heated in the oven at 90^{o}C for 15 minutes to inactivate the added enzymes in the diet.

In experiment 2, 7 different diets containing 0% PKM as control (T1), 10% PKM (T2), 10% mannanase A-treated PKM (T3), 10% mannanase B-treated (T4)

Table 1. Ingredient composition of experimental diets (g/kg)

Diet 1 (D1)	Diet 2 (D2)	Diet 3 (D3)	Composition
Untreated PKM	Mannanase A- PKM	Mannanase B- PKM	915
Palm oil	Palm oil	Palm oil	40
Limestone	Limestone	Limestone	16
Salt	Salt	Salt	5
Premix	Premix	Premix	4
Celite	Celite	Celite	20

Table 2. Experimental diets composition (g/kg)

Feed Ingredients	Starter Diets				Grower Diets			
	T1	T2	T3	T4	T1	T2	T3	T4
Palm kernel meal	0	100	100	100	0	100	100	100
Maize	512	460	460	460	560	470	470	470
Full fat soybean	250	250	250	250	250	250	250	250
Fish meal	130	130	130	130	100	100	100	100
Rice bran	86	42	42	42	70	58	58	58
Palm oil	0	0	0	0	0	5	5	5
Dicalcium phosphate	16	12	12	12	14	11	11	11
Premix	2	2	2	2	2	2	2	2
DL- methionine	2	2	2	2	2	2	2	2
L-Lysine	1	1	1	1	1	1	1	1
Salt	1	1	1	1	1	1	1	1
Calculated:								
AME (MJ/kg)	12.97	12.85	12.85	12.85	13.19	13.02	13.02	13.02
Protein	228	229	229	229	212	214	214	214
Methionine	6.0	5.9	5.9	5.9	5.7	5.7	5.7	5.7
Cysteine	3.7	3.7	3.7	3.7	3.5	3.6	3.6	3.6
Lysine	12.8	12.8	12.8	12.8	12.7	12.7	12.7	12.7
Calcium	11.8	11.7	11.7	11.7	10.5	10.1	10.1	10.1
Phosphorous	8.7	8.6	8.6	8.6	8.4	8.2	8.2	8.2

were offered for performance study. The pre-treated PKM was heated in the oven at 90 ^{o}C for 15 minutes to inactivate the enzyme prior to its mixing with the diets. The study was conducted for 42 days and the birds were fed their respective diets *ad libitum*. Faecal discharge was collected for three consecutive days for faecal digestibility and ileal digesta was taken from Mackel's diverticulum to 1 cm before caeca for ileal protein digestibility.

Statistical analysis

A completely randomized design was used in each of these two experiments. Three treatment diets with five replicate cages and four different diets with five replications were used for trial 1 and trial 2 respectively. Data were analyzed by analysis of variance using Minitab software package. Differences among treatments were tested for significance by using Tukey Test (Steel and Torrie, 1980).

Results and Discussions

Data of effect of predigestion on nutrient digestibilities and broiler performance are shown in Tables 3 and 4. It has been well recognized that most of the dietary fibre in PKM is in the form of indigestible mannan (Daud and Jarvis, 1992). Efficacy of using an enzyme to increase feed digestibility has been long recognised by nutrisionists in various feedstuffs. However, all the data of the efficacy of enzyme were based on direct addition of enzyme onto the diet. Direct application of enzyme suffers from enzyme damage when the diet was pelleted due to heat exposure. Accordingly, predigestion where the low quality feedstuffs undergo degradation process in the digestion chamber prior to mixing the diet, was able to minimise the inacticity of enzyme during pelleting or feed production.

The AME of PKM in this present study was low, being 6.0 MJ/kg. Treatment with mannanase increased AME of PKM by 24 to 27%. This improvement may partly be due to an increased digestibility of crude fibre when PKM was treated with mannanase. Even though the activity of the mannanases used in this current study was different, digestibilities and AME of the PKM were statistically the same between two mannanase-treated PKMs in experiment 1. It can be speculated here

Table 3. Effect of predigestion on nutrient digestibilities in experiment 1

Parameters	D1	D2	D3	SEM
Dry matter digestibility (%)	36.7^{a}	42.4^{b}	41.1^{ab}	1.7
Crude Fibre digestibility (%)	17.2^{b}	27.4^{a}	29.3a	3.0
Faecal protein digestibility (%)	52.0	58.0	54.5	2.1
Ileal protein digestibility (%)	52.1^{b}	58.3^{a}	54.9^{b}	0.8
Apparent Metabolizable Energy (MJ/kg)	6.00^{b}	7.61^{a}	7.43^{a}	0.43
Faecal moisture (%)	72.2	71.4	71.9	0.8

Note: Values with the different superscript within a column are significantly different (P<0.05).

Table 4. Effect of predigestion of PKM with mannanase on body weight gain, feed intake and FCR in experiment 2

Parameters	T1	T2	T3	T4	SEM
Body weight gain (g)	2009^{ab}	1865^{b}	2083^{a}	1988^{ab}	21.6
Feed intake (g)	3615^{a}	3417^{b}	3692^{a}	3659^{a}	28.2
FCR	1.80	1.83	1.78	1.84	0.01

Note: Values with the different superscript within a column are significantly different (P<0.05).

that the mannanase A activity of 800 U/g may be enough to optimally increase digestibilities and AME of this enzyme treated PKM. Further increased activity of 1100 U/g for mannanase B did not affect feed digestibility and AME of PKM greater than that for mannanse A.

Although the digestibility of protein either by using the total faecal collection or the illeal digesta method was nearly the same, coefficience of variance of faecal protein digestibility tended to be higher than those of illeal protein digestibility (8.5 vs 5.8%). It can be said here that problem of measuring protein digestibility in the faeces was not only as a matter of contaminated faecal protein with urine and the microbes outflow from the large intestine, but also this procedure sufferred from high variance of the data. It is possible that high coefficience of variance of faecal protein digestibility found in this current study was partly due to variation in total amount of microbe and urine outflow to the faeces and this may not be found in illeal digesta.

Addition of 10% PKM in the broiler diet did not negatively affect the body weight of birds, compared to the birds fed the control diet. These data are consistent with the results reported by Panigrahi and Powell (1991). The data also clearly suggest that the use of 10% PKM in the broiler diet could be promoted in PKM producing countries where the PKM was abundantly available. There was a trend that treating PKM with mannanase slightly increased bodyweight gain but this improvement was not statistically significant. The efficacy of this enzyme treatment technology become evident when the birds were fed on the 10% PKM in the diet. In this particular diet, treatment of PKM with mannanase A improved body weight gain significantly and the body weight of birds fed a mannanase A-treated 10% PKM in the diet exceed the body weight of birds fed the control diet (2009g vs 2083 g). In conclusion, this enzyme treatment technology can be effectively used to increase feed digestibility and AME of PKM and improved body weight gain, particularly within the inclusion rate of 10% PKM in the broiler diet.

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Reduction of *Salmonella typhimurium* in the Caecum of Broiler Offered Rations Containing Banana Peel or Palm Kernel Meal

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Abstract

Oligosaccharides in digestive tract stimulate the growth of some intestine lactobacilus bacteria and reduced phatogenic bacteria. Kernel palm meal (KPC) and banana peel (BP) are high in mannan and fructose based polisaccharides. Limited ferementation of these producs may produce olygosaccarides. The objective of this research was to evaluate the effect of the dietary inclution of fermented palm kernel meal and banana peel on Salmonella typhimurium in the caecum of broiler. Experimental diets were: P0= Ration without BP, PKM, Bifidobacterium bifidum (Control); P1= Ration 2.5% BP; P2= Ration 2.5 % PKM; P3= Ration 2.5 % BP + B.bifidum, P4= Ration 2.5 % PKM+ B.bifidum. Ration conained 22% protein and 3050 kcal ME/kg. Experimental diets were allocated in a completely rendomized design with 5 replicates of 5 DOC each. At 14 days old, broiler were infected by Salmonella typhimurium and offered experimental diets.Weight gain, final body weight, carcass weight, carcass precentage, feed consumption and convertion, viscerals percentage and total colony of S.typhimurium in secum were evaluated. Weight gain, final body weight, carcass weight, carcass precentage, feed consumption and convertion, viscerals percentage of broiler offered ration contained BP, PKM and B. bifidum were not different from those of control. Percentage of liver, proventikulus, and jeujenum of broiler in P1 were smaller (P<0.05) than those of control. Total colony of S.typhimurium in secum of broiler in P1, P3, and P4 were smaller (P<0.05) than those of control. It was conluded that inclution of benana peel 2.5% in the broiler rations without B.bifidium inhibited S.typhimurium growth and increased body weight, weight gain, carcass weight, and improved feed convertion. Addition of B.bifidium into the ration did not improve the usefulness of banana peel as a source of prebiotic.

Keywords: broiler, banan peel, palm kernel, S. typhimurium, B. bifidum,

Introduction

The presence of gastrointestinal pathogenic bacteria such as *Salmonella typhimurium* results in many problems in broiler farming and products (Ohl & Miller. 2001). *Salmonella typhimurium* is one of the common bacteria reducing nutrient utilization, disturbing some biological functions, growth and increasing mortality of chicks. Maintaining the balances of gastrointestinal micro flora is an important effort. Dietary addition of antibiotic is a common practice in reducing the persence of gastrointestinal pathogenic bacteria. However, the residual antibiotic in animal products is a major concerns, since it results in the resistance of bacteria wihch is harmful for the consumer. Application of prebiotics in poultry ration is an alternative to replace the nutritional fuction of antibiotics.

Dietary addition of mannan and fructose olygosaccharides have been demonstrated to have similar beneficial effects with an antibotic in maintaining a balance gastrointestinal micro flora and improve productivity in broilers (Kim *et al*., 2011). Mannan containing polysaccharides from palm kernel meal (PKM) could be used as an alternative to replace antibiotics in preventing the colonization of *Salmonella typhimurium* in poultry (Tafsina *et al*., 2007). Banana peel is an agricultural by product wich is avalable from banana processing activity. Controled hidrolysis of PKM and banana peel containing mannan and fructose polysacharides, may produce mannan and fructoce olygosaccharides. The present experiment aimed et evaluating the effect of the dietary inclution of fermented PKM and BP on *Salmonella typhimurium* in the caecum of broiler.

Materials and Methods

The total of 125 day old chick (DOC) of Cobb CP 707 strain were allocated radomly into five dietray treatments in a completely rendomized design with 5 replicates of 5 DOC each. Experimental diets were: P0= Ration without BP, PKM, *Bifidobacterium bifidum*; P1= Ration 2,5% BP; P2= Ration 2,5 % PKM; P3= Ration 2,5 % BP + *B. bifidum*, P4= Ration 2,5 % PKM+ *B. bifidum*. Ration contained 22% protein and 3050 kcal ME/kg. Chick in all treatments were offered P0 ration and kept in a collony cage facilitated with two 100 watt of ball lamps as heaters. Banana peel and palm kernel meal used in the experiment were ground and mixed with filtrate obtained from the fermentation of either BP or PKM. The fermentation was conducted for 24 hours accoding to the modified method of Tilley dan Terry (1963). Source of bacteria used in the method was the mixture of bacteria isolates instead of fresh rumen liquor. At 14 days old, broiler in all treatments were infected by *Salmonella typhimurium* and offered experimental diets. Feed and water were given *ad libitum S. typhimurium* and *B. bifidum* were administered orally on day 14. *S. typhimurium* was administered at level of 1 x 10^4 cfu and *B. bifidum* 1x10^5 cfu. Weight

gain, final body weight, carcass weight, carcass precentage, feed consumption and convertion, viscerals percentage and caecal *Salmonella typhimurium* were observed. Feed consumption and body weight were determined weekly. Caecal *Salmonella typhimurium* was observed on day 28.

Results and Discussion

Live Weight and Carcass

Mean of final live weight, carcass weight and carcass percentage of of broiler infected *S. typhimurium* and offered different rations with or wthout *B. bifidum* were presented in Table 1. Mean of final and carcass weight of broiler was the highest when BP was included in the diet. However further inclution of *B. bifidum* in a ration containing BP reduced the final weight of the broiler. Mean of carcass percentage were not affected by dietary treatments. The result indicated that feeding broiler with a ration containing BP for 14 days reduced the negative effect of *S. typhimurium* infection in broiler. The result suggested that the fermented BP contained active

Table 1. Mean of Final Live Weight, Carcas Weight and Carcas Percentage of Broiler offered Ration Containing BP or PKM on 28 days

	Dietary Treatments				
	P0	P1	P2	P3	P4
Final live weight, g	$871^{AB} \pm 49$	$943^{B} \pm 67$	$858^{AB} \pm 42$	$812^{A} \pm 68$	$843^{AB} \pm 60$
Carcass weight, g	$527^{AB} \pm 49$	$584^{B} \pm 45$	$516^{AB} \pm 39$	$493^{A} \pm 51$	$525^{AB} \pm 34$
Carcass percentage, %	60.39 ± 2.42	61.77 ± 0.65	60.10 ± 1.41	60.73± 1.95	62.31 ± 0.79

Note: Means with different superscript differ significantly (P<0.05); P0= Control; P= Ration 2.5% BP; P2= Ration 2.5% PKM; P3= P1 + *B. bifidum*; P4= P2 + *B. bifidum*.

Table 2. Mean of Feed Consumption, Daily Gain and Feed Conversion Ratio of Broiler offered Ration Containing BP or PKM on 28 days

	Ditary Treatments				
	P0	P1	P2	P3	P4
Consumption (g/bird)	1190 ±101	1166 ±40	1233 ±80	1167 ±145	1165 ±51
Weight Gain (g/bird)	739 ±43	765 ±53	750 ±56	721 ±90	709 ±35
FCR (consumption/ ADG)	1.61 ±0.11	1.53 ±0.06	1.65 ±0.06	1.63 ±0.15	1.65 ±0.12

Note: P0= Control; P= Ration 2.5% BP; P2= Ration 2.5% PKM; P3= P1 + *B. bifidum*; P4= P2 + *B. bifidum*.

substances such as fructose olygosaccarides, but the olygosacharides from fermented BP did not support the growth of *B. bifidum*.

Feed Consumpsion, Weight Gain and Feed Conversion Ratio

Mean of feed consumption, weight gain (WG) and feed conversion ratio (FCR) were presented in Tabel 2. Mean of total feed intake, weight gain and feed conversion of broiler infected *S. Typhimurium* and offered different rations with or wthout *B. bifidum* varied from 1164-1233 g per bird, 708-764.97 g per bird and 1.53-1.63, respectively. There were no significant different ($P<0.05$) in total feed intake, weight gain and feed conversion among treatments. Inclution of BP without *B. bifidum* tended to increase weight gain and improved feed conversion. The result indicated that inclusion of BP, KPM and *B. bifidum* in broiler infected by *S. typhimurium* was not effective. However, the improvement of the fermentation method of BP and PKM and the increase in inclusion level of fermented product may improve the response of broiler.

Wiryawan et al. (2005) reported that supplementation of FOS at level of 2.5% from garlic increased weight gain of infected *S. typhimurium* bird. The present result indicated that BP and PKM suppressed the negative effect of *S. typhimurium* on growth but the level could not optimize nutrient metabolism to create the better weight gain and feed conversion ratio.

Mean of Percentage of Visceral Organ

Mean of percentage visceral organ is presented in Tabel 3. Inclution of BP, KPM and *B. bifidum* in diet of broiler from 14 to 28 day old, had significant effect on their percentage of liver, gizzard and proventriculus. Percentage of liver, gizzard and proventriculus of broiler offered diet P1 had the lowest values. The result indicated that BP inclution in the diet reduced the negative effect of *S. typhimurium* infection in broiler. *Ferket et al*. (2002) reported that infection of *S. typhimurium* could swell liver organ. Therefore dietary inclution of 2.5% BP (P1) stimulated the balance growth of intestinal micro flora and suppressed *S. typhimurium* growth. Reduction of *S. typhimurium* growth may maintain the normal condition and fuction of liver and digestive tract.

Total Colonies of Salmonella typhimurium

Data of total number of *S. typhimurium* collonies was shown in Table 4. Mannan and fructose olygosaccharides produced short chain fatty acids and lactic acid (Bantora & Ditya, 2012). These organic acids have antimicrobial property to inhibit pathogenic bacteria. The data indicated that the most effective treatment to reduce *S. typhimurium* collonies was P1 and P4 with total number of *S. typhimurium* collonies was 1.58×10^3 and 2.4×10^3, respectively.

Table 3. Mean of the Percentage of Visceral Organ to Live Weight (LW) of Broiler offered Ration Containing BP or PKM on 28 days

Visceral Organ (% LW)	Dietary Treatment				
	P0	P1	P2	P3	P4
Liver	3.17±0.62^{b}	2.46±0.18^{a}	2.97±0.45^{ab}	2.73±0.13^{ab}	2.80±0.24^{ab}
Gizzard	2.47±0.32^{ab}	2.15±0.18^{a}	2.30±0.18^{ab}	2.57±0.35^{b}	2.33±0.18^{ab}
Proventriculus	0.73±0.20^{b}	0.57±0.07^{a}	0.66±0.07^{ab}	0.67±0.11^{ab}	0.70±0.11^{b}
Lymph	0.12±0.04	0.11±0.04	0.14±0.04	0.12±0.04	0.09±0.04
Heart	0.52±0.04	0.50±0.03	0.57±0.06	0.54±0.06	0.57±0.08
Bile	0.15±0.20	0.11±0.04	0.10±0.05	0.13±0.04	0.10±0.06
Kidney	0.79±0.11	0.89±0.14	0.82±0.14	0.83±0.08	0.88±0.12
Pancreas	0.44±0.12	0.41±0.04	0.42±0.06	0.39±0.05	0.44±0.06

Note: Means with different superscript differ significantly (P<0.05); P0= Control; P= Ration 2.5% BP; P2= Ration 2.5% PKM; P3= P1 + *B. bifidum*; P4= P2 + *B. bifidum*.

Table 4. Number of *S. typhimurium* collonies in Cecum of Broiler offered Ration Containing BP or PKM on 28 days

Parameter	Treatment				
	P0	P1	P2	P3	P4
Number of colonies (cfu)	155 x 10^3	1.58 x 10^3	160 x 10^3	10 x 10^3	2.4 x 10^3

Note: P0= Control; P= Ration 2.5% BP; P2= Ration 2.5% PKM; P3= P1 + *B. bifidum*; P4= P2 + *B. bifidum*.

Data showed that BP inclution in a broiler diet stumulated the growth of *B. bifidum* and therefore reduced caecal *S. typhimurium* colonies. Dietary inclusion of PKM had no effect on *S typhimurium* growth in the caecum. However, inclution of *B. bifidum* in the diet containing PKM reduced caecal *S. typhimurium* colonies. The result indicated that inclution of either BP or PKM facilitated the growth of *B. bifidum as* probioticand.

Conclusions

Dietary inclution of 2.5% fermented banana peel was effective to reduce colonization of *S. typhimurium*. Dietary inclution of both banana peel and palm kernel meal stimulated the growth of *Bifidobacterium bifidum* suppressing *S.*

typhimurium growth in broiler. Therefore banan peel and palm kernel meal were potential sources of oligosaccharides.

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II. FEED AND NUTRITION
Sub Theme: Ruminant

Oil Palm Fronds (OPF) as Potential Affordable Source of Feeds for Ruminants Small Holder Farms

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Abstract

Presently, the most important constraints faced by the ruminants industry are not only the high cost of feeds but also their shortage. The shortage is especially so in the supply of palm kernel cakes (PKC) since 95% of PKC produced in Malaysia were exported to European Union (EU) countries for their dairy industries (Sabah Veterinary Services, 2009). Most of the cattle industries (about 47%) in Malaysia are run by small holders and traditional farmers. With the sufficiency level of 24.4%, it can be considered low as far as food security level is concerned. The current challenges faced by both small holders and traditional farmers are the high cost of feedstuff for the ruminants industry such as cattle and goats. The main reason for the slow growth of the ruminant industry is due to the high demand and shortage of PKC and at the same time the high cost of soya bean meal (SBM), the best alternative. Oil Palm Fronds (OPF) on the other hand are possible substitutes as affordable feeds for ruminants in cases where forages and fodder are limited. Furthermore, with the huge planted hectare of oil palm in Malaysia and Indonesia, the potential for constant supply of oil palm fronds is huge. Studies have shown the recommended levels of OPF in the total mixed rations (on dry matter basis) are 50% for beef cattle and 30% for dairy cattle and goats. Although the energy level is only 5.6M.E/MJ/kg and the crude protein (CP) is 4.7%, the OPF can be considered reliable due to their constant supply and with the dry matter production of 9.7mt/hectare/year can be considered sustainable feed for the ruminant industry. This paper reviews the potential of OPF as an alternative and affordable source of feeds for ruminants for smallholders and traditional farmers to sustain the growth of industry in Malaysia and Indonesia.

Keywords: cattle, feeds, Oil Palm Fronds (OPF), small holders, traditional farmers

Introduction

The issues at hand with the ruminant industry in Malaysia

In Malaysia, natural forages are freely grown and the extensive system is widely practiced by the smallholders. The livestock graze on poor quality native pastures such as carpet grass (*Axonopus compresses*) and *Paspalum spp* which are the common vegetation grown under the waste land and under the tree crop. The slow growth of the livestock industry in Malaysia is mainly due limited forage species and the lack of technical knowhow among the small holders. In addition, the high cost of feed and shortage of palm kernel cake (PKC) supply also contributed to the constraints (Sabah Veterinary Services, 2009). The prospects of increasing farmland for grass and fodder are simply not favorable (Joseph, 1991) because the national third agricultural policy (NAP3) stated that any conversion of tropical rainforest to pasture for grazing had not enough justification in terms of environment or economic viability. The ruminants industry then was left to be carried out by the traditional farmers except for feedlot projects which were implemented in big scales by the private sector or individuals. The potential of biomass products especially from oil palm trees need to be given special attention since this is the most logical source of affordable feed.

Oil Palm Fronds (OPF) Availability as Source of Feeds for Ruminants

Previously the oil palm fronds are abundant as waste materials left rotting within the stacking rows and mainly used to recycle as composting fertilizers and for soil conservation. After the introduction of cattle and goat integration programs in oil palm plantations, the potential of OPF has been observed when the cattle and goats were seen grazing on the oil palm fronds. Usually the cattle and goats would turn to oil palm fronds if the forages in the grazing area were not available. This happened when the oil palm reached the stage where the canopy became extensive thus preventing forages to grow due of poor lighting. Taking advantage of this, with the assumption of the average of economic life span of oil palm of 25 years, this would give a huge and promising supply of OPF for the ruminants industry. With the present increase in oil palm exports and rising revenue, it is very likely and most surely that there will be a tremendous increase of oil palm areas in both Malaysia and Indonesia. This in turn will provide a good opportunity to harness the biomass byproduct which includes oil palm fronds. The best thing about oil palm fronds is that they are available at all times when the pruning, harvesting and replanting are being carried out. The oil palm fronds can be taken by cattle and goats as feeds either green or conserved as silages in combination with other ingredients as total mixed ration (Abu Hassan and Ishida 1991). The oil palm fronds are similar to rice straws in that they have fibrous characteristic.

Availability of Oil Palm Fronds

Oil palm fronds are available at all times when the pruning, harvesting and replanting have been carried out (Table 1).

Oil Palm Fronds from pruning and routine harvesting

The total dry weight of fronds can also be obtained from routine pruning and harvesting (Table 2). OPF are available regularly in terms of dry weight and seem to be more sustainable resources of biomass compared to other oil palm biomass such as palm press fiber (PPF), palm kernel cake (PKC), oil palm trunks (OPT) and other biomass products such as soya bean meal (SBM), rapeseed and maize.

Comparison Cost of Other Biomass Products with Oil Palm Fronds

The cost of oil palm fronds pellets (OPFP) is lower compared to PKC and SBM (Table 3). The feed conversion rate (FCR) for 7 kg OPFP produced an average daily gain weight (ADGW) of 0.8 kg to 1.1 kg for Kedah Kelantan Cattle which cost in the range of USD 1.33 to produce 1kg ADGW (Mardi 2008). Compared to PKC rations of 80% PKC, 17.5% grass/hay, 1.5% limestone and 1% mineral premix (M Wan Zahari and A .R Alimon 2004) the cost was USD 1.70.

Table 1. Availability of fronds during felling at replanting in million tons in 2009

Location	Oil palm (mature ha)	Fronds dry weight (mil tons)
Peninsular Malaysia	2,489,814	1.80
Sabah	1,361,598	0.99
Sarawak	839,748	0.61
Total	4,691,160	3.40

Sources: Based on 5% replanting of mature areas at 14.47t/ha of dry weight of fronds at felling taken from Chan 1999.

Table 2. Total Availability of fronds from annual pruning and harvesting in million tons in 2009

Location	Oil Palm (mature ha)	Fronds dry weight (mil tones)
Peninsular Malaysia	2,489,814	25.91
Sabah	1,361,598	14.17
Sarawak	839,748	8.74
Total	4,691,160	49.36

Sources: Based on 10.4tones dry weight/ha from pruning and harvesting taken from Chan 1999.

Table 3. Cost Comparison of OPF, PKC and SBM

Palm Kernel Cake (PKC)	Total Cost	Soya Bean Meal (SBM)	Total Cost	Fresh oil palm Fronds (OPF)	Oil Palm Frond Pellet (OPFP)
Material cost Range USD 150- 174 Based on the CPO price zone USD 903- 1000	USD 174	Material Cost	USD 472-520	Free	USD 180
Delivery cost USD 53-66	USD 66		USD 66		USD 10
Total	USD 240		USD 586	NIL	USD190

Sources: Mohammad Amizi Ayob *et al*. (2011)

Nutritive Potential of Oil Palm Fronds as Ruminant feeds

OPF contain less crude protein compared to the SBM and PKC (Table 4). The expeller PKC and SBM showed higher crude protein (CP) and metabolizable energy (ME). Although OPF only contain 4.7% CP and 5.7mj/kg ME, the cost of OPF is still much cheaper compared to the both PKC and SBM. Furthermore, OPF is abundant, free and available at all times. The ADGW in cattle is not much different when fed with OPF compared to PKC with exception of SBM and this was shown in a study of Brahman cattle when fed with PKC and OPF showed the ADGW of 0.75kg and 0.65kg respectively and when fed with SBM showed the ADGW of between 1.2kg to 1.3kg (Alimon and Hair Bejo 1995). Even though the ADGW was slow compared to PKC and SBM, the big difference, OPF was much cheaper.

Table 4. Comparison of Nutritive Value of By- Product (%)

By product	CP	CF	NDF	ADF	EE	ASH	TDN	DM	ME (MJ/kg)
Soya Bean Meal (SBM)	48	7.0	14.0	10.0	18.8	5.5	78	90	13.3
Oil Palm Fronds (OPF)	4.7	38.5	78.7	52.9	2.1	3.2	45	30.2	5.70
Palm Kernel Cake (PKC)	17.2	17.1	74.3	55.6	1.5	4.3	65	89	11.3

CP: Crude Protein, CF: Crude Fibre, NDF: Neutral Detergent Fibre, ADF: Acid Detergent Fiber, EE: Ether Extract, TDN: Total Digestibility Nutrient, DM: Dry Matter, ME: Metabolizable Energy.
Sources: Alimon and Hair Bejo (1995), and Baize, John C (2000).

The other potential of OPF is that it can be mixed in mixed rations thus reducing cost in the usage of PKC and SBM (Wan Zahari *et al* 2003). This will then make animal feeds affordable to traditional farmers which mostly dominated the ruminants industry in this region.

Conclusion

OPF has a huge potential as substitute of ruminants feeds due to their availability and sustainable supply at all times compared to other by products. Continuous promotion of OPF as value added product for livestock feeds will give a higher impact potential during shortage of the livestock feed. The lower cost of livestock feed is necessities due to the Ruminants industry in Malaysia and South East Asia were dominantly accommodate by the traditional farmers and small holders. This can sustain the ruminants industry and contribute the food security in red meat production. Further research of the mixed ration with OPF needs to be carried out to observe for other potentials of this byproduct.

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Biodegradation of coffee husk substrate during the mycelia growth of *Pleurotus ostreatus* and the effect on in vitro digestibility

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Abstract

The aim of this studies were conducted to evaluate culturing of mushroom P.ostreatus on coffee husk in solid state fermentation as means of improving the nutritive value of coffee husk for ruminant animals. The influence of P.ostreatus on coffee husk biodegradation was investigated. The dry matter and composition changes of coffee husk substrate for P.ostreatus cultivation were analysed on day 0, 30 and 60 after seeding. The profile of cellulose, hemicellulose and lignin were changed when it was used by P.ostreatus. Meanwhile their rate of change varied at different growing day. The increase of protein content and the reduction of lignocellulose content increase dry matter digestibility of coffee husk substrate. This fact could provide an alternative of biofermentation product based on coffee husk substrate which is safe for environment.

Keywords: biodegradation, coffee husk, digestibility, substrate, P. ostreatustion

Introduction

Pleurotus ostreatus is one of the popular cultivated mushroom. It can be cultivated on a wide range of lignoselulosic substrates such as wheat straw, cocoa husk and cotton stalks (Fazaeli *et al.,* 2004; Li *et al*., 2001; Alemawor, 2009). *Pleurotus ostreatus* belongs to white rot fungi which are able to degrade lignin because produce ligninolytic extracellular enzymes, such as laccases, lignin peroxidases and Mn peroxidases (Kerem *et al.,* 1992; Chang and Miles, 2004).

The ability of *P.ostreatus* degrades a wide variety of lignoselulosic substrates, enabling it to play an important role in managing organic wastes whose disposal is problematic. *Pleurotus* species have been used by human for their nutritional value,

medicinal properties, transformation of wastes into animal feed and other beneficial effects (Hadar and Arazi, 1986; Gregori *et al.* 2007; Adamovic *et al*., 1998).

Coffee husk is the major byproducts produced during the operation of coffee cherry to get coffee grain by sun drying (Fan *et al*., 2004). In coffee-producing regions, coffee husk is barely utilized. Therefore, it is considered the most abundant pollutant material. Coffee husk has potency as a source of ruminant feed. The protein content is 9.2-11.3% equally to rice bran protein (±10.4%) and has metabolic energy around 3.356 Kcal/kg (Zainudin and Murtisari, 1995). The content of lignin is 35.0-40.0% (Fan and Soccol, 2005). The digestibility of these materials are limited by the presence of lignin which prevents access of hydrolytic enzymes to cellulose and hemicelluloses.

Application of *Pleurotus ostreatus* is worth considering for improving the nutritive value of coffee husk. This study was carried out to asses the effect of a solid state fermentation involving *Pleurotus ostreatus* on the nutrition composition of coffee husk and to evaluate in vitro digestibility. In addition, fermentation period on the process was evaluated.

Materials and Methods

Coffee husk were obtained from coffee hulling plant at Rejang Lebong Residence Bengkulu Province. Coffee husks were air-dried to moisture content 10-15%. The solid state substrate were prepared with the composition adopted from sawdust standard medium (Herliyana *et al.* 2008). The mushroom substrate may be defined as a kind of lignocellulosic material supports the growth, development and fruiting of mushroom. The substrate were consisted of 82,5% coffee husk, 15% rice bran, 1,5%gips and 1,0% $CaCO_3$. The clean water were added to the substrate as much as 60-65% (v/w). All these components were placed in polypropylene bag in amount 400 gram per bag. Each bag was closed with a small cotton plug inserted in the middle of its opening. The bags were sterilized at 121^{o}C for 30 minutes. After cool, each of bag was seeded with 15 gram (3,75%) of *Pleurotus ostreatus* spawn. All spawned bag were placed in growing room with the temperature was 22-28^{o}C and relative humidity 60-80%. After 30 days, the substrate was fully colonized, and on 60 days primordial started to appeared.

The content of protein was analyzed using Kjeldahl method. The cell wall components (NDF, ADF, Lignin, cellulose and hemicelluloses) were analyze using deterjent analyze method as described by Goering and Van Soest (1970). *In vitro* dry matter digestibility was evaluated according to Tilley and Terry method (1963).

The treatment was the fermentation time consisted of 0 untreated), 30 and 60 days after seeding. The nutrient composition changes were described descriptively. For the dry matter measurement the treatment was arranged in Block Randomised Design (3x4). The rumen inoculum were obtained from four cattles as block.

Significant differences were calculated using Duncan's multiple range test following analysis of variance.

Result and discussion

The celluloses, hemicelluloses and lignin are the main sources of carbon and energy for *P.ostreatus* growth, while protein serves as the nitrogen source. Their degradation and utilization can greatly affect *P.ostreatus* growth and resulting feed value of the substrate. The change of nutrient composition contents during the *P.ostreatus* mycelia growth period are shown in Table 1.

There were increasing of protein content and decreasing of fiber fraction (lignin, NDF and ADF) produced by biofermentation. The decreasing of fiber fraction is the indication that *Pleurotus ostreatus* can degrade the cell wall component of coffee husk.

The decreasing of NDF and ADF from coffee husk suggested that these fungi could utilize the cell wall component as carbon source and energy for growth. The decreasing of NDF and ADF contents of treated coffee byproduct has been reported by Penaloza *et al*., (1985). The decreasing of NDF, ADF and ADL in the first 30 days of mycelia growth were 2.339%, 4.586% and 19.874%, respectively. Meanwhile in 60 days, the decreasing of NDF, ADF and ADL were 16.587%, 15.036% and 31.161%, respectively from the initial value.

The fermentation time was important to improve the nutritive value of straw. The longer fermentation period led greater depletion of carbohydrate source of coffee husk by fungi. This condition could improve the digestibility of coffee husk as result of the changes in non structural carbohydrate to structural carbohydrate ratio. Decreasing of lignin in coffee husk could be a result of lignin degrading enzymes produced by *Pleurotus* (Hong *et al*., 2003). These result are supported by the report from Widiastuti *et al*. (2008) who noted ligninolityc enzyme activities followed the pattern of lignin disappearance from substrate and directly corrected with time of its disappearance. Plat and Hadar (1983) noted that during the mycelia growth period, *P.ostreatus* mycelia were more capable to degrade lignin, and the degradation of lignin played an important role in mycelia development.

The rapid decreasing of hemicellulosic component in 30 days fermentation showed that hemicelluloses were the first substrate utilized by mycelia as the carbon and energy sources at the beginning phase of growth. The decreasing of hemicelluloses was 31,578% from initial value in 30 days fermentation. This suggest that hemicellulose is more easily degraded than cellulose and lignin. *Pleurotus ostreatus* mushroom secreted enzym to demolish the easier used compound. *Pleurotus ostreatus* needs a carbon source which is easier to metabolize (Crawford, 1981). Hemicelluloses were degraded easier than cellulose and lignin (Perez, 2002).

The cellulose content increased 35.574% in 30 days and 27.063% in 60 days.

Biofermentation broke the lignocelluloses bond. Delignification has important role in mycelia growth which cleavage polysaccharide component (cellulose and hemicelluloses) (Agosin and Odier, 1985). This component will be utilized by fungi as substrate for their growth (Hatakka, 2004).

During the mycelia growth, the protein content increased 0.927% in 30 days and 17.220% in 60 day fermentation. Mycelia in 60 days were thicker than 30 days. Fungal cell in mycelia contributed the protein content of subtrate because 60 and 70% of nitrogen present in the fungal cell is protein (Chang and Miles 2004). The higher protein content in 60 days in the substrate were prepared to transferable nitrogen into fruit bodies. The extensive formation of primordia in 60 days indicated the end of the vegetative growth phase of *P.ostreatus*. As coffee husk substrate was degraded and nutrient used by *P.ostreatus*, the total organic matter of substrate decreased (Table 1).

The increasing of protein content and the decreasing of lignocelluloses of coffee husk after fermentation showed that Coffee husk could be used as substrate *P.ostreatus* cultivation. The improving nutrition value after fermentation especially on 60 days indicated that the substrate can be used as a product feed.

In vitro dry matter digestibility tests for ruminant were conducted for the digestibility of untreated and treated coffee husk. Four replication were conducted and the result are shown in figure 1. Average dry matter digestibility (Table 1) increased significantly 4.983% in 60 days fermentation and decreased 14.435% in 30 days fermentation from untreated coffee husk. The possibility of this condition is that in 30 days fermentation the higher level of cellulose made digestibility lower.

Table 1. Changes of nutrient contents and average *in vitro* dry matter digestibility of coffee husk substrate during *Pleurotus ostreatus* mycelia growing (0, 30, and 60 days fermentation) (as % dry matter)

Nutrient contens (%)	0 days (Untreated)	(Treated) 30 days after seeding	(Treated) 60 days after seeding
Organic matter	93.710	92.950	86.599
Crude Protein	10.360	10.456	12.144
NDF	95.177	92.950	79.390
ADF	87.184	83.186	74.075
Hemicelluloses	7.993	5.469	5.3170
Cellulose	19.514	26.456	24.795
Lignin	65.421	52.419	45.035
Dry Matter Digestibility (%)	29.518 ± 1.249^{a}	25.257 ± 0.721^{b}	30.989 ± 1.263^{c}

Different superscript in the same row means significantly different ($P<0.05$)

It suggested that on 30 days, the degradation of lignocellulosic component was not optimal yet. Therefore, it could be acceptable to use the coffee husk substrate after *P.ostreatus* cultivation on 60 days fermentation as ruminant feed.

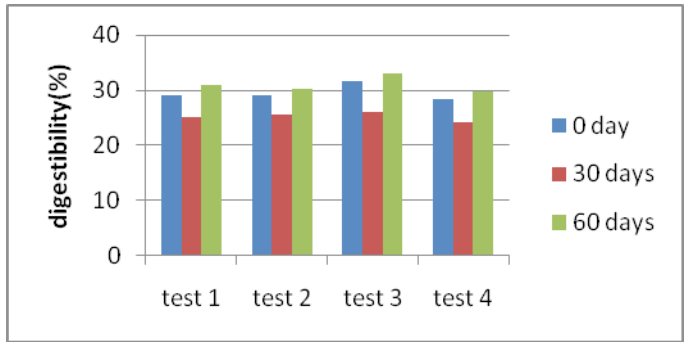

Figure 1. *In vitro* dry matter digestibility of coffee husk during *Pleurotus ostreatus* mycelia growing

Conclusion

It was concluded that protein content and cell wall components in coffee husk substrate changed during *Pleurotus ostreatus* mycelia growing period. In 60 days of fermentation times, cellulose, hemicelluloses and lignin contents in the substrate were decreased and protein content increased as compared with the untreated coffee husk. This could contribute to the increasing in dry matter digestibility of the substrate. It is suggested to use the coffee husk substrate as a ruminant feed especially in 60 days fermentation.

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In vitro Fermentation and Bacterial Protein Synthesis in the Different Diets Supplemented with Lerak Extract plus Mineral (Ca, P, Mg, S)

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Abstract

Bacterial protein supply is usually low in animals fed with high forage ration because of the lack of sources of minerals in the rumen. The aim of this study was to evaluate the use of lerak extract plus mineral (Ca, P, Mg, S) on fermentation and bacterial protein synthesis in the in vitro fermentation with different ratios of forage and concentrate. The design of experiment was factorial block design (3x3) with 2 factors which were: ratio of forage and concentrate (70:30, 50:50, 30:70) as the first factor and the type of supplements (0, 1mg/ml lerak extract and 1mg/ml lerak extract + minerals) as the second factor. Total volatile fatty acid (VFA), NH3 concentration, population of protozoa, and bacterial protein synthesis were measured at 4 h incubation. Dry matter and organic matter digestibility were evaluated after 48 h incubation. The result showed that there was no interaction effect between ratio of forage to concentrate and the type of supplements. The different ratios of forage and concentrate had no effect on dry matter and organic matter digestibility, and NH3 concentration. The increase of concentrate ratio in the diet reduced population of protozoa, but increased total VFA and bacterial protein synthesis. The addition of 1 mg/ml lerak extract without minerals significantly decreased ($P<0.05$) population of protozoa and increased ($P<0.05$) bacterial protein synthesis but no effect on dry matter and organic matter digestibility, NH3 concentration, and total VFA production compared to the control. However, the addition of lerak extract plus mineral (Ca, P, Mg, S) had no effect on all parameters measured. In conclusion, bacterial protein synthesis increased by supplementation of lerak extract without mineral addition.

Keywords: bacterial protein synthesis, fermentation, mineral, Sapindus rarak

Introduction

Rumen microbial population has an important role in the digestion of feed fiber by ruminants. The rumen has a lot of variety of microbial communities such as protozoa, bacteria, fungi, and viruses. Interaction between protozoa and bacteria sometime has disadvantage because of predation of bacteria by protozoa. This predation can cause the reduction of bacterial population and affect the growth of ruminants. It has been known that microbial protein in the rumen, especially bacterial protein, is the major good quality protein resource for ruminants (Pathak, 2008).

Bacterial protein supply is usually low in animals fed by high forage ration because of the nitrogen/protein deficiency in the diet. In addition, forage based diets often lack of some minerals required for the synthesis of microbial protein in the rumen. To overcome this problem, a strategy is required to improve the quality of forage with mineral supplementation and to reduce the population of rumen protozoa. Defaunation by using whole fruit lerak (*Sapindus rarak*) extract modified bacterial composition and increased the growth of some bacteria, especially *Prevotella ruminicola* and *Ruminococcus albus*, and stimulated propionate production (Suharti *et al*., 2011). Our previous *in vivo* study showed that the use of lerak extract for beef cattle with high forage diets increased VFA production without a significant increase in microbial protein synthesis (Suharti *et al.,* 2010) which may be due to mineral deficiency in the high forage based diet, especially Calcium (Ca), Phosphor (P), Magnesium (Mg) and Sulfur (S).

This study was conducted to evaluate the use of lerak extract plus mineral (Ca, P, Mg, S) on fermentation and bacterial protein synthesis in the *in vitro* fermentation with different ratios of forage and concentrate.

Materials and Methods

Whole Fruit Lerak Extract Preparation

The lerak fruits (including seed) were harvested from Central Java Indonesia. Whole fruit lerak extract was extracted by using maceration method (Wina *et al*., 2006). The extract was freeze-dried the freeze-dried extract was dissolved in distillate water just before being used as extract of whole fruit lerak

In vitro Fermentation

In vitro fermentation was conducted according to Tilley and Terry method (1963). The rumen fluid for this experiment was obtained before morning feeding from the rumen of fistulated Ongole crossbred beef cattle fed commercial concentrate and elephant grass (50:50, DM). The rumen fluid was squeezed through a double-layer cheesecloth for *in vitro* experiment. The commercial concentrate consisted of rice bran, molases, bread industry waste, groundnut meal, cassava waste,

and wheat pollard. Elephant grasses were harvested from the Faculty of Animal Science Farm, Bogor Agricultural University, and then were dried in the oven and milled. The design of experiment was factorial block design (3x3) with 2 factors i.e., ratio of elephant grass and concentrate (70:30, 50:50, 30:70) as the first factor and type of supplements (0, 1mg/ml lerak extract and 1mg/ml lerak extract + minerals mix) as the second factor. Mineral mix was composed of Ca 0.54%, P 0.37%, Mg 0.23%, and S 0.1%. The levels of lerak extract used in this experiment were based on our previous study showing that the addition of 1 mg/ml lerak extract increased fermentation activities. Five hundred milligrams of substrate (Concentrate, elephant grass and supplement) according to the treatments were put into a 100 ml fermentation tube. Forty milliliters of McDougall buffer was added, followed by 10 ml of rumen fluid. The McDougall buffer contained, per 6 liters, $NaHCO_3$ (58.8 g), $Na_2HPO_4.7H_2O$ (42 g), KCL (3.42 g), NaCl (2.82 g), $MgSO_4.7H_2O$ (0.72 g), $CaCl_2$ (0.24 g) and H_2O. The mixture was stirred and flushed with O_2-free carbon dioxide. The tubes were then sealed with rubber corks fitted with the gas release valve. All fermentation tubes were incubated in a shaker water bath at 39°C.

Sampling and measurement

Total volatile fatty acid (VFA), NH_3 concentration, protozoal population, and bacterial protein synthesis were measured from liquid sample taken at 4 h incubation. Dry matter and organic matter digestibility were evaluated after 48 h incubation. After 4 h of incubation, samples of rumen aliquot were taken for protozoa counting under a microscope (Ogimoto & Imai, 1981). The contents of fermentation tubes were shaken and 0.5 ml aliquot was mixed with 0.5 ml methyl green formaldehyde saline solution containing 35% formaldehyde, distilled water, methyl green and NaCl. The stained sample was kept at room temperature and the population of protozoa was counted directly by using a counting chamber under a microscope (40×). Ammonia concentration was analyzed by using micro diffusion Conway and total volatile fatty acid production was analyzed by steam distillation (General Laboratory Procedure).

Microbial protein synthesis was determined based on Lowry assay according to Makkar *et al*. (1981). The strained rumen liquor was shaken in a magnetic stirrer (400 rpm) for 45 s to remove the microbes adsorbed on the feed particles. It then was centrifuged at 408 × g for 5 min for removing protozoa and remaining feed particles. Aliquots of 10 ml of rumen liquor, obtained after removing the feed particles and protozoa, were taken, and 2.5 ml of 64.5% TCA and 3.8 ml of 2/3 N sulfuric acid were added to each sample. Aliquots were then centrifuged at 15000 rpm for 20 min. Supernatants were discarded, and cells obtained were washed with McDougall's buffer and the mixtures were then centrifuged. The precipitates obtained after washing with distilled water were suspended in 30 ml of .25 N NaOH, heated in boiling water bath for 10 min, and protein was estimated according to Lowry method *(*Lowry's *et al.,* 1951*)*.

Statistical Analysis

Statistical analysis of the data was carried out by ANOVA using General Linear Procedure. Computation was performed using SPSS 13.0 for windows evaluation version.

Results and Discussion

Population of Protozoa

The population of protozoa were significantly reduced ($P<0.05$) with the addition of lerak extract. Supplementation of mineral mix to the lerak extract did not affect the population of protozoa as compared to the control treatment at 4 h incubation. The different ratios of forage to concentrate did not affect the population of protozoa (Table 1). There was no interaction effect between the addition of supplement (lerak extract or lerak extract plus mineral supplementation) and ratio of forage to concentrate on the population of protozoa.

The reduction of protozoal population was caused by saponin content in the lerak extract. It is known that saponin could inhibit the growth of protozoa due to binding activity of saponin to sterol that composed protozoal membrane (Patra *et al*., 2006). Among all rumen microbes, protozoa are almost susceptible to saponin-induced changes in cell membrane properties (Moss *et al.,* 2000). Hu et al. (2005) reported the reduced protozoal numbers was due to the saponin treatment. The addition of mineral mix (Ca, P, Mg, & S) to lerak extract did not significantly reduce protozoal population compared with lerak extract alone. These may be due to the presence of mineral that could stimulate the growth of protozoa and improve protozoal survival.

Bacterial Protein Synthesis (BPS)

There was no interaction effect between the addition of lerak extract supplementation and the different ratios of forage to concentrate on bacterial protein synthesis. The addition of lerak extract or lerak extract + mineral increased bacterial protein synthesis significantly ($P<0.05$). The different ratios of forage to concentrate also affected bacterial protein synthesis significantly ($P<0.05$). Bacterial protein synthesis increased when the level of concentrate was increased in the ration (Table 1).

The increased bacterial protein synthesis may be due to the reduced protozoal population in the presence of lerak extract supplementation. Protozoa has an important role in the turnover of microbial biomass. The digestion of bacteria by protozoa leads to a direct decline of efficiency of microbial growth. It was known that protozoa often digested bacteria to fulfill their protein requirement (Guiterez, 2007). Inhibition of protozoal growth, allows some bacteria to grow and increase bacterial protein supply in the rumen.

Table 1. Protozoal population, bacterial protein synthesis and fermentation characteristic with the addition of lerak extract or lerak extract + mineral in different diets

Parameters	Type of Supplementation			Diet Ratio (F:C)		
	0	lerak extract	lerak extract + mineral mix	70:30:00	50:50:00	30:70
Protozoa (Log 10 CFU)	4.37±0.09[a]	4.18±0.15[b]	4.24±0.05[a]	4.34±0.06	4.27±0.09	4.19±0.06
BPS* (mg/10 ml)	95.90± 9.57[a]	137.49±20.92[b]	114.39±24.61[ab]	92.38±29.12[a]	119.66±24.14[ab]	135.70±12.18[b]
NH3 (mM)	8.76±1.07	9.82±0.78	10.08±1.22	8.60±0.29	9.58±1.11	10.48±1.22
Total VFA (mM)	158.08±14.16	148.55±8.87	138.51±15.27	148.88±5.84[a]	128.81±7.98[b]	167.45±8.87a
DMD (%)	57.86±1.73	55.48±3.11	55.26±1.4	54.16±3.27	56.90±1.14	57.53±3.36
OMD (%)	60.75±1.44	60.20±2.23	54.27±0.88	54.89±3.39	55.99±0.91	59.23±1.28

*BPS=Bacterial Protein Synthesis, F= Forage, C= Concentrate
Means in the same row with different letters are significantly different (P<0.05).

The different diets also affected the bacteria protein synthesis. Bacterial protein synthesis tended to increase in line with the increase in concentrate ratio in the rumen. This may be due to the sufficiency of energy protein ratio (C/N) in the higher concentrate ratio that stimulates bacterial protein synthesis.

Fermentation Characteristic

Ammonia Concentration. At 4 h of incubation, there was no interaction between the addition of supplement (lerak extract or lerak extarct plus mineral supplementation) and the ratio of forage to concentrate on ammonia concentration. Ammonia concentration was not affected by either lerak extract or lerak extract + mineral supplementation or different ratios of forage to concentrate (Table 1). The addition of saponin from lerak extract did not affect protein degradation in the rumen.

Total VFA Production. The addition of lerak extract or lerak extract + mineral did not affect total VFA production. However, total VFA production was significantly affected by forage:concentrate ratio in the diet. Total VFA production increased when the level of concentrate in the ration was 70%. There was no interaction effect between lerak extract supplementation and different ratios of forage to concentrate (Table 1). The higher concentrate ratio in the diets could stimulate VFA production by rumen microbe as concentrate feed contains high carbohydrate that a major substrate for VFA production.

Dry Matter and Organic Matter Digestibilities. There was no interaction effect between the addition of lerak extract supplementation and the different ratios of forage to concentrate on dry matter and organic matter digestibilities. The addition

of lerak extract or lerak extract + mineral did not affect dry matter and organic matter digestibilities. The different ratios of forage to concentrate also did not affect dry matter and organic matter digestibilities (Table 1). These results showed that the reduction of protozoa caused by saponin did not alter the digestibility of feed in the rumen. Although protozoa have a role in feed degradation, inhibition of protozoa by saponin did not affect *in vitro* dry matter and organic matter digestibilities.

Conclusion

There was no interaction effect between ratio of forage to concentrate and the type of supplements on protozoal population and bacterial protein synthesis. The addition of 1 mg/ml lerak extract with or without minerals decreased protozoal population and increased bacterial protein synthesis without effect on dry matter and organic matter digestibilities, NH_3 concentration, and total VFA production as compared to control. The different ratios of forage and concentrate had no effect on dry matter and organic matter digestibilities and NH_3 concentration. The increased concentrate ratios in the diet reduced protozoal population but increased total VFA production and bacterial protein synthesis. Bacterial protein synthesis increased by supplementation of lerak extract without mineral addition.

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Ruminal Fungi Colonisation of Stem Tissue of Untreated and Urea Treated Rice Straw Varieties

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Abstract

The study was conducted to investigate the digestibility untreated and urea treated rice straws varieties from ruminal fungi colonization in the rumen of sheep. The study used straw from 3 varieties of rice namely Illabong, Dongara and Yrm varieties which represented medium and low quality of straw. Each varieties of straw were treated with urea prior incubation in the rumen. Approximately 5mm of 10 cross section of untreated and urea treated straw stem internodes were taken below flag leaves. The materials was placed in nylon bag and incubated in the rumen of fistula sheep for 24, 48 or 72 hours. After withdrawn from the bag bags were washed then samples were removed from bags and observed under scanning electron microscopy (SEM). Observation under SEM revealed that sporangia ruminal fungi had colonised the Dong and Yrl varieties after both 48 and 72 hours incubation. The substrate of Ilb was colonised after 24 and 72 hours incubation. All population tended to be lower after 72 hours incubation than the shorter incubation. Urea treatment decreased the time required for ruminal fungi colonisation. All treated samples were colonised after 24 hours of incubation. From this study can be concluded that digestibility variation due to urea treatment could be explained from ruminal fungi colonisation but not with rice straw varieties.

Keywords: digestibility, rice straw, ruminal fungi, urea treatment, varieties

Introduction

Rice straw is a crop residue that widely available in tropical countries One of the factors that affect the quality of rice straw is varieties. Nutritive quality of rice straw can be evaluated by biological methods and chemical method. Yulistiani *et al.* (2000) reported that different part and varieties has different chemical components content and in vitro digestibility. Dry matter degradability of rice straw varieties in the rumen of sheep was also affected by varieties, botanical fraction and urea treat-

ment (Yulistiani *et al.*, 1998). However, Yulistiani (2010) reported that stem tissue structure between varieties could not be differentiate using scanning electron microscopy observation, but this observation could detect the extent of tissue degradation of untreated and urea treated rice straw varieties after 24 hours incubation.

The primary fibre degrader in the rumen was cellulolytic bacteria (Chen *et al*., 2008). However, the utilization of poor quality, high fibre crop residues by ruminants is enhanced by ruminal fungi (Gordon and Phillips, 1995). Fungi have the ability to colonize lignified cell walls and to weakens fibrous plant tissues in the rumen (Akin and Borneman, 1990) and the ability to degrade the structural components of plant cell walls, due to its ability to produce xylanase and cellulose (Bahramian *et al*., 2011), therefore the ruminal fungi play an important role in the digestibility of fibre in the rumen (Gordon and Phillips, 1995). The objective of this studies were to investigate using scanning electron microscopy the ruminal fungi colonization on untreated and urea treated rice straw varieties incubated in the rumen of sheep.

Materials and Methods

Two fistulated sheep were used in this experiment, the sheep were fed a maintenance ration consisted of 50% oaten chaff and 50% Lucerne chaff supplemented with mineral mixed. The untreated and urea treated stems of the lower part of three varieties of rice straw Dongara (Dong), Ilabong (Ilb) and Yrl obtained from Yanco Agricultural Institute, Yanco, Leeton, N.S.W. were studied. These three varieties was chosen represented for high, medium and low quality from evaluation on their in vitro organic matter digestibility (IVOMD) (Yulistiani *et al*., 2000). The straws were treated with urea at level 40g urea/kg straw DM. Approximately 5 mm of 10 cross sections of untreated and urea treated rice straw stems internodes were taken. The samples were placed in nylon bags and incubated in the rumen for 8, 24 an 48 hours in reverse order. All bags were withdrawn simultaneously and gently washed under running tap water for 30 minutes. Control sample (0 hr incubation) were prepared similarly. For observation under SEM, samples were prepared according to the method of Akin *et al (*1984). Counting of ruminal fungi carried out by observing longitudinal section of samples using Phillips XL 20 scanning electron microscope (SEM), and printed on video print. All video images were captured at the same magnification with a data bar of 500 μm. At least two samples per incubation time and two areas per sample were evaluated. Sporangia that were attached to plant material within the delineated area were counted. The presence of sporangia was verified by comparing un incubated and incubated materials. The ruminal fungi population per cm^2 was calculated by dividing the total numbers of sporangia by the total area and multiplying by 100 data were presented descriptively.

Results and Discussion

The number of fungi colonising of untreated and urea treated straw internode are presented in Table 1. These result showed that, sporangia of ruminal fungi had colonized the untreated Dong and Yrl varieties after samples had been incubated for 48 and 72 hours. Fungi were absent or were present in extremely small numbers, in samples of both varieties after incubation for 24 hours. However, in Ilb variety which had medium quality (Yulistiani *et al*., 2000) sporangia were present after 24 and 72 hours incubation, but not after 48 hours. In 2 of 6 means values of observations fungal population decreased after 72 hours of incubation. Ruminal fungi colonised the thick cell walls sclerenchyma and small vascular bundles of rice straw internodes (Figure 1). Lack of ruminal fungi colonisation of Yrl variety are shown in Figure 2 in contrast to colonisation in Dongara and Illabong varieties.

Tabel 1. Total number of fungal sporangi/cm^2 on rice straw stems before and after treatment with urea (mean at least two observation)

Varieties	Treatments	Incubation time (hours)		
		24	48	72
Dong	untreated	0	0.13	0.36
	urea treated	2.1	1.93	0.63
Ilb	untreated	0.71	0	0.91
	urea treated	0.22	0.06	0.03
Yrl	untreated	0	0.23	0.20
	urea treated	0.5	0.17	0.21

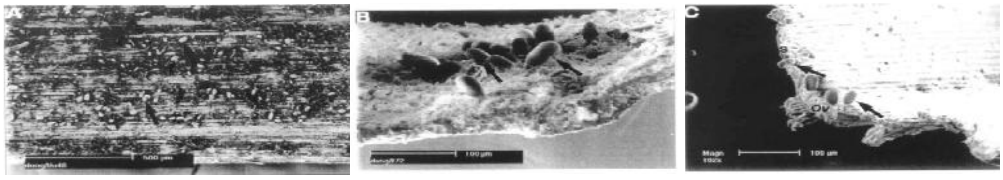

Figure 1. Scanning electron microscopy of ruminal fungi (arrow) colonization on the internode of rice straw. (A. After incubation for 24 hours (magnification 70x); B. After incubation for 72 hours (magnification 234 x); C. Ruminal fungi colonized lignified tissue of the small vascular bundles/ov and schlerenchyma/s) (magnification 162x).

From previous study it was reported that from various variety of straw evaluated showed that the lower part of the straw from Dong had the highest IVOMD and DM degradability (Yulistiani *et al*. 2000). The number of ruminal fungi colonising

untreated Dong and Ilb samples was also higher than that for Yrl, which has a lower IVOMD than either of these varieties. In addition, the fungal population numerically was higher in the treated, than the untreated, straw. This results suggest that there is a tendency for ruminal fungi to colonize the more digestible straw in higher numbers. The results on ruminal fungi population in this study could not be statistically analysed because there was no sample replication for sheep. Even though the sheep were fed on a similar diet it appeared that there were differences in diet preferences for different part of the diet. The differences in ruminal population could therefore be due to differences between sheep or to substrate.

The ruminal fungi population in the untreated dong variety extremely small after the straw had been incubated in the rumen for 24 hours, while that of urea treated Ilb was higher after the same incubation time (Table 1), eventhough the IVOMD and DM degradability of Dong were higher than those of Ilb (Yulistiani *et al*., 2000; 1998). This indicates that tissue degradation may have occurred in the presence or absence of ruminal fungi. This might indicates that the fungal population was not as active in fibre digestion as bacteria in mixed rumen microbial population, and this results agrees with the conclusion of Chen *et al*. (2008).

Ruminal fungi preferentially colonized lignified tissues of sclerenchyma and the small vascular bundles (Figure 1). Similar results has been observed by Grenet and Barry (1988). In spite of this, these walls did not degrade significantly, as previously reported by Yulistiani (2010). On the other hand, Rezaeian *et al.* (2005) reported in *in vitro* pure culture the higher fungal biomass of sodium hydroxide treated barley straw was higher and followed by the higher degradation of cellulose than that of untreated straw. Therefore, in mixed rumen microbes, the development of large numbers of sporangia on fibre may not indicate that ruminal fungi have a substantial role as a forage digester.

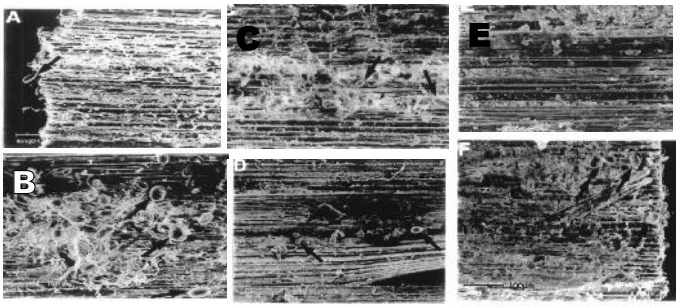

Figure 2. Scanning electron microscopy of a longitudinal section of internodes of rice straw varieties after incubation in the rumenfor 24 hours, with presence (arrow) or absence of ruminal fungi. (A. Untreated Dongara variety; B. Urea treated Dongara variety; C. Untreated Illabong variety; D. Urea treated Illabong variety; E. Untreated Yrl variety; F. Urea treated Yrl variety)

Urea treatment reduced the time required for fungi to colonise stem tissue. All area treated samples had been colonized by ruminal fungi after 24 hours of incubation. However, only with the Dong variety was the number of fungi in the treated straw higher at all incubation times compared to untreated straw (Tabel 1; Figure 2). On the other hand the IVOMD and dry matter loss (at all incubation time) of all rice straw varieties was increased after urea treatment (Yulistiani *et al*., 2000; 1998). These results indicate that digestibility increased even in the absence of ruminal fungi colonization.

Conclusion

Observation using scanning electron microscopy shows that ruminal fungi colonisation could not explain the variation of rice rice straw varieties and the effect of urea treatment.

Acknowledgment

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Reducing Methane (CH_4) Emission of Sheep Fed a Diet Supplemeted With Coconut And Palm Oil

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Abstract

An experiment was conducted to investigate the effect of polyunsaturated fatty acids supplementation on the methane (CH4) production and performance of sheep. Twenty sheep with the average body weight of 20 kg were used in this experiment. The sheep were randomly divided into four groups and each group received different ration. There were four treatments tested in this experiment i.e., R0 (basal ration + 3% coconut oil/saturated fatty acids); R1 (basal ration + 2% coconut oil + 1% crude palm oil/unsaturated fatty acids); R2 (basal ration + 1% coconut oil + 2% crude palm oil); R3 (basal ration + 3% crude palm oil). The basal ration consisted of 50% grass + 50% concentrate with nutrient content of 14% crude protein, 5% fat and 70% TDN. Experimental results showed that the supplementation of polyunsaturated fatty acids into the rations tend to reduce methane production in the rumen. The supplementation also increased the body weight gain, and digestibility of the rations, but it decreased feed consumption. It can be concluded that the addition of polyunsaturated fatty acids into the ration could improve the efficiency of energy utilization of sheep, and consequently improve the animal's performances.

Keywords: coconut oil, methane, palm oil, polyunsaturated fatty acids, ruminants

Introduction

Methane as one of end-products of fermentation process in the rumen is continuously produced in the rumen by microorganisms such as *Ruminococcus albus, R. flavefaciens, Selenomonas ruminantium* and by methanogenic species from reducing CO_2 (Yokoyama and Johnson, 1993). High methane production by animal, besides contributing to potential global warming, also indicates that feed utilization efficiency is low. Ruminants lose 5-12% of the dietary energy ingested as gases (mainly CH_4) depending on the type of diets and the level of intake (Reid *et al*., 1980).

To inhibit the formation of methane the addition of oil into diet seems to be more prospectus will not leave residues in animal products. Supplementation of unsaturated oil into ruminant diet perhaps will reduce methane emission from animals. Unsaturated oils may serve as an effective H sink in the rumen by binding H (Byers and Schelling, 1993). Palm oil contains 50% unsaturated fatty acids (APOC). Therefore it is a good source of unsaturated fatty acids. The objective of this experiment was to investigate the effect of polyunsaturated fatty acids (palm oil) supplementation into ruminant diet on the ruminal methane emission and the performance of the sheep.

Materials and Methods

The experiment was conducted *in vivo* using twenty sheep with average body weight of 18.26 ± 1.94 kg. The animals were randomly divided into four groups consisting of five animals each group in completely randomized design. Each group of animals received different ration, and the length of animal experiment was four months.

The basal ration consisted of 50% grass + 50% concentrate with nutrient content of 14% crude protein, 5% fat and 70% TDN. Four levels of oil supplementation were tested in this experiment, i.e., R0 (basal ration + 3% coconut oil/saturated fatty acids); R1 (basal ration + 2% coconut oil + 1% crude palm oil/unsaturated fatty acids); R2 (basal ration + 1% coconut oil + 2% crude palm oil); R3 (basal ration + 3% crude palm oil). Parameters measured including: rumen methane production, rumen fermentation products, feed consumption, body weight gain, feed conversion and nutrient digestibility (dry matter, organic matter, and crude protein, crude fiber).

Methane gas was determined using a closed-circuit respiration chamber. Meanwhile, nutrients digestibility was determined using total collection method. Concentration of ruminal VFA was measured using steam destilation method, and concentration of ruminal NH_3 was measured using Conway micro-diffusion method (General Laboratory, 1966)

Data were subjected to ANOVA (Steel and Torrie, 1980) using SPSS computer program.

Results and Discussion

Feed Intake and Body Weight Gain

Addition of unsaturated fatty acids gave inconsistent results of feed (dry matter) intake of sheep (Figure 1) and statistically was not different ($P>0.05$) among the treatments. Sheep fed R2 had the highest dry matter intake. It is often reported that unsaturated oil is more toxic to the microbial rumen than saturated oil (Henderson, 1973), and inhibits growth of microbes (Palmquist and Jenkins, 1980). Wachira *et*

al. (2002) reported that feeding unprotected fish oil (unsaturated oil) to sheep caused a decrease in feed intake and body weight gain. This agrees with the present results, especially with the results of sheep fed R1 and R3.

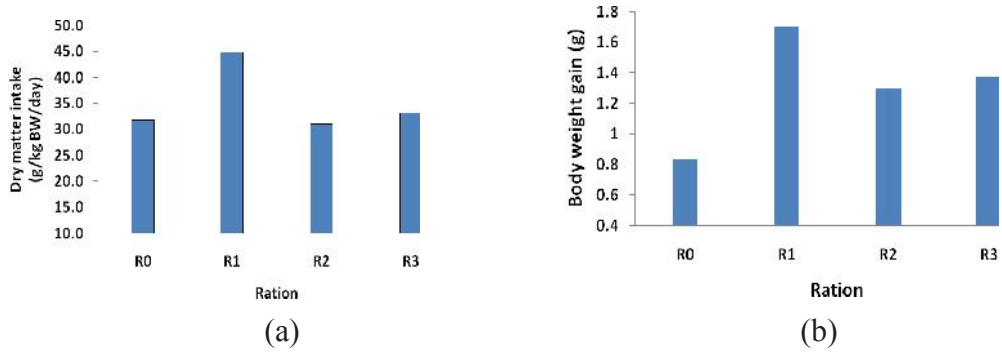


Figure 1. Effects of additional fat into the diets on (a) dry matter intake and (b) body weight gain of sheep. (R0: basal ration + 3% coconut oil/saturated fatty acids; R1: basal ration + 2% coconut oil + 1% crude palm oil/unsaturated fatty acids; R2: basal ration + 1% coconut oil + 2% crude palm oil; R3: basal ration + 3% crude palm oil).

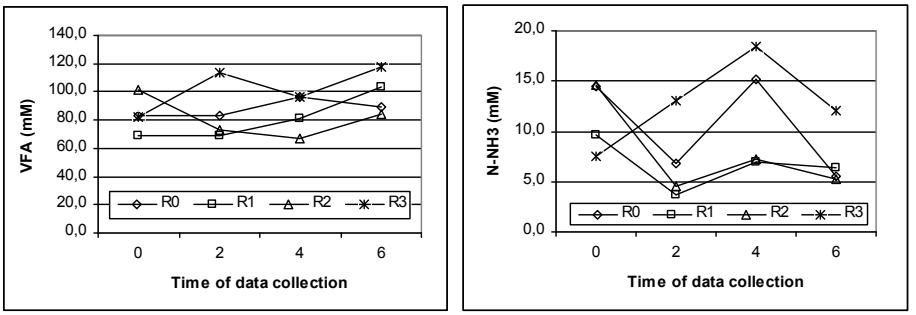
Addition of unsaturated fatty acids improved body weight gain of sheep (Figure 1), eventhough statistically was not different. The highest body weight gain was achieved by animals fed R1 (Basal diet + 2% saturated oil + 1% unsaturated oil), followed by R3, R2 and R0. Body weight gain) is influenced by level of feed intake and characteristics of feed. Except sheep fed R2, those sheep fed unsaturated oil (R1 and R3) had lower feed intake than those fed saturated oil (R0). This may indicated that higher body weight gain of sheep fed R1, R2, and R3 was due to the supplementation of unsaturated oil by improving feed efficiency (low methane production).

Ruminal VFA and N-NH_3

Ruminal VFA concentration was not significantly ($P>0.05$) affected by the treatments and it ranged from 65 to 120 mM (Figure 2). This range is in normal concentration and is sufficient to support rumen microbial growth and to supply energy for the animals. There is no negative effect of unsaturated oil supplementation on the carbohydrates fermentation in the rumen, and yet the R3 (ration containing 3% unsaturated oil) had the highest VFA concentration in the rumen. Other research results also showed that total volatile fatty acid concentrations was not affected by source and amount of oil (Ohajuruka, *et al*., 1991).

The concentration of N-NH_3 was in normal range (4–18 mM) and enough to support rumen microbial activities and to synthesize rumen microbial protein. This

result also means that protein degradation in the rumen was not affected by the supplementation of unsaturated oil into the rations. This is in line with Ohajuruka, *et al*. (1991) who reported that ammonia-N was not affected by source and amount of oil.



(a) (b)

Figure 2. Effects of additional fat into the diets on (a) ruminal VFA and (b) ruminal N-NH_3. (R0: basal ration + 3% coconut oil/saturated fatty acids; R1: basal ration + 2% coconut oil + 1% crude palm oil/unsaturated fatty acids; R2: basal ration + 1% coconut oil + 2% crude palm oil; R3: basal ration + 3% crude palm oil).

Feed Digestibility

Effect of oil addition into the diet on feed digestibility was shown in Figure 3. Feed digestibility had tendency to increase by the addition of unsaturated oil into the diet. Eventhough it was not significantly different. In contrast, crude fiber digestibility reduced by the addition of unsaturated oil into the rations. Oil supplementation may decrease forage digestibility by disturbing normal condition of rumen microbe when given at level above 5% of the ration (Preston and Leng, 1987). This is more pronounced with unsaturated oil, because unsaturated oil is more toxic to the microbial rumen than saturated oil (Henderson, 1973). The increase in feed digestibilities in this experiment indicated that the addition of 3% unsaturated oil into sheep rations did not cause negative effect on the fermentation process in the rumen.

Methane Production

Unsaturated oil addition into the ration tend to lower methane production by sheep (Figure 4), eventhogh statistically was not different. Unsaturated oils may also serve as an effective H sink in the rumen by consuming H (Byers and Schelling, 1993). Therefore, methane formation competed with the saturation of unsaturated oils for H sink. It implies that when dietary unsaturated oils are given to ruminant, in line with H availability in the rumen, saturation occurs and methane production decreases as shown by the results in this experiment (R1, R2 and R3). The results are supported by Chikunya *et al*. (2004) who reported that biohydrogenation in the

rumen of linoleic acid (18:3 n-6; 85-90 %), linolenic acid (18:3 n-3; 88-93 %), docosahexaenoic acid (22:6 n-3; 91 %) and EPA (20:5 n-3; 92 %) was extensive.

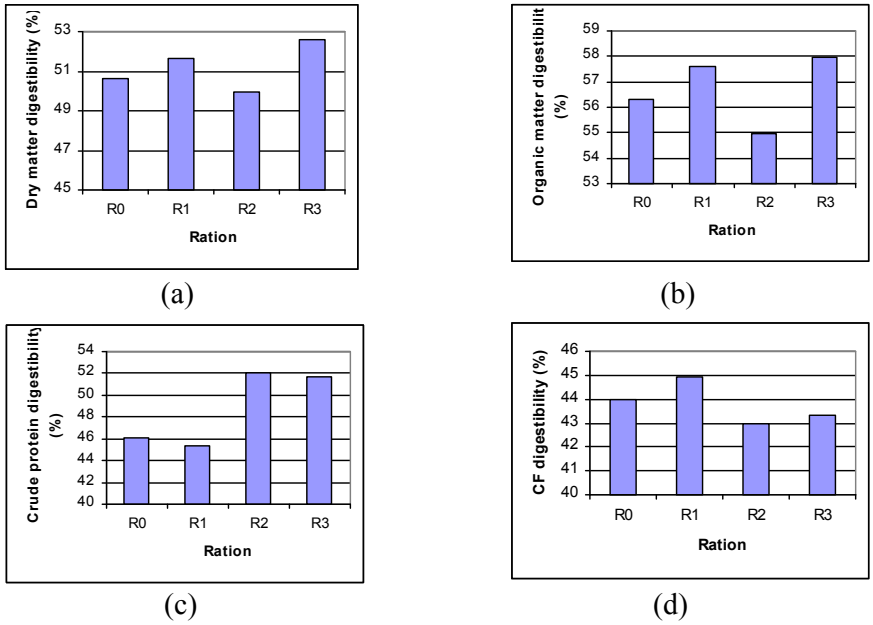


Figure 3. Effects of additional fat into the diets on feed digestibility (%) of sheep: (a) dry matter digestibility, (b) organic matter digestibility, (c) crude protein digestibility, and (d) crude fiber digestibility. (R0: basal ration + 3% coconut oil/saturated fatty acids; R1: basal ration + 2% coconut oil + 1% crude palm oil/unsaturated fatty acids; R2: basal ration + 1% coconut oil + 2% crude palm oil; R3: basal ration + 3% crude palm oil)

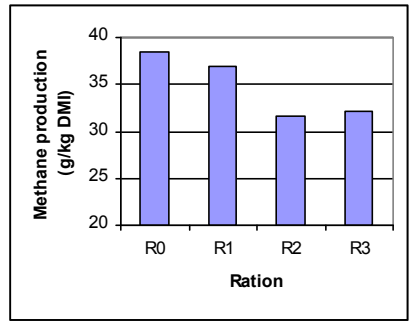


Figure 4. Effects of additional fat into the rations on methane (CH_4) production of sheep, (R0: Basal diet + 3% saturated fat; R1: Basal diet + 2% saturated fat + 1% unsaturated fat; R2: Basal diet + 1% saturated fat + 2% unsaturated fat; R3: Basal diet + 3% unsaturated fat).

Conclusion

The supplementation of polyunsaturated fatty acids into sheep rations improved feed utilization and efficiency indicated by higher body weight gain and feed digestibility, and lower methane production. Addition level of 3% of polyunsaturated fatty acids did not cause negative effect but it is not sufficient to show significant results on the sheep performance.

Acknowledgement

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In Vitro Digestibility of Lampoyangan Grass (*Panicum sarmentosum Roxb*) in Form of Hay and Silage

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Abtract

The study aim was to determine in vitro digestibility of silage and hay of Lampoyangan grass (Panicum sarmentosum Roxb) which were fermented and dried in defference of length.This wild grass live in Tondo Panicum sarmentosum Roxb Grasses made in silage and hay forms were obtained from the wild grasses that lived in Tondo Village, District of Eastern Palu, Palu Municipal. In this study the grass was made as silage and hay forms as a treatment followed 2 x 3 factorial pattern of Completely Randomized Design the length of fermentation for silage was 30, 45 and 60 days and the length of drying for hay was 2, 4 and 6 days. Observed variables were in vitro dry matter (DMD) and organic matter (OMD) digestibilities of Lampoyangan grass both silage and hay treatments. The finding results showed that the length of fermentation in silage treatment did not affect DMD and OMD of the grass, but significantly affected DMD and OMD of Lampoyangan grass in hay treatment (P<0,05). Four days of drying was the best time to get the effective time for making hay of Lampoyangan grass. Actually the DM and OM digestibilities of Lampoyangan grass silage treatment were higher than of hay treatment.

Keywords: digestibility, fermentation, hay, Lampoyangan grass, silage.

Introduction

Lampoyangan grass (*Panicum sarmentosum Roxb*) is one type of promising grass that grows and spreads naturally on dry land in Tondo Village (Amar, 2003; (Amar *et al*., 2005). This grass grows better than other grasses, either planted as a crop monoculture or mixed with *Panicum maximum*, or planted with Desmanthus (Tarsono *et al*., 2009). Lampoyangan grass will become the best grass in the future and considering as superior grass in the dry land areas due to its high capability for shade tolerance and drought resistance.

Production of *Panicum sarmentosum Roxb* grass are often surplus than, 145 kg/ha (Tarsono *et al*., 2009) and the content of crude protein and crude fiber were 12.80% and 33.82%. Respectively for the grass before flowering (Tarsono and Amar, 2007). Those advantages are usefull anticipating of the shortage of forage production in the transition period of rainy to dry season. Overproduction during the best growth can be utilized, that is in preserved forms of silage or hay. Factors that may affect the quality of silage and hay are: plant age, origin or type of forage, storage temperature, level of withering and maturity or cutting length (Regan, 1997 and Driehuis *et al*., 2001). The best time to cut the plants for making silage or hay is in the vegetative phase, before the formation of flowers (Reksohadiprodjo, 1995, and Regan, 1997). The plant growth phase at the time of making silage and hay affects the digestibility of dry matter and organic matter (Harrison *et al*., 1994). Saloko (2006) reported that *Panicum sarmentosum Roxb* grass that got the addition of preservatives in the level 22.5% of dry matters and fermented for 30 days resulted in the succeed silage percentage of 94.58%, while 4–day drying time was the best of *in vitro* digestibilities in cassava leaves (*Manihot esculenta Crantz*) as reported by Zubaidah (2005). To examine whether a given feed material is good enough to support microbial growth and enzymatic processes in animal body, the way that is so long to be considered as the most convenient, accurate and relatively quick is through *in vitro* assays. The assays are carried out outside the animal body by using simulations that are similar to processes that occur in the body. Digestibility studies on *Panicum sarmentosum Roxb* grass that involve the fermentation length in silage process and the drying length on in hay prosses are still in the lack of interest. Therefore, the aim of this research was to obtain an overview and recommendations about *in vitro* digestibilities of silage and hay of *Panicum sarmentosum* Roxb grass with differences of fermentation and drying length.

Materials and Methods

Materials

The material of this study was *Panicum sarmentosum Roxb* grass obtained from wild grasses that lived in Tondo village, District of Eastern Palu, Palu Municipal.

The study was designed with a Completely Randomized Design of factorial pattern consisting of two factors (2x3). namely silage and hay with three levels of silage treatments, namely the length of fermentation 30, 45 and 60 days respectivelly and the hay treatments were the length of drying 2, 4 and 6 days, respectively. The treatments that showed a significant effects were followed by Duncan test.

Methods

Panicum sarmentosum Roxb grass that had been chopped (± 20 cm from ground level), was taken to the laboratory and then withered to about 65% water

content. The grasses were cut into pieces about 1-2 cm. a pieces were then treated in accordance with the length of fermentation for silage and the length of drying for hay. The use of preservatives that have been determined, that was 22, 5% of the dry matters (Saloko, 2006). Samples were taken at random and then divided into two groups, that was 50% to be made for silage with the length of fermentation 30, 45 and 60 days and 50% to be made for hay with the length of drying 2, 4 and 6 days. In making silage cans were filled and compacted with a press so that there were no air cavities in between, then given with the pieces of *Panicum sarmentosum Roxb* grass. Cans were sealed and given with a tape (insulation) in order to be airtight. While, in making hay three repetitions were made for each treatment so that there were 9 cans (silos), so it was with making hay, the grass divided according to the number of treatments and then drying was performed by using a drying rack in the sun. After the silage and hay have been made in accordance with each of treatments then they were continued by drying in oven 55^{o}C. After drying, the sample was milled using a Wiley mill with a sieve of 3 mm diameter hole, then followed by in vitro digestibility test, follow the procedures Tilley and Terry (1963) in (Harris, 1970).

Observed Variables

The observed variables were *in vitro* dry matter (DMD), and organic matter digestibilities (OMD) based on methods that have been developed by Tilley and Terry (1963).

Results and Discussion

In vitro dry matter digestibility (DMD) and organic matters of *Panicum sarmentosum Roxb* in forms of silage and hay. The value of in vitro dry matter digestibilities (DMD and OMD) of *Panicum sarmentosum Roxb* made in form

Table 1. Dry matter digestibilities (DMD) and organic matter digestibilities (OMD) of *Panicum sarmentosum* Roxb grass in silage and hay form

Fermentation Length (days)	DMD (%)	OMD (%)
30	57.51 ± 1.57	56.13 ± 0.76
45	57.52 ± 1.57	56.14 ± 0.76
60	57.38 ± 2.07	56.55 ± 1.53
Drying Length (days)		
2	54.51 ± 1.57	54.15 ± 0.76
4	57.21 ± 1.57	56.13 ± 0.76
6	56.18 ± 2.07	56.12 ± 1.53

showed that the treatment length of ensilage and hay process has no significant effect on DMD and OMD (Table 1).

In vitro dry matter and organic matter digestibilities of *Panicum sarmentosum Roxb* grass in silage forms with different fermentation lengths showed nearly same levels of digestibilities, with the fermentation lengths 30, 45 and 60 days, respectively; for DMD was 57, 51% $\pm$ 1.57^{a}; 56.52 $\pm$ 0.76^{a}; 57.38 $\pm$ 1.57^{a}; and for OMD 56.13 $\pm$ 0.76^{a}; 56.14 $\pm$ 2.07^{a} and 56.55 $\pm$ 1.53^{a}. This indicates that the fermentation lengths did not affect crude fiber components either in fermentations for 30 days, 45 or 60 days. One of the factors that influence digestibility in both DMD and OMD is components of crude fiber, mainly cellulose, hemicellulose, and lignin (Van Soest, 1982). Cellulose, hemicellulose are parts of plants that are difficult to digest, while lignin is a part of plants that can not be digested at all (Anggorodi, 1984).

The four-day drying length is was significantly higher than the 2-day drying length, but there is was no significant difference with the 6-day drying length. This is due to the feed material that has a longer drying will be more difficult to be degraded by rumen microbes because the heating process can protect some proteins of *Panicum sarmentosum Roxb* grass. The same thing was stated by Abidin and Hendratmo (1983) in Zubaidah (2005) that the feed material that has undergone heating could protect some proteins against microbial fermentation in the rumen. *In vitro* dry matter and organic matter digestibilities of Panicum sarmentosum Roxb grass decrease with the length of drying, because the longer drying the harder components of crude fiber, among those are ADF, NDF, cellulose, hemicellulose, and lignin contents those are components of plant cell wall. The plant cell wall components are parts that are difficult to digest, even the lignin component can not be digested at all.

The length of the longer fermentations is was unable to improve both in vitro DMD and OMD. This is possible because the used grass to have the same relative age. One of the factors that influence both the level of DMD and OMD is the age of the plant, for which the older plant the higher crude fiber content, so that both *in vitro* DMD and OMD made in silage form with different fermentation lengths have the same percentage rate (Muck, 2009). Feed substance components that are easily digested such as protein decrease, while the components that are difficult to digest such as ADF, NDF, cellulose, and lignin increase with increasing plant age. Whiteman (1980) stated that increasing plant age, the proportion of parts that can be digested such as carbohydrates, proteins and other cell contents tended to decrease, whereas the proportion of which were difficult to digest such as lignin, cuticle, and silica increased. Similarly, Crowder and Chheda (1982) stated that the digestibility value differences of a forage in relation to changes of chemical composition, fibrous parts, lignin, and silica content which arose as a result of differences in species and genotype, growth rate, environmental conditions, place to grow, and management system.

Based on the values of *in vitro* organic matter digestibility (OMD), *Panicum sarmentosum Roxb* which will be utilized in fresh form or preserved in dry form (hay) should be cut at the age of 40 days, in order to obtain *in vitro* digestibility of organic matter is optimal. Just as in vitro dry matter digestibility (DMD), Panicum sarmentosum Roxb grass made in silage form with different fermentation lengths showed no significant effect on in vitro organic matter digestibility (OMD), but that were made in hay form showed significant effect ($P < 0.05$). Similarly, *in vitro* dry matter digestibility (DMD) of *Panicum sarmentosum Roxb* grass as well as *in vitro* organic matter digestibility (OMD) of *Panicum sarmentosum Roxb* grass, because the organic matter content was calculated based on the dry matters, then the existence of 4-day drying length drying influence on DMD, the same influence also occurred on OMD. Determination of plant dry matter when burned, then what remains is ash (inorganic material) (Anggorodi, 1984; Reksohadiprodjo, 1995; and Tillman *et al*., 1989).

Conclusion

1. *Panicum sarmentosum Roxb* made in silage form, the amount of fermentation time showed no significant effect on *in vitro* dry matter (DMD) and organic matter digestibilities (OMD).
2. *Panicum sarmentosum Roxb* grass made in hay form with different drying lengths showedsignificant effect. Four-day drying length is the best time to get the effective time for making hay of *Panicum sarmentosum Roxb* grass.
3. DM and OM digestibilities of *Panicum sarmentosum Roxb* grass made in silage form are higher than those in hay form, but they have no statistically significant differences.

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Improving Production Performance of Peranakan Ongole Cows and Nutrient Digestibility of Rice Straw Based Diet with Energy-Protein Supplementation Given Separately or in Complete Feed

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Abstract

Climate is the limiting factor for availability of good quality forage in Rembang regency. Rice straw is then given as an alternative feed for Peranakan Ongole (PO) cows. However, this feed is low in nutrient content and quality has low digestibility and low palatability. Overcoming this problem can be done by designing good quality supplements, such as nutrient rich supplement (SKN) that can be mixed with concentrate, or can be formulated in a complete feed. Therefore, this experiment was conducted to study responses of PO cows to improvement of rice straw based diet with supplementation and complete feed. The experiment was carried out in a randomised block design with 16 cows were allocated randomly into 4 groups. The treatments were R1 (rice straw), R2 (R1 + rice bran 2 kg/head/day), R3 (R2 + SKN 0.4 kg/head/day), and R4 (complete feed). The data were analysed using analysis of variances and differences among treatments were examined with contrast orthogonal test. The results showed that the use of rice bran, SKN and complete feed improved quality of rice straw as based diet which subsequently improved nutrient intakes. The treatments were significant on dry matter and crude protein digestibilities ($P<0.05$), but did not affect organic matter and energy digestibility, body weight gain and feed efficiency. The highest income over feed cost was obtained by using R2. It is concluded that giving complete feed (R4) produced the best performance of PO cows. SKN can still be used to improve rice straw based diet if the amount is increased.

Keywords: complete feed, peranakan ongole cows, rice straw, SKN, supplementation

Introduction

Rembang Regency is the 4th rank for beef cattle production in Central Java Province, populations were 115,220 heads in 2009 with Peranakan Ongole (PO) was one of cattle breeds raised by farmers (Government of Rembang Regency, 2011). Cattles are used to meet local requirement for meat, and exported to other regencies in Java Island. However, climate is the limiting factor for raising the cattle and for the availability of good quality forage; this was because: 1. its location in low land area in the north coastal area of Java island; 2. having tropical climate (maximum temperature 33 ^{o}C and average temperature 23 ^{o}C); 3. short period of wet season (4-5 month/year) with long period of dry season, and 4. scarcity in rain drops in a year (average rain drops 1 039.36 mm in 2009) (Agricultural and Forestry Office, Rembang Regency, 2009).

Rice straw is given as an alternative feed for PO cows, but it has low nutrient content and quality, low digestibility and low palatability (Sutardi, 1981). One solution to overcome the problem is by designing good quality supplements (Suryahadi *et al*., 2003; Leng, 1993), i.e. nutrient rich supplement (SKN, *suplemen kaya nutrien*) containing good quality of energy, protein and mineral sources, that can be mixed with concentrate, or can be formulated in a complete feed. These supplements are expected to provide nutrients lacking in rice straw and can be given in a small amounts to improve rice straw utilisation. These can be formulated by using feed sources available locally in Rembang Regency or nearby cities (CENTRAS, Centre for Research and Community Service, IPB, - Government of Rembang Regency, 2010). Fish meal from locally fish industry and leguminous leaf meals (leucaena, *Leucaena leucocephala*; and turi, *Sesbania grandiflora*) are undegradable protein sources, and degradable protein sources are cassava meal (*Manihot esculenta*) and rice bran cassava leaf meal and rice bran had protein that were less degradable than fish meal, soybean meal and the other two legume leaf meal; so would you mind to leave the previous statement as it is(Sutardi *et al*., 1983; Soenarso, 1984). Rice bran and molasses are highly fermentable energy sources than rice straw. Mineral sources are also provided. These are expected improve animal performance. Therefore, this experiment was conducted to study responses of PO cows to improvement of rice straw based diet with supplementation and complete feed.

Materials and methods

Mature PO cows (16 heads) were used (aged : 2-6 years old and initial body weight, BW : 304.31 $\pm$ 30.1 kg), divided into four groups and kept in an individual cage. Feeds were rice straw (*Oryza sativa*), leaf meal containing cassava, leucaena, and turi, fish meal, rice bran, molasses and mineral mixed from Rembang, Tuban and Pati districts.

Four treatments were developed based on observation in feeding practise conducted by farmers in Rembang Regency: R1=Rice straw (*ad libitum*); R2= R1+rice bran as energy supplement (2 kg/head/day); R3=R2+SKN (0.4 kg/head/day); supplement was given separately, and R4= Rice straw base complete feed in which rice straw and energy - protein supplement (SKN) was mixed and given as a single feed. Complete feed: rice straw 40, rice bran 30.5, fish meal 8.5, cassava leaf meal 5.7, leucaena leaf meal 3.0, sesbania (turi) leaf meal 0.3, molasses10, vegetable oil 1 and mineral mix 1 (% dry matter, DM basis).

Variables measured were feed and nutrient intakes, digested nutrients, nutrient digestibilities, body weight gain (BWG), feed or ration efficiency ratio (FER), and income over feed cost (IOFC). Randomised block design (4 treatments and 4 replications), analysis of variance and contrast orthogonal were applied (Steel and Torrie, 1993).

Results and Discussion

The study was done in a community farm taken care by 18 members and located in Tanjung village, Sulang district, Rembang Regency (July to September 2010). PO cattles (mature bulls 2 heads, mature cows 16 heads, and calves 4 heads) were mostly kept for fattening and breeding programmes. As keeping cattle is the secondary income, the farmers look after the cattle traditionally and give feeds

Table 1. Nutrient composition of ration

Nutrient composition1	Treatment			
	Rice straw (R1)	Rice straw + supplement		Complete feed (R4)
		Rice bran (R2)	Rice bran + SKN (R3)	
Dry matter (DM, % fresh sample)	37.99	50.44	52.05	60.44
Ash (% DM)	17.40	17.19	17.08	18.75
Organic matter (% DM)	82.60	82.81	82.92	81.25
Crude protein (% DM)	4.21	5.92	6.48	11.80
Ether extract (% DM)	1.44	2.48	2.71	3.52
Crude fibre (% DM)	32.50	31.02	30.44	25.80
Nitrogen free extract (% DM)	44.45	43.39	43.30	40.12
Ca (% DM)	0.42	0.30	0.41	2.65
P (% DM)	0.28	0.54	0.52	0.29

[1] Calculated on the basis of data from Sutardi (1981) and proximate analysis by the Laboratory of Research Centre of Natural Resources and Biotechnology, Bogor Agricultural Institute (2011).

available seasonally having low quantity and quality which were insufficient to support production and reproduction of animals.

R1 had the lowest nutrient contents, excepted for CF content (Table 1). Rice bran supplementation (2 kg/head/day) improved nutrient contents in R2 with further improvements were obtained in R3 and R4. These affected intakes of rice straw DM, total nutrients (DM, OM, and CP) ($P<0.01$) and TDN ($P<0.05$), but did not af-

Table 2. Intake, digested nutrient, nutrient digestibility of rice straw based diet, body weight gain and feed efficiency ratio

Variables	Treatment			
	Rice straw (R1)	Rice straw + supplemen		Complete feed (R4)
		Rice bran (R2)	Rice bran + SKN (R3)	
Intakes (kg/head/day):				
Fresh	9.28 ± 1.43	8.75 ± 0.57	9.15 ± 0.67	10.08 ± 1.63
Rice straw (DM)[1]	3.52 ± 0.56[a]	2.60 ± 0.16[b]	2.63 ± 0.32[b]	1.87 ± 0.31[c]
Total dry matter[1]	3.52 ± 0.56[c]	4.42 ± 0.16[b]	4.76 ± 0.32[b]	6.09 ± 0.90[a]
Total organic matter[1]	2.91 ± 0.46[c]	3.66 ± 0.13[b]	3.95 ± 0.26[b]	4.95 ± 0.73[a]
Total crude protein[1]	0.15 ± 0.02[c]	0.26 ± 0.01[b]	0.31 ± 0.01[b]	0.72 ± 0.11[a]
TDN[1,2]	2.11 ± 0.41[b]	2.53 ± 0.38[b]	2.75 ± 0.20[a]	2.94 ± 0.38[a]
Digested nutrient (kg) :				
Digested DM	2.32 ± 0.48	2.73 ± 0.49	2.97 ± 0.25	2.94 ± 0.41
Digested OM	2.05 ± 0.40	2.40 ± 0.37	2.61 ± 0.19	2.72 ± 0.35
Digested CP1	0.03 ± 0.02[a]	0.13 ± 0.04[b]	0.15 ± 0.02[b]	0.45 ± 0.06[c]
Digested energy (MJ)	38.81 ± 7.57[a]	46.62 ± 6.90[a]	50.61 ± 3.64[b]	54.09 ± 6.92[b]
Digestibility (%):				
Dry matter (DM)[1]	65.41 ± 3.65[a]	61.84 ± 10.09[a]	62.57 ± 6.17[a]	48.65 ± 6.94[b]
Organic matter (OM)	69.99 ± 3.17	65.62 ± 9.09	66.34 ± 5.56	55.34 ± 6.05
Crude protein (CP)[1]	18.55 ± 8.60[a]	50.40 ± 13.25[b]	49.27 ± 8.95[b]	62.67 ± 4.88[c]
Digestible energy (DE, MJ/kg BK)	10.97 ± 0.48	10.55 ± 1.39	10.65 ± 0.88	8.93 ± 0.93
BWG and FER				
BWG (kg/head/day)	0.06 ± 0.30	0.29 ± 0.43	0.31 ± 0.12	0.66 ± 0.34
FER (%)	2.08 ± 8.36	6.56 ± 10.06	6.34 ± 2.29	10.78 ± 5.57

[1]Means with different superscript in the same row differ significantly at ($P<0.05$); [2]TDN (total digestible nutrient) was estimated using this formula: TDN = digested crude protein + 2.25 digested ether extract + digested crude fibre + digested nitrogen free extract (Sutardi, 1980).

fected fresh feed intake (Table 2). Increases in total energy and CP intakes followed ascending order: R2, R3 and R4.

Treatments affected digested energy and CP ($P<0.05$) with the lowest in R1 (Table 2), and the highest in R4 ($P<0.05$). Digested nutrients were also improved by rice bran supplementation (R2), but its combination with SKN (R3) only increased digested energy. The increase in CP digestibility ($P<0.05$) occurred in a similar trend, but reverse results were found in DM and OM digestibility, and digestible energy (DE). The lowest results in energy digestibility were complete feed ($P<0.05$), and the highest were rice straw (R1). As bulky feeds with high CF content, rice straw filled the digestive tract stimulating satiety sensation in faster rate, slowering passage rate, decreasing its DM intake and increasing its digestion.

Supplementation improved BWG and FER biologically (Table 2) that were similar to others (Purnomoadi *et al*., 2007; Arifin *et al*., 1998). The same effects also occurred in IOFC, but in descending order: R2, R3 and R4 (Table 3) due to the high cost of supplement (SKN) increasing the price of complete feed with a greater use of SKN.

Table 3. Income over feed cost for cattle given rice straw based diet and supplement

Variables	Treatment			
	Rice straw (R1)	Rice straw + supplement		Complete feed (R4)
		Rice bran (R2)	Rice bran + SKN (R3)	
Rice straw DM intake (kg/head/day)	3.52	2.60	2.63	-
Rice bran DM intake (kg/head/day)	-	1.82	1.82	-
SKN DM intake (kg/head/day)	-	-	0.31	-
Complete feed DM intake (kg/head/day)	-	-	-	6.09
Total feed cost (Rp/day)[1]	704.83	2,339.04	3,241.37	12,926.03
Body weight gain (BWG, kg/head/day)	0.06	0.29	0.31	0.66
BWG values (Rp/head/day)[2]	1,412.4	6,465.25	6,710.55	14,421.55
Income over feed cost (Rp/head/day)	707.57	4,126.21	3,469.18	1,495.53

[1]Feed price in July 2010: rice straw= Rp. 200,-/kg; rice bran= Rp. 1000,-/kg; SKN= Rp. 2890,-/kg; complete feed= Rp. 2122,50/kg; [2]Cattle selling price= Rp. 22000,-/kg live weight.

Supplementation produced good effects through the increase in availability of good quality nutrients (energy sources, combination between degradable and undegradable protein and mineral addition) for rumen microbes to improve its ability to ferment rice straw based diet and synthesis its protein and for ruminants to digest the nutrients intensively in post ruminal digestive tract (Suryahadi *et al*., 2003). Lack of significant effects on BWG and FER was due to small amount of supplement (SKN) given to the animals and variation in responses among cattles.

Conclusion

Biological effects of rice straw based diet supplemented with rice bran has increased efficiency of nutrient utilisation, especially energy. Further increases were obtained by supplementing with SKN. Given rice straw, rice bran and SKN in a complete feed produced the best performance based on efficiency of energy utilisation (TDN and DE values) and BWG, and the best IOFC. The highest IOFC produced by using rice bran as supplement was due to the high price of SKN and the highest cost of producing complete feed. It is recommended to improve nutrient quality and quantity of supplements, to reformulate SKN composition and ratio between SKN and rice straw in complete feed, and to produce supplements at low cost in order to increase income over feed cost for the farmers.

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Effect of Waste Products on Ruminal Microbe Population and Rumen Charateristics *in Vitro*

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Abstract

Certain tropical plants may have a nutritional value beyond simply their nutrient content, i.e. as rumen-manipulating agents that can be used as an alternative mean to solve problems in animal nutrition and livestock production in Indonesia. The objective of the present study was to measure the nutrient and phytochemical contents, and their effects on rumen microbial population and rumen fluid characteristics in vitro, of different parts of Uncaria gambir leaves and Cacao (Theobroma cacao L). Determination of nutrient content according to AOAC procedures (1995), fiber fractions (NDF and ADF) according to van Soest et al. (1991), phytochemical (alkaloid, phenol, terpenoid, saponin and tannin) content qualitatively and the effect of plant fractions on rumen microbial fermentation were evaluated in in vitro batch culture of rumen fluid. Parameters observed were total rumen ciliate population, total bacteria, cellulolyt ic bacteria, total VFA, ammonia N concentration, and pH. There was no significant effect (P>0.1) of phenolic and tannin present in Uncaria gambir on total microbial population, although the presence of saponin indicated a tendency to reduce the number of protozoa. However, it gave a significant effect (P<0.01) on VFA total and ammonia N concentration compared to control. Meanwhile, fractions of cacao waste (cacao pod and leaves) resulted in significant effect (P<0.01) on pH, ammonia N concentration and VFA total. But, the presence of phenolic (tannin) and alkaloid (theobromine) has no significant effect on total ciliate, total bacteria and cellulolytic bacteria and therefore they may have the potential as beneficial manipulators of rumen fermentation.

Keywords: rumen characteristics, ruminal microbe population, Uncaria gambir, Theobroma cacao

Introduction

Certain tropical plants may have a nutritional value beyond simply their nutrient content, i.e. as rumen-manipulating agents that can be used as an alternative mean to solve problems in animal nutrition and livestock production in Indonesia. The

objective of the present study was to measure the nutrient and phytochemical contents, and their effects on rumen microbial population and rumen fluid characteristics *in vitro*, of different parts of *Uncaria gambir* leaves and Cacao (*Theobroma cacao* L). Determination of nutrient content according to AOAC procedures (1995), fiber fractions (NDF and ADF) according to van Soest *et al*. (1991), phytochemical (alkaloid, phenol, terpenoid, saponin and tannin) content qualitatively and the effect of plant fractions on rumen microbial fermentation were evaluated in *in vitro* batch culture of rumen fluid. Parameters observed were total rumen ciliate population, total bacteria, cellulolytic bacteria, total VFA, ammonia N concentration, and pH. There was no significant effect (P>0.1) of phenolic and tannin present in *Uncaria gambir* on total microbial population, although the presence of saponin indicated a tendency to reduce the number of protozoa. However, it gave a significant effect (P<0.01) on VFA total and ammonia N concentration compared to control. Meanwhile, fractions of cacao waste (cacao pod and leaves) resulted in significant effects (P<0.01) on pH, ammonia N concentration and total VFA. But, the presence of phenolic (tannin) and alkaloid (theobromine) has no significant effect on total ciliate, total bacteria and cellulolytic bacteria and therefore they may have the potential as beneficial manipulators of rumen fermentation.

Materials and Methods

This experiment utilized the waste plant fraction at several stages during the agricultural production of the main export commodities from West Sumatera Indonesia i.e. *Uncaria gambir* (fresh leaves, steamed leaves, moulded leaves) and Cacao (leaves, cacao pod, and cacao skin). Determination of nutrient content was conducted according to AOAC procedures (1995), fiber fractions (NDF and ADF) was determined according to van Soest *et al*. (1991), phytochemical (alkaloid, phenol, terpenoid, saponin and tannin) content was determined qualitatively and the effect of plant fractions on rumen microbial fermentation was evaluated in *in vitro* batch culture of rumen fluid. Experimental design used was Randomized Block Design with 4 treatments and 4 repeats as block. Parameters observed were total ruminal ciliate population, total bacteria, cellulolytic bacteria, total VFA, ammonia N concentration, and pH in a rumen microbial batch fermentation system.

Results and Discussion

There was no significant effect (P>0.1) of phenolic and tannin present in *Uncaria gambir* on total microbial population, although the presence of saponin indicated a tendency to reduce the number of protozoa. However, it gave a significant effect (P<0.01) on VFA total and ammonia N concentration compared to control. Meanwhile, fractions of cacao waste (cacao pod and leaves) resulted in significant

Table 1. Effects of fractions of *Uncaria gambir* and *Theobroma cacao* on microbial number (log 10/ml) and ruminal fluid characteristics

Treatments	Parameters					
	Total bacteria	Cellulolytic bacteria	Protozoa	$N\text{-}NH_3$ (mg/100ml)	VFA (mM)	pH
Control	9.74	8.56	3.97	17.22	58.34	6.88
Cacao leaves	10.44	9.37	2.13	32.22	138.6	6.92
Cacao pod	10.24	9.20	3.16	29.60	142.7	6.70
Cacao seed skin	10.00	8.98	4.24	29.92	174.4	6.55
Pressed Uncaria leaves	8.72	8.39	3.88	33.27	130.69	6.82
Young Uncaria leaves	8.98	8.90	2.97	40.89	175.37	6.90
Moulded Uncaria leaves	8.85	8.80	4.18	42.28	182.14	6.79

effects ($P<0.01$) on pH, ammonia N concentration and VFA total. But, the presence of phenolic (tannin) and alkaloid (theobromine) has no significant effect on total ciliate, total bacteria and cellulolytic bacteria.

Conclusions

Results from the present *in vitro* studies suggest both agricultural wastes from *Uncaria gambir* and *Theobroma cacao* had no substantial effects on microbial numbers and ruminal fermentation characteristics and therefore may have the potential as beneficial manipulators of ruminal fermentation. However, further *in vivo* studies are required to study the effect on livestock performance.

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Evaluation of Complete Ration Silage on Performance and Quality of Goat Meat

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Abstract

Major constrains of animal production development in Indonesia are the availability and quality of feeds, particularly in dry season. This study was aimed at studying the effect of silage in complete ration on weight gain and quality of goat meat. The experimental design in this study was complete randomized design consisting of 4 treatments and 4 replications using 16 of one year old goats. Ration consisted of R1(forage), R2 (forage and concentrate were given separately), R3 (complete hay), and R4 (complete ration silage). Feeds were given to meet the nutrient requirements of the animals, i.e. 3% of body weight, and drinking water was given ad libitum. The variables measured were nutritional composition of rations, blood plasma cholesterol, slaughtered and carcass weights, as well as carcass percentage of the goat. Results showed that the quality of goat meat determined by its levels of protein, fat, and cholesterol was affected by the rations. Meat produced by R4 (complete ration silage) were better than the others, containing 21.97% protein, 1.9% fat, and 75.41 mg/dl cholesterol. This R4 goat performance was also showing higher slaughtered weight (22.70 kg) with a carcass weight of 9.86 kg and carcass yield of 43.44% out of slaughtered weight and income over feed cost (IOFC) of Rp. 495,768. Therefore, the complete ration containing silage was considered as quantitatively better than other treatments in giving a better performance and meat quality of the goat.

Keywords: silage, cholesterol, carcass weight, carcass percentage

Introduction

The scarcity in availability and poor quality of feeds are major constraints faced in development of livestock rearing in Indonesia. This condition is more pronounce in the problem of feed management, especially during the dry season; abundance in wet or rainy season. Some agricultural-industry by products, such as

palm oil sludge and cassava waste are available throughout the year; however, since they are not managed properly, their availabilities are not stable. Other reason for this problem is inefficient technology (ex. drying) in handling the feed increasing it to high price.

To lessen this fact, it is necessary to find an effort that technologically and economically wise. Providing rations for ruminants have to consider their nutrient requirement with regard to their physiological status, such as weight gain. To obtain higher gain, it needs higher concentrate level. Providing feeds in silage had shown some improvement for the livestock (ZoBell *et al.* (2004).

The objective of this experiment was to evaluate the application of complete ration containing silage toward the performance and meat quality in local goat.

Materials and Methods

There were one year- 16 male goats allocated into 4 treatments in completely randomized design. The treatments were: roughage only (R1), split roughage and concentrate (R2), dried complete ration (R3), and complete ration containing silage (R4). Water was served *ad libitum*. Feed ingredients used are: sagu waste, yellow corn, pulp out, waste of shrimp, corn strow, rice strow, rice bran and grass field

The rations were formulated isocaloric and isoprotein, crude protein 13%, crude fiber 18 % and dry matter 62,74 % based on the recommendation of Nutrient Requirement of Sheep (NRC, 1994). The rations were given for three months. Proxymate analyses of rations and meat were conducted in Laboratory of Feed Science and Technology in Animal Science Faculty, Bogor Agricultural University. Variables observed in this experiment were ration consumption, average daily body weight gain (ADG), feed conversion, carcass weights, and nutrient quality of goat meat, and IOFC (Income Over Feed Cost).

Data were analysed using analysis of variance (anova); if there were differences, it would be tested for Least Significant Difference (Steel and Torrie, 1993).

Results and Discussion

Consumption and performance of local goat

Consumption of the rations was significantly different ($P<0.05$), R1 was the highest (1.21 kg/d) that was different from R2 (1.10 kg/d); while hay consumption (R3) and the silage (R4) were not different (1.00 kg/d).

Silage was different from hay in terms of odor (acid), texture (wet), and nutrients (complete what does it mean with complete). However, the silage was better in improving the goat performance in this study, supporting that this ration was good enough in nutrients and palatability (Parakkasi, 1995). Ration conversion of the rations were significantly different ranging from 6.58 to 15.87 ($P<0.05$). The lowest

Table 1. Averages of ration consumption (kg/d), ration conversion, average daily gain (ADG) of the goat (kg/d)

Variables	Ration			
	R1	R2	R3	R4
Consumption (kg/d)	1.21[a]	1.10[b]	1.00[c]	1.00[c]
Conversion	15.87[a]	9.82[b]	7.24[c]	6.58[d]
ADG (kg/hr/d)	0.07[a]	0.11[b]	0.13[c]	0.15[d]
Slaughtered weight (kg)	19.79[a]	20.50[a]	21.40[b]	22.70[c]
Carcass (kg)	7.74[a]	8.35[b]	9.15[c]	9.86[c]
Carcass percentage (%)	39.11[a]	40.78[a]	42.75[b]	43.44[b]

Different superscript among rations were significantly different (P<0.05)

one was found in ration containing silage (R4), while the highest was in control diet with roughage only (R1); this was due to the fact that they had different nutrient (14.38 % crude protein, crude fiber, 17.02% and Bet-N 26.59 % in the silage) .

Average daily gains were different significantly (P<0.05), R4 was the highest (0.152 kg/d). This silage ration with its complete nutrients has stimulated rumen microorganism and metabolism, causing better daily gain of the goat (Pilliang and Djojosoebagia, 2006).

Slaughtered weight of fasted goat was found to be the highest in R4 (22.70 kg/d), while the highest was in the R1 only 19.79 kg/d. Gaili *et al.* (1992) stated that ration affected slaughtered weight, carcass weight, and subcutaneous fat deposition. Therefore, it determined the quality of the carcass. Carcass weights of the goat were about 7.74 to 9.86 %, the lowest was found in R1, while the highest was in the ration containing silage (R4). Carcass weight corresponded in positive manner to the slaughtered weight. Production of carcass was affected by the mass of muscle, fat, and bone during the growth period (Hammond *et al*., 1991).

Percentages of carcasses were 39.11- 43.44 %; R1 was the lowest, while R4 was the highest. This ration had correlation between the live weight and slaughtered weight, in which R4 was the highest too how is the correlation positive. This data was similar to other findings reported by Hasnudi (2005), that carcass composition increased with increasing carcass weight.

Quality of goat meat

There were no significant differences among rations; however, the silage showed higher carcass protein (21,47 %), while the control was the lowest. Cholesterol concentration was found to be the highest in R2 (118.18 mg/dl), while the lowest was in control ration (R1). The R4 was showing a moderate cholesterol level of 75.41mg/dl. Mechanism of this is the silage had lower fat content, but higher

Table 2. Average Nutrient Composition of the Goat Meat

Variables	Treatments			
	R1	R2	R3	R4
Dry matter (%)	24.07	26.10	25.63	27.21
Ash (%)	1.25	0.84	1.15	1.24
Crude Protein (%)	18.68	19.65	20.51	21.47
Fiber (%)	0.77	4.08	4.15	3.66
Fat (%)	1.65	2.05	1.14	1.90
Beta-N (%)	2.48	2.15	3.29	2.59
Cholesterol mg/dl	55.73	118.73	80.41	75.41

fiber, therefore, this fibre will bind pancreatic bile better, excreted into feces. This condition will stimulate liver to produce bile acid out of the available cholesterol, such that the cholesterol level in the body will decrease. This is based on the fact that the cholesterol content in a feed or ration will be metabolised as acetyl co-A (Krisnantuti and Yenrina, 199l). Because in the silage added shrimp waste animal protein saouces rich in omega3 and yellow corn vegetable protein source rich in omega6 which are hypolipidemik, taht lower cholesterol. How it works to inhibit the synthesis and transfer are bad LDL cholesterol and increasing HDL cholesterol because of it is anti aggregation.

Income over feed cost (IOFC)

Analysis of income over feed cost (IOFC) was designed to evaluate the cost and net return of applying these rations in local goat during the experiment. R4, the complete ration containing silage, gave the highest net return, representing by Income Over Feed Cost (IOFC) for as much as Rp. 495,768,- /60 days/head. This high net return was supported by the efficiency of using the ration as indicated by

Table 2. Average Nutrient Composition of the Goat Meat

Variables	R1	R2	R3	R4
Ration price (Rp/kg)	750	1,500	2,000	2,000
Feed conversion	15.67	9.87	7.24	6.58
Ration cost (Rp/kg meat)	11,752	14,730	14,480	13,160
Meat price (Rp/kg)	35,000	35,000	35,000	35,000
Net income (Rp/kg meat)	23,240	20,270	20,520	21,840
Meat production (kg)	19.79	20.52	21.40	22.70
I O F C (Rp.)	460,078	415,535	439,120	495,768

the feed conversion (6.58). Seemingly, the more improved the ration, it will produce better carcass and net return, eventually.

Conclusions

The complete ration containing silage was considered the most efficient ration on the basis of the performance (9.86 kg carcass, 22.70% carcass of live weight, and 43.44% carcass of slaughtered weight) and meat quality of goat carcass containing 21.97% protein, 1.9 % fat, and 75.41mg/dl cholesterol, with the IOFC of Rp 495,768,-.

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The Potency of Sugar Cane Waste Product for Supporting Sustainable Beef Cattle Feed Resouces at Integrated Farming Center in Solok Regency, West Sumatra

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Abstract

The research was intended to study the availability and nutrient content of waste product of sugar cane (bagasse, molasse and sugar cane top) in integrated beef cattle with sugar mill and sugar cane farming. Based on the data, it was formulated the ration for beef cattle, and known the carrying capacity of the sugar cane area for supporting feedstuff for beef cattle. The methodology of research was survey, proximate analysis and linear programming. Based on the survey, it was found that the production of bagasse was 27.60 ton/Ha/year (46% of sugar cane production); molasse was 1.27 ton/ha/year (1.99% of sugar cane production); and sugar cane top was 10.57 ton/ha/year (43.42% of sugar can production). Nutrient contents of bagasse were 79.01%, 2.15%, 40.45%, 1,44%, and 50.33% for dry matter (DM), crude protein (CP), crude fiber (CF), fat (F) and nitrogen free extract (NFE) respectively. Dry matter, CP, CF, F and NFE contents of molasses were 13.29 %, 12.33%, 0.63%, 17.83% and 63.98%, respectively. The content of sugar cane top was 27.29%, 7.59%, 40.39%, 1.51% and 40.67% for those parameters, respectively. The result of ration formulation based on the nutrition content for beef cattle (CP 11 %, and TDN 62.5%) was sugar cane top 25%, bagasse 30%, molasses 5 %, and other feedstuffs 40 %. The other feed stuffs were rice brain 15 % and coconut meal 15 %, and waste of tofu 10%. The area could support fodder for 1,478, 8,403 and 398 beef cattles every year, based on availability of sugar cane top, bagasse, and molasses respectively.

Keywords: bagasse, molasses, sugar cane top, beef cattle

Introduction

The sustainable feed resource is the important factor to support Indonesian Government Program to self supply of beef cattle in 2014. Forage may constitute an important fodder component to meet the maintenance requirements of ruminants, especially for farmers who practice intensive farming. Nowadays, the farmers

began to face the difficulties to get the fodder because of limitation of area to plant the forage. Most of the land is used for others; e.g.; plantations, agricultures, horticultures, buildings, etc.

In Solok Regency, West Sumatra, There is a center of traditional sugar plantation that is located in Talang Babungo Village. It has 415 hectare area of sugar plantation that is owned by 530 farmers. The farmers resulted 3280 ton traditional sugar every year. Besides that, there are by- products of plantation that is potential for the source of fodder; namely sugar cane top, bagasse and molasses. Unfortunately; the potency is still wasted, whereas there are about 500 beef cattles that are depend on traditionally fed with grass by the farmers.

Based on the problem, it is needed to study the potency of the by-products as source of fodder of beef cattle. As the preliminary studies, we want to know the production and the nutrient content of the byproducts. Besides that, we tried to formulate the ration of beef cattle with using the products. Based on the formula, we could calculate the carrying capacity of the by-products to support availability of fodder for beef cattle in the integrated farming.

Materials and Methods

The materials used in the present research were sugar cane top, bagasse, molasses and chemical materials for proximate analysis. The device was measuring tape, GPS, scale (capacity 550 kg) and a set of proximate analysis instrument. Besides that, it was used sugar cane mill (3 rollers type) with a 24 HP engine.

The data of availability of byproducts were obtained by survey where sampling was collected by stratified random sampling. Strata of the samples were based on altitude of the area; i.e.; 1000-1100 m and 1100-1200 m above the sea level. The nutrition content of the products was be determined by proximate analysis. Linear programming was applied for ration formulation with sugar cane byproducts as main fodder. Based on the availability and ration formula could be determined the carrying capacity.

Results and Discussion

Productions of bagasse, sugar cane top and molasses

The production of bagasse, sugar cane top and molasses in Talang Babungo village, Solok regency, West Sumatra is presented in Table 1. On the table show that the total production of sugar cane was influenced by the altitude of the land; the higher the location, the higher the production. The average of production was 63.69 Ton/Ha/year. The production of bagasse was 27.70/ha/year (43.42% of total production). The production of sugar cane top was 10.57 ton/ha/year (16.79 % of total production); meanwhile the production of molasses was 1.17 ton/ha/year

(1.19% of total production). The production is equivalent with 19.4 ton/ha/year, 2.9 ton/ha/year, and 0.2 ton/ha/year dry matters of bagasse, sugar cane top and molasses accordingly. There were 415 Ha area of sugar cane plantation in location of the research, so it can be predicted that the availability of bagasse, sugar cane top and molasses in Talang Babungo village were 8069, 1197, and 65 ton of dry matters/ year.

Nutrient content of the by-products

The nutrient content of the by-products is presented in Table 2. The bagasse contains highly dry matter (DM) and crude fiber (CF), but lower in crude protein (CP) and fat. Sugar cane top contains DM and CP relatively the same as grass, so it can be used for substituting the grass, but CF is relatively higher. The molasses is

Table 1. The production of bagasse, sugar cane top and molasses in Talang Babungo Village, Solok Regency, West Sumatra

Criterion	Production		
	Altitude 1000-1100 masl*	Altitude 1100-1200 masl*	Average
Total sugar cane production (ton/ha/year)	53.88	73.50	63.69
Bagasse production			
ton/ha/year	23.11	32.30	27.70
% of total production	42.89	43.95	43.42
Sugar cane top production			
ton/ha/year	9.68	11.47	10.57
% of total production	17.97	15.61	16.79
Molasses production			
ton/ha/year	1.20	1.15	1.17
% of total production	2.23	1.56	1.90

Note: *meter above the sea level

Table 2. Nutrient content of the by-products of sugar cane plantation

Kind of the by-products	Nutrient content (%)				
	DM	CP	CF	Fat	NFE
Bagasse	79.01	2.15	40.45	1.44	50.33
Sugar cane top	27.29	7.59	40.39	1.51	40.67
Molasses	13.29	12.33	0.63	17.83	63.98

the best fodder as source of energy because contains the highest nitrogen free extract (NFE) of those by-products. Based on the nutrient content of those by-products, it was need to add other fodder to formulate the ration for beef cattle.

Formulation of Ration

Ration formulation of beef cattle used bagasse, sugar cane top, molasses and others fodder generate formula as being shown in Table 3. In the table show that 60 % of the ration consists the by-products of sugar cane, and the remaining comprise other feedstuffs. The rice brain, coconut meal and waste of tofu function to cover insufficiency of the nutrient requirement derived from those by-products. Thus; the by-products mainly serves as substitution of grass that main component of forage resource in Talang Babungo.

Carrying Capacity

Based on the ration formula, it can be calculated the carrying capacity of the by-products that produced in Talang Babungo to support the nutrient requirement of

Table 3. The ration formula of beef cattle based on by-products of sugar cane as main component*

Kind of fodder	Percentage (%) of DM
Sugar cane top	25
Bagasse	30
Molasses	5
Rice brain	15
Coconut meal	15
Waste of tofu	10
Total	100

Note: *The requirement of CP = 11% and TDN 62.5%.

Table 3. The ration formula of beef cattle based on by-products of sugar cane as main component*

The kind of by products	Production (ton/year)	The requirement (ton/cattle/year)	Carrying capacity
Sugar cane top	4,387	2.968	1,478
Bagasse	11,496	1.368	8,403
Molasses	486	1.219	398

The assumptions: The total area of the sugar cane plantation is 415 Ha. The average of beef cattle weight is 300 kg. The requirement of dry matter is 3% of live weight.

beef cattle. The carrying capacity based on availability of the by-products presented in Table 4. In the table, it can be seen that based on availability of bagasse, sugar cane top and molasses can cover the needs of feed for 8 403, 1 478, and 398 cattles respectively. Thus; the availability of molasses was the limiting factor of exploiting the by-products as the feed resource of beef cattle in Talang Babungo.

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The Effect of Essential Oils of Spearmint on the *in Vitro* Rumen Fermentation, Growth, and Deaminative Activity of Amino Acid Fermenting Bacteria

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Abstract

The aim of this study was to assess the effect of essential oils of spearmint (SEO) on the in vitro gas production (GP) kinetics, fiber digestibility and proteolysis by rumen microbial community using Menke syringes. Asymptotic GP, half time of GP and the sigmoidisity degree of the GP curve were determined using a typical growing lamb diet. Ammonia production and in vitro fiber digestibility in response to different doses SEO was measured. The effect of this oil on the deaminative activity of mixed rumen microbes and a recently isolated amino acid-fermenting bacterium were studied. SEO decreased asymptotic GP and increased degree of sigmoidicity. While In vitro fiber digestibility was negatively affected by SEO, the concentration of ammonia in fermentation syringes increased. The tested oil inhibited ammonia production and the specific rate of ammonia production by the isolate (Clostridium SPP. MT8). Similarly, such results were observed for ammonia production by mixed rumen microbes. Overall, SEO had an antimicrobial activity and anti-proteolysis effect, and therefore modulating effect on rumen fermentation.

Keywords: bacteria, deaminatvie activity, growth, in vitro, spearmint

Introduction

In ruminants, nitrogen retention is inefficient because of deamination of amino acids in the rumen, which leads to the excess of NH_3 in the blood stream that is converted to urea and lost. Antibiotic growth promoters (i.e., ionophores) have been successfully used to enhance N utilization efficiency in ruminants. These antibiotics inhibit the growth of a group of rumen bacteria called the ammonia hyper-producing bacteria (HAP), which have the capacity to produce ammonia despite their relatively low number compared to other predominant rumen bacteria (Russell *et al*, 1988). Recently, antibiotic use in animal ration has faced many criticisms due to the emergence of antibiotic resistance. An alternative to antibiotics is plant

natural compounds that can be used as feed additives. Essential oils (EO) are one of category of compounds, which have been considered for their potential to modulate the rumen fermentation. Spearmint (*Mentha spicata*) is a medicinal plant which has many uses in Iranian ethnomedicine. The antimicrobial effect of its essence against a broad spectrum of microorganisms has been documented (Mkaddem *et al*., 2009). However, little information is available about the effect of this EO on fermentation parameters of mixed rumen microorganisms and activity of ruminal amino acid-fermenting bacteria. Therefore, the aim of this study was to assess the effect of different doses of spearmint EO (SEO) on the fermentation parameters of mixed rumen microbes and the activity of an amino acid-fermenting bacterium isolated from the rumen of Mehraban sheep.

Materials and Methods

Rumen inoculum was taken from four ruminally fistulated mature Mehraban sheep fed a diet containing 70% alfalfa hay and 30% concentrate plus mineral and vitamin supplements. For kinetics measurement 200 mg substrates (i.e., atypical diet for growing lambs) were weighed into 100 ml glass syringes and subsequently filled with 30 ml buffered rumen fluid. Gas volume was recorded at 1 to 144 hours after incubation. The kinetic parameters were estimated using mono-phasic model (Groot *et al*., 1996) of $GP=A/(1+(B/t)^s)$ where GP is the cumulative gas production (ml/g incubated OM), A is the estimated asymptotic gas production (ml/g incubated OM), B is the time (h) after incubation at which half of the asymptotic gas production has been reached and S represents a constant that determines the sharpness of the switching characteristic of the profile. In a separate run of incubation fiber digestibility (IVFD) and ammonia were measured in syringes containing 500 mg of the substrates and inoculated with 40 ml of buffered rumen fluid according to Makkar (2010).

In another trial an amino acid fermenting bacteria was isolated from the rumen of Mehraban sheep and identified as described by Flythe and Andris (2009). The accession number of isolated bacteria (Clostridium SPP. MT8) was JN804563. Also, a suspension of mixed ruminal bacteria was provided (Flythe, 2009). The effect of essences on the growth of isolated bacteria was evaluated by measuring optical density (absorbance at 600 nm) of stationary phase cultures after 24 hours of incubation (39ºC). Ammonia production and protein of pure culture of bacteria were determined at 0 and 6 hours of incubation. Specific rate of ammonia production was calculated from these two values using the averages of 0 and 6 hours protein samples. The ammonia production by mixed rumen microbes were measured by differences between the values of 0 and 24 hours incubations. At the beginning of all trials, different dosed of SEO (0, 250, 500, 750 and 1000 mg/L) were added to the fermenting media. The data were analyzed using generalized linear model ANOVA

procedures (SAS, 8.1). For all analyses, specific orthogonal contrasts were used to test 1) control vs. the average of SEO doses and 2) linear (L), quadratic (Q) and cubic (C) effects of EO doses on parameters.

Results and Discussion

The effect of SEO on the fermentation parameters are shown in Table 1. Inclusion of SEO decreased asymptotic GP and controls were significantly different from treatments. Half-time was decreased with the increased concentration of SEO, while addition of SEO increased the switching factor. SEO had led to the increase in the ammonia concentration. However, IVFD were markedly decreased by inclusion of SEO especially at the highest doses. The decrease in GP in response to inclusion of SEO is indicative of their antimicrobial effects, which have been demonstrated previously (Taghavi-nezhad *et al*., 2011). The degree of sigmoidicity represents the possible lag process occurring at the early stages of incubation (Groot *et al*. 1996). Also, a more sigmoidal GP curve indicates that incubated substrates have lower nutritive value than the exponential mode (Groot *et al*., 1996) or an altered fermentation (France *et al*., 2000).

In general, lowered GP as a result of SEO shows that this EO have a general and dose dependant antimicrobial activity. Trends to sigmoidicity with addition of SEO point out a specific inhibitory effect against some microorganisms in addition to its general effect. The decrease in IVFD shows the negative effect of these essences on the degradation of organic matter and structural carbohydrates at high doses.

The effects of different doses of SEO are shown in Table 2. The growth of isolated bacterium was decreased by SEO. Also, ammonia production, microbial protein and specific rate of ammonia production were lowered by inclusion of SEO.

Table 1. The effect of different doses of SEO on the kinetics of *in vitro* ruminal fermentation

Parameters	SEO doses					Contrasts			
	0	250	500	750	1000	Control vs. SEO	L	Q	C
A	507.4	473.1	443.6	370.8	264.4	***	***	***	NS
B	12.3	10.1	11.0	9.2	5.4	***	***	***	*
S	1.16	1.18	1.2	1.3	1.9	***	***	***	*
Ammonia	5.88	6.08	6.81	7.38	6.36	***	***	***	***
IVFD	0.33	0.34	0.27	0.22	0.16	***	***	**	NS

A: asymptotic GP (ml/g OM); B: half time of asymptotic gas production (h); S: regulating the switching characteristics of the GP profiles; L: linear; Q: quadratic; C: cubic; * P<0.05; **: P<0.01 *** P<0.001; NS: non-significant; SEO: spearmint essential oils. IVFD: in vitro fiber digestibility.

Table 2. The effect of different doses of ZEO and SEO on the ammonia production (mmol), microbial protein (µg) and specific rate of ammonia production (mmol/mg of protein) by isolated bacterium

Parameters	SEO doses					Contrasts			
	0	250	500	750	1000	Control vs. SEO	L	Q	C
Growth of bacteria (O.D)	0.416	0.262	0.181	0.095	0.051	***	***	***	NS
Ammonia	6.7	5.1	2.7	1.98	2.84	***	***	**	NS
Microbial protein	504.4	281.1	334.6	317.7	303.1	***	**	*	*
Specific rate of NH_3 production	14.02	19.48	7.83	6.21	9.6	NS	*	NS	**

O.D: optical density after 24 hours of incubation; SEO: spearmint essential oils. L: linear; Q: quadratic; C: cubic; * P<0.05; **: P<0.01; *** P<0.001; NS: non-significant

Addition of SEO had a negative effect on the ammonia production by the mixed rumen bacteria suspension and at the highest doses of SEO (750 and 1000 µg/ml) small amounts of ammonia were produced (Figure).

The inhibitory effects of EO on amino acid fermenting bacteria have been cited previously (McIntoch *et al*., 2003). Carvone (the main constituent of spearmint) is suggested to exhibit its antimicrobial activity via disrupting metabolic energy status of cells (Burt, 2004). However, the small amount of ammonia production at the highest doses of SEM instead of complete inhibition of bacterial growth could be due to the facilitated diffusion and other transport mechanisms in absence of ion-motive forces (Flythe and Russel, 2005).

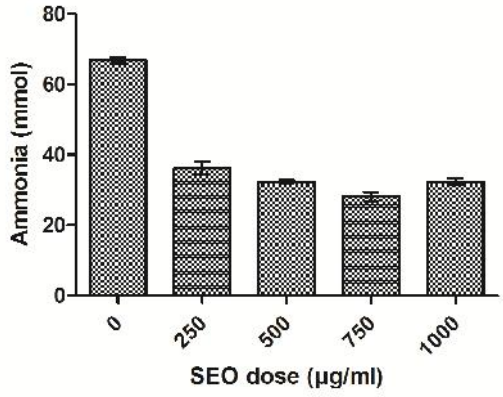

Figure 5. The effect of SEO on ammonia production by mixed rumen microbes

Conclusions

One important aspect of using phytochemicals in ruminants nutrition is the improvement of nitrogen utilization efficiency which can be achieved by lowering proteolysis and ammonia production in the rumen. Decreased ammonia production by mixed rumen microorganisms and a pure culture of a novel amino acid-fermenting bacterium showed that SEO has the potential to manipulate protein metabolism in the rumen. At the lower doses,SEO can modulate rumen fermentation without negatively affecting organic matter and fiber degradation. Further research is needed to elucidate *in vivo* effects of this EO, such as lactation and fattening performance of ruminants.

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Effect of Energy and Protein Contents of Dietary Having the Same Synchrony Index on Local Beef Cattle Performance

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Abstract

Macronutrients such as energy and protein affect rumen microbial growth and efficiency of microbial protein synthesis which can be increased by synchronizing energy and N-protein released in the rumen. The experiments were conducted to study the optimum energy and protein content of diet that were synchronized in releasing energy and N-protein in the rumen of native beef cattle. Randomized block design with a factorial of 3 x 2 was used in this experiment. The first factor was three diferent crude protein (CP) levels i.e. 10, 12, and 14 %; the second factor was two deferent levels of TDN (total digestible nutrients) (65 and 70 %). Eighteen local cattles were arranged into three groups on the basis of average body weight of the animals. Each group was fed six types of diet that was different in levels of CP or TDN. The diets had the same synchrony index, namely 0.560. The results showed that, with the exception of crude fat digestion, there was no effects of interaction between protein and energy on variables measured. Compared to diet with 70% TDN, diet with 65% TDN produced higher rumen microbial N, consumption and digestion of nutrients, and N retention (P<0.05). Diet with protein level of 12% tend to have better allantoin concentration in the urine, consumption and digestion of nutrients, N retention and blood urea nitrogen (BUN) than that of 10% or 14%. It can be concluded that the diet having 65% TDN and 12% protein with synchrony index of 0.560 generate more efficient N synthesis of rumen microbes and average daily gain of local cattle.

Keywords: synchrony index, degradation, N retention, intake of nutrients, digestion

Introduction

The intentions of synchronizing the release of N and energy from diets in the rumen are to maximize microbial protein synthesis from the capture rumen degradable protein (RDP), to reduce the requirement for expensive undegradable protein (UDP), to minimize losses of ammonia from the rumen, and to minimize energy cost for converting the excess ammonia into urea and for excreting urea in

urine, and to improve animal performance (Sinclair *et al*, 1993; Gustafsson *et al*, 2006).

The available energy in the rumen (ruminally degradable organic matter) is the most limiting factor for ruminal N utilization (Shabi *et al*, 1998). But these may need to take second place when looking at more recent finding (Block, 2006). Maeng *et al*. (1999) demonstrated that supply of crude protein (CP) or nitrogen improved microbial efficiency to greater extent than did either fiber or starch.

The experiments were conducted to study the optimum energy and protein content of diet that were synchronized in releasing energy and N-protein in the rumen of native beef cattle (sapi pesisir)

Materials and Methods

The feedstuffs used to formulate the treatment diet were presented in Table 1. The rumen-fistulated cattle was used to determine ruminal degradability coefficient of organic matter (OM) and protein of the feedstuff, namely by mean of the equation: $p = a + b\,(1 - e^{-ct})$ (Orskov and McDonald, 1979); where p= the amount degraded at *t* time; *a*= the rapidly soluble fraction; *b*= potentially degradable fraction; *c*= the rate of degradation of fraction *b*; and *t*= time (h). From hourly quantity of OM and protein degraded, a synchrony index of nitrogen to organic matter was then calculated by the following equation:

Synchrony Index= $\dfrac{25 - \sum_{1-24} \dfrac{\sqrt{(25 - hourlyN/OM)^2}}{24}}{25}$ (Sinclair *et al*. 1993), where 25= 25 g N/kg organic matter trully digested in the rumen.

Table 1. Chemical composition and sinchrony index of feedstuff (%)

	Field grass	Rice brand	Corn meal	Coconut peal	Fish meal
Dry matter (DM)	35.6	87.8	85.8	89.2	87.2
Organic matter (OM)	94.3	90.8	99.1	79.7	59.8
Crude protein (CP)	10.2	13.0	7.7	17.6	22.7
Crude fiber (CF), %	27.8	11.6	0.9	9.7	11.2
Ether extract (EE), %	2.0	8.6	3.5	9.7	3.4
Total digestible nutrients (TDN), %	63.7	66.8	81.9	65.3	12.3
Sinchrony index[1)]	0.538	0.277	0.660	0.827	-0.167

[1)] synchrony index is calculated according to Sinclair *et al.* (1993) modified by Hermon *et al*. (2008) concerning efficiency of microbial protein synthesis in the rumen to be 20g N/kg OM fermented.

Table 2. Composition and sinchrony index of treatment diets (% of DM)

	10P65E	10P70E	12P65E	12P70E	14P65E	14P70E
Field grass	86.3	43.4	32.9	22.2	13.2	9.0
Rice brand	-	6.3	6.1	23.1	21.7	32.6
Corn meal	6.7	38.0	28.3	29.1	20.0	19.0
Coconut peal	6.5	8.1	23.7	23.0	37.6	36.8
Fish meal	0.01	3.7	8.5	2.1	7.0	2.2
Mineral	0.5	0.5	0.5	0.5	0.5	0.5
OM	93.1	93.0	88.6	90.3	86.1	87.5
CP	10.4	10.4	12.4	12.0	13.9	13.6
CF	24.7	14.3	13.4	11.6	10.8	10.3
EE	2.6	3.6	4.7	5.7	6.7	7.3
TDN	64.7	68.5	64.7	68.6	64.7	67.3
Sinchrony index[1)]	0.562	0.562	0.562	0.562	0.562	0.564

10P65E= diet of 10%CP, 65% TDN; 10P70E= diet of 10%CP, 70%TDN; 12P65E= diet of 12%CP, 65%TDN; 12P70E= diet of 12%CP, 70%TDN; 14P65E= diet of 14%CP, 65%TDN; 14P70E= diet of 14%CP, 70%TDN.

Six treatment diets prepared following the randomized block design of a 2 x 3 factorial (Table 2). The first factor is the level of TDN in diets, namely 65 and 70%. The second factor is the level of protein in diets, namely 10, 12, and 14%. All these diets had the same relative synchrony index.Each diet was given to three cattle (1 to 1.5 years of age and weighing 90-135 kg), which were randomly placed in individual cages. The diet given 2 times daily with the same dose at 8.00 and 16.30. The study was conducted for 35 days, 14 days for a period of adaptation to diet treatment, 14-day fattening period and 7 days before the end of the study for the collection period. In the collection period, measurements were daily consumption and collection of feces and urine. Blood samples to determine blood urea nitrogen (BUN) were taken from coccygeal vessels at the last sampling day. Urine was used to determine the retention of N and purine derivate which was then used to calculate the efficiency of rumen microbial protein synthesis/ rumen microbial production (Chen and Gomes, 1992). Rumen fluid was taken from rumen-fistulated cattle fed diet treatment, namely before feeding in the morning and 3, 6, 9 h after feeding. Data were analyzed for variance of randomized block design using the GLM procedure SAS (2004).

Result and Discusion

Table 3 shows that the production of rumen microbial protein was influenced by diet energy and was not affected by diet protein, corresponding to the statement

Karsli and Russell (2002). Rumen microbial production of the diet with 65% TDN was higher than that of the diet with 70% TDN (P <0.05), while the diet with 10% protein tend to yield higher microbial production than the diet with the other proteins. This might occur because of the diet contained more forage (Table 2), so that the dilution rate in the rumen was high (Stern and Hoover, 1979), and because of lower fat content (Table 2) (Van Soest, 1982).

The greater rumen microbes may increase activity of nutrients fermentation including CF. There is a close correlation between CF and DM digestibility in the rumen (Varga and Hoover, 1983) and it is estimated that most of the diet will be fermented in the rumen, it will increase the digestibility of DM, including nutrient contained in it (Table 2). There was also a close relationship between the consumption and digestibility (Poppi *et al*., 2000), therefore the diet with 65% TDN or the diet with 10% protein would be more consumed than the diet with 70% TDN and the diets with protein 12 or 14% (P <0.05).

Table 3 shows that the diet with 65% TDN produced a higher N retention compared to the diet with 70% TDN (P<0.05), while the diet with 12% protein tend to produce higher N retention compared to the diet with protein of 10 or 14%. Although N retention was high, the diet with 12% protein had a lower ADG (204 g / day) compared with the diet with 14% protein (207.7 g/day). This may be happen

Table 3. Effect of treatment diets on variables

	CP (%)			TDN (%)		Interaction: CP vs TDN
	10	12	14	65	70	
Consumption:						
DM; kg	2.0	2.1	1.8	2.3[a]	1.6[b]	ns
CF; kg	0.4	0.3	0.2	0.4[a]	0.2[b]	ns
Digestion : DM; %	69.3[a]	62.5[a]	58.8[b]	67.9[a]	59.2[b]	ns
CP; %	68.7[a]	63.3[b]	60.4[b]	63.3[a]	61.0[b]	ns
CF; %	64.3[a]	36.7[b]	33.5[b]	56.9[a]	32.9[b]	ns
RMP[1)]; g N/day	14.3	12.4	10.2	16.3[a]	8.3[b]	ns
N retention; g	3.6	5.7	4.3	6.2[a]	3.0[b]	ns
ADG; g/day	169.7	204.0	207.7	219.7	167.9	ns
BUN; mg/dl	37.1	39.6	34.7	36.2	38.1	ns
VFA; mM	112.9	93.5	99.6	98.9	105.1	s
NH_3; mg/100ml	17.0	15.4	18.6	16.6	17.5	ns
PER; %	72.5	79.8	79.0	84.5	76.3	ns

[a,b] deferent subscript in the same row and nutrient was significant (P<0.05); s= significant; ns= not significant; [1)]RMP= rumen microbial production.

because some of N retened within diet with 12% protein was converted to BUN to meet energy needs, this was corresponded to the statement of Bani *et al*. (1991). High BUN concentration was likely not derived from the excessive rumen NH_3, because its concentration was low compared to the diets of 10 and 14% protein. The Diet with 12% protein had a higher PER than diets with 10 or 14% protein.

Conclusion

It can be concluded that the rumen microbial production was influenced by the availability of energy for its body protein synthesis, although the diet was formulated synchronously to release N-protein and energy in the rumen. Local cattle diet containing 65% TDN and 12% protein with 0.560 synchronization index had the optimal rumen microbial production, protein efficiency ratio and average daily gain

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Fermentability and Digestibility of Ration Containing Crude Curcin Extract of *Jatropha curcas* L. Seed Meal

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Abstract

Jatropha (J.) curcas L. seed meal (JCSM) is a byproduct of J. curcas L. oil extraction. This JCSM contains high protein concentration (37.56% DM) and ether extract (35.02% DM) which makes a potential source of animal feed. However, JCSM utilization can be limited by the presence of antinutrients such as curcin or lectin, and phorbolester. These antinutrients, especially the curcin, can be digested differently by microbes from the rumen fluid of cattle and buffalo. Therefore, an experiment was conducted to study fermentability and digestibility (in vitro) of ration that contain crude curcin extracted from Jatropha curcas L. seed meal by microbes from the rumen fluid of cattle and buffalo. Factorial randomised block designs were used in fermentability (4x2x2), and digestibility (4x2) studies; three replications were used in both experiments. The treatments were levels of crude curcin extracted from JCSM (0, 1, 2 and 3% v/w) that were added into rations as factor A, microbes from the rumen fluid of cattle and buffalo as factor B, and incubation times (0 and 3 h) as factor C in fermentability study; factors A and B were also applied as treatments in digestibility experiment. Variables measured were ammonia and total VFA concentrations, total bacterial and protozoal populations, and dry matter (DM) and organic matter (OM) digestibilities. Data were analysed using analysis of variance, and differences in treatment means were examined with contrast or polynomial orthogonal. The results showed that effects of addition levels of crude curcin extracts into rations were not significant on all variables measured, except the total protozoal population ($P<0.01$). There were differences between the rumen fluid of cattle and those from the rumen fluid of buffalo on total bacterial population, DM and OM digestibilities ($P<0.01$). Ammonia and total VFA, and total bacterial population were increased at 3h incubation time ($P<0.01$). It is concluded that addition of crude curcin extracts from JCSM up to 3% (v/w) did not produced negative effects on ration fermentability and digestibility, except for protozoal population. The greater numbers of bacteria in the rumen fluid of buffalo than those in the rumen fluid of cattle cause higher DM and OM digestibilities.

Keywords: curcin, digestibility, fermentability, Jatropha curcas L. seed meal

Introduction

Jatropha (*J*.) *curcas* L. seed meal (JCSM) is a byproduct of *J. curcas* L. oil extraction and has potential source as animal feed due to its production potential and nutrient contents as protein and energy sources. In Indonesia, the production was about 0.4 ton/ton dry seed with 200 - 300 l oil production (Brodjonegoro *et al*., 2005). JCSM without husk contained 86.26% dry matter (DM), 37.56% crude protein (CP), 35.02% ether extract (EE), 7.23% crude fibre (CF), 12.47% nitrogen free extract (NFE),7.71% ash, 16.30% NDF, 15.86% ADF, 4.51% lignin and 0.01% silica (DM basis). Nut husk inclusion in oil extraction increased DM and fibre fractions, but reduced CP and EE contents; the husk contained high fibre fractions (Tjakradidjaja *et al*., 2007). The fibrous fractions and presence of curcin and phorbolester as the main antinutrients (Martinez-Herrera *et al*., 2006; Makkar *et al*., 1998) limited its use, but antinutrient effects varied among JC provenances, location, *etc* (Makkar and Becker, 1997; Makkar *et al*., 1997).

JCSM is, then, more suitable for ruminants than for monogastric. The presence of rumen microbes, especially the bacteria, has enable the ruminants to degrade CF or antinutrients developed by adaptation to feeds that were consumed; however, these varied among rumen microbes and ruminants (McDonald *et al*., 2002). Tolerance of rumen microbes from goat, sheep, cattle and buffalo to JCSM antinutrients had been studied (Tjakradidjaja *et al*., 2008; Tjakradidjaja *et al*., 2010). Goat rumen bacteria were more able to degrade JCSM and tolerate its antinutrients within 0-12 h fermentation with proteolytic bacteria are important in degrading antinutrients having protein structure. These led to extract curcin from JCSM and study its effects on ration fermentability and digestibility. Its addition up to 3% (v/w) did not affect fermentability, rumen fluid of goat had greater total bacterial population with no change in protozoal population at 3 h incubation, but lower DM and OM digestibility compared to sheep (Tjakradidjaja *et al*., 2011). The effects can be different using rumen fluids of large ruminant. Therefore, an experiment was conducted to study fermentability and digestibility (*in vitro*) of ration containing JCSM crude curcin extract by microbes from cattle and buffalo rumen fluids.

Materials and Methods

Materials were JCSM with husk, crude curcin extract, cattle rumen fluids obtained from slaughter house in Bogor and the buffalo from fistulated animal in BATAN, and ration. The ration was elephant grass (*Pennisetum purpureum*), ground corn and concentrate= 50 : 25 : 25% w/w). Crude curcin was extracted with Stirpe *et al*. (1976) method modified due to limitations in availability of experimental apparatus. Fermentability study was done following the first stage of Tilley and Terry method (1963) modified by Sutardi (1979); the two stage method was used

in digestibility study. Ammonia and total VFA concentration was determined, respectively, by micro diffusion Conway and steam distillation method (General laboratory procedure, Department of Dairy Science, 1966). Ogimoto and Imai (1981) method was used to count total bacterial (serially dillution method) and protozoal populations.

Three factors applied in fermentability study; factor A:JCSM crude curcin extract levels added into rations (0, 1, 2 and 3% v/w), factor B:rumen fluid sources (cattle and buffalo), and factor C:incubation time (0 and 3 h). Only factor A and B were treatments in digestibility study. Variables were concentrations of ammonia and volatile fatty acid (VFA), total bacterial and protozoal populations, and DM and organic matter (OM) digestibility coefficients. Experimental design and statistical analysis (analysis of variance, and orthogonal) were carried out based on Steel and Torrie (1993). Factorial randomised block design 4x2x2 and 4x2 were, respectively, used in fermentability and digestibility experiment with rumen fluids (three replications for each animals) were used as blocks.

Results and Discussion

Crude curcin extract and its effect on nutrient composition of rations

The extract in this experiment (370 ml/250 g dry weight) was smaller than that obtained by Tjakradidjaja *et al*. (2011) that was due to differences in JCSM sample and experimental condition during extraction. Addition levels at 1, 2 and 3% (v/w) were equal to addition of 0.67, 1.33 and 1.99% JCSM into treatment rations which were greater than that used by Ahmed and Adam (1979), 0.25% JCSM. Extract also contained saponin at 0.2% (Laboratory of Biofarmaka, IPB, 2008) that may also influence treatment effects. Extract addition slightly reduced DM (89.66 to 83.41-85.22%), NFE (55.13 to 51.65-52.10%) and TDN (68.71 to 66.75-67.77 %) contents of treatment rations, but increased CP (13.98 to 14.8 -15.56%) and CF (18.64 to 19.82-20.79%) contents; the effects did not follow linear patterns with extract levels. Increase in CP content was due to addition of protein structure of curcin, glycoprotein (Makkar and Becker, 2004; Juan *et al*., 2002; Aregheore *et al*., 2003).

Effects of treatments on fermentability, microbial population and digestibility

Table 1 showed that extract addition levels decreased significantly total protozoal population ($P<0.01$). Rumen fluid sources produced significant effects on microbial numbers ($P<0.01$). Incubation for 3 h increased ammonia ($P<0.01$) and total VFA ($P<0.05$) concentrations and total bacterial numbers ($P<0.01$) without changing protozoal numbers.

Results indicate that rumen bacteria were able to degrade protein including the extract, and ferment energy sources. The present ammonia concentration was

Table 1. Effects of treatments on all variables

Treatments	Variables					
	Ammonia (mM)	VFA (mM)	Bacterial population (x 10^8 colony forming unit/ml)	Protozoal population (x 10^5 cel/ml)	DM digestibility (%)	OM digestibility (%)
Curcin level addition[1,2]						
0% (v/w)	21.12±6.84	149.82± 12.95	0.98±0.77	0.78±0.05^{Aa}	41.62±5.43	39.97±6.50
1% (v/w)	21.26±4.92	155.77± 12.22	0.86±0.69	0.64±0.02^{Ab}	39.45±3.52	37.30±4.76
2% (v/w)	21.60±4.33	159.18± 12.26	0.50±0.11	0.57±0.02^{Bc}	36.33±5.55	34.24±7.32
3% (v/w)	22.19±3.26	164.85± 9.36	0.46±0.22	0.51±0.00^{Bc}	37.86±5.78	34.72±5.91
Rumen fluid sources[1,2]						
Cattle	21.63±0.83	153.19± 6.76	0.26±0.05^{A}	0.48±0.15^{A}	35.23± 2.56^{A}	32.23± 2.92^{A}
Buffalo	21.46±0.19	161.62± 5.87	1.14±0.47^{B}	0.77±0.08^{B}	42.40± 2.19^{B}	40.89± 2.56^{B}
Incubation time[1,2]						
0 h	18.12± 1.50^{A}	149.13± 7.31^{a}	0.38± 0.06^{A}	0.61±0.10	-	-
3 h	24.96± 0.67^{B}	165.68± 0.67^{b}	1.02±0.49^{B}	0.64±0.13	-	-

[1]Means with different superscript in capital letter within column differ significantly at (P<0.05)
[2]Means with different superscript in capital letter within column differ significantly at (P<0.01).

greater than that obtained from JCSM fermented by cattle and buffalo rumen fluids (Tjakradidjaja *et al*., 2008). Ammonia concentration had also been used to show ability of rumen microbes to degrade ricin from castor seed meal (*Ricinus communis* L.) at levels 0.42-1.68 mg/ml within 3-12 h incubation (de Oliviera *et al*., 2010), but rumen microbes could not degrade phorbolester (Makkar and Becker, 2010). Ability of rumen microbes degrading antinutrient with protein structure still depended on its concentration. Protozoal number reduction also occurred in other study (Tjakradidjaja *et al*., 2011). Protozoal cell was damaged and lysed by curcin and saponin (Fardiaz, 1992; Juan *et al*., 2002) due to greater sensitivity of protozoal cell, eukaryotes, to curcin and saponin than bacterial cell, prokaryotes (Makkar *et al*., 1998; de Oliviera *et al*., 2010). Sample used caused differences in microbial numbers between the ruminants. Less microbial population in cattle rumen fluid

because cattle was not fed properly before being slaughtered; the greater microbial numbers in buffalo was due to regular feeding. Other factors were differences in growth rates, enzyme activities (Bathia *et al*., 1980; Pradhan, 1994), and degree of resistancy to antinutrients (curcin and saponin) which may be greater in microbes from buffalo than cattle; however, these still needs a further clarification.

Differences in rumen fluid affected DM and OM digestibilities ($P<0.01$) with greater result was obtained from the buffalo (Table 1). DM digestibility was comparable to those found by Hakim (2002) for ration containing grass and concentrate (38-46%). The greater microbial population and its ability to ferment nutrients from rumen fluid of buffalo provided more nutrients that were more easily digested by enzymes in the post ruminal organ.

Conclusion

Addition of JCSM crude curcin extracts up to 3% (v/w) did not produce negative effects on ration fermentability and digestibility, except for protozoal population. The greater numbers of bacteria in rumen fluid of buffalo than cattle caused higher DM and OM digestibilities.

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Comparison Between Portable and Static Types of Silo on Silage Quality of Total Mixed Ration Containing Ramie Leaves (*Boehmeria nivea*, L. GAUD)

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Abstract

Ensiling is an alternative method to optimize utilization of seasonal available ramie leaves (Boehmeria nivea, L. Gaud) in ruminant daily feed but there is still lack of information available on the impact of silo type to the silage quality. The study was aimed to compare portable tower silo (plastic container 200 l) and static trench silo (1 ton capacity) effects on physical (odor, texture, moisture, color and spoilage), fermentative (pH, DM, VFA, DMD, CP, NH3, CPD, WSC and fleigh number) and utilities (rumen ration fermentation and degradation) characteristics of silage produced. The results showed that trench silo was less effective in giving good physical characteristics of silage compare to the plastic container (9% vs 2.59% of spoilage respectively). Fermentative characteristic of silage produced in plastic container was excellent (fleigh number 118) while trench container produced good grade silage (fleigh number 74). All silage pH were less than 4.4. Utilities characteristic of silage were not affected by the type of silo. Both silage were highly fermentable and digestible to ruminant (>71% OMD). Plastic container produced better physical and fermentative characteristics of total mixed ration contained ramie leaves, but utilities characteristic of silage produced in both type of silo were equal.

Keywords: ramie leave, ruminant, silage, silo, total mix ration

Introduction

Productivity of cows in the developing countries was only 22% of cows in the developed countries (Speedy and Sansoucy, 1989). The first factor limiting the milk production and productivity was nutrition aspects such as increasing difficulty in providing the bulk, high price, and fluctuated availability and quality of feed required by cattle. Alternative source of seasonal available high quality agro-industrial by-product such as ramie leaves (*Boehmeria nivea*, L. Gaud) have been

studied (Despal, 2007). The leaves contained 16% (Despal *et al*., 2011) to 21% of CP (Duarte *et al*., 1997), that is equal to lucerne or alfalfa (Ferreira *et al*., 2007; DeToledo *et al*., 2008).

Because of its seasonal availability, conservation technique should be applied (Mayne and O'Kiely, 2005). Several laboratory scale conservation methods of ramie leaves including time and additive used for ensiling (Despal *et al*., 2011a) and time and drying technique for hay making (Asti *et al*., 2009) have been optimized. Comparison of the nutrient values of ration containing dried and ensiled ramie leaves were also tested *in vitro* (Despal *et al*., 2011b). From the previous experiments, it is concluded that ensiling method conserved better nutrients of ramie leaves and produced better feed utilities for ruminant compare to drying method.

To be able to provide supply of nutrients required by dairy cattle continuously, ensiling the leaves as total mixed ration on larger capacity of silo can be an alternative. Unfortunately, there is limited available information of suitable silo types for smallholder dairy cattle farmer, especially in humid tropical developing countries.

The study was aimed at comparing portable tower silo (plastic container 200 l) and static trench silo (1 ton capacity) effects on physical characteristics of the silage produced (odor, texture, moisture, color and spoilage) and ensiling characteristics (pH, dry matter, volatile fatty acid, dry matter degradation, crude protein, ammonia, crude protein degradation, water soluble carbohydrate and fleigh number) as well as the silage utilization by ruminant (ruminal ration fermentation and digestibilities) *in vitro*.

Materials and Methods

Depending on capacity of the silo (100 kg for tower and 350 kg for trench silos), total ration have been mixed homogenously out of 58.8% of 2 cm length chopped elephant grasses, 24.5% of 2 cm chopped ramie leaves, 1.3% rice bran, 3.7% pollard, 5.6% corn meal, 2.4% soybean oil meal and 3.7% coconut oil meal to produce 32.36% dry matter (DM), 66% total digestible nutrients (TDN), 19% crude protein (CP), 1.71% calcium (Ca) and 0.4% phosphorus (P) of nutritional content of the ration. The mixed materials were placed into the silo. The airs were pushed out of the silo by compressing the materials manually. The silos were then sealed. Ensiling was let for 5 weeks anaerobically at room temperature.

Qualities of silage produced were compared based on physical characteristics (odor, texture, moisture, color and spoilage) descriptively. Ensiling characteristics of both silage were compared based on pH, DM, volatile fatty acid (VFA), DM degradation (DMDs), CP, ammonia (NH_3), CP degradation (CPD), water soluble carbohydrate (WSC) and fleigh number (FN) variables of the silages. Utilities characteristics of the silage for dairy cattle were compared based on ruminal

fermentability of the silage to produce VFA and NH_3 and their DM digestibility (DMDr) and organic matter digestibility (OMD) *in vitro*.

Physical characteristics of the silage were described quantitatively. Scale (+1) were given to the least desired and (+4) to the most desired physical characteristic of the silage. Measurements of pH were done according to Naumann and Bassler (1997) procedure. The amounts of 10 g of silages were mixed with 100 ml distilled water using mid speed blender for 1 min. Supernatants were separated and the pH was measured using calibrated pH meter. The supernatants were stored frozen until it were used for determination of silage VFA (using steam distillation method) and NH_3 (using Conway micro diffusion method) concentrations.

Degradations of DM during ensiling were calculated by subtracting DM in the material from DM in the silage. Degradations of CP during ensiling were quantified from NH_3 produced from the degradations. Analyses of DM were conducted using oven heat, while CP contents were measured using Kjehldal method (Naumann and Bassler, 1997). Water soluble carbohydrates were determined using Phenol Method according to Singleton and Rossi (1965), while, FN were calculated according to formula described by Idikut et al. (2009), where NF = 220 + (2 x %DM – 15) – (40 x pH).

Ruminal fermentabilities were conducted according to General Laboratory Procedure (1966). The VFA ruminal concentrations were determined using steam distillation method, while ruminal NH_3 concentrations were determined using Conway micro diffusion method. *In vitro* digestibility trials were done following Tilley and Terry (1963) two-stage technique.

Observations of ensiling characteristics were conducted following completely randomised design, while utilities characteristics observation used randomised block design. Each treatment was repeated thrice. The data obtained were analyzed using Varian analysis if the assumptions were fulfilled. For those which were not, descriptive analyses were used instead.

Results and Discussions

Physical, ensiling and utilities characteristics of silage produced in tower (plastic container) and trench types of silo were showed in Table 1. Tower silo (plastic container) produced better physical characteristics of silage by means of lighter color, more acidic odor, better texture, less moisture and spoilage (Haustein, 2003). Ammonia odor was not found in the tower silo, but in the trench silo. Lower amount of clotted silage (0.003%) was found in the tower silo compare to the trench which was reach up to 1%. Ammonia odor and clotted silage found in trench silo showed spoilage microorganism activities during and after ensiling which was more favor in higher moisture silage (Saun and Heinrich, 2008). Therefore, moisture control to reduce water activity such as wilting or the use of absorbent substrate (Despal *et*

Table 1. Physicals, ensiling and utilities characteristics of the silages

Variables	Trench silo	Tower silo
Physical characteristics		
Color	+3 (Brownish green)	+4 (Yellowish green)
Odor	+3 (Acid + ammonia)	+4 (Acid)
Texture	+3 (3.5 kg clotted silage)	+4 (3 g of clotted silage)
Moisture	+3	+4
Spoilage	+2 (9%)	+4 (2,59%)
Ensiling characteristics		
pH	4.38 ± 0.10	3.60 ± 0.54
DM (%)	22.07 ± 0.46^{b}	28.89 ± 1.19^{a}
VFA (mM)	8.05 ± 2.72	5.12 ± 2.62
DMDs (%)	10.56 ± 0.46^{a}	3.74 ± 1.19^{b}
CP (%)	17.24 ± 6.97	19.03 ± 4.82
NH_3 (mM)	0.81 ± 0.40	0.84 ± 0.11
CPD (%)	4.69 ± 0.91	4.56 ± 1.04
WSC (% BK)	2.20 ± 0.12^{a}	1.37 ± 0.08^{b}
NF	74.00 ± 3.92^{b}	118.78 ± 21.5^{a}
Utilities characteristics		
VFA (mM)	201.65 ± 6.99	213.41 ± 30.7
NH_3 (mM)	19.25 ± 6.47	18.81 ± 0.72
DMDr (%)	71.06 ± 1.83	73.40 ± 1.17
OMD (%)	71.62 ± 1.67	73.25 ± 1.45

Different superscript in the same line means significantly different ($P<0.05$)

al., 2011a) might be possible to successfully control undesirable microorganisms sucs as *Clostridium*. In wet silages (22% DM), the most important microorganisms growing were bacterial strains which lead to higher ammonia-N and pH increases (Nussio, 2005) such as those were found in the trench silo.

Ensiling characteristics of the silage in tower silo were also better than those of the trench by means of higher DM and CP contents and NF value of the silage and lower pH, DMDs, CPDs, WSC and VFA content of the silage (significantly or just tent to). More rapid drop in silage pH was found in the tower silo than those in the trench. These were esensial for minimising proteolysis and successful ensiling (Saarisalo and Jaakkola, 2005). Lower degradation of DM and CP in tower silo was caused by more rapid decreasing of pH which was produced by more active convertion of WSC into lactic acid (mainly) by activity of lactic acid bacteria

(LAB). These condition inhibited harmful microorganism growth rate, therefore, less substances were degraded and higher nutrient could be conserved. These efficient forage conservation system that minimise quantitative and qualitative losses had always been the importance emphasised in ensiling research technique (Mayne and O'Kiely, 2005).

Degradation of DM (DMDs) in this study was 3.74% for tower and 10.56% for thrench silos. The lower DMDs in the tower type silo might also be caused by the higher bulk density of the tower (100 kg/200 l or equal to 500 kg/1000 l) compared to trench silo which was only 350 kg/m^3 or equal to 350 kg/1000 l. The equal losses of DM were observed by Ruppel (1992) which reported DM losses of 202 and 100 g/kg for silage bulk densities of 160 and 360 kg/m^3, respectively.

Silage value index described in NF value showed that silage produced in tower silo resulted in significantly higher NF score (118.78) than those in the trench silo (74.0). According to Idikut *et al*. (2009), silage produced in the tower silo could be categorized into very good silage (NF > 85), while trench silo produced good quality silage (60 < NF < 80).

Utilization of both silages by ruminant (*in vitro*) did not show statistical different in their characteristics. Both silages were categorized highly fermentable. Concentration of VFA found in rumen fluid after incubation of the total mixed ration were above optimum level of 80–160 mM for microbial growth requirement. Fortunately, the excess of VFA were synchronized by excess of NH_3 (> 12 mM) which were expected could improved microbial growth leading to higher microbial activities and protein synthesis. The high activity of feed degradation in the rumen were shown by high DMDr and OMD of the silage (> 70%).

Conclusions

Total mixed ration containing ramie leaves silage produced in both silos were in the grade of good to very good qualities with high effiency of forage conservation systems. Both silo produced equal utilities characteristics of silage for ruminant, however, tower silo (plastic container) produced better physical and ensiling characteristics of silage with higher nutrients could be conserved.

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Blood Metabolite Statues of Local Sheep Fed With *Indigofera* sp.

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Abstract

Blood metabolite statues represent blood nutrients which will be metabolized by the cell. Low nutrient statues of local sheep fed with sole grass caused low performance. The aim of this experiment was to evaluate blood metabolite statues of growing male local sheep through improvement of ration containing Indigofera sp. Eight local sheep consisted of four UP3-jonggol and four garut growing sheep, were used in this experiment for three months. The rations were pellet concentrate containing 30% of Indigofera sp with 15% crude protein (CP) and 73.80% total digestible nutrient (TDN). The animals were reared in individual cages and given water and feed ad libitum. At the end of the experiment, blood was drained and collected from jugular vein using 5 ml spoit. The blood were analysed for the concentrations of plasma glucose, urea and total cholesterol using diagnostic KIT Merck Dya-Cys following laboratory standard procedure. The design of this experiment using simple T-test, the mean data was compared for all parameters. Result showed that plasma glucose concentration was not significance (77.95 vs 85.89 mg%) among the local sheep, while concentrations of blood urea-N (BUN) and plasma cholesterol in jonggol sheep (73.31 mg% and 77.18 mg%) were significant higher than in garut sheep (55.99 mg% and 58.97 mg%). It was concluded that jonggol sheep have higher plasma cholesterol and BUN compared to the garut sheep which fed by 30% Indigofera sp. in the ration.

Keywords: cholesterol, garut and jonggol sheep, glucose, Indigofera sp., urea-N

Introduction

Lamb meat has high cholesterol concentration which caused metabolic syndrome disease *(atherosclerosis)*. It is important to manage system in order to get good quality of meat without reducing the productivity. Feeding management can solve the problem through diversification kind of forage utilization. Cholesterol concentration in sheep (80 mg%) was higher than in beef (74 mg%). Blood metabolite status, represents blood nutrients, are produced by cell metabolism and will affect

meat quality. Low nutrient status of local sheep fed with sole grass caused low blood metabolite status and their performance *Indigofera* sp. is one of legume which has potential to be used and has 27% protein and 15.5% fiber content, meanwhile the productivity is around 4,096 kg dry matter (DM)/ha (Abdullah, 2010). Fatty acid composition of *Indigofera* sp. is low in poly-saturated fatty acids, so it is promising to be used as the best part in the ration for producing good lamb meat. Substitution poly-saturated fatty acid with poly-unsaturated fatty acid could reduce total cholesterol concentration, including the concentration of LDL-cholesterol (Marsic and Yodice, 1992). On the other hand, local sheep reared under the tropical rainforest with sole mixed-grass, had low blood profil status and low performance (Astuti *et al*., 2009). So far, it was reported that the concentrations of serum trigliceride, glucose and total protein of those sheep had only 18,71 mg% , 51,86 mg % and 6,40 mg%, respectively. Meanwhile Wiryawan *et al*. (2010) reported that jonggol sheep had low performance (40 g/h/d) caused by unbalance nutritional status and high daily temperature and humidity. There is no study in utilisation of *Indigofera* sp. for local sheep (garut and -jonggol) to improve poor feeding management; so it is expected that feeding the legume will improve metabolite profile and lamb meat quality.

The aim of this experiment was to evaluate blood metabolite statues of growing male local sheep (garut and jonggol) fed with pellet ration containing 30% *Indigofera* sp.

Materials and Methods

Eight local sheep consisted of four jonggol and four garut growing sheep (average body weight, 20 kg), were used in this experiment for three months. The rations were pelleted containing 30% of *Indigofera* sp. and 70% of concentrate with total ration contained 18% crude protein, 15.40% fiber, 3.14% Extract Ether, 42.43% starch and 73.80% total digestible nutrient (TDN). The animals were reared in individual cages and given water and feed *ad libitum*. At the end of the experiment, blood was drained and collected from jugular vein using 5 ml syringes. Whole blood was separated by centrifugation the blood at 10.000 x g for 10 minutes to obtain the serum. The serums were analyzed for the concentrations of glucose, blood urea nitrogen (BUN) and cholesterol using assay kit DyaSys catalogue number 101592 as standard procedure with spectrophotometer. The data were compared using T-test between two local breeds and presented as figures.

Results and Discussion

The physiological and biochemical differences between individuals and breeds, which had different types of blood profile are used for indicating response to the

Table 1. Metabolite profile of local sheep fed with *Indigofera* sp.

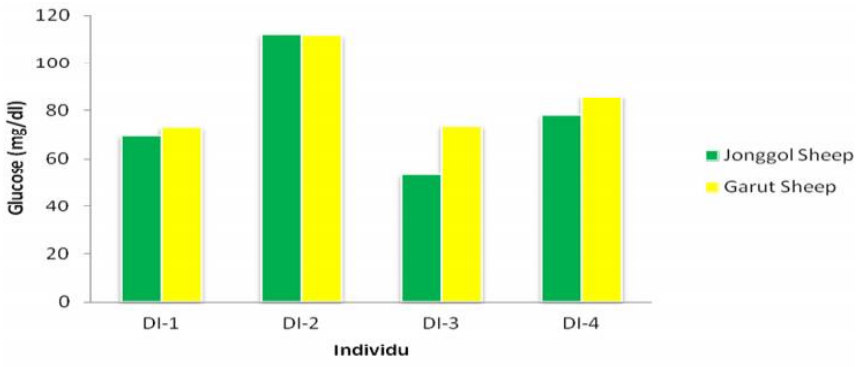
Parameters	Jonggol sheep	Garut sheep
Glucose (mg%)	77.95±24.73	85.89±17.87
Blood Urea (mg%)	73.31±4.18[a]	55.49±7.46[b]
Cholesterol (mg%)	77.17±15.27[a]	58.97±9.74[b]

Means in the same row followed by different letters are significantly different (P<0.05)

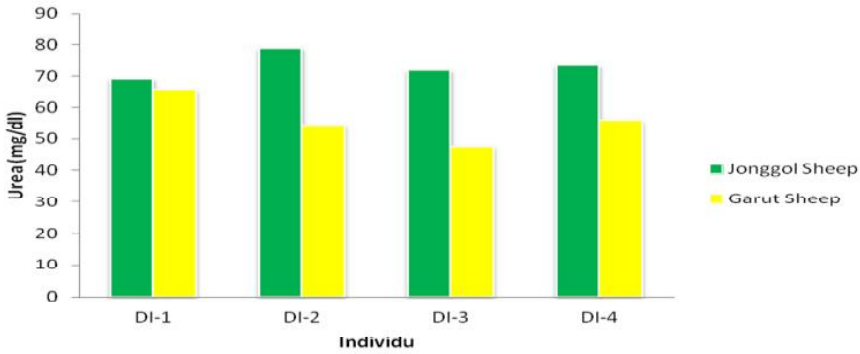
ration containing *Indigofera* sp. Results showed that serum glucose concentration was not significance among two local breeds (jonggol: 77.95 mg% and garut: 85.89 mg%), while concentrations of serum urea-N and cholesterol in jonggol sheep (73.31 mg% and 77.18 mg%) were significantly higher than in garut sheep (55.99 mg% and 58.97 mg%) (P<0.05).

The glucose concentration of both local sheep fed with *Indigofera* sp. showed in good condition compared to the healthy sheep. Riis (1983) reported that the normal glucose concentration of healthy sheep was 35–60 mg%. These ranges covered for growing sheep (58 mg %), pregnant sheep (47 mg%) and lactating sheep (59 mg%). The composition of *Indigofera* sp. ration with high starch (42%) value and rapidly metabolized may increase glucose concentration in both breeds. In ruminant blood metabolite profile, glucose concentration is low, but it is very important to support nutrient for the brain, nervous systems and active organs such mammary gland (Riis,1983).

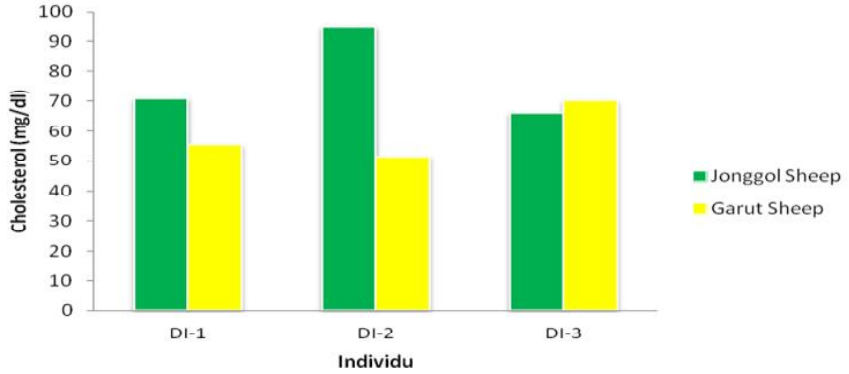
Blood urea nitrogen (BUN) is representing metabolite exes of protein. Jonggol sheep which are usually reared in grazing pasture with low quality of forage has better response to the high quality ration compared to garut sheep (kept in semi intensive farming system). The condition caused a compensatory metabolism which can improve the blood metabolite status in a short time. The evaluation during three months observation showed that the concentration of BUN in UP3-jonggol growing sheep were higher than in garut growing sheep.



Graph 1. Glucose profile of UP3-jonggol and garut growing sheep



Graph 2. Urea-N profile of UP3-jonggol and garut growing sheep



Graph 3. Cholesterol profile of UP3-jonggol and garut growing sheep

Cholesterol status of UP3-jonggol growing sheep were higher than garut growing sheep in the same age fed with *Indigofera sp.* and both higher than other breeds. Cox-Ganser *et al.* (1994) reported that blood compositions in sheep grazing Brassicas had average value of 68 mg/dl, 20 mg/dl and 64 mg/dl for glucose, BUN and cholesterol, respectively. Some factors affecting to the cholesterol concentration were feeding, age, physiological state, health and breed. Cholesterol is needed for the steroids hormone precursor in growing animals (Riis, 1983).

Miresan (2003) reported that serum cholesterol in difference breeds of mountain sheep in grassing area were 48.00±2.66 mg/dl (tsigai); 51.60±4.30 mg/dl (merino) and 48.40±2.37 mg/dl (corriedale), while for the BUN were 39.80±2.13 mg/dl, 31.00±1.51 mg/dl and 37.00±1.67 mg/dl, for tsigai, merino and corriedale, respectively. Different breed cause different fate of nutrient metabolism causing variation in nutrient metabolite status and deposition. Sudarman *et al*. (2006) reported that utilization of 1.5% Ca-saponified lemuru oil in ration could reduce meat cholesterol-LDL concentration in sheep up to 32% and increased feed efficiency up to 36%, while the concentration of triglyceride was around 29 mg%.

Conclusions

Analysis of biochemical parameters of local sheep fed with 30% *Indigofera* sp. to the blood metabolite profile showed that their values are in normal physiological status. Jonggol growing sheep has higher blood urea-N and cholesterol serum concentrations compared to garut growing sheep.

Acknowledgement

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Analysis of the Kinetics Fermentability, Degradability, and Nutritive Value of Soybean Groats and Lemuru Fish Oil Protected by *in-Vitro*

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Abstract

Objective of the research was to compare kinetics fermentability, degradability and nutritive value of protected soybean groats and lemuru fish oil using in-vitro study. Soybean groats were protected using formaldehyde whereas the lemuru fish oil was protected by Ca-salt. The protected soybean groats and lemuru fish oil were given as supplements of 5% and 10% of cattle's dry matter ration, respectively. The experiment was conducted in the cow house experiment, the Laboratory of Biochemistry Faculty of Gadjah Mada University and the Laboratory of Nutrition and Animal Feed Faculty of Agriculture, Animal Husbandry Department, Sebelas Maret University. Fermentability and degradability of the rations were observed at 0, 3, 6, 9, 12 hours after incubation in the rumen fluids. The collected data were analyzed to calculate their mean and standard deviation, and mentioned descriptively. Results of the research showed that the kinetics of pH, VFA, NH3 and the production of ruminal microbes of soybean groats share the same pattern. Protected lemuru fish oil indicated higher kinetics in pH, VFA and microbial protein production, but lower for NH3 results. The protected soybean groats have a higher dry matter consumption compared to that of lemuru fish oil, but its organic matter and crude protein digestibility is lower. It was concluded that suplementation of protected soybean groats and lemuru fish oil gave similar kinetics fermentation, rumen degradability as well as nutritional values to the cattle rations therefore could be used as PUFA feed additive sources in cattle ration.

Keywords: degradability, fermentability, lemuru fish oil, protection, soybean groats

Introduction

Implementation of productivity improvement method of beef cattle through the use of Poly Unsaturated Fatty Acid (PUFA) fodder source material is very important

to take note of these days. Unsaturated fatty acids can improve the function of the corpus luteum (Mattos *et al*., 2000), ovarian follicle (De Fries, 1998). Beef cattle on feedlot fed with protected PUFA fodder source material that contain vegetable and animal oils produced beef with high linoleic acid content (Gillis *et al*., 2004), and with improved quality of beef (Scollan *et al.*, 2001 and Riyanto *et al*., 2011). PUFA source material can be derived from animals and vegetables. Both linoleic and linolenic acids in the form of Saturated Fatty Acid (SFA) in the rumen escape the process of biodehydrogenase (Lourenc *et al*., 2010). The fat consumed by cattle beef will be hydrolyzed by microorganisms in the rumen and attached to feed particles and microbial fermentation in the rumen thereby disrupting the structural carbohydrates (Bauman *et al*., 2003). To extract post-ruminal PUFA, protective treatment is needed. Soybean protection is conducted using, among others, non-enzimatic microwave irradiation (Nobar, 2011), and formaldehyde (Madsen, 1982). The current research is conducted to evaluate the fermentability and degradability kinetics of basal ration (fermented straw and basal concentrate) with protected PUFA fodder source material of lemuru fish oil and soy groats

Materials and Methods

The research is conducted at Laboratory of Nutrition and Feed Animal Science Department of Agriculture Faculty of Sebelas Maret University, Laboratory of Biochemistry and Chemistry of Pusat Antar Universitas of Gadjah Mada University, and at the Laboratory of Biochemistry, Faculty of Animal Science of Gadjah Mada University.

Two ruminally fistulated Ongole breed cattle were employed in this study as the ruminal fluid donors. Both of them were fed with fermented straw (40%) and concentrate (60%). The latter is composed of a mixture of basal concentrate, soy groats, and protected lemuru fish oil. The lemuru fish oil protection method refers to that of Cabatit (1979) cit Widiyanto (2008), that is, by saponification using KOH and $CaCl_2$. Soy groat protection method is conducted in accordance with that of Widyobroto *et al*., (1999), that is, by spraying 37% formaldehyde that constitutes 2 percent of dry feeding material. Basal feed (control) contain 12.2% crude protein, 5.2% crude fat, 16.9% crude fiber, and 55.2% TDN.

The study parameters include ruminal pH, VFA (*Volatile Fatty Acids*) production, NH_3 concentration, microbial protein kinetics, consumption (dry materials, organic materials, and crude protein), and digestibility (dry materials, organic materials, and crude protein). Measurement of ration fermentability is conducted in specified time to determine the ruminal kintetics, 0, 3, 6, 9 and 12 h after feeding. The collected data were analyzed to calculate their mean and standard deviation, and mentioned descriptively.

Results and Discussion

The kinetics of ruminal fluid pH, NH_3 concentration, VFA total production, and microbial protein are illustrated in Figures 1 and 2 as the results of lemuru fish oil and protected soy bean groats supplementation in basal feed.

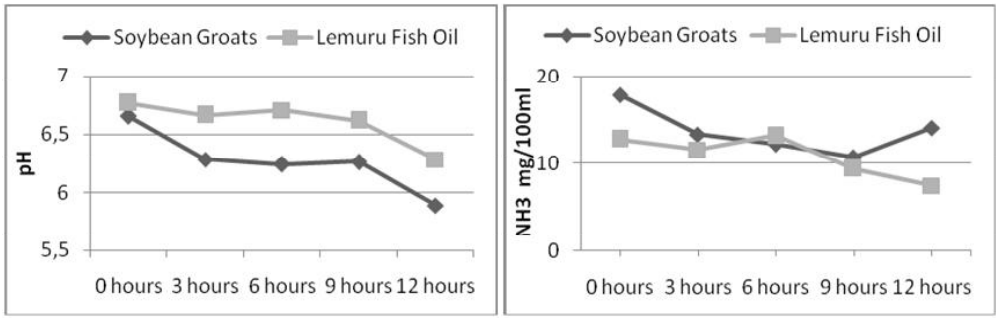

Figure 1. pH Kinetics and NH_3 Concentration

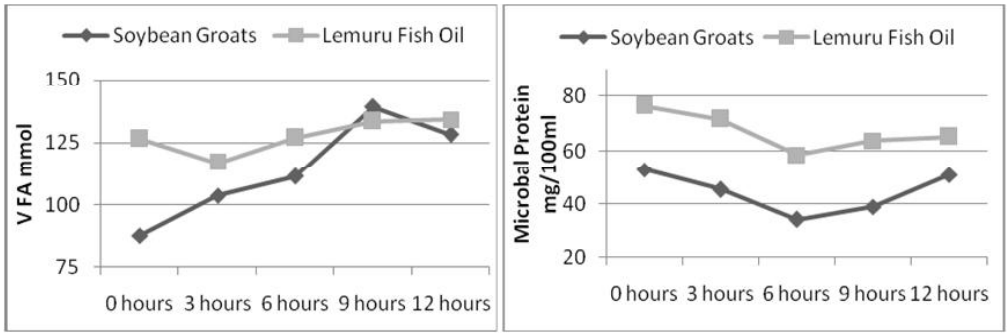

Figure 2. VFA Production and Microbial Protein

The pH kinetics of the protected soy groats is 6.26±0.24 (5.89-6.65) and of the protected lemuru fish oil is 6.60±0.17 (6.28-6.76) (Figure 1). The fluctuative pattern of pH decrease on SG and lemuru fish oil treatment as PUFA source fodder is related to its organic substance content. Ørskov (1992) higher non-fiber organic substance made the pH fluctuation in ruminal liquid possible. While the mean concentration of NH_3 of soy bean groats supplementation is 13.61±2.65(10.69-17.77) mg/100ml higher than that of NH_3 with lemuru fish oil supplementation 10.89±2.39 (7.51-13.17), both are still within the normal range (Figure 1). It is in accord with what Leng (1980) suggested that NH_3 concentration ranges from 1 to 34 mg/100ml, for maximum growth and activity of microbe the necessary NH_3 concentration ranges from 5.0 to 23.5 mg/100ml. VFA Concentration of lemuru fish oil 127.46±6.89 (116.83-133.96) mmol is higher than that of soy bean groats 114± 20.02 (88.21-139.01) mmol with similar kinetics pattern (Figure 2). The VFA ruminal fluid

kinetics, after reaching an optimum concentration, decreases irregularly. This is related to the absorption of VFA as an energy source through the rumen wall; the lower the ruminal pH, the higher the VFA absorption (Owens and Goestch,1988). Microbial protein production in diets supplemented with protected lemuru fish oil is 67.06±6.97 (58.53-76.37) mg/100ml, and the that supplemented with protected soy bean groats is 44.58±7.73 (34.50-52.80) mg/100ml (Figure 2). It is caused by the synchronization of the nitrogen source provision required by the microbes in the form of NH3 and VFA. Lemuru fish oil can provide a source of microbial protein precursor compilers more than soybean groats. According to Henning *et al*., (1993), the efficiency of microbial protein synthesis is influenced by the availability of all precursors in sufficient concentration in the ruminal fluid. The microbial protein compiler precursors are NH_3 as N-source, energy, carbon skeleton, mineral and other nutritional elements.

Consumption and digestibility of dry materials, organic materials and crude protein from protected soybean groats and lemuru fish oil obtained from this research are shown in Table 1. The protection carried out by coating the micro granules of vegetable oils containing unsaturated fatty acids using protected formaldehyde shown to increase the amount of unsaturated fatty acids deposited in the ruminant tissues (Scott and Ashes, 1993). This treatment prevents the process of unsaturated fatty acids biohydrogenase in the rumen and also increases the amount of feed intake. The efficacy of triglycerides or fatty acid added to ruminants' diets depends on their digestibility and their influence on the digestibility of other nutrients in the diet. Because energy intake results from dietary energy concentration and intake of dry matter (DM), the influence of the supplemental fat on food consumption also has to be taken into account (Voigt, dkk., 2006). Organic material consumption in the forms of protected soybean groats and lemuru fish oil is in accord with the dry material

Table 1. Intake and degradibility parameter of protected supplemented soybean groats and lemuru fish oil

Parameters	Soybean groats protected	Lemuru fish oil protected
Intake :		
Dry matter (g/d)	6260.85±103.50	5965.87±1613.93
Organic matter (g/d)	5550.24±81.84	5569.29±1501.58
Crude protein (g/d)	896.05±24.43	770.00±219.82
Degradibility :		
Dry matter (%)	59.28±3.80	54.00±15.55
Organic matter (%)	65.01±3.29	71.8±711.22
Crude protein (%)	66.59±11.03	80.48±6.63

consumption. Consumption of organic material is closely related to the consumption of dry matter; the more consumption of dry matter, the more consumption of organic material (Van Soest, 1994). The increasing amount of ruminel microbes allows them to work more effectively to fermentatively degrade the components of crude fibers, and thereby increasing the dry matter digestibility of the consumed feed.

Higher digestibility of protein compared to that of dry matter and organic matter account for the availability of nutrients in cow diets. This means that the provision of rations with a high fat content did not affect the protein, organic matter and dry matter digestibility. Mahadevan, dkk., (1983) the rates of in vivo ammonia appearance and in vitro proteolysis were highly correlated (r--0.966, $P<0.01$) between 1130 h and 1200 h. These rates of rumen ammonia appearance reflected the in vivo rate of proteolysis. Both bacterial and protozoa protein content decreased significantly at the higher levels of formaldehyde treatment

Conclusions

It was concluded that suplementation of protected soybean groats and lemuru fish oil gave similar kinetics fermentation, rumen degradability as well as nutritional values to the cattle rations therefore could be used as PUFA feed additive sources in cattle ration

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Combination Effect of Clove and Cinnamon Oil on *in Vitro* Rumen Gas and Methane Production

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Abstract

Clove and cinnamon oils were used for rumen manipulation in ruminant animal production. Their major component, eugenol and cinnamadehyde were proved to optimize rumen metabolism and microbial composition. The objective of this study was to evaluate combination effect of clove and cinnamon oil on rumen gas and methane production by using an in vitro rumen fermentation system. Three ruminal cannulated cows were used as donors of ruminal fluid and were individually penned indoors. The cattle were fed daily total mix ratio (tmr – 60% concentrate 40% alfalfa hay) and had free access to water at all time. Ruminal fluid for in vitro rumen fermentation system was prepared as in vitro hohenheim gas test method. The treatments were 1) control, 2) cinnamon oil 300 ppm, 3) clove oil 300 ppm and 4) combination of clove and cinnamon oil which were assigned in order to factorial design. The results indicated that insoluble gas fraction (b; 40.8, 46.91, 40.36 and 47.46 ml respectively) and potential of extent of gas production (|a|+ b; 43.01, 51.03, 42.96 and 50.45 ml respectively) were significantly different ($p < 0.01$) between treatments. Soluble gas fraction (a) and rate of gas production (c) were not different between treatments. Clove oil, cinnamon oil and their combinations affected methane production and both essential oil decreased methane production (18.15 ml/ g dm vs 11.80 ml/ g dm and 11.55 ml/g dm, $p<0.05$). However there were no additive or synergistic effect when they were used together (11.80 ml/g dm vs 11.55 ml/g dm). These results explained that some essential oils were used together as blend oil may not give additional beneficial effect.

Keywords: clove oil, in vitro rumen fermentation system, orange peel oil, rumen gas production, rumen methane production

Introduction

Public awareness of the potential health risk and environmental problem caused by the excessive use of in-feed antibiotics, growth hormones and some pharmaceutical food production lead to prohibition of some antibiotics since 1998 in EU member state. Some aromatic herbs and essential oils which have been used for animal health management may substitute the using of growth promoters such as antibiotics and hormones. Current interest in ruminant animal production industry is focused on trying to improve production efficiency and reduce environment effect (Methane emission and ammonia excretion) with using essential oils. Clove and cinnamon essential oils have been evaluated at some doses. Their major component, eugenol and cinnamaldehyde were proved to optimize rumen metabolism and microbial composition.

Some *in vitro* research of clove essential oils and euogenol oil from doses 3 to 5000 mg/L *in vitro* fermentation culture was reported had effect on all of rumen fermentation products (Busquets, 2005a) decreasing VFA concentration (Busquet, 2005b; Castillejos *et al*., 2006). *In vivo* experiments using used blend oils contain eugenol (Agolin ruminant) 0.5 g/day/cow which had no effected on dry matter intake and milk yield, but increased fat contain in milk (Santos, 2010). Cinnamon leaf essential oil (500 mg/l) containing 76% eugenol decreased N-ammonia concentration, molar of branched-chain VFA (Fraser et al., 2007), acetate but increased propionate and butyrate at doses 312 mg/l (Busquet *et al*., 2005b). Cinnamon leaf oil (250 mg/l) also reduced methanogenesis activity of rumen bacteria and methane concentration in the fermentation gases, without altering total VFA (Chaves *et al*., 2008). *In vivo* studies reported that supplementation of cinnamaldehyde (200 mg/kg of dry matter) had no effect on dry matter intake, gain, feed efficiency, carcass characteristics, meat quality and rumen N-ammonia concentration in growing lamb fed barley or corn-based diets (Chaves, 2008).

The combination between essential oils or their components may result in additive and/or synergistic effects that may enhance efficiency of rumen microbial fermentation and nutrient utilization in ruminants. Thus, this research was evaluated the effects of clove (eugenol), cinnamon(cinnamaldehyde) and their combinations in the *in vitro* fermentation system.

Materials and Methods

Three ruminal cannulated cows were used as donors of ruminal fluid and were individually penned indoors. The cow was fed daily total mix ratio (TMR – 60% concentrate 40% alfalfa hay) twice at 08.00 and 14.00 h and had free access to water at all time.

Clove (CO) and cinnamon (CIN) essential oils were extracted using methanol

from clove bud and cinnamon bark. The chemical composition of clove and cinnamon essential oil samples indicated that eugenol contain in clove oil is 97.26% and cinnamaldehyde contain in cinnamon oil is 17.79%. The TMR that was used for ruminal animal donor, was also used as a substrate for *in vitro* rumen fermentation system. The TMR contained protein 18.81%, ADICP 9.22 %CP, NFE 49.87%, crude fibre 19.77%, NDF 38.22 and ADF 29.24%

The treatments were 1) control, 2) Cinnamon oil (CIN) 300 ppm, 3) Clove oil (CO) 300 ppm and 4) Clove oil (CO) 300 ppm + Cinnamon Oil (CIN) 300 ppm. Addition of CO, CIN and their combination were evaluated by *in vitro* Hohenheim Gas Test (HGT). The method of the HGT system is described in detail by Menke and Steingass (1979).

Anaerobic techniques were used in all procedures during the rumen fluid transfer and incubation period. Rumen fluid was collected from rumen-fistulated cows before morning feeding after 2 weeks feed adaptation. Filtered Rumen fluid was added to buffer medium in proportion 1 : 2 v/v, keep with carbon dioxide flushing for 10 -15 minute before starting to fill up the syringes. CO and CIN oils 300 ppm added to mixed rumen fluid and buffer medium as a treatments of this experiment. Through the inlet of the HGT glass syringes which substrate is placed inside it , 30 ml incubation mixed medium (rumen fluid, buffer medium and CO,CIN or combination CO-CIN) then dispensed into the pre-heated HGT glass syringe (39 ^{o}C) with the help of a semi-automatic pipette. The incubation was conducted for 96 hours inside the modified water bath (39 ^{o}C). Buffer medium containing some solution which is described by Menke and Steingass (1979).

Gas production data was collected at 3, 6 , 9, 12, 24, 48, 72, 96 hours. After 6 h of incubation methane gas was measured using a catharometer methane sensor OLC20 (Oldham ®, USA). The methane sensor was calibrated at 0–100% methane at gas produce at the time, and was calibrated again using pure methane gas (99,7%). Cumulative gas production data were fitted to the model of Orskov and Mcdonald (1979):

$$y = a + b(1-exp^{-ct})$$

where : a - gas production from immediately soluble fraction (ml). b- gas production from the insoluble fraction (ml). [a + b] – potential gas production (ml). c – gas production rate constant for the insoluble fraction (ml/h). t – incubation time (h). y – gas produce at time t (ml)

Estimation of metabolic energy of TMR was calculated by equations from Alderman (1985) using proximate values of TMR and from Boguhn et al (2003) using gas production value. The predicted value of ME of TMR for this experiment is 2.70 Mcal/Kg.

A two (CO0-CO300 ppm) by two (CIN0-CIN300) factorial arrangement in a completely randomized designed was used to compare gas production, gas produc-

tion kinetics and methane production using the General Linear model (GLM) of the SAS. The significance of differences between individual means wre determined using Duncan's multiple comparation test.

Results and Discussion

Soluble gas fraction (a) and rate of gas production (c) were not different between treatments. These data suggested that a lag phase due to delay in microbial colonization of the TMR may occur in the same time of incubation. The fermentation TMR by using CO, CIN and their combination had significant effect on insoluble gas fraction (b), potential of extent of gas production (|a|+b), gas production after 96 h incubation and methane gas production after 6 h incubation (Table 1). The value for a intercept for all treatments was same .

The addition of CIN 300 ppm and combination between CO and CIN resulted in higher value of gas volume at asymptote (b) than control treatment but athe addition

Table 1. Methane production at 6 hour incubation, characteristic and cumulative gas volume production throughout 96 hour of rumen incubation with TMR, CO, CIN, and combination between CO-CIN at 300 ppm

Gas Parameter	CO-0		CO300		SE	P < 0.05		
	CIN-0	CIN300	CIN-0	CIN300		CO	CIN	CO*CIN
Gas characters :								
a	-2.22	-4.12	-2.60	-2.99	0.66	0.59	0.11	0.28
b	40.80^{b}	46.91^{a}	40.36^{b}	47.46^{a}	1.17	0.97	0.00	0.68
c	0.08	0.08	0.09	0.07	0.01	0.36	0.36	0.11
[a] + b	43.01^{b}	51.03^{a}	42.96^{b}	50.45^{a}	1.97	0.27	0.03	0.28
Gas Production :								
6 h	12.50^{b}	14.21^{a}	12.86^{ab}	12.53^{b}	0.44	0.16	0.15	0.04
24 h	31.28^{b}	36.17^{a}	30.75^{b}	34.59^{a}	0.59	0.1	0.00	0.39
48 h	37.31^{b}	41.69^{a}	37.27^{b}	42.13^{a}	0.97	0.84	0.00	0.81
96 h	38.74^{b}	42.87^{a}	37.70^{b}	44.56^{a}	1.76	0.85	0.01	0.45
pH	6.78	6.76	6.76	6.74	0.01	0.19	0.14	1.00
ME (MCal/ KgDM)*	2.25^{b}	2.30^{a}	2.25^{b}	2.29^{a}	0.01	0.10	0.00	0.39
Methane %	26.55^{a}	21.11^{b}	23.18^{b}	18.86^{c}	0.79	0.01	0.06	0.09
(ml/g DM)	18.15^{a}	11.80^{c}	14.90^{b}	11.55^{c}	0.01	0.02	0.07	0.08
MR (%)		34.99	17.91	36.36				

CO= Clove oils, CIN = Cinnamon Oils, MR= methane reduction, ME*= predicted by equation from Boguhn, J. *et al.* (2003), Same letter at the same row indicate to no difference bettween treatment (P>0.05).

of CO 300 ppm had same value with control. The gas volume at asymptote (b) represented the fermentation of the insoluble fraction. This result might indicated that the addition of CO at 300 ppm had no negative effect on digestibility of insoluble fraction of TMR, because CO at level 0.25 ml and 0.50 ml could inhibit CMCase, xylanase and acetylesterase activity (Patra, 2010). The addition of CIN 300 ppm and combination between CO and CIN could increased digestibility insoluble fraction of TMR due to high value of gas production at asymptote (b) and total gas production at 96 h after incubation. There is no interaction effect between CO and CIN on gas production at asymptote (b) value ($P > 0.05$).

The Addition of CIN 300 ppm and combination between CO 300 ppm and CIN 300 ppm had potential extent of gas production value higher than control, but the addition of CO had same value with control. These results indicated that the addition CIN and combination CO and CIN affect digestibility of insoluble fraction of TMR. Gas production 24 h after incubation increased with the addition of CIN and combination CO-CIN which it might efficiency of energy using from TMR. Addition of CIN 300 ppm and combination CO and CIN had predicted metabolism energy after 24 h of incubation higher than control. Menke et al (1988) suggested that gas volume at 24 h after incubation has a relationship with metabolizable energy in feedstuff.

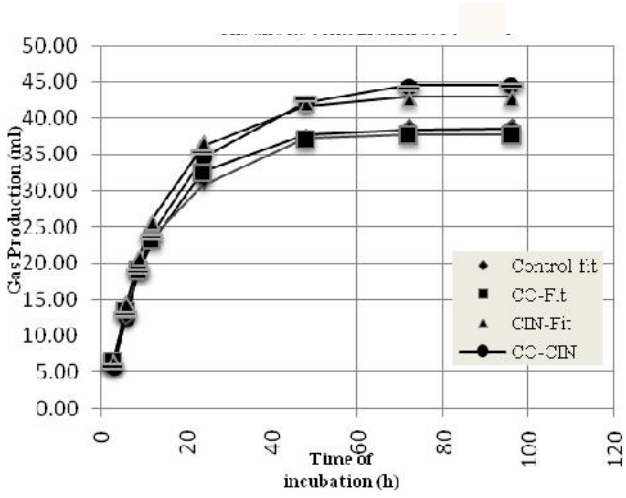

Figure 1. Cummulative gas volume estimated by $Y = a + b [1-exp(- ct)]$ throughout 96 hour. substrat is TMR added with CO, CIN and its combination at 300 ppm

Available energy of TMR affected on gas production and methane emission from rumen fermentation. Addition of CIN 300 ppm, CO 300 ppm and combination CO 300 ppm and CIN 300 ppm reduced methane production after 6 h incubation from control (34.99%, 17.91% and 36.36%, respectively). Methane reduction value of addition CIN 300 ppm had twice value from addition CO 300 ppm, but addition combination of CO and CIN had same methane reduction value with the addition

CIN 300 ppm. It indicated that there was no synergy effect between CO 300 ppm and CIN 300 ppm at in vitro gas production test.

Conclusions

The addition of cinnamon 300 ppm, clove 300 ppm and their combination affected on methane reduction. Methane reduction value of addition cinnamon was twice higher than addition of clove, but there had no synergy effect of methane reduction value of addition combination between cinnamon and clove. Methane gas reduction of addition clove, cinnamon and their combination due to efficiency of energy available in TMR from insoluble fraction of TMR.

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Effects of Feeding Different Level of Dietary Protein with or without Probiotics or Ionophores on Performance of Growing Kids

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Abstract

The study examined the effects of high and low input feeding system on nutrients ingestion, digestibilities, nitrogen (N) retention, blood urea nitrogen (BUN), metabolic hormones and economics of weight gain in growing kids. Eighty male beetal goats of 6 month of age were randomly divided into ten groups, eight goats in each group in completely randomized design. Nine isocaloric diets with varying crude protein (CP) levels with or without ionophores and probiotics were formulated. Diets containing 12, 16 and 20% CP were designated as low protein (LP), medium protein (MP) and high protein (HP) diets, respectively, while each of these CP diets when supplemented with ionophores @ 20ppm or probiotics (Yea Sec, 0.1%) were denoted as LPI, MPI, HPI and LPP, MPP,HPP, respectively. One group was fed berseem hay (FOD) only as a representative of traditional feed. The study lasted for 3 months. Higher dry matter (DM) and CP intake and digestibilities by kids fed LP, MP and HP diets were observed than those fed FOD diet. Similar trendfor DM and CP intakes were noticed by supplementation of ionophores or probiotics. Blood glucose, BUN, N balance, tri-iodothyronine and thyroxin concentrations were higher in goats fed LPI, MPI, HPI diets than those fed LP, MP, HP and LPP, MPP and HPP diets. Outcome of the study indicated that feeding growing kids on high input feeding system compared to, regardless supplementation of ionophores or probiotics, traditional feeding system increased nutrients intake, utilization, N balance and growth with better profit margin.

Keywords: blood metabolites, growing kids, nutrient rich system, nutrient utilization, weight gain

Introduction

Diet and feeding regimens are considered important factors which not only influence growing small ruminants (sheep & goats) productivity and profitability.

Nutrition of growing ruminants plays a pivotal role to enhance mutton production and also has strong association with quality and quantity of carcass. Balanced diets can improve their growth without affecting sensory quality of mutton and also reduce the time to slaughter with increased dressing percentage (Ryan *et al*., 2007). Crude protein (CP) and energy are the major nutrients which directly affect the growth of small ruminants and manipulating these nutrients can help optimize their growth performance. Nutrient utilization at ruminal level, in nutrient rich feeding system, can further be improved by adding suitable feed additives like ionophores and probiotics. Ionophores have been reported to increase the efficiency of feed utilization in growing ruminants by improving dry matter and protein digestibilities (Potter *et al.,* 1976a; Raun*et al.,* 1974). Probiotics have also been reported to increase weight gain in small ruminants by enhancing nutrient utilization at ruminal level (Abd El-Ghani, 2004).Productivity and economic index of growing male *beetal* goats under tropical environmental conditions, maintained on high input feeding system (high dietary energy and protein with or without feed additives) versus conventional feeding system (fodder only) is limited. Therefore, the present study was planned to examine and compare the nutrients intake, digestibility, growth, blood composition, nitrogen balance and growth of growing male *beetal* goats fed on high input feeding system and traditional feeding systems.

Materials and Methods

Eighty male *beetal* goats (6 month old) were randomly divided in ten groups with eight animals each. Nine isocaloric diets with three levels of crude protein (12, 16 and 20%) with or without ionophores (@ 20ppm) and probiotics (0.1% of ration) were formulated (Table 1). These diets were fed to nine groups of lambs while tenth group was offered fodder (berseem hay) only. The goats were fed *ad libitum* and weighed weekly to know the weight gain and its economics. The study lasted for 90 days. Feed intake was recorded daily. Total collection method was used to determine the nutrient [(dry matter (DM), crude protein (CP) neutral detergent fiber (NDF), acid detergent fiber (ADF)] digestibilities. Feces were collected daily, dried at 55°C, bulked and mixed at the end of each collection period. Urine samples were acidified with 50% H_2SO_4 and stored at -20 °C for laboratory analysis. Feed and fecal samples were analyzed for DM and CP as per description of AOAC (2003) while NDF and ADF by the method described by Van Soest *et al*. (1991). Nitrogen (N) balance was calculated using equation described by NRC (2001).Blood glucose was analyzed by method described by Davies *et al*. (2007), while triiodothyroxin (T3) and thyroxin (T4) concentrations were analyzed by the methods of Todini *et al*. (2007). Blood urea nitrogen (BUN) was measured by procedure described by Bull *et al*. (1991).

The data collected were subjected to statistical analysis using ANOVA under completely randomized design (SAS, 1997) and Tukey`s significant difference test was used to compare means (Steel and Torrie, 1984).

Table1. Ingredients and Chemical composition of diets fed to growing goats

INGREDIENTS	Diets[1]									
	FOD	LP	MP	HP	LPI	MPI	HPI	LPP	MPP	HPP
Corn grains	-	25	25	25	25	25	25	25	25	25
Wheat bran	-	18	10	10	18	10	10	18	10	10
Rice polishing	-	18	18	18	18	18	18	18	18	18
Wheat straw	-	12.5	9.75	8	12.5	9.75	8	12.5	9.75	8
Cotton seed meal	-	7	12.5	12.5	7	12.5	12.5	7	12.5	12.5
Canola meal	-	7	12.5	12.5	7	12.5	12.5	7	12.5	12.5
Molasses	-	7	6.1	6.25	7	6.1	6.25	7	6.1	6.25
Urea	-	0	0.15	1.625	0	0.15	1.625	0	0.15	1.625
Vegetable oil	-	1.5	2	2.125	1.5	2	2.125	1.5	2	2.125
NaCl	-	1	1	1	1	1	1	1	1	1
DCP	-	2	2	2	2	2	2	2	2	2
$NaHCo_3$	-	1	1	1	1	1	1	1	1	1
Probiotics (Yea sac) %	-	-	-	-	-	-	-	0.1	0.1	0.1
Ionophores (Monensin) ppm	-	-	-	-	20	20	20	-	-	-
Total	100	100	100	100	100	100	100	100	100	100
Chemical composition, %										
Dry matter	89.8	89.8	89.9	90	89.79	89.9	90	89.79	89.9	90
Crude protein	18.9	12	16	20	12	16	20	12	16	20
Total dig. Nutrients	60.6	70	70	70	70	70	70	70	70	70
Neutral detergent fibre	51.1	29.9	28.5	27.3	29.92	28.5	27.3	29.92	28.5	27.3
Acid digestible nutrients	41.1	16.4	15.9	15.1	16.4	15.9	15.1	16.4	15.9	15.01

[1]FOD, LP, MP, HP, LPI, HPI, HPI, LPP, MPP and HPP stand for fodder (berseem hay), low protein, medium protein, high protein, low protein ionophores, medium protein ionophores, high protein ionophores, low protein Probiotics, medium protein Probiotics and high protein Probiotics, respectively.

Results and Discussion

Nutrients Intake

Higher DM intake was observed by goats fed LP, MP and HP diets than those fed FOD diet (Table 2). The DMI increased linearly in goats fed diets with gradual increase in dietary CP concentration supplemented with probiotics. Linear trend for CP intake was observed with increasing the dietary CP concentrate in goats fed diets containing ionophores and probiotics supplementation. The ADF intake was higher by goats fed FOD diet than those fed LP, MP and HP diets regardless

of ionophores and probiotics supplementation. Higher nutrients intake by goats fed gradual increasing dietary CP concentration than those fed FOD diet was due to lower NDF content of these diets because high dietary NDF generally reduced nutrient intake by imparting rumen fill effect (Sarwar *et al*., 1991).Higher DMI by goats fed diets with gradual increase in dietary CP concentration than those fed FOD diet was consistent with the findings of Damry *et al*. (2001) who reported higher DM intake in animals fed concentrate diets than those fed fodder. The increased dietary CP concentration might have increased the amount of available nutrients, required for microbial growth (DelCurto *et al*., 1990).

Nutrients digestibility and nitrogen balance

The DM digestibility was higher in goats fed LP, MP and HP diets than those fed FOD diet (Table 2). The CP digestibility was also higher in goats fed LP, MP and HP diets than those fed FOD diet. The N balance was significantly higher in goats fed HPI and HPP diets than those fed FOD diet (Table 2). Higher nutrient digestibility by goats fed gradual increasing CP concentration than those fed FOD diet might be attributed to better availability of energy and protein nutrients. Synchronized availability of sufficient N and keto-acids (carbon skeleton) at ruminal level might have improved rumen microbial fermentation leading to better digestion of nutrients in goats fed varying CP concentration than those fed FOD diet. Higher, CP, NDF and ADF digestibilities in lambs fed HP, HPI and HPP diets might be due to more digestible CP, NDF and ADF contents of these diets. Higher N balance by goats fed gradual increasing dietary CP concentration than those fed FOD diet was due to higher N intake and its degradation. Higher N balance in HP, HPI and HPI diets than those fed FOD diet in the present study was consistent with the observations of Dabiri and Thonney (2004) who reported increased N balance in ruminants fed concentrate based diet than those fed fodder. Atti *et al*. (2004) noticed increased N retention in ruminants fed diets with gradual increasing dietary CP concentration.

Blood Metabolites

Goats fed HP, HPI and HPP diets had higher T4 level than those fed LP, LPI and LPP diets (Table 3). An increasing tendency in blood glucose was observed in goats fed LP, MP and HP diets. The BUN was significantly lower in goats fed FOD diet than those fed diets with varying dietary CP concentration with or without ionophores and probiotics. Higher T4 concentration in goats fed diet with increasing dietary CP concentration was supported by Todini *et al.* (2007) who observed that lambs fed high nutrient diet had higher plasma T4 concentration than those fed low nutrient diets.Unaltered blood glucose levels in diets with increasing CP concentration with or without ionophores and probiotics were inconsistent with Anthony *et al*. (1986). Furthermore, in the present study, non-significant difference in blood glucose is consistent with findings of small ruminants fed yeast supplemented

Table 2. Nutrients intake, digestibilities and nitrogen balance by goats as influenced by fodder versus intensifying dietary crude protein with or without ionophores and probiotics

Items	Diets[1]										SE	Main Contrasts[2]				Protein	
	FOD	LP	MP	HP	LPI	MPI	HPI	LPP	MPP	HPP		F vs C	CI vs CP	C vs CI	C vs CP	L	Q
Nutrients intake , g/d																	
Dry matter	706.35	892.06	890.48	965.08	906.35	912.70	912.70	906.35	928.57	987.30	25.19	*	NS	NS	NS	*	NS
Crude protein	133.43	107.05	142.48	193.02	108.76	146.03	182.54	108.76	148.57	197.46	3.66	*	NS	NS	NS	*	NS
Neutral Detergent fibre	360.24	266.9	253.79	263.47	271.18	260.12	249.17	271.18	264.64	269.53	7.60	*	NS	NS	NS	NS	NS
Acid Detergent fibre	289.60	146.57	141.32	145.24	148.91	144.85	137.36	148.91	147.36	148.59	4.4	*	NS	NS	NS	NS	NS
Nutrients digestibility																	
Dry matter ,%	49.57	58.00	65.29	64.43	61.14	66.57	69.57	63.29	66.00	68.57	2.77	*	NS	NS	NS	NS	NS
Crude protein,%	68.71	71.15	72.61	73.55	71.51	72.11	73.65	71.59	72.66	73.98	1.69	*	NS	NS	NS	NS	NS
Neutral Det. fibre,%	39.43	42.71	43.57	47.43	46.43	46.14	48.29	47.43	48.29	49.86	3.24	NS	NS	NS	NS	NS	NS
Acid det. fibre,%	33.29	37.71	38.71	36.00	41.00	38.29	35.97	41.86	41.29	40.43	2.27	NS	NS	NS	*	NS	NS
Nitrogen balance, g/d	11.56	10.18	13.6	17.53	10	13.71	18.41	9.97	13.8	18.52	1.85	*	NS	NS	NS	*	NS

LP, MP and HP represent low (12%), medium (16%) and high (20%) dietary crude protein (DCP) concentrations, respectively. Affixes of "I" and "P" in remaining diets indicate supplementation of ionophores and Probiotics with similar low, medium and high DCP contents, respectively. Diet FOD reflects berseem hay. [2] F VS C= Fodder versus Concentrate (LP, MP, HP), CI VS CP= concentrate ionophores (LPI, MPI, HPI) versus Concentrate Probiotics (LPP, MPP, HPP), C VS CI= Concentrate verses concentrate ionophores, C VS CP= Concentrate verses concentrate Probiotics, L= linear, Q= Quadratic, * ($P<0.05$), NS=Non significant

Table 3. Blood metabolites and growth in goats as influenced by fodder versus intensifying dietary crude protein with or without ionophores and probiotics

Items	Diets[1]										SE	Main Contrasts[2]				Protein	
	FOD	LP	MP	HP	LPI	MPI	HPI	LPP	MPP	HPP		F vs C	CI vs CP	C vs CI	C vs CP	L	Q
T3 (n mol/L)	1.01	1.16	1.16	1.51	1.20	1.36	1.14	1.19	1.30	1.47	0.20	NS	NS	NS	NS	NS	NS
T4 (n mol/L)	54.57	57.57	62.00	63.86	57.14	56.00	67.00	59.86	60.14	65.71	4.94	NS	NS	NS	NS	*	NS
Blood glucose (mg/dl)	50.57	60.57	62.14	66.71	67.57	69.43	68.86	59.57	67.57	67.00	4.75	NS	NS	NS	NS	NS	NS
Blood Urea N, mg/dl)	12.57	14.29	16.71	20.43	16.14	17.43	22.14	15.29	15.14	22.71	1.94	*	NS	NS	NS	*	NS
Daily gain (g)	75	149	159	175	153	169	161	162	131	150	18.88	*	NS	NS	NS	NS	NS
FCR	11.04	6.41	5.94	6.69	6.64	5.83	6.32	5.90	8.68	7.65	1.27	*	NS	NS	NS	NS	NS
Economics[3]	1.79	1.0	1.07	1.23	1.04	1.05	1.16	0.94	1.56	1.40	0.22	*	NS	NS	NS	NS	NS

[1]LP, MP and HP represent low (12%), medium (16%) and high (20%) dietary crude protein (DCP) concentrations, respectively. Affixes of "I" and "P" in remaining diets indicate supplementation of ionophores and Probiotics with similar low, medium and high DCP contents, respectively. Diet FOD reflects berseem hay. [2] F VS C= Fodder versus Concentrate (LP, MP, HP), CI VS CP= concentrate ionophores (LPI, MPI, HPI) versus Concentrate Probiotics (LPP, MPP, HPP), C VS CI= Concentrate verses concentrate ionophores, C VS CP= Concentrate verses concentrate Probiotics, L=linear, Q=Quadratic, * ($P<0.05$), NS=Non significant

[3]Cost of feed to produce 1Kg live weight (U$)

(Galip *et al.,* 2006) and ionophores (Yang *et al.,*2003). Increasing tendency of BUN concentration with increasing the dietary CP concentration has also been supported by other workers (Hristove *et al*., 2004; Castillo *et al*., 2001; Armentano *et al*., 1993; Jia *et al*., 1995). Blod urea nitrogen BUN concentration with increase in dietary CP is generally influenced by multiple factors like dietary concentrate, dietary ruminally degradable protein, range of dietary CP, adaptation period and age of ruminant animals etc (Castillo *et al*., 2001; Armentano *et al*., 1993; Jia *et al*., 1995). Similar findings were reported by Yang *et al*. (2003) who noticed that ionophores supplemented diets had no effect in goats.

Growth Performance and Economics

Goats fed increasing dietary CP concentration gained more weight than those fed FOD diet (Table 3). Gradual increase in weight gain was observed in goats receiving diets with increasing dietary CP concentration. Cost of feed to produce one kg live weight and feed conversion ratio were higher in goats fed FOD diet than those fed any of experimental diets with or without ionophores and probiotics (Table 3).The findings of higher daily weight gain by goats fed diets with gradual increase in dietary CP concentration than those fed FOD diet were consistent with the finding of Murphy *et al*. (1994) who reported higher daily weight gain in small ruminants fed concentrate than those fed fodder diets. Furthermore, Haddad *et al*. (2001) observed higher growth rate in lambs fed diets containing high dietary CP (16 and 18%) than those fed diets containing low dietary CP contents (12 and 14%). Unaltered FCR by goats fed diets with gradual increasing dietary CP concentration were consistent with the finding of Duff *et al*., (1994) who reported that gain to feed ratio remained unaltered in ionophores supplementation. Likewise, Raeth-Knight *et al*. (2007) reported that probiotic supplementation had no effect on feed to gain ratio in dairy animals.

Conclusion

Outcome of the study indicated that feeding growing kids on high input feeding system compared to traditional feeding system increased nutrients intake and utilization, N balance and growth with better profit margin.

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Evaluation of Nutrient Digestibility of Goats Fed on Biofermented Cocoa Pods Using *Phanerochaete chrysosporium* Supplemented by Mangan (Mn) and Calsium (Ca)

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Abstract

An in vivo experiment was conducted to evaluate biofermented cocoa pods to substitute forages for goats. The experiment was carried out using a Randomized Block Design on a total of 15 goats with 5 treatments and 3 blocks. Ration was designed iso-protein (50 g/day) and TDN (359 g/day) using cocoa pod (CP) or napier grass (NG) as forages and concentrate. The treatments consisted of concentrate plus : 80% NG (R1); 60% EG (R2); 30% EG and 30% untreated CP (R3); 30% EG and 30% bio fermented CP supplemented Mn and Ca (R4), and 30% NG and 50% biofermented CP supplemented Mn and Ca (R5). Variables measured were feed consumption, nutrient digestibility and average daily gain. Data were analyzed with analysis of variance and followed by Duncan`s multiple range test. Results showed that ration contained 30% biofermented CP supplemented by 100 ppm Mn and 1 190 ppm Ca, 30% napier grass and 40% concentrate resulted in a better goat performance and more profitable than other rations. Goats receiving such ration had more advantage on dry matter consumption (560.33 g/day/head), dry matter digestibility (70.48%), organic matter digestibility (74.31%), feed conversion (5.50), average daily gain (101.79 g/day/head) and income over feed cost (Rp 1 192.80) compared to the other treatments. It was concluded that cocoa pod biofermented with Phanerochaete chrysosporium supplemented by Mangan (100 ppm Mn) and Calsium (1 190 ppm Ca) is potential to be used as forages for substituting napier grass for feeding goats during growing period.

Keywords: cocoa pod, biofermented, Phanerochaete chrysosporium fungi, nutrient digestibility

Introduction

Cocoa pod is a biggest cocoa by-product. Some researchers utilized cocoa pods as animal feed for cows (Smith *et al.* 1988; Amirroneas 1990; Laconi 1998), sheep (Smith and Adegbola 1985), broiler (Donkoh *et al.* 1991; Sobawima and Longe 1994; Olubamiwa 2002; Tequia 2004), layer (Osei *et al.* 1991) and rabbit (Ridwan *et al.* 1993). Utilizing cocoa pods on ruminant decrease feed digestibility and animal performance (Smith and Adegbola 1985; Smith *et al.* 1988).

Constrains utilizing cocoa pods as animal feed are low protein and high fiber contents. High lignin content and hard structure of cocoa pods can reduce feed consumption, digestibility and animal performance.

Utilizing cocoa pods as feed can improve by pretreatment with *Phanerochaete chrysosporium*. *P. chrysosporium* bioconversion can reduce tightness lignocellulose bond and feed lignin content. Fermentation cocoa pods with *P. chrysosporium* and supplement Ca and Mn for 10 days result better digestibility and cellulose lignin ratio.

Materials and Methods

Main materials used were cocoa pods, manganese sulfat ($MnSO_4 \cdot H_2O$), calsium chloride ($CaCl_2$) and *P. chrysosporium* fungi. *P. chrysosporium* fungi IFO 31249 were obtained from Pusat Penelitian Kimia-LIPI Kawasan PUSPIPTEK Serpong. Fungus were maintained on millet for 10 days.

Diet composed to fulfill nutrient requirement goats with weight 10kg and daily gain 100 g day^{-1}. Nutrient requirement base on 50g day^{-1} total protein and 359g day^{-1} total digestibility nutrient (NRC 1981). Feed dry matter consumption estimated as big as 645g day^{-1}.

Fresh cocoa pods chopped and dried (dry matter $\pm$35%). Dried Cocoa pod was supplemented with 100 ppm Mn dan 1190 ppm Ca and inoculated with 0.9% *P. chrysosporium* (Bonnen *et al.* 1994). Cocoa pod was fermented for 10 days.

Fifty goats with an average live weight of 11.60±1.54 kg were used to compared utilization of cocoa pods in randomized complete design. Treatment did in 3 periods were adaptation period for 30 days, preliminary period for 15 days and data collection for 28 days. Diets for data collection period were ad libitum based on voluntary feed intake on preliminary period. Measured and analyzed variables were feed and nutrient consumption (g kg^{-1} $BW^{0.75}$ day^{-1}), average daily gain (g day^{-1}), nutrient digestibility (total collection method) and feed conversion.

Data on feed intake, growth, feed conversion, feed digestibility were analyzed statistically by analysis of variance (ANOVA) and Duncan's multiple range test was used to separate the means.

Table 1. Composition diet experiment (%)

Feedstuffs	Treatment				
	A	B	C	D	E
Napier grass (NG)	80.0	60.0	30.0	30.0	30.0
Cocoa pod (CP)	0.0	0.0	30.0	0.0	0.0
Fermented cocoa pod	0.0	0.0	0.0	30.0	50.0
Rice bran	9.5	29.5	29.5	29.5	9.5
Corn	2.0	2.0	2.0	2.0	2.0
Coconut Meal	2.0	2.0	2.0	2.0	2.0
Soybean Meal	2.0	2.0	2.0	2.0	2.0
Molasses	3.2	3.2	3.2	3.2	3.2
Salt	0.5	0.5	0.5	0.5	0.5
Feedmix	0.8	0.8	0.8	0.8	0.8
Total	100.0	100.0	100.0	100.0	100.0
Diet Nutrient (% DM)					
Dry matter	84.88	86.31	86.29	86.25	84.78
Crude protein	10.18	11.29	11.32	12.89	12.86
TDN	58.93	62.37	61.59	62.59	59.29

Results and Discussion

Feed Intake

The level of intake and the characteristics of digestion depend closely on the feeding behaviour in goats reared in the goat house (Morand-Fehr 2005). Feed intake can expressed in g day^{-1} (Lallo 1996), g BW^{-1} day^{-1} (Aregheore 2000) or g kg^{-1} $BW^{0.75}$ day^{-1} (Ananda *et al.* 1996; Mandal *et al.* 2005). Dry matter intake (Table 2) were 433.92 - 560.33 g $days^{-1}$ or 60.40 - 78.49 g kg^{-1} $BW^{0.75}$. Some researches had been reported that dry matter intake by goats were 41.5 (Kondo *et al.* 2004), 46.0 (Ananda *et al.* 1996), 57.1 (Santra *et al.* 1998) and 61.8 g kg^{-1} $BW^{0.75}$ day^{-1} (Aregheore 2006). The variation of feed intake caused nutrient content, particularly feed protein and energy (Lallo 1996), sex (Aregheore 1995) and feed composition (Aregheore 2006). Dry matter intake increased with increasing feed protein. However, no significant ($P>0.05$) differences were obtained among treatments. Lallo (1996) reported dry matter intake by goats increased in step increasing feed protein content.

Feed Digestibility

Dry matter digestibility range from 55.36 to 70.48% (Table 3). Tuah *et al.*

Table 2. Average nutrient consumption (g day^{-1})

Nutrient	Treatment				
	A	B	C	D	E
Dry matter	433.92^{a}±8.40	499.29^{a}±59.05	537.93^{a}±24.04	560.33^{a}±57.07	549.83^{a}±21.15
Organic matter	368.27^{b}±7.13	427.69^{ab}±50.59	467.41^{ab}±20.89	492.31^{a}±50.14	481.82^{a}±16.16
Crude protein	45.17^{b}±0.87	58.92^{ab}±6.97	63.48^{a}±2.84	71.55^{a}±7.29	66.09^{a}±14.56

Means within each treatment for each variable of different superscript differ (P<0.05).
A: 80% NG + 20% concentrate; B: 60% NG + 40% concentrate; C: 30% NG + 30% CP + 40% concentrate; D: 30% NG + 30% Fermented CP + 40% concentrate; C: 30% NG + 50% Fermented CP + 20% concentrate.

Table 3. Nutrient digestibility (%) experimental diet

Nutrien	Treatment				
	A	B	C	D	E
Dry matter	55.36^{b}±7.01	66.99^{a}±5.82	64.13^{a}±6.21	70.48^{a}±2.47	68.32^{a}±2.80
Organik matter	59.25^{b}±6.74	70.27^{a}±5.24	68.37^{a}±5.66	74.31^{a}±2.17	71.28a±2.55

Means within each treatment for each variable of different superscript differ (P<0.05).
A: 80% NG + 20% concentrate; B: 60% NG + 40% concentrate; C: 30% NG + 30% CP + 40% concentrate; D: 30% NG + 30% Fermented CP + 40% concentrate; C: 30% NG + 50% Fermented CP + 20% concentrate.

(1995) reported that dry matter digestibility of cocoa pods on sheep range from 44.67 to 59.02%, tend to decrease with increasing level of cocoa pods in the diet. The difference was caused by goat capability in digest fiber fraction compare sheep.

There were difference (P<0.05) ini dry matter digestibility of goats fed napier grass. The digestibility of dry matter of diets of B, C, D and E were not different (P>0.05). Diet contain 30% fermented cocoa pod had highest digestibility value, about 70.48%. Diet D contain higher crude protein but lower fiber content. Tuah et al. (1995) reported that digestibility of cocoa pods with level 30% in sheep diet as big as 49.37%. This difference is caused by change of characteristic of fermented cocoa pods and ability of livestock in digesting fibrous feed. Fermentation of cocoa pods with *P. chrysosporium* and addition of Ca and of Mn can decrease lignin content and improve crude protein. Degradation of lignin content give to access to microbe for the degrade of cellulose, hemiselulosa and other feed component.

Average Daily Gain

Average daily gain of goat was 81.19 g day^{-1} (Table 4) lower than target was 100 g day^{-1}. Lower feed protein and energy cause daily gain was low. Protein requirement for goat with body weight 13.47kg and daily gain 100 g was 85.99 g day^{-1}. Estimated dry matter, protein and TDN requirement are in Table 5.

Diet D contain 30% napier grass, 30% fermented cocoa pods and 40% concentrate give highest daily gain was 101.79 g. This result better than *et al.* (1995) that

Table 4. Initial body weight, daily gain, and feed efficiency

Nutrient	Treatment				
	A	B	C	D	E
Initial body weight (kg)	13.88±0.58	12.72±1.14	13.50±0.91	13.73±1.06	13.52±3.48
Final body weight (kg)	15.53±0.67	14.88±1.29	15.85±0.95	16.58±1.11	15.87±3.57
Daily gain (g)	58.95^{d}±3.09	77.38^{c}±5.46	83.93^{b}±1.79	101.79^{a}±1.79	83.93^{b}±3.09
Feed conversion	7.38^{a}±0.51	6.45^{ab}±0.63	6.42^{ab}±0.41	5.50^{b}±0.47	6.52^{ab}±1.20

Means within each treatment for each variable of different superscript differ (P<0.05).
A: 80% NG + 20% concentrate; B: 60% NG + 40% concentrate; C: 30% NG + 30% CP + 40% concentrate; D: 30% NG + 30% Fermented CP + 40% concentrate; C: 30% NG + 50% Fermented CP + 20% concentrate.

Table 5. Estimation of requirement of dry matter, protein and TDN for daily gain 100 g

Requirement	Treatment				
	A	B	C	D	E
Dry matter (g day^{-1})[a]	748.73	716.07	738.00	744.53	738.47
Protein					
Metabolic body weight (kg $BB^{0.75}$)	7.19	6.73	7.04	7.13	7.02
CPm (g day^{-1})[b]	41.92	39.24	41.04	41.57	40.93
CPg (g day^{-1})[b]	45.00	45.00	45.00	45.00	45.00
Total protein (g day^{-1})	86.92	84.24	86.04	86.57	85.93
Protein in diet (%)	11.61	11.76	11.66	11.63	11.64
Energy					
TDNm (g day^{-1})[b]	216.42	202.57	211.90	214.61	211.30
TDNg (g day^{-1})[b]	161.00	161.00	161.00	161.00	161.00
Kebutuhan TDN (g day^{-1})	377.42	363.57	372.90	375.61	372.30
TDN in diet (%)	50.41	50.77	50.52	50.45	50.42

CPm= Protein for maintainance (5.83 g kg-1 BB0.75); CPg= protein for gain (0.45 g for 1 g body gain); TDNm: TDN for maintainance (30.1 g kg-1 BB0.75); TDNg: TDN growth (1.61 g for 1 g body gain).
[a] 2.8 (%) x BW (kg) + 0.36 kg (NRC 1981); [b] Mandal *et al.* (2005).

obtain 55 g on 30% cocoa pods in diets. Utilizing fermented cocoa pods (D) give better result than nonfermented cocoa pod (C) and napier grass (A). However, these feed contain same proportion (30%) in the diet resulted different daily gain, 101.79, 83.93 dan 77.38 g for diet D, C and A, respectively.

Feed conversion influence body gain. Smaller feed conversion value indicated better feed quality. Feed conversion on this experiment range from 50 to 7.38. This result better than cocoa pod conversio on sheep was 12.23-17.74 (Lallo 1995). Feed conversion of diet A significantly ($P<0.05$) bigger than others.Diet contain 30% fermented cocoa pods result lowest feed conversion was 5.50. Lower feed conversion and higher body gain influence economical value of feed. Analysis of feed cost on benefit did calculated *Income Over Feed Cost* (IOFC). Better daily gain on diet that contain fermented cocoa pod menghasilkan highest income (Rp 1 192.80) than others.

Conclusion

Utilization 30% fermented cocoa pods that combination with 40% concentrate and 30% napier grass result dry matter consumption (560.33 g day^{-1}), dry matter digestibility (70.48%), organic matter digestibility (74.31%), feed conversion (5.50), daily gain (101.79 g day^{-1}) *income over feed cost* (Rp 1 192.80) is better and more profitable than napier grass and non fermented cocoa pods.

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Nutritive Values of Forages Evaluated Using a Mixed Bacteria Isolated From the Rumen Liquor of Buffalo

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Abstract

Fresh rumen liquor (FRL) is commonly used in in vitro studies to evaluate the nutritive values of feed for ruminants. However, the type and activity of microbes in the FRL vary with feed and time. To reduce the variation of results, a mixed bacterial isolates (MBI) may be used as an alternative to repalce the FRL. This study was aimed at evaluating the nutritive value of forages using MBI and FRL. Sampels of Penisetum sp., Panicum sp., Brachiaria sp., Setaria sp., Paspalum sp., Calliandra sp., Leucaena sp., Indigofera sp. and Gliricidia sp. were fermented in vitro using either MBI or FRL for 3 and 48 hours to determine NH_3, total volatile fatty acids (VFA), dry matter (DMD) and organic matter digestibility (OMD) based on two steps digestibility method. Paired t-Test was applied to compare the results of using MBI and FRL. Dry matter and organic matter digestibility of the forages were lower when they were determined using MBI than those determained using FRL. The forages DMD and OMD determined using MBI and FRL had significant correlation. Digestibility of dry matter and organic matter were affected by fiber and protein content of the forages. The results sugested that to determine the nutritive values of the forages, in in vitro studies, the MBI could be used to replace FRL with an adjusment to the result.

Keywords: buffalo, forage, isolation, liquor, mixed bacteria

Introduction

Fresh rumen liquor is commonly used in *in vitro* studies to evaluate the nutritive values of feed for ruminants (Dhanoa *et al*., 2004). However the type and activity of microbes in the rumen liquor varies with feed and time after feeding (Yanhong Chen *et al*. 2011). This study aimed at evaluating the posibility of using a mixed bacteria isolated from rumen buffalo to determine the nutritive value of forages.

Materials and Methods

Fresh rumen liquor (FRL) of cattle obtained from a slaugther house and a mixed of six types of bacteria isolated from buffalo rumen (MBI) were used as treatments of microbial sources in an *in vitro* study. Sampel of *Penisetum sp., Panicum sp., Brachiaria sp., Setaria sp., Paspalum sp., Calliandra sp.*, *Leucaena sp.*, *Indigofera sp. and Gliricidia sp.* were fermented *in vitro* for 3 hours to determine NH_3 and VFA, and for 48 hours to determine dry matter and organic matter digestibility based on two steps disgetibility method (Tilley and Terry, 1963) using either FRL or MBI. Proximate analysis was applied to determine the nutrient content of forages. Concentration of NH_3 and VFA in the fermentation media were determined accoding to microdifusion Conway method and steam distillation method, respectively. Paired t-Test was applied to compare the different effect of two treatments. Correlation between nutrient content of forages on their fermentative characteristic was evaluated (Steel and Torrie, 1980).

Results and Discussion

Nutrient content of forages are shown in Table 1. Forages varied in their nutritive content. Ash and crude fiber content of grasses were higher than those of leguminous forages, but the leguminous had higher protein content. Crude fiber is the

Table 1. Nutrient Content of Various Grass and Leguminous Forages Used in an *In Vitro* Digestibility Study Using Fresh Rumen Liquor (FRL) and Mixed Bacterial Isolates (MBI).

Forages	Dry Matter	Ash	Crude Protein	Crude Fiber	Ether Extract	NFE
Penisetum purpureum	26.58	7.37	9.43	34.10	2.07	47.03
Panicum maximum	23.67	9.69	9.71	39.58	0.95	40.07
Brachiaria humidicola	23.73	4.96	9.24	38.88	1.47	45.45
Setaria splendida	10.42	9.25	14.48	44.89	1.78	29.60
Paspalum notatum	25.84	6.42	9.96	36.52	2.14	44.96
Leucaena leucocephala	27.05	0.32	24.29	27.19	3.32	44.88
Calliandra calothyrsus	28.42	0.22	19.53	27.48	1.52	51.25
Indigofera sp.	25.56	0.31	25.87	18.72	3.79	51.31
Gliricidia sepium	18.26	0.34	22.11	22.02	2.25	53.28
Mean	23.28	4.32	16.07	32.15	2.14	45.31
Standard deviation	5.64	4.06	6.92	8.77	0.90	7.18

most limiting factor determining the digestibility of dry and organic matter of feeds. Crude fiber of the forages varied from 18.72% to 44.89% indicating that coefficient digestibility might represent the ability of bacteria in both the mixed bacterial isolates and fresh rumen liquor in degrading the fiber component of the forages.

Table 2. Coieficient Digestibility of Dry and Organic Matter of Grasses and Leguminous Forages in an *In Vitro* Study Using Fresh Rumen Liquor (FRL) and Mixed Bacterial Isolates (MBI)

Forages	% CDDM		% CDOM	
	FRL	MBI	FRL	MBI
Penisetum purpureum	47.64	19.37	46.19	16.66
Panicum maximum	37.09	20.20	34.33	15.72
Brachiaria humidicola	38.14	21.60	36.81	21.41
Setaria splendid	42.09	22.56	39.70	18.78
Paspalum notatum	33.93	20.19	32.01	17.71
Leucaena leucocephala	54.28	53.13	50.58	49.23
Calliandra calothyrsus	37.24	33.87	35.12	30.36
Indigofera sp.	67.18	67.47	65.05	63.55
Gliricidia sepium	56.19	46.54	51.16	40.23
Mean	45.98^{a}	33.88^{b}	43.44^{A}	30.41^{B}
Standard Deviation	11.19	17.76	10.76	17.07

Note: Means with different superscripts differ ($P<0.01$)

Concentration of $N\text{-}NH_3$ and VFA in the filtrate of fermentation media contained grasses and leguminous forages are indicated in Tabel 3. MBI as a source of bacteria produced more $N\text{-}NH_3$ than FRL did. High concentration of $N\text{-}NH_3$ in MBI treatment indicated that bacteria in the media had less ability to convert the $N\text{-}NH_3$ into microbial protein.

Coefficient digestibility of DM and OM of forages determined using MBI and FRL had significant correlation (Table 4). Bacteria from MBI and FRL degraded the forages components in the different extent, resulted in different values of the coefficient digestibility of DM and OM. Dry and organic matter digestibility in MBI treatment was reduced by increasing content of $N\text{-}NH_3$ in the media. Coefficient digestibility of DM and OM were reduced by the increase of ash and crude fiber of the forages, but they were stimulated by the crude protein and ether extract of the forages. Ash content migh represent undegradable lignin in fiber component.

Table 3. Concentration of NH_3 and VFA in the Filtrate of Fermentation Media Composed of Grasses and Leguminous Forages in an *In Vitro* Study Using Fresh Rumen Liquor (FRL) and Mixed Bacterial Isolates (MBI)

Foarges	N-NH_3(mM)		VFA (mM)	
	FRL	MBI	FRL	MBI
Penisetum purpureum	4.43	23.33	168.36	148.25
Panicum maximum	5.94	21.48	185.29	146.52
Brachiaria humidicola	3.69	22.82	214.77	178.86
Setaria splendid	10.40	24.02	165.35	150.46
Paspalum notatum	3.96	21.40	102.04	245.41
Leucaena leucocephala	6.63	11.60	167.00	177.00
Calliandra calothyrsus	4.56	9.92	199.00	152.00
Indigofera sp.	9.84	13.29	190.00	174.00
Gliricidia sepium	8.82	12.38	216.00	201.00
Mean	6.47^b	17.80^a	178.65	174.83
Standard Deviation	2.61	5.82	34.59	32.23

Note: Means with different superscripts differ (P<0.01)

Table 4. Correlations Between the Coefficient Digestibility (CD) of Dry Matter (DM), Organic Matter (OM), N-NH_3 Concentration in Fermentation Media, Ash. Crude Protein (CP), Crude Fiber (CF), and Ether Extract (EE) Content of Forages

	CD-DM-FRL	CD-DM-MBI	CD-OM-FRL	CD-OM-MBI
CD-DM-MBI	0.883 (0.002)			
CD-OM-FRL	0.995 (0.000)	0.868 (0.002)		
CD-OM-MBI	0.874 (0.002)	0.996 (0.000)	0.865 (0.003)	
N-NH_3-FRL	0.648 (0.059)	0.558 (0.118)	0.622 (0.074)	0.519 (0.152)
N-NH_3-MBI	-0.506 (0.164)	-0.787 (0.012)	-0.467 (0.205)	-0.770 (0.015)
Ash	-0.592 (0.093)	-0.818 (0.007)	-0.569 (0.110)	-0.825 (0.006)
CP	0.785 (0.012)	0.949 (0.000)	0.754 (0.019)	0.932 (0.000)
CF	-0.772 (0.015)	-0.876 (0.002)	-0.755 (0.019)	-0.867 (0.002)
EE	0.847 (0.004)	0.851 (0.004)	0.857 (0.003)	0.866 (0.003)

Note: Pearson correlation (P-Value). Fresh Rumen Liquor (FRL). Mixed Bacterial Isolates (MBI).

Conclusion

Dry matter and oragnic matter digestibility determined using MBI indicated the nutritive value of the forages. Threfore, MBI was possible to replace FRL in *in vitro* studies to evaluate the nutritive values of forages.

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Greenhouses Gases Emissions from Dairy Cattle in Indonesia

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Abstract

Dairy cow population in Indonesia has currently reached 487,715 head and located mainly on Java Island, (East Central and West Java Provinces). Indonesian dairy cows contributed 3.8% to the Indonesian total greenhouse gas production, while in global level, dairy sector contributed about 4.0% to the total global anthropogenic GHG emissions. This study assesses the greenhouse gases (GHG's) emissions from dairy cattle sector in Indonesia. The objective of this study was to compare GHG's from Indonesian dairy cows using the IPCC (2006) guideline Tier 1 and Tier 2 method. The data was obtained from field observations in KUNAK dairy cattle village, Bogor as well as previous studies. Cow's body weight, milk production, dry matter intake and dry matter digestibility of feed data were used in the model. Manure management system in dairy cattle was also observed. The results showed that total methane emissions calculated using Tier 1 was relatively higher than that calculated using Tier 2. Methane emission from the enteric fermentation and manure management of dairy cows calculated by Tier 1 method were 33.16 and 15.12 Gg year^{-1} respectively, while by Tier 2 method were 25.74 and 0.12 Gg year^{-1} respectively. Direct and indirect N_2O (nitrous oxide) emissions from manure were 256,381, and 2,299 kg year^{-1} respectively. it is concluded that the total greenhouse gas emission from dairy cattle sector in Indonesia were 1,187 (Tier 1) or 671 (Tier 2) Gg CO_2-eq.

Keywords: dairy cattle, greenhouse gases, methane, N_2O emission

Introduction

Dairy farm in Indonesia has an important role in supplying the milk demand. Current dairy cow population reached 487.715 head and produce approximately 827,000 tones of milk production per year or 26.5% of national demand which reached 2.7 million liters per year. Dairy cow population is mainly located on Java island.

Based on Thalib *et al* (2008) report, dairy cow in Indonesia contribute as much as 3.6% of greenhouse gases compared to the total livestock population. This figure is lower compared with the average contribution of the world which reached 4% globally.

Inventory of greenhouse gas emissions (GHG's) for the livestock sector is highly dependent on the determination of emission factor on each animal. IPCC (2006) has provided guidance in calculating the greenhouse gas in the various sectors. However, emission factors in each sector should be developed in each country.

Based on IPCC (2006) methane emission factor for dairy cattle South East Asia region was 68 kg/head/year. The emission factor is relatively high compared with rill conditions. Therefore it is necessary for the calculation of emission factors based on the real conditions in the field.

The objective of this study was to compare greenhouse gases emissions from Indonesian dairy cows using calculated the IPCC (2006) guideline Tier 1 and Tier 2 methods.

Materials and Methods

Data Collection

The study used primary and secondary data. The primary data was obtained from field observations which have done in dairy cattle production area (KUNAK Cibungbulang), Bogor. The data included cow's body weight, milk production, dry matter intake and dry matter digestibility of ration as well as manure management system in dairy cattle was also observed. The dairy population was cited from Directorate General of Livestock and Veterinary (2011) as secondary data.

Methodology

The calculation of methane (CH_4) and nitrous oxide (N_2O) emissions were calculated based on IPCC Guidelines (2006). The methane emissions were calculated based on Tier 1 (default) and Tier 2 (survey based modified) models. The IPCC (2006) defined that the methane conversion factor (Ym) was 6.5 $\pm$ 1%, indicating that Ym is at the high end of the range when digestibility of feed is low and *vice versa*. Considering the wide range in feed digestibility all over the world we incorporated a range of Ym values according to the following formula: Ym = 9.75 – 0.05 x digestibility. In Model 2, Ym is then used in the following formula: CH_4 emission = (annual feed intake * Ym/100) * (18.55/55.65).

Nitrous oxide (N_2O) emissions (direct and indirect emission) were calculated according IPCC Guideline (2006). Conversion CH_4 to CO_2 was 23, while conversion N_2O to CO_2 was 296 (IPCC 2001). The data were analyzed using statistical descriptive.

Results and Discussion

Dairy Cattle Population

Base on the Table 1, dairy cow population in Indonesia was mainly located in Java Island. Province which has the largest dairy population was East Java Province, which was about 47.4% of the total population, followed by Central Java (25.2%) and West Java (24.7%). The population in other provinces are relatively small, less than 1%.

Table 1. Dairy population in Indonesia 2010

Province	Population (head)	%
East Java	231,408	47.4
Central Java	122,489	25.1
West Java	120,475	24.7
DI Yogyakarta	3,466	0.7
DKI Jakarta	2,728	0.6
North Sumatera	2,642	0.5
South Sulawesi	2,198	0.5
West Sumatera	857	0.2
Other Provinces	1,452	0.3
Total	487,715	

Source: Directorate General of Livestock and Veterinary (2011)

Methane Emission

According to IPCC default (Tier 1), methane emission factor from enteric fermentation of dairy cattle in South Asia region is 68 kg/head/day. Based on the survey results the average of cow body weight was 390 kg and dry matter intake was 11.9 kg/head/day, therefore the methane emission factor was 53 kg/head/day. This methane emission factor was smaller than the IPCC default (Tier 1).

Table 2 showed that methane emissions from enteric fermentation and manure management calculated using Tier 1 model were 33.165 and 15.119 Gg/year respectively, whereas, the calculation of the respective emission using Tier 2 model were 25.738 and 0.120 Gg/year. Methane emission calculation using Tier 2 was lower than Tier 1 model. Enteric fermentation contributed 68.7% to total methane emission from dairy cattle sector.

Methane emissions from livestock sector depend on the number of livestock population. Compared to other livestock, dairy cows contributed only 3.6% to total methane emission. Beef cattle were the major contributor (53.8%) followed

Table 2. Methane emission from enteric fermentation and manure management calculated by Tier 1 and Tier 2

Province	Tier 1			Tier 2		
	Enteric fermentation (Gg/year)	Manure management (Gg/year)	Total (Gg/year)	Enteric fermentation (Gg/year)	Manure management* (Gg/year)	Total (Gg/ year)
East Java	15.74	7.17	22.91	12.21	0.06	12.27
Central Java	8.34	3.80	12.13	6.46	0.03	6.49
West Java	8.19	3.74	11.93	6.36	0.03	6.39
DI Yogyakarta	0.24	0.11	0.34	0.18	0.00	0.18
DKI Jakarta	0.19	0.09	0.27	0.14	0.00	0.15
North Sumatera	0.18	0.08	0.26	0.14	0.00	0.14
South Sulawesi	0.15	0.07	0.22	0.12	0.00	0.12
West Sumatera	0.06	0.03	0.09	0.05	0.00	0.05
Other Provinces	0.10	0.05	0.14	0.08	0.00	0.08
Total	33.17	15.12	48.28	25.74	0.12	25.89

* Calculated using Qurimanasari (2011).

Table 3. Nitrous oxide emission from enteric fermentation and manure management calculated by Tier 1 and Tier 2

Province	Direct N_2O emission (kg/year)	Indirect N_2O emission (kg/year)	Total N_2O emission (kg/year)	Total CO2-Eq (Gg/year)
East Java	121,646	1,091	122,737	36.33
Central Java	64,390	577	64,967	19.23
West Java	63,331	568	63,899	18.91
DI Yogyakarta	1,822	16	1,838	0.54
DKI Jakarta	1,434	13	1,447	0.43
North Sumatera	1,389	12	1,401	0.41
South Sulawesi	1,155	10	1,166	0.35
West Sumatera	451	4	455	0.13
Other Provinces	763	7	770	0.23
Total	256,381	2,299	258,680	76.57

by buffaloes (12.6%), goats (10.6%), pigs (7.4%), and sheep (7.0%) (Thalib *et al*., 2008). During 2003-2007 period, Indonesian methane emission ha increased by 1.9% per year.

Table 3 showed that the N_2O emission from dairy cattle in Indonesia. The N_2O emissions mostly come from direct emissions of N_2O. The direct and indirect N_2O

emission were 256,381 and 2,299 kg/year respectively. The total N_2O emission was 258,680 or equivalent to 76.57 Gg CO_2 per year.

Conclusions

Total methane emissions from dairy sector in Indonesia was 48,28 Gg/year (1,111 Gg CO_2 eq) while N_2O emissions were 258,680 kg/year or 76.57 Gg CO_2-eq. Thus the total GHG's emission from dairy sector in Indonesia were 1,187 Gg/year. Methane emissions calculations based on survey results was lower than the IPCC default.

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Managerial and Nutritional Strategies to Minimize Lactational and Reproductive Losses in Heat-Distressed Dairy Cows

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Abstract

Heat stress (HS) is a welfare issue and has detrimental effects on lactation and reproduction. When environmental temperature exceeds 25 °C or when thermal-humidity index exceeds 75, cows exhibit HS signs. Feed intake, milk production, and fertility decrease and immune system is compromised in heat-distressed dairy cows. Managerial approaches cover barn design (stocking density, ventilation, shading, and sprinkling water) and animal's handling and mating (synchronization and timed-artificial insemination), which are intended to cool down the cow. Nutritional approaches are intended to reduce heat production by the body and covers access to unlimited clean and fresh water, feeding at night, increasing feeding frequency, feeding fat, reducing forage percentage and increasing concentrate, K, Na, and Mg percentages in the ration, balancing protein fractions, modifying particle size, formulating alkaline diet, and supplementing cow with buffer and yeast cultures. In conclusion, both managerial and nutritional approaches help maintain body temperature so that lactation and reproduction potential can be achieved during hot weather.

Key words: dairy cow, heat stress, lactation, management-nutrition, reproduction

Introduction

Heat stress (**HS**) is a costly problem in dairy operations because of its deleterious effects on performance, lactation, and reproduction in dairy cows. It is also an important welfare issue. Managerial approaches are intended to cool the cow and nutritional approaches are intended to compensate reduced dry matter intake (**DMI**). Environmental modifications to minimize HS, coupled with an appropriate nutritional program are necessary to maintain intake and minimize lactational and reproductive losses. The purpose of this review is to emphasize managerial and nutritional strategies to reduce adverse effects of ambient environmental temperature on performance and productivity in dairy cows.

Heat Stress

All animals have a thermal comfort zone (**TCZ**), which refers to a range of temperature that allows the animal to exhibit normal physiological status. This range for the dairy cows is between -13 to 25ºC with a body temperature of 38.5-39.3ºC. Environmental temperatures below and above this zone is a significant stressor to the cattle and interfere with expression of their genetic potentials. Temperature-humidity index (**THI**) reflects the combined effects of ambient temperature and relative humidity, which is calculated as follows:
$THI = T_{air} - [0.55 - (0.55 \times RH / 100)] \times (T_{air} - 58.8)$, where T_{air} = air temperature (°F), RH = relative humidity (%) or **THI = 0.72 x (W+D) +40.6, where W = wet bulb temperature (**°C) and D = dry bulb temperature (°C).

The THI values less than 70 are considered comfortable, 71-80 mild and 81-90 moderate stressful, and a value greater than 90 causes extreme distress with cows being unable to maintain thermo regulatory mechanisms or normal body temperatures (Table 1). When temperatures exceed 25°C or when THI exceeds 70, cows experience HS, which is accompanied by reduced dry matter intake (**DMI**), lower milk production, decreased milk fat percentage, decreased fertility, depressed immune system, and reduced activity as well as increased maintenance requirement, body temperature (> 39.3 °C), and risk for mastitis and laminitis. These can vary by 1) severity of the environmental conditions 2) level of milk yield and quantity of feed consumed 3) stage of lactation, 4) size of the cow, 5) cooling management, 6) exercise requirements, and 7) breed and color.

For the cow, there are two sources of heat: the environmental temperature and the heat produced internally from basal nutrient metabolism. Heat produced from nutrient metabolism is lesser factor than environmental heat sources. Solar radiation and elevated ambient air temperature are primary environmental heat sources. Environmental temperature is aggravated by high relative humidity and lacking air movement.

Table 1. Temperature-humidity index for dairy cows

°C	Relative humidity (%)													Stress level
	40	45	50	55	60	65	70	75	80	85	90	95	100	
23.9	No stress					72	72	73	73	74	74	75	75	Mild
26.7	73	3	74	74	75	76	76	77	78	78	79	79	80	Medium
29.4	76	77	78	78	79	80	81	81	82	83	84	84	85	
32.2	79	80	81	82	83	84	85	86	86	87	88	89	90	Severe
35.0	83	84	85	86	87	88	89	90	91	92	93	94	95	
37.8	86	87	88	90	91	92	93	94	95	97	98	99		
40.6	89	91	92	93	95	96	97							

Mechanisms by which the dairy cow dissipates body heat and maintain body temperature include conduction, convection, radiation and evaporation. The first three depend on a relatively large differential between body and environmental temperatures, and the last one depends on relative humidity. When the environmental temperature is close to the body temperature, especially in the presence of high relative humidity, all the cooling mechanisms are compromised.

Adverse Effects of Heat Stress on Lactation and Reproduction

Heat-distressed dairy cows exhibit altered acid-base status resulting from panting (> 80 breaths/min) and sweating, ways of evaporative cooling. Sweating and panting are accounted for two and one-thirds of evaporative water loss, respectively. Increased the loss of CO_2 via respiration reduces the blood concentration of H_2CO_3 and consequently, increases blood pH (*respiratory alkalosis*). Compensation for the respiratory alkalosis involves increased urinary HCO_3^- excretion, leading to a decline in blood HCO_3^- concentration. At 35°C, water loss by sweating is estimated to be 150g/m^2 body surface/h.

Reticulo-rumen motility decreases. Consequent decrease in the rate of digesta passage is associated with lower production of volatile fatty acid, with a high percentage of acetate. While blood flow to digestive tract and other internal tissues decreases, blood flow to the skin surface increases. In general, urine volume increases.

The effect of high environmental temperature on cow performance is mediated through the body temperature. Decreased productivity is linked mostly to depressed DMI (Table 2), which is an attempt to reduce heat production from the digestion and metabolism of nutrients. Intake for Holstein and Jersey cows is negatively correlated with minimum and maximum daily THI (-0.63 and -0.62, Holsteins; -0.62 and -0.55, Jerseys). In NRC prediction model (1981), DMI for a 600-kg cow producing about 27 kg of milk will decline by 8.2% (18.2 kg at 20°C to 16.7 kg at 35 °C) and maintenance requirement will increase by 20% (Table 2). At 40 °C, maintenance increases by 32% and DMI decreases by 56% as compared to cows reared in TCZ. Briefly, a 0.56°C increase in temperature above 38.61°C causes 1.8 and 1.4 kg decreases in milk yield and total digestible nutrients.

Even under TCZ, reproductive performance of dairy cows is low due to increased milk production associated with prolonged and severe negative energy balance (**NEBAL**). The adverse of effects of HS on reproduction is related directly to the increase in body temperature and development of NEBAL. Infertility is one of the most important obstacles for sustainable dairy production in tropical and subtropical countries. Development of NEBAL and existence of catabolic profile (low blood glucose, insulin, insulin-like growth factors, and growth hormone concentrations and high non-esterified fatty acids, β-hydroxybutyrate, and glucagon) lead to silent

and/or short estrus and impair folliculogenesis, which eventually result in reduced conception rate and prolonged calving interval.

Heat stress suppresses synthesis of reproductive hormones, reduces quality of oocytes, and interferes with folliculogenesis. Damaged somatic cells within the follicles interfere with decrease estradiol synthesis that influences expression of estrus through increased secretion of ACTH, ovulation, and the corpus luteum. Heat stress, in general, does not dramatically influence days to first ovulation and estrus, but prolongs days open and increases services per conception (> 2.5) due to early embryonic loss and/or reduced conception rate (< 40%). Also, 1°C increase in rectal temperature is associated with 20-30% reduction in blood flow to uterus. Undernourished uterine tissues and increased uterine temperature (> 40°C, normally 38.6°C) compromise embryo survival (Figure 1). In case of successful pregnancy in heat-distressed dairy cows, the result is a delivery of calves with low birth weight.

Table 2. Effect of environmental temperature (°C) on performance of dairy cows

Temperature	Prediction				
	Maintenance (% of requirement at 10 °C)	DMI needed (kg/d)	DMI (kg/d)	Milk yield (kg/d)	Water intake (l/d)
-20	151	21.3	20.4	20.0	51.1
-10	126	19.8	19.8	25.0	57.9
0	110	18.8	18.8	27.0	64.0
10	100	18.2	18.2	27.0	64.0
20	100	18.2	18.2	27.0	68.1
25	104	18.4	17.7	25.0	73.8
30	111	18.9	16.9	23.0	79.1
35	120	19.4	16.7	18.0	120.0
40	132	20.2	10.2	12.0	106.0

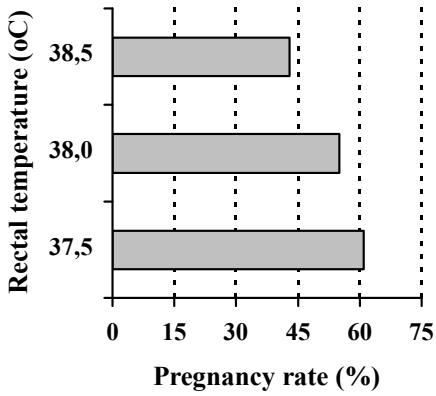

Figure 1. Pregnancy rate of dairy cows that have different rectal temperature at time of breeding

Managerial Strategies

Modifying the environment is the most effective way to reduce HS. Providing shade is the cheapest and best way to decrease radiant energy, but it does not decrease air temperature. Shade with a 2.5 cm-insulation (3.7-4.6 m^2/head) should be installed on 2.1-4.4 m away from the cow. It is shown that total heat load can be reduced by 30-50% with well-designed shade and that there is a 10% gain in milk yield simply by shading cows. As compared with cows in no shade, shaded cows have lower rectal temperature (38.9 and 39.4 ºC) and less respiratory rate (54 and 82 breaths/min), and produce 10% more milk (Table 3).

During periods of high temperature and/or humidity, however, shade alone is rarely enough. Thus, additional evaporative systems, such as ventilating and sprinkling are needed. Using fans and sprinklers improve DMI by 7-9% and milk yield by 8.6-15.8% and reduce rectal temperature by 0.44-0.56 °C and respiration rate by 17.6-40.6%. Sprinkling cows helps reduce body temperatures by increasing evaporative cooling (Figure 2) and ground temperature, as well. Intermittent sprinkling is

Table 3. Effect of shading on performance of dairy cows

Measurement	Shade	No shade
Rectal temperature (°C)	39.2	40.8
Respiration (count/min	83	133
Dry matter intake (kg/d)	20.7	16.8
Milk yield (kg/d)	19.4	17.0

suggested to not to cause humidity, sprinkling of 2-3 min every 30 min.

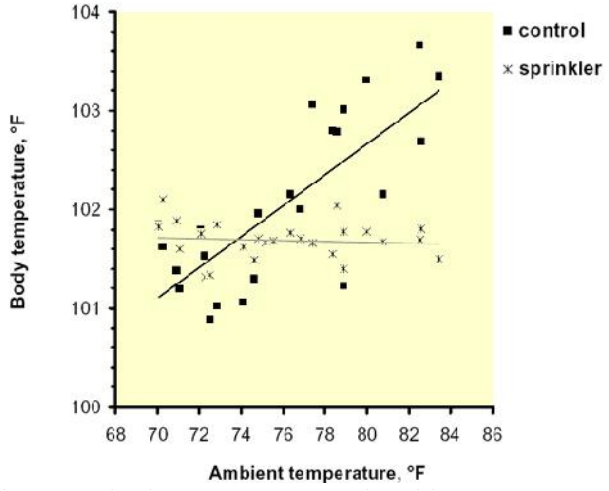

Figure 2. Relationship between body temperature and ambient temperature for cows that are not cooled and are sprinkled for 90 min before afternoon milking

Cows should not be handled in hot weather, especially after 10:00 hr because handling increases the body temperature about 0.5-3.5 °C. If needed, cows should be handled between midnight and 08:00 hr. Holding and processing areas should have shade and sprinklers.

Nutritional strategies to maintain reproductive performance seem less effective than managerial strategies. A number of reports show that cooling and shading increase estrus duration and pregnancy rate and reduce calving interval and percentage of cows with silent estrus by 50%. Thus, before and after artificial insemination, cows must be kept as cool as possible. Through estrus synchronization and timed-artificial insemination, breeding season should be switched to cooler days of the year in order to minimize reproductive losses.

Nutritional Strategies

The need for water increases sharply as the temperature increases (Table 2) because of water loss from sweating and panting. Provision of unlimited quantity of clean, fresh, and cool water to the cow is the most important nutritional strategy. Normally, a cow consumes 2-3 L of water for each kg DMI and an additional 3-5 L of water for each kg of milk yield. As the environmental temperature increases from 4.5 to 26.7 °C, the water consumption increases from 23 to 31 L in dry cows, 60 to 100 L in cows producing 18 kg milk, and 98 to 170 L in cows producing 30 kg milk. Although cooling water to below 15°C helps dissipate body heat, offering water at 20-27°C is recommended during hot weather. Waterers should be placed under shade and on the way to milking parlors.

The heat production of metabolic functions accounts for about 31% of the energy intake in a 600-kg cow producing 36 kg 4% fat-corrected milk. Heat production increases as DMI and milk yield increase. Energy expenditure for maintenance during hot weather increases. Metabolic heat production increases after feeding, at different levels depending upon feedstuffs in ration. Heat increment during metabolism accounts for two-thirds of endogenous heat production. The order of heat increment for nutrients, from the lowest to highest, is fat < concentrate < fiber. When heat production is reduced, less is going to be dissipated and energy utilization efficiency is improved. Thus, during hot weather diets should be formulated to contain fat (5-6%), high quality (digestible) fiber (a bit reduced rate), and concentrate (a bit increased rate). That is, *the ration should be heated up to cool the cow down* in order to maintain intakes of nutrients due to depressed DMI (Table 4).

In order not to cause acidosis, milk fat depression, off-feed conditions, and laminitis, diets should contain more than 55-60% concentrate. The percentages of nonstructural carbohydrates (**NSC**), acid detergent fiber, and neutral detergent fiber should be 33-38% at maximum, 19% at minimum, and 30% at minimum, respectively. Nonstructural carbohydrates can be increased to 40% when the diet contains

Table 4. Dietary modifications during hot weather*

Dry matter intake (% depression)	0	5	10	15	20
Respiration rate (count/min)	< 75	80	85	90	95
Dry matter intake (kg/d)	24.1	22.9	21.7	20.5	19.3
Energy (NEL, Mcal/kg)	1.59	1.70	1.81	1.92	2.00
Mcal/d	38.4	38.9	39.4	39.5	40.4
Crude protein (CP, %)	17.4	18.1	18.9	19.8	20.8
Rumen undegradable protein (% of CP)	39.4	42.7	45.7	48.7	51.7
Neutral detergent fiber (%)	35.0	31.0	28.0	26.0	25.0
Fat (%)	0.0	1.0	2.0	3.0	4.0
Minerals (%)					
Ca	0.46	0.70	0.73	0.78	0.82
P	0.41	0.43	0.45	0.49	0.51
Mg	0.25	0.27	0.28	0.30	0.31
K	1.00	1.15	1.30	1.45	1.60
Na	0.18	0.23	0.28	0.33	0.38
S	0.20	0.21	0.22	0.23	0.24

*Estimated for a 650 kg cow producing 45 kg milk with 3.6% fat and 3.2% protein.

high quality forage. In particle size separator, long (> 1.90 cm), medium (0.79-1.90 cm), and short (< 0.79 cm) particles should constitute 6-10, 30-50, and 60-60%, respectively.

Both deficient and excess crude proteins have detrimental effects on fertility. Both cases also cause an increase in body heat production resulting from reduced digestibility in association with reduced rumen motility and excessively formed NH_3 in association with a more than 60% ruminally degradable fraction, respectively. Excessive NH_3 requires additional energy for hepatic detoxification.

Through sweating cows lose K (human does Na). Thus, K requirements increase during hot weather. Supplementing heat-distressed cows with K (1.5-1.6% DM), Na (0.5-0.6% DM), and Mg (0.35-0.4% DM) is suggested. Providing alkaline diet reduces HCO_3^- loss and compensates K loss, suggesting that dietary cation-anion difference (Na + K - Cl - S) should be positive. Due to lowering fiber/increasing NSC in the ration, supplementing heat-distressed cow with $NaHCO_3$ (150-200 g/day/head or 0.75-1.0% of DM), niacin (6 g/d), yeast cultures, and fungal products (*Aspergillus oryzae*) is recommended. These supplements stabilize rumen fermentation and reduce risk for milk fat depression and other fermentation related disturbances through increasing fiber digestion, volatile fatty acid production, turnover rate of

lactic acid, and numbers of cellulolytic bacteria as well as minimizing variations in rumen pH and ammonia formation.

Effectiveness of excessive supplemental vitamins A and E in heat-distressed dairy cows is controversial. Trace minerals (Zn and Cu) should be increased to minimize oxidative stress and boost immune potency. Other nutritional approaches to alleviate heat stress include 1) provision of total mixed ration, 2) feeding at night and after milking, 3) increasing feeding frequency to provide fresh and cool ration, and 4) adding water to ration.

Conclusion

Heat stress has detrimental effects on lactation and reproduction, which are major income sources in dairy operations. Managerial and nutritional strategies should be applied simultaneously to cool the cow down and reduce body heat production and maintain intakes of nutrients.

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Performance of Friesian Holstein Cross Post Colostrums' Calves Reared Under Free Choice Feeding System

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Abstract

The aim of this experiment was to evaluate the free choice feeding technique on performance of Frisian Holstein Cross POST colostrums calves on early weaning programs. Six FH calves were divided into two feeding systems. Half of them were fed with mix diet and another half were subjected to free choice diet. Mix diet consists of 38.65% corn, 28.98% wheat brand, 28.98% soybean meal and 3.39% mineral mix. The free choice diets consisted of corn, wheat brand, soybean meal, coconut meal and mineral mix served in separate feeding buckets. The data obtained were analyzed using analysis of variance and any significant differences were subjected to T-Test. There were no significant different on dry matter intake, total digestible nutrient, fiber, Ca and P intakes, weight of weaning calves and feed efficiency between the treatment, but the treatment were significantly affected the protein intake, milk intake, weaning time and body weight gain. It was concluded that the free choice diets technique provide nutrients to support a good performance of FH calf on early weaning program.

Key words : free choice diet, Friesian Holstein, performance, rearing

Introduction

Although post colostrums calves rearing program by a large farm who have good records of production history is one of business opportunity to provide good qualities of bulls and cows replacement stocks, but the program is high-risk. This is caused by the fact that raising calves from birth to weaning is one of the most difficult periods of cattle husbandry. The greatest risk in this period is disease and mortality factors. The important strategy to reduce the risk is through feeding management. Management of feeding that appropriate with calf requirement before weaning will affect its production performance later on.

Nowadays, variation program to formulate rations have been used by many feed producers, but the programs do not guarantee the balance between the ration

prices with their economical impact to the farmer. To find a suitable feed formula in the field, free choice feeding technique can be used.

Free choice feeding technique would provide a freedom for livestock to choose. In this case, pre-weaning calf will choose feed using their instinct. Keskein et al. (2004) stated that free choice feeding technique improved animal welfare through nutrients requirement fulfillment. Free choice feeding technique also provided the opportunity for calves to make their own feed formula to support their rumen development and make them comfort (Nicol, 1997).

This study was designed to get the most proper starter ration formula for calves rearing program which could satisfy calves requirements, enhance calves performance and improve efficiency of feed utilization.

Material and Methods

This study used 6 male weaning calves of Frisian Holstein (FH) hybrid with initial body weight of 38.34 ± 2.34 kg. The calves were reared in individual cage. Two feeding systems were employed as treatments, namely 1) the free choice feeding system (FCFS) and 2) the complete mix feeding system (CMFS). In both systems, similar feed ingredients were offered. They were maize, pollard, Soybean meal, coconut meal, salt, $CaCO_3$, premix and fresh milk.

The calves were divided into two groups and eachgroup consisted of three calves (as replications). The first group was subjected to the FCFS treatment, while second group was given the CMFS treatment. The calves were kept in individual cages and observed for 46 days.. The feeds were offered ad libitum every day from 6:00 am to 07:00 pm. Each calf was given 4 litres milk, twice a day, 2 liters in the morning and 2 liters in the afternoon. Drinking water were provided ad libitum.

Results and Discussion

Dry Matter and Nutrient Intake

Average of feed and nutrients intake of both feeding systems was shown in table 1. The data analysis showed that the treatments had no effect on the dry matter intake (DMI). The DMI of CMFS treated calves were 710.12 g/head/day or equal to 1.47% body weight (BW) while DMI of FCFS treated calves were 940.83 g/head/day (2.02% BW)\ These intakes satisfied DMI requirement of calves according NRC (2001) feeding standard. According to NRC (2001), dry matter requirement for 30 - 60 kg BW calf with 0.4 - 0.6 kg average daily gain (ADG) was 560-1040 g/head/day (1.4% -1.7% BW). The FCFS treated calves obtained different ingredient compositions from the CMFS treated calves. Proportion of soybean meal consumed by FCFS treated calves was higher (80%) than consumed by the CMFS (28.98%), while the proportions of corn and pollard consumed by the FCFS treated calves

were lower (15% and 4%, respectively) than the CMFS treated calves (38.65% and 28.98%, respectively).

The calves fed with FCFS had opportunity to choose ingredients with high protein content such as soybean meal to satisfy their protein requirement. The ingredient was consumed much more than corn, pollard, and coconut meal which contain less protein. Although, maize, pollard, and coconut meal contained less protein than soybean meal, their nitrogen free extract (NFE) content was higher. Forbes (1995) stated that high content of NFE in feed lowered its consumption. He also proved that soybean meal had better palatability than other materials. Table 1 showed that CP consumption of FCFS treated calves were significantly higher ($P < 0.05$) than of the CMFS treated calves, but in both systems, consumption of total of digestible nutrients (TDN), crude fiber (CF), Ca, and P were not significantly different. Sutardi (1981) stated that CP requirement of 1–4 months calves with 30-64 kg BW were 120-210 g/head/day, while according to NRC (2001), the CP requirement for calves with 30 - 60 kg BW and 0.4-0.6 kg ADG were 141-217 g/head/day. Ration consumed by the CMFS treated calves satisfied their CP requirement based on Sutardi (1981) and NRC (2001) recommendations. The calves consumed 195.98 g CP/head/day. The FCFS treated calves however, consumed CP more than their requirements (303.84 g/head/day).

TDN content of feed ingredients ranged from 67.9% to 83.2% while TDN of milk was 129%. According to NRC (2001), TDN requirement for calf with 30–60 kg BW and 0.4–0.6 kg ADG was 0.82-1.21 kg. TDN consumptions in this study were 669.29 g/head/day (CMFS treatment) and 742.29 g/head/day (FCFS treatment). These results indicated that protein requirement for livestock kept in tropical region was different from livestock in temperate regions.

Although ruminants have the ability to digest fiber with their microbe's help, but calves do not have such ability because their rumen functions have not fully developed. Therefore, their ability to digest fiber is still low. Boga (2009) showed

Table 1. Average of nutrient consumption of concentrate starter and milk (g/head/day)

Consumption	Treatments					
	CMFS			FCFS		
	Starter	Milk	Total	Starter	Milk	Total
CP	68.31	127.67	195.98 ± 6.42[a]	176.17	127.67	303.84 ± 54.98[b]
TDN	247.08	419.21	669.29 ± 21.78	323.08	419.21	742.29 ± 103.94
CF	24.05	0	24.01 ± 2.26	21.15	0	21.15 ± 6.87
Ca	0.68	4.34	6.96 ± 0.08	1.36	4.34	9.65 ± 0.45
P	2.36	3.53	5.98 ± 0.28	2.75	3.53	6.30 ± 1.03

Different superscript in the same line means significantly different ($P<0.05$)

that calves offered free choice feeding system formulated ration using their instincts which contained high protein but low fiber.

According to Sutardi (1981), Ca and P requirement for calf with weight 30 - 64 kg was 6.14 - 10.8 g/head/day and 4.09 - 7.22 g/head/day, respectively. In both treatments, Ca and P requirements were fulfilled. Ca and P consumption of CMFS treated calves were 6.96 g/head/day and 5.98 g/head/day, respectively. While the FCFS treated calves consumed 9.65 g/head/day and 6.30 g/head/day of Ca and P, respectively. Thompson (1978) recommended that level of Ca in growing male calf ration was 4.32 g/head/day at the first stage of feeding and 2.16 g/head/day at the end. While levels of P in the ration was 3.33 g/head/day at the first stage of feeding and 1.62 g/head/day at the end.

Weanings Time and Weight, Body Weight Gain, and Feed Efficiency

Weanings time and weight, body weight gain, and feed efficiency of the calves were shown in Table 2. A calf can be weaned if the calf can consume 0.5-0.7 kg/head/day of calf starter concentrate (Jones and Heinrichs, 2007; Imran, 2009). Weaning in this study was based on the consumption of 750 g/d fresh weight of starter ration for 3 consecutive days. The free choice feeding system provides a more rapid weaning time than the complete mix feeding system (days 31st vs. 44th). The FCFS treatment allowed the calves to select the preferable feed ingredients to be consumed according to their needs.

Initial and weaning weights in the CMFS treatment were 39 ± 3 and 57 ± 4 kg, respectively. While, the FCFS were 38 ± 2 and 55 ± 1 kg. Boga (2009) stated that weight gain of calves fed under FCFS was higher than the calves fed under the CMFS. The ADG of calves were affected by the feeding system ($p < 0.05$), which showed that the FCFS were higher than CMFS (553.76 vs. 418.97 g/head/day). The higher of calves ADG under FCFS than CMFS were caused by the higher proportion of soybean meal consumed as a protein source (80% vs. 28.98%).

Table 2. The effect of treatment on the weaning time and weight, weight gain, milk consumption and feed efficiency

Variables	Treatment	
	Mix	Free Choice System
Weaning time (day)	44±1^{a}	31±1^{b}
Wean weight (kg)	57±4	55±1
Body weight gain (g/head/day)	418.97 ± 0.06 ^{a}	553.76±0.05^{b}
Feed efficiency	0.60±0.01	0.61±0.09

Different superscript in the same line means significantly different ($P<0.05$)

CP consumptions were higher in FCFS treatment than CMFS (303.84 vs. 195.98 g/head/day) that significantly influenced the calves ADG. It was in line to the Parakkasi (1999) statement that higher protein content in ration resulted higher ADG. In opposite, higher content of CF in ration resulted lower ADG.

Feed efficiency in both treatments showed no significant different. The results were suspected from the indifferent of corn and soybean meal digestibility. Milk consumption of calves kept under FCFS was significantly less than CMFS (115 vs. 168 liters). These results were related to the shorter weaning time for FCFS calves than the CMFS.

Conclusion

Free choice feeding system produces a ration formula consisted of 15% maize, 4% pollard, 80% soybean meal, and 1% coconut meal which contained 85.66% DM, 37.45% CP, 4.62% CF and 82.17% TDN. Cafeteria feeding system produces faster weaning times, and higher body weight gain in compare to the complete mixed feeding system, but do not different in feed efficiency.

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Palm Kernel Cake (PKC): A Potential High Energy Feed for Farm Animals

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Abstract

Production of PKC in Malaysia is in abundance throughout the year and has increased more than 43% since 1999 (1.62 million metric tonnes) to 2011 (2.39 million metric tonnes) respectively. Palm kernel cake (PKC) is a protein source leftover from pressed nuts of the palm fruit. PKC is rich in both protein (16-18%) and energy (9-14 MJ/Kg) and is regarded as a potential protein source of farm animals feed. This paper discussed on the rate of energy conversion, cost effectiveness and availability of PKC as a supplementary food ingredient for ruminants, non-ruminants and fishes. Price comparison between soybean cake and PKC showed that soybean cake cost 550.0 US$/MT while PKC is 150 US$/MT. This difference indicated that PKC is a reasonably good economic feed for better growth and fattening rate to farm animals (cattle, sheep, goat, pig, chicken and ducks) and farm fishes (cat fish and other). Countries in Asia, Asia-Pacific, South America and Africa are presently using PKC as an alternative feed for various farm animals. PKC is rich in protein, due to bio-conversion effects which doubled the nutritive value and protein concentration of PKC to 32% protein. Besides that, PKC is highly palatable for ruminants, non-ruminants and fishes because of its distinctive carbohydrate source namely 56.4% mannose, 11.66% glucose, 3.77% xylose and 1.4% galactose. PKC has high fibre content (16%), high phosphorus to calcium ratio and other essential elements like magnesium, iron and zinc recommended in animal optimal growth. A value-added quality of PKC is that it is free from Aflatoxins and other toxins of E.Coli, S.aureus and Salmonellaspp., that could be harmful for animal growth and productivity. Its sufficient concentration of vitamin E, acts as natural anti-oxidants which helpsin synthesizing female reproductive hormones. As a supplement of high energy feed, PKC can be used under various concentrations for different farm animals such as, for beef cattle up to 90%, dairy cattle 50%, swine 25-30%, poultry 20-25%, sheep and goat 30-40% and fish 20-30% of total ration. Therefore, this paper will provide

an outlook of PKC as a potential feed source that may meet the requirements as an alternative feed ingredient for farm animals and fishes.

Key words: energy conversion and cost effective, farm animals, palm kernel cake (PKC), protein concentration

Introduction

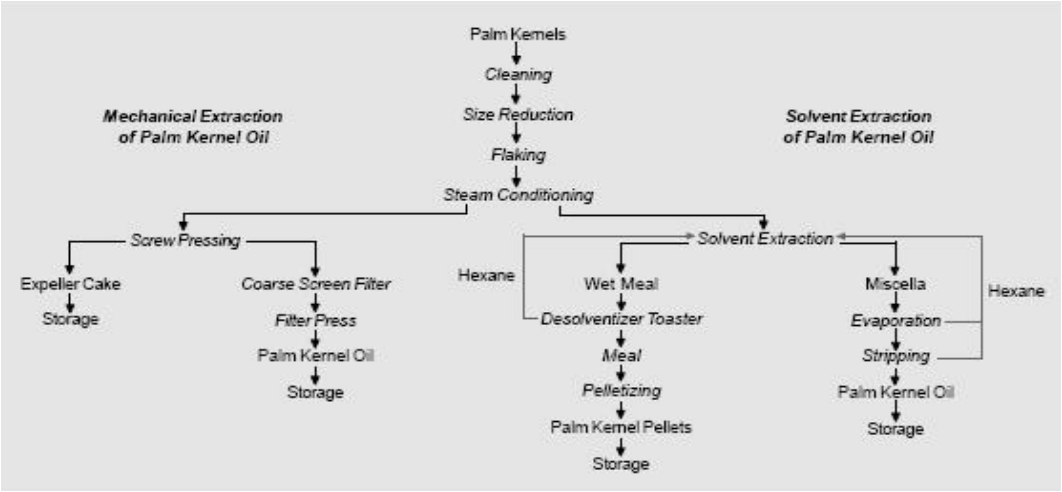
The oil palm industry in Malaysia has expanded rapidly from 60,000 ha in 1964 and reached 5 million hectares in 2011 and increased 3% from 4.85 million hectares recorded in previous year (MPOB, 2011). Therefore the palm kernel cake also increased 6.5% from 2.22 million tons in 2006 to 2.39 million tons in 2011 (MPOB 2011). Most of Palm kernel cake was exported to European Union Countries and China, which represents 93% of the total production of palm kernel cake in Malaysia. Most of the EU countries consumed the palm kernel cake as potential livestock feed for dairy cattle industry. The exports of palm kernel cake were increased 910,000 metric tons to 2.39 million metric tons in 2011 to the European Union Countries and China (MPOB 2010).

Literature Study

The palm kernel cake is the biomass residue which obtained from the crushing of palm kernels to extract the kernel oil .The palm kernel was crushing with 2 methods depending on the size of the plant throughput which is shown in the figure 1 (Yusoff, 2000). The mechanical method was using the traditional method which needs higher power consumption and has high maintenance cost due to wear and tear of the screw expeller. Normally the palm kernel with low capacity is suitable with this method. The other method for crushing the palm kernel is the solvent extraction method. This method was used only with high capacity of palm kernel. The oil residue in the palm kernel cake is about 1% compared to the mechanical method where the oil residue left in the screw expeller more than 6%.

The nutrition value in (Palm Kernel Cake) PKC contains 16-18% of crude protein, 13-16% crude fibber, 4-6% fat and the metabolisable energy estimated at10.3MJ/kg. The available nutrient contain of Palm Kernel Cake is a suitable and valuable source of feed for ruminants (Yeong *et al*., 1981). The growth performance of various breed of cattle with 100% of palm kernel cake and the combination with other ration ingredients contribute the average daily gain weight ranges 0.5-1.2 kg/head/day and the Droughtmaster cattle contribute average daily gain weight(ADGW) at the rate 0.75kg//head/day at small holder feedlot model (Jelan *et al*., 1991).

Figure 1



Sources: Yusof , M.S.A (2000)

Discussion

The palm kernel cake can be considered as reliable supply compared to the other by product. The other advantageous of palm kernel cake are lower cost compared to Soya bean Meal. Price comparison between soybean cake and PKC showed that soybean cake cost 550.0 US$/MT while PKC is 150 US$/MT (Ayob *et al.,* 2011). This difference indicated that PKC is a reasonably good economic feed for better growth and fattening rate to farm animals (cattle, sheep, goat, pig, chicken and ducks) and fishes (cat fish and other). The PKC is free from aflatoxins and safe for animal . In additions the PKC are also free from pesticide, chemicals, heavy metals and dioxins (Codjo, 1995). The PKC is difficult to become moldy due to high dry matter content encourages the growth microorganisms. PKC also very palatable and contain high vitamin E which acts as natural anti-oxidants helps in the synthesis of female reproductive hormones. The value added characteristic quality is the PKC are free from Aflatoxins, and other toxins of*E.Coli*, *S.aureus* and *Salmonella*spp., that could be harmful for animal grow and productivity.

Conclusion

PKC is a high energy and protein containing alternative feed source for all kinds of ruminant, non-ruminant (monogastric) animals and fishes. It is easily affordable to the small and large scale farmers all over the world and constantly available in the market.

Table 1. PKC in Animal and Aquaculture Feed

	Beef Cattle and Buffaloes	Dairy Cattle	Sheep and Goats	Poultry
Inclusion levels in Feed	80% in feed	30-50% in feed	30%	Up to 20%
Advantage	May give Live Weight Gain of 0.6-0.8 kg/day and 1-1.2 kg day for local (Kedah-Kelantan) and Mafriwal respectively	A cow may yield 10-12 liters milk/day. With good formulation can even give higher yields	Good and cheap source of energy for sheep and goats	FCR of 1:0.48 was reported for broilers fed palm kernel expeller (PKE) at 35 days of age (Onifade and Babatunde, 1998)
Disadvantage	-	-	Long-term feeding of PKC at high inclusion level (>80%) can cause Cu toxicity in sheep	Inclusion of PKC at levels >20% was reported to reduce egg production and egg quality (Yeong *et al*., 1981)

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Rumen Fermentation Characteristics and Methane Production in Sheep Fed a Total Mixed Ration Containing Coffee Residue

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Abstract

The objectives of this experiment were to determine the effect of coffee residue (CR) as a replacement of kleingrass hay in total mixed ration (TMR) on rumen fermentation characteristics and methane production in sheep. Four wethers with initial body weight (46 ± 7.2 kg) were used in a 4 × 4 Latin square design. The wethers were allotted to one of four TMR at 55 g dry matter (DM)/kg body weight -0.75/day. Four TMR used in this experiment were (CTL) Kleingrass hay + wheat + oat (50 : 25 : 25, on DM basis) as control; (LCR) : Kleingrass hay + wheat + oat + CR treated Bio-PKC (45 : 25 : 25 : 5, on DM basis); (MCR) Kleingrass hay + wheat + oat + CR treated with Bio-PKC (40 : 25 : 25 : 10, on DM basis); (HCR) Kleingrass hay + wheat + oat + CR treated with Bio-PKC (30 : 25 : 25 : 20, on DM basis). Bio-PKC was applied at level of 2% (w/w). The result showed that rumen pH value was maintained from 6.05 to 6.25. There was no significant (P>0.05) difference in rumen pH value by inclusion of coffee residue into TMR. The NH3-N concentration and protozoa number were similar (P>0.05) among treatments. There was no difference in methane production among treatments when expressed as l/kg DMI and l/day/BW-0.75. The study suggests that forage can be replaced by coffee residue treated with BIO-PKC up to 20% of the diet DM in ruminant feed.

Key words: total mixed ration, coffee residue, methane, sheep, rumen

Introduction

Appropriate use of relatively inexpensive agricultural and industrial by-products is of paramount important for profitable livestock production. However, high cost and low availability of conventional livestock feedstuff frequently demand consideration of by-product even if efficiency of utilization is low. In the beverage industry, wastes from coffee grounds have increased rapidly in recent years. Approximately 200.000

ton of coffee ground are produced annually in Japan. Although a small proportion of those wastes are converted into raw compost material, most are generally incinerated (Wakasawa *et al*., 1998; Xu *et al*., 2007).

There is increasing demand for the efficient use of food by-products because of economic and environmental concerns. Coffee grounds usually contain 14.5% CP, 18.4% ether extract (EE), 68.8% neutral detergent fiber (NDF), and 54.8% acid detergent fiber (ADF) (Xu *et al*., 2007). Santoso *et al*. (2011) reported that coffee waste contain 13.2 of crude protein (CP), 68.1% of NDF, 45.2% of ADF, 16.5% of EE and 0.08% of caffeine. Therefore, coffee ground could possibly be a source of nutrients for ruminants Xu *et al.* (2007).

It is difficult to recycle those wastes as animal feeds, because they contain high moisture and considerable amount of secondary metabolite such as caffeine and tannin which may reduce appetite and protein digestibility of the feeds. However, the secondary metabolites have been reported to mitigate rumen methane emission. In the previous study, Santoso *et al*. (2011) reported that *in vitro* CH_4 production in coffee waste substrate was lower by 70% as compared timothy grass hay substrate. Therefore, the fermented residues processed to prevent aerobic deterioration with the mixed microbial products might be significant means in environmental and resource recycling aspects for sustainable agriculture. The objectives of this experiment were to determine the effect of coffee residue treated by Bio-PKC as a replacement of kleingrass hay in TMR on rumen fermentation characteristics and methane production in sheep.

Materials and Methods

Animal and Treatments

Four wethers with initial body weight (46 ± 7.2 kg) were used in a 4 × 4 Latin square design. The wethers were individually housed in metabolic cages and fed the four total mixed ration (TMR) at 55 g DM/kg body weight $(BW)^{-0.75}$/day to meet maintenance energy requirements. Half of the TMR were fed at 08:00 h and the other half at 16:00 h. Water and sodium chloride block were freely available throughout experiment. Four TMR were used in this experiment, namely: CTL : Kleingrass hay + wheat + oat (50 : 25 : 25, on DM basis) as control; LCR : Kleingrass hay + wheat + oat + CR treated with Bio-PKC (45 : 25 : 25 : 5, on DM basis); MCR: Kleingrass hay + wheat + oat + CR treated with Bio-PKC (40 : 25 : 25 : 10, on DM basis); HCR: Kleingrass hay + wheat + oat + CR treated with Bio-PKC (30 : 25 : 25 : 20, on DM basis). The BIO-PKC was applied at level of 2% (w/w). The experiment was conducted in 4 periods with four wethers per treatment per period. Each period was consisted of 7 days adaptation, 2 days respiratory trial and followed by 1 day for rumen fluid collection. Refusals were weighed daily before the afternoon feeding. Body weight was measured before the afternoon feeding at the beginning and end of each period.

Respiratory Trial

Respiratory trial is conducted during 2 days of each period. Oxygen consumption, and carbon dioxide and methane production by each animal are monitored by an open circuit respiratory system using a hood over the animal's head.

Rumen fluid collection

Rumen fluid (20 ml) were collected from each wether via fistula by using a 50 ml hand syringe immediately before feeding (0) and at 1, 2, 4, 6, 8 h after feeding on the last day of each period. The pH and ORP was measured immediately by using a pH meter (D-51, Horiba Ltd., Japan). Sample was frozen at –10 °C for further analysis of ammonia nitrogen and volatile fatty acids (VFAs) concentrations. Concentrations of individual VFAs were analyzed using a gas chromatography (GC 2014, Shimadzu, Japan). Concentration of NH_3-N was analyzed according to method of Conway and O'Malley (1942). One millilitre of rumen fluid was mixed with 4 ml of Methyl Green Formalin Saline (MFS) to count protozoa number.

Sample Analyses

Dried samples were used to determine DM, ash and CP according to procedure of AOAC (1995). Procedure of Van Soest *et al.* (1991) was used to determined concentrations of NDF, ADF and acid detergent lignin (ADL). NDF was determined without the use of ∝-amylase and sodium sulphite.

Statistical Analysis

Data of gas emission and fermentation characteristics were subjected to analysis of variance for a Latin square design using GLM procedure of SAS (SAS Institute Inc., Cary, NC). When significant effects (*i.e.*, $P<0.05$) of the treatment occurred, Duncan's multiple range test were used to determine differences between treatments. Significance was declared at $P<0.05$, and a tendency toward significance was declared at $0.05<P<0.10$.

Results and Discussion

The chemical composition of TMR used in this experiment is shown in Table 1. Increasing concentration of coffee residue in the TMR tended to reduce DM, hemicellulose contents and to increase ADF content. The OM, CP, cellulose and GE contents of all TMR were similar 94.3 to 95.5%, 13.8 to 13.9%, 14.9 to 15.0% and 19.1 to 20.0%, respectively. In a previous study, Santoso *et al*. (2011) reported that coffee waste contained 0.08% of caffeine. Similar value of 0.13% of caffeine in coffee grounds has been reported by Bartley *et al*. (1978).

Table 2 shows methane production in sheep fed TMR containing coffee residue. There was no significant ($P>0.05$) difference in methane production among

Table 1. Chemical composition of experimental TMR containing coffee residue

	TMR			
	CTL	LCR	MCR	HCR
DM (%)	88.3	83.9	79.6	70.9
	----------% of DM----------			
OM	94.3	94.6	94.9	95.5
CP	13.9	13.9	13.8	13.8
NDF	47.0	47.1	47.3	47.7
ADF	17.5	18.5	19.5	21.5
Hemicellulose	29.5	28.7	27.8	26.1
Cellulose	14.9	14.9	14.9	15.0
GE (MJ/kg of DM)	19.1	19.3	19.5	20.0

Table 2. Methane production in sheep fed TMR containing coffee residue

	TMR				SEM	P
	CTL	LCR	MCR	HCR		
CH_4 (l/d/kg $BW^{0.75}$)	1.49	1.36	1.55	1.63	1.44	0.79
CH_4 (l/kg DMI)	26.61	28.77	29.45	30.32	0.44	0.70

treatments when expressed as l/kg DMI and l/day/$BW^{0.75}$. Increasing concentration of coffee residue in TMR resulted in a higher methane production when expressed as l/day/$BW^{0.75}$. This result could be attributed to increased ADF content in the TMR. Moss (1994) revealed that digestible ADF, cellulose and hemicellulose are important variables influencing CH_4 production in the rumen. In a previous study, Santoso *et al*. (2011) found that *in vitro* CH_4 production in coffee waste substrate was lower by 70% as compared timothy grass hay substrate.

Table 3 summarizes the rumen pH value, concentrations of NH_3-N and VFA in the rumen of sheep fed the TMR containing coffee residue. Inclusion of coffee residue in the TMR had no effect (P>0.05) on pH value, protozoa number, concentrations of NH_3-N, total VFA, acetate and propionate. Average of pH values in the rumen of sheep fed coffee residue varied from 6.06 to 6.26, which are in the optimal pH range of 6.7 ± 0.5 required to maintain normal cellulolysis (Van Soest, 1994) and required for microbial protein synthesis (Russell *et al*. 1992). Total VFA concentration in the rumen of sheep fed TMR containing coffee residue (LCR, MCR and HCR) was relatively higher than of the control sheep. This result suggesting that inclusion coffee residue up to 20% of DM in TMR did not inhibit rumen microbial fermentation. This finding was also supported by there was no significant

Table 3. Rumen fermentation characteristics in sheep fed TMR containing coffee residue

	TMR				SEM	P
	CTL	LCR	MCR	HCR		
pH	6.20	6.26	6.06	6.17	0.05	0.18
N-NH_3 (mg/100 ml)	74.9	75.8	73.1	69.7	20.48	0.99
Protozoa (10^6 cell/ml)	1.5	1.1	1.2	1.6	0.17	0.23
Total VFA (mM)	60.7	75.0	68.0	78.7	7.38	0.40
Acetate (mol/100 mol)	78.0	73.1	78.4	75.2	1.95	0.28
Propionate (mol/100 mol)	18.1	23.7	18.0	19.4	1.66	0.15
Butyrate (mol/100 mol)	2.7[a]	1.9[b]	2.4[a]	2.7[a]	0.15	0.02

Means in the same row followed by different letters are different (P<0.05)

effect on DM digestibility of sheep due to inclusion coffee residue in TMR (data not shown). In the previous study, however, Bartley *et al*. (1978); Xu *et al*. (2007) reported that the ruminal fluid from Holstein steers or sheep receiving coffee ground had significantly lower concentration of total VFA than those receiving no coffee ground. Proportion of butyrate in sheep fed LMR was (P<0.05) lower than those fed other TMR.

Conclusion

Rumen methane production was similar in sheep fed all TMR. Total VFA was relatively higher in sheep fed TMR containing coffee residue than those fed TMR without coffee residue. Forage can be replaced by coffee residue treated with Bio-PKC up to 20% of the diet DM in ruminant feed.

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Ongole Crossbreed Performance Given Silage of Cattle Rumen Contens as a Feed Substitute for Grass

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Abstract

The waste from the slaughterhouse is usually removed in a certain place while it can be used to feed the cattle. The use of rumen content as one of the alternative feed help to farmer to provide the feed easily, conserve the environment and support the development program, particularly in the urban area. The research is aimed at identifying the effect of the cattle rumen content distribution as the subtitute for the grass on the performance of the beef cattle. 12 SimPO oxen aged 1.5 - 2 years were adopted in the research which lasted for 8 weeks (2 months) with the feeding of 3% of the weight based on the dry matter and the drink was given ad libitum. The treatment consisted of subtituting the cattle rument content silage for the grass. The treatment consisted of T0 = giving 100% grass, T1= giving 25% cattle rumen content silage and 75% grass, and T2 = giving 50% cattle rumen content silage and 50% green grass. The ratio of grass to concentrate was 20% : 80%. The variables of the study were the average daily gain and feed conversion. The result indicated that the treatment was not significantly related with the average daily gain and feed conversion. It can be concluded that the substitution in part of the grass with the rumen content silage up to 50% exerted no effect on the average daily gain and feed conversion.

Keywords: beef, cattle rument content, silage

Introduction

Until recently the proliferation of beef cattle had converged into one production, - the meat (Parakkasi, 1999). Feed plays important rule in the cattle's need of nutrition for growth to produce the meat maximally. The growth of the cattle was affected by several factors, namely the race, sex and quality and quantity of the feed. Tillman *et al.* (1998) argued that the cattle growth rate heavily depends on the

amount of the consumed feed eaten. The lack of the feed represents the obstacle in the development process.

The Indonesian season, which consists of rainy and dry season, was associated with the difference ways in providing the feed for the cattle. In the rainy season, the provision of the feed for the cattle is much easier, while it is difficult to find the leaves in the dry season if not they are expensive. When it is necessary for the breeder to buy the feed, they had the reduced income since they have to buy the feed.

One of the slaughterhouse waste which can be used as the cattle feed was the cattle rument content. The use of cattle rumen content from the slaughterhouse has been reported by Messermith (1973) who used the rument content as the matter for ration preparation up to 15% could yield the average daily gain, feed consumption, feed efficiency and conversion which was unsignificantly different with the control.

The waste from the slaughterhouse is usually disposed. It is expected that 24.5 kg rument content or 3.8 kg per cow was produced each day since it contains 15.5% dry matter (Witherow dan Lammers, 1976 cited in Utomo *et al*, 2007). Overall, 46,525,500 kg fresh rumen content was produced from all slaughterhouse in Indonesia. The Data on the population and the number of slaughterhouse in Indonesia was presented in Table 1.

The use of rumen content as an alternative feed to fulfill the feed requirement help the feed provision, the environment conservation, and support the development program, partcularly in the urban area. One of the ways to remove the bad odor, to prevent the decay (reservation) and the maintain the nutritive value is to make it into silage, that is fermetation with the main product is the lactid acid.

The aim of the research was to identify the effect of silage substitution of cow rumen content in the grass basal feed on the performance of beef oxen which were fatten in the fattening effort, consisting of daily weight increase and feed conversion. The research was closely related with the use of industrial waste from the slaughterhouse in order to gain the provitable and efficient outcome. In addition, it was related with the use of slaughterhouse waste for the alternative feed cattle replacing the green leaves without reducing the quality and production of the cattle fattening.

Table 1. Cattle Population and Slaughterhouse in Indonesia (,000 cow)

	2004	2005	2006	2007	2008
Population	10,504	10,569	10,875	11,515	12,257
Beef cuts	1,733	1,654	1,800	1,886	1,899

(Directorate General of Animal Husbandry, 2011)

Materials and Methods

Location and Time

The research was conducted from August 1st, 2011 to November 1st, 2011. The research took place in Giwangan Slaughterhouse in Yogyakarta and in the fattening farm in Jarum Village, Kayuloko, Sidoharjo district in Wonogiri Regency. The test on the feed nutrient content was conducted in the bio-chemistry laboratory of Gadjah Mada University.

Materials

The rumen content. The rument content from the cattle used in this research was obtained from Giwangan slaughterhouse in Yogyakarta. The green rumen content was selected since it was indicated that the cattle consumed the green feed.

The silage of cattle rument content. Before conducting the silage on the cattle rumen content, the research determined the chemical composition of the basic matter in conducting the silage, namely the cattle rument content, soft and molases using proximate analysis. The preparation consisted of 64.60% rumen, 35.40% soft, creating dry matter from the 35% silage, plus 8% molases from the silage raw amatter and inoculated by *Lactobacillus plantarum* 0.1 % from silage matter which was put in the black vacuum *polyethylene* (Utomo *et al.*, 2007). The fermentation took 21 days. The resulted silage was subjected to proximate analysis to identify the chemical composition of it.

Stall. The research used 12 individual stall. The size of each stall is 100 x 150 $cm^{2.}$ the base for the stall was cement which was then covered with rubber to prevent the cattle for being slipped. The wall was made of brick and the roof-tile was used for the ceiling.

Tabel 2. Nutrient Composition of Feed Materials Making Up The Ration (% DM)

	King Grass	Rumen content silage	Consentrate
Dry Matter (DM)	21.25	28.90	84.21
Crude Protein (CP)	8.81	12.73	4.41
Crude Fiber (CF)	22.60	27.16	25.53
Organic Matter (OM)	85.47	84.81	77.06
TDN	60.44	65.11	53.55
Ash	14.53	15.19	22.94

The result of Bio-chemistry Laboratory Analysis in Husbandry Faculty, Gadjah Mada University

Equipments. The equipment used in this research consisted of a set of stall equipment, *vacum cleaner,* black *polyethylene* bag, plastic rope, *Rudweight* cattle scale with the capacity of 1,250 kg with the sensitivity of 1 kg and Goat cattle feed scale with the capacity of 15 kg and the sensitivity of 50 gr and a set of laboratory equipment for analyzing the feed.

The ingredient of ration. The ingredients for preparing the ration consisted of king grass, rument content silage and nutrisi fit(R) concentrat. The nutrition composition was presented in Table 2.

Methods

The research lasted for 13 weeks. The first three weeks was used to prepare the silage of rument content, 1 week later was used to feed adaptation and next 8 weeks was used to examine the distribution of rument content silage. The consentrat feed was given twice a day at 8 in the morning and at 6 in the afternoon while the rument content silage was given in the noon - from 12 to 13 pm. The drinking water was given *ad libitum* while the research took place.

The Cattle Grouping

The population of the research consisted of 12 SimPO oxen. The weight of the cattle ranged from 350 to 400 kg. The cattle was assigned into three feed treatments.

The Pattern of Feeding

The control feeding consisted of the grass to concentrat : concentrate ratio of 20% : 80%. King grass was used in this treatment, while the concentrat was the same for all treatments- the product of nutrisi fit. The feeding pattern in the research consisted of three kinds of treatments:

P0 : control (no cattle rument content silage).

T1 : the cattle rument content silage was 25% and the grass 75%.

T2 : the cattle rumen content silage was 50% and grass 50%.

The silage of cattle rument content was used to subtitute the grass.The fattening treatment was conducted in 8 weeks (2 months) and previously the 2-week adaptation period was conducted to adapt the cattle to the physical environment and the feed. The feed distributed to the cattle was 3% of the weight based on the dry matter, while the drink was given ad libitum. The scaling was conducted once in a week to identify the daily average weight gain in a week, while the scalling of the feed and the remains was conducted every day during the research (8 weeks)

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Data Analysis

Oneway ANOVA using random design was adopted to analyze the average

daily gain and feed conversion. When the significant values were obtained, they were then subjected to *Duncan multiple range test*/DMRT (Christensen, 1996).

Results and Discussion

Average Daily Gain

The following table listed the average daily gain for each ox Table 3 also listed the result from the three treatments. The result of statistical analysis on the treatment of cattle rument content silage in the beef cattle was not significantly related with the average daily gain.

The average daily gain of T1, T2, and T3 were 0.92 ± 0.02, 0.97 ± 0.05, and 0.96 ± 0.03 kg/ox/day, respectively. There is not significantly different because nutrient composition of feed materials for all the treatment was not different to.The average daily gain resulted in this research was different with that of Yudhanto (2008) who conducted research on the Ongole cattle. The average daily gain for Nutrient composition of feed materials making up the ration and SimPO cattle were 0.58 and 1.05 kg/ox/day, respectively.

The average daily gain was also subjected to covarian analysis for identifying the effect of initial weight on the ADG. The covarian analysis on the initial average daily gain indicated that Fcount < Ftable, meaning that it was not *significant.* It is clear that the substitution of some green plants with cattle rument content silage exerted no significant effect on the average daily gain.

Types of feed, consumption and composition of chemical composition influenced the growth, protein consumption and energy which produced the more rapid pace of growth (Soeparno, 2005). It is clear that the factor affecting the growth and the development of the cattle included the feed, sex, hormon, age, environment and climate.

According to Tillman *et al*. (1998) the weight gain takes place when the consumed feed is beyond the requirement for living. If the main need is fulfilled, the excessive nutrition was then stored as the flesh and fat tissues (Cullison, 1970).

Table 3. Average Daily Gain (kg/ox/day)

	Treatment		
	T0	T1	T2
1	0.96	0.94	0.96
2	0.92	1.05	1.00
3	0.91	0.96	0.91
4	0.91	0.92	0.98
Average	0.92 ± 0.02	0.97 ± 0.05	0.96 ± 0.03

Table 4. Feed Conversion

	Treatments		
	P0	T1	T2
1	13.55	15.35	13.30
2	14.00	13.55	13.20
3	15.28	14.31	14.29
4	14.39	13.44	13.01
	14.30 ± 0.69	14.16 ± 1.46	13.45 ± 0.70

Feed Conversion

The data of daily average feed conversion per ox from the three treatments is presented in Table 4. The statistical analysis on the treatment of cattle rument content silage se among the beef cattle insignificantly affected the feed conversion. The average feed conversions of T1, T2, and T3 were 14.30 ± 0.69, 14.16 ± 1.46 and 13.45 ± 0.70, respectively. There was no difference in the dry matter consumption and average dily gain among the three treatments and thus, there was no difference in the feed conversion among the three treatments.

The resulted feed conversion in this research was slightly higher than that of Ngadiyono's (1995) studi on the PFH oxen with the ration of concentrat and king grass was (70 : 30), resulting the conversion value of 10.8 kg/ox/day and that of Suwignyo's (2003) research on ACC oxen with the fermented rice straw and concentrate with the resulted feed conversion of 9.6-11.4 kg/ox/day.

The lower value of feed conversion, according to Tillman *et al.* (1998) means the more efficient the consumption of the feed. According to Campbell dan Lasley (1985), the feed conversion was affected by the cattle's ability to digest the feed, the adequacy of the feed for maintaining the living requirement, the growth and other body function as well as the type of consumed feed. According to Pond *et al*. (2005) nutrient was directly related with the growth rate and the body composition during the development. The energy was used to fulfill the requirement for maintaining, protein development and fat deposition.

Conclusion

It can be concluded that the substitution in part of the grass with the rumen content silage up to 50% exerted no effect on the average daily gain and feed conversion.

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Performances and Meat Cholesterol Content of Fat Tail Sheep Fed Diets Supplemented with Sardinella Fish Oil Based Ca-soap Mixed with Herbal

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Abstract

Previous results showed that sardinella fish oil based Ca-soap supplementaion at level a of 3.0% in ration reduced meat LDL cholesterol of lamb by 49% and improved feed utilization efficiency by 13%. However, sardinella fish oil based Ca-soap had bad fishy smell causing low palatability of feed and reduced feed intake. In Indonesian tradition, fishy smell is ussualy overcomed by applying herbal. This experiment aimed to elaborate herbal meal addition (ginger, turmeric, and beluntas leaf) into sardinella fish oil based Ca-soap in reducing its fishy smell, and subsequently improve its palatability. Sixteen male fat tail sheep were allocated into four experimental treatments with four replicates and arranged in a block randomized design. The experimental period lasted for 14 weeks and the animals had free access to feed (concentrate: native grass in ratio of 1:1) and water. At the end of experiment, two sheep from each treatment were slaughtered. The treatments were T0 (without herbal addition), T1 (with turmeric addition), T2 (with ginger addition), and T3 (with beluntas leaf addition). Herbal Ca-soap, at level of 3%, were mixed thoroughly with feed ingredients to produce concentrate diet. The variables measured were feed intake, daily weight gain, feed conversion ratio, blood triglicerides and meat cholesterol content. The results showed that addition of sardinella fish oil based Ca-soap mixed with herbal slightly reduced feed intake and daily gain, but improved feed efficiency utilization. Herbal addition reduced cholesterol, but increased triglicerides content of blood plasm. Herbal addition, except for beluntas leaf, also reduced cholesterol content of meat. It is concluded that sheep fed sardinella oil based Ca-soap with turmeric addition had better performance and lower cholesterol of meat.

Key words: calcium-soap, herbal, meat cholesterol, sardinella fish oil

Introduction

Ruminant meat contains high saturated fatty acids (laurate, myristate, and palmitate) that caused high cholesterol in the blood plasm (Grande, 1975). Substitution saturated fatty acid with polyunsaturated fatty acid could reduce total cholesterol, including LDL-cholesterol (Marsic and Yodice, 1992). Lamb contains higher cholesterol than beef, i.e. 94 mg/100g vs 87 mg/100g. People that consumed food containing high cholesterol can cause *atherosclerosis* leading to coronary heart disesase. Omega-3 polyunsaturated fatty acids (PUFA) can reduce risk of atherosclerosis (IGER, 2003). Source of PUFA containing high omega-3 concentration is fish oil, e.g., from sardinella fish. Sardinella fish oil is easy to obtain in Indonesia. It is a waste product of fish canning industry spreaded out in eastern part of East Java Province.

Results of the previous experiment showed that feeding sardinella oil based Ca-soap at level of 3.0% decreased LDL cholesterol of lamb by 49% (Sudarman *et al*., 2008[a]) and feed utilization efficiency increased by 13% (Sudarman *et al*., 2008[b]). This indicates that lipid profile of lamb can be altered by feeding Ca-soap. However, feeding Ca-soap caused feed intake to decrease. This was probably caused by bad fishy smell of Ca-soap causing the palatability of diet to decrease.

When cooking fish materials in Indonesia, it is a tradition to add herbals to overcome the fishy smell. The herbals that are usually used are ginger (*Zingiber officinale* Rosc.), turmeric (*Curcumae domestica*) and beluntas leaf (*Pluchea indica* Less).

The objective of this experiment was to evaluate the effects of herbal addition into sardinella oil based Ca-soap on the reduction of its fishy smell. This will, subsequently, improve palatability of the diet and performance of sheep, and reduce low cholesterol lamb.

Materials and methods

Sixteen male growing fat tail sheep (approx. 8 m.o.) were allocated into four experimental diets with four replicates and arranged in a block randomized design. They were reared in individual cage for two months. Feed (concentrate : native grass in ratio of 1:1 dry matter base) were given *ad libitum* (110% of previous day intake) at 07.00 and 17.00. The animals had free access to water. The ingredients of concentrate were pollard, cassava wate meal, coconut meal, rice bran, palm kernel meal, soybean meal, molasses, $CaCO_3$, urea, and DCP. Nutrients composition based on proximate analysis was 16% crude protein, 5% of extract ether, 10% crude fiber and totally had70% TDN.

The experimental treatments were T1= basal diet (containing 5% fat), T2= T1 + 3% Ca-soap with turmeric meal, T3= T1 + 3% Ca-soap with ginger meal, and T4=

T1 + 3% Ca-soap with beluntas leaf meal. Ca-soap complex was made based on the method used in the previous experiment (Sudarman *et al*., 2008[b]) and the herbal were added before mixing with other feed ingredients for making concentrate.

At the end of experiment, blood samples from two sheep of each treatment were collected from jugular vein for analyzing cholesterol and trigliceride contents of blood plasm. Total cholesterol was measured by the CHOD-PAP method. Triglyceride determinations were performed using the GPO-PAP method. Subsequently, two sheep of each treatment were slaughtered for analyzing meat cholesterol content using Lieberman Burchard method (Kleiner and Dotti, 1962).

Data of sheep performances were subjected to Analyzes of Variance (ANOVA) and any different means were further tested using LSD (Steel and Torrie, 1980). Other data were analyzed using descriptive statistics. The variables measured were dry matter intake, weight gain, feed conversion ratio, blood plasm cholesterol and triglicerides, and meat cholesterol content.

Results and discussions

Range of ambient temperature and humudity during the experimental study were 22.2-33.8 °C and 83.7-92.3%, respectively.

Sheep Performances

Data of dry matter intake, daily weight gain and feed conversion ratio (FCR) were shown in Table 1. Dry mattter intake of sheep of all treatment groups were not significantly different. This indicated that herbal addition was not able to improve the palatability of feed added sardinella oil based Ca-soap. Intake of sheep in this experiment (4.0% BW vs 2.5% BW) was higher than that of sheep in previous results (Sudarman *et al*., 2008[b]). This discrepancy was possibly due to the difference in breed of sheep used. Previous experiment used thin tail sheep breed with average body weight of 16.9 kg, while the present experiment used thick tail sheep breed

Table 1. Performance of sheep fed sardinella fish oil base Ca-soap with different herbal addition (± SD)

Treatments	Dry matter intake (g/head/day)	Daily gain (g/head/day)	FCR
T0	1,080 ± 63	148 ± 26	7.44 ± 1.33
T1	1,058 ± 106	162 ± 21	6.60 ± 0.95
T2	1,021 ± 128	148 ± 41	7.14 ± 1.29
T3	1,041 ± 40	131 ± 15	8.02 ± 0.80

Note: T0 (basal diet), T1 (T0 + turmeric), T2 (T0 + ginger), and T3 (T0 + beluntas leaf).

with average body weight of 25.2 kg. Daily weight gain of sheep of all treatment groups were also not significantly different, but turmeric addition resulted in 9.5% daily gain higher than that of control group. Feed utilization efficiency (FCR) was slightly better for those given sardinella oil based Ca-soap added with turmeric, eventhough it was not statistically significantly different.

Plasm Cholesterol and Triglicerides

Cholesterol and triglicerides of blood plasm were presented in Table 2. Blood cholesterol of sheep fed Ca-soap with herbal addition were slightly lower than that of control group. Blood composition including blood cholesterol was always in dynamic condition. It is affected by type of feed ingested. Blood triglicerides of sheep given Ca-soal with all herbal addition were consistently lower than that of control group.

Table 2. Blood cholesterol and and trigliserides of sheep fed sardinella fish oil based Ca-soap with different herbal addition (± SD)

Treatments	Plasm Cholesterol (mg%)	Plasm Trigliserides (mg%)
T0	83.86 ± 11.46	16.85 ± 5.35
T1	74.41 ± 11.60	34.83 ± 20.47
T2	60.63 ± 27.02	30.34 ± 14.27
T3	80.71 ± 15.29	32.02 ± 8.68

Note: T0 (basal diet), T1 (T0 + turmeric), T2 (T0 + ginger), and T3 (T0 + beluntas leaf).

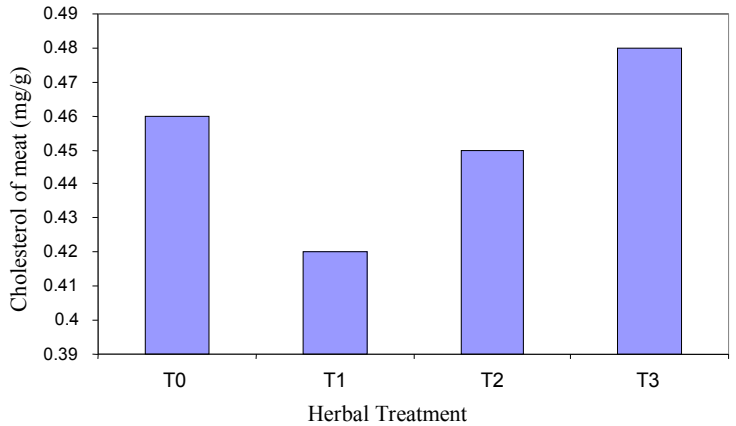

Figure 1. Cholesterol contents of meat of sheep fed experimental diets: T0 (basal diet), T1 (T0 + turmeric), T2 (T0 + ginger), and T3 (T0 + beluntas leaf)

Meat Cholesterol

Meat cholesterol contents of all groups are presented in Figure 1. Giving Ca-soap with herbal addition into diet did not consistently affect meat cholesterol content. Turmeric addition was able to reduce meat cholesterol better than with the addition of other herbals. Previous results (Sudarman *et al*., 2008[a]) showed that adding sardinella oil based Ca-soap at level of 3% without herbal addition decreased LDL cholesterol of 49%.

Conclusion

Herbal addition is not able to improve palatability of diet supplemented with Ca-soap fish oil. Sheep fed sardinella oil based Ca-soap with turmeric addition had better performance and lower cholesterol of meat.

Acknowledgement

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Diversity of Domestic Grasses for Sheep Browse in the Coastal District Gebang, Cirebon Residence

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Abstract

In general, sheep browse in Indonesia comes from the availability of domestic grasses (Family Poaceae and Cyperaceae), just an effort to make agronomic improvements is still very limited. Therefore we need basic research on domestic forage to determine its potential as a forage cultivation. The purpose of this study is to identify potential sources of herbaceous species forage of sheep. Research carried out by the method of survey, observation, collection of plants by way of example, shooting, maintenance ex-situ, making herbarium and species identification. In total there are 39 types of forage from the Family Acanthaceae, Cyperaceae, Leguminosae, Liliaceae and Poaceae. Types grasses are found, divided into three belts: Belt-1 (0-1 km from coast) consisting of: 8 of the Family Poaceae species and 9 species of Cyperaceae Family. The dominant species of livestock are available for Xerochloa Cheribon (Steud.) Ohwi and Chloris barbata Swartz. Belt-2 (1-2 km) consisting of 13 species of the Family Poaceae and 4 species of Cyperaceae Family. The dominant species of livestock are available for Cynodon dactylon (L.) Pers., Paspalum conjugatum Berg. and Cyperus scariosas R. Br. Belt-3 (2-3 km) consists of: 12 species of the Family Poaceae and two species of Cyperaceae Family. The dominant species of livestock are available for Paspalum conjugatum Berg., Cynodon dactylon (L.) Pers. and Eleusine indica (L.) Gaertn. Getting away from the beach Family Poaceae is dominant as the compared to the potential forage Cyperaceae Family. Based on field observations and statements of farmers, sheep prefer grasses of the Poaceae Family.

Keywords: domestic grass, coastal, sheep

Introduction

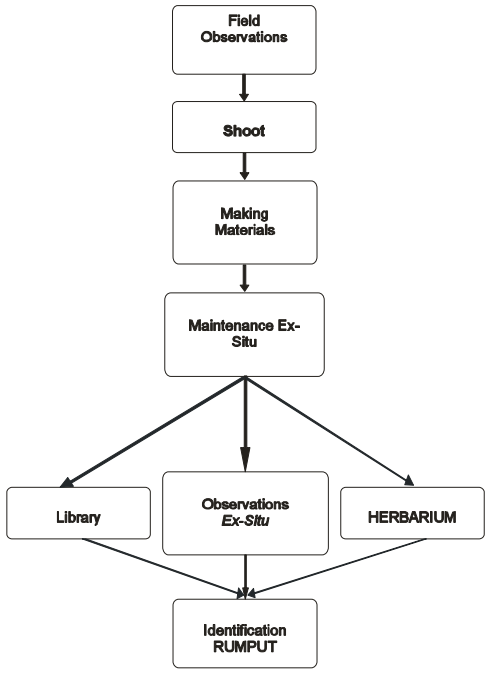
Lamb is one type of animal that has the potential to meet the needs of animal protein, because lamb can easily accepted by all people and religion, especially in Indonesia. The problem that usually encountered in the development of sheep farming is the low productivity due to low availability of forage quality, especially grass.

Fresh fodder as feed is one of the main base for support, especially for livestock farms both large and small ruminants, which every day takes quite a lot of forage, because more than 60% of all ruminant livestock feed consumed is fresh, both in the form of fresh or dried. Haryanto (2004) stated that reduced the carrying capacity of natural resources (feed) to the business of cattle due to conversion of agricultural land, as well as changing patterns of farming became one of the causes of declining livestock population. Besides being used for forest and coastal tourism, coastal areas can be utilized as a forage-producing areas of quality forage.

Materials and Methods

Research material is locally grown forage grass in the District Gebang and tools were used quadrant measuring 0.5mx 0.5m, knives, plastic bags, stationery, alcohol 70%, scrap paper, and label. The method used in this study is a survey research method to conduct interviews with some sheep ranchers farm-related conditions, as well as direct review of the diversity of forage grasses. Gebang district is divided into three Belts based on distance from shore observations are: Belt 1st (0-1 km from the loast);-2nd Belt (1-2 km from the coast), and Belt-3rd (2-3 km from the coast).

Data obtained from field survey and sub-cultivated in a descriptive profile includes general picture of the state of research sites, as well as the characteristics of sheep farming in the study site. Data processing method used is the identification of forage grass.



Results and Discussion

Gebang district, at is lowland areas, with an average height of 6 meters above sea level. The temperature of 28ºC - 32 ºC. Humidity is 83.07 - 86.1%.

Sheep was dominantly (5200 head) maintained in District Gebang, compared to other ruminants (cattle, buffaloes and goats). Lamb is generally a side business of farmers' fields. Types that are kept are fat tail and a thin tail.

Sheep maintenance system in the District Gebang consists of two systems, namely intensive and semi-intensive. Semi-intensive maintenance system are found in 1st Belt (0-1 km from the coastline). In this system, farmers tending their flocks during the day starting at 08.00 am and will impound the animals at 16.00 pm. They choose to release or indulgence in flocks on the there are many forage grasses that can be used for feeding their animal. Mr. Tono, one of the owners of sheep farmers in 1st Belt has a unique habit of feeding forage for livestock. During the day from 08.00 am until late afternoon at 16.00 pm he graze the livestock on the coast, while in the evening he gave them extra feed that is api-api leaves (*Avicennia marina* (Forsk.) Vierh.) that grow in coastal areas of Gebang district. Based on his story, giving that leaves did not have a negative effect on his sheep.

Intensive systems are found in the observation Belt 2nd and 3rd is the agricultural Belt. In the intensive systems, animal cages all day on the grounds because peasant-farmers are busy in the fields all day and if the cattle was grazed, they feared it could damage agricultural crops around. In the zone or the distance is not directly adjacent to the coast (Belt-2nd and Belt-3rd), a pattern that is widely used for livestock grazing is a intensive maintenance system. The pattern of forage supply in this zone is to use the pattern "cut and carry". Cattle were fed twice a day in the morning and afternoon. Feed given to cattle sheep is a 100% field grass that grow around the edge of the road, river, rice fields and plantations. Forage is cut with a sickle or crescent. The breeders take the cutting of browse into sacks and carry the grass clippings by using a bicycle and becak.

According Djajanegara *et al*. (1993), in tropical area sheep and goats are usually kept by the breeder (farmers, ranchers) with semi-intensive system. The ranchers graze their cattle or to graze in the afternoon and impound their animals at night. Impounding the animals at night for security reasons and so that is not lost or stolen.

Based on interviews with some of the breeders in the district Gebang about the utilization of fresh fodder (Table 1.) there are three types of forage grass that is most preferred *Dactyloctenium aegyptium* (L.) Ritch., *Echinochloa colonum* (L.) Link., and *Eriochloa polystachya* H. B. K.. Note also that grasses, there is some fresh grass with sheep preferred degree among *Brachiaria eruciformis* (J. E. Smith) Griseb., *Brachiaria subquadripara* (Tan) Hitche., *Chloris barbata* Swartz., and *Paspalum conjugatum* Berg.. In general, feeds such as legumes forage preferred by sheep. However, the granting of a legume forage such as *Leucaena leucocephala* LAMK,

Table 1. Field and forage grass species and the degree of favorite feed supplements by sheep

Local name	Latin Name	Family	Degree of favorite
-	*Cyperus babakan Steud.*	Cyperaceae	-
Waling	*Cyperus elatus L.*	Cyperaceae	-
Teki	*Cyperus rotundus L.*	Cyperaceae	+
Teki	*Cyperus scariosas R. Br.*	Cyperaceae	+
Teki	*Cyperus trinervis R. Br.*	Cyperaceae	+
Nyiur-nyiuran	*Cyperus iria (L.) Rikl.*	Cyperaceae	-
-	*Ficinea Sp.*	Cyperaceae	-
-	*Fimbristylis acuminata Vahl*	Cyperaceae	-
-	*Fimbristylis hookeriana Bacek*	Cyperaceae	-
-	*Fimbristylis schoenoides (Retz.)*	Cyperaceae	-
-	*Fimbristylis tomentosa Vahl*	Cyperaceae	-
-	*Fimbristylis vahlii (Lamarck) Link.*	Cyperaceae	-
-	*Mapania Sp.*	Cyperaceae	-
-	*Agropyron repens (L.) Beauv.*	Poaceae	-
Suket reketek	*Brachiaria eruciformis (J. E. Smith) Griseb.*	Poaceae	++
-	*Brachiaria subquadripara (Tan) Hitche.*	Poaceae	++
-	*Chloris barbata Swartz.*	Poaceae	++
-	*Crysopogon aciculatus (Retz.) Trin*	Poaceae	+
Grintingan	*Cynodon dactylon (L.) Pers.*	Poaceae	+
Tapak jalak	*Dactyloctenium aegyptium (L.)*	Poaceae	+++
-	*Digitaria ciliaris (Retz.) Koel.*	Poaceae	+
Jampang piit	*Digitaria nuda Schuamch.*	Poaceae	+
Tuton	*Echinocloa colonum (L.) Link.*	Poaceae	+++
Jajagoan	*Echinocloa stagnina (Retz.) Beauv.*	Poaceae	+
Godong ulo	*Eleucine indica (L.) Gaertn.*	Poaceae	+
Bebekan	*Eragrotis tenella (L.) Beauv.*	Poaceae	+
Suket peronan	*Eriochloa polystachya H. B. K.*	Poaceae	+++
Meniran	*Panicum paludosum Roxb.*	Poaceae	+
Lempuyangan	*Panicum repens L.*	Poaceae	+
Paitan	*Paspalum conjugatum Berg.*	Poaceae	++
-	*Xerochloa cheribon (Steud.) Ohwi.*	Poaceae	+
Lamtoro	*Leucaena leucocephala LAMK*	Leguminoceae	++
Gamal	*Gliricidia sepium (Jacq.) Steud.*	Leguminoceae	++
Turi	*Sesbania grandiflora L. PERS*	Leguminoceae	++

Table 1. Continued

Local name	Latin Name	Family	Degree of favorite
Kaliandra	*Calliandra calothyrsus Meissn.*	Leguminoceae	+
Jerami padi	*Oryza sativa L.*	Poaceae	+
Daun bawang	*Allium cepa L. Rank.*	Liliaceae	-
Daun jagung	*Zea mays L.*	Poaceae	-
Daun api-api	*Avicennia marina (Forsk.) Vierh.*	Acanthaceae	++

Source: Primary data processing 2011
Table info: - : disliked; + : a biliked; ++ : preferred; +++ : highly preferred

Gliricidia sepium (Jacq.) Steud., and *Sesbania grandiflora* L. PERS is rarely do because of its availability is very little or limited in District Gebang.

Sometimes the animals were given additional fresh forage during the rest of the farm post-harvest due to the very large stock availability at the time, but the farmers claim they are reluctant to provide livestock forage because the rest of agriculture often cause digestive health problems in livestock such as the provision of fresh onions that often cause diarrhea to their sheep

Bredeers in the district Gebang very rarely provide additional feed their sheep in the form of concentrates and pulp out because according to the farmers, the provision of concentrates and pulp for their sheep only give additional cost of maintaining the sheep. Because sheeep is not the main business but only a side business of farming.

Zoning for the sampling of grass in this study were divided into three belts based on distance from the coast and the election of District Gebang point sampling forage grasses based on consideration of the many ranchers who graze on the site. 1st Belt is the belt at a distance of about 0-1 km from the coast, the 2nd Belt is the belt at a distance of about 1-2 km from the coast, and 3rd Belt is the sampling belt at a distance of 2-3 km from the coast. In Figure 1 can be seen that there is a pattern of distribution of type of forage grass field that is unique.

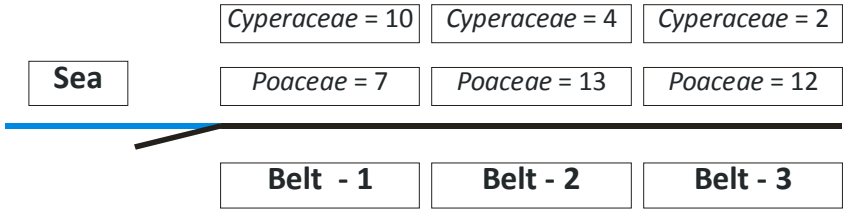


Figure1. The Pattern of Distribution of Vegetation Types Based on the Distance from the Beach (Source: Primary data 2011).

The farther the distance from the coast will be more spacious with the type of vegetation found Poaceae getting closer to shore and vice versa is more found in type Cyperaceae fresh field. Poaceae is a member of the tribe of flowering plants. In general, a hallmark of plant Poaceae trunked jointed, was crowned flowers, and ribbon-shaped leaves. Interest-tekian or Cyperaceae puzzle is one of the members of the tribe of flowering plants. Interest rates are the closest relatives of grains (Poaceae) and have many similarities. The difference between simply Poaceae and Cyperaceae can be seen in cross-sectional shape on the trunk. Poaceae has a cross section of rod-shaped oval or round, while Cyperaceae has a triangular cross-section rod. Most abundant grass in 1st belt is *Xerochloa cheribon* (Steud.) Ohwi. by percentage botanical composition about 57.0%. On the field 2nd belt is dominated by grass *Cynodon dactylon* (L.) Pers. with a percentage of the botanical composition about 41.83%. while on the most dominating 3rd belts based on the botanical composition of *Paspalum conjugatum* Berg. with a percentage of the botanical composition about 43.39%.

Conclusion

There are differences in the amount of forage grass species in each Belt in the District of Gebang, with total forage grass species that grow in the District Gebang reached 31 species of Poaceae and Cyperaceae Family.

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Physical Characteristic and Palatability Test of Biscuit Feed for Sheep

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Abstract

The objective of this study was to determine the physical characteristic and palatability of corn plant waste after formed as a biscuit feed. Experimental design used in this research was Completely Randomized Design with 6 treatments and 3 replications. The treatment were : R1(100% field grass), R2(50% field grass + 50% corn leaf), R3(100% corn leaf), R4(50% field grass + 50% corn husk), R5(50% corn leaf + 50% corn husk) and R6(100% corn husk). The results were subjected to ANOVA and Contrast Orthogonal Test (Steel and Torrie, 1991). Biscuits feed variables measured were moisture, water activity, water absorption, density, and palatability. The results of this research indicated that the treatment of biscuit feed made from field grass and corn plant waste gave highly significant effect (P<0,01) on water content. According to SNI (2000) water content of feed should not be more than 14%. Water content in this study ranged from 11-12%. Thus, biscuit is more durable and can storage for long period. The treatments was significant effect (P<0,05) on water absorption,but was not significant effect on water activity, density and palatability. Biscuits containing field grass and corn leaf was preferred by sheep.

Keywords: corn plant waste field grass, biscuit feed, physical characteristic, and palatability

Introduction

Central Bureau of Statistics (2009) reported that corn plant production in Indonesia is 4.2 tons / ha. The proportion of waste corn per cent dry matter consisted of 50% trunk, 20% leaf, 20 % corncob and 10% husk.

Corn plant waste used in this research consisted of corn leaf and corn husk then formed into biscuits through the process of heating and pressing. Corn leaf and corn husk have a higher palatability than corncob and trunk of corn (Wilson

et al., 2004). Biscuit is a dry product that has relatively long lasting storage period and easy to handle on the way (Whiteley, 1971). The objective of this study was to determine the physical characteristic and palatability of corn plant waste after formed as a biscuit feed.

Materials and Methods

The experiment used eighteen heads of thin tail sheep with the average body weight was around 21.66 ± 0.87 kg. The experimental sheeps were maintenance individually.

Experimental design

The experimental design used in this research was Completely Randomized Design with six treatments and three replications, the treatments were biscuit composition i.e: R1= 100% field grass, R2= 50% field grass + 50% corn leaf, R3= 100% corn leaf, R4= 50% field grass + 50% corn husk, R5= 50% corn leaf + 50% corn husk, R6= 100% corn husk. The data method used was Analysis of Variance. The differences among treatments were examined with orthogonal contrast test (Steel and Torrie, 1993). The variables that would be measured were:

a) Water Activity. Aw meter is calibrated prior to use a solution of barium chloride (BaCl2). Measurement of water activities conducted by entering the feed biscuits to the Aw meter and left for 1 hour and then do the reading.
b) Moisture content of biscuits made by weighing a sample of about 3 grams of feed as the initial weight. The sample is dried in an oven at a temperature of 105 ° C until constant weight. Water content were calculated using the formula (SNI, 1991):

$$\text{Mouisture} = \frac{\text{Initial weight biscuit feed(g)} - \text{Ovendry weight (g)}}{\text{Initial weight biscuit feed (g)}} \text{ x } 100$$

c) Water absorption. Measurement of water absorption is done by measuring the sample weight before and after the biscuit feed water immersion for 5 minutes. Water absorption were calculated using the formula :

$$\text{WA (\%)} = \frac{B2 - B1}{B1} \text{ x } 100$$

Information :
WA= Water absorption(%)
B1 = Weight sample of dry air biscuit feed a(g)
B2 = Weight sample of biscuit feed after immersion(g)

d) Density were calculated using the formula (Widarmana, 1977):

$$K\ (g/cm3) = \frac{W}{\pi r2 \text{ x } T}$$

Information:
K = Density (g/cm^3)
W = Weight sample biscuit feed (g)
T = Thick sample biscuit feed (cm)
Π = 3,14
r = radius of biscuit feed (cm)

e) Palatability test is based on the modification of the method Kaitho et al. (1997) with adaptation for 5 days and measurement of palatability tests for 2 days. Provision of biscuits made from 06:00 to 12:00 am as much as 100 grams. The results of the preference of animal can be identified by a reduction in the provision of biscuits with remaining biscuits. Palatability test was modified from previous research refers to research Kaitho (1997).

Result and Discussion

The color of corn leaf biscuit greener than the field grass biscuit and corn husk biscuit have a more brown color. Differences of each treatment biscuits can be seen in Table 1. The color of the field grass and biscuits corn plant waste generated varies between green and brown as well as a combination of both. The difference is due to the differences in the use of forage for biscuits. Although there are differences in color, the sheep could not distinguish colors because the sheep are color blind (Pond et al., 1995). However, the sheep still eat biscuits was given because of the nutrient factor required. After heating and pressing processes, biscuits produced generally have a brown color. Brown color is due to the browning reaction in non enzimatis the reaction between organic acids with reducing sugars and amino acids between the reducing sugars or maillard reactions occur causing caramel odor due to heating

Table 1. General Characteristic of Biscuit Field Grass and Corn Plant Waste

Biscuit	Texture	Density	Color	Odor
R1	Rough	Compact	Brownish green	Fragrant
R2	Rough	Very crumb	Brownish green	Fragrant
R3	Rough	Crumb	Green	Fragrant
R4	Rough	Compact	Greenish brown	Fragrant
R5	Rough	Compact	Greenish brown	Fragrant
R6	Rough	Very Compact	Brown	Fragrant

Information : R1= 100% filed grass; R2= 50% field grass + 50% corn leaf; R3= 100% corn leaf; R4 = 50% field grass + 50% corn husk; R5= 50% corn leaf + 50% corn husk; R6= 100% corn husk.

of feed materials (Adawyah, 2007; Winarno, 1992). The diameter of biscuit is 7 cm with a thickness of 1 cm can be eaten by the sheep.

Nutrient Composition of Biscuit Field Grass and Corn Plant Waste

Biscuit of R3 (corn leaf biscuit) have a fairly high protein content which reached 16.12% in contrast to biscuit of R1 (field grass biscuit) which has the lowest crude protein content (12.89%) than other treatments.

The high crude fiber in all the biscuits with a range of 27.25% -42.49% indicates that biscuit field grass and corn plant waste can sufficient requirement for ruminants because it has a crude fibers more than 20%. Biscuits of R4 (a combination of field grass with corn husk) has a crude fiber content of the highest of 42.49%. High levels of crude fiber is most likely caused by the thick cell walls of plants from a field grass and corn husk (Roslinda and Afdal, 2005), that was indicated by the high crude fiber on a biscuit of R1 and biscuits of R6, which reached 41.33 % and 38.12%.

Physical Characteristic of Biscuit

Water Content. Water content in this study ranged from 11-12%. The results of variance showed that differences in the use of feed highly significant ($P < 0.01$) affect the water content of biscuits. Biscuit 100% corn leaf have the highest water levels (R3), and this is because the biscuit have fewer cavities so that the evaporation is slow. Biscuits R2 has the lowest water content (11.06%) because they have more cavities and large that evaporation is running fast, this is indicated by a very crumb texture. In addition, the differences that occur are influenced by differences in initial water content of the raw materials used. Retnani *et al.* (2009) mention the wafer with the composition of the field grass has a cavity less evaporation occurs more slowly. According to SNI (1992) water content of feed should not be more than 14%. Water content in this study ranged from 11-12%. Thus, biscuit is more durable and can storage for long period.

Water Activity. Water activity to determine the minimum limits of microorganisms that can grow in feed. The result of analysis of variance showed that there were not significant different in the use of feed ingredients affect the activity of water. Water activity in the study ranged from 0.69-0,70. Water activity is one important factor in determining the quality of feed. Activity of microorganisms and enzymes can be control at 0,70 of water activity, so the feed is not easy to mold (Syarief and Halid, 1993).

Water Absorption. Biscuits of field grass and corn plant waste in this study provide significant effect ($P < 0.05$) on water absorption. Based on the results of orthogonal contrasts, the average value of the lowest water absorption found in biscuits of R2 (a

combination of field grass with corn leaf), while the higher water absorption occurs in the biscuit of R1 (field grass), R4 (a combination of field grass with corn husk) and R5 (corn husk combination with corn leaf). The biscuits of R4 (a combination of field grass with corn husk) has the highest water absorption which is 514.48% and it can be interpreted more compact and hard enough, but has a good ability in the process of softening by saliva during chewing by ruminant and easily inflate and be easily degraded by rumen microbes, thereby increasing the rate of emptying of the rumen (Siregar, 2005).

Biscuits of R4 (a combination of field grass with corn husk) has a high crude fiber content that is equal to 42.49% and has a rough texture. The high crude fiber content showed that the biscuits are capable of binding water because of the OH bond in water with the fibers on the biscuits. Siregar (2005) stated that there is a positive relationship between water absorption fraction of particles with chemical composition of the crude fiber.

Density. The density of fiber-rich feed ingredients have a highly variable value (Toharmat *et al*., 2006). The results of analysis variance showed no significantly different effect on density, it can be said that the use of different treatment having the same effect on the density of biscuits. The range value of density between 0.44 -0.52 g/cm^3 to 0.47 g/cm3 average. However, it is descriptive it can be seen that the lowest density of biscuits (0.44 ± 0.03 g/cm3) contained in the biscuit of R2 (a combination of field grass with corn leaf) and the highest (0.52 ± 0.03 g/cm^3) found in biscuits of R5 (corn husk combination with corn leaf).

Table 2. Average Consumption of Biscuit Field Grass And Corn Palant Waste

Biscuit	Palatability Test Period	
	Fresh ingredients	Dry Matter
	----------g/head/6 hour----------	
R1	83,33	74,68
R2	70,83	61,75
R3	73,33	64,25
R4	68,33	60,89
R5	68,33	59,7
R6	61,67	54,7
Total	425,83	375,95
Average	70,97	62,66

Information : Different superscripts in the same column indicate significantly different (P <0.05).
R1= 100% filed grass; R2= 50% field grass + 50% corn leaf; R3= 100% corn leaf; R4 = 50% field grass + 50% corn husk; R5= 50% corn leaf + 50% corn husk; R6= 100% corn husk.

Palatability

Palatability is describe of the characteristic of the feed such as mirrored by organoleptic appearance, smell, taste (sour, salty, sweet, bitter), texture and temperature, giving rise to the stimulation and attraction of livestock to consume (Yusmadi *et al*., 2008). According Umiyasih *et al*. (2008) corn leaf and corn husk have a high palatability. However, the results of the study showed that the average palatability is high enough from the period of adaptation to the testing period (Table 2). Descriptively, palatability of R1 and R3 biscuits have a highest value of 83.33 g/head/day and 73.33 g/head/day or 74,68 g/head/day to 64,25 g/head/day in dry matter.

Conclusions

Biscuits containing field grass and corn leaf was preferred by sheep.

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Optimizing Vitamin-Mineral Supplementation in King Grass-Based Rations to Maximize Productivity of Bali Cattle

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Abstract

Bali cattle have a great potency to supply national meat demand which is increasing progressively every year. The main constrain in Bali cattle farming is the deficiency of trace minerals on native grass resulting in low Bali cattle productivity. The present study was done to determine the optimum vitamin-mineral supplementation in King grass-based rations to maximize productivity of Bali cattle steers. Randomized Complete Block Design used in this study consisted of four treatments and five groups based on differences in live weight cattle. Treatments consisted of: S0 = concentrate as much as 5 kg + King grass given ad libitum, S1, S2, and S3 = S0 successively added 0.1%, 0.2% and 0.3% vitamin-mineral in concentrate. Variables observed were nutrients intake, deposition of nutrients, energy retention, live weight gain of the animals, and feed efficiencies. The data were analyzed by analysis was of variance, and regression analysis used to predict the optimal level of supplementation. Results showed that vitamin-mineral supplementation significantly (P <0.05) affected all the observed variables. Supplementation levels of 0.2 to 0.3% can reduce the consumption of nutrients, but supplementation levels of 0.1 to 0.3% increased deposition of nutrients, energy retention, feed efficiency, and increased a live weight gain of Bali cattle steer up to 14% (0.58 vs. 0.66 kg/ day) compared to those cattle without supplements. It is concluded that vitamin-mineral supplementation of 0.1 to 0.3% in ration based on King grass can increase deposition of nutrients, energy retention, feed efficiency, and live weigh gain of Bali cattle steers. Based on regression analysis, it is obtained that the optimum level of vitamin-minerals supplementation in concentrate was 0.16% which can produce maximum live weight gain of Bali cattle steer fed King grass-based rations.

Keywords: bali cattle, supplementation, vitamin-mineral

Introduction

Bali cattle has a great potency to supply the national meat demand which is increasing progressively every year. However, the main constraint of fattening Bali

cattle agribusiness is the limited forages available for the farm and trace mineral deficiency in the native grass.

King grass and concentrates are usually given to cattle by farmers in Bali, but still need to be supplemented with minerals to maximize the productivity of cattle because plants of tropical feed are deficient in trace minerals (Kaunang, 2004). Therefore, the productivity of Bali cattle can still be improved by feeding an adequate and balanced nutrients .

The present study was done to determine the optimum vitamin-mineral supplementation in King grass-based rations to maximize productivity of Bali cattle steers.

Materials and Methods

This study consisted of a series of field and laboratory experiments. Field trial was conducted in the Serongga village, Gianyar regency. Laboratory analysis was conducted at the Lab. of Nutrition, Fapet - Unud, and Lab. of Analytical Udayana University.

The experiment was conducted in individual cages. Cage is designed to meet the maintenance requirements of fattening Bali cattle. Required 20 individual cages to accommodate 20 steers of Bali cattle with an average live weight of 319 kg or with the range of 279-367 kg steers.

Ration treatments consisted of King grass and concentrates supplemented with pignox (commercial product as a source of vitamins and minerals). There were four treatments in concentrate rations i.e., S0 is concentrate without pignox addition, while concentrate on the S1, S2, and S3 supplemented with 0.1%, 0.2% and 0.3% pigox (Table 1).

Table 1. Nutrient content of diets

Nutrient	Concentrates				King grass
	S0	S1	S2	S3	
Dry matter (%)	87.64	87.64	87.64	87.64	24.80
Organic matter(%)	64.13	64.13	64.13	64.13	71.84
Crude protein (%)	12.13	12.13	12.13	12.13	5.01
Crude fiber (%)	7.76	7.76	7.76	7.76	27.20
Energy (GE. Mcal/kg)	3.22	3.22	3.22	3.22	3.39
Sulfur (S. ppm)	685.50	694.09	702.68	711.28	-
Zinc (Zn. ppm)	45.09	65.09	85.09	105.09	26.12

Description: S0, S1, S2, and S3= vitamin-mineral supplementation in concentrate with 0%, 0.1%, 0.2% and 0.3% pignox, respectively.

Variables observed were nutrients intake, deposition of nutrients, energy retention, live weight gain of the animals, and feed conversion ratio Energy retention was calculated by deposition of nutrients and deposition of nutrients was calculated by converting live weight gain with body composition (by urea space technique). Based on this nutrient deposition, it can be calculated the energy retention with the provisions of 1g of fat deposition equivalent to 9.32 kcal, while the deposition of 1 g protein equivalent to 5.5 kcal (Ørskov and Ryle, 1990). So the retention of energy per day per cattle can be calculated by summing the energy content of the deposition of body fat and protein per cattle per day.

The data obtained were analyzed by analysis of variance, and regression analysis used to predict the optimal level of supplementation (Steel and Torrie, 1986).

Results and Discussion

Vitamin-mineral supplementation significantly ($P < 0.05$) affected dry matter intake, protein intake, energy intake, deposition of nutrients, energy retention and live weight gain of Bali cattle (Table 2).

There is a clear relationship between vitamin-mineral supplementation with live weight gain of Bali cattle, following regression quadratic equation: $Y = 0.583 + 0.959\ X - 2.95\ X^2$ with a coefficient of determination (R^2)= 0.414* with the understanding of X= supplementation of vitamin-mineral (%), Y= live weight gain of Bali cattle (kg/day) as seen in Figure 1. From this regression equation can be pre-

Table 2. Dry matter and nutrients intake, nutrients deposition, retention of energy and live weight gain of Bali cattle fed King grass-based rations with vitamin-mineral supplementation

Variables	Supplementation Treatment			
	S0	S1	S2	S3
Dry matter intake (kg/h/d)	6.65^b	6.58^b	6.29^a	6.18^a
Protein intake (g/kgW$^{0.75}$/d)	7.92^b	7.87^b	7.44^a	7.32^a
Energy intake (Kcal/kgW$^{0.75}$/d)	271.52^b	267.47^b	254.54^a	247.33^a
Protein deposition (g/ kgW$^{0.75}$/d)	1.18^a	1.31^b	1.30^b	1.19^a
Fat deposition (g/ kgW$^{0.75}$/d)	2.6^a	3.0^b	2.9^b	2.7^a
Retention of Energy (NEp. Kcal/kgW$^{0.75}$/d)	31.14^a	34.92^b	34.64^b	32.06^a
Feed Conversion Ratio (FCR)	11.48^b	10.06^a	9.68^a	10.18^a
Live weight gain (kg/d)	0.58^a	0.66^b	0.65^b	0.61^a

Values with different superscript in the same line means significantly different ($P<0.05$).
NEp= Net energy for production.

dicted the optimum vitamin-mineral supplementation was 0.16% which produces the maximum live weight gain of Bali cattle of 0.66 kg/day.

Rations tested in this study met the nutrient requirement which consisted of 5 kg concentrate and an average of 15 kg king grass per cattle per day, so that these rations contained dry matter (DM) 40.51%, crude protein (CP) 10.31%, and energy (GE) 3.27 Mcal/kg, equivalent to 56.29% of TDN (Total Digestible Nutrients). However, the results of this experiment showed that feed intake and nutrient levels such as dry matter and energy significantly ($P<0.05$) decreased when given vitamin-mineral supplementation in concentrate, especially at supplementation of 0.2 to 0.3% (Table 2). This decline in consumption may be caused by an imbalance of nutrients in diet, especially minerals. Decrease in consumption level has not yet led to nutrient-deficient cattle and this can be proved by live weight gain of cattle during the experiment, although feed intake decreased with increasing levels of vitamin-mineral supplementation.

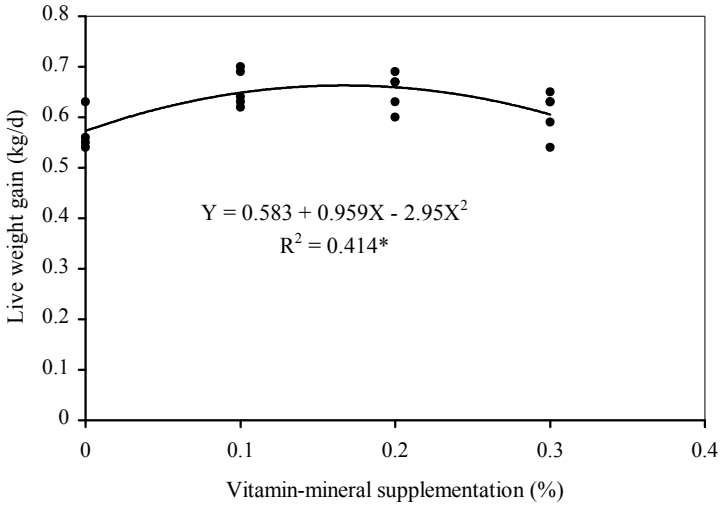

Figure 1. The relationship between vitamin-mineral supplementation with live weight gain of Bali cattle fed King grass-based rations

Vitamin-mineral supplementation from 0.1 to 0.2% in concentrate gave a positive influence on energy utilization and productivity of Bali cattle fed King grass-based rations which were characterized by higher energy retention and live weight gain (Table 2). This was supported by higher levels of ammonia ($N\text{-}NH_3$) and propionic acid, lower methane emission and higher rumen microbial protein synthesis at the level of supplementation from 0.1 to 0.2%. Rumen microbial protein as a major source of amino acids for the host animals, so the higher rumen microbial protein production of higher protein deposition in the body of cattle. Meanwhile, the lower the methane gas production means less energy is wasted so that more energy is stored in the form of animal protein and fat (Partama *et al*., 2010).

High deposition of nutrients, energy retention and live weight gain in cattle with vitamin-mineral supplementation of 0.1% due to the sufficient and balanced nutrients in the ration. Concentrate with a vitamin-mineral supplementation of 0.1% containing balanced ratio of N:S, and contains enough minerals Zn and S (Table 1). S is an essential mineral in amino acids synthesis contains sulfur, and is needed in large numbers for microbial protein synthesis. Meanwhile, Zn minerals involved in metalo enzyme synthesis such as DNA and RNA polymerase, alkaline phosphatase, amylase and neutral protease (Jouany, 1991).

The research was supported by N-NH_3 concentration sufficient to support the relatively high concentration of VFA (from 166.33 to 198.06 mM), consequently would strongly support the rumen microbial protein synthesis (Partama *et al*., 2010). Stern *et al* (2006) states that the rumen bacteria can use protein and carbohydrates as energy sources. Carbohydrate is the main energy source for bacteria, and can also be used as a carbon skeleton that combines with ammonia (NH_3) to rumen microbial protein synthesis.

Vitamin-mineral supplementation of 0.1% in concentrate gave the best effect in productivity of Bali cattle fed King grass-based rations. This shows that supplementation at the level of 0.1% pignox in the rations contained enough nutrients and balanced. Mineral content of Zn in concentrate was 65.09 ppm (Table 1), slightly higher than the recommendation of Georgievskii (1982), i.e., 40-60 ppm due to differences in cattle breeds used.

Conclusions

Vitamin-mineral supplementation at 0.1 to 0.3% in rations based on King grass can increase deposition of nutrients, energy retention, feed convertion ratio, and live weigh gain of Bali cattle steers. Based on regression analysis, the optimum level of vitamin-minerals supplementation in concentrate was 0.16%.

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Performance and Milk Quality of the Lactating Dairy Cow Consuming *Ganoderma lucidum,* Organic Chromium and CLA as Feed Supplement

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Abstract

The aim of this study was to evaluate the use of feed supplement containing Ganoderma lucidum (GL), orgCr (organic Chromium) and CLA (Conjugated linoleic acid) from roasted soy bean by measurement of performance and milk quality of the lactating dairy cow. Two experiments were carried out in different time based on randomized block design. The first experiment used nine of the lactating dairy cow that consume three rations: R0: control; R1: R0 + GL; R2: R0 + GL + orgCr. The second experiment used twelve of the lactating dairy cow that consume four rations: P0: control; P1: P0 + GL + CLA; P2: P0 + CLA + orgCr; P3: P0 + GL + CLA + orgCr. The parameters for cow performance were consumption, average of daily gain, digestibility and milk production; while milk quality were protein, fat and total solid of milk. The first experiment results were the use of R2 increased as much two times of average of daily gain, 16% of milk production and 22% of milk fat. The second experiment results were P1 significantly increased 13% of the consumption and 18% of digestibility ($P<0.05$). The highest of milk production 4% FCM (9.09 kg/d) was achieved by consume P3 in which milk fat, protein and total solid significantly increased 29%, 21% and 7%, respectively from control ($P<0.05$). As conclusion, the performance and milk quality of the lactating dairy cow could be improved by addition of feed supplement containing Ganoderma lucidum, organic Chromium and roasted soybean as a source of CLA.

Keywords: chromium, CLA, dairy cow, Ganoderma lucidum, milk

Introduction

Good management of rearing the lactating dairy cow is an important step for continuity farm to meet high quality product. The animal in this period should get adequate feed to fulfill the requirement. There are some conditions affect to the quantity and quality of milk production such as low quality and availability of forages, insufficient amount and quality of concentrate, and the climate change. To maintain the stability of its production, there is one alternative way such as feeding management control by giving the feed supplement in certain circumstances like in transition and lactation periods. Feed supplement should be appropriate to its function in animal body. One of feed supplement which popular for human is fruiting body of *Ganoderma lucidum* mushroom. This mushroom having high capability as one of pharmacological substances like immunomodulator due to containing ganoderic acid, triterpenoid, β-D-glucan, etc, that function to improve the immunity system of human (Jin, 2000; Sjabana, 2001).

In our previous research, when sheep consumed 15-30% of *G. lucidum* mycelium for one month, blood lymphocyte increased around 24% compared than control, consumption of dry matter, organic matter and nitrogen retention also increased, but the average of body weight gain decrease by increasing of mycelium in rations (Amirroenas *et al.*, 2002). Based on those results, in this research the use of *G. lucidum* as feed supplement was enriched by addition of organic Chromium. Moreover, source of CLA (*conjugated linoleic acid*) which made from roasted soy bean was added also to the supplement for improving the animal productivity. The aim of these researches were to evaluate the use of feed supplement containing *Ganoderma lucidum* (GL), orgCr (organic Chromium) and/or CLA through measurement of performance and milk quality of the lactating dairy cow.

Materials and Methods

Two experiments were carried out in different time which each of experiment used randomized block design for 8 weeks. Both of experiments used similar type of dairy cow in different number. In the first experiment, nine of the lactating dairy cow with body weight average 392.74 $\pm$ 22.7 kg and 3–9 liter milk production per day were used, and in the second experiment, twelve of the lactating dairy cow with body weight average 380-450 kg and 3–16 kg milk production day were used.

In daily rearing, the cow given the basal ration that suitable to their lactation periods. Both of the experiments used supplement substances such as the fruiting body of *Ganoderma lucidum* and organic Chromium (orgCr), while CLA (*conjugated linoleic acid*) was used in the second experiment only. There were two kinds of substrate that used for producing of organic Chromium (orgCr). To produce orgCr in the first experiment, an-organic Chromium was incorporated into rice straw as a

substrate in fermentation process using *Ganoderma lucidum,* while another orgCr in the second experiment was produced by similar way with soy bean as a substrate in fermentation process using *Rhizopus sp*. For the second experiment, source of CLA was obtained by dry frying the soy bean in 15 minutes. All of supplement substances were added to the lactating dairy cow rations with composition as below, R0= basal ration as a control; R1= R0 + *Ganoderma lucidum* (GL); R2= R0 + GL + orgCr, for the first experiment, and P0= basal ration as a control; P1= P0 + GL + CLA; P2= P0 + CLA + orgCr; P3= P0 + GL + CLA + orgCr, for the second experiment. Doses of supplement substances were GL 100 ppm/day, orgCr 3 ppm/kg DM ration, and CLA 5% of ration total fat. All rations fulfilled the requirement of 14% crude protein and 67% TDN for the lactating dairy cow, where ratio of concentrate and forage was 50:50. For both of experiments, two kinds of parameter were measured such as performance that consisted of dry matter consumption (DMC), average of daily gain (only in the first experiment), dry matter digestibility (DMD) and milk production. The other parameter was milk quality that consisted of protein, fat, total solid and solid non fat. The results data of experiment were analyzed by ANOVA of randomized block design and Duncan's test (Steel and Torrie, 1993).

Results and Discussion

The results of feed supplement effect of ration containing *G. lucidum* (GL), orgCr and CLA to the performance and milk quality of the lactating dairy cows in difference time of experiment were written in Table 1. In the first experiment, addition of GL and also orgCr (R1 and R2) showed no different effect to dry matter consumption (DMC) and dry matter digestibility (DMD), but significantly increased around 13% of DMC and 18% of DMD when roasted soy bean were added to the supplements (P1 and P2) ($P<0.05$). Linoleic acid from soy bean can be used as a precursor of CLA in the rumen. Or-Rashid *et al*. (2011) reported that the major isomer of CLA synthesized from linoleic acid was 9*c*11*t*-CLA isomer and the second largest was 10*t*12*c* produced predominantly by the bacterial suspension (12 hours of incubation in rumen). Bacteria and protozoa can hydrogenize CLA isomer of stearic acid (18:0) through the reduction of 18:1 isomers. However, lipids including polyunsaturated fatty acids (PUFA) that present in ruminant forage/ration undergo extensive hydrolysis and biohydrogenation in the rumen. Hence, in the presence of PUFA at 50 μg ml^{-1} inhibited grow of cellulolytic bacteria. Toxicity to growth was ranked EPA (eicosapentaenoic acid) > DHA (docosahexaenoic acid) > LNA(alpha linolenic acid) > LA (linoleic acid) (Maia *et al.*, 2007). Increasing of digestibility was corresponding to increasing of the consumption. In addition of CLA together with orgCr and *G. lucidum* in P2 and P3, there were occurred slight decreasing of digestibility also consumption.

In the first experiment, there was two times increasing of the average daily

gain when dairy cows consumed R2. This increasing might affected by addition of chromium (Cr) to the supplement. As a *Glucose tolerance factor* (GTF), Cr will interact to insulin and cell receptor, then induce glucose enter cell, after that change to energy then used for protein synthesis, maintaining cell and growth of tissue in the body (Vincent and Davies, 1997).

There was no different effect of GL addition (R1) in the ration to milk production and milk production in 4% FCM than control, but increasing percentages of those milk parameters were occurred in addition of orgCr in R2 as much 16% and 30%, respectively. These results were more increase in the second experiment by addition of orgCr and CLA in P1 (72% and 80%), P2 (85% and 102%) and P3 (91% and 112%), and also were higher than Adawiah (2006) when used similar amount of roasted soy bean as a source of CLA in the ration, and found increasing of milk production and production of 4% FCM on dairy cow were 14.96% and 17.79%, respectively than control.

In case of milk quality especially in solid non fat for both of experiments, there were no different effect, also in milk protein and total solid (R1 and R2), but increased by addition of orgCr (P2 and P3). Milk protein and solid non fat from the first experiment were suitable to SNI 01-3141-1998 (2.7% and 8.0%), but their percentages were higher in the second experiment. Increasing of milk protein of

Table1. Effect of the feed supplement containing *Ganoderic lucidum*, organic Chromium and CLA to performance and milk quality of the lactating dairy cow

Parameters	The First Experiment			The Second Experiment			
	R0	R1	R2	P0	P1	P2	P3
Performance:							
Consumption (kg/d)	14.60±0.46	14.31±0.60	14.65±0.33	7.44±0.32^{c}	8.45±0.69^{a}	8.35±0.43^{a}	7.87±0.65^{b}
Digestibility (%)	75.10±0.8	78.80±1.0	78.20±0.5	49.40±0.5^{c}	58.20±0.8^{a}	57.80±0.5^{a}	55.20±0.7^{b}
Avg of daily gain (kg/d)	-0.03±0.48	-0.11±0.30	0.03±0.34	-	-	-	-
Milk production (l/d)	5.43±1.93	5.28±3.08	6.30±4.24	3.97±2.12	6.81±4.20	7.34±5.14	7.60±2.38
Milk production 4% FCM (kg/d)	5.20±1.77	5.41±2.41	6.74±4.43	4.28±2.01	7.72±4.81	8.65±6.00	9.09±2.83
Milk Quality:							
Protein (%)	2.90±0.60	2.50±0.30	2.70±0.10	3.40±0.60^{b}	3.80±0.20^{a}	4.00±0.10^{a}	4.10±0.50^{a}
Fat (%)	3.70±0.60	4.50±1.00	4.60±0.20	4.10±0.20^{b}	4.80±0.30^{b}	5.20±0.10^{a}	5.30±0.90^{a}
Total Solid (%)	12.00±0.80	12.30±1.00	12.60±0.20	12.40±0.80^{b}	12.80±0.30^{b}	13.10±0.40^{a}	13.30±0.80^{a}
SNF (%)	8.20±0.30	7.70±0.20	8.00±0.10	8.30±0.90	8.00±0.30	7.90±0.40	8.00±0.60

Different superscript in the same line means significantly different ($P<0.05$).
R0= basal ration as control; R1= R0 + Ganoderma lucidum (GL); R2= R0 + GL + orgCr;
P0= control; P1= P0 + GL + CLA; P2= P0 + CLA + orgCr; P3= P0 + GL + CLA + orgCr

P1, P2 and P3 were 11.8, 17.6 and 20.6%, respectively. Those results in this experiment were higher than 11.44% when used the similar amount of roasted soy bean (Adawiah, 2006).

In both of experiments, milk fat showed higher than SNI (3.0%). The addition of GL and orgCr increased the milk fat in R1 (21%) and R2 (22%) than control (R0), and were higher when added by orgCr and CLA in P1 (17%), P2 (27%) and P3 (29%) than control (P0). In this case, those results also were higher than 18.54% that found by Adawiah (2006). There are two substances affect to the quality of milk i.e. orgCr and source of CLA. Cr caused increasing of nutrient flow rate from the blood to the cell, availability of substrate for milk production also increase by addition of orgCr and then facilitate the insulin to activate some enzyme for synthesize of milk component (Pechova and Pavlata, 2007; Mertz, 1977). Addition of roasted soy bean as CLA source in the ration, support increasing of protein and energy sources. By roasted process might protect the soy bean from degradation by rumen microbes then absorbed in the digestive tract post rumen and transfer the nutrients for milk synthesize (Adawiah, 2006). From those phenomenon's suggesting the use *G. lucidum* as a supplement should be added by orgCr and/or CLA to increase the milk production.

Conclusions

The conclusion of two experiments were the feed supplement containing *Ganoderma lucidum* should added by the organic Chromium-Tempeh and roasted soy bean as source of CLA (*conjugated linoleic acid*) to achieve the highest of performance and milk quality on the lactating dairy cow.

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III. ANIMAL MANAGEMENT AND PRODUCTION

Factors Affecting to Biosecurity Adoption on Laying Hen Farmers

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Abstract

The present study was undertaken to identify the factors that influence biosecurity adoption on laying hen farmers. Sidrap district, South Sulawesi province was chosen as the place for the research because beside it was famous as the center of laying hen farms, it also became one of six districts in South Sulawesi which suffered from Avian influenza outbreak in 2005. Total sample were 60 respondents. The sample were choosen through random sampling from two subdistricts which were the most populous of layer smallholders, namely Baranti and Maritengngae. Data were obtained through observations and interviews using a questionnaire. Data were analyzed using a score based on biosecurity status. Biosecurity status was obtained based on the adoption of biosecurity measures which consisted of 9 stages: farm inputs, traffic onto farms, distance from sources of pathogens to shed, exposure of farm, biosecurity at farm boundary, biosecurity between farm boundary and shed, biosecurity at the shed door, traffic into the shed and susceptibility of the flock. Multiple regression model was employed to analyse the data. The study revealed that the adoption biosecurity were associated with gender, age, education, farming experience, farm-income, family size and social capital. These variables contributed 20.00% variation in biosecurity adoption on laying hen farmers. However, only farm income, family size and social capital were the major factors affecting to the adoption of biosecurity ($P \leq 0.05$).

Keywords: adoption, biosecurity, farmers, laying hen

Introduction

Laying hen is one of animal protein sources in the form of meat and egg. The Indonesian government has implemented various programs to increase the production of chicken to fulfill the demand for meat and egg which always increase in relation to the population growth. However, the implementation of the program faces problems, one of them is Avian influenza outbreak. To overcome these problems,

biosecurity measures has been declared to be applied to poultry farms in all provinces in Indonesia.

Biosecurity is the key of the poultry industry in preventing the spread of disease and infections. Biosecurity is made up of three components: segregation, cleaning and disinfection (FAO, 2008). Adoption is a a mental process through which an individual passes from hearing about an innovation to its adoption (Rogers, 1962 in Karki and Bauer, 2004). There are five of adoption stages namely: awareness, interest, evaluation, trial and error, and the last is adoption (Ban and Hawkins, 1999).

There are number of factors that influence the extent of adoption of technology, such as characteristics of attributes of technology, the adopters or clientele, which is the object of change, the change agent (extension worker, professionals, etc); and the socio-economic, biological and physical environment in which the technology take place (Cruz, 1987 cited by Chi and Yamada, 2002). Eze and Okudu (2008) stated that farm income, stock and educational levels were the most valuable variables determining the poultry farmers technology adoption potential. The classification performance of the model was 83.33%. Olele and Emah (2007) found that level of eduction, age of farmers, farm size, farm income and extension contact were the major determinants of fish production technologies adoption at 0.05 level of significance. Teklewold *et al.* (2006) argued that farmers' decision on adoption of poultry technology was positively affected by sex of the household head, family size, availability of supplementary feed, credit and extensions service and extent of expected benefit from poultry and negatively affected by market problem. Munasib and Jordan (2011) concluded that community involvement had positive effect on the decision to adopt sustainability agricultural practice, and it also had a positive effect on the extent to which farmers adopt these practices. Padmaja and Bantilan (2008) stated that build up social capital played an important role in influencing impacts from the technology because of the ways in which social network and social relationship facilitated technology disemination.

Sidrap district is famous as the most populous of layer farms in South Sulawesi province. Total layer smallholders were 1,334 with the population were 3,439,556 chickens (Dinas Peternakan Kabupaten Sidenreng Rappang, 2011). In 2005, Sidrap district became one of six districts in South Sulawesi province which suffer from Avian influenza outbreak and affects to several loss from their layer farms (Kristanti, 2009). This survey was conducted in Sidrap district South Sulawesi province to know factors affecting adoption of biosecurity measures on laying hen farmers.

Materials and Methods

In this paper, the multiple regression model was used to determine the factors influencing the adoption of biosecurity on laying hen farmers. The adoption level

was calculated from Farm Biosecurity Status Score (FBSS) adopted from Patrick and Jubb (2010). The dependent variable was the adoption index which was exspressed as a percentage of adoption level measures out of a specific maximum of bioseurity measures (Rahman, 2007). The empirical model was specified as:

$$Yi = \beta o + \beta 1GENDERi + \beta 2AGEi + \beta 3EDUCi + \beta 4EXPRi + \beta 5SOCAPi + \beta 6FAMSIZEi + \beta 7FARMINCi + \varepsilon i$$

- Yi was the dependent variable. It was expressed as a percentage of biosecurity measures adopted out of 9 risk stages. The independent variables used in the model with their expected signs are presented below:
- GENDER: was expressed as a binary variable with 1 if the farmer was male, 0 otherwise. Expected sign for gender was ambiguous.
- AGE: was expressed as the length of their life (year). Age was assumed to have negative effect on adoption.
- EDUC: was expressed as the periode of farmers have formal education (year). Education was hypothesized to have a positive effect on adoption.
- EXPR: was expressed as the length of farmers took care of their poultry (year), experience was assumed to have a positive effect on adoption.
- SOCAP: was expressed as farmers' trust with their community (score), social capital was assumed to have a positive effect on adoption.
- FAMSIZE: was expressed as number of family of farmers (person), family was assumed to have a positive effect on adoption.
- FARMINC: was expressed as amount of revenue from chicken and egg selling, income was assumed to have a positive effect on adoption.

Total sample were 60. Ten percent (10%) of the sample were choosen from two subdistricts with the most populous layer smallholders, namely Maritengngae and Baranti subdistricts which had total population of 600 layer farmers. The survey was conducted by trained enumerators in 2010. A pre-test questionnaire with closed-ended questions was used to capture information from laying hen farmers on socio-economic characteristics such as farmers characteristics, the farm, and adoption of biosecurity measures including 9 stages. The multiple regression model were estimated using SPSS version 16.

Results and Discussion

Adoption index

Adoption index was expressed as a percentage of measures out of a specific maximum of bioseurity measures. The research revealed that the most highly adopted of biosecurity measures was traffic onto the farm (75.2%). While the least adopted of biosecurity measures was biosecurity at farm gate (42.1%). The mean adoption

index was 63.4%, implies that 6 out of 10 laying hen farmers had adopted biosecurity measures. This index was higher than that of Musaba (2010) and Rachman (2007) findings which was 56.0% and 55.87% respectively.

Results of the multiple regression analysis

The multiple regression analysis was performed to know factors that affect the adoption of biosecurity measures by laying hen farmers. The result of the regression analysis was presented in Table 1.

The coefficient of determination (adjusted R-square = 0.20) indicated that 20.0% variation in the overall adoption index of biosecurity measures could be explained by seven independent variables included in the model. The results in Tabel 1 showed all the coefficients have the expected signs, except education and family size. Only those coefficients associated with statistically significant variables at the 5-percent level were discussed.

The coefficient of farm income was found to be significant ($P \leq 0.05$) and positively related to adoption level. Controlling other factors, the coefficient was 2.35. This mean that the addition farm income by Rp 1,- would increased adoption of biosecurity by 2.35%. This findings were accordance with those of Eze and Okudu (2008), Olele and Emah (2007), and Supradit *et al*. (2006).

The coefficient for family size was found to be significant ($P \leq 0.05$) and negatively related to adoption level. Controlling for other factors, the coefficient was -2.78. This mean that the addition of 1 person of a family, would reduced 2.78% adoption rate of biosecurity. A negative sign for family size suggested that adoption was higher among smaller family size. This might because larger family size would increase the spread of disease than the small ones. As it was known that human ac-

Table 1. Multiple regression analysis of factors affecting to adoption of biosecurity measures on laying hen farmers

Variables	Standarized coefficients	Standar Error	T-value	Sig
Constant	46.53	16.99	2.74*	0.01
Gender	0.20	6.30	1.61	0.11
Age	- 0.27	0.12	- 1.91	0.06
Education	- 0.01	0.33	- 0.02	0.99
Experience	0.20	0.22	1.68	0.10
Farm income	0.33	0.00	2.35*	0.02
Family size	- 0.34	0.89	- 2.78*	0.01
Social capital	0.26	0.22	2.18*	0.03

Adjusted R^2= 0.20; F= 3.16*; N= 60; * significance level at 5%.

tivities were the main route for the spread of the virus (Bleich *et al*., 2009). It was implied that the less the people entered the farm, the less the spread of the virus. This results contrast with that of Teklewold *et al*. (2006).

Social capital has a significant ($P \leq 0.05$) positive effect on adoption of biosecurity measures. Controlling for other factors, the coefficient was 2.18. This entails that increased social capital by 1%, can lead to increased adoption of biosecurity measures by 2.18%. This findings were accordance with those of Munasib and Jordan (2011) and Padmaja and Bantilan (2008).

Conclusions

Econometeric analysis using multiple regression model showed that biosecurity adoption was positively affected by farm income and social capital, however negatively affected by family size ($P \leq 0.05$). The other factors namely gender, age, education and farm experience had no significant effect on adoption of biosecurity measures in the study area.

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The Perception of Beef Cattle's Farmers on Implementation of Artificial Insemination in Three Central Areas of Beef Cattle in Indonesia

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Abstract

The general objective of the research is to describe and analyze implementation of adopted artificial insemination (AI) innovation on slaughter cattle's farmers. Specifically, is to describe and analyze the perception of slaughter cattle's farmers on AI. The locations of the research are Geger District of Bangkalan Regency and Mantup District of Lamongan Regency in East Java Province and Penebel District of Tabanan Regency in Bali Province. This research was designed as a correlation descriptive survey by both quantitative and qualitative approach. The number of sample is 240 respondents -who are the acceptors of AI- and by approach of purposive sampling method. Data collection was completed with questionnaires, interview and field observation. Kruskal-Wallis Test and U Mann-Whitney Test were applied to analyze the data sample. The result of the research showed that the perception of the farmers on most of AI's aspects are significantly different among locations of the research, with the exception of the phenotype (physical appearance) of breed cattle and the artificial insemination services by inseminators. To be suggested that socialization or extension on AI should be conducted by different approaches to fit the characteristic of internal and external and AI's perception of the farmers.

Keywords: perception, artificial insemination

Introduction

Artificial insemination (AI) had been introduced to Indonesia since year 1950s. The technology have been tested and applied widely in year 1969, however, the policy of implementation just have first been enforced by Indonesian government on year 1976. At that time, the implementation of AI was meant to improve dairy and beef cattle's production and productivity.

Artificial insemination as a reproduction technology was an instrument to achieve breeding goal. According to Gordon (2004), the AI were a proven technology

and became the most applicable and important reproduction technology since twentieth decade. It was so because the AI technology was relatively affordable and applicable. According to Skjervold (1982), during the last two decade, the AI technology had become the most important breeding technique and this technique has given a new dimension to cattle breeding activity. In general, the AI was aimed at: 1) to improve genetic quality of offspring; 2) to faster the distribution of high breed gene among its offspring; and 3) to improve efficiency of high performance bull utilization (Foote 1981; Gordon 2004).

The AI technology have replaced natural mating method which were applied by the farmers for centuries. Natural mating method have became a major part of social and culture of Indonesian farmer. Therefore, the farmer responses to the AI technology transformation process were not as simply. It was influenced by several factors and the process took times. According Lionberger and Gwin (1982:5), factors that influenced farmer response to an introduction of innovation were (1) individual, (2) situation and (3) characteristic of the innovation factors. Furthermore, the response to each innovation was different between person to person and between communities to community, so to its measures was also different. So far, according to van den Ban and Hawkins (1999:140), in most diffusion innovation research, only a few concerns have been given to a massive changing in social structure or community's way of life. Institutional and community changing were also rarely been investigated. In fact, such social changing were very important, especially to vilage community. According to Rogers (2003:11), innovation was an idea, conduct, or object which were considered new to an individu or a group. Innovation characteristic, as they meant, will explain the different speed of the innovation adoption processed. The innovation characteristics were 1) relative advantages, 2) compatibility, 3) complexity, 4) trialability and 5) observability (Nasution 2002:125). After almost 4 decades of the AI introduction, phenomena of community respons to the technology were still vary. The farmer were grouped into AI- :1) minded; 2) accepted; 3) tried, and 4) rejected.

Research problem which was become focus of observations were how the beef cattle farmer perception on AI. In general, objectives of this research were to get the information rate of adoption on AI innovation, particularly to know: 1) internal and external characteristics of beef cattle farmer and 2) beef cattle farmer perception on AI.

Materials and Methods

The research were desaigned as a correlational descriptive survey research using quantitative and qualitative approaches. The research were conducted in three regencies. Lamongan and Bangkalan regencies represented area of local cattle breeds namely Ongole cross (PO) and Madura, respectively. While, Tabanan regency

represented area of Indonesian indigenous cattle breed namely Bali Cattle. Research population were taken from all beef cattle farmers of AI acceptors from Mantub District of Lamongan Regency, Geger District of Bangkalan Regency and Penebel District of Tabanan Regency. The total amount of 240 farmer samples were taken from the three locations (80 samples from each location). Primary data were collected through respondens interview based on quisionaire that have been prepared and tested earlier as well as from other sources. Statistical data analyses were conducted using 1) descriptive statistic and 2) *Kruskal-Wallis Test* and *U Mann-Whitney Test* to test the different means of samples (Santoso 2004).

Results and Discussions

Internal characteristics of beef cattle farmer

Internal characteristics of beef cattle farmer parameters included several indicators as shown in Table 1. Result of *Kruskal-Wallis Test* of the samples showed that in general, internal characteristics of beef cattle farmer were significantly different between different locations, except for the motivation to use the AI.

External characteristics of beef cattle farmer

External characteristics of beef cattle farmer parameters included indicator as shown in Table 2. *Kruskal-Wallis Test* result showed that in general, indicator of external characteristics of beef cattle farmer were significantly different between the locations, except for the information availability.

Beef cattle farmer perception on the AI

Perception of beef cattle farmer on the AI was the means were given to accept or rejected the AI based on process of self observation or experience by individual farmer. The farmer perception on AI innovation included technical (type of cattle breed, physical characteristics, goal of breeding/AI, goal of breeding/AI, inseminator services, oestrus symptoms), socio-cultural (social norm system, cattle farmer institution, social structure), economic (production improvement by AI, relative profit) and government regulation (cross, pure-breed, cross and pure-breed/mix) aspects of beef cattle breeding.

Evaluation results on the reliability perceptions which constructed all the indicators showed a coeffiecient of VE = 0.21. Based on their degree of probability, several perception indicators were more dominant to form perception parameters. They were type of cattle breed (39%; R^2=0,20), inseminator services (13%; R^2=0.20), oestrus symptoms (11%; R^2=0.32), social structure (20%; R^2=0.32), production improvement by the AI (7%; R^2=0.61), relative profit (19%; R^2=0.54) and pure-breed program regulation (28%; R^2=0.28).

Table 1. Indicator distribution of internal characteristic of beef cattle farmer

Internal characteristics	Bangkalan		Lamongan		Tabanan		Total	
	total (person)	%	total (person)	%	total (person)	%	total (person)	%
Farmer age (years)								
· young (25-33)	1	1.3	8	10.0	5	6.3	14	5.8
· adult (34-51)	51	63.7	57	71.3	41	51.3	149	62.1
· older (52-68)	28	35.0	15	18.7	34	42.4	77	32.1
Farmer education								
· low (<finish elementary school)	79	98.7	53	66.2	45	56.2	177	73.8
· middle (finish high school)	1	1.3	25	31.3	33	41.3	59	24.6
· high (finish college)	0	0.0	2	2.5	2	2.5	4	1.6
Farmer experience of beef cattle keeping (years)								
· less experienced (1-11)	9	11.3	21	26.3	10	12.5	40	16.7
· experienced (12-33)	71	88.7	38	47.5	40	50.0	149	62.1
· highly experienced (34-55)	0	0.0	21	26.2	30	37.5	51	21.2
Number of cattle owned (AU)								
· few (0.5-1.9)	49	61.3	29	36.2	51	63.7	129	53.8
· enough (>1.9- 4.9)	31	38.7	50	62.5	29	36.3	110	45.8
· many (>4.9-7.5)	0	0.0	1	1.3	0	0.0	1	0.4
Farmer orientation (income)								
· part time (<30%)	68	85.1	46	57.5	37	46.2	151	62.9
· branch of business (30-70%)	9	11.2	12	15.0	31	38.8	52	21.7
· main business (>70%)	3	3.7	22	27.5	12	15.0	37	15.4
AI motivation used								
· external factor (extrincsic)	47	58.7	42	52.5	56	70.0	145	60.4
· self motivation (intrincsic)	33	41.3	38	47.5	24	30.0	95	39.6
Membership of AI group								
· non member	68	85.0	78	97.4	80	100	226	94.2
· member/committee	12	15.0	2	2.6	0	0.0	14	5.8
Degree of cosmopolitan (freq/ month)								
· low (0-2)	43	53.0	34	42.4	76	95.0	153	63.6
· mild (3-8)	37	47.0	32	40.1	4	5.0	73	30.6
· high (9-12)	0	0.0	14	17.5	0	0.0	14	5.8
Income from selling cattle (mio. rupiah/year)								
· low (<8.6)	78	97.5	55	68.7	65	81.3	198	82.5
· middle (8.6-25.7)	2	2.5	21	26.2	15	18.7	38	15.8
· high (>25.7-42.9)	0	0.0	4	5.1	0	0.0	4	1.7

Table 2. Indicator distribution of external characteristics of beef cattle farmer

External characteristics	Bangkalan		Lamongan		Tabanan		Total	
	total (person)	(%)	total (person)	(%)	total (person)	(%)	total (person)	(%)
AI institution								
· low (1 institution)	75	93.7	64	80.0	67	83.7	206	85.8
· middle (2 institutions)	5	6.3	10	12.5	13	16.3	28	11.7
· high (>2 institutions)	0	0.0	6	7.5	0	0.0	6	2.5
AI equipments								
· not available	22	27.5	76	95.0	41	51.3	139	57.9
· less available (only 1 set)	52	65.0	3	3.7	15	18.7	70	29.2
· highly available (more than 2 sets)	6	7.5	1	1.3	24	30.0	31	12.9
Cattle market assurance								
· low (only 1 choice)	37	46.3	79	98.7	58	72.5	174	72.5
· middle (2 choices)	43	53.7	1	1.3	22	27.5	66	27.5
Extention services intensity (freq/6 months								
· rarely (<2 times)	48	60.0	79	98.7	51	63.7	178	74.2
· frequent (3-5 times)	32	40.0	0	0.0	6	7.5	38	15.8
· intensive (≥6 times)	0	0.0	1	1.3	23	28.8	24	10.0
AI information availability								
· difficult (only 1)	14	17.5	11	13.7	2	2.5	27	11.2
· easy (2)	43	53.7	56	70.0	62	77.5	161	67.1
· very easy (> 2)	23	28.8	13	16.3	16	20.0	52	21.7
AI information sources (type)								
· low (only 1)	29	36.2	26	32.5	15	18.8	70	29.2
· middle (2 types)	48	60.0	42	52.5	48	60.0	138	57.5
· high (> 2 types)	3	3.8	12	15.0	17	21.2	32	13.3

Based on *Kruskal-Wallis* test, the results showed that there was significant different of farmer perception among location except for the indicators of physical characteristic of cattle replacement stock and inseminator services. To know which location were different from others, *Mann Whitney U test* have been conducted. The test were run between Bangkalan and Lamongan regency, Bangkalan and Tabanan regency as well as Lamongan and Tabanan regency. The result showed that the perception of the farmer among location were significantly different about the AI.

On physical characteristic of cattle breed, the result showed that 71.2% of respondents at least agreed that the cattle breed should have great body score with great physical performance and 84.6% respondens agreed that the cattle should have ideal or proporsional body condition. High perception were shown by respondens

agreement on statement that physically, stock cattle should have great condition with heavy body weight (90.8%). The physical characteristics which can easily be seen and ideal condition of cattle body part might be the caused of why respondens perception on physical characteristics of the cattle did not show significant different among research locations.

In giving insemination services, 97.5% of respondens stated that inseminator always ready to give services anytime. To the AI charge or cost should be paid by the farmer, about 93.3% of the respondens said that the charges were equivalent to the benefit they received. Insignificant differents among research locations in the inseminator service qualities explained that insemination services were part of the AI system that operated nationally. Regulation of the AI application nationally were equipped with minimum technical standard of services that should be provided by an inseminator in giving the AI services.

The farmer perceptions on the AI between: 1) Bangkalan and Lamongan Regencies were statistically different, except for inseminator services and regulation of cross breed indicators; 2) Bangkalan and Tabanan Regencies in general also showed statistically different, except for the inseminator services, oestrus symptom and social norm system indicators; 3) Lamongan and Tabanan Regencies in general also showed significantly different, except on physical characteristic of stock, breeding goal, inseminator services, social structure and regulation of pure and cross breed, which were not significantly different between the two location.

Conclusions

In general, internal and external characteristic of beef cattle farmer were differ, except for their motivation to use the AI and the availability of AI information.

The significant indicators to contruct beef cattle farmer perception on the AI were type of stock, physical characteristic of the stock, inseminator services, oestrus symptom of cows, social norm system, social structure, improvement of production, relative income, regulation toward cross and pure breed program. The dominan factors as perception construction to the AI were improvement of production, relative income/profit, oestrus symptom, social structure, regulation toward pure breed, type of stock breed and inseminator services, respectively.

In general, the farmer perception about the AI showed different respons between locations of the research, except for indicator of physical characteristic of stock breed and indicator of inseminator services.

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The Characteristic of Farming System for The *Walik* Chicken in West Java, Indonesia

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Abstract

The Walik chicken is one of rare indigenous chicken breeds found in Indonesia. The limited information on the management practices of the Walik chickens applied by the farmers in Indonesia cause the difficulity to predict their potency of conservation and utilization. Therefore, this study investigated the characteristics of the farmers, and the practice of the Walik chicken farming, and their potency of utilization. Thirty three (33) farms from Sumedang District, West Java were surveyed. Their main occupations of the farmers were labor in agricultural field (46.15%), private sector (30.77%), and house mother (23.08%). A mean flock size of Walik chicken per household was 4.15, varying from 2-7 chickens. Farmers applied non traditional (73%) by using commercial medicaments, and traditional (27%) health care and management to prevent and control diseases of the chickens. However, none of the farmers applied the vaccination program. Traditionally, most of the farmers (64%) selected the chicken breeds by their own traditional knowledge. Broken rice, rice hulls, and household by product were feedstuffs provided for the chickens. The mean of egg production, and hatchability of semi-intensive were 11 eggs/clutch/hen (31 eggs/hen/year), and 92,71%, respectively. The Walik chicken farming is important for household income. Improvement in rearing management practices should be done to increase the productivity of the Walik chicken. Giving education for improved management system is also an alternative strategy to increase chicken productivities and household income.

Key words: Walik chicken, farming system, egg production, hatchability

Introduction

In Indonesia, Walik chicken breed that owns a frizzling type of feather is considered as endangered population, reason for which we consider that a special attention should have been given to save it. The unique characteristics of frizzle causes the chickens are more tolerance to high temperature, however this properties are not utilized yet in Indonesia since there is limited data on their characterization and identification of their characteristics, farming system applied, and their utilization. The very small population of this breed in a certain warm geographic areas of Indonesia, such as Sumedang District, causes the chickens are unfamous breed recognized by the people.

The Strategic Priority 5 of the *Global Plan of Action* (FAO, 2007) adopted by the International Technical Conference on Animal Genetic Resources for Food and Agriculture, acknowledges the contribution of livestock keepers in indigenous and local production systems to the domestication, development, maintenance and conservation of animal genetic diversity. Moreover, Weigend and Romanov (2001) argued that the possession of chicken traits in relation to current and future value and socioecultural importance is one of the crucial inputs for decisions on chicken conservation and utilization. The limited information on the management practices of the *Walik* chickens applied by the farmers in Indonesia cause the difficulity to predict their potency of conservation and utilization. Therefore, this study investigated the characteristics of the farmers, and the practice of the *Walik* chicken farming, and their potency of utilization.

Materials and Methods

The purposive sampling method was choosen to select the study areas of the research. The initial survey to identify the individual households kept the *Walik* chickens was done by interviewing to the head of villages, and the oldest people in a society who know well the people in the study areas as described on the snow ball methods. Thirty three (33) farms from 3 sub-districts (Padanaan, Palasah, Ujungjaya dan Keboncau), Sumedang District, West Java were surveyed in June-August 2009. The data of farm characteristics, household flock characteristics, farming system, and chickens performances and chickens utilization were recorded during the research, and then analyzed descriptively.

Results and Discussion

Farmers Characteristics

Thirteen farmers were devided into 2 classes of productive age, 9 (69.23%) and 4 (30.77%) farmers composed as productive age (17-55 years old), and unproductive age (>55 years old) respectively (Tabel 1). The youngest was 38 and the oldest was

68 years old). The range of their ownership was 98-5.110 m^2. Their main occupations were labor in agricultural field (46.15%), private sector (30.77%), and house mother (23.08%).

The chickens farming system applied by the farmers were extensive (7.69%), semi intensive with scavenging and backyard system (84.62%), and intensive (7.69%), wherein the women were predominantly (61.54%) as the keeper of the chicken under the scavenging, and backyard system. Women, who have to do a lot of the work involved in caring for the chickens, have good mothering instincts and provide chicken meats and eggs that can be used for sold in the market. However,

Table 1. The Characteristics of Farmers Surveyed

No	Farmer Characteristics	Number of farms surveyed		Farming System (Farm)		
		Farm	%	Semi Intensive*	Intensive	Extensive
1	Age					
	17 - 55 year	9	69.23	8	1	-
	> 55 year	4	30.77	4	-	-
2	Sex					
	Male	5	38.46	3	1	1
	Female	8	61.54	8	-	-
3	Education background					
	Illiteracy	1	7.69	1	-	-
	Elementary School	9	69.23	8	-	1
	Junior High School	1	7.69	-	1	-
	Senior high school	1	7.69	1	0	-
4	Occupation					
	Labor in agricultural field	6	46.15	6	-	-
	Household mother	3	23.08	3	-	-
	Private sector	4	30.77	2	1	1
5	Farming system					
	Intensive	1	7.69	-	-	-
	Semi Intensive*	11	84.62	-	-	-
	Extensive	1	7.69	-	-	-
6	Purpose of farming					
	Household consumption	1	7.69	1	-	-
	Trade	12	92.31	10	1	1

Note: *Scavenging and backyard system

due to the low input management system, chicken productivities appears still very low.

Most of the farmers (45%) stated that the *Walik* chicken farming is important for their income since the main purpose of *Walik* chicken farming is for trading (92.31%) as meat and egg producers, instead of for household consumption (7.69%). Some farmers sometimes had difficulities in trading since most consumers often judge the *Walik* chicken as the unhealthy chickens due to the frizling type of their feather. However, others earn high price in trading since some consumers also prefer the chickens as an exotic birds, and for cultural and religion purposes. The data provided by local Livestock Department Services of Sumedang District, West Java (2009) does not break down the population for each type of chicken, but it is believed that *Kampong* is the most popular indigenous chicken since other indigenous chickens are only occasionally found in certain areas and their population is low.

Rearing Management, Flock Size adn Egg Production Potency

A mean flock size of *Walik* chicken per household was 4.15, varying from 2-7 chickens. During study, the *Kampong* chickens were also kept by the farmers, and predominant (133 head; 66.52%) to *Walik* chickens (60 head; 29.56%). The *Kate* chickens were also kept by the farmers (10 head; 4.92%). For men, chicken farming is only the second job therefore men usually had limited work power which allows only for part-time activities therefore the chicken population, and productivities were still low. Women stated that limited space and money for chicken farm invesment are the reasons they do not increase their chicken population. In another work, Muladno and Thieme (2009) found that the reasons for not having larger flocks of local chickens include among others are the limited space in their house's yard, not enough money to invest in a chicken farm and, and limited work power which allows only for part-time activities.

Under semi extensive system, the chickens are housed in an open-fenced area, which resembles a ranch and is usually built in the backyard of the farmer's house. In some cases, colony cages are provided to allow chickens to sleep at night. In most cases, there are no cages available and the chicken sleep everywhere on the farm, such as kitchen, around farmer houses.

Farmers provide feed and drink regularly 1 time to 3 times a day. Broken rice, rice hulls, and kitchen waste were feedstuffs provided for the *Walik* chickens under extensive and semi-intensive system. Besides, the chickens also scavenge for feed insects, worms, grasses and vegetables around the farmer houses. Farmers applied non traditional (73%) by using commercial medicaments, and traditional (27%) health care and management to prevent and control diseases of the chickens. However, none of the farmers applied the vaccination program. Therefore, the mortality of chicks is usually high during 3-6 months of age due to diseases (mainly New Castle Diseases and Coryza), and also predator.

Most of farmers (64%) bought the chickens from local markets, whereas the rest (36%) got the chickens as a present from their relatives. Traditionally, most of the farmers (64%) select the chicken breeds by their own traditional knowledge. Despite the absence of recording, farmers often memorize the ancestry of their chickens in great detail and over several generations. Introduction of good native chicken breeding practice (Deptan 2006) is therefore still needed to improve better undertanding of the farmers on chicken selection.

The mean of egg production, and hatchability of semi-intensive were 11 eggs/clutch/hen (31 eggs/hen/year), and 92,71%, respectively. This egg production was lower compared to another indiginous chciken such as *Kampong* chicken (59 eggs/hen/year) (Diwyanto & Prijono 1996). In general, under natural condition, the *Walik* chicken brood for between 21-23 days, and chicks remain with their mothers for a period of 2-3 months, after which period the hen will start the next egg laying period. Poor nutrition and the absence of disease prevention or control measures contribute to this low production. Diwyanto *et al.* (1996) also reported that the productivity of local chicken in semi intensive and intensive systems is better than in extensive system. However, Mansjoer (1989) found that the chicken maintained under intensive system were inefficient in their feed consumption and had higher feed conversion ratios than commercial chicken breeds. Improvement in rearing management practices should be done to increase the productivity of the *Walik* chicken.

Conclusions

The *Walik* chicken farming is important for household income. The chickens rearing system applied by the farmers were extensive, semi intensive with scavenging and backyard system, and intensive, wherein the women were predominantly as the keeper of the chicken under the scavenging, and backyard system. Improvement in rearing management practices should be done to increase the productivity of the *Walik* chicken.

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Utilization of *Datura Metel* Linn. Leaf to Decrease Transportation Stress in Sheep

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Abstract

Transportation resulted in serious stress at livestock. Datura metel Linn. contains a natural anesthesia compound. This research aimed to study the affectivity of administration of the leaf extract to decrease the negative effect of transportation stress in sheep. First experiment aimed to determine the doses of leaf extract. Twenty one of hamsters were administered the leaf extract at concentration of 0, 5, 10, 15, 20, 25% and no dilution. They were allowed to pass trough rotary wheel for 1 minute at 20, 30, 60, 120, and 180 minutes after offering the extract. Second experiment was designed to test the affectivity of administration of the leaf extract just before the sheep were exposed to transportation stress. Third experiment aimed to study the method of extraction of the leaf. Five size of the leaf particle of >60, 60-40, 40-30, 30-20, and <20 meshes were dissolved in water for 10 minutes. Data was processed with descriptive analysis. All treatment indicated anesthesia sensation up to 180 minutes. Sheep offered the leaf extract had lower degree of transportation stress than no treatment. Mesh size of <20 gave the highest solubility. The conclusion was that the leaf extract of Datura metel Linn. reduced the effect of transportation stress in sheep.

Key words: Datura metel Linn., stress, sheep, transportation

Introduction

Transportation causes stress in sheep. Fernandez *et al.* (1996) and Rajion *et al*. (2001) reported live weight loss and increase in neutrophil-lymphocyt ratio and glucose in plasma. *Datura metel* Linn. can be used as an anesthetic, anodyne, anti-asthmatic, antispasmodic, antitussive, bronchodilator, hallucinogenic, hypnotic, and mydriatic material (Emboden 1979; Duke and Ayensu 1985). It contains hyoscyamine, hyoscine dan atropine (Chopra 1986). Extracts of *Datura metel* Linn. flower which was equivalent to 3-5 g anesthetic drug produced general anesthesia in 5 minutes (Duke and Ayensu 1985).

Materials and Methods

Experiment 1

Twenty one of hamsters were administered with the leaves extracts of *Datura metel* Linn or fed unextracted leaves. Extracts concentartions administered were 0, 5, 10, 15, 20, 25%, each extract as a treatment was administered to three hamsters. The effort of the hamster to rotate the rotary wheel had tested for one minute at 0, 10, 20, 30, 60, 120 and 180 minutes after administration of the extract.

Experiment 2

Ten sheep were used in this experiment. Five sheep were administered extract leaves and 5 others were not administered the extract (control). All sheep were transported for approximately 5 hours. Their live weight was determined before and after transportation. Blood samples were collected after transportation.

Experiment 3

Datura metel Linn. leaves were dried and ground to >60, 60-40, 40-30, 30-20 and <20 mesh to evaluate their solubility. Samples of 10 g each were wrapped in gauze and dipped into water for 10 minutes. Dry matter of samples was measured by removed from the gauze and dried in an oven. Descriptive method was used to analyze the experimental data (Walpole, 1993).

Results and Discussion

Experiment 1

Figure 1 showed, the performance of the hamsters to rotate the rotary wheel for 1 minute. The control group of humster rotated the wheel for 39,92 rotattion/minute which was higher than did the hamster groups administered extract leaves. The result indicated that *Datura metel* Linn. contained substances affected the nerves sytem of hamster resulting in low effort to rotate the rotary wheel. A similar effect was reported by Duke and Ayensu (1985), that *Datura metel* Linn., especially leaves and seeds coul be used as an anesthetic, anodyne, antiasthmatic, antispasmodics, antitussiv, bronchodilator, hallucinogenic, hypnotic, and mydriatic.

Anesthetical effect of the leaves extract administration continued up to 180 minutes (Figure 2.). The rotation of rotary wheel per minute for control group of humster was higher than the average of that for other groups (29.00 vs 39.92).

The results showed that anesthetic agent contained in *Datura metel* Linn. leaf effected on hamster nerve. Previously study by Duke and Ayensu (1985) showed that the extract of *Datura metel* Linn. flower could be used as an anesthetic agent equivalent to 3-5 g and produced general anesthesia in 5 minutes after oral administration and the effect continued upto 5-6 hours later.

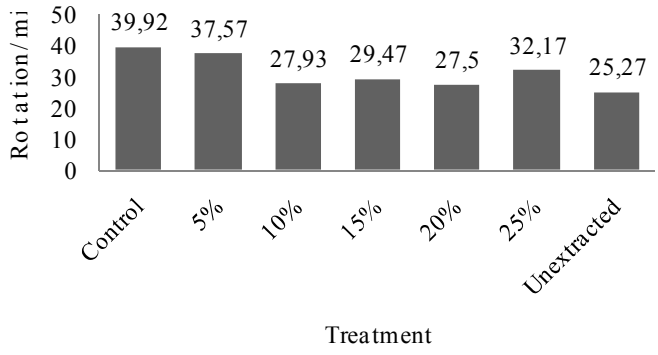

Figure 1. Total rotation of the rotary wheel rotated by hamster for one minute after administration of the leaves extract of *Datura metel* Linn.

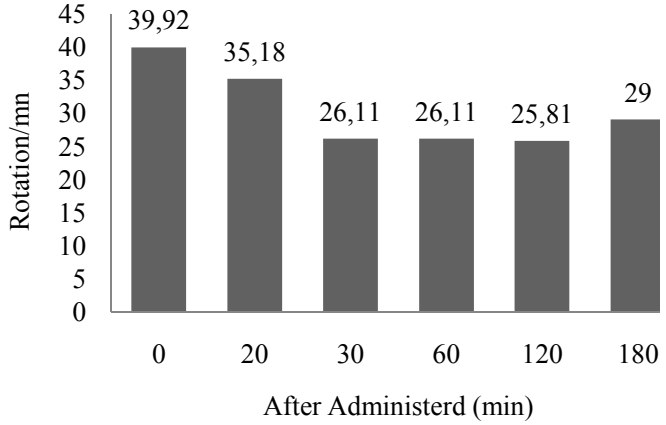

Figure 2. Total rotation of the rotary wheel rotated by hamster after administration of the leaves extract of *Datura metel* Linn.

Experiment 2

The effect of *Datura metel* Linn. leaves extract on blood profile of sheep after transport-stressed for 5 hours is showed in Figure 3. The average of neutrophiles : lymphocites ratio and blood glucose in blood of sheep group administered the extract of *Datura metel* Linn leaves were lower than that of control. The ratio of neutrophiles : lymphocites and blood glucose are good indicator of transport-stress in animal. Rajion *et al.* (2001) reported that transport-stresses increased neutrophiles:lymphocites ratio and glucose in blood.

The lowest effect of transport-stress was observed in the group of sheep administered leaves extract. They had lower live weight loss than the control group did (Figure 4.). Gortel *et al.* (1992) reported that sheep exposed to transport stress released a large amount of electrolyte ions in urine and feces resulted in reduction in live weight and carcass quality.

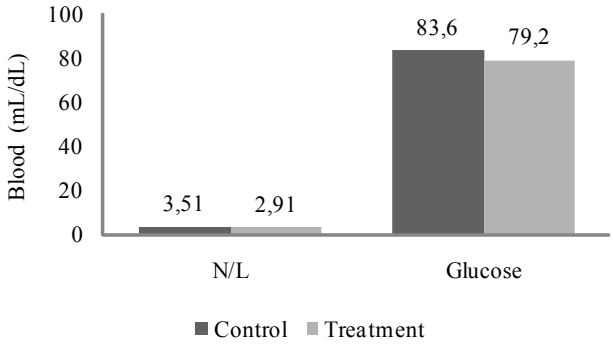

Figure 3. Profile of neutrophile:lymphocites (N/L) ratio and glucose in blood of sheep exposed to transport-stressed for 5 hours

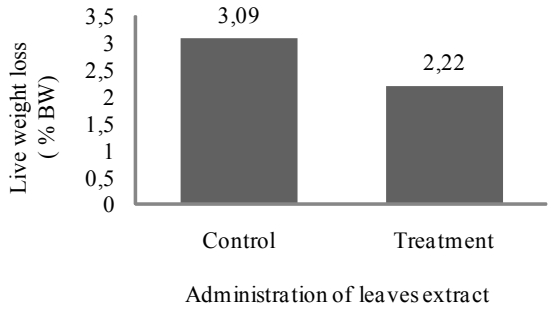

Figure 4. The effect *Datura metel* Linn. leaves extract administration on live weight loss of sheep exposed to transport-stress for 5 hours

Experiment 3

Solubility test indicated that the mesh size of dry leaves affected the solubility of the soluble part of the dry leaves of *Datura metel* Linn (Figure 5). The smaller of mesh size, the lower the part of the solute. This was due to the finer particles would close the pores of gauze causing delayed the movement of water out into the mesh bag, so that decreased the chance of water in dissolving the part of *Datura metel* Linn. leaves.

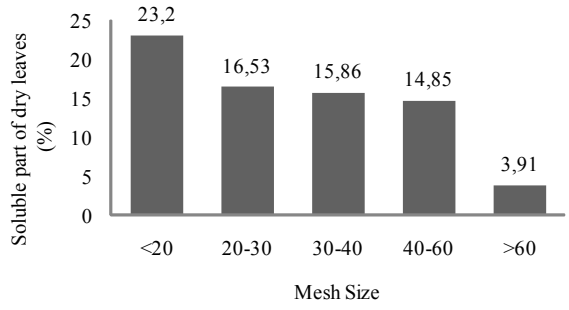

Figure 5. Soluble part of the dry leaves of *Datura metel* Linn. ground into various mesh size

Conclusion

Leaves extract *of Datura metel* Linn. could be administered to reduce the negative effect of transport-stress in sheep.

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Effect of Climate Change on Livestock Production in Pakistan

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Abstract

The climate change is affecting the animal productivity especially in resource poor localities in the region. While most of the areas suffer from the insufficiencies of the data, a few scientific measurements in some areas have proved that the effect of climate changes are adversely affecting the livestock production directly due to heat stress or indirectly due to changes incorporated in the ecosystem. Some of these may include reduced agricultural productivity, deteriorated health due to shortage of feed resources, water quality, global warming, shrinking glaciers, erratic weathers, river floods, disease prevalence, disrupted ecosystem and increase in the frequency of natural hazards and disasters. Pakistan still suffers more than 30 % Crude Protein and TDN deficiency for large and small ruminants in its feed balance. The exact Information on the losses are very much undermined and not realized by many or sometimes sorely lacking. A detailed understanding is warranted to anticipate the future impacts of the climate change in different areas on the productivity of the ruminants. Unless these losses are realized and scientifically proved, the measures needed or taken will attain less significance. Our studies revealed that there is a drastic effect on temperature change on water buffaloes and their calves. When ambient temperature reached to 32-47° C with a mean relative humidity of 33-75%, the physiological norms of the buffalo calves were significantly affected and their weekly body weight decreased as 43 kg as compared to 46 kg under open air tree shade than inside a shed with showers plus ceiling fans, body temperature was higher 101.6ºF that 101.0ºF, respiration rate was higher 28 to 26 per minute, and the pulse rate increased to 53-54 per minute under treatment with open air tree shade as compared to inside with ceiling fans and showers. This paper will delineate in length some of the effects on livestock production due to climate change in a country like Pakistan where 35-40 million rural people derive their livelihood from livestock rearing. Mitigation measures will also be suggested to save productivity losses to ensure the food supply from animal origin for the exploding population.

Keywords: climate change, heat stress, livestock productivity, mitigation, Pakistan

Introduction

Pakistan has experienced the worst drought of its history in the last decade of the last millennium which lasted until 2004. She has been ranked 16 regarding countries most vulnerable to environment change (Maplecroft, 2010). Out of total 79.6 million hectares (MH), 27 % is cultivated area i.e. about 21 (MH), containing 19.12 (MH) are irrigated and 3.67 (MH) are rainfed. The irrigated area consumes about 80% of the country's freshwater. Figure 1 shows this distribution along with 62% of the country areas are rangelands (Quraishi et al. 1993). Agriculture sector being the back bone of the economy will be more probable stake holder of aftermaths of environmental changes. The climate change affected the livestock in two ways (i) by affecting the forage production, (ii) directly affecting the livestock kept under different production systems. The losses suffered by the animals not only due to heat stress but also in the form of disasters in the previous years. This thematic paper explains the possible problems faced by the livestock due to heat stress, impact of climate change and the different ways and means to mitigate the effect of environment on livestock productivity.

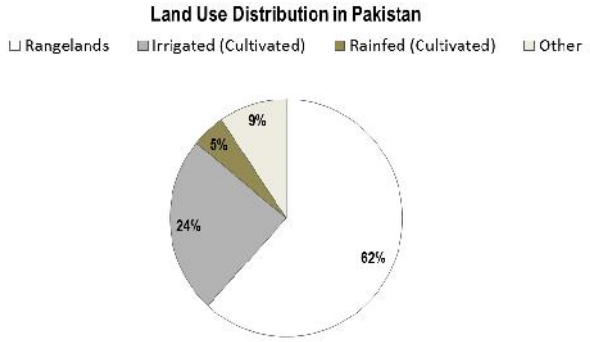

Figure 1. Showing the different classification of the areas of Pakistan

The Climatic Zones and Water Resources of Pakistan

The environment and livestock production systems are closely related (Nardone and Gibon, 2000). The agriculture sector depends on the availability of the water for irrigation and annual precipitation. Based on the annual precipitation six Agro-Ecological Zones (AEZ) has been defined by Quraishi *et al*. (1993), Chaudhry and Rasul (2004) is summarized in the Table 1. Environment changes are affecting the glaciers in the northern part of the country and affecting the Indus Basin system badly. In combination with the predicted heavy rainfall, it likely to exacerbate the already serious problems of flooding and draining, especially in the lower parts of the Indus basin in the next few decades, when the glacial reservoirs will be empty, and there are likely to be dramatic decreases in river flows (World Bank, 2005).

Table 1. Classification of the areas according to precipitation

Sr #	Type of area	Amount of Precipitation (mm)
1	Humid	>1000-2000
2	Sub-Humid (wet)	700-1000
3	Sub-Hummid (dry)	>500-700
4	Semi-Arid (wet)	300-500
5	Semi-Ard (dry)	200-300
6	Arid	Less than 200-250

Source: Adopted from Quraishi *et al*. (1993)

Environmental Temperature and Rain Pattern

Farooqi *et al.* (2005) have further analyzed the climate change perspective in Pakistan and concluded that annual mean surface temperature is on consistent rising trend since the beginning of 20th century. Charlotte (2011) further added that the major risk from the climate change in South Asia is increased summer precipitation, intensity in temperate regions, increase flash-flood prone areas and further added that the arid and semi-arid regions would be drier in summer, which could lead to severe droughts.

Effect of Droughts and Floods

Pakistan had the fully operational irrigation system and enough water from the rivers flowing through it. But quite recently the water was seldom enough to meet the requirement of end users. The population growth had started putting unprecedented pressure on the irrigation system (UN, 2001). Livestock population is affected greatly by the droughts and their growth has been declined in last decade especially during 2003-04 (SBP, 2003-04). The researchers of Balochistan have also reported that drought has affected whole of the country; Balochistan province being the worst-hit and resulted in the loss of life (Shafiq and Kakar, 2007). During the recent years, due to the change of precipitation, the floods have adversely affected the country especially Punjab and Sindh. A latest report by FAO (2012) confirmed these notions by explaining that 116,000 heads of livestock died by the flood hit in the Sindh province.

Effect of Heat Stress on Dairy Animals

The most drastic effect of heat stress is in the form of change in the energy metabolism and partitioning coped with decreased in DMI. This results in the low

milk production response. There is 20-30% increase in the maintenance energy requirement and heat stress combined with the DMI decrease by 10-20% in the commercial dairy herds (Chase, 2006). Formulation for adequate nutrient intake challenging for heat stress cow depends on optimizing then rumen undegradable protein to improve milk production response. The forage level must decrease due to high heat of increment (West, 1999). De Rensin and Scaramuzzi (2003) found that appetite and dry matter intake are reduced so by this way it prolonged the postpartum negative energy phase in dairy cows. Tao *et al*. (2011) found that cooling of heat stress can increase milk production (28.9 vs 33.9 kg/day) and lower milk protein (3.01 vs 2.87%). The physiological change regarding milk synthesis during heat stress may be due to hepatic glucose preferentially used for process other than milk synthesis (Baumgard *et al*., 2011). Climatic factors for example temperature, precipitation frequency and severity of extreme events affects the livestock and crop yield (Thornton *et al.,* 2008). Temperature Humidity Index (THI) has been used as indicator of heat stress. The critical values of THI is 72 (Igono et al. 1992) while the studies of Dikeman and Hanson, 2009 showed that dry bulb temperature could also be used to predict the rectal temperature of lactating Holstein cows in sub-tropical environment. The increase in thermal load above the thermal neutral zone affects the animal performance. The animal fails to dissipate excess heat to maintain homeothermy (West, 1999). Mukherjee et al. 2011 found that the decrease in lymphocytes in heat stress cows. Physiologically there will be increase in rectal temperature and respiration rate due to increase in ambient temperature beyond the thermoneutral zone (Chase, 2006). Gwazdauskas (1985) found that estrous hormones were found lower during heat stress and resulted in shortening of estrus duration. He further investigated that lower fertility in heat stressed male cattle are due to the impaired spermatogenesis and testosterone during exposure to hyperthermia. Baumgard and Rhoads (2007) said that there is negative effect of heat stress on a variety of dairy parameters, including milk yield and reproduction causing a significance of financial burden. Increase in thermal load decreases the reproductive efficiency (Fuquay, 1981, Imtiaz Hussain *et al*., 1992). Most of the reproductive problems are decrease duration and intensity of estrus (Her *et al*., 1988), lower conception rate (Stott *et al*., 1972) and high embryo mortality (Wise *et al*., 1988).

The change in environmental temperature affects the animal body. Many scientists have investigated the dynamics of animal body changes due to heat stress. The earlier studies on heat stress in Pakistan were conducted at the University of Agriculture, Faisalabad, under PL-480 schemes (Qureshi *et al*., 1978). They concluded that there is drastic effect of temperature change on Buffalo (*Bos Bubalus bubalis*). From five years (1973-78) long trials, they concluded that combined use of showers and fans as a thermal relief measure in dairy buffaloes was found to be significantly useful as compared to the use of fans or showers alone as improved milk yield and the occurrence of estrus was more pronounced in given treatment of

combined use of fans and showers. Similar observations were recorded in the follow up studies (Younas et al. 1979) indicated that under the environmental temperatures of 32-47ºC with a mean relative humidity of 33-75 %, the physiological norms of the buffalo calves were significantly affected and their weekly body weight decreased as 43 kg as compared to 46 kg under open air tree shade than inside a shed with showers plus ceiling fans, body temperature was higher 101.6 ºF than 101.0 ºF, respiration rate was higher 28 to 26 per minute, and the pulse rate high which was 53 to 54 per minute under treatment with open air tree shade as compared to inside with ceiling fans and showers. The final conclusion from these studies was that declining effect of thermal stress on certain components of milk and blood, however, could not be solely ascribed due to heat stress. The growth of buffalo calves as determined from increase in their body weights manifested an interesting contrast in the effect of various treatments used. The highest average body weight was observed in group of calves provided ceiling fans alone as thermal relief whereas in trials with adult buffaloes, combined use of fans showered proved better. The influence of thermal stress on blood picture of buffalo-calves was also seen. Blood glucose, total lipids and phospholipids content were found to consistently increase whereas, cholesterol levels decreased in the entire group with exception of group 1 in which a slight increase was observed (Younas et al. 1982).

In a follow up study, the lactating and cycling Holsteins in each of two summers were assigned randomly to pens in a free-stall barn either with or without overhead fans to study the effect of fan cooling on certain endocrine and behavioral responses during the estrous cycle (Younas *et al*., 1993). Rectal temperatures were lower in the group cooled by fans than in the control group each summer. Luteal progesterone secretion tended to be greater in the fan group each summer; area under the luteal phase curve was significantly higher than for controls during the second summer. There was tendency for more pre-ovulatory surges of LH and higher estrous responses rates in the fan group during the second summer. Thus, fan cooling of lactating dairy cows for several weeks before anticipated breeding provides potential for more efficient reproductive performance during the summer.

Conclusions

The countries like Pakistan require change in policies regarding the livestock production. Keeping in view the effects of climate change, the production systems in the country need to be re-visited and revolutionized. There is no best way than managing the dairy animals in a wise and economical way during the hot summers enabling them to dissipate their body heat and facilitating the animals comfort as much as possible. Correct management decisions will enable the dairy animals to grow faster, pronounced estrual behavior, improved conception rates, reproduce on time and produce their maximum when they are wet. The measures like prediction

of monsoon; protection from rain and sun; feeding management like grazing during cool hours, offering succulent varieties, decreased DMI, use of silage and hay, nutrient density, avoiding excess with normal rumen function; provision of fresh and clean water; genetic selection; housing management like forced/tunnel ventilation, sprinkling, showering, misting; use of ecological modeling and innovations with appropriate strategic management decisions; risk analyses and devising some innovative methods to provide comfort to the animals can help in minimizing the effect of heat stress on dairy animals.

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Performance of Pre-weaning Javanese Thin-Tail Lambs under Semi-Intensive Management at Different Age and Sex

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Abstract

The study was conducted to determine growth traits of thin tail pre-weaning lambs that produced in semi intensive management system. 61 animals (30 ewes and 31 lambs) were used in this study that located at Jonggol Animal Science Teaching and Research, Faculty of Animal Science, Bogor Agricultural University. Birth weights (BW), average daily gain (ADG) and weaning weight (WW) of lambs were affected by sex and ewes age. The highest of BW lambs was obtained from 2-year-age ewes (2,316±349 g), but ADG and WW of lambs from 3-year-age of ewes were highest than others (85.1±32 g/day and 7,385±2,351 g, respectively). BW of female was higher than male lambs (2,264±438 g and 2,149±716 g) but ADG and WW of male (80±24 g/day and 6,969±2,078 g) was higher than female lambs (69±26 g and 6,469±1,788 g). The WW was significantly (P<0,01) correlated by BW and ADG with r value were 0.67 and 0,96 respectively.

Keywords: correlation, performance, semi-intensive, thin-tail lamb

Introduction

Growth is one of important aspect in livestock production. Good growth performance will boost production. One phase in livestock production process is animal pre-weaning, where animal growth process in this phase would affect the productivity of post-weaning production. In sheep, if pre-weaning performance such as birth weight, body weight gain and weaning weights are high, then further growth will be high too (Riggio *et al.,* 2008). Marquez *et al.,* (2012) added that some limiting factors that cause failure to achieve a high level of performance in sheep production are the low birth rate, high mortality rate, low body weight gain and poor management.

In Indonesia as a tropical country, sheep are generally managed by extensive and semi-intensive system, where the supply of feed nutrients dependon the pasture.

Furthermore, those management systems without concentrate supplementation would implicate body weight development and daily weight gain of pre-weaning lambs (Gauly *et al.,* 2004). Yilmaz *et al.* (2007) mentioned that male lambs showed better pre-weaning growth than female lambs that reared in extensive system. Nevertheless, study focusing on semi-intensive sheep management in Indonesia generally is still limited. This study was conducted to identify growth performance of pre-weaning lambs in semi-intensive management system.

Materials and Methods

The study was conducted in Jonggol Animal Science Teaching and Research Unit (Jastru), Faculty of Animal Science, Bogor Agricultural University (IPB) Indonesia. This area is located at 106,53^{o} E and 6,53^{o} N and at an altitude 145 m, where monthly rainfall is 65 mm with minimum, maximum temperature and humidity is 20,8 ^{o}C, 32,9 ^{o}C and 91,7 respectively.

Sheep production in Jastru was reared in 55 ha of *B. humidicola* rangeland and managed by semi-intensive. Thirty pregnant ewe and thirty one young growing lambs were used in this study. Lambs and its mother were grazed from 09:00 am until 4:00 pm every day without any concentrate supplementation. *Ad libitum* watering was allowed to all animals during grazed. Animals were housed at night in colony stable. Type of grass in pastures was dominated by *B. humidicola* with nutrients composition of crude protein, fiber, fat, ash and NFE were 11.5, 41.2, 0.2, 7.2 and 42.75 of dry matter basses respectively.

During four months thirty pregnant ewes with different of ages were observed until birth. Data of lambs werecollected type of birth, birth weight, body weight gain and weaning weight. The data were analyzed descriptively. Correlations were analyzed among data by Pearson correlation analysis in order to see the relationships between variables (Walpole, 1995).

Results and Discussion

Birth Weight

Result showed that birth weight of female lambs was higher than male lambs (Table 1). Based on age of ewe, it was found that one-year-old ewes had the lowest lamb's birth weight. Gardner *et al.* (2007) mentioned that birth weight was correlated to maternal characteristics. Sheep in the first parity generally have not been the maximum growth of reproductive organs. The first pregnancy leaves a 'physiological imprint' in the uterus and enables greater blood volume expansion during the second pregnancy and will facilitate greater fetal in subsequent pregnancies (Campbell and MacGillivray, 1984).

Average birth weights of male and female Javanese thin-tail lambs in this

Table 1. Birth weight of lambs by its sex and age of ewes (g)

Age of ewe (year)	Sex of lamb		Average (n)
	Male (n)	Female (n)	
1	-	2,007±563 (3)	2,007±563 (3)
2	1,900±0 (1)	2,420±300 (4)	2,316±349 (5)
3	2,303±887 (4)	2,258±666 (4)	2,280±727 (8)
4	2,097±720 (7)	2,285±362 (8)	2,197±545 (15)
Average	2,149±716 (12)	2,264±438 (19)	-

study were 2.1 and 2.3 kg, respectively. These results werehigher than the study conducted by Reese *et al.* (1990), Grace *et al.* (2007), Gunawan and Noor (2006) and Suryapratama (1990) who resulted the lamb birth weight 1.5 kg, 1.9 kg, 1,7 kg and 1.9 kg, respectively. Heriyadi (2007) mentioned that the standard birth weight of garut sheep breed wasbetween 2.0 to 3.2 kg. It showed that in semi-intensive management without supplementation of additional feed still produce a good birth weight of lambs.

Growth Rate

The male lambs hadhigher body weight gain compared to female 80.3 g/h/d and 69.9 g/h/d, respectively (Table 2), although male lambs had less birth weight (Table 1). Mandal *et al.* (2012) concluded that growth of lamb's body weight is strongly influenced by gender. Tuah and Baah (1985) also added that skeletal growth of male lambs is higher than the female so that growth of male would be higher than female lambs. Growth also is affected by hormonal system. Testosterone produced by male animals would promote body tissue growth of male lambs (Macit, 2002).

Average body weight gains (BWG) of male and female Javanese thin-tail lambs in this study were 80,3 and 69,9 g/h/d respectively. The study conducted by Reese *et al.* (1990) only has 56 g/h/d of Javenese thin-tail lamb without feed supplementation of its ewe.

Table 2. Body weight gain of lambs by its sex and age of ewes (g)

Age of ewe (year)	Sex of lamb		Average (n)
	Male (n)	Female (n)	
1	-	41.7 ±21.5 (3)	41.7±21.5 (3)
2	73.0±0.0 (1)	75.88±7.58 (4)	75.3±6.6 (5)
3	86.4±26.0 (4)	83.7±42.0 (4)	85.1±32.3 (8)
4	77.8±27.8 (7)	63.3±15.72 (8)	74±24 (15)
Average	80.3±24.8 (12)	69.9±26.9 (19)	-

Weaning weight

Weaning weight has a positive relationship with birth weight of lambs. Sheep which have a high birth weight will generally have a high ability to live through the critical period after birth and will have a high weaning weight. Table 3 shows that male lambs had higher body weight than female lambs. It could relate to the body weight gain was higher in male lambs (Table 2), so that the weaning weight is higher.

In this study the weaning weights of males and females lamb reached 6.9 kg and 6.4 kg, lower than the study conducted by Grace *et.al.* (2007) and Gunawan and Noor (2006) who reprted that the data were 8.0 kg and 10.3 kg respectively. The result might be caused by feeding management. Lambs in both studies were kept by addition of intensive concentrate feed, so that nutrition requirements are likely met. This study indicates that semi-intensive management required additional feed or concentrate in order to increase animals' productivity.

The correlations between birth weight, body weight gain and weaning weight showed a very close positive relationship between these three variables ($P<0.01$). The highest correlation was found between body weight gain with weaning weight ($r= 0.97$) (Table 4). Rahmat *et al.* (2007) also obtained that correlation between birth weight and body weight gain was high in Javanese thin-tail lambs.

Table 3. Weaning weight of lambs by its sex and age of ewes (g)

Age of ewe (year)	Sex of lamb		Average (n)
	Male (n)	Female (n)	
1	-	4,533±1,841 (3)	4,533±1,841 (3)
2	6,300 (1)	6,975±741 (4)	6,840±709 (5)
3	7,488±2,437 (4)	7,275±2,464 (4)	7,385±7,385 98)
4	6,769±2,156 (7)	6,535±1,384 (8)	6,644±1,722 (15)
Average	6,969±2,078 (12)	6,469±1,788 (19)	-

Table 4. Pearson's correlation (r) between birth weight, body weight gain and weaning weight of lambs

Variable	Birth weight	Body weight gain
Birth weight	-	-
Body weight gain	0.458**	-
Weaning weight	0.672**	0.965**

** very significant ($P<0.01$).

Conclusions

Maintenance of sheep in semi-intensive systems without additional feed still had a low success rate in its production. Nevertheless, addition of concentrate feed is needed to improve the performance of lambs produced. In this study, birth weight, average body weight gain and weaning weight of lambs were affected by its sex and age ewes. There was a very close relationship performance between birth weight, body weight gain and weaning weight of lamb reared on semi-intensive system.

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IV. ANIMAL PRODUCT'S TECHNOLOGY

Protein Quality of Fermented Beef by *Lactobacillus plantarum* 1B1

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Abstract

This study was conducted to investigate the proteolytic activity and protein characteristics of fermented beef. The effect of different mechanical treatments on beef, (sliced and ground beef) was compared. The contents of amino acid were analyzed using HPLC (high performance liquid chromatography) method. The results indicated that Lactobacillus plantarum 1B1, isolated from beef, had better proteolytic activity on sliced fermented beef if compared to ground fermented beef, and improved crude protein on sliced fermented beef. Sliced and groud fermented beef contained 15 components of amino acids which consisted of threonine, tyrosine, methionine, valine, phenylalanine, isoleucine, leucine and lysine as essential amino acid and aspartate, glutamate, serine, histidine, glycine, alanine and arginine as non-essential amino acids. Leucine was the highest among essential amino acids content and glutamate was the highest among non-essential amino acids content. The SDS PAGE profiles showed the beef protein molecule weight range of 94.71 - 13.43 kD. The numbers of protein band of fresh beef, sliced and ground fermented beef were 12, 7 and 7. In conclusion, fermentation process on raw beef by Lactobacillus plantarum could improve amino acid availability because of its proteolytic activity on beef protein.

Key words: fermented beef, Lactobacillus plantarum, protein quality

Introduction

Dark cutting beef is often known as dark, firm and dry (DFD) beef. It occurrs as a result of depletion of muscle glycogen reserves prior to slaughter. Glycogen is used as an energy source for muscle contraction and relaxation. Lactic acid is a by-product of glycogen utilization by the muscle when energy is produced in a stress event. In Indonesia, DFD beef frequently happen in slaughter houses due to unstandardized slaughtering practice procedure. Several studies have shown that quality improvement of DFD beef quality could be improved through fermentation

process by lactic acid bacteria, such as *Lactobacillus plantarum (L. plantarum)*. The fermentation might extend the shelf life and appearance of meat products. The addition of starter *L. plantarum* 1B1 in beef fermentation is expected to perform on the proteolytic activity of beef. The aim of this research was to investigate the quality of protein of fermented beef by *L. plantarum* 1B1.

Materials and Methods

L. plantarum 1B1 was obtained from Laboratory of animal product and technology, Faculty of Animal Science, Bogor Agricultural University. The strain was isolated from local beef and was identified using 16S rRNA gene sequencing (Arief, 2011).

The treatment used in this experiment was type of beef fermentation. DFD beef (pH value : 6.2) was used in this experiment. Sliced and grinded beef with inoculated by *L. plantarum* 1B1 for fermentation. Starter culture of 10% w/w *L. plantarum* 1B1 were inoculated into fermentation process. The sliced beef was arranged in a baking dish coated with aluminum foil and covered with plastic top surface. The ground beef was put in a plastic bag, flaked with a thickness of approximately 3 mm, packed and arranged in a baking dish. These treated meat groups were then incubated for 24 h at 37°C, smoked for nine hours at a temperature of 30°C.

Variables observed included the content of crude protein (AOAC, 2005), amino acid composition using HPLC (AOAC, 2005), proteolytic activity of *L. plantarum* 1B1 (Bergmeyer and Gawehn (1983), and SDS-PAGE electrophoresis of protein from fermented beef (Fadda *et al.*, 1998).

The experiment was set up in a completely randomized design with three replication. The data were analyzed using ANOVA, and if found any differences, they were further tested using Tukey test (Steel and Torie, 1995). Amino acid composition and SDS Page electrophoresis were described by descriptive analysis.

Results and Discussion

Crude protein of fermented beef

Proteins in food generally determine the quality of a product primarily derived from meat. Isolates of *L. plantarum* 1B1 used to achieve optimization of beef fermentation with the assumption that these bacteria would be more adaptive to beef. Crude protein and proteolytic activity of of fermented beef is presented in Table 1.

The crude protein of fresh DFD beef was 70.72 % db. If compared with fresh beef, fermentation process could increase crude protein content of fermented beef, because protein from cell wall of *L. plantarum* affected the crude protein content of fermented beef. Crude protein content of sliced fermented beef was higher than

Table 1. Crude protein and proteolytic activity of fermented beef

Parameters	Sliced fermented beef	Ground fermented beef
Crude protein (% db)	81.77 ± 2.46[a]	72.13 ± 2.84[b]
Proteolytic activity (U/g)	0.11 ± 0.04	0.09 ± 0.03

Different superscript in the same line means significantly different (P<0.05)

that of ground fermented beef. Grinding lossed sarcoplasmic protein of beef it could reduce total protein content of ground beef.

Proteolytic activity of fresh beef was not detected (0), it means that natural proteolytic enzyme of beef was not active. Increased proteolytic activity occurred after the fermentation took place in fermented beef. This proved that the isolates of *L. plantarum* 1B1 had proteolytic activity on beef protein. *L. plantarum* 1B1 had extracelullar protease enzyme in the de_man Rogosa Sharp broth 0.041 U/ml. According to Fadda *et al.* (1998) that some strains of *Lactobacillus* bacteria such as *L. casei* and *L. plantarum* can produce proteolytic enzymes in fermented meats.

Molecular weight protein of fermented beef

Fermentation could degrade beef protein, caused by proteolytic activity of L. plantarum 1B1, as shown in Figure 1.

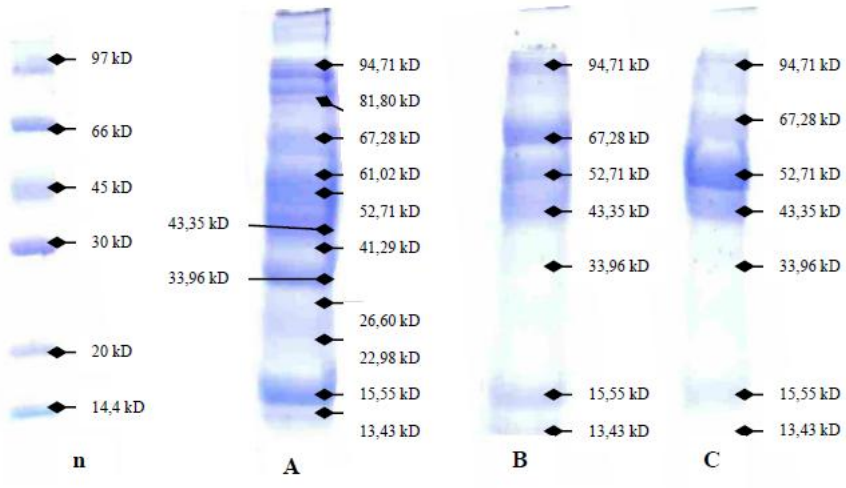

Figure 1. SDS-PAGE electrophoresis bands of beef protein (n= marker; A= fresh DFD beef; B=sliced fermented beef; C=grinded fermented beef).

This type of protein bands detected in fermented beef were closely related to the level of functional protein damage. Reduction in protein occurred in a protein bands with a molecular weight of 81.80 kD, 61.02 kD, 41.29 kD, 26.60 kD and 22.98 kD.

These were due to faulty conformation of the protein after fermentation and protein structure changes into a more simple peptides and amino acids. Fadda *et al.* (1999) showed the degradation of the protein bands because of proteolytic activity of *L. plantarum* that occured in protein with molecular weight 200 kD (myosin), 66 kD and 43 kD (actin). In this research, molecular weight protein bands were (35 to 50 kD) hydrolyzed, whereas myosin and actin partially hydrolyzed after the inoculation of *L. plantarum.* These results further reinforce the fact the degradation of protein beef into simpler form after fermentation.

Amino acid composition of fermented beef

Amino acid composition of sliced fermented beef was different than ground fermented beef by descriptive analysis. Nonessential amino acids were detected by HPLC analysis of the aspartic acid, glutamic acid, serine, histidin, glycine, arginine and alanin, while the essential amino acids detected were treonin, tyrosin, methionin, valin, phenilalanin, isoleusin, leusin and lysin. The quantitative results can be seen in Table 2.

The results of HPLC analysis showed an increase in amino acid content after fermentation. This occurred as a result of the proteolytic enzymes of *L.plantarum*

Table 2. Amino acid composition

Amino acid	Percentage of amino acid content (% w/w)			Increasing of amino acid in fermented beef compared than fresh beef (%)	
	Fresh DFD beef	Sliced fermented beef	Grinded fermented beef	Sliced fermented beef	Grinded fermented beef
Aspartic acid	1.66	2.93	2.56	76.50	54.22
Glutamic acid	3.42	5.64	4.79	64.91	40.06
Serin	0.79	1.31	1.09	65.82	37.97
Histidin	0.70	1.02	0.91	45.71	30.00
Glysin	0.97	1.47	1.41	51.55	45,36
Threonin	0.98	1.58	1.27	61.22	29.59
Arginin	1.57	2.18	1.98	38.85	26.11
Alanin	1.08	1.79	1.60	65.74	48.15
Tyrosin	0.67	0.99	0.79	47.46	17.91
Methionin	0.47	0.72	0.36	53.19	-23.40
Valin	0.90	1.46	1.48	62.22	64.44
Phenilalanin	0.73	1.16	1.07	58.90	46.58
I – leusin	0.90	1.50	1.29	66.67	43.33
Leusin	1.48	2.40	2.09	62.16	41.22
Lysin	1.45	1.90	2.08	31.03	43.45

1B1. Fermentation by *L plantarum* 1B1 could increase amino acid percentage of both fermented beef if compared to fresh DFD beef. Lactic acid produced from the fermentation process caused a decrease in muscle pH beef. This decline continued until the pH reached its optimum value. It then activated proteolytic enzymes of *L.plantarum* 1B1. The enzyme degraded Z-line on myofilamen, eliminating cross-bridge of actomyosin, and amino acid in actomyiosin could be released. In general, amino acid percentages of sliced fermented beef was higher than ground fermented beef. It was supported by crude protein of sliced fermented beef, that was higher than ground fermented beef (Table 1).

Conclusions

Fermentation by *L. plantarum* 1B1 on beef could increase crude protein of beef, while sliced fermented beef had a higher protein content than ground fermented beef. *L. plantarum* 1B1 had proteolytic activity due to fermentation. Fermentation could degrade protein structure, as shown by reduction of protein bands with a molecular weight of 81.80 kD, 61.02 kD, 41.29 kD, 26.60 kD and 22.98 kD. Fermentation by *L plantarum* 1B1 could increase amino acid percentage of both fermented beef. Amino acid percentages of sliced fermented beef was higher than ground fermented beef. In general, sliced fermented beef had better protein quality than ground fermented beef.

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Cashmere Quality of Raeini Goats Kept by Nomads in Iran

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Abstract

This paper assesses the cashmere quality and its variation in Raeini herds to determine the scope for improvement. In April 2010 fleece weights (FW) and midsize fleece samples were taken from a total of 686 male and female cashmere goats of 1, 2 and 3 years of age belonging to 29 herds. The herds were randomly chosen in the summer grazing area of nomads within 20 km of the city of Baft, province of Kerman, South of Iran. Cashmere yield (CY) was determined from the weight of dehaired cashmere to weight of shorn f Cashmere fibers were analyzed using an OFDA instrument. A general mixed linear model including sex, age and sex by age interaction as fixed effects and herd as random effect was used to analyze the data and measure the relationships between different cashmere characteristics and fleece attributes. The overall means ± standard deviations were for fleece weights (FW) 507±183 g, cashmere yield (CY) 56.5±12.2%, mean fiber diameter (MFD) 19.7±1.5 µm, fiber diameter standard deviation (FDSD) 4.5±0.6 µm, fiber curvature (FC) 62.9±8.5 °/mm and staple length (SL) 54.2±7.0 mm, respectively. Herd effect was significant for all traits except for SL and sex by age effect was only significant for MFD. One year old males and females had finer cashmere than older goats. FW and FDSD were higher in males and CY and FC was higher in young animals. Pearson correlation between MFD and FC, FDSD and MFD, MFD and FW was -0.647, 0.399 and 0.211 respectively. Raeini cashmere is white, has an excellent SL and FC but is relatively coarse. Given the differences between and within herds there seems to be substantial scope to improve the commercial value of Raeini cashmere.

Key words: fiber curvature, fiber diameter, fleece weight, staple length

Introduction

40% of the 25 million goat population in Iran is kept by nomads in a habitat of about 59% of the total area of the country (Ministry of Agriculture, 2004). Iran together with Afghanistan is the third largest producer and exporter of cashmere in the world, after China and Mongolia (FAO, 2009). More than 50% of the Iranian cashmere is produced by Raeini goats in Kerman province. Dehaired cashmere is one of the finest and softest luxury natural fibers of the world used mainly for clothing providing warmth and lightness (Watkins and Buxton, 1992). Iranian cashmere is generally designated as 2-3 μm coarser than Chinese and Mongolian cashmere (Petrie, 1995) and is therefore cheaper (Phan and Wortmann, 2000; Schneider, 2011). Ansari-Renani (2001) showed that cashmere produced by three different Iranian breeds, Raeini, Nadoushan and Birjandi, was indeed coarser but also longer than cashmere from China and Mongolia.This paper studiedcashmere quality and its variation in Raeini flocks in the main cashmere producing region in Iran in order to determine the scope for improvement.

Materials and methods

Twenty nine Raeini nomad flocks were randomly selected within about 20 km of the city of Baft and a stratified fiber sampling was organized. Samples were collected from four randomly selected goats of each sex (females, males) by age (1, 2 and 3 years) combinations; in total 686 samples were obtained; Sampling was conducted in early spring (mid-April 2010), prior to the seasonal moult and regular annual shearing period.

The raw cashmere samples consisting of undercoat and guard hair were sent to the Alrun Fiber Laboratory in Almaty, Kazakhstan for analyses. The dehaired cashmere was minicored into 2 mm snippets, washed in solvent, dried, reconditioned and then tested using an optical fiber diameter analyzer (OFDA 4000 in the mode of an OFDA 100). Based on more than 4300 individual fiber measurements, mean cashmere fiber diameter (MFD, μm), fiber diameter standard deviation (FDSD, μm) and fiber curvature (FC, $^{\circ}$/mm) were measured. Cashmere staple length (SL, mm) was obtained as the mean of three staples. Analysis of variance was performed using a mixed linear model (Mixed Procedure of SAS, 2008).

Results and Discussion

Table 1 shows the overall means, standard deviations and ranges for the six traits considered across all sampled animals. Table 2 presents flock averages for these traits. In this case some of the variation may be due to different management, shearing dates, genetic quality, or other environmental or genetic factors. As expected, across animal and across flock means were almost equal but standard deviations and ranges are smaller for flock averages. The flock averages not necessarily

Table 1. Overall means, standard deviations (s.d.) and ranges of fiber characteristics for Raeini goats

Trait	No of animals	Mean	s.d.	Minimum	Maximum
Fleece weight (g)	643	507.3	182.6	100	1,250
Cashmere yield (%)	686	56.5	12.2	9.5	87.1
Mean fiber diameter (µm)	686	19.7	1.5	14.9	25.2
Fiber diameter standard deviation (µm)	686	4.5	0.6	3	7.4
Fiber curvature (°/mm)	686	62.9	8.5	33.9	93.6
Staple length (mm)	686	54.2	7	40	79

Table 2. Flock average means, standard deviations (s.d.) and ranges of fiber characteristics for Raeini goats

Trait	No of flocks	Mean	s.d.	Minimum	Maximum
Fleece weight (g)	29	505.6	94.3	306.3	678.1
Cashmere yield (%)	29	56.5	4.5	49.9	72.6
Mean fiber diameter (µm)	29	19.7	0.6	18.4	20.5
Fiber diameter standard deviation (µm)	29	4.45	0.19	4.03	4.91
Fiber curvature (°/mm)	29	62.9	3.3	57.8	69.3
Staple length (mm)	29	54.2	1.4	52.2	57.1

represent a typical Raeini flock because of the deliberate stratified sampling in our study.

Males had on average 139.9 g ($P<0.0001$) higher fleece weight than female goats but there was no significant difference in fleece weight between goats at different age. Males had also slightly higher cashmere yields than females (2.6% points, $P<0.003$) and there was a slight reduction of yields with age (58.3, 56.1, and 55.3%, $P<0.019$).

Results indicate that overall cashmere diameter was 19.7±1.5 µm. In a FAO publication Iranian cashmere was described as having a range of diameter of 17-21 µm and that it is chiefly used for weaving (Petrie, 1995).

All samples had a curvature greater than 34 °/mm with 17% between 34 and 60°/mm, 61% between 61 and 75 °/mm and 22% between 76 and 94 °/mm. Compared with cashmere of China, Tajikistan and Kyrgyzstan with mean fibre curvature of 46, 46, and 58 °/mm (McGregor *et al*., 2009); cashmere of Raeini goats would be

considered as highly curved and long which is preferred for woven worsted yarn products.

Significant strong negative relationship (-0.647, P<0.0001) was found between mean fiber diameter and fiber curvature (Figure 1). This negative relationship in cashmere goats of Kyrgyzstan and Australia was 51 and 39% respectively (McGregor and Butler, 2009; McGregor *et al*., 2009). Average cashmere staple fiber length was 54.2 mm (Table 1) with no age or sex effects. The actual distribution of staple length and FD of samples in Figure 2 shows that there is no strong relationship between these two characteristics.

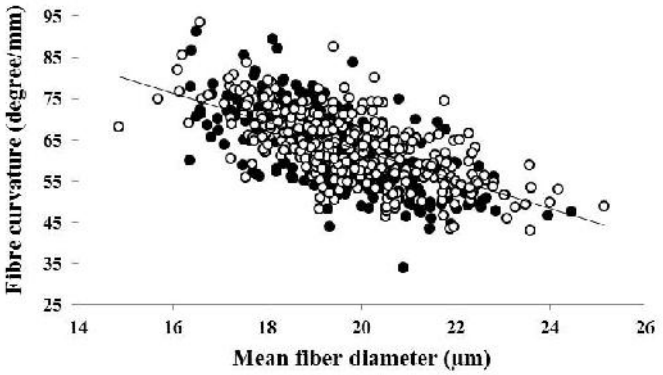

Figure 1. The relationship between mean fibre diameter and fibre curvature from individual goats. Symbols: does (●); bucks (○).

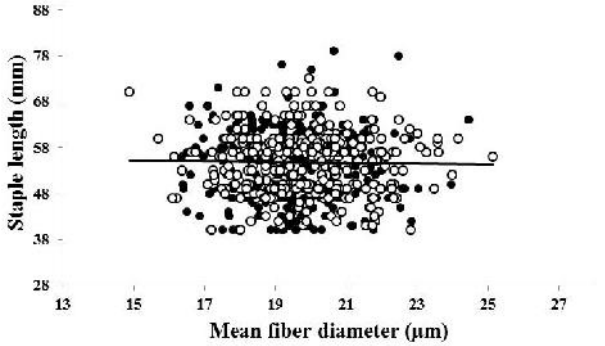

Figure 2. The relationship between mean fibre diameter and staple length from individual goats. Symbols: does (●); bucks (○).

Development options for cashmere production and conclusion

Raeini cashmere can be characterized as long and highly curved however steps must be taken to improve fibre diameter to capture higher prices in the international markets. Significant differences were found between goats and between flocks in-

dicating the potential to improve cashmere quality and the need for adopting proper management and selection methods. This may be achieved through selection of goats with finer cashmere taking care of maintaining the excellent cashmere staple length and curvature. Moreover, sorting the clip in fiber diameter lines would certainly improve cashmere quality; cashmere fleeces from one year old goats and that of fine older goats should be kept separate from the coarser cashmere fleeces after harvesting and before packaging. Furthermore, nomad producers do not comb their goats to harvest shed fibres, instead they shear 1-2 months after onset of shedding. Results from previous studies indicate that 30% of cashmere is lost during shedding season and if not harvested it would be wasted (Ansari-Renani *et al*., 2011). Introducing combing would increase the weight and commercial value of cashmere.

However, at present no price differential is paid to the producers for fine cashmere, as a major portion of cashmere produced is exported without any added value through processing. Cashmere harvesting and buying takes place over a short period of time in spring. The nomad producers and small-scale domestic traders are not aware of world market prices for different cashmere quality classes. As a result of the current marketing system and lacking infrastructure nomad producers do not achieve good prices and have little incentive to produce better quality cashmere.

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Properties of Salt Coagulated Cheese Produced by Calcium Chloride and Calcium Propionate

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Abstract

The project was carried out to clarify the physical, chemical and microbial properties of cheese coagulated by salt solutions. Salt coagulated cheese was produced by adding 4.2% by volume salt solution into boiled whole raw milk. Two salt solutions of calcium chloride and calcium propionate were studied at 3 different concentrations, including 5, 10 and 20% (w/v). The cheese curds were then pressed for 1 h at room temperature, cut into small cubes and kept at refrigerated temperature for physical, chemical and microbial analysis. In general, the yield of calcium propionate cheese was slightly higher than that of the calcium chloride cheese. The calcium propionate cheese had higher moisture content and pH value together with lower salt content and hardness compared to those of the calcium chloride cheese. The highest salt content of 1.27 $\pm$ 0.13% was found in the calcium chloride cheese added with 20% salt solution, whereas the highest hardness (4.03 $\pm$ 0.12 N) was discovered in the calcium chloride cheese supplemented with 5% salt solution. Different types and concentrations of salt solutions did not significantly affect the whiteness of the cheese sample ($p>0.05$). The number of psychrotroph bacteria in different cheese samples was in the range of 3.48 – 5.48 log cfu/g, while the presence of yeast and mould in all the cheese samples was lower than 1.49 log cfu/g.

Keywords: calcium chloride, calcium propionate, salt coagulated cheese, salt concentrations

Introduction

Cheese is one group of fermented milk based food products that are produced in wide range of flavours and forms throughout the world (Fox and McSweeney, 2004). The production of cheese involves 3 basic principles, including coagulation of milk, reduction in moisture content and ripening (Nielsen, 2004). Coagulation of milk can be carried out by the action of rennet enzyme and/or acidification (Fox and McSweeney, 2004; Nielsen, 2004).

Milk coagulation was a complex process, which was affected by calcium addition, phosphate addition, pH, ionic strength and temperature (McMahon *et al*., 1984). Coagulation of milk during cheese production is influenced by concentration of casein and milk fat. When acid was used to coagulate the milk, the acid dissolved the colloidal calcium phosphate of the casein micelles and neutralized the electric charge of the particles, which caused the micelles to be aggregated (Walstra *et al*., 2006). The addition of salt solution would also destabilize the casein micelle. Pastorino *et al*. (2003) mentioned that adding salt to milk or casein systems promoted dissociation of calcium and phosphate from casein micelles into the solution.

Calcium chloride was occasionally added during cheese making to reduce the lag time between enzyme addition and coagulation. The addition of the salt solution could also reduce the requirement of rennet by 50% (McMahon *et al*., 1984). McMahon *et al*. (2005) found that an increase in the calcium content of non fat Mozzarella cheese caused the protein bundles became larger and denser with a corresponding increased in serum pockets as water was excluded from the protein network matrix.

This study was concentrated on the production of cheese coagulated by salt solutions. Two calcium salts, including calcium chloride and calcium propionate, were investigated.

Materials and Methods

Production of Salt Coagulated Cheese

Raw milk from a local dairy cooperative in Chiang Mai was purchased and delivered to the laboratory within 30 min under refrigerated condition. An amount of 1,200 ml raw milk was boiled, added with 50 ml of calcium salt solution, either calcium chloride (Foodfill, Bangkok, Thailand) or calcium propionate (Kemira Chem Solutions b.v., Holland, Netherland), and continued to be heated for a further 5 min to ensure the coagulation was completely be carried out. Each of the calcium salt solution was studied at 3 concentration levels, which were 5, 10 and 20% (w/v). The separated liquid (cheese whey) was then separated using cleaned double layers cheese cloth. The cheese curds were collected and pressed in a small scale pressing equipment for 1 h at room temperature to promote more removal of the cheese whey. At the end of the pressing time, the cheese curd was cut into small pieces (approximately 2x2x2 cm^3) and kept in refrigerated temperatures until the time of analysis. Each treatment was prepared in triplicate.

Physicochemical and microbial analyses of salt coagulated cheese

Yield of salt coagulated cheese was calculated based on the amount of the final cheese curd (after pressing) divided by the amount of raw milk used and multiplied by 100. The amounts of salt, total titratable acidity and moisture content of salt

coagulated cheese were determined using methods of AOAC (2000). The measurement of pH, colour and texture of salt coagulated cheese were carried out using a pH meter (Consort C830, Belgium), a colorimeter (Minolta CR-300, Japan) and a texture analyzer (Texture Analyser model TA.XTPlus, Stable Micro Systems, UK), respectively.

For microbial enumeration, total plate count, the count of yeast and mould and psychrotroph bacteria were carried out based on procedures published by Harrigan (1998).

Results and Discussion

The yield of salt coagulated cheeses was more affected by the type of calcium salt solution rather than the concentrations of salt solutions (Fig. 1a). The cheese curd coagulated with calcium propionate produced higher yields compared to those of the curd added with calcium chloride. A cheese yield between 10.1 and 10.4% had been reported by Heino *et al*. (2010) for Edam cheese milk, whereas Hydamaka *et al*. (2001) found a yield of 47.7 to 74.6% for heat and acid coagulated cheese from ultrafiltered milk retentates. Differences in the cheese yield were mainly affected by different pressing time and pressure.

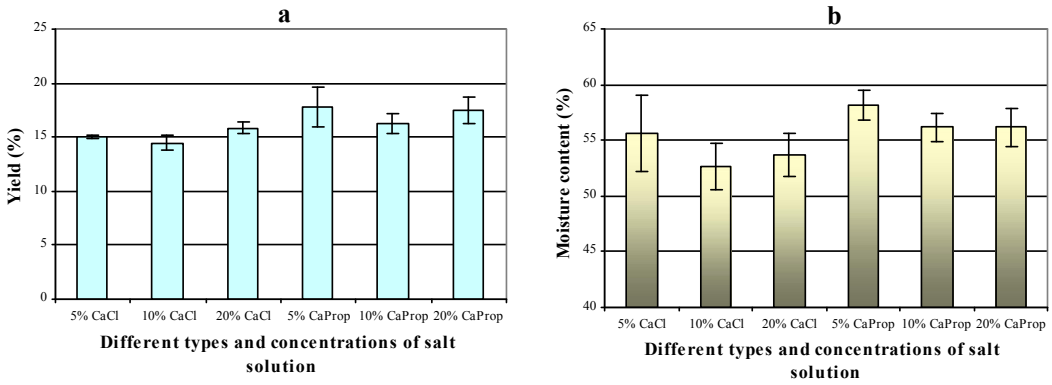

Figure 1. Yield (%) (a) and moisture content (%) (b) of salt coagulated cheese affected by different types and concentrations of calcium salt solutions

A higher cheese yield produced by calcium propionate was contributed to a higher moisture content found in the cheese samples (Fig. 1.b). The lowest moisture content of 52.67 $\pm$ 2.07% was found in the cheese coagulated with 10% calcium chloride. Okpala *et al*. (2010) reported a moisture content of 63.1 $\pm$ 0.8% for fresh cheese, whereas Dave *et al*. (2003) found moisture contents between 53.2 and 57.8% for direct acidification of Mozzarella cheese. Discrepancy in the moisture content of different cheeses was affected by the pressing condition, raw milk composition,

type and strength of coagulant and processing parameters (Hydamaka *et al*., 2001; Dave *et al*., 2003).

Cheese samples coagulated with calcium propionate significantly had higher pH values compared to those produced with calcium chloride (Fig. 2a). The pH of calcium chloride cheese samples was similar to the report of Singh *et al*. (2007), who found that the addition of calcium salts (50 mg/100ml) in milk reduced the pH of milk to be about 6.3 that led to destabilization of the milk. The high pH found in this study might also be affected by the absence of microorganisms and acid addition during its production.

The amount of salt measured as sodium chloride showed that the cheeses made from calcium chloride contained higher amounts of salt compared to those produced by calcium propionate (Fig. 2b). The highest amount of salt (1.27 $\pm$ 0.13%) was significantly found in the cheese coagulated with 20% calcium chloride. This value was similar to the salt content in the Mozzarella cheese produced by direct acidification (Dave *et al*., 2003).

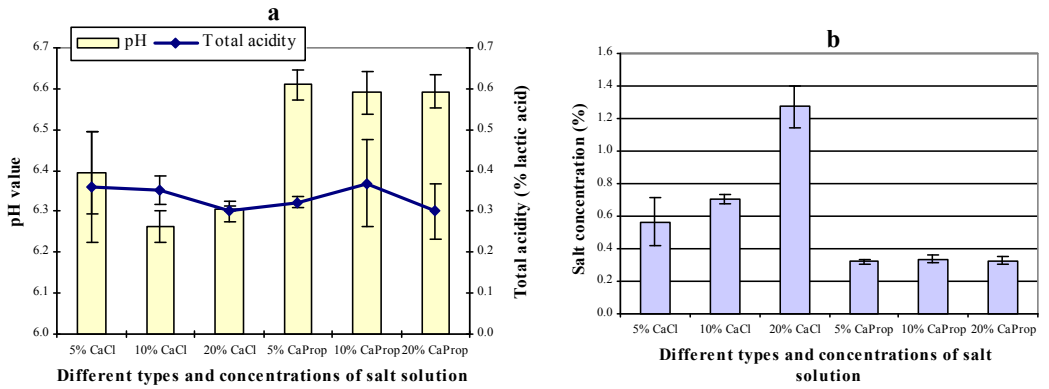

Figure 2. pH, total acidity (% lactic acid) (a) and salt concentration (%) (b) of salt coagulated cheese affected by different types and concentrations of calcium salt solutions

The colour of different salt coagulated cheeses was not affected by different types and concentrations of salt solutions (Fig. 3a). The cheeses had a light, almost pure white colour with slightly green and yellow colour directions. This finding was almost similar to the fresh cheese produced by rennet coagulation (Okpala *et al*., 2010).

The hardness of salt coagulated cheeses was affected by different types of salt solutions. Cheeses coagulated with calcium chloride had higher hardness values than those made from calcium propionate (Fig. 3b). The highest hardness value was found in the cheese produced by 5% calcium chloride. A fresh cheese produced by rennet coagulation was reported to have a hardness value of 2.54 $\pm$ 0.09 N (Okpala *et al*., 2010).

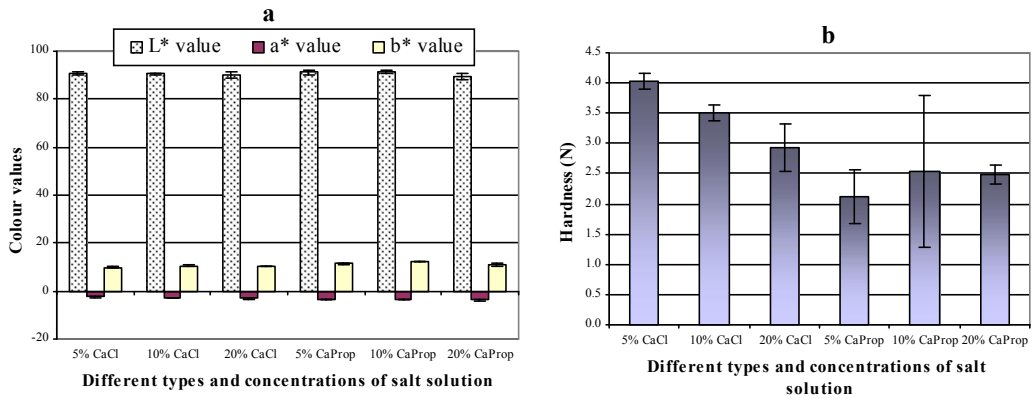

Figure 3. Colour values (a) and hardness (N) (b) of salt coagulated cheese affected by different types and concentrations of calcium salt solutions

Different salt coagulated cheeses contained a total microbial count between 5.00 ± 0.42 and 7.48 ± 0.00 log cfu/g, a total number of yeast and mould of less than 1.49 ± 1.42 log cfu/g and a total psychrotroph bacterium in the range of 3.48 ± 0.00 to 5.48 ± 0.00 log cfu/g. The high number of total microorganisms in the cheese could be contributed from the concentration factor of the raw milk during the boiling process. High pH values and moisture contents of the salt coagulated cheeses would be other factors that might support the survival of microorganisms during the pressing period for 1 h at room temperature. The majority of microorganisms in salt coagulated cheeses were represented by psychrotroph microorganisms.

Conclusions

From the collected data, it could be concluded that coagulation of raw milk could be produced by a combination of heat treatment and calcium salt solutions. The addition of calcium chloride created better cheese characteristics, indicating a better effect of the salt solution to destabilize the casein micelles. With a lower pH value of the calcium chloride cheese, the cheese had higher salt content and hardness value than those of the cheese produced with calcium propionate.

Acknowledgement

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Wool Fibre of Local and Crossbred Sheep: Production, Processing Technique and Performance

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Abstract

Local sheep in Indonesia are mainly raised to produce meat. In fact some of local sheep also actually produce strong (harsh) wool that have not been utilized as some farmers do shearing the sheep for sanitation purposes. Studies on wool production and technology of local sheep in Indonesia have not been reported. This field research was aimed to study (i) wool production and the fineness of local sheep and crossbred sheep wool, (ii) simple wool processing technique and (iii) Processing performance of both type of wool. The data of wool production were collected in Bogor by using 12 adult female sheep for each of local and crossbred sheep. The results showed that wool production of local sheep was lower than crossbred sheep (605.55 ± 22.98 g/head/year and 2911.75 ± 108.82 g/head/year, respectively). Wool fibres of local sheep were much coarser than crossbred (35.06 ± 6.14 μ and 22.94 ± 0.88 μ, respectively). This study also found that simple wool processing technique was able to be used for local sheep wool. This process steps included (i) sorting 1 (ii) washing (iii) drying (iv) sorting 2 (v) combing (vi) yarning, (vii) whitening, (viii) designing (ix) coloring and (x) weaving. It was found that processing performance of crossbred sheep wool was likely better than local sheep wool. It was concluded that both wool types produced by local and crossbred sheep were able to be processed to make different quality of yarn with simple processing technique. It is recommended that the technique can be applied to develop wool processing small industry to increase value added of sheep farming business as well as source of income for the community.

Keywords: fiber diameter, local sheep, processing, wool production

Introduction

Local sheep in Indonesia are mainly raised to produce meat and some additional and economic product such as sheep skin/hide. Some of local sheep, however, also actually produce strong (harsh) wool that have not been utilized, as some farm-

ers shear the sheep for sanitation purposes. This type of sheep are local crossbred sheep that have been in Indonesia for decades and well adapted in certain areas. The crossbred sheep were originated from the importation of subtropical sheep from Netherlands and other sheep producer countries, some decades ago. Studies on wool production and technology of either local or its crossbred sheep in Indonesia have not been many reported. Parakkasi *et al* (1994) reported that wool growth and fibre diameter of Priangan sheep were 0,30 g/cm2/day and 51,47 µm, respectively, while Syamyono *et al* (2002) found that the wool production of Priangan sheep was 391,5 ± 90,2 g/head/year, and sheep fibre diameter (FD) were 30,13±13,11 µm for their wool and 130,44±20,58 µm for kemp (rough wool). On the contrary, wool growth of subtropical dual type sheep were much higher as reported by Lupton *et al*. (2004) that Dorset produced wool of 2,3-4,1 kg/head/year. Similarly the FD of the sheep was also much finer than Priangan sheep (FD of Dorset was around 31,5±6,45 µm, Finsheep 27,5±6,08µm, Romanov 27,7±17,46 µm, Texel 34,1±7.32 µm, and Montadale 29,3±5,98 µm) (Lupton *et al.,* 2004).

This field research was then aimed to (i) study wool production and fibre diameter of local sheep and crossbred sheep in both sex (ii) identify wool processing technique and (iii) to study processing performance by using loss percentage of wool staple during wool processing in both local and crossbred sheep at different sex.

Materials and Methods

Materials

- Local thin tailed sheep: 5 heads of each male and female adult sheep (2 years old) were used from Sekati sheep farmer group, Ciomas Bogor.
- Crossbred sheep: 5 heads of each male and female adult crossbred sheep (Merino x Dorset) were used from a sheep fattening commercial farm, Gunung Putri, Bogor.
- Equipments: wool shearing scissors, scale, micrometer, small scissor, holed ruler, carder, yarn maker, plastic bags, detergent and disinfectant.

Methods

- Wool production: sheep were shorn throughout the body by using manual shearing scissor special for sheep wool/hairs. The greasy wool was then weighed. The date of last shearing was recorded according to secondary data from the farmer group (for thin tailed local sheep) or enterprise (for crossbred sheep).
- The fineness of wool was determined by measuring fiber diameter (FD). Wool sample was taken by clipping the wool staple at 5 different areas (1 cm^2 each) by putting the staple clip at a hole area of a ruler. FD of one sample was the average of 4 wool fiber having the mosr coarser (2 fibers) and the most finest (2 fibers). By using a micrometer the fiber was measured at the base of fibre with a normal

pressure.

- Identification of wool processing technique: Direct observation was conducted to record the technique applied in a wool processing used in wool handicraft group in Indramayu. Timing, materials and procedures of the technique were identified.
- Processing Performance/Processing loss of wool staple. Samples were weighed at each step of wool processing by using a scale. Percentage of wool loss is defined as percentage wool loss of total wool before being processed in each step.
- Statistical Analysis: data between local and crossbred sheep were compared with descriptive analysis, as they were collected from different locations and different management system. T-test was used to compare the differences between sheep sex on the processing performances.

Results and Discussion

Wool Production and Fiber Diameter

Sheep are characterized to have wooly typed hairs, some breed of sheep produce good wool, others are just harsh wool. Results of this study show that in local sheep there were no significant differences between sex on wool production (589.3 ± 85.43 and 621.8 ± 105.94, respectively in male and female sheep). FD was also similar between male and female sheep (39.4 ± 1.87 and 30.72 ± 4.98, respectively) ($p>0.05$) (Table 1). Wool production in crossbred sheep was around 4-5 much higher than wool production in local sheep, although statistically these data cannot compared as the wool were from different locations. However for local sheep, they are given a good standard of sheep farming system in the village. Wool growth starts at the base of wool follicle from a root of follicle called dermal papilla where nutrients input is supplied to the follicle through the blood vessels to dermal papilla.

Similarly, wool of crossbred sheep were finer than local sheep wool (Table 1). According to wool standard, the crossbred FD in this study can be categorized as medium wool type and the local sheep wool was as strong/coarse wool. Genetically sheep have wool type, meat type as well as dual purpose (Cottle, 1994). Crossbred

Table 1. Wool production and fiber diameter of local and crossbred sheep in both sexes

Sheep breed	Sex	Wool production (Greasy weight) (g)	FD (mμ)
Local sheep	Male	589.3 ± 85.43	39.40 ± 1.87
	Female	621.8 ± 105.94	30.72 ± 4.98
Crossbred Sheep	Male	2,834.8 ± 360.99	22.32 ± 1.47
	Female	2,988.7 ± 453.56	23.56 ± 2.04

sheep used in this study were between Merino (wool producers) and Dorset (meat type), it is therefore the wool was identified as medium type, unlike most Merinos have fine wool.

Wool processing technique

This study shows that the wool processing technique was quite simple. There were 10 steps identified in the processing, including (i) sorting 1 (ii) washing (with detergent and disinfectant) (iii) drying (iv) sorting 2 (v) combing (vi) yarning, (vii) whitening/bleaching, (viii) designing (ix) coloring and (x) weaving. More simple steps were shown in Figure 1.

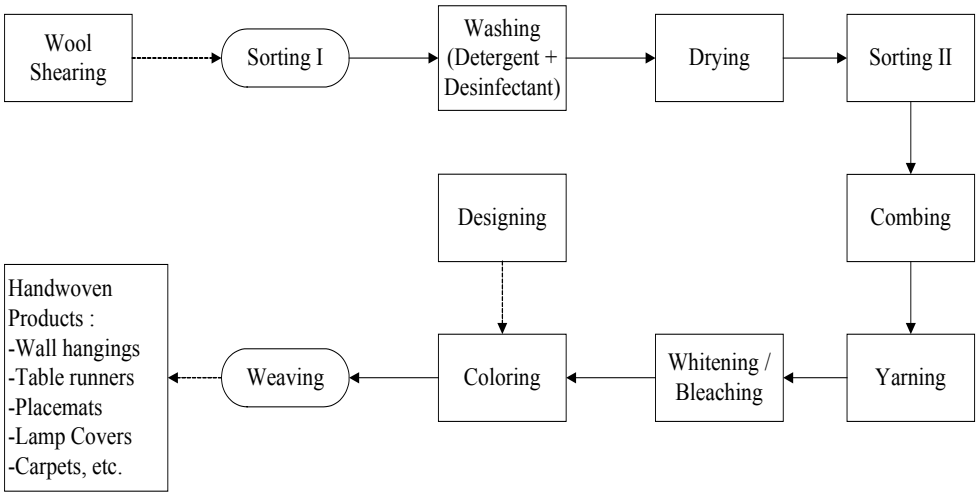

Figure 1. Diagram of steps in wool processing technique

In first step, sorting was applied by cleaning and throw away any dirts/strange materials sticked on the wool, such as dry feces, soils, dry grass etc. Washing process was started by soaking the wool in water for 24 hours to partition sticky wool fibres. Then soaking and cleaning with detergent for 2-3 hours (100 g detergent/10 liters of water) was applied to the greasy wool. The next step was soaking for one hour and cleaning with disinfectant (10 cc detergent/10 liter of water). Drying procedure was proceeded by putting the wool under sun until dried for 2-3 days depending on the climate condition. The dried wool were then sorted again by separating the wool fibres by hands and hand carders. The next process was combing the wool fibers by using drum carder several times. The combing wool staples were then process to become wool yarn by yarning the wool using non-machine yarning tool. Yarning needs experience to practice to be a skillful yarning technician. To clean any left wool grease produced by sebaceous glands in order to make cleaner and more white, whitening the yarn was then applied by boiling the wool for 2 minutes in solution of

2 liters of water, 10 cc of peroxide acids (H_2O_2) and 2 tea spoons of detergent, then rinsing with clean water and dried under indirect sunshine. Coloring the wool yarn was applied by boiling the wool yarn in a solution of 10 liters of water, 0.3 liters of vinegar concentrate for 1 hour, rinsing and then drying. Type of color depends on the design of the woven handicrafts. The last step was weaving the yarn by using non-machine weaver. This technique needed a special skills involving patience, accuracy and arts.

Processing Performance

Wool staples from both breed were processed according to the technique identified in this study. The loss of wool during processing is important to study the efficiency of wool process that will determine the profit of its business. The results show that there were no significance differences of processing loss between sheep sex at any processing steps in both sheep breeds ($p>0.05$) (Table 2). However, when comparing sheep breed, local sheep wool was likely to have more loss in all processing steps. For local sheep wool, the average loss percentage in sorting 1, washing/drying, sorting 2, carding and yarning were 5.8; 45.7; 12.1; 16.1; 12.8 %, respectively, whereas for crossbred the average lost in the steps were 1.52; 31.2; 4.12; 13.1; 6.6 %, respectively at the wool processing steps. This findings may indicate that processing performance of crossbred sheep wool was better than local sheep wool.

Wool fibres can be processed during especially carding, yarning and weaving when the wool staples need to be strong. Keratin protein in wool follicles make the strength, in addition the waviness of wool making flexibility during the process to avoid breakage of fibres. Finer wool would have better wool processing performances (Leeder, 1984). Wool processing performance such as the loss during processing depends on breed, sheep nutrition and climate conditions (Hynd, 1989).

Table 2. Processing loss of wool in local sheep and crossbred sheep in both sex

Sheep Breed	Sex	Processing Loss (%)				
		Sorting 1	Washing + Drying	Sorting 2	Carding	Yarning
Local sheep	Male	3.74 ± 1.45	38.52 ± 8.63	12.52 ± 8.55	16.58 ± 4.74	17.44 ± 8.08
	Female	7.76 ± 6.00	52.84 ± 9.62	11.6 ± 4.74	15.58 ± 4.67	9.36 ± 1.67
Crossbred Sheep	Male	1.18 ± 1.52	27.40 ± 4.25	5.14 ± 1.41	13.90 ± 6.55	5.30 ± 4.20
	Female	1.86 ± 2.47	34.98 ± 6.18	3.10 ± 1.84	12.30 ± 7.61	7.94 ± 2.53

Conclusions

Wool production of local sheep was likely less than in crossbred sheep. Fiber diameter of crossbred sheep was clearly finer than in local sheep wool. Wool

processing technique was quite simple and reliable to process local and crossbred sheep wool. This process steps were (i) sorting 1 (ii) washing (iii) drying (iv) sorting 2 (v) combing (vi) yarning, (vii) whitening, (viii) designing (ix) coloring and (x) weaving. Wool in local sheep was able to process, however processing performance of crossbred sheep wool was likely better than local sheep wool. It is recommended that the technique can be applied to develop wool processing small industry to increase value added of sheep farming business as well as source of income for the community.

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Fiber quality of carpet-wool sheep breeds

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Abstract

This experiment was aimed to identify characteristics and comparative advantage of Iranian sheep breed wool. Fiber characteristics of 270 male and female sheep of 1, 2, 3 and 4 years of age belonging to Afshari, Zandi, Mehrabani, Lori and Baluchi sheep breeds were studied. Samples of fiber were taken from the left midside of sheep and analyzed using standard objective measurements for staple length (SL), mean fiber diameter (MFD), coefficient of variation of fiber diameter (FDCV), inner coat fiber ICF, outer coat fiber (OCF), kemp fiber (KF) and clean wool production (CWP). A general linear model was used to analyze the data usin SAS package. The mean (s.e) for SL, MFD, FDCV, ICF, OCF, KF and CWP were 110.8±0.1 mm, 36.9±0.5 µm, 50.2±0.8 %, 84.2±0.6 %, 9.8±0.4 %, 5.9±0.4 % and 69.3±0.7 respectively. MFD of 22% of all wool samples les was between 22 – 30 µm. MFD of a further 36 and 42 percent of wool samples was between 31 – 37 µm and coarser than 37 µm . SL of 12 % of wool samples was shorter than 100 mm and 38 % of samples between 100 – 120 mm and 50 % of samples were longer than 120 mm. There is substantial scope to improve the quality of fiber produced by Iranian sheep breeds through genetic selection.

Keywords: fiber diameter, fiber types, sheet, staple length, wool

Introduction

Sheep population of Iran was 53.8 million heads in 2008 which ranks 5th in the world (FAO, 2010) with 27 well defined breeds. This population of sheep produces annually about 400,000 tones of meat, 820,000 tones of milk, 60,000 tones of wool, 22 million skin pelts and 188,000 tones of guts (Ministry of Agriculture, 2009). More than 1.6 million people are directly involved in sheep breeding with significant role in the economy and livelihood of rural and nomadic societies.

The fleece of sheep grows from specialized follicles in the skin. While primary follicles bear medullated outer coat coarse fiber (hair) and provide mechanical

protection, secondary follicles produce non-medullated inner coat fine fiber or true-wool which provide thermal protection (Nixon, et al. 1991). Carpet wool quality and value is primarily determined by fiber diameter and length which reflect the degree of wool growth and fineness respectively. Presently, little technical data is available on Iranian sheep fiber characteristics. Accordingly, the present work was designed to identify fiber characteristics and development options for future utility.

Materials and methods

A total of 212 sheep (75 males and 137 females) of Afshari, Zandi, Mehrabani, Lori and Baluchi breeds respectively from Zanjan, Qom, Hamedan, Lorestan and South Khorasan provinces were used in this study. The sheep grazed all year but their diets were supplemented during winter with limited amount of forage and grain (containing 15 g N kg^{-1} dry matter and 9.1 MJ) and were housed at night during severe weather conditions. Sheep were grouped into 4 age groups: 1, 2, 3 and 4 years old.

About 10 g of fiber containing hair, kemp (medullated) and true wool (non-medullated) from the left mid-side site was cut from a 5 × 5 cm square close to the skin using regular scissors. Each sample was separately packaged and labeled with ear tag number, age, gender and the breed of the sheep.

To determine the percentage of clean wool weight, net bags containing samples without contaminants were weighed immediately, immersed in three scouring bowls solution containing 0.3% of Na_2CO^3 and 0.1% of soap and water and stirred for 15 minutes at a temperature of 52±3 ^oC. This procedure was repeated once more but only with warm water. Washed samples were oven-dried and weighed and the percentage of clean wool weight was estimated. The mean fiber diameter of the washed wool sample was measured using a projection microscope (Chapman, 1960). The average staple length for each wool sample (in triplicates) was measured to the nearest 0.1 cm.. The number of non-meduallated inner coat fiber, meduallated outer coat hair fiber and medullated kemp fiber was measured (IWTO, 1952). Analysis of variance was performed using a general linear model (GLM) of SAS package (SAS, 1996). Differences between means were tested using Duncan's new multiple range test.

Results and Discussion

For the measured wool characteristics total mean and standard errors are provided for different breeds, genders and ages (Table 1), and different ranges of fiber are shown (Table 2). 22 and 36% of all wool samples had a fiber diameter between 22-30 and 31-37 μm respectively. A further 42% of the wool samples were coarser than 37 μm. All samples were longer than 80 mm but shorter than 147 mm with 12% less than 100 mm, 38% between 100 and 120 mm and 50% longer than

Table 1. Mean (±s.e.) of yield (Y), mean fiber diameter (MFD), coefficient of variation of fiber diameter (FDCV), staple length (SL), non-medullated inner coat fiber (ICF), medullated outer coat fiber (OCF) and medullated kemp fiber (KF)

		No	SL (mm)	MFD (μm)	FDCV (%)	ICF (%)	OCF (%)	KF (%)	Y (%)
Mean			110.8±0.1	36.9±0.5	50.2±0.8	84.2±0.6	9.8±0.4	5.9±0.4	69.3±0.7
			**	NS	**	NS	NS	NS	**
Sex	Male	75	113.7±0.1^{a}	36.2±0.7	47.4±1.3^{a}	86.0±1.0	9.4±0.8	4.8±0.6	72.0±1.0a
	Female	137	116.9±0.2^{b}	37.1±0.6	51.3±0.9^{b}	83.5±0.8	10.0±0.5	6.4±0.5	68.3±0.9b
			*	*	**	**	NS	**	*
Age	1	53	104.7±0.2^{a}	34.2±0.6^{b}	47.2±1.5^{b}	87.3±1.2^{a}	8.9±0.9	3.8±0.6b	73.4±1.1a
	2	59	112.6±0.2^{b}	38.1±0.8^{a}	53.3±1.1^{a}	82.0±1.0^{b}	10.5±0.6	7.1±0.6a	67.0±1.2b
	3	44	113.3±0.2^{b}	36.2±1.0^{ab}	49.2±2.3^{ab}	85.4±1.7^{ab}	8.8±1.0	6.1±1.0a	68.7±1.8b
	4	56	122.1±0.3^{c}	37.9±1.0^{a}	49.3±1.6^{ab}	83.5±1.4^{ab}	10.4±0.9	6.2±0.9a	69.3±1.4b
			*	*	*	*	*	*	*
Breed	Afshari	68	110.7±0.1^{b}	39.5±0.6^{a}	53.6±1.0^{b}	77.1±0.8^{c}	14.0±0.5^{a}	8.7±0.5^{a}	64.4±1.0^{a}
	Zandi	21	120.0±0.2^{c}	30.6±0.7^{b}	43.4±1.3^{c}	93.7±1.9^{a}	6.1±1.9^{b}	0.8±0.3^{b}	63.2±1.6^{b}
	Mehrabani	41	90.1±0.1^{a}	42.1±1.2^{a}	60.7±2.2^{a}	84.8±1.4^{b}	5.5±0.7^{b}	9.5±0.9^{a}	76.5±1.3^{a}
	Lori	31	120.9±0.2^{c}	32.8±0.8^{b}	39.7±1.7^{c}	91.6±1.5^{a}	7.4±1.1^{b}	1.0±0.5^{b}	74.0±1.8^{a}
	Balouchi	51	130.3±0.1^{d}	31.0±0.4^{b}	42.8±1.2^{c}	92.7±0.6^{a}	6.1±0.5^{b}	1.2±0.2^{b}	75.6±1.3^{a}

* and ** significantly different at P<0.01 and P<0.05 respectively

Table 2. Classification of the incidence (%) of sampled wool based on mean fiber diameter (MFD), staple length (SL) and non-medullated inner coat fiber (ICF).

	MFD	%	SL	%	ICF	%
	22-30	22	<100	12	<65	5
All Breeds	31-37	36	100-120	38	65-80	28
	>37	42	>120	50	>80	67
	22-30	5	<100	0	<65	9
Afshari	31-37	36	100-120	60	65-80	48
	>37	59	>120	40	>80	43
	22-30	60	<100	0	<65	5
Zandi	31-37	35	100-120	62	65-80	5
	>37	5	>120	38	>80	90
	22-30	3	<100	85	<65	5
Mehrabani	31-37	24	100-120	15	65-80	15
	>37	73	>120	0	>80	80
	22-30	35	<100	0	<65	0
Lori	31-37	52	100-120	10	65-80	13
	>37	13	>120	90	>80	87
	22-30	43	<100	0	<65	0
Baluchi	31-37	55	100-120	4	65-80	4
	>37	2	>120	96	>80	96

120 mm. 67% of all samples had a percentage of inner coat fiber greater than 80 while 28 and 5% of wool samples had a non-meduallated inner coat fiber percentage of 65-80 and less than 65 respectively.

Percentage of wool fiber types

The 9.8 % medullated OCF in the present study is similar to that reported for Arabi (10.9 %) (Ashmawi and El-Azzawy, 1980) lower than those in the Awassi sheep in Jordan and Iraq (12%) (Tabbaa *et al*. ,2001, Al-Azzawi, 1977) and higher than those in the Ossimi (5.3%) and Rahmani (2.3%) sheep (Maria *et al*., 1992). There was no significant difference in inner coat fiber percentage between males and females of Iranian breeds of the present study, in agreement with the findings of Jordanian Awassi sheep breed. Tabbaa et al. 2001 reported 11 and 10% medullated inner coat fiber for male and female Awassi lambs respectively. One year old sheep had highest percentage of inner ICF percentage and decreased with age. Tabbaa *et al*. (2001) and Seoudy *et al*. (1973) also reported that the percentage of inner coat decreased with advancing age in Awassi and Barki sheep breeds. One year old sheep

had the lower percentage of kemp fiber than older sheep, a finding in agreement with Awassi sheep (Tabbaa *et al*., 2001).

Large variation in the percentage of ICF (58.9-98.6) and undesirable KF (0-33.6) demonstate the possibility for improving Iranian sheep fleece quality by selection. Farmers need to be trained on selection criteria for breeding rams based on subjective wool assessment to avoid keeping breeding males with high kemp percentage.

Mean fiber diameter and staple length

Zandi and Balouchi breed had the finest and longest wool while Mehrabani sheep had the coarsest and shortest with 73% of wool samples coarser than 37 µm. While Baluchi and Zandi wool is used in making fine carpets, Mehrabani wool is used in very rough and bulky appearance carpets indicating the need for breeding programs to decrease the fiber diameter and increase the staple length.

Results indicated that overall fiber diameter was 36.9 µm which is comparable to Middle Eastern carpet wool sheep breeds, 36.0 µm for Awassi (Tabbaa *et al.,* 2001), 31.0 µm for Barki (Seoudy *et al*., 1973), 35.4 µm for Ossimi and 31.5 µm for Rahmani (Maria *et al*., 1992). A significant effect of age on fiber diameter is in agreement with younger animals (Sidwell *et al*., 1971) and the Barki and Merino breed crosses in Egypt (Seoudy *et al*., 1973). The impact of age could be associated with larger body size and reduced skin follicle density and competition for nutrients and therefore fiber diameter of older sheep increases. In contrast with the present study, increasing age had no significant effect on fiber diameter in Awassi and Arabi sheep (Tabbaa *et al*., 2001, Al-Azzawi, 1977, Ashmawi and El-Azzawy, 1980) possibly because the animals were younger.

The staple length of 110.8±0.1 mm of Iranian sheep breeds of the present study is lower than that of Awassi sheep in Jordan 140.0 mm (Tabbaa *et al*., 2001) but higher than Arabi (Ashmawi and El-Azzawy, 1980) Ossimi and Rahmani sheep (Maria *et al*. 1992). A significant effect of age and sex on staple length is in agreement with Tabbaa *et al*. (2001), Sidwell *et al*. (1971) and Azzawi (1977).

Fiber shedding

It was observed that Afshari sheep was the only breed that had fiber shedding. Shedding is also common in double-coated British sheep breeds such as primitive Wiltshire and Soay sheep (Slee, 1963) and feral sheep such as Merino breed in Arapawa Island (Orwin and Whitaker, 1984) however the level of shedding which causes complete wool casting in latter breeds is much higher.

Conclusion

It is apparent that there are differences between Iranian sheep breeds in the way

the fibers they produce which contributes to different fleece characteristics such as fiber diameter, staple length and the level of medullation. Hand made carpet weavers and manufacturers prefer finer wool with lower fiber diameter and medullation for making highly notted/mm^2 and softer carpets. Significant difference is found between sheep in wool characteristics indicating the potential to improve wool quality and the need for adopting proper management and selection methods.

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Training Programme of Biogas to Minimize Environmental Pollution in the Tempok Village Sub Tompaso District

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Abstract

The cattle in the village Tempok traditionally maintained, in the sense that no caged animals. On the afternoon of cattle grazing in the garden, the evening brought home and left on the home page. The problem, waste of cattle can cause environmental pollution. Based on the problems of making biogas training was conducted with the aim to increase knowledge and awareness of peasant farmers in minimizing environmental pollution and produce biogas reactor. The goal of this group was the farmers' cattle Pinatoroan and Samperongan. The method used of the application of science and technology was extension and training program of making biogas. Waste of cattle produces methane (CH4), which can increase greenhouse gas emissions. One of the activities that can be done is processing of biogas as an effort to improve environmental quality. Biogas reactor is made of 2 pieces and has successfully seen the fire coming out of the stove. This activity is done to reduce greenhouse gas emissions produced from waste of cattle. The result of the application of science and technology was to increase knowledge and awareness of peasant farmers in minimizing environmental pollution. Biogas reactor produces gas as a fuel substitute. Benefits derived from these activities is to reduce expenditures for kerosene, reducing the dependence of fuel wood, the home page be clean, pleasing to the eye and reduce odor.

Keywords: biogas, cattle waste, environment

Introduction

According to Putro (2007), global energy crisis caused world oil prices reached U.S. $ 70/barrel. This condition influenced the life of Indonesian including rural people of the districts Tompaso. There is a need to provide an alternative energy supply through development non-fuel energy technologies which are environmentally friendly.

Based on joint decision of the Minister of Home Affairs and Minister of Agriculture, No. 54 of year 1996: 304/KPTS/L.P.120/4/96, about Guidelines for Implementation of Agricultural Extension, a program to improve farmer groups, based on local conditions and potential resources, and considering the strategic environment that influence it, have been run (Department of Animal Husbandry, 1998). The program was primarily intended for low income rural households. One energy technology in accordance with the requirements of the rural households was biogas technology. According to Srisertpol *et al*. (2010), biogas was one kind of energy and sustainable development that were essential to energy and environmental planning. Biogas from cattle waste could substitute kerosene which were expensive and scarce in rural area.

In District Tompaso there were two groups of cattle farmers, namely group of cattle farmers Pinatoroan and Samperongan. The groups maintained their cattle traditionally and extensively. On the morning until late afternoon the cattle were let grazing in the field. In the afternoon, around 18:00 o'clock, the cattle were brought back and let slept in their home yard. The system caused environmental problem due to unmanaged of the cattle dunk(El-Hadidi and A-Turki, 2007).

Based on these problems, we conducted a program to use cattle waste to make biogas. The purpose of program was to train members of the cattle farmer groups to convert their cattle waste into biogas. This program were consisted of two activities namely extention service and training. These activities were done as efforts to increase awareness of the cattle farmers in minimizing environmental pollution.

Materials and Methods

Based on the background and the problems above, extention service and training for groups of cattle farmers Semporongan and Pinotoroan have been conducted. Pinotoroan group consisted of 23 members while the Samperongan group have 20 members. In livestock development, especially beef cattle, extension service take an important role especially in strengthening of farmer groups and increase adoption of farm technology (Abdullah, 2008). Extension service that have been conducted in the rural Tempok were aimed at changing of the farmer behavior toward a better direction (Pambudy, 1999). Materials and media used were brochures and LCD projector. After the extension services, the farmers were trained in making biogas reactor and how to produce biogas. Materials and equipment used was waste of cattle, two old drum container, hoses, and gas stove. Extension service have been successfully carried out can be seen from the compactness of the group members in response to the manufacture of biogas. Technology adoption is measured from the biogas reactor has been successful in producing a flame.

Results and Discussion

The number of cattle owned by members of the Semporongan group was 55 and Pinatoroan group owned 64 cattle. The cattle were privately owned by the group. The cattle released waste daily. Unmanaged cattle waste produced methane (CH_4), which increased greenhouse gas emissions (GHG). Methane was a greenhouse gas that accumulates in the atmosphere due to human activities (Masse *et al*., 2003). Therefore, cattle farming have been blamed to cause global warming.

Livestock waste was a potential source of CH_4 emissions (Moss, 1993 in Masse *et al.*, 2003). Therefore, it should be converted into biogas. According to Yiridoe et al. (2009), production of biogas in general, was considered financially feasible if it was made from 50 cows or 200 sows.

In average, a family energy needs for cooking was 2000 liters per day. According Putro (2007), household cooking energy needs can be met from waste of 3 cattle. Therefore, biogas produced by the group was considered financially feasible (numbers of cattle owned by the group were more than 50 with average of cattle owned was 3).

Biogas technology has been introduced and developed quite a long time in Indonesia (Widodo *et al.*, 2009). Biogas technology can be applied to the scale of household, commercial or village (Eze, 2009). Bond and Templeton (2011) explained that the biogas contains 50-70% CH_4 and 30-50% CO_2. In nature, methane gas was always there, but there was a need for equipment and specific conditions to accelerate the formation of gas (Putro, 2007).

Biogas reactor was a device that can process waste into biogas. Each biogas reactor unit had been made from two drum container. The other two drums were used to build a gas reservoir. Cattle waste was mixed with water in the ratio 1: 1, stirred until dissolved and then inserted into the biogas reactor. Biogas reactor was made simply to be accessible to the farmers (Figure 1). Lo *et al*. (1984) noted that unwillingness of North American farmers to adopt the biogas technology were due to the high capital investment for construction of biogas. The earlier reactor had been made for converting pig waste (Adl *et al*, 2012).

A larger drum with a capacity of 200 liters were filled with water. The drum served as a control gas formation. Then a smaller drum with a capacity of 120 liters were then be put into the larger. The drum were fed with fresh cattle waste every day. The biogas process could reduce the ratio of carbon to nitrogen (C/N) 21.82 to 14.19 (Chen *et al*, 2010).

The biogas were resulted after 3-4 weeks of cattle waste convertion in the biogas reactor. Biogas was produced by bacteria that convert organic material in the absence of oxygen (anaerobic process) (Putro, 2007). This process took place during processing or fermentation. The resulted gas was consisted mainly out of CH_4 and CO_2. If the content of CH_4 gas was more than 50%, then the mixture was highly

flammable gas. The CH_4 gas content in the biogas produced from cattle waste in this training were about 60%.

Figure 1. Biogas Reactors using Drum (Oley *et al*., 2009)

The biogas reactor was connected to the reservoir gas, methane gas generated out through the hose to the gas reservoir. The resulting methane gas can come out through the hose from the gas reservoir to the gas stove. After 4 weeks, the gas can be heated up and used for cooking. Biogas production could partially replace fossil fuel energy so as to reduce the environmental impact. Biogas was cleaner fuels and renewable energy (Schievano et al, 2009). Furthermore, Barnhart (2012) said that household-scale biogas technology could be used for cooking as a substitute to firewood and improved human health and the environment.

Training of making biogas for cattle farmers in the village of Tempok very beneficial to the availability of fuel energy. As a result, household expenditures for kerosene, which was increasingly expensive and scarce, could be suppressed. In addition, this activity could be beneficial for reduction of environmental pollution. According to Simpson (1979), biogas production may also benefited from reduction of flies and mosquitoes reproduction cycle. While, Aklaku et al (2006) explained that the presence of biogas as an energy source would free the farmer from the dependence on wood fuel, reduced bad smell and the presence of animal pests such as flies. According Biyatmoko and Wijokongko (2011), an important benefit of biogas as a fuel alternative was because of it was cheap, the raw materials were easily available, and because it was environmentally friendly. Methane gas that will burn and destroy ozone could be optimally utilized as a source of fuel in rural communities.

According Amjid et al (2011), the opportunity cost of women increased in the presence of biogas and gave a positive impact on households. But its application as an alternative energy source was limited because of several problems including costly investment for development of each farmer. Widodo et al (2009) conducted a

study to develop a biogas reactor for scale of the group farmers. In this case the development of the village Tempok need government intervention. According Biyatmoko and Wijokongko (2011), there was an urgency for socialisation of biogas uses and improving public perceiving in biogas utilization. This condition, especially in rural communities, including improvement of capacity in technical and management digester care.

Conclusion

Application of science and technology can improve farmer knowledge and awareness of in minimizing environmental pollution. The availability of two units of biogas reactor in the Tempok Village produced gas that can be used as a fuel substitute for petroleum. Benefits derived from these activities were reduction of expenditures for kerosene, reducing the dependence on fuel wood, produced a better environment for the farmer by means of cleaner yard and less smell of cattle waste.

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Microbiological Characteristic and Antimicrobial Activity of Koumiss Against *Salmonella typhimurium* and *Mycobacterium tuberculosis*

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Abstract

Koumiss is a traditional fermented milk product originated from the Central Asian steppes and mostly produced from mare milk by spontaneous fermentation of lactose to lactic acid and alcohol. Koumiss's starter cultures consist of lactic acid bacterias (Lc. lactis and Lb. acidophilus) and yeast (Sc. cereviceae). Koumiss is believed to pose health promoting properties, which is mainly related to the ability of the starter to produce vitamins of the B-group and antimicrobials. Koumiss could also served as functional food that was showed by its capability to produce the antimicrobial substrate that inhibit pathogenic bacterias. The objectives of this research were to identify microbiological characteristics of koumiss and to study its antimicrobial activity towards pathogenic bacterias such as S. Typhimurium ATCC 14028 and M. tuberculosis H37RV. The experimental design used on this research were non parametric of cohran test for M. tuberculosis H37RV and descriptive analysis for S. Typhimurium ATCC 14028. Variables observed were diameter of inhibition zone of the antagonistic assay used well diffusion method agar for S. Typhimurium ATCC 14028. In addition, the Lowenstein Jensen (LJ) modification agar was used for study the inhibition of M. tuberculosis H37RV. The result showed, koumiss could decrease the total of coliform. The average of koumiss's inhibition zone in different storage time toward S. Typhimurium ATCC 14028 was ± 7.801 mm. It was bigger than filtrate which was ± 6.002 mm. The average of diameter showed the antimicrobial activity of koumiss against S. Typhimurium ATCC 14028. The result of Cohran test showed the growth of M. tuberculosis H37RV could obstructed with modification of LJ extra koumiss stored for 4, 6, and 8 days. The conclusion of this research that koumiss was effective to against pathogenic bacterias such as S. Typhimurium ATCC 14028 and M. tuberculosis H37RV.

Keywords: antimicrobial activity, koumiss, mare's milk, pathogenic bacteria

Introduction

Milk is animal products contain a variety of potential nutrients that the body needs. Physical and chemical composition of mare's milk is different from cow's milk, goat, buffalo and camels. Mare's milk has a low fat content is 1.6% and high lactose of 6.1% Chandan et al. (2008). in Indonesia mare milk called wild horse milk is widely produce in West Nusa Tenggara (NTB). This milk is a naturally fermented milk product that has a liquid consistency without pasteurization treatment.

Koumiss is made by fermentation with a mixed microflora, which contains different lactic acid bacteria and yeasts that use for the treatment of tuberculosis in Russia. Bacteria are commonly used as starter cultures are producing antimicrobial substrates that have antagonistic properties against pathogenic bacteria. The number of high antimicrobial substrates will play a more powerful in inhibiting pathogenic bacteria, especially *Salmonella typhimurium* and *Mycobacterium tuberculosis* bacteria.

Materials and Methods

Preparation of Koumiss starter culture

The first step for making a starter koumiss is pasteurized mare milk at 65 °C for 30 minutes, then cooled to a temperature of 28 °C. Koumiss starter culture made by dividing the three equal parts, one part milk with *Lc. lactis* D-01, one part milk inoculated with *Lb. acidophilus* Y-01 and then incubated at 37 °C for seven hours and one part milk inoculated with *Sc. cereviceae* at 25 °C for five hours. *Lc. lactis* D-01, *Lb. acidophilus* Y-01 and *Sc. cereviceae* as much as 3%-5% (v/v) mived into the mare pasteurize milk. The results of a mixture of milk and starter cultures were incubated at 28 °C for 24 hours to form the desired starter (modified Rahman et al., 1992).

Koumiss manufacture

Koumiss made by pasteurized at 65 °C for 30 minutes and after the temperature reached 28 °C were inoculated with a starter (30%). Incubation was performed again at 28 °C for 42 hours (Rahman *et al*, 1992).

Characteristics of microbiological koumiss

Pipetted one ml koumiss, put in a petri dish, then poured with 15-20 ml of sterile medium and homogenized. Petri dishes were incubated with the situation reversed in an incubator temperature of 37 °C for 24 hours (Fardiaz, 1992). Microbial colonies formed was calculated based on the Standard Plate Count (SPC).

The antimicrobial activity of Koumiss against Salmonella Typhi-murium

The inhibition of antimicrobial activity against the *Salmonella* typhi-murium made by the well agar diffusion method (Wiryawan *et al.*, 2009). This method performed by spreading the bacteria standard 0.5 Mc. Farland without equally diluted, cut well with a hole punch or cork borer (5 mm), coated with Bacteriological media koumiss used to avoid seep at the bottom of the well. A total of 50 µl koumiss pipetted into the well, then the cup is placed in the refrigerator and then incubated at 37 °C for 24 hours.

The antimicrobial activity of Koumiss against Mycobacterium tuberculosis

Inoculation of bacterial suspension begins with the preparation of *Mycobacterium tuberculosis* H37RV with a standard concentration of 0.5 Mc. Farland and diluted to 10^3 cfu/ml. Dilution made by adding 1% of bacteria (v/v) into five ml of NaCl sterile. A total of 100 ml bacterial suspension was inoculated into the media of resistance that has been prepared. Incubation media tubes with horizontal position with the angle of 30^o to the incubator at 37 °C for one night with the lid loose. After incubation, the tube caps are tightened and enforced tube into a vertical position. Colony growth readings performed at day 28 and 42 (Sjahrurachman, 2008).

Statistical Analysis

Testing treatment for *M. tuberculosis* H37RV is day 0, 2, 4, 6 and 8. Analysis of data for *M. tuberculosis* H37RV using non-parametric design, Cohran test. Statistical models are used as follows:

Cohran Test (Daniel, 1990)

$$Q = \frac{c\,(c-1)\left(\sum_{j=t}^{c} C_j^2\right) - (c-1)N^2}{(cN) - \sum_{i=1} R_i^{\,2}}$$

Information:

Q = Cohran statistics

C = Number of replication

N = Total number of treatment

R = Total number of replication

Results and Discussion

Microbiological characteristics of Koumiss

Koumiss microbiological charac-teristics observed a total coliform, total microorganisms (TPC), total lactic acid bacteria and yeast total. Microbiological characteristics of koumiss in this study had 9.67 $\log_{10}$ cfu TPC/ml, coliform> 1 $\log_{10}$ cfu/ml, LAB 10.13 $\log_{10}$ cfu/ml and 9.72 $\log_{10}$ cfu yeast/ml.

The number of yeast colonies during storage ranged 9-11 $\log_{10}$ cfu/ml. This amount is less than the number of LAB colony during storage ranged 8-12 $\log_{10}$

cfu/ml. LAB and yeasts grew together form a symbiosis in the koumiss like kefir grains. Yeasts in the kefir grains serves to maintain the integrity and viability of microflora populations. Essential amino acids and growth factors for lactic acid bacteria produced by yeast, whereas the metabolites of LAB is used as an energy source. Symbiosis between the LAB and the yeast is making kefir into a stable product (Farnworth and Mainville, 2003).

The inhibitory activity of antimicrobial Koumiss againts S. typhimurium ATCC 14028

The diameter inhibition of koumiss against *S. typhimurium* ATCC 14028 increased on the day of the storage (H8). Koumiss has a pH of 3.87 ± 1.77 0.004 ± 0.032 and TAT. The optimum pH value for growth of *S. typhimurium* is 6.5 to 7.5 (Cox, 2000), the growth of *S. typhimurium* ATCC 14028 koumiss been restrained by a low pH. Diameter inhibition of koumiss again *S. typhimurium* ATCC 14028 with a spread plate method is smaller than the pour plate method. The population of *S. typhimurium* ATCC 14028 on a spread plate method is 10^8 cfu/ml, whereas the pour plate method 10^6 cfu/ml.

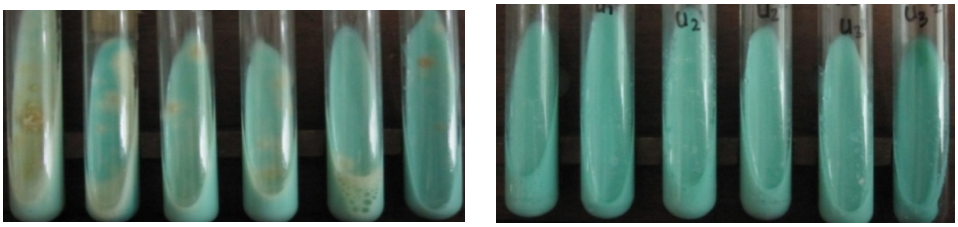
Antimicrobial activity can be observed in the diffusion test wells influenced by several factors, such as: (1) the type and size of the tube, (2) the type of agar, pH and salt content, (3) the ability of substances to diffuse into the agar, (4) characteristics of the media and (5) type of test bacteria used (Branen, 1993).

Inhibition of the filtrate and koumiss by category Morales et al. (2002) included the intermediate category. Intermediate category is the category with inhibitory response against bacterial pathogens that need to be treated with high doses of antimicro-bials. One way is by doing regular therapy with these antimicrobials.

The inhibitory activity of antimicrobial Koumiss againts M. tuberculosis H37RV on variety storage times

Control indicates the growth of *M. tuberculosis* with means proportion of 1.00 *M. tuberculosis* H37RV grown on Lowenstein Jensen media controls that are genuine growth medium *M. tuberculosis*. Growth of *M. tuberculosis* H37RV in the 8th week of observation is presented in Figure 2.

Growth of *M. tuberculosis* H37RV in the 8th week of observation for the control treatment highly significant ($P<0.01$) with the addition of koumiss treatment on day zero storage time (H0), four days (H4), six days (H6) and eight days (H8). Aditama (1999) suggest that the inhibitory properties of fermented compounds are bacteriostatic, because *M. tuberculosis* still can grow when the acidity is removed until it reaches a neutral pH.



control (n=6) *koumiss* H8 (n=6)

Figure 2. Growth of *M. tuberculosis* H37RV in the Storage Koumiss Days (H2), (H4), (H6) and (H8)

Conclusion

Koumiss with storage treatment can inhibit bacterial growth of *S. typhimurium* ATCC 14028 in the range of inhibition zones varying, while the antimicrobial activity of koumiss effective in inhibiting the growth of bacteria *M. tuberculosis* H37RV after the product has a minimum of four days of storage.

Acknowledgment

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Potency of Wool Handicrafts Production in Indonesia

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Abstract

Wool from local sheep have not been utilized and become a waste when the farmers shear the wool for sanitation purposes. The wool of local crossbred sheep can be still processed for a handwoven handicrafts. Study of the wool processing in Indonesia are still very limited, it was therefore this study was conducted to investigate the potency of the product itself and the economic potency of this product. This was a case study in a wool processing small industry in Indramayu, West java. Wool materials used were from local crossbred sheep. Wool product potency evaluated were types, capacity and favorability of the product through primary and secondary data from observation, questioners and references. Economic potency was analyzed with descriptive and financial analysis. Descriptive analysis was used to describe value chain and labor absorption. Financial analysis used indicator of NPV (net present value), IRR (internal rate of return) and PBP (payback period. The results show that the wool processing products were mostly wool woven wall hangings (size of 60 x 120 cm2) which were only produced at 4 % of its production potency. The consumers liked the products as they were beautiful as natural fiber product and had high value of arts. The wool handicraft production also had good economic potency in terms of its NPV, IRR and PBP. In addition of financial profit, the small industry also provided jobs to community. It is concluded that this wool handicraft small industry can be recommended to develop as a profitable small business in Indonesia.

Keywords: economy, hand woven handicrafts, potency, product

Introduction

Wool from local sheep in Indonesia have not been utilized and become a waste when the farmers shear the wool for sanitation purposes. For crossbred sheep that have been well adapted for decades (called as local sheep) still had potency to process. They had much finer fiber diameter (FD) around 25 mμ and produce around 3-4 kg/year of greasy wool (Lupton *et al*. 2004), as they were crossing sheep

between dual purpose sheep (meat and wool type, such as Dorset, Texel or Merino) and native local sheep that had FD more than 40 mμ and produced only 0,4 kg/year of greasy wool (Parakkasi *et al*., 1994; Syamyono *et al*., 2003). There had been small industry in Indramayu, West Java, that processed this type of crossbred sheep wool and had been quite successful to run its business until national crisis occurred in 1999, the small business was collapsed. At present, national economy has been progressively achieved. It is therefore, this current work was conducted to study present potency of paper discussed work was conducted to research that had been conducted as a case study in the wool processing small industry was reported based on present conditions, to show its great potency to develop as a profitable business. This objectives of this study were to study product and economic potency of wool processing small industry in Indonesia.

Materials and Methods

Product Potency of Wool Handicraft

To study this wool product potency, a case study in wool small industry in Indramayu was conducted. Some parameters of this potency were:

a. Type of wool products: Questioners were used and direct observation were conducted in this study. Respondents of the questioners were the owner of the small industry and some experienced employees in the industry for technical matters of the products.
b. Percentage of products capacity: was the capacity of wool products made in the industry was compared to availability of raw materials based on secondary data of sheep population.
c. Product Favorability: Ten female academic staff at faculty of Animal Science IPB became the respondents for the favorability and marketability of the products.

Economic Potency of Wool Handicraft

Data on Economy potency were analyzed with descriptive and financial analysis. Descriptive analysis were used to describe value chain and labor absorption. Financial analysis used indicator of NPV (net present value), IRR (internal rate of return) and PBP (payback period).

$$NPV = \sum_{t=1}^{n} \frac{Bt - Ct}{(1+i)^t}$$

$$IRR = i_1 \frac{NPV_1}{NPV_1 - NPV_2}(i_2 - i_1)$$

Where:

NPV_1 (NPV_2) = NPV with discount factor i_1 (i_2)

i = discount factor; Bt = benefit year-t; Ct = cost year-t

Results and Discussion

Products Potency of Wool Handicraft

Type and characteristic of wool products. The results of this case study show that the wool product produced were woven handicrafts which could be included wall hangings, prayer mats, lounge cover, bags, hand phone cover, place mats, lamp cover and carpets. It was reported that among the products, wall hangings were dominantly produced. Others were made based on special order from certain consumers. The wall hangings were made in the size of 60x120 cm^2, with different design such as contemporary, ethnic, natural pictures depending on location of wall hangings would be placed. The product can be placed in a living room, dining room, bedroom or lobby inside houses, offices or hotels. This type of product is an art work, it is therefore that beside quality of materials, design is very important to consider before making the products. One example of wall hangings is shown in Figure 1.

Figure 1. Wool handicraft product as a wall hanging with an ethnic design.

Percentage of products capacity. At present population of local crossbred sheep producing meat as well as coarse wool (Merinos x Texel x local sheep) were concentrated in West Java in Banjarnegara and Wonosobo. In Banjarnegara itself the local sheep population was around 107.272 heads and it was predicted that there were 20.000 heads of local crossbred sheep including 10.000 heads of adult sheep producing around 30 tones of coarse wool (Yamin *et al.,* 2009). The wool could be processed to make for 18.000 pieces of wall hanging (60x120 cm^2) per year. This study found that Indramayu wool handicraft group produced only 60 pieces per month or 720 pieces per year. This only contributed around 4% of product capacity compared to its potency. This means that the small business can be further developed

by increasing wool production and quality as well as preparing professional craft men and businessmen in this area.

Product Favorability. The results show that the product of wool handicraft were mostly wall hangings are basically art materials, therefore design of the product should be interesting, beautiful and touchful as well as it must have characters. The results of this case study showed that 80 percents of the respondents liked the products very much. Main reason of the answer was that the materials were from natural fibers of Indonesian local sheep coarse wool which were amazing things to see an unexpected product of wool in Indonesia.

Economic Potency of Wool Handicraft

Economic potency of wool handicraft was illustrated from market pontency, financial feasibility and labor absorbtion of wool handicraft industry.

Market potency. Hand made wool handicraft was an art product, therefore its price was relatively expensive. The price of this product varied, depending on design of handicraft (motif), raw materials used, level of difficulties and period/length in making the handicraft.

The average of the price for the wall hanging at craftmen size 60 x 120 cm^2, was Rp 220,000,-/piece. The products were sold by the craftmen around 48.89% to souvenir shop, 22.22% to inter-regional middlemen trader, and 28.89% sold directly to consumers visiting the craftmen workshop. At retailer (souvenir shop), the selling price of those product varied depending on design. Minimum selling price was Rp 280,000,-. The more interesting of its looks, the selling price would be more expensive (Figure 2).

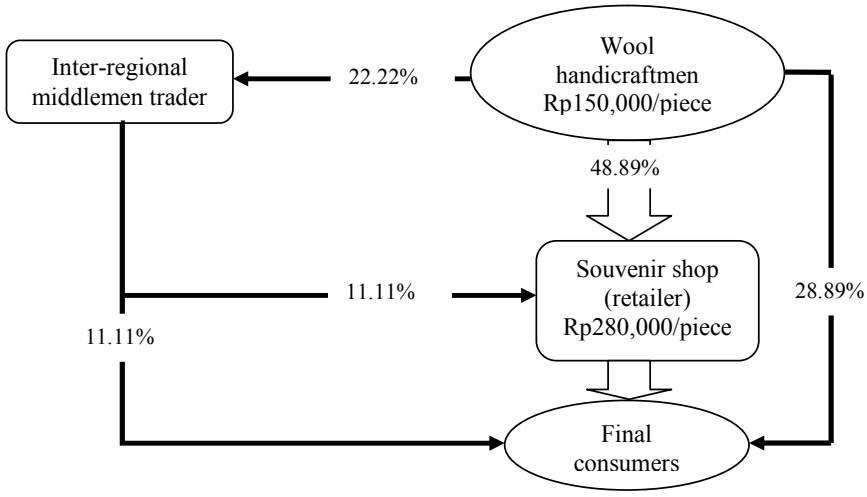

Figure 2. Market chain of wool handicraft wall hangings

The market destinations of the product were outside Indramayu such as Jakarta, Bandung and other city shopping centers in West Java Province. Souvenir shops were located in other tourism cities outside West Java (e.g. Jogjakarta, Bali) were also a potential market for the products. Beside domestic market, international markets were also potensial for this product market.

Financial feasibility and labor employment. Gittinger (1986) stated that financial analysis is an analysis to compare cost and benefit obtained to determine whether a project is profitable for period of the project. Handwoven craft industry need capital invesment for building, non-machine spinner and equipments. Financial feasibility analysis need to be calculated to know wether the investment was economically feasible or not. Invesment and variable cost of handwoven craft industry (in the case of Indramayu with the assumption of production capacity of 60 pieces per month) were calculated.

Investment needed for producing 60 pieces of wall hangings per month was Rp 89.82 million with operational cost of Rp 217 404,-/piece. Total production cost per piece was Rp 258 494, and selling price was Rp 280 000, then net profit will be Rp 21 505,-. This net profit could be higher if the selling price is higher by selling to better promotion, more exclusive target and better design.

Based on those technical coefficient and prices, it obtained financial feasibility indicators, as shown in Table 1. Internal rate of return (IRR) was 36%, meaning that handwoven craft industry financially feasible because the IRR was greater or the sama with discount level (Kadariah *et al.,* 1999). At interest rate of 15%, invesment of handwoven craft industry obtained net present value (NPV) of Rp 107 654 046,- with the total capital invesment of Rp 116 511 900,-. This indicates that the business is feasible because its NPV was larger than 0 (Kadariah *et al.,* 1999).

In addition of financial profit, handwoven craft industry also provided job to spinning and woven labors for 5 and 3 mandays per piece, respectively for the two activities. At production capacity of 60 pieces per month, handwoven craft industry employed 25 female labor of spinner and 15 women of weaver, with 20 working days / month, respectively each. Labor wage of weaver (Rp 25.000/mandays) was higher than spinner (Rp 15.000/mandays), because it needs special skills for weaving.

Table 1. Indicators of financial feasibility of handwoven craft industry

Indicator	Unit	Value
IRR	%	36
NPV (15% interest rate)	Rp	107 654 046
Pay back period (15% interest rate)	year	4
Capital (Investment + 2 month operational cost)	Rp	116 511 900

Conclusions

Development of Wool Handicrafts Production in Indonesia had great potency, either the product itself or its economic potency. The product was an art work using natural wool fibre from local sheep, was beautiful and interesting. Economic potency was also good. Wool processing products were mostly wool woven wall hangings, the small industry only used 4% of its production potency. The consumers liked the products as they were beautiful as natural fiber product and had high value of arts. The wool handicraft production also had good economic potency in terms of its NPV and IRR. It is concluded that this wool handicraft small industry is recommended to develop to become a profitable small business in community.

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Physical, Chemical, and Microbiological Characteristics of Healthy Drink that Contains Honey and Duck Egg Yolk in Difference Age

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Abstract

Egg as a source of protein has many benefits. All parts of the egg could be used as a source of food; for instance, egg yolk is used as an ingredient for herbal drinks. Mixing raw eggs in drinks like herbal medicine, energy drinks or food have become a habit for some people. The addition of egg yolk into drinks such as herbal medicine had to be supervised because it usually used raw eggs. Raw eggs are easily contaminated by bacteria during storage. The objective of this research was to analyze physical, chemical and microbiological characteristics of herbal drinks that contain duck egg yolk and honey. Complete Randomized Design with two-factor factorial pattern was used in this experiments design. The first factor was the treatment of honey addition (addition of honey and without addition of honey) and the second factor was the difference in age of the egg (day 2, day 5, and day 8). Data was processed by using ANOVA, then the results that showed significant effect was further analyzed by using Tukey's test. Data on the microbiological properties were analyzed descriptively. The temperature increased significantly with honey addition. pH decreased significantly with honey addition and increased significantly during storage. The interaction between honey addition and the difference in age of the egg had significant effect in viscosity, water contents and protein contents of herbal drink. Honey was not only known as calorie source but also it had ability to reduce the amount of microbial contamination in herbal drink that contained egg yolk. The result of microbiological test showed that duck egg yolk was safe to eat until day 8 of storage.

Keywords: duck egg yolk, herbal drinks, honey, storage

Introduction

Egg is one of the animal origin food nutritious because it contains nutrients needed by human body such as proteins with a complete amino acid, fat, vitamins, minerals and have a high digestibility. Despite of as a source nutrition for humans, food

derived from animal is a food source for microorganisms. Eggs as a source of animal protein should be guaranteed safety for consumers, because eggs are perishable food. One is the use of eggs as an ingredient for health drinks. Mixing raw eggs in drinks like herbal medicine, energy drinks or food have become a habit for some people. The addition of egg yolk into drinks such as herbal medicine had to be supervised because it usually used raw egg. Raw eggs are easily contaminated by bacteria during storage. As a health drink, duck egg yolk usually mix with honey. It is necessary to sudy of physical, chemical and microbiological characteristics health drink that contains honey and duck egg yolk in difference age.

This objective of this research was to study physical, chemical and microbiological characteristics health drink that contains honey and duck egg yolk in difference age.

Materials and Methods

Location and Time

This research was done in the Integrated laboratory at Faculty of Animal Husbandry, Bogor Agricultural University, in August-December 2010.

Materials

Egg samples used in this study were duck eggs which different age (day 2, day 5 and day 8). Egg samples used were obtained from farms in Leuwiliang, Bogor.

Procedures

Samples of duck eggs wiped with alcohol before broken. After a broken egg, each egg was separated between albumen and yolk. Duck egg yolk was treated by adding honey in the ratio 2:1. The yolk was added with honey, whipped homogeneously. The characteristics studied were physical, chemical and microbiological.

Parameters were measured

Physical properties testing conducted on the study include color, viscosity and temperature. Testing chemical properties measured were pH value (AOAC, 1995), protein content, and moisture content (SNI 01-2891-1992). Microbiological quality included: total plate count (TPC), *Salmonella*, *Escherichia coli* and *Coliforms* (DSN, 1992).

Experimental Design

Complete Randomized Design with two-factor factorial pattern was used in this experiments design. The first factor was the treatment of honey addition (with and without addition of honey) and the second factor was the difference in age of the egg (day 2, day 5, and day 8). Data was processed by ANOVA, then the results

that showed significant treatment effect was further analyzed by using Tukey's test. Data on the microbiological properties were analyzed descriptively.

Results and Discussion

Microbiological Characteristics

Based on Table 1 can be assumed that health drink contained honey and duck egg yolk had lower of total plate count than without the addition of honey. Based on the SNI 01-7388-2009 (DSN, 2009) until the eighth day, duck egg yolks are safe for consumption.

According to Tonks (2003), honey has antimicrobial activity or anti-bacterial, because honey has a moisture content that is relatively low at less than 20% and a high sugar content. The condition do not support to the growth of microorganisms due to osmotic effects that can kill microorganisms. Honey has a low pH levels that can inhibit the growth of microbes, has a large osmotic pressure and the carbon to nitrogen ratio is high (Rosita, 2007). In addition, honey can inhibit the growth of microorganisms through a compound of hydrogen peroxide produced.

Contamination of *Salmonella* and *E. Coli* were not found in duck egg yolks with or without the addition of honey. However, *Coliform* contamination was found in duck

Table 1. Microbiological characteristics of duck egg yolk with or without addition of honey in difference age

Difference age	Addition of honey	Without addition of honey
Total Plate Count …….....……..CFU / g……..		
Day 2	< 30×10^1 ($1x10^1$)	< 30×10^1 ($1.5x10^1$)
Day 5	< 30×10^1 ($6,5x10^1$)	< 30×10^1 ($12.5x10^1$)
Day 8	< 30×10^2 ($0,9x10^2$)	< 30×10^2 ($1.1x10^2$)
Coliform …….....….....CFU / g……...……		
Day 2	< 30×10^1 (1×10^1)	< 30×10^1 ($2x10^1$)
Day 5	< 30×10^1 ($1,5x10^1$)	< 30×10^1 ($2.5x10^1$)
Day 8	< 30×10^1 ($0,6x10^1$)	< 30×10^2 ($0.4x10^2$)
Salmonella …….....……. / 25 g……...……..		
Day 2	Negatif	Negatif
Day 5	Negatif	Negatif
Day 8	Negatif	Negatif
E. coli …….....…..CFU / g……....……..		
Day 2	< 30×10^1 (0×10^1)	< 30×10^1 (0×10^1)
Day 5	< 30×10^1 ($0x10^1$)	< 30×10^1 ($0x10^1$)
Day 8	< 30×10^1 ($0x10^1$)	< 30×10^1 ($0x10^1$)

egg yolks with or without the addition of honey. *Coliform* contamination in duck egg yolk without the addition of honey was higher than the addition of honey. *Coliform* increased during storage. Until the eighth day, both the duck egg yolks with the addition of honey or without the addition of honey were not in accordance with SNI 01-7388-2009 (DSN, 2009) which states in food Coliform limit is 3 CFU / g. Honey could inhibit the growth of pathogenic bacteria such a*s E. coli, Salmonella* Typhimurium*, Listeria monocytogenes, Bacillus cereus* and *Staphylococcus aureus* (Taormina *et al.,* 2001).

pH value

The analysis showed that the pH range duck egg yolk significantly different ($P < 0.05$) by differences in age of duck eggs and highly significant ($P < 0.01$) by the addition of honey treatment (Table 2). There is no interaction between factors A (addition of honey) and factor B (aged eggs) to the pH value of egg yolk. pH value of duck egg yolks with the addition of honey was lower than without the addition of honey. The addition of honey made reducing of pH value because honey has pH at 3.65 – 4.96 and duck egg yolk has pH at 6-6.5.

Differences in the age of eggs used also showed differences in the pH of the yolk. pH increased during storage, this might be caused by H_2O and CO_2 evaporation in the eggs. Evaporation of CO_2 in the egg caused by compounds that break down into NaOH, $NaHCO_3$, and NaOH will decompose back into ions Na^+ and OH^- (Silverside and Scott, 2000).

Table 2. pH value of duck egg yolk with or without addition of honey in difference age

Difference age	Addition of honey	Without addition of honey	Average
Day 2	5.78±0,04	6.08±0,02	5.93^{b}
Day 5	5.83±0,04	6.15±0,08	5.99^{ab}
Day 8	5.86±0,09	6.19±0,05	6.02^{a}
Average	5.82^{B}	6.14^{A}	

Note: Means in the same coloumn with different superscript differ significantly $P<0.05$)
Means in the same row with different superscript very differ significantly ($P<0.01$)

Temperatures

Duck egg yolk temperature affected highly significant ($P < 0.01$) by the addition of honey treatment, but did not differ ($P > 0.05$) by differences in age of eggs and also there is no interaction between both factors. Average temperature can be seen in Table 3. Temperature of duck egg yolks with the addition of honey was higher than without the addition of honey. Honey contains carbohydrates especially fructose which is nutrients as an energy source.

Viscosity

The treatment of honey addition, age of the egg and interaction had highly significant effect on viscosity (P<0.01) (Table 4). The addition of honey increased on viscosity of health drink, but viscosity decreased during storage.

Water Content

The treatment of honey addition and age of the egg had no significant effect on the water content. The average of water content was 42.97% (Table 5).

Table 3. Temperature of duck egg yolk with or without addition of honey in difference age

Difference age	Addition of honey	Without addition of honey	Average
Day 2	24.13±0.12	23.77±0.40	23.95
Day 5	24.03±0.25	23.7±0.20	23.87
Day 8	23.97±0.15	23.53±0.32	23.75
Average	24.04^{A}	23.67^{B}	

Note: Means in the same row with different superscript very differ significantly (P<001).

Table 4. Viscosity of duck egg yolk with or without addition of honey in difference age

Interaction	Factor A x Factor B	Viscosity (dpa.s)
1	KT_0 H2	$7.33±0.58^{A}$
2	KT_0 H5	$4.70±0.26^{B}$
3	KT_0 H8	$3.73±0.25^{C}$
4	KT_1 H2	$1.83±0.21^{D}$
5	KT_1 H5	$0.93±0.06^{E}$
6	KT_1 H8	$0.73±0.06^{E}$

Note: Means in the same column with different superscript very differ significantly (P<001).

Table 5. Water content (%) of duck egg yolk with or without addition of honey in difference age

Difference age	Addition of honey	Without addition of honey	Average
Day 2	42.58±2.63	42.64±0.46	42.61
Day 5	42.92±0.87	43.13±1.32	43.03
Day 8	43.32±1.74	43.23±1.11	43.28
Average	42.94	43.00	

Table 6. Protein content (%)of duck egg yolk with or without addition of honey in difference age

	Addition of honey	Without addition of honey	Average
Day 2	13.07±1.79	18.50±0.36	15.79
Day 5	11.89±0.43	17.77±1.00	14.83
Day 8	12.75±2.69	18.13±1.18	15.44
Average	12.57^{B}	18.13^{A}	

Note: Means in the same row with different superscript very differ significantly (P<001).

Protein Content

The treatment of honey addition had highly significant effect on protein content (P<0.01) (Table 6). Protein content of duck egg yolks with the addition of honey was lower than without the addition of honey. Decreasing of protein content of duck egg yolk with honey addition was caused by increasing of water content.

Conclusion

Honey was not only known as calorie source but also it had ability to reduce the amount of microbial contamination in herbal drink that contained egg yolk. Based on total plate count test, duck egg yolk was safe to eat until day 8 of storage. Otherwise based on coliform test, duck egg yolk wasnot safe to eat until day 8 of storage.

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Microbiological Quality of Probiotic Yoghurt Jelly Drink During Storage in Refrigerator

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Abstract

As a diversification product, probiotic yoghurt can be used as raw material to make a jelly drink. The jelly drink of probiotic yoghurt could be useful as a health drink because it contains lactic acid bacteria, probiotic and fiber. The objective of this research was to analyze the microbiological quality of the jelly drink made by probiotic yoghurt as raw material during storage in a refrigerator. Randomized complete design with three repetition was used as experimental design. The treatment was storage time of the product in refrigerator (day 0, day 7, day 14, day 21 and day 28). Data was processed by using ANOVA, then the results that showed significant treatment effects was further analyzed by using Tukey's test. The storage time decreased significantly (P<0.01) Total Plate Count (TPC), total lactic acid bacteria, total of Bifidobacterium longum and total of Lactobacillus acidophilus. The storage time influenced significantly to the number of mold and yeast in the product (P<0.01). Hygiene and sanitation of environment during processing and storage must be evaluated, because coliform was found in the product aged of 28 days storage. Base on the number of lactic acid bacteria observed, it could be concluded that probiotic yoghurt jelly drink may be regarded as a functional food. Due to the acidity of the product, the incorporation of antifungi is needed to preserve the probiotic yoghurt jelly drink from the growrh of mold and yeast during storage.

Keywords: jelly drink, lactic acid bacteria, probiotic, storage, functional foods

Introduction

Yoghurt is made by fermented milk and has many benefits, it can be can be consumed by people with lactose intolerance. Probiotic yoghurt is a yoghurt that resulted from a fermentation of milk by using yoghurt starter culture e.g *Streptococcus salivarius* spp., *S. thermophilus* and *Lactobacillus delbrueckii spp. Bulgaricus* that combined with probiotic bacteria such as *Bifidobacterium longum* and *Lactobacillus acidophilus*. A beverage of jelly drink made by probiotic yogurt could be

useful as health drink. It contains lactic acid bacteria and fiber. As a health drink, probiotic yoghurt jelly drink has high nutrition and water content. Consequently, it is a perishable food.

The objective of this research was to analyze microbiological quality of jelly drink made by probiotic yoghurt as raw material during storage in refrigerator.

Materials and Methods

Location and Time

This research was done in the laboratory of dairy processing at the Faculty of Animal Science, Bogor Agricultural University, in 2007.

Procedures

Stages of research were: (1) process of making yogurt probiotic, (2) process of making jelly drink with yogurt probiotic, and (3) storage. Based on viscosity and sensorial value in preliminary test, the best concentration of jelly powder for the jelly drink was 0.8%.

Parameters

Measured parameters in this research were total plate count, total lactic acid bacteria, total of *B. longum*, total of *L. acidophilus*, total of mold, total of yeast and total of coliform during storage (day 0, day 7, day 14, day 21 and day 28) in refrigerator (4-7 ^{o}C).

Experimental Design

Randomized complete design with three repetition was used in this experiment. The single factor of this experiment was storage duration (day 0, day 7, day 14, day 21 and day 28).

Results and Discussion

Microbiological quality of probiotic yogurt jelly drink during storage is shown in Table 1.

Total Plate Count and Total Lactic Acid Bacteria

Total plate count consists of total lactic acid bacteria , total of pathogen bacteria and total fungi. The number of bacteria decreased during observation. Storage time influenced significantly to decreased the number of lactic acid bacteria in the product. To be effective, probiotic strains must retain their functional health characteristics, including the ability to survive transit through the stomach and small intestine. then to colonize the human gastrointestinal tract (Tuomola *et al*. 2001). The optimum

Table 1. Microbiological quality of probiotic yogurt jelly drink during storage

Variable	Days of storage				
	0	7	14	21	28
	Log 10 cfu/ml				
Total Plate Count	$9.3^{A} \pm 0.01$	$9.00^{A} \pm 0.03$	$8.93^{A} \pm 0.01$	$8.34^{B} \pm 0.04$	$8.43^{B} \pm 0.03$
Total Lactic Acid Bacteria	$9.22^{A} \pm 0.04$	$8.94^{B} \pm 0.04$	$8.86^{B} \pm 0.01$	$7.39^{C} \pm 0.02$	$7.25^{D} \pm 0.05$
Total of *B. Longum*	$9.30^{A} \pm 0.01$	$9.00^{A} \pm 0.04$	$8.93^{A} \pm 0.03$	$7.98^{B} \pm 0.02$	$7.39^{C} \pm 0.06$
Total of *L. acidophilu*s	$9.25^{A} \pm 0.06$	$8.91^{B} \pm 0.04$	$8.59^{C} \pm 0.21$	$7.40^{D} \pm 0.06$	$7.30^{D} \pm 0.02$
Total of mold	< 1*	$1.86^{B} \pm 0.05$	$2.58^{C} \pm 0.07$	$2.95^{D} \pm 0.01$	$3.09^{E} \pm 0.02$
Total of yeast	< 1*	$1.54^{B} \pm 0.06$	$1.83^{C} \pm 0.16$	$2.20^{D} \pm 0.03$	$2.33^{D} \pm 0.03$
Total of coliform	< 1*	< 1*	< 1*	< 1*	1.00

Note: Means in the same row with different superscript very differ significantly (P<0.01).

number of probiotic bacteria required to provide the desired health or nutritional benefits for consumers is not known (Gilliland *et al*., 1989). Counts higher than 7 Log_{10} CFU/g have been suggested by Viderola and Reinheimer (2000) in order to ensure probiotic effects.

The decrease in the total amount of lactic acid bacteria until day 28 was still above the minimum of lactic acid bacteria as probiotic (7.25 Log_{10} CFU/g). The growth rate of lactic acid bacteria based on Table 1 was -6.5 X 10^4 generations per minute. These results indicated the presence of growth inhibition compared to the optimal growth rate at 35-45 ^{o}C temperature was 0.014 generations perminute (Widowati and Misgiyarta, 2002; Tamime and Robinson, 1999).

Total B. Longum

B. longum is a probiotic bacteria that has been recommended by Generally Regarded as safe (GRAS). The treatment of storage had a highly significant effect on the total of *B. longum* (P <0.01). The total of *B. longum* decreased during storage was caused by bacteriocin that from *S. thermophilus B. longum* was anaerobi bacteria (Holt *et al.*, 1994), therefore the existence of oxygen could hold the bacteria growth. *B. longum* population in the probiotic yoghurt jelly drink until the end of storage (7.39 log_{10} cfu/g) still fulfilled the standard probiotic bacteria requirement in food based on Shah (2000) which is 6.00 log_{10} cfu/g.

Total L. acidophilus

The treatment of storage had a highly significant effect on total of *L. acidophilus* ($P < 0.01$). *L. acidophilus* population in product decreased after 14 days of storage. It may have been caused by H_2O_2 accumulation from bacteria metabolism. Shah (2000) said that probiotic bacteria viability in yoghurt was influenced by production of H_2O_2. *L. acidophilus* is one of the *Lactobacilli* groups that could accumulate H_2O_2 in the product, because it was a negative catalayzed bacteria (did not have catalayzed enzyme to break H_2O_2 into O_2 and H_2O). Its bacteria population still fulfilled the standard probiotic bacteria based on Samona and Robinson (1994) *in* Viderola *et al*.(2000) which is a minimum 6.00 $\log_{10}$ cfu/g until the end of storage time (28 days).

Total Mold and Yeast

The treatment of storage had a highly significant effect on total of mold and yeast ($P < 0.01$). Total of mold and yeast increased during storage. Deteriorated microbes such as mold and yeast were less sensitive to the environment factor, so it is possible to grow and expand (Rahman *et al.,* 1992). Robinson (1981) said the maximal number of total mold is 2 $\log_{10}$ cfu/ml, and the maximal number of total yeast is 3 $\log_{10}$ cfu/ml. The yoghurt probiotic jelly drink was safe until 14th day for customers.

Total Coliform

Coliform is usually indicitave of poor process and contamination after food processing (Stringer and Dennis, 2000). Total coliform started to grow at 28th day. Robinson said the maximal number of total coliform is less more 1 log 10 cfu/ml. The yoghurt probiotic jelly drink was not safe until 28th day for customers.

Conclusion

Storage time had a higly significant effect on the microbiological characteristics of the yoghurt probiotic jelly drink. This product was still a functional probiotic food until 28th day. Otherwise based on total mold, yeast and coliform this product was safe until 14th day for customers.

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Tenderness and Cooking Loss of Yearling Brahman Cross and Mature Ongole Cross Beef Treated Tenderizing Method

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Abstract

The application of tenderizing methods such as enzyme, internal endpoint temperature, and thawing method on beef from different age and breed of cattle are important to investigate. The objectives of this research were to study effect of papain enzymes, internal endpoint temperatures and thawing methods on Warner-Bratzler shear force (WBSF) and cooking loss of meat from yearling Brahman Cross and Ongole Cross 3-4 years old used as young and mature beef respectively. Young frozen beef was thawed for 24 hr in refrigerator or soaking in ambient temperature water for 30 min. Old beef was only thawed used first thawing method. Cooking loss and WBSF were evaluated after beef were treated with or without soaking in papain enzyme solution for 30 min and continued with boiling until internal endpoint temperature 80 °C, 90 °C or 100 °C. The result showed that papain could improve tenderness of mature beef if reach internal endpoint temperature 90 °C, but could not at young beef. Cooking loss of young and mature beef that were boiled until internal endpoint temperature 80 °C or 90 °C had no differences. Thawing for 24 hr in refrigerator with internal endpoint temperature 80oC resulted in the most tenderness and the lowest cooking loss in young beef. In conclusion; the use of papain enzyme to improve tenderness was effective for mature beef, and internal endpoint temperature 80 °C or 90 °C was better than 100 °C to get lowest cooking loss at young beef neither mature beef. In this research beef from mature Ongole Cross (Indonesian local breed) has lower WBSF value (more tender) than beef from young Brahman Cross (Australian Brahman Cross).

Key words: beef tenderness, cooking loss, tenderizing method

Introduction

Tenderness is the main factor that influences meat palatability and consumer decision for choosing the meat (Brooks *et al*., 2000). Beef tenderness was affected by breed (Huffman *et al*., 1967; Slanger *et al*., 1985), age (Shorthose and Harris, 1990; Huff-Lonergen *et al*., 1995), feed (Ponnampalam *et al*., 2002), aging (George-Evin

et al., 2004), chilling and rate of cooking (King *et al*., 2003). Papain enzyme was proteolytic enzyme that could improve the meat tenderness (Huffman *et al*., 1967; Ionescu *et al*., 2008). Ionescu *et al*. (2008) reported that papain, besides bromelin, led to a limited hydrolysis of beef meat proteins, to a loss of physical integrity of muscle and connective tissue, accompanied by a high solubility of structural proteins, and to an improvement of the beef meat tenderness. Addition end point of internal temperature in cooking also plays a part in beef tenderizing (Parrish *et al*., 1973; Liu and Berry 1996; George-Evin *et al*., 2004). The other factor that could affect beef tenderness before cooking is thawing method.

Information about meat tenderizing method was necessary to produce beef tender. Besides that, cooking loss also to be concern as beef cooking characteristic that influence consumer acceptance. Tenderizing treatments such as enzyme, internal temperature, and thawing method on beef from different age and breed of cattle have to be investigated. The objectives of this research were to study the effect of papain enzymes, internal endpoint temperatures and thawing methods on Warner-Bratzler shear force (WBSF) and cooking loss of meat from yearling Brahman Cross and Ongole Cross 3-4 years old used as young and mature beef respectively.

Materials and Methods

Beef that used in this research were got from round of male yearling Brahman Cross as young beef, and Ongole Cross 3-4 years old as mature beef. Both of meats were frozen until evaluation procedures were held. Frozen young beef was thawed with different method. Beef that still covered in plastics packaging were thawed for 24 hr in *refrigerator* or soaked in ambient temperature water for 30 min. The mature beef was only thawed by first thawing method. After thawing, the packaging were removed from samples, and were treated with or without soaking in papain enzyme solution (2% crude extract; w/v) for 30 min that continued with boiling until reach internal endpoint temperature (IET) 80 ^{o}C, 90 ^{o}C or 100 ^{o}C.

Tenderness was measured by WBSF (kg/cm^2). Beef samples boiled according to each treatment, and then min of three 1.27 cm diameter and length 3-4 cm of samples cores were taken parallel to the muscles fiber. Then the cores were shared by Warner-Bratzler shear. Cooking loss represented weight losses after cooking that was measured as sample weight margin before and after boiled.

The effect of IET (80^{o}C, 90^{o}C dan 100^{o}C), papain enzyme (with and without), and thawing method (soaking 30 min in water and refrigerator for 24 hr) on WBSF and cooking loss of young beef from Brahman Cross were arranged in factorial design 3 x 2 x 2 with 3 replication and used randomize complete design as basic design. While effect of internal endpoint temperature (80 ^{o}C, 90 ^{o}C dan 100 ^{o}C), papain enzyme (with and without) on WBSF and cooking loss of Ongole Cross studies used factorial design 3 x 2 with 3 replication and randomize complete design

as basic design. Analysis of variance was used to analyze effect of the treatment, and mean differences among treatment was analyzed using Tukey test.

Results and Discussion

Beef thawed at refrigerator (4-6 °C) for 24 hr was more tender (was indicated by lower WBSF at P<0.05) than beef soaked for 30 min at ambient temperature water (Table 1). This fact indicated that beef tenderness was caused by the damage of meat fiber as consequence of ice crystal formation, and the damage of meat fiber thawed at refrigerator was more intensive than that thawed in water. This explanation make reference to Linares *et al.* (2005) that reported that slow thawing as it happened in refrigerator causes more damage through ice crystallization in the meat.

Actually, enzyme papain and IET treatment didn't affect the tenderness of young beef from yearling Brahman Cross (Table 1). That case was different with WBSF value of beef from mature Ongole Cross. WBSF value of beef from mature Ongole Cross significantly affected (P<0.05) by interaction of IET and papain enzyme treatment. Non-papain treatment resulted in the lowest WBSF (the most tender) at endpoint internal temperature 100 °C, while if use papain treatment, the lowest WBSF (the most tender) reached at IET 90 °C (Table 2).

Table 1. Warner-Bratzler sheared force of young beef treated by papain enzyme, different thawing method, and internal endpoint temperature (kg/cm2)

Thawing/Enzyme	Internal Temperature		
	80 °C	90 °C	100 °C
Soaking, 30 min			
Non-papain	5.32 ± 0.98	4.19 ± 0.36	6.40 ± 2.07
Papain	6.89 ± 1.28	4.83 ± 0.76	5.07 ± 0.38
Mean		5.42 ± 1.33[a]	
Refrigerator, 24 hr			
Non-papain	4.09 ± 1.12	4.36 ± 0.15	4.47 ± 0.22
Papain	4.71 ± 0.40	5.40 ± 0.81	4.84 ± 1.13
Mean		4.62 ± 0.80[b]	
Mean of internal temperature	5.17 ± 1.40	4.65 ± 0.71	5.19 ± 1.27
Mean of Papain			
Non-papain		4.77 ± 1.23	
Papain		5.27 ± 1.05	

Note: Different superscript in the same column indicate significant different (P<0.05).

This study indicated that papain application to improve tenderness was effective for mature beef. The result could be explained according to report of Ionescu *et al*. (2008) that papain showed hydrolytic activity on the connective tissue, leading to a better tenderization of the adult beef meat. Another fact from this research showed that beef from mature Ongole Cross (Indonesian local breed) has lower WBSF value (more tender) than beef from young Brahman Cross (Australian Brahman Cross) (Tabel 1 and 2).

Cooking loss of young beef from Brahman Cross was affected by interaction among IET, thawing method and use of papain enzyme ($P<0.05$). The lowest cooking loss reached by IET 80°C for both of papain and thawing treatment, and 90 °C for all the treatment, except thawing by soaking for 30 min in ambient temperature water and without papain using (Table 3). Cooking loss of mature beef from Ongole Cross significantly affected ($P<0.05$) by interaction between papain enzyme and IET of cooking (Table 4). Internal endpoint temperature 80°C with or without papain enzyme treatment resulted lower percentage of cooking loss, and no difference with IET 90°C with or without enzyme. This research showed that increasing of IET

Table 2. Warner-Bratzler sheared force of mature beef treated by papain enzyme and internal endpoint temperature (kg/cm2)

Enzyme	Internal Temperature		
	80 °C	90 °C	100 °C
Non-papain	3.91 ± 0.15^{a}	3.82 ± 0.50^{a}	2.64 ± 0.42^{ab}
Papain	3.02 ± 0.48^{ab}	2.22 ± 0.52^{b}	3.73 ± 0.64^{a}

Note: Different superscript in the same raw and column indicate significant different ($P<0.05$).

Table 3. Cooking loss of young beef treated by papain enzyme, different thawing method, and internal endpoint temperature (%)

Thawing/Enzyme	Internal Temperature		
	80 °C	90 °C	100 °C
Soaking, 30 min			
Non-papain	37.90 ± 3.75^{bcd}	42.70 ± 4.10^{abc}	47.49 ± 6.21^{ab}
Papain	35.24 ± 6.93^{bcd}	39.35 ± 6.41^{abcd}	46.80 ± 5.87^{ab}
Refrigerator, 24 hr			
Non-papain	29.16 ± 4.95^{d}	37.84 ± 4.63^{bcd}	43.59 ± 6.16^{ab}
Papain	28.68 ± 3.49^{d}	39.07 ± 2.84^{abcd}	50.96 ± 7.23^{a}

Note: Different superscript in the same raw and column indicate significant different ($P<0.05$).

Table 4. Cooking loss of mature beef treated by papain enzyme and internal temperature (%)

Enzyme	Internal Temperature		
	80 °C	90 °C	100 °C
Non-papain	23.86 ± 5.09^{d}	32.33 ± 3.87^{bcd}	42.38 ± 7.21^{a}
Papain	28.51 ± 2.31^{cd}	35.95 ± 3.63^{abc}	41.03 ± 4.57^{ab}

Note: Different superscript in the same raw and column indicate significant different (P<0.05).

increased percentage of cooking loss both of young and mature beef. The result accord with George-Evan *et al*. (2005) reported that the increasing of IET increased cooking time and cooking losses.

Conclusions

The use of papain enzyme to improve tenderness was effective for mature beef, and internal endpoint temperature 80°C or 90 °C were better than 100 °C to get lowest cooking loss at young beef neither mature beef. In this research beef from mature Ongole Cross (Indonesian local breed) has lower WBSF value (more tender) than beef from young Brahman Cross (Australian Brahman Cross).

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V. SOCIAL ECONOMICS AND POLICY IN ANIMAL PRODUCTION

Productivity of *Kalung* Crickets (*Gryllus bimaculatus*) Cultivation (Case Study in Central and East Java)

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Abstract

The research was conducted to analyse technical and economical of Kalung crickets cultivation productivity and to analyse any factors that influence them. The research was conducted in Central Java (Demak, Kudus, and Purwodadi) and East Java (Tulungagung, Kediri, and Porong) from March 3rd until March 12nd, 2010. The research consisted of two stages: (1) determination of research area and the number of samples and (2) data collection. Survey method was used in this research and the samples were selected purposively based on farm scale and farmer's experience. The farm's productivity, income and R/C ratio were analysed descriptively. The average of cricket's production in Central and East Java were 9.78 and 12.69 tons/year respectively. Technical productivity in both provinces was not different, in contrary to economical productivity. The average income in Central Java was IDR 134,714,300.00 or 72.21% from IDR 186,566,666.00 revenue, while in East Java was IDR 149,899,333.00 or 58.56% from IDR 255,960,000.00 revenue. The R/C ratios in both provinces were more than one which meant the enterprises is profitable and feasible economically. The value of R/C ratio in Central Java (3.5) was higher than East Java (2.6). Low R/C ratio value in East Java was caused by high cost in feed, equipment and cage. The farmers should utilize local resources optimally.

Key words: income, kalung crickets (Gryllus bimaculatus), productivity, R/C ration

Introduction

Crickets are animal from insects class that has great potential to be developed as a protein resource in livestock feed. Some researchers have showed that cricket has high protein content (61.58%) with a fairly complete amino acid (Novianti, 2003). Tremendous potential of crickets is what makes the community of Central Java and

East Java Province attempted to cultivate cricket so that the area is renowned as a centre for the cultivation of crickets. The research objectives were to evaluate the productivity of the cultivation of *Kalung* crickets technically and economically as well as the factors that affect the productivity of *Kalung* crickets cultivation.

Materials and Method

The research was conducted in the community of *Kalung* cricket's cultivators in Central Java (Purwodadi, Demak, Kudus) and East Java (Tulungagung, Kediri, Porong). Research was carried out for two weeks, from March 3 to 12, 2010.

The materials used in this study were stationery, thermometers, cameras, and questionnaires to obtain primary data cultivation of *Kalung* crickets in the farmer's community. Primary data was collected using a questionnaire through direct interview with respondents. The respondents were purposively selected in each region; one person was taken of each region. Selected farmers are cricket's cultivators that have greatest and longest cultivated for more experienced and skill of the cultivation of crickets. Secondary data was obtained from the literature and report documents from related government agencies.

This research was designed as a survey. Descriptive analysis was used to describe the characteristics of cricket's cultivation techniques, cricket's productivity, and income analysis.

Results and Discussion

Kalung Crickets Cultivation Techniques

Kalung cricket's hatching eggs are usually derived from their own cultivation or some were obtained from cicada eggs merchants. Egg harvesting is done using a strainer or sieve to separate the eggs from the sand and dirt. Harvesting is done every day. Eggs that have been harvested then incubated for hatching. Meanwhile the media is returned to the nesting box for brood stock maintenance. This must be done to anticipate the possibility if not the entire parents spawning. Examination of spawning media is done every 3-4 days. The characteristic of a good quality cricket's eggs are cream-colored, translucent, shiny, clean, not dingy, and warm when wrapped (Paimin, 1999; Paimin *et al*., 1999). Cricket's cultivators use cloth, sand, and sawdust for hatching media. The relative humidity required for hatching eggs range between 65-80%, with air temperature 26 ^{o}C. Temperature and relative humidity needs to be maintained to prevent the hatching failure caused by poor egg quality, unsuitable moisture and many predators. Nymph's maintenance is important in cricket's cultivation.

Cultivators in Central Java use cages made of bamboo and plastic, while in East Java they used cages made of wood and plywood. There are two type of box,

open and closed or compound and no compound. Selection of the type is more due to the ease of feeding and drinking, maintaining, harvesting, cleaning cages and for the prevention from predator. The crickets are placed in an area free from direct sunlight and complemented with a hiding place, made of dried banana leaf or egg tray. Observation in the field showed that the cage cleanliness was maintained properly. Farmers always change the hiding media and the cultivator used pedestal in feeding their crickets. Cages were also prevented from predators such as lizards, ants, mice and other animals by covering the cage with gauze, cloth, anti-ants chalk or putting mat containing kerosene or used motor oil on each foot cage. The average size of the cage for 4000 crickets per cage in Central Java was 250x112x50 cm and 230x112x58 cm in East Java. Cage density in both provinces was lower than the density reported by Widyaningrum (2001) that was 5000 crickets in every cage. This cage density affected the cricket's mortality. Problems that affect cricket's mortality are low hatchability, cage's density, unsuitable temperature, dwarfism, cannibalism, disease and death-smelling diarrhea.

Crickets feed on Central and East Java consisted of concentrate and forage. Cultivators used laying quail or broiler feed for cricket's concentrate. Forages used by farmers in Central and East Java, were banana stems, squash, cassava leaves, thorns cottonwood leaves, cabbage leaves, grass, mustard greens, fruits and stems of papaya. Variation of forage feed depended on the forage resources available in each area. Vita chick and vitamins were commonly given when the environmental conditions of cultivation was not good.

Cricket's Productivity

Technical productivity of the cultivation of *Kalung* crickets in Central and East Java vary in each production period. This is due to many factors that influence cultivation such as temperature and humidity environment, predators, egg quality, feed quality, and cultivator's management skills (Widyaningrum, 2001; Praditya, 2003; Fitriyani, 2005). The average production in Central Java was 9.78 tons/year, which was lower than in East Java (12.69 tons/year) (Table 1). Average production in East Java crickets was higher due to better management of cultivation.

Income Analysis

Farm income analysis (Table 1) showed that the average income per year cultivators in Central Java was Rp134,714,300.00 and Rp149,899,333.00 for cultivators in East Java. Feeding cost in Central Java was Rp8,000,000.00 and in East Java was Rp27,712,000.00. The cost of feed in East Java needs to be reduced through the use of local resources to increase benefit. Equipment cost in East Java is also higher (Rp17,892,000.00) than in Central Java (Rp216,000.00). The analysis showed that in Central Java the largest proportion of variable cost was cost of labor (46.29%) and hatching eggs (30.23%), whereas in East Java was the cost of feed (26.13%),

Table 1. The characteristics and productivity of kalung cricket's cultivation

Indicator	Central Java	East Java
Average production	9.78	12.69
Scale	Large	Large
Average initial scale (box)	18	4
Average scale during research (box)	38	28
Business typology	Main (33,3%) Part-time (66,7%)	Main (100%)
Capital	Self-equity	Self-equity
Harvest time	Every 32 days	Every 27 days
Harvest frequency	8 times/year	More than 10 times/year
Packaging material	Plastic bag	Plastic bag & box
Packaging size	2 kgs/pack	According to consumer's demand
Marketing target	Collectors	Retailers, consumers
Marketing area	Inside the region	Inside & outside the region
Average farm gate price (Rupiah/kg)	19,166.67	19,500.00
Payment system	Delayed (in 1 weeks)	Cash & delayed (in 2 days)
Revenue (Rupiah)	186,566,666	255,960,000
Cost (Rupiah)	51,852,366	106,060,666
Profit (Rupiah)	134,714,300	149,899,334
Revenue/Cost Ratio	3.5	2.6

labor (25.08%), and equipment (16.87%). The percentage of hatching eggs cost in Central Java was high because 33.3 per cent cultivators bought eggs from cicada eggs merchants. Production cost in *Kalung* crickets was affected by farm scale, and wage rate in each region. Fixed costs consist of depreciation of cage and equipments. The average cricket's prices in Central Java was Rp19,166.67/kg, while in East Java, the average price was Rp19,500.00/kg. The farmers stated that they will get a break-even if the price of crickets was not less than Rp10,000.00 per kg. Revenue-Cost Ratio (R/C) of *Kalung* cricket's cultivation in Central Java and East Java was 3.5 and 2.6, respectively. Value of R/C ratio is more than one indicates that the cultivation of *Kalung* crickets that cultivators in Central Java and East Java run this business profitable and viable, despite fluctuations in the price of each harvest period (Hernanto, 1993; Soekartawi, 1995).

Conclusion

The productivity of *Kalung* cricket's cultivation in Central Java and East Java provinces technically was not different. Farm income analysis showed that *Kalung* cricket's cultivators in East Java get higher income than cultivators in Central Java. The R/C ratio in both location was more than one, means that *Kalung* cricket's cultivation is economically profitable and feasible to run.

Cricket's farmers in East Java are necessary to save the cost of feed, through the use of local resources without reducing the productivity of crickets. Similar research can be done by larger number of farmers and areas included so we can get more accurate data to increase productivity and income of cricket's cultivators in the future.

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Public Perception in Thai Native Chicken (Pradu Hang-Dum Chiang Mai) via Food Contests

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Abstract

Thai native chicken variety Pradu Hang-Dum Chiang Mai (PD-CM) was developed and bred in the year 2002-2007 from a joint-cooperation between the Department of Livestock Development (DLD), Ministry of Agriculture and Cooperatives, and Thai Research Fund (TRF). The chicken meat had lower fat, cholesterol and triglyceride contents as compared to those of crossbred and broiler meats. In addition, the appearance of the whole chicken meat was appraised by some of the Northern Thai people, particularly the hill tribes, for religious ceremony purposes. A survey data from 207 consumers revealed that chicken's meat was the second popular meat for cooking. For northern people, the most favorite dish prepared from the meat was "Kang Kai", which was a thick-chili soup with green cabbage. This present study was aimed to promote the PD-CM chicken as a native chicken to Thai people and to encourage the utilization of the meat as a source of delicious and healthy meat through food competitions. Three food contests were organized in Chiang Mai within several months interval. Based on the audience assessment, the contests tended to increase the perception of the PD-CM chicken in all targeted sample groups. The perception of the ordinary people who attended the contest was increased from 25.5% in the first contest to 69.4% in the last contest. A survey in both farmers who produced chicken and administrative staffs of DLD indicated that the perception percentages were also improved.

Key words: food contest, native chicken, Pradu Hang-Dum Chiang Mai

Introduction

Thai domestic fowl is diverse in terms of both phenotype and variety. Twelve varieties of Thai native chicken were characterized by The Department of Livestock Development (DLD), Ministry of Agriculture and Cooperatives and distributed to

chicken-raising farmers (Amnuay *et al*., 2010a). However, without controlling of cross breeding, some of these purebred chickens were reduced in number.

Pradu Hang-Dum Chiang Mai (PD-CM) is one of the Thai native chickens which faced the same situation of problem relevant with the number of purebred. Therefore, the variety was recalled under a well organized research work during the year 2002-2007 from a joint-cooperation between the DLD and Thai Research Fund (TRF). The work was aimed to conserve the variety and to utilize the meat as a high quality protein source (Amnuay *et al*., 2011).

Naturally, most of the Thai native chickens are fighting cock. Therefore, they are raised mainly for sport not for food materials. However, the quality of the native chicken's meat in particular PD-CM was much better than those of crossbred and broiler. This was because the meat of PD-CM contained lower content of fat, cholesterol and triglyceride (Sanchai *et al*., 2011). In addition, the appearance of the whole chicken meat was appraised by some of the Northern Thai people, particularly the hill tribes, for religious ceremony purposes. From these potential characteristics, the PD-CM meat was chosen as a frontier food material from native chicken to be introduced to consumer. Acceptability of the consumer could lead to the marketing opportunity and consequently sustainability of purebred variety.

The food contest was proved to be one of the most effective activities for promoting consumer perception. The enjoyable food prepared from PD-CM meat and impressive environment might increase public perception in the chicken as food material. Therefore, this present study was aimed to promote the PD-CM chicken as a native chicken to Thai people and to encourage the utilization of the meat as a source of delicious and healthy meat through food competitions.

Materials and Methods

Survey on utilization of chicken meat as food ingredient

A survey was carried out at 5 fresh markets in Chiang Mai province around one month before the first food contest started by interview. A questionnaire was developed and tested before the survey. Two groups of consumer (each of 100) were randomly selected. The first group was the people originally from Northern part of Thailand and the second group was the people from other parts of the country.

Public perception in PD-CM via food contests

The food contests were organized 3 times in Chiang Mai. The menus used in the contests were based on the previous survey. Sensory properties of boiled PD-CM meat (from 3 month's old chicken with a weight between 1.0 and 1.5 kg) were determined from panelists who attended the contest. A perception assessment about PD-CM was also carried out from the audience attended in each contest. In the first food contest 94 audiences were interviewed, whereas in the second and third contests the number of people interviewed was 107 and 124, respectively.

Results and Discussion

Utilization of chicken's meat as food ingredient

A survey data in Chiang Mai province from 207 consumers revealed that the consumer from both Northern and other parts of Thailand preferred to consume the meat from pork and chicken as food materials (Table 1). A traditional Northern style hot and spicy soup with vegetables namely *Gang Kare* was the most popular menu to be prepared from chicken's meat in the Northern of Thailand, while people from the other parts of the country preferred to use chicken's meat in a hot and spicy (thick and clear) soup called *Tom Yum* (Table 2).

The meat from native chicken was accepted more than broiler's meat by Thai consumer (Apichai, 1993; Amnuay, 2010b). This trend was similar to that of consumer in China (Tang *et al*., 2009). Results from this study indicated that flavor, nutritional values and firmness of the meat were important factors as positive properties of the native chicken's meat while supply of the meat, price and stickiness produced the negative properties (Figure 1). Scientific evidences for this matter have been previously reported (Sanchai *et al*., 2003; 2011).

Public perception in PD-CM via food contests

The food contests were organized 3 times in the city of Chiang Mai. The first contest was carried out on April 7th, 2010 at Northern cultural market, Faculty of Agriculture, Chiang Mai University. The second competition was organized on

Table 1 Popular meat for food preparation in Chiang Mai

Meat types	Number Chosen				Scored	Ranking
	1st rank	2nd rank	3rd rank	4th rank		
Northern						
Chicken	18	52	41	8	318	2
Pork	70	32	14	3	407	1
Beef and buffalo	4	11	18	86	171	4
Fish	27	24	46	22	294	3
Others						
Chicken	19	36	21	5	231	2
Pork	42	23	11	5	264	1
Beef and buffalo	5	2	13	61	113	4
Fish	15	20	36	10	202	3

Notice: Scored = (the no of the 1st rank x 4) + (the no of the 2nd rank x 3) + (the no of the 3rd rank x 2) + (the no of the 4th rank x 1).

Table 2. Popular menu prepared from chicken meat in Chiang Mai

Menu	Number Chosen				Scored	Ranking
	1st rank	2nd rank	3rd rank	4th rank		
Northern						
Yum	28	27	31	26	281	2
Gang Kare	33	34	24	21	303	1
Gang Om	18	32	40	22	270	3
Steamed chicken	33	19	17	43	266	4
Others						
Tom Yum	27	22	26	5	231	1
Gang	14	30	14	22	196	3
Grilled chicken	9	17	17	37	158	4
Fried chicken	30	11	23	16	215	2

Notice: Scored = (the no of the 1st rank x 4) + (the no of the 2nd rank x 3) + (the no of the 3rd rank x 2) + (the no of the 4th rank x 1).

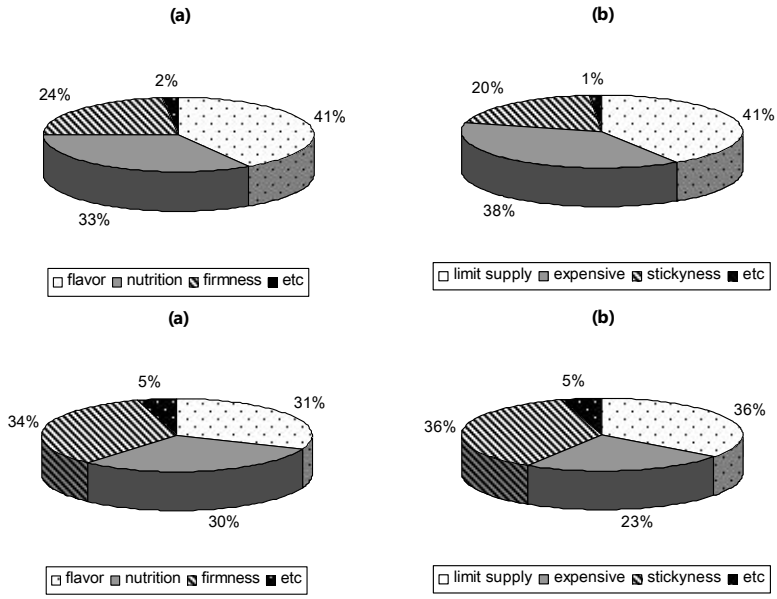

Figure 1. Opinions about advantage (a) and disadvantage (b) of native chicken's meat as food ingredient of people from Northern (top) and other parts (bottom) of Thailand

December 4th, 2010 at Agricultural Fair, Royal Flora Botanic Garden and the last one was accomplished on January 22nd, 2011 at Big C Supermarket. The last competition was done with a cooperation with Mae Heia Municipality.

Sensory properties of the boiled PD-CM meat that were evaluated by the panelists who attended the food contests are shown in Table 3. The overall acceptability of the meat was very good as a result from its texture and taste. These properties of the chicken were well in an agreement with previous finding observed by Amnouy *et al.* (2010). However, all volatiles might evaporate during a cooking process resulted in low score for odor attribute (Sanchai *et al*., 2011).

Table 3. Sensory properties of boiled PU-CM meat

Sensory attribute	Scored
First food contest (95 panelists)	
Texture	4.03 ± 0.37
Odor	3.67 ± 0.64
Taste	4.02 ± 0.40
Overall acceptability	4.16 ± 0.41
Second food contest (107 panelists)	
Texture	4.04 ± 0.45
Odor	3.79 ± 0.71
Taste	4.09 ± 0.69
Overall acceptability	4.18 ± 0.72
Third food contest (122 panelists)	
Texture	4.02 ± 0.67
Odor	3.97 ± 0.76
Taste	4.13 ± 0.74
Overall acceptability	4.17 ± 0.76

Notice: values are expressed as mean ± standard deviation
Scored 1=poor, 2=fair, 3= good, 4= very good and 5= excellent

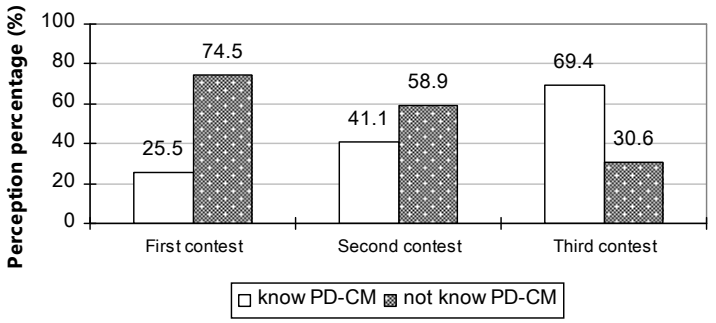

Figure 2. Evolution of perception in PD-CM chicken during the food contests

The perception of the public about PD-CM was increased after the food contests as showed in Figure 2. The obtained data indicated that a joyful activity that was incorporated with sensory trial of food products promoting good impression and enhanced the perception of the products (Pitoon, 1994; Siritorn, 2002).

Conclusions

Chicken's meat was one of the popular meats for Thai food. Flavor, nutritional value and texture of Thai native chicken's meat contributed to its quality. However, the supply in the market and price produced a negative feed back for its market potential. The meat quality of PD-CM was acceptable especially in terms of sensory. The food contest with entertainment activities dramatically improved the public perception in PD-CM chicken.

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Trade Performance of Meat and Meat Preparation Sector in Malaysia: The Case of Non-Ruminant

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Abstract

Over the years Malaysia has undergone a substantial and persistent food trade deficit. It is continue to grow at an alarming rate as the figures climbed to a staggering RM 10.1 billion in 2008. Registered at 3.4 billion in 2005, it swelled to RM 4.9 billion in 2000. If this detrimental trend continues unimpeded, the food trade deficit is expected to reach RM12.4 billion by 2010 with food imports at RM27.3 billion, far outweighing exports worth RM14.9 billion. This phenomenon has to be arrested, which requires Malaysia to boost its food exports and it is a need of the policy makers, practitioners, producers and the government agencies to undertake appropriate action related this issues. This study is to evaluate the related factors and to evaluate the competiveness of 10 food commodities in the meat and meat preparation sector. The data cover non ruminant commodities in the HS 6 digits group which are expected to have high potential in the future. The studies will be based on the concept of revealed comparative advantage (RCA). The basic logic behind RCA is that it evaluates comparative advantage on the basis of a country's specialization in (net) exports relative to some reference group. A different interpretation of comparative advantage is furnished by Vollrath indices, which are adopted in this study. Vollrath offers three alternative specifications of revealed comparative advantage, they are; relative export advantage (RXA), relative import advantage (RMA) and relative trade advantage (RTA). From this studies contributed and demonstrated the potential performance of sub selected sector in the non ruminants.

Keywords: meat preparation, non-ruminants, sub sectors, trade meat performance

Introduction

Malaysia has undergone a substantial and persistent food trade deficit. It continues to grow at an alarming rate as the figures climbed to a staggering RM 10.6 billion in 2009. In line with the global food crisis in 2008, it has heightened government awareness and brought this issue to the forefront of public interest. This phenomenon has to be addressed, which requires Malaysia to boost its food exports and to substitute imports. There is a need to determine the local commodities that are capable of substituting imported products and at the same time, searching for varieties of potential products for export. This is particularly important for the food processing industry, as the scheduled liberalization of trade under the World Trade Organization (WTO) and the ASEAN Free Trade Area (AFTA) would produce greater challenges and stiff competition to Malaysian food producers. Thus, to penetrate a wider range of foreign markets, along with the effort to curtail the food trade deficit, it is crucial for Malaysian food processors to identify food sub-sectors that are internationally competitive and viable.

The meat and meat preparation sector in Malaysia has been chosen as to date, it is mainly an imported item. It is the aim of this study to evaluate the competitiveness of 10 food commodities in the meat and meat preparation sector (division 01) covering the period of 1997 to 2008. The data cover only the non ruminant commodities in the HS 6 digits group which are expected to have high potential in the future.

Our investigation is based on the concept of revealed comparative advantage (RCA). Vollrath offers three alternative specifications of revealed comparative advantage. The major difference between the Balassa index and the Vollrath indices is that the latter eliminate country and product double-counting. Time series data were obtained from Global Trade Atlas (GTA) and International Financial Statistics (IFS).

The remainder of this paper is structured as follows: Section II reports a review of some of the related literature. Section III discusses the methodology. Section IV reveals the findings and the conclusions are summarized in Section V.

Recent Studies on Competitiveness and Comparative Advantage

For many decades, the concept of competitiveness has been widely used in economic research and economic policy from various points of view. A large and growing body of literature attempts to assess the issue theoretically and empirically. The definition of competitiveness itself may also vary with respect to the level considered (Havrila & Gunawardana, 2006). The diversity of the concepts and measures largely pertains to the variety of policy analysis needs, perspectives and objectives of the research.

Recently, a study carried out by Bojnec and Ferto (2009) attempted to investi-

gate the level, composition, and differences in agro-food relative trade advantages/ disadvantages for eight Central European and Balkan countries on the European Union (EU) markets and their implications for food policy. Higher and more stable relative trade advantages were found for bulk primary raw agricultural commodities and less so for consumer-ready foods, implying competitiveness shortcomings in food processing and in international food marketing. Duration analysis showed that the EU enlargement has had a negative impact on agro-food relative trade advantages for all eight analyzed countries. Estimations imply that the duration of agro-food relative trade advantages were the highest for Hungary and Poland, and also for Bulgaria in differentiated products, indicating their agro-food trade potential in the EU-15 markets.

Bojnec and Ferto (2006) examined the comparative advantage and competitiveness of Hungarian and Slovenian agro-food trade in the EU markets. Applying a highly disaggregated trade dataset, they described the pattern of agro- food trade in Hungary and Slovenia using the Balassa index. Their findings indicated that both countries have lost their comparative advantage for a number of product groups over time. The indices of specialization have tended to converge. For particular product groups, the indices displayed greater variation. They are stable for product groups with a comparative disadvantage, but product groups with a weak to strong comparative advantage show significant variation.

With the aim to move the attention away from advanced industrialized economies, Uchida and Cook (2005) examined the trends for trade and technological specialization among the East Asian developing economies. The analysis is confined to seven East Asian economies: Hong Kong, South Korea, Singapore, Indonesia, Malaysia, the Philippines, and Thailand. Important differences are found in the patterns of specialization, and in the relationship between them among the advanced East Asian economies and those catching up. The country level analysis indicated that a difference in the patterns of competitive advantage among the East Asian economies was greater for technology than for trade. Cumulative or path-dependent technological change was found to be important in Hong Kong, South Korea, and Singapore.

As too little attention has been paid to examine the developing countries' comparative advantage in services, Seyoum (2007) attempted to fill this gap by analyzing the competitiveness of selected services: business, financial, transport and travel services in developing countries in relation to that of the rest of the world based on three indices of revealed comparative advantage. Strong comparative advantages exist for many developing countries in transport, and travel services. There is substantial room for improvement in financial and business services. Trade liberalization and lack of adequate preparation appears to have resulted in a weakening of their comparative advantages over the years. However, their revealed comparative advantages remain, by and large, stable and do not show a fundamental shift in the structure of their comparative advantages.

Methodology

A different interpretation of comparative advantage is furnished by the Vollrath indices, which offer three alternative specifications of revealed comparative advantage. They are relative export advantage (RXA), relative import advantage (RMA), and relative trade advantage (RTA), expressed as,

$$RXA_{ij} = \frac{(X_{ij} / X_{nj})}{(X_{ir} / X_{nr})} \quad (1), \quad RMA_{ij} = \frac{(M_{ij} / M_{nj})}{(M_{ir} / M_{nr})} \quad (2), \text{ and} \quad RTA_{ij} = RXA_{ij} - RMA_{ij} \quad (3)$$

Where X are the exports and M are the imports of sector (or product) i of country j, n is the rest of the products and r representing the rest of the world. According to Vollrath, positive values of the RTA index indicate a comparative advantage, whereas negative values indicate otherwise.

Vollrath (1991) pointed out that the appraising of comparative advantage at aggregate and dis-aggregated levels can 'identify the overall direction and drive in which a country's investment and trade should take in order to exploit international differences in product and factor supply and demand' as well as 'to evaluate socially desirable specialisation patterns along narrow product lines'. He further argued that the estimation of comparative advantage may be particularly beneficial when considering trade between countries with different factor endowments.

Results and Discussion

The analysis of the Vollrath indices (RXA, RMA and RTA) outlined the trading specialization of Malaysia in the non ruminant sector. Through analysing Malaysia's indices, the results that we obtained were somewhat ambiguous. In the relative export advantage index (RXA), as presented in Table 1, we can see that the duck products (Duck, Goose & Guinea Fowl Meat & Meat Offal Prepared or Preserved Excluding Livers, Ducks, Geese And Guinea Fowls, Domestic, Whole, Frozen and Duck, Geese or Guinea Fowl Cuts And Edible Offal, Domestic, Frozen) have the highest value which are all more than 1. The rest, especially chicken products, can be considered to have a comparative disadvantage. The relative import advantage (RMA) shows that none of this sub sector has RMA values of more than 1. The highest value for Malaysia is observed in, Duck, Geese or Guinea Fowl Cuts and Edible Offal, Domestic, Frozen which represents the major importing product in this sector. As indicated above, negative values (positive) indicate a competitive trade disadvantage (advantage). Thus, from the table it is apparent that Malaysia is competitive in the production of non-ruminant sub sector. Of the 10 commodities in meat and meat preparation, all the positive values are less than 1.

Table 1. Average relative export, relative import and relative trade advantage for selected meat and meat preparation sectors in Malaysi

HS Code	Commodity	RXA	RMA	RTA
HS 020711	Chickens and Capons, Whole, Fresh or Chilled	0.7961	0.0043	0.7918
HS 020712	Chickens and Capons, Whole, Frozen	0.5531	0.1470	0.4062
HS 020713	Chicken and Capon Cuts and Edible Offal, Fresh or Chilled	0.7833	0.0017	0.7815
HS 020724	Turkeys, Whole, Fresh or Chilled	0.0565	0.0091	0.0473
HS 020732	Ducks, Geese or Guinea Fowls, Domestic, Whole, Fresh or Chilled	0.4570	0.0013	0.4558
HS 020733	Ducks, Geese and Guinea Fowls, Domestic, Whole, Frozen	21.334	0.0283	21.053
HS 020735	Ducks, Geese/Guinea Fowl Cuts & Edible Offal, Excluding Fatty Livers, Fresh or Chilled	0.0757	0.0229	0.0528
HS 020736	Duck, Geese or Guinea Fowl Cuts and Edible Offal, Domestic, Frozen	12.677	0.3509	0.9168
HS 160232	Chicken & Capon Meat & Meat Offal Prepared or Preserved Excluding Livers	0.1123	0.0183	0.0943
HS 160239	Duck, Goose & Guinea Fowl Meat & Meat Offal Prepared or Preserved Excluding Livers	190.304	0.0338	189.967

Source: Author's calculation

Conclusion

The analysis provided here revealed that Malaysia is generally very competitive in the non ruminant sector. A good competitive performance was observed in HS 160239 (Duck, Goose & Guinea Fowl Meat & Meat Offal Prepared or Preserved Excluding Livers) which is confirmed by both its relative export advantage (RXA) and relative trade advantage (RTA) indices, whereas the RTA index shows strong competitiveness. Malaysia demonstrates a good performance in this selected sub sector. A great deal of attention should be given, through aggressive research and development of new products and production techniques, to maintain and improve the competitiveness of the Malaysian food sector.

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Integration of Cattle-coconut Farming in South Minahasa Regency

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Abstract

Coconut plantation is a source of income of South Minahasa community. Land under coconut plantation is utilized for the development of cattle farming in an integrated cattle-coconut plantation system. System integration is maintained in cattle under coconut trees, the land planted with forage and cattle waste used as fertilizer. While non-integration system is the land under a coconut tree is used for forage and cattle waste is used as fertilizer. The problem is how the benefits of system integrated cattle-coconut. The objective of this study was to analyze the benefits of system integrated cattle-coconut. District and Subdistrict purposively determined by consideration of having the largest cattle population. Number of respondents consisted of 86 of farmers are determined based on the ownership of at least 2 cattle and had to sell cattle. Data analysis was using descriptive analysis. Coconut lands are managed either by owners or tenants amounted to 10 935 trees (an average of 165.68 trees per respondent). Coconut land for grazing cattle borrowed amount to 2250 trees (an average of 112.50 trees per respondent). The results showed that the average farmer earned income non integrated system of Rp 16,583,767.54 per year. The average income earned on the system integration of Rp 21,658,525.52 per year. In conclusion, cattle-coconut plantation integration system provides benefits such as availability of feed resources under coconut, improve soil fertility and as an alternative source of income.

Keywords: cattle, coconut, integrated

Introduction

Coconut is one of the agricultural commodities that dominate in South Minahasa regency. Coconut is a source of income of most people in the region and it is

sold in the form of copra. According to Supadi and Nurmanaf (2006), coconut as a strategic commodity has a social role, cultural and economic life of society. Land under coconut farmers utilized for the development of beef cattle. Waste of food crops is a source of feed, whereas cattle manure used for soil fertility improvement under a coconut tree. This farming system is known as crop-livestock integration.

System of crop-livestock integration has many advantages such as availability of food resources, reduce the cost of weed control, improved soil fertility, increase crop yields and principal divides the risk of loss (Mansyur *et al*., 2009). These benefits can increase the productivity of land is higher, thus providing greater benefits for the farmer. Integrated of farming is effort related, mutually supportive, mutually reinforcing and mutually beneficial (synergistic). Ramrao (2006) concluded that the integrated farming system is the most profitable.

According Channabasavanna *et al*. (2009) that the Integrated Farming System are very productive and profitable. Since 1977, the integrated farming system has been claimed to reduce land degradation and productivity compared with conventional rice-based system. Integrated livestock farming is the development of the livestock resource use that can reduce the risk of having the principles of sustainability efforts (Soedjana, 2007). In this case, Rajasekaran *et al*. (1991) introduced a system of natural resource management for sustainable agricultural development.

The problem of cattle farming in South Minahasa is that the cattle is traditionally maintained by grazing system that tied under the coconut trees and move around. Based on these problems, this study aimed to analyze profitability of the cattle–coconut integration farming in South Minahasa.

Materials and Methods

The research was conducted in South Minahasa Regency using the survey method. The Minahasa Regency was purposively selected for the study as the Regency was a centre for coconut production and cattle farming in North Sulawesi. The districts in South Minahasa was determined by purposive sampling; Sinonsayang and Tenga districs were the districts with the largest cattle population (BPS South Minahasa, 2011). Peasant farmers in every village of the sample was restricted to coconut farmers who owned at least 2 (two) heads of cattle and had to sell cattle. There were as many as 86 respondents. The type of data used were cross section and time series data. The data collection techniques were by interviews with cattle farmers and direct observation in the field. The collected data were analysed using descriptive analysis method.

Results and Discussion

The results showed that in South Minahasa the number of coconut trees owned

by farmer ranged between 30 - 1000 trees for a total of 13.185 trees. Coconut lands were managed either by owners or tenants that amounted to 10.935 trees, or an average of 165.68 trees per farmer. Meanwhile the borrowed land for cattle grazing was managed by the tenants and coconut trees numbered 2250 or an average of 112.50 trees per farmer. Coconuts was processed into copra. Coconut production per tree was about 20-40 pieces. To produce 100 kg of copra, 400-450 coconuts were required depending on the size of the coconuts. The copra prices prevailing in the study area ranged from Rp 570.000 to Rp 980.000 per 100 kg copra. The price would be different when the farmers sold the copra to the coconut oil factory which greatly affected the income of the farmer.

Cattle sales made in the "blantik" market in the village Ongkaw; the trader who arrived at the site was a farmer and sold the cattle to other farmer. The price of cattle depends upon the price of beef which is about Rp 50.000-Rp 70.000 per kg. Income from livestock enterprises that consume waste and grass that are not qualified. If the land under coconut trees used to grow quality grass then the income would be higher. Land use under the coconut to serve as a cover crop forage. According to Rahim (2006), cover crops is an act of conservation at the time instead of the growing season.

The average land area for maize cultivation was 0.9 ha and the planting of corn was in a 1-3 year period. Most of the farmers' cattle (66 respondents or 76,74%) planted corn under coconut trees with an area of 0,71 ha on average. The number of respondents who grew corn instead of under the coconut trees as much as 20 respondents (23,26%) with an area of dry land on average 0.87 ha. The income of the farmer from the three farms which were not integrated can be seen in Table 1.

In Table 1, it turns out that the average income earned per respondent of Rp 15.899.081,29 per year. This income is obtained by the system of diversification of farming systems. According to Rota and Sperandini (2010) that the system consists of components of plant diversification and free-living animals at the same time. In this case, the integration of crops and livestock is primarily to minimize risk and resource recycling.

Table 1. Average relative export, relative import and relative trade advantage for selected meat and meat preparation sectors in Malaysi

Sources of Income	Amount (Rp/Year)	Average (Rp/Year/Respondent)	%
Coconut Farming	871,987,077.30	10,137,896.25	63.77
Cattle Farming	64,174,413.10	746,214.11	4.69
Corn Farming	206,180,500.00	2,397,447.67	15.08
Labor of Cattle	225,107,000.00	2,617,523.26	16.46
Total Income	1,367,448,990.40	15,899,081.29	100

The integrated production process showed that land under coconut trees could be used for fodder crops (forage or legume). Dolev and Kimhi (2010), land area is a determinant factor of the viability of agriculture. One Ha of land under coconut trees covering an area of 0,8 ha planted with forage grass seed needs of 16,000 cuttings. The average land area owned, managed and borrowed by farmers according to the results of this study was 0.71 ha of grass cuttings Brachiaria mutica requiring as many as 11,360 cuttings. Technological innovation in the animal feed crop-livestock Integration Systems Waste-Free (SITT-BL) according to Haryanto (2009) provides an exciting opportunity to clean green and agricultural development. Grasses that can be generated as much as 85.2 tons / year is equivalent to 6.67 AU/year, with cut and carry system.

If the land under coconut trees planted forage then the respondent may obtain income from these forages. If the grass produced can be sold to other farmers then the respondent will earn income of Rp 35.328.093.00 per year per respondent.

Cattle manure in the study area was only allowed on agricultural lands and not used as compost. In an integrated production process then all the existing waste utilized by the principle of zero waste. In this case, no waste is wasted and the manure can be processed to generate income for farmers and their families. Inefficient use of inputs according to Asche *et al* (2008) may worsen the environmental impact. Fleckinger and Glachant (2011) suggested that each manufacturer must collect and process-related waste products.

Some research indicates that a cattle can produce as much as 10 kg of faeces per cattle per day. Impurities can be processed into compost by 3 kg. If the price of compost is assumed to be Rp 3.000 per kg in a day then the revenue that the amount of Rp 9.000. The average of ownership of 3.4 cattle will produce 10.2 kg of compost for the revenue obtained is Rp 11.169.000/year. Compost can be expressed as an alternative income for farmers who had only left the plantation lands or in the yard. Another advantage is the compost can be used by farmers to substitute artificial fertilizer prices higher. The benefits of compost is to improve

Tabel 2. Farmer Income on Integration Cattle-Coconut Farming in South Minahasa Regency

Sources of Income	Amount (Rp/Year)	Average (Rp/Year/Respondent)	%
Coconut Farming	871,987,077.30	10,137,896.25	16.90
Cattle Farming	64,174,413.10	746,214.11	1.25
Forage Farming	3,038,215,998.00	35,328,093.00	58.88
Compost Business	960,534,000.00	11,169,000.00	18.61
Labor of Cattle	225,107,000.00	2,617,523.26	4.36
Total Income	5,160,018,488.40	59,998,725.62	100

soil fertility owned by farmers in the study area. Organic fertilizer / compost derived from mixed Chromalaena and manure can replace about 50% of chemical fertilizers (Urea and SP-36) (Abdullah and Puspitasari, 2007). Provision of organic materials from manure and crop residues can improve soil physical properties (Prasetyo and Suriadikarta, 2006). The income of farmers as a respondent in an integrated cattle-coconut farming can be seen in Table 2.

As shown in Table 2, the average income of the farmer obtained an integrated farming system was Rp 59.998.725,62 per year. This income was greater than the farming of cattle-coconut that is not integrated. According Salendu and Elly (2011) that sustainable livestock development in North Sulawesi could be implemented by developing models of coconut-cattle integration. Rota and Sperandini (2010) suggested that the high integration of crops and livestock are often considered as a step forward. Ahmed et al (2011) states that the pattern of integrated farming is the best farming system in terms of resources, efficiency, productivity, production and food supply.

Conclusion

Based on the research results it could be concluded that the income received by farmers with cattle-coconut integration system was greater than that with the non-integration system. Cattle-coconut integration system provides benefits such as availability of feed resources under coconut, improve soil fertility and as an alternative source of income.

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Applicative Model in Utilizing Mulberry Plant as a Worth Feed Resource for Increasing Farmers' Income

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Abstract

Integration of mulberry plant and livestock can be optimal when implement the applicable management that promote benefits to the farmers, so it can support its sustainability. The aim of this research was to improve farmers' income as well as animal productivity by exploring some applicative models. There were three applicative models which were tested: the P1 Model= introduction of two goats into the silkworm farming system to enable them to utilize mulberry leaves that are not consumed by the silkworms; the P2 Model= Using the whole mulberry plants as feed ingredients for two feedlot cattle; the P3 Model= Selling mulberry leaves produced for concentrate ingredient. Parameter measured was net income generated from each model. Result of the study indicated that integration models of mulberry plant with livestock have their own uniqueness to be applied. The highest income for farmer was obtained when the whole mulberry plant was used as feedstuff for feedlot cattle (the P2 model). Income of Silkworm farmers also increased when applying the P1 model, which introduced two goats for each box of silkworm rose. But in a certain occasion, it was more beneficial for the farmers to harvest and dry mulberry plant then sells it for concentrate ingredient (the P3 model). In conclusion, the main factor to be considered in choosing one particular model to be implemented is mainly determined by ability of the farmers to procure cattle or goat to be raised in the mulberry plant–livestock integration model without ignoring the forecast of dry and rainy seasons.

Keywords: Integration model, mulberry, livestock, income, farmers

Introduction

Utilizing locally available feedstuff can be considered as a strategic and wise choice. The choice is giving a chance to enhance nation self-sufficiency in livestock sector. Mulberry plant as a local feed resource has a potency to be a value feedstuff

due to its potential production, its nutrient content and its well adaptable growth. Estimate production of mulberry leaves could reach 19 tones DM/ha/year, which is higher than the yield of such other legumes as *Gliricidia sepium* with production 7-9 tones DM/ha/year (Horne et al., 1994). Nutrient contents of mulberry are crude protein 22-23%; total sugars 8-10%, minerals 12-18%, ADF 35%, NDF45.6%, hemicellulosess 10-40%, cellulose 21.8% (Datta et al., 2002). Based on the nutrient contents and high production of leaves, it is potential to substitute concentrate in ruminant feeding system (Doran et al., 2006).

An experiment to evaluate the potency of mulberry leaves for substitution of concentrate on rice straw based feeding system has been conducted. The result of the study indicated that 50% of mulberry leaves could be used as the optimum level for substitution of concentrate. This level increased the ruminal bio-fermentation (Syahrir et al, 2009).

A good management in crop-livestock integration should prioritize the benefit for farmer so that it could support sustainable increase in livestock production. Therefore a study on integrated farming system, especially applicative model on the integration of mulberry plants and livestock becomes important. The purpose of this research was to build a management model of the integration of mulberry plant and livestock system in order to increase farmer income and to support sustainable livestock production.

Materials And Methods

The study was carried out in three spots of lands of mulberry plant estate, Enrekang Regency. The size of each land section was approximately 3,000 m^2 (the size is equivalent to an area of a mulberry estate required to raise three boxes of silkworms). Each section of land was assigned to one of the three applicative models of integrated mulberry plant and livestock as treatments. The models were:

The P1 Model = The introduction of two goats into one box of silkworm farming so that the goats can utilize mulberry leaves which are not consumed by the silkworms.

The P2 Model = Replacing the silkworm farming with fattening two beef cattle

The P3 Model = Farmers produce mulberry leaves that will be sold as ingredient for concentrate. The selling price will be adjusted similar to the market price of mulberry leaves.

Field study was conducted for six months consisted of three periods (two periods for raising silkworm and one period for beef cattle fattening). The whole cost used for raising silkworms and for feed supplement (other than mulberry leaves) of the P1 and the P2 models were calculated as the production cost. Similarly, income generated from selling the cocoon and livestock of both models was considered as

the income. Parameters measured were total production cost and income of each applicative model. Data were analyzed descriptively.

Results And Discussion

All of the three models showed certain uniqueness. The selection of one particular model to be applied by farmers will be strongly influenced by each farmer's capital for obtaining cow or goat as well as the season and condition of mulberries. Each of the resulting applicative models is described below:

The P1 Model

This applicative integration model of silkworm farming with goat is intended to maximize the utilization of mulberry. In this integration model, farmers raise silkworms by providing an area of ± 1000 m^2 of mulberry plants.

Some of the mulberry plants were not utilized during the silkworm farming, because farmers always provide an area of mulberry plantation that exceed the necessary amount needed, anticipating a condition of ineffective mulberry plants. In addition, the mulberry leaves left over from feeding on the silkworms are usually available. The introduction of goats for silkworm farmers can optimize mulberry plant utilization, leading to an increase of the farmer's income.

During the 6 months of mulberry farming activity, the introduction of two goats increased the farmer's income by Rp 633,000. This additional revenue almost equals to the main income obtained from sole silkworm farming of Rp 643,667 (Table 1), giving total revenue for the farmers of Rp 1,277,000. Another advantage gained from the introduction of two goats into silkworm farming was the availability of composted manure from goat feces which helped reduce the production cost of mulberry planting.

The P2 Model

Applicative model which replace silkworms with fattening two beef cattle was used by farmers who possess a land of mulberry plants to raise one box of silkworm. The descriptive data can be seen in Table 1, in which during the 6 months of study, when the whole mulberry plant was used as feedstuff for feedlot cattle, the average income for the farmers was Rp 1,833,000. The income was even larger if the cost of grass supplies used to feed cattle which is usually calculated as a maintenance cost of Rp 950,000/farmer, was included as farmer's income.

In addition to the income obtained from the purchase and sale price of the fattening cattle, farmers also benefited from the feces production of the cattle. The feces produced by one beef cattle was approximately 3 kg DM/day, therefore each farmer can acquire more profit from as much as 6 kg DM/day of feces production. This can replace most of the cost of fertilizer used on mulberry plants.

Table 1. The average production cost, gross income, and net income of silkworm farmers applying either the P1,P2, or P3 models

No	Description	Value
The P1 Model		
Main income from silkworm		
	Production cost :	
	- Raising period I (Rp)	128,000
	- Raising period II (Rp)	183,000
	Total cost (Rp)	311,000
	Gross income (Rp)	954,667
	Net income (Rp)	643,667
Additional income from inclusion of goat		
	Production coast:	
	- Price of goat (Rp)	1,666,667
	- Vaccination and medicine (Rp)	100,000
	Total cost (Rp)	1,766,667
	Gross income (Rp)	2,400,000
	Net income(Rp)	633,333
	Total net income (Rp)	1,277,000
The P2 Model		
Cattle fattening		
	Production cost :	
	- Cattle (Rp)	9,066,667
	- Grass (Rp)	950,000
	- Vaccination and medicine (Rp)	100,000
	Total production cost (Rp)	10,116,667
	Gross income (Rp)	12,000,000
	Total net income (Rp)	1,883,333
The P3 Model		
Harvest I	Fresh mulberry leaves production (kg)	526
	Dry mulberry leaves production (kg)	149
	Net income* (Rp)	446,000
Harvest II	Fresh mulberry leaves yield (kg)	973
	Dry mulberry leaves yield (kg)	224
	Net income* (Rp)	673,000
Total production	Fresh mulberry leaves (kg)	1,499
	Dry mulberry leaves (kg)	373
	Total net income (Rp)	1,119,000

*Price of mulberry leaves meal = Rp. 3.000/kg

The P3 Model

The application of the P3 model that involves the production of raw material in the form of mulberry plant biomass by mulberry plant farmers, which will then be sold as feed ingredients for concentrate, is another viable alternative. During the 6 month period of research, the income that farmers gained from the sale of dried mulberry leaves usually used for feeding 1 box of silkworms was Rp. 1,119,000. (Table 1). This result was higher than the income of farmers who solely farm silkworms, which amounted to Rp. 643,667, but slightly lower than the income of mulberry farmers who farm silkworms and also introduced two goats, which amounted to Rp 1,277,000 (Table 1).

The application of the P3 model is effective for farmers who face certain problems which resulted in incapability for them to farm silkworms for a certain period of time. All this time, during the periods where mulberry farmers are incapable of farming silkworms due to certain constraints, the mulberry plants are left to grow and are only trimmed when the farmers are ready to nurture silkworms.

Conclusion

Application of each of the Integration models of mulberry-livestock has its own uniqueness. The conclusions that could be drawn from the application of those three models are:

1. Utilization the whole mulberry leaves as feedstuff for fattening beef cattle (the P2 Model) generates the highest income for the silkworm farmers.
2. Revenue for the silkworm farmers increases when applying the P1 Model, which is introducing two goats for every box of silkworm.
3. When mulberry farmers face a problem particularly in a certain condition, they could harvest and dry the mulberry plant then sell it as ingredient for leaf meal concentrate (the P3 Model).

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VI. ANIMAL HEALTH AND DISEASE PREVENTION

Spatio-Temporal Epidemiology of Highly Pathogenic Avian Influenza H5N1 Outbreaks in East Java, 2009-2011

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Abstract

Outbreaks of highly pathogenic avian influenza (HPAI) subtype H5N1 have occurred in Indonesia since February 2004. The outbreaks still can be found in many areas of the country up to now. This study described the spatial and temporal patterns of the HPAI subtype H5N1 outbreaks in East Java, Indonesia from 2009-2011. The aim of the study is to to describe the spatial and temporal patterns of the HPAI H5N1 outbreaks reported between January 2009 and December 2011 and to compare endemic areas of HPAI H5N1 in East Java. According to the report, HPAI H5N1 outbreak increase up to 167% in average each year. This study described the spatial pattern through three endemic areas in East Java which were defined as West, Central and East region. As the result, GSTAR modeling gave further information that aside being correlated spatially, the series also patterned temporally. West and East region are both affected by other regions, while Central region only being affected by the last time condition of certain location.

Keywords: avian influenza, epidemiology, H5N1, Indonesia, spatio-temporal analysis

Introduction

Outbreaks of highly pathogenic avian influenza (HPAI) subtype H5N1 have occurred in Indonesia since February 2004. Therefore the disease has spread and now becoming endemic in large parts of the country. The first step in controlling the spread of HPAI virus is to understand the infection dynamics of the virus in the environment and have insight into the causes of the disease process. An understanding of the complex epidemiological system that involves the frequent interactions between the various infection reservoirs and hosts is needed to be able to break the transmission cycle. Clustering of disease events provides clues to the causes of the disease process, and may assist in formulating disease prevention and control programs (Ekong *et al*., 2012).

The objectives of this study therefore were (1) to describe the spatial and temporal patterns of the HPAI H5N1 outbreaks reported between January 2009 and December 2011; and (2) to compare endemic areas of HPAI H5N1 in East Java. This information may be useful in planning prevention, surveillance and control strategies in HPAI H5N1 virus high-risk areas and also to direct future research into HPAI H5N1 epidemiology in the country.

Materials and Methods

Study area and study period

East Java occupies a land area of 46.428,57 km^2 and comprised of 29 regencies and 9 cities with number of people 37.476.757 in the year of 2010. Among three years, from January 2009 to December 2011 inclusive, the outbreaks of HPAI H5N1 were recorded in this areas. Of these, 20 commune-level outbreaks are clustered, then divided to three regions, West, Central, and East, as endemic areas. Furthermore, it is defined as study areas.

Data sources and case definition

From January 2009 to December 2011, we used data obtained from an household-report that was conducted by the Department of Animal Health of East Java Province. The HPAI H5N1 surveillance relies on poultry households level recognizing sick or dead poultry in a flock and then reporting the details of these events to commune veterinarians. In this study, the index household in each commune, that is the first case ever recorded and diagnosed to be H5N1 positive in the commune, was recruited as the case and controls were defined as households that had poultry not showing clinical signs of disease.

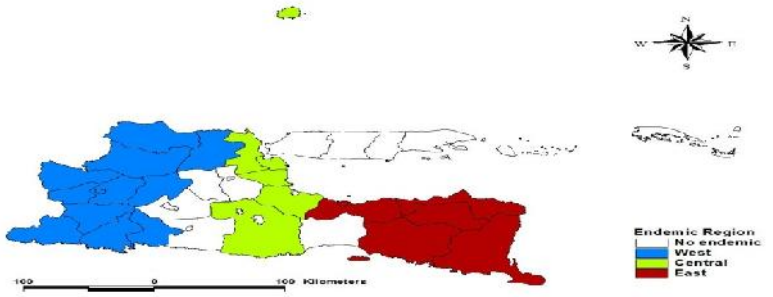

Figure 1. Endemic Region of Avian Influenza of East Java through 2009-2011

Data analysis

The data were restricted only for a certain areas which were endemic areas. From 29 regencies and 9 cities, there were 20 regencies that highly endemic of H5N1. Those endemic areas are clustered into three regions that are West, Central

and East region. The data analyses have three major components: descriptive analysis, spatial methods, and modelling. The descriptive analyses were done by using Microsoft Excel. The spatial data was visualized using ArcVIEW GIS 3.2. And modeling method was done by SAS.

Spatio-Temporal

All data have a more-or-less precise spatial and temporal label associated with them. A purely spatial model usually has no causative component in it; such models are useful when a space-time process has reached temporal equilibrium, or when short term causal effects are aggregated over a fixed time period. Data that are close together in space (and time) are often more alike than those that are far apart. A spatial model incorporates this spatial variation into the generating mechanism, in contrast to a nonspatial model (Cressie, 1993). Spatial-temporal models arise when data are collected across time as well as space.

Generelized Space Time Autoregressive (GSTAR)

In practical problems, GSTAR model is frequently applied to geology and ecology [10]. The generalized space-time autoregressive model of order ($p;\lambda_1,...,\lambda_p$), shorten by GSTAR($p;\lambda_1,...,\lambda_p$), is one of space-time models characterized by autoregressive terms lagged in the pth order in time and the order of ($p;\lambda_1,...,\lambda_p$) in space (Nurhayati,)

Spatial dependent in GSTAR model is expressed by weight matrix. Let $\{Z(t) : t = 0,\pm1,\pm2,...\}$ be a multivariate time series of N components. In matrix notation, the GSTAR model of autoregressive order p and spatial orders $\lambda_1,\lambda_2,\ldots,\lambda_p$, GSTAR ($\lambda_1,\lambda_2,\ldots,\lambda_p$) could be written as (Borovkova *et al*., 2002) :

$$Z(t) = \sum_{s=1}^{p}[\Phi_{s0} + \sum_{k=1}^{\lambda_s}\Phi_{sk}w^{(k)}]Z(t-s) + e(t) \qquad (1)$$

Where :

$\phi_{s0} = diag(\phi_{s0}^1,...,\phi_{s0}^N) and\ \phi_{sk} = diag(\phi_{sk}^1,...,\phi_{sk}^N)$

weights are choosen to satisfy $w_{ii}^{(k)} = 0\ and \sum_{i\neq j} w_{ij}^{(k)} = 1$. Selection or determination of space weight is one of the main problems at GSTAR modeling. Some methods for determining space weight have been proposed to the application of GSTAR model. One of the methods is uniform weight, i.e. $w_{ij} = 1/n_i$, where n_i number of spaces or locations where are located near to location *i* (Borovkova, 2002).

Results and Discussion

In total, 478 outbreaks were recorded from 20 regencies as endemic areas in East Java through 2009 until 2011. In that mean time East region had the largest

number of incidents (Table 1). The incident reports of HPAI H5N1 are increased 167% in average each year.

Table 1. Overall percentage of HPAI outbreaks in three region through the study period (*n*=478)

Region	2009 (*n*=45)	2010 (*n*=113)	2011 (*n*=320)	2009 – 2011 (*n*=478)		
	N %	*N* %	*N* %	*N* %	Mean	95% CI
West	20 45	4 4	80 25	104 22	6	[0.11]
Central	10 22	33 29	95 30	138 29	4	[2.6]
East	15 33	76 67	145 45	236 49	7	[4.10]

n: number of HPAI outbreaks; %: percentage of HPAI outbreaks; CI: confidence interval of %; *N*: number of outbreaks by region

Spatial Pattern

The spatial pattern of three endemic areas is described as Figure 2. Total incident through 2009 until 2011 being summarized in seven classes then each regency/city is colored according to the class. The most infected areas are: Magetan, Madiun and Lamongan regencies with outbreak more than 36 cases, as we can see from the darkest color.

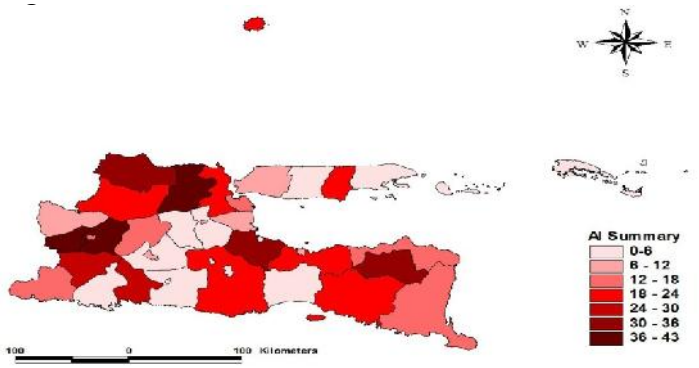

Figure 2. Endemic Mapping of Avian Influenza of East Java through 2009-2011

GSTAR Model

Through this study period, the highest outbreaks occur in 24th until 28th period which are January until April 2011. This pattern can be seen in overall location. According to the time series plot across 2009-2011 we can see that outbreaks in East region tend to be higher than the two others.

After being transformed due to stationarity in variance, autocorrelation function of the three regions is shown in Table 2 (MACF). The (+) and (-) signs are indicating the lags which are significantly different from 2*standard error. Through MACF we can conclude that the series are stationary in mean.

Table 2. Schematic representation of Cross Correlation and Partial Cross Correlation for Three Regions

MACF	Region \ Lag	0	1	2	3	4	5	6	7	8	9	10
	West	+..	+..	…	…	…	…	…	.-.	.-.	…	…
	Central	.++	.++	--+	…	…	…	…	.-.	…	…	…
	East	.++	.++	.++	…	…	…	.-.	.-.	.-.	.-.	…
MPACF	Region \ Lag	0	1	2	3	4	5	6	7	8	9	10
	West	+..	…	…	…	…	…	…	…	…	…	…
	Central	.+.	…	…	…	…	…	…	…	…	…	…
	East	..+	…	…	…	…	…	…	…	…	…	…

+ is > 2*std error, - is < -2*std error, . is between

According to Multivariate Partial Auto Correlation Function (MPACF) (Table 2) the chosen tentative model to be tested is GSTAR(1_1) with AIC (Akaike Index Criterion) -8.73. After being weighted uniformly for each region, parameter testing is done using linear regression analysis. The models for each region are computed as the following matrix and Table 3.

Table 3. Models for Each Region

Region	Models
West [$Z_1(t)$]	$Z_1(t) = 0.55 * Z_1(t-1) + 0.12 * Z_2(t-1) + 0.12 * Z_3(t-1) + e_1(t)$
Central [$Z_2(t)$]	$Z_2(t) = Z_2(t-1) + e_2(t)$
East [$Z_3(t)$]	$Z_3(t) = 0.26 * Z_1(t-1) + 0.26 * Z_2(t-1) + 0.53 * Z_3(t-1) + e_3(t)$

As we can see in Table 3 every region is affected by the last time condition. West and East regions both are affected by other regions, whereas Central region only is affected by the location itself in the last condition. We can use the models for forecasting as well.

Conclusions

The data analyses provide the evidence of spatio-temporal of HPAI H5N1 outbreaks at the household level. The number of reported incidents increases more likely twice each year. According to the number of outbreaks, study area is clustered into three regions. From these regions, the East has the largest number of outbreaks during study period (49%). GSTAR method gives a model for each region. The models show that sWest and East region are both affected by other regions, while Central region only being affected by the last time condition of certain location.

Acknowledgement

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Anatomy and Morphometry of Reproductive Organ of Male Mouse Deer (*Tragulus javanicus*)

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Abstract

Mouse deer (Tragulus javanicus) is one of endangered animals that are distributed in tropical forests of Southern Asia, including Java, Sumatera, and Borneo Islands. The mouse deer population is predicted to decrease, as a side effect of high conversion of their habitat into human needs land. However, conservation of this mouse deer is urgently required. Unfortunately, we still lack of scientific information relating to its reproductive system, including anatomical and morphological data. Understanding of the reproductive system is required in developing a suitable technology in mouse deer breeding. This technology will be applied in deer conservation and improvement of their population. The aim of this study was to characterize anatomy and morphometry of male reproductive organs of mouse deer. This study was conducted in Field Laboratory Unit of the Faculty of Veterinary Medicine, Bogor Agricultural University (IPB). Growing mouse deer aged 2 to 3 years old and weighed 1.8-2.0 kg were used in this experiment. The reproductive organs of male mouse deer were observed, measured, weighed, and documented. The collected data were tabulated and analyzed descriptively as follow: Penis type of mouse deer was fibroelastic, length of testis was 12.33±2.89 mm and weigh was 0.81±0.17 g, Vas deferens was 113±3.60 mm, Ampula was 17.33±2.87 mm, Vesicularis gland was 18.00±3.46 mm and 5.7±1.10 mm in dimension. The Mouse deer had gland bulbourethralis, with 8.26±1.02 mm in length and 0.86±0.04 g of weight, and length of its free penis of preputium was 58.33±10.41 mm. In conclusion, the male reproductive organ of mouse deer was similar to reproductive organ of other domestic livestocks, they had similarity in dimension of various organs, including primary organ (gonad or testis), accessories gland (ampula, gland vesicularis, and bulbourethralis prostate), channel part, which was consisted of epididymus, vas deferens and urethra, and also organ copulatoris or exterior organ, called penis. On the other hand, penis showed different characteristics from other livestock's' penis in its morphology. Their penis showed a clockwise turn spiral-like form, with the

number of rotations was two and a half and showed branch form, which the function was not yet known.

Key words: Male mouse deer, reproductive organ, anatomy

Introduction

Mouse deer (*Tragulus javanicus*) is the smallest ruminant animals in the world. This animal is only found in tropical forests in southern Asia, including the islands of Java, Sumatra, and Kalimantan. The population of mouse deer is thought to decline due to habitat destruction and conversion into agricultural uses and hunting activity as well as the threat of predators that can prey on it.

Natural breeding of mouse deer in captivity has not succeeded yet. Factors causing the failure of mouse deer breeding in captivity are not known, but most likely it is caused by a lack of knowledge or information about the reproductive biology of this animal.

Information about the anatomy and morphometry of male mouse deer reproductive organs has not been widely reported. Male reproductive system is an important factor in the success of animal breeding or animal reproduction technology. Knowledge of such information is one of the important and decisive factors in achieving the success of animal breeding in captivity. The purpose of this study was to examine the anatomy and morphometry of the male mouse deer reproductive organs to support the animal breeding and reproduction for conservation and cultivation.

Materials and Methods

Time and Location

This study was conducted in May and October 2009 at the Field Laboratory of Reproductive Rehabilitation Unit (URR), Department of Clinic, Reproduction, and Pathology, Bogor Agricultural University.

Materials

Three healthy and mature (had canine teeth) male mouse deer with body weight ranged 1.8-2.0 kg were used in the experiment.

Measurement and Weighing of the Reproductive Organs

This study used three male mouse deer that were not adapt and finally died in captivity. The dead animals were then prepared to obtain their reproductive organs.

These organs were cleaned from fat and laid in its original position in the body. They were observed, documented, measured, and weighed and the data were tabulated and analyzed descriptively according to Toelihere (1993).

Testes. The length of the testes was measured by placing a measuring tape at the end of the testes from one side to another with or without the caput and cauda epididymis. Diameter of the testes was measured by using a microcaliper at the largest part of the testes, and then weighed using an analytical balance.

Vas deferens. The length of the vas deferens was measured by placing the measuring tape at the end of the cauda epididymis, and the tape was then pulled until it reached the end before the enlargement of the vas deferens to the ampulla. Vas deferens diameter was measured by using microcaliper before it was weighed.

Ampulla of vas deferens. Ampulla of vas deferens length was measured from the initial enlargement of the vas deferens to the border with vesicularis gland. The diameter of ampulla was measured at the largest part before it was weighed.

Vesicularis gland. The longest part of the vesicularis gland was considered as the length, while the shortest was considered as the width. Diameter of the vesicularis gland was measured with a microcaliper. The two glands had been separated from the main organ before they were weighed.

Prostate. Measurement of prostate length, diameter, and weight were similar to those of vesicularis gland.

Bulbourethralis (Couper). Measurements of bulbourethralis were similar to those of vesicularis glands.

Penis. Measurements of total penis length was started from the base of the penis (the radix) up to the penis free end, and length measurement was also conducted for penis parts such as the penis glans and prepuce. Diameter of the penis was measured at the largest part of the organ.

Data obtained from each measurement were tabulated and the average was calculated and analyzed descriptively.

Results and Discussion

In general, parts of a male deer reproductive organs were similar to those of other domestic livestock; they were the primary sex organs (male gonads or the

testes), complementary sex glands (the ampulla, the vesicularis gland, the prostate, and the bulbourethralis), and channels consisting of the epididymis, the vas deferens and the urethra, and the external genital organs or copulatoris organ called the penis.

Testes. Male mouse deer had a pair of testicles which were wrapped by the tunica albugenia protected by the scrotum on the outside. The testes functions to produce spermatozoa in a process called spermatogenesis and produces testosterone hormone in the interstitial cells (Leydig) (Hafez & Hafez 2000; Toelihere 1987). Mouse deer testes length was 12.33 ± 2.89 mm, diameter was 8.20± 1.92 mm, and weight was 0.81± 0.17 g (Table 1).

Table 1 Morphometry of male mouse deer reproductive organs

Organs	Remarks	Mouse Deer
Testes without scrotum	Length (mm)	12.33±2.89
	Diameter (mm)	8.20±1.92
	Weight (g)	0.81±0.17
Vas deferens	Length (mm)	113±3.60
	Diameter (mm)	2.0
	Weight (g)	0.48±0.04
Ampulla	Length (mm)	17.33±2.87
	Diameter (mm)	2.0
	Weight (g)	0.07±0.01
Vesicularis gland	Length (mm)	18.00±3.46
	Diameter (mm)	5.73±1.10
	Weight (g)	0.29±0.09
Prostate (body)	Length (mm)	17.33±2.52
	Diameter (mm)	6.53±0.06
	Weight (g)	0.43±0.07
Bulbourethralis gland	Length (mm)	8.27±1.02
	Diameter (mm)	5.47±0.85
	Weight (g)	0.86±0.04
Penis	Total length (mm)	142.33±14.74
	Free-preputium length (mm)	58.33±10.41
	Penis glans (mm)	44.33±2.08
	Diameter (mm)	4.0

Epididymis. The epididymis is a structure that is elongated and tightly attached to the testis. The mouse deer epididymis consisted of caput in the anterior, corpus in the dorsal, and cauda epididymis in the posterior.

Vas deferens. Vas deferens connected the epididymis with the accessoryes glands, serves as a channel of transport of spermatozoa from the epididymis to the ampulla. The length of the mouse deer vas deferens in this study was 113 ± 3.60 mm which was shorter than that of timor deer (452.0 ± 0:44 mm) (Nalley 2006) or sheep (24 cm) (Hafez 1987).

Ampulla of vas deferens. Ampulla of vas deferens is the magnification of the end of the vas deferens before vesicularis gland. The length of the mouse deer ampulla (17.33 ± 2.87 mm) was smaller than that of Timor deer (72.53 ± 2.39 mm) (Nalley 2006) or sheep (70 mm) (Hafez 1987).

Vesicularis gland. There was a pair of vesicularis glands attached to the dorsolateral edge of vesica urinary neck. Mouse deer vesicularis gland length and diameter were 18.00 ± 3.46 mm and 5.73 ± 1.10 mm, respectively. This was smaller than the vesicularis gland of timor deer (45.36 ± 1.42 mm) or sheep (40 mm).

Bulbourethralis gland (Cowper). In the mouse deer, there was a pair of bulbourethralis glands with a length of 8.26 ± 1.02 mm, diameter of 5.47±0.85 mm and weight of 0.86 ± 0:04 g. Bulbourethralis gland is also very clearly seen in horses and pigs. Nalley (2006) reported that these glands were not found in Timor deer.

Penis. The penis is a male copulation organ and is established by the erectile tissue. The penis of mouse deer was fibroelastic which is similar to that of cattle, so that the penis corpus and glans were only slightly enlarged during erection.

Mouse deer penis was composed of radix and the corpus had a sigmoid flexura which was similar in general to other ruminants. Free-preputium penis length in mouse deer (58.33±10.41mm) was longer than the penis of Timor deer (35.38±0.88mm) (Nalley 2006) or sheep (40 mm) (Hafez 1987). Characteristics of the mouse deer penis had the features that could distinguish it from other animals, such as the tip of the penis which form spiral rotation in clockwise direction with two and a half spins. Function of this rotated penis tip is not yet clearly known. The same feature was also found in pig's penis, but opposite direction with a total spin of one and a half.

Conclusion

Mouse deer penis is characterized by a flexura sigmoidea, fibroblastic type with a very distinctive penis tip, which forms a spiral with a spin number of two and a half in clockwise direction.

Acknowledgements

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Effectiveness of *Lactobacillus acidophilus* 2B4 as Biocontrol to Prevent *Salmonella enteritidis* Infection on Laying Hens

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Abstract

Laying hen is raised and selected to produce eggs. Poultry could be infected by several kinds of Salmonella enterica such as S. enteritidis as a specific bacterium which is carried by chickens. Salmonella enteritidis can depress the hen weight by dehydration and contaminate the egg which is very dangerous for human health. Regarding this negative effect, the preventive treatment to eliminate Salmonella enteritidis contamination in eggs becomes a major concern. This research aimed to determine the optimum dosage and frequency applied of probiotic L.acidophilus to avoid contamination of Salmonella enteritidis in ovary and egg of laying hens. The result showed that probiotic treatment could increase feed consumption, egg production, however, in the other hand decreased the feed conversion. Among other, probiotic treatment was effective to reduce the population of Salmonella enteritidis in ovary and egg of laying hens. The optimum dosage of L.acidophilus probiotic was within population of 109 cfu/ml and frequency of twice a day was the most effective as biocontrol.

Key words: L. acidophilus, laying hen, probiotic, Salmonella enteritidis

Introduction

Food security is a major issue in national development program. Food is a basic requirement for the fulfillment of - human rights for everyone. Food safety issues are major concern in public health policy. Foodborne disease and food contamination incidents occurred in various countries, not only in developing countries where sanitation and hygiene conditions are generally poor, but also in developed countries. One of the emerging pathogen is *Salmonella enteritidis* transovarian contamination of grade A eggs. These eggs are contaminated since the start of its formation in the body because its parent is *infected* by *S.enteritidis* in ovaries (Gantois *et al.*, 2009).

Biosecurity via biocontrol is needed to apply in layer chicken farms as preventive effort.

Lactobacillus acidophilus 2B4 has been proven as probiotic (Arief, 2011). This strain could inhibit the growth of *S. enteritidis* isolated from the ovary of laying hen by in vitro analysis (Ulupi *et al*., 2009). Application of probiotic *L. acidophilus* 2B4 as a biocontrol agent for the prevention of contamination of eggs by *S. enteritidis* transovarian is very necessary, especially in laying hens. The aim of this research was to find out the dosage and frequency of probiotic *L. acidophilus* 2B4 treatment to prevent contamination of *S. enteritidis* in ovarium and egg of layer hen

Materials and Methods

Sixty layer hens were divided into 4 Treatments. Treatment 1 (R1) was laying hens without oral administration of *L. acidophilus* 2B4 and *S. enteritidis* (as control). R2 was laying hens without *L. acidophilus* 2B4, but were infected by *S. enteritidis*. R3 was laying hens administered of *L. acidophilus* 2B4 once per day (dosage 1 $x10^9$ cfu/ml) and were infected by *S. enteritidis*. R4 was laying hens with oral administration twice per day (dosage 2 $x10^9$ cfu/ml) and were infected by *S. enteritidis*. *L. acidophilus* 2B4 was given via drinking water for 20 days, and *S. enteritidis* was given by oral administration for 5 days (day 5-day 9). Each treatment consisted of 15 laying hens. , A total of 3 hens of each treatment were slaughtered for parameters evaluation. in day 5, 10, 15 and 20.

Parameters observed were performances of layer hens and Salmonella contamination on ovarium and eggs. Analysis of Salmonella contamination was done by qualitative analysis according to BAM (2007). The samples were incubated on selective media on Lysine Desoxycholate xylose media (XLD) Agar, Hectoen Eteric Agar (HEA), and Bismuth sulfite agar (BSA). These three selective media were incubated at 35 ± 2 °C for 24 ± 2 hours. After incubation typically appearance was observed whether there was a growing colony. The analysis followed the biochemical tests using triple sugar early Iron (TSI) and Lysine Iron Agar (LIA) in italics. Typical colonies that grew on the three specific medias XLD Agar, HE agar and BS. Each loop was inoculated using a sterile needle on TSI agar and LIA agar.

The experimental was completely random designed with 3 replications. Data were analyzed using ANOVA and then further subjected to Tukey test (Steel and Torrie, 1995) if there were any differences.

Results and Discussion

Performance of layer hens

Performance of layer hens were affected by probiotic administration as described in Table 1.

Table 1. Performance of layer hens

Performance	R1	R2	R3	R4
Feed consumption (g/hen/day)	111.81[a]	112.80[a]	118.29[b]	118.54[b]
Egg production (%)	67.64[a]	70.31[a]	75.91[b]	81.97[c]
Egg weight (g/egg)	59.43	58.89	59.57	59.58
Feed conversion	3.34[a]	3.48[a]	3.36[a]	2.72[b]
Mortality	0	0	0	0
Haugh Unit (HU)	96.40[(AA)]	94.00[(AA)]	94.59[(AA)]	95.30[(AA)]

Different superscript in the same line means significantly different (P<0.05), except HU

Probiotic treatment affected the amount of feed intake. R4 treatment indicated more efficient than other treatments. There was a difference between R2 and R4 treatments. Egg production of R4 was highest than the others. This was presumably because use of feed consumed by chickens R2 to improve declined condition due to the administration of *Salmonella enteritidis*. While at R2 and R4 the improved function of the body condition has been taken over by the probiotic *L. acidophilus* 2B4. R4 was better than R3. It meant that probiotic given twice per day was more effective to enhance performance than R2.

Based on the weight of eggs, there were not significant differences in all treatments. This finding was consistent with the Indonesian National Standard (1995) which states the criteria and the weight of eggs for egg consumption are extra large (more than 60 grams), large (55-60 g), moderate (51-59 grams), small (45-50 grams), and extra small (less than 46 grams). Viscosity of the albumin can be characterized by higher albumin (Sirait, 1986). The high albumin is used to determine the value of Haugh Units of eggs. The higher Haugh Unit value indicates the higher quality of the eggs (Sudaryani, 2000). Haugh Unit values of this treatment were more than 90 for each treatment, therefore, Haugh Unit value of each treatment was categorized into AA (USDA, 1964). *Salmonella* contamination on ovarium and egg of layer hens was described in Table 2.

At day-5, addition of probiotic *L. acidophilus* 2B4 could decrease *Salmonella* in layer hens (R3 and R4). After infection of *S. enteritidis* by oral administration at day 5-9, Salmonella could also be decreased by addition of *L. acidophilus* 2B4 twice per day. R4 was better than R2 and R3. *S. enteritidis* is invasion bacterium that invades from gastrointestinal tract to blood and reach ovarium. After Salmonella invasion, addition of probiotic *L. acidophilus* 2B4 twice per day (R4) could reduce Salmonella on ovarium at day-20. It was better than addition of probiotic only once per day (R3). Administration of probiotics enhances intestinal antibodies to alien antigens, and reduce colonization of pathogens in gastrointestinal tract (Haghighi *et al*. 2006).

Table 2. *Salmonella* infection on ovarium and egg

Treatments	*Salmonella* infection on ovarium (%)			
	day-5	day-10	day-15	day-20
R1	66.67	66.67	66.67	66.67
R2	66.67	66.67	100	100
R3	33.33	33.3	100	100
R4	0	0	100	33.3
	Salmonella infection on eggs (%)			
R1	33.33	33.33	66.67	66.67
R2	33.33	33.33	100	66.67
R3	33.33	33.33	66.67	0
R4	0	33.33	33.33	0

Administration of probiotic *L.acidophilus* 2B4 could reduce *Salmonella* contamination on egg after infection *S. enteritidis* on day-10. Adddition of *L. acidophilus* 2B4 twice per day (R4) was better than once per day (R3). There was correlation between *Salmonella* contamination on ovarium and egg. That proved that *Salmonella* invasion could be spred by vertical contamination from ovarium to egg.

Conclusions

Administration of probiotic *L. acidophilus* 2B4 could increase feed consumption and egg production of layer hens which was contaminated by *S. enteritidis*. Administration of *L. acidophilus* 2B4 twice per day was more effective than once per day for 20 days treatments to reduce feed conversion. *L. acidophilus* 2B4 also could reduce *Salmonella* contamination on egg and ovarium. It proved that *L. acidophilus* 2B4 was effective used as biocontrol to prevent *Salmonella enteritidis* infection on laying hens

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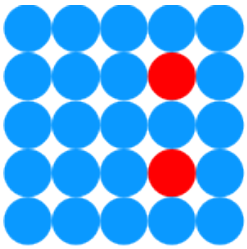

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