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PREFACE

It is a great pleasure for us that the Proceeding of the 1st International Seminar on Animal Industry has been successfully completed. The proceeding consists of 68 papers, among them 8 papers from invited speakers, 30 papers from oral presentation, and 23 papers from poster presentation. Papers from the invited speakers were not further reviewed and some of them were not in full papers. The papers from participants included in this proceeding were reviewed by experts in the related field. If the reviewed papers required substantial correction, they were sent back to the authors for correction. However, due to time limitation, if the reviewed papers need only non-substantial correction, the reviewing process were considered sufficient after few corrections were done by the reviewers.

In this opportunity, the Editors would like to thanks all paper contributors (invited speakers, oral presenters, and poster presenters) for their collaboration and support, so that this proceeding can be finally completed. Thanks is also delivered to all sponsors which provide financial support. We are also in debt to all reviewers and organizing committee of ISAI for their hard work and time outpouring from the preparation until the finalization of the proceeding.

Hopefully, the experiment results presented in this proceeding will be useful as a guidance to improve animal production and animal industry especially in Indonesia, and to direct science and technology development of animal science in the very near future.

Bogor, March 2010
On behalf of Editors,

Prof. Dr. Ir. I Komang G. Wiryawan
Chief editor

FOREWORD FROM CHAIRMAN OF ORGANIZING COMMITTEE

Assalamu'alaikum warahmatullah hiwabarakatuh and selamat pagi

Selamat datang di Indonesia, selamat datang di Bogor, dan selamat datang di IPB International Convention Centre to attend The First International Seminar on Animal Industry 2010 which is held officially by Faculty of Animal Science, Bogor Agricultural University.

Today and the day after tomorrow we will gather and discuss comprehensively about the development and current progress of animal industries in several countries; present current research results related to the improvement of efficiency and productivity; improve stakeholder's perspective on potency, prospects, and limitation in developing animal industry in Indonesia. Several topics including recent feed technology, development in animal reproduction for sustainable use of animal genetic resources, future animal production system for anticipating global warming will be presented in this seminar.

In this seminar, outstanding scientist from Germany, United State of America, Japan, Malaysia, and Indonesia will share their expertise; and 88 research papers will be discussed in the parallel session as well as presented in the poster session. In total, approximately 150 participants are registered.

For our foreign scientist visiting Indonesia for the first time and our domestic guests visiting Bogor for the first time, we encourage you to go around Bogor and surrounding areas with attractive places such as botanical garden, presidential palace, puncak, Indonesian Safari Garden, Beautiful Indonesian Miniature Garden, and many others.

This seminar would never happen without any total support from key persons in Bogor Agricultural University and our team in the Faculty of Animal Sciences. For their important roles, we would like to sincerely thank Rector of IPB Prof. Dr. Herry Suhardiyanto, Dean of Faculty of Animal Science Dr. Ir. Luki Abdullah, Senate Members of Faculty of Animal Science, all steering committee members as well as organizing committee members.

We would like also to thank all institutions and private companies contributing significantly to the success of this international seminar, namely: IPB, Departemen Pertanian, Tabloid Agrina, PT Telkom Indonesia Tbk., PT Kaltim Prima Coal Tbk., PT Aneka Tambang Tbk., PT Indocement Tunggal Prakarsa Tbk., PT Napindo Media Ashatama, PT Bank Mandiri Tbk., and PT. Galur Prima Cobbindo. Finally, on behalf of organizing committee, I apologize all of you if you find uncomfortable during your involvement in this seminar. Thank you very much and have a very furitful discussion in this two-day seminar.

Wabillahi taufiq wal hidayah

Wassalamualaikum warahmatullahi wa barakatuh

Bogor, November 23rd, 2009

Chairman,

Prof. Dr. Ir. Muladno, MSA.

REMARKS FROM
THE DEAN OF FACULTY OF ANIMAL SCIENCE

Assalamu'alaikum Warahmatullahi Wabarakatuh

First of all, let us pray to Allah SWT the Almighty for His blessings bestowed to all of us.

As the Dean of Faculty of Animal Science, it is surely a great pleasure for me to welcome all of you on the 1st International Seminar on Animal Industry 2009, entitled "Sustainable Animal Production for Food Security and Safety". This seminar is organized by the Faculty of Animal Science, Bogor Agricultural University.

As one of the faculties of animal science in Indonesia, it is our responsibility to take a real action for developing animal production for food security and safety. In this seminar, we expect to have interesting discussion on current animal research especially on efficiencies and productivities of livestock; perspectives of stakeholders on potencies, prospect and constraints on animal industry; animal biotechnology, animal business in global era; and other relevant topics. We hope that this seminar supplies a scientific recommendation to government and non government institutions on policy development of food security and safety, improvement of international linkage for solving the problems in animal production, strengthen the international collaborative research and the exchange of information.

In this special occasion I would like to express appreciation to Ir. Suswono, MMA., the Minister of Agriculture for support and encouragement. We also extend our gratitude to the Directorate General of Livestock Services, who has given the support to this seminar. I would like to express my appreciation to the invited speakers and other speakers both oral and poster presentation, who are willing to share their experience and vision with us. To the contributors, sponsors and exhibitors I would like to express our great thank to every effort which have been done to make this event successful. Last but not least, please accept my gratitude for the members of steering committee and organizing committee, without their effort and hard work, this meeting will never be carried out. Please, enjoy the seminar and hopefully you will get the benefits of this scientific and professional gathering. Thank you very much.

Wabillahi taufiq wal hidayah,

Wassalamualaikum warahmatullahi wa barakatuh

Bogor, November 23rd, 2009

Dean,

Dr. Ir. Luki Abdullah, M.Sc.Agr.

OREWORD FROM
RECTOR OF BOGOR AGRICULTURAL UNIVERSITY

After a publication of the World Bank's report few years ago, entitled "Agriculture for Development", agricultural development has regained much attention by many governments. Since then, for developing countries with suitable agro-climatic condition like Indonesia, the attention seems to be more than before. Agricultural development has then been viewed as a good way of reducing poverty—a problem widely shared not only by developing countries but also developed ones and, as stated in the Millennium Development Goals, to be alleviated by all these countries. More attention to agricultural development has been also due to occasional extreme weather events resulted by the global warming which threat food security.

Facing the MDGs commitment of reducing poverty and anticipating threat to food security, many governments including of Indonesia have put more efforts to develop sub-sectors of the agricultural sector. The current Government of Indonesia, which will serve the nation in the period 2009-2014, has prioritized food security and agricultural development to cope with uncertainties resulted from the climate change/global warming and to reduce poverty. The government, which has so far been focused mainly on food crop and estate plantation sub-sectors, by now places more serious efforts to develop livestock sub-sector. This is an important opportunity to all stakeholders of the sub-sector.

The livestock sub-sector and animal industry are seen by many Indonesian economists, including myself, as to have great potential to the economy of Indonesia. The livestock sub-sector and the animal industry have the nature of labor intensive. They provide employment for unskilled up to skilled workers in a great number. Livestock sub-sector requires much less land if compared with the other sub-sectors of agriculture, and hence could be run by many small holding farmers. If this has been conducted successfully, not only food security will improve, farmers' income will also increase, reducing the incidence of poverty. The animal industry can be developed in many areas adjacent to the farmers and the market, and hence could improve rural-urban linkages in addition to the high value added obtained. As this takes place, the current problem of rural unemployment would reduce and rural-urban income disparity would be corrected.

In order to manifest the aforementioned potential, we need a lot of knowledge—empirical as well as theoretical one. The knowledge is transformed to become technology and know how. And as the sub-sector as well as the animal husbandry dynamically progress, the knowledge must be generated continuously. Improvement in technology and know how must be disseminated to the farmers and industrial actors time to time. The knowledge has been, and will be, generated by researchers or academicians. Experiences and lessons learned have been, and will be, accumulated by the industrial and government people. Sharing the knowledge and presenting experience or lessons learned are the main objectives of the International Seminar on Animal Industry 2009. And this book of proceeding summarizes results of continuous works for generating knowledge and experience relevant to livestock sub-sector and animal husbandry by the researchers from universities and government research institutes as well as research institutes of the related industry, from various countries including Indonesia. There are many useful materials contained in the articles of the book. They would be useful for upgrading materials for teaching as well as for igniting improvement of performance of the animal industry.

Bogor Agricultural University—which is "*Institut Pertanian Bogor*" in *Bahasa Indonesia*—has been very happy to host the International Seminar on Animal Industry 2009. We hope that the event will continue and be carried out regularly, and that more knowledge and lessons learned become available for supporting development of the livestock sub-sector and animal industry in the future. For the Organizing and Steering Committees of the International Seminar on Animal Industry 2009 as well as the Dean of Faculty of Animal Husbandry of Bogor Agricultural University, we would like to express our thanks for the successful seminar and the hard work to prepare this book of proceeding. Thanks are also extended to the writers whose articles are presented in this book.

Bogor, November 23rd, 2009

Bogor Agricultural University,

Vice Rector for Resources and Development

Professor Hermanto Siregar

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Monday, November 23rd, 2009

Time	Ballroom 3	
	Event	**Speaker**
08.00-09.00	Registration	
09.00-10.00	Opening Ceremony • Organizing Committee Report • Welcome Address from Rector of the Bogor Agricultural University • Opening and Keynote Speech by Deputy Minister of Agriculture	Prof. Dr. Ir. Muladno, MSA. Prof. Dr. Ir. Hery Suhardiyanto, M.Sc. Dr. Ir. Bayu Krisnamurthi, MS
10.00-10.15	Coffee Break and Poster Session	
10.15-10.45	Plenary 1: Breeding for Sustainable Future (AnGR)	Orlando Fernandez, DVM, FPCCP
10.45-11.15	Plenary 2: Nutritional Strategies to Enhance Efficiency and Production of Chickens Under High Environmental Temperature	Ahmad Mujahid, PhD, Ichiro Hagimori, Kazuaki Takahashi and Atsuro Matsuda
11.15-11.45	Plenary 3: Anticipating the Outbreak of Zoonotic Infectious Diseases Related to Animal Industry	Dr. Drh. Retno Dewi Bagja
11.45-12.15	Plenary 4: New Development of Animal Production in Indonesia	Prof. Dr. Ir. Toto Toharmat, M.Agr.Sc.
12.15-13.30	Lunch	
13.30-14.00	Poster Session	

Time	Room C Genetics, Breeding and Reproduction Moderator: Prof. Dr. Ir. Cece Sumantri, M.Agr.Sc.	Room D Feed and Nutrition Moderator: Dr. Ir. Sumiati, M.Sc.
14.10-14.25	**Anneke Anggraeni:** Genetic Polymorphism of the Kappa-Casein Gene in Holstein-Friesian Dairy Cattle in West Java Province	**Retno Murwani:** Effect of Mungbean as Local Feed Ingredients to Substitute Soybean Meal in the Diet on the Performance of Broilers
14.25-14.40	**Yuni Erwanto:** Identification of Pig Using Polymerase Chain Reaction-Retriction Fragment Length Polymorphism for *Halal* Authentication	**Riyadh Abbas Al-kirshi:** The Chemical Composition and Nutritive Value of Mulberry Leaf Meal as a Protein Source in Poultry Diets
14.40-14.55	**Almira Primasari:** Identification of Growth Hormone Releasing Hormone Gene in Local Buffalo (*Bubalus bubalis*) using PCR-RFLP	**Lovita Adriani and Andi Mushawwir:** The Effect of Ration with Antibiotics (*Virginiamycin*) and Temulawak (*Curcuma xanthorriza Roxb*.) on Performances and Income over Feed Cost of Broiler
14.55-15.10	**Jakaria:** Identification of Growth Hormone (GH) Gene MspI and AluI Locus Polymorphism in Indonesian Cattle Breeds	**Atapattu, NSBM:** Effect of Dietary Chili Powder on Growth Performance and Serum Cholesterol Contents of Broiler Chicken
15.10-16.00	**Coffee Break, Poster Session and Exhibition**	
	Genetics, Breeding and Reproduction **Moderator: Prof. Dr. Ir. Sri Supraptini Mansjoer, M.S.**	**Feed and Nutrition** **Moderator: Prof. Dr. Ir. Wiranda G. Piliang, M.Sc.**
16.00-16.15	**Tatik Suteky and Dwatmadji:** Effects of Work on Reproductive Performance of Bali Cattle under the Oil Palm Plantation in Bengkulu	**Dewi Apri Astuti:** Physiological Status, Blood Profile and Body Composition of Sheep Fed with Ca-Saponified Lemuru Oil Coated by Herbs
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	of Grading-up Between Local Goat and Boer Goat	nation of Microbial Growth in the Rumen Simulation Technique (Rusitec)
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Tuesday, November 24, 2009

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08.30-09.00	Plenary 6: BROILER Chicken Welfare: WHAT DO THEY WANT AND WHAT DO WE WANT?	Prof. Dr. Zulkifli Idrus
09.00-09.30	Plenary 7: The global market of organic animal products – chances and risks	Prof. Dr. Gerold Rahmann
09.30-10.00	Coffee Break and Poster Session	
10.00-10.30	Plenary 8: Future of Domestic Ducks in Rice Field	Dr. Lertrak Srikitjakarn
10.30-11.00	Plenary 9: Development of Indonesian policy in contributing sustainable production	Dr. Tjeppy D. Soedjana, M.Sc.
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	Animal Production Moderator: Prof. Dr. Ir. Pollung H. Siagian, M.S.	**Feed and Nutrition Moderator: Dr. Ir. Asnath M. Fuah, M.S.**
14.30-14.45	**Mohd Amizi Bin Ayob and Mohd Azid Bin Hj Kabul:** Cattle Integration in Oil Palm Plantation through Systematic Management	**Iyep Komala:** The effect of Garlic (*Allium sativum*) Extract on the Growth of Bacteria Isolated from Uterus Dairy Cattle
14.45-15.00	**Mohamad Yamin:** Increasing Local Sheep Growth Performance through Rapid Selection at Fattening Farm	**Taufik Budi Pramono and Dyahruri Sanjayasari:** Effect of Protein Level and Energy Protein Ratio on the Growth Broodstock Performance of Senggaringan Fish (*Mystus nigriceps*)
15.00-15.15	**Agnes Wahyuni:** Detection of Enterobacter sp from Dairy Cow's Milk in Boyolali and Sleman	**Anita S. Tjakradidjaja:** Effectivity of *Jatropha curcas* Seed Meal Fermented with Various Moulds as Protein Source for Male Mice (*Mus musculus*)
15.15-15.30	**Prima Puji Raharjo:** Lambing Type and Ewe Age on Milk Yield of Local Sheep at UP3 Jonggol (Jonggol Animal Science Teaching and Research Unit)	**Syahriani Syahrir:** The effect of Mulberry Leave Extract Fermentation in Feed to Performance of Mice
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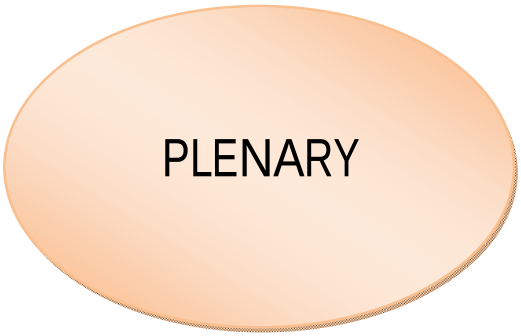
Thursday, November 25, 2009

Time	Event
07.00-15.00	**Excursion:** • PT Indolakto • Peternakan Domba "Tawakal Farm" **Note:** Those who want to participate in excursion, an additional fee will be charged


PLENARY

Breeding for Sustainable Future

O. Hernandez
Cobb Vantress Inc.

Man has been selecting chickens ever since their domestication. More intensified and concentrated efforts by industrial breeding companies for the past several decades supported by research in academia as pointed out by Siegel *et al*. (2006) have contributed to dramatic improvements in methods and progress due to selective breeding. Improvements in growth, yield, and feed efficiency of broilers within the last 40 to 50 years are well documented (Havenstein *et al*., 2003). Such dramatic improvements would not have been deliverable unless breeders had a keen interest in health and well-being of their products.

Modern broiler survival and good health are keys to efficient production. Primary breeders are well aware that selecting for better health and well-being along with economic traits such as faster growth rate, higher levels of meat yield, and improved efficiency of feed utilization are critical to balanced long-term genetic progress of their pure lines as well as to increased production efficiency of broiler products for the broiler industry.

Cobb collects and selects on over 50 phenotypic observations per pedigree candidate at various ages. Over 50% of these collections are involved with evaluation of each bird's health, welfare, and fitness. Some examples of these traits are various chick defects, various broiler age skeletal and leg abnormalities, feather cover, various physiological measures of heart and lung functions, and specific causes of mortality. Large pedigree populations, massive data collection infrastructure, integration of better technologies in evaluation of phenotypes, and sophisticated data analysis capability have allowed geneticists to perform selections that are balanced for both economic and welfare traits.

Cobb's internal as well as world-wide sponsored research has facilitated geneticists to make science-based breeding decisions. Each pedigree line per product available to primary breeders exhibits their own unique characteristics that are enhanced by selective breeding and positioned in special mating schemes to produce the product and welfare performance that our customers demand. Additionally, most if not all primary breeding companies now offer different products for different markets that exhibit varying levels of performance and behavior to fit customer needs. Future expansion of these products and creation of new products by breeding companies will be in large dictated by both our customers and consumers. An elaborate library of gene pools has been assembled by primary broiler breeders to address development needs of today and future products demanded by consumers around the world. Genomics would facilitate genetic markers as an additional tool for geneticists to better select their stocks in the near future.

Nutritional Strategies to Enhance Efficiency and Production of Chickens Under High Environmental Temperature

A. Mujahid[1], I. Hagimori[1], K. Takahashi[2] and A. Matsuda[3]

[1]NAS Laboratory, Narita; [2]Graduate School of Agricultural Science, Tohoku University, Sendai; [3]Sumitomo chemical, Co., Ltd. Tokyo, Japan

ABSTRACT

Climate model projections indicate that the global surface temperature will probably rise a further 1.1 to 6.4°C during the twenty-first century; therefore, with this rise in global average temperature significant impact on efficiency, production, morbidity and mortality can be expected on birds and animals. Presently, high environmental temperature exposure is of major concern for poultry industry especially in the hot region of the world because of the resulting poor growth performance, immuno-suppression and high mortality. Different methods are available for decreasing the heat production using various nutritional strategies to alleviate stress in high temperature-exposed chickens. The nutritional strategies are designed after considering the factors such as type of birds, age of birds, stage of production, duration of heat exposure, intensity of heat exposure and health of the birds. The nutritionists can base their strategy on less heat-production, increased nutrient intake, decreased energy wastage, and reduction in heat-induced oxidative stress and damage in birds to overcome the deleterious effects of high temperatures on metabolism, physiology, feed efficiency, production performance and health. This can be accomplished by traditional nutritional strategies to reduce heat stress by feeding good quality feed with high digestibility and nutrient density, adding fat as an energy source, balancing and provision of additional amino acids, and supplementing with vitamins, minerals and glucose. Recently, there are new concepts for nutritional strategies to focus on redox status of the chickens and to decrease the oxidative stress and damage on exposure to high environmental temperature. None of these strategies are effective alone in terms of growth, feed efficiency, livability, meat quality, stress tolerance or immune response, therefore, a combination of the nutritional strategies may help to alleviate the deleterious effects of heat stress and improve the chicken performance under high environmental temperature.

Key words: heat stress, chicken, nutrition, oxidative stress, oxidative damage, uncoupling protein

REVIEW

During the last century global surface temperature increased 0.74±0.18°C, and climate model projections summarized in the IPCC report indicate that the global surface temperature will probably rise a further 1.1 to 6.4°C during the twenty-first century (IPCC, 2007). There is growing evidence that climate-health relationships pose increasing health risks for humans under future projections of climate change and the warming trend over recent decades has already contributed to increased morbidity and mortality in many regions of the world (See review by Patz *et al*., 2005). Same is true for birds and animals, where significant impact on efficiency, production, morbidity and mortality can be expected with rise in global average temperature.

Presently, high temperature exposure is of major concern for poultry industry especially in the hot region of the world because of the resulting poor growth performance, immunosuppression and high mortality (Bottje and Harrison, 1985; Young, 1990; Mujahid *et al*., 2005, 2009b). The continuous selection for fast growth has been associated with increased susceptibility of broilers to high temperature (Geraert *et al*., 1993; Cahaner *et al*., 1995; Berong and Washburn, 1998). Exposure of chickens to high temperature cause significant changes in physiological responses (Harrison and Biellier, 1969; Altan *et al*., 2003, Toyomizu *et al*., 2005, Mujahid *et al*., 2009b). Thermal stress exerts its deleterious effects on feed intake and body weight gain (Geraert *et al*., 1996) as well as on carcass yield, carcass protein, muscle ca-

lorie content and mortality rates (Smith, 1993, Tankson *et al*., 2001).

As ambient temperatures increase within the thermoneutral range, birds initially utilize sensible heat loss mechanisms to control body temperature with little or no loss in growth or production. However, under moderate or severe heat stress birds minimize heat production since the major route of heat loss, evaporation of water from the respiratory tract (panting), requires considerable energy expenditure. Birds respond by reducing their metabolizable energy (ME) and feed intake to reduce thermogenesis. Although ME intake has been shown to decline at an increasing rate with increasing ambient temperature it does so more rapidly than the corresponding decline in metabolic heat production. Therefore, less energy would be available for production processes, as ambient temperature increased.

Many factors influence the response of chickens to change in environmental temperature. Intensity of environmental temperature, humidity, radiant heat, wind velocity, duration of exposure and previous acclimatization of the birds influence the response of chickens to high temperature exposure. Presently, birds are increasingly being subjected to environmental temperatures that are above their comfort zone. Additionally, birds are growing faster and producing more than ever before, and are thus heavier and more productive than previously at any given age with marked change in their metabolic activities. Birds in general, perform well within a relatively wide temperature range, 10-27°C (Milligan and Winn, 1964; de Albuquerque *et al*., 1978; Mardsen and Morris, 1987). Highest growth rate of broiler chickens occur in the range of 10-22°C, while maximum feed efficiency is at 27°C (Kampen, 1984). The ideal optimum temperature range is different for growth and feed efficiency, e.g., feed efficiency in laying hens reduced below 21°C, while egg production and growth rate are reduced at temperature below 10°C. Exposing chickens to high temperature significantly decrease the feed intake, although high environmental temperature have significant and direct impact on performance and feed efficiency that are unrelated to feed intake, e.g., exposure of laying hens to 21 and 38°C temperature, 40-50% reduction in egg production and egg weight at 38°C is only due to reduced feed intake, while the reductions in shell thickness and shell strength are mainly due to high temperature (Smith and Oliver, 1972). Similarly, only 63% reduction in broiler chicken growth on exposure to heat stress is due to reduced feed intake (Dale and Fuller, 1979). In addition to the effect of high temperature on feed intake, heat stress-exposure results in increased mitochondrial superoxide production, reduced ATPase activity, and oxidative damage to body proteins and fats, resulting in cellular metabolic changes and growth reduction (Mujahid *et al*., 2005, 2007a-b; Feng *et al*., 2008).

The metabolic and nutritional status affect the tolerance of chickens exposed to high ambient temperature and interrelationship exists between nutritional status and resistance to acute heat stress (McCormick *et al*., 1979; Garlich and McCormick, 1981). Recently, the nutritional strategies are of increasing interest to decrease the heat production and thus alleviate the heat stress in chickens on exposure to high environmental temperature. The nutritional strategies are designed after considering the factors such as type of birds, stage of production, age of birds, duration of heat exposure, intensity of heat exposure and health of the birds. The nutritionists can base their strategy on less heat-production, increased nutrient intake, decreased energy wastage, and reduction in heat-induced oxidative stress and damage in birds to overcome the deleterious effects of high temperatures on metabolism, physiology, feed efficiency and production performance.

Traditional Nutritional Strategies to Reduce Heat Stress

It has been proposed that the adverse effects of high temperature on production performance may be alleviated by following dietary modifications:

Feed Density

Although, ME requirement decreases with increasing temperature above 21°C, mainly due to a reduction in energy requirement for maintenance, the requirement for production is not influenced by environmental temperature (Daghir, 1995). Using high energy rations for chickens have been common in warm regions probably to overcome the negative effects of decreased feed intake

and to reduce the heat increment. Under severe heat stress, ME requirements increase due to the need for the bird to dissipate body heat by respiratory heat loss. Adding fat, lysine and methionine has been shown to improve the performance in hot weather (Micklebury *et al*., 1966; McNaughton and Reece, 1984; Jiang *et al*., 2007). The higher fat content of the diet contributes to reduced heat production, since fat has a lower heat increment than either proteins or carbohydrates. High environmental temperature increases food passage time (Wilson *et al*., 1980) while fat has shown to decrease the rate of food passage in GIT (Mateos *et al*., 1982), thus increasing the nutrient utilization. Therefore, the addition of fat to the diet also appears to increase the energy value of the other feed constituents (Mateos and Sell, 1981). Alterations in dietary ME concentration had a limited influence on food and nutrient intake and egg mass output of hens in early lay kept at 10-24, 6-16 or 25-35°C temperatures. Even the highest intakes of ME and protein achieved at hot temperatures failed to increase egg mass output to the values attained on any diet at low temperatures (Scott and Balnave, 1988). Increases in energy and calcium intake helped partially to maintain normal egg production, egg weight, and prevent egg shell deformation on exposure of laying hens to high temperature (Tanor *et al*., 1984). However, when chickens are reared in a warm environment the body weight response to increased dietary energy level will occur only when adequate amino acid levels are supplied (McNaughton and Reece, 1984). Increasing dietary ME at particular amino acid:ME ratios significantly improve growth and food utilization of broilers kept at 18-26 and 25-35°C ambient temperatures during the finishing period. The optimum amino acid:ME ratio varies with dietary ME concentration in the hot, but not in the moderate environment. Relatively greater increases in food intake and growth rate occur in the hot environment when dietary ME increases and the amino acid:ME ratio decreases. Increasing the dietary protein at particular ME concentrations had little or no effect on the food intake and growth rate of birds kept at high temperatures. The rectal temperatures of birds in the hot environment increase with age and, towards the end of the finishing period, when higher energy diets are fed (Sinurat and Balnave, 1985).

Amino Acid

A well-balanced amino acid supply should minimize the energy cost of excreting surplus nitrogen and might therefore help the chicken to cope with heat stress. At high ambient temperatures, there is a decrease in protein synthesis (Geraert *et al*., 1996), probably due to reduced plasma amino acid concentration and to lower energy supply (Temim *et al*., 2000), as observed in broiler chicken muscle tissue. In addition, heat stress decreases plasma T3 concentration and increases plasma corticosterone, both changes known to reduce protein deposition through alterations in protein turnover in birds and other species (Yunianto *et al*., 1997). Under conditions of heat stress, diets in which excess protein had been minimized performed significantly better than conventional diets. An interaction was therefore shown to exist between amino acid balance and environmental temperature. Exposure to high environmental temperature has been shown to influence amino acid digestibility and significantly decrease the uptake of certain amino acids from intestine (Wallis and Balnave, 1984; Balnave and Olivia, 1991; Brake *et al*., 1998). Additionally, high ambient temperatures affect the ideal amino acid balance for broilers (Brake *et al*., 1998; Chamruspollert *et al*., 2004) and increasing the dietary Arg:Lys ratio improves broiler performance at high temperatures (Brake *et al*., 1998). An interaction also exists between dietary NaCl and Arg:Lys ratio and dietary NaCl could affect the apparent ileal digestibility of Arg and Lys at certain Arg:Lys ratios (Brake *et al*., 1998; Balnave and Brake, 2001; Chen *et al*., 2005). In particular, Brake *et al*. (1998) observed that at a low dietary NaCl concentration (1.2 g/kg), the feed conversion ratio and body weight gain of heat-stressed broilers were significantly improved with increasing Arg:Lys ratios, but at a higher dietary NaCl concentration (2.4 g/kg), no such response occurred. Recently, it has been shown that increasing the amino acid levels in the diet of chickens reared under high temperature conditions improve their performance as compared to the birds fed with recommended levels at thermoneutral temperature (Corzo *et al*., 2003; Jiang *et al*., 2007).

Vitamins

Ascorbic acid (Vitamin C) has been shown to improve chicken performance at high temperature and birds experience a less severe stress response after exposure to high temperatures when they are provided dietary ascorbic acid (Mahmoud *et al*., 2004). Optimum responses in growth, feed efficiency and/or liveability in broilers under heat stress were reported to occur with supplements of about 250 mg ascorbic acid / kg (Kutlu and Forbes, 1993). Vitamin A and E in combination (15,000 IU retinol and 250 mg dl-□-tocopheryl-acetate/kg diet), reduced malondialdehyde concentration (an indicator of lipid peroxidation) more than half in serum and liver of heat stress-exposed broilers, but showed less effect when fed alone (Sahin *et al*., 2002). Ascorbic acid and chromium have similar effects when fed at high temperatures, and a combination of ascorbic acid (250 mg/kg diet) and chromium (400 µg Cr/kg of diet) may offer a potential protective management practice in preventing heat-stress related depression in performance of broiler chickens (Sahin *et al*., 2003). In laying hens, studies have shown that the livability and production of heat-stressed laying hens can be improved by supplementing their diet with ascorbic acid or vitamin E (Njoku and Nwazota, 1989; Cheng *et al*., 1990; Bollengier-Lee *et al*., 1999; Puthpongsiriporn *et al*., 2001). Requirements for thiamine has been shown to be significantly increased for chicks grown at 32.5°C, as compared with chicks raised at 21°C (Mills *et al*., 1947).

Minerals

Dietary modifications in mineral concentration offer a practical way to alleviate the effect of high environmental temperature on chicken performance. Mineral supplementation may reduce the consequences of heat stress in birds and have beneficial effects on production performance and meat quality.

Increased mineral excretion is one of the major consequences of heat stress. Retention rates of phosphorus, potassium, sodium, magnesium, sulfur, manganese, copper, and zinc are lowered in broilers raised at high cycling ambient temperatures (24-35°C) compared with those housed at 24°C (Belay and Teeter, 1996). Egg weight and eggshell strength decline at high environmental temperature. Also, the lower concentrations of plasma calcium and inorganic phosphate in hens exposed to 30°C compared to 18°C (Usayran and Balnave, 1995) may provide some evidence of an increased requirement for these minerals in heat-stressed laying hens. Stress causes secretion of epinephrine and corticosteroids and results in Mg loss (Seelig, 1980, 1981). Mg-aspartate supplementation increases the body weight of chickens during heat stress (Donoghue *et al*., 1990). Zinc supplementation results in an improved live weight gain, feed efficiency, and carcass traits, as well as a decrease in serum MDA concentrations in chickens reared at high temperature (Kucuk *et al*., 2003).

When broiler chickens are exposed to heat stress (34°C), plasma sodium values reduces after 6 h while no such change occurs after 12 or 18 h of exposure. Potassium levels are lower by 6 or 12 h of heat stress and no differences in blood calcium levels are observed between the control and heat-stressed chickens (Mujahid *et al*., 2009b). Exposure to different durations of heat stress results in significant decreases in the levels of blood HCO_3- and pCO_2 and significant concomitant increase in blood pH which is however, dependent on duration of heat stress exposure (Toyomizu *et al*., 2005, Mujahid *et al*., 2009b).

Panting during heat stress to dissipate body heat may result in an increased loss of carbon dioxide and a consequent depletion of blood bicarbonate (Gorman and Balnave, 1994) which can induce respiratory alkalosis and this may be exacerbated by high RH that makes respiratory heat loss less efficient. Blood alkalosis limits growth rate of chickens reared under high environmental temperature and the induced respiratory alkalosis can be partially alleviated by dietary modifications (Teeter *et al*., 1985). Therefore, it is theoretically possible that heat stress may induce a metabolic requirement for the bicarbonate ion (Teeter *et al*., 1985). One of the means for alleviating the problem of respiratory alkalosis associated with panting has been to supplement the diet with sodium bicarbonate. Metabolizable anions, such as bicarbonate, carbonate and acetate, are all capable of neutralizing acid and raising blood pH. This procedure has the additional merit of improving the shell quality of eggs from heat-stressed laying hens (Balnave and Mu-

heereza, 1997). However, it has been reported that the dietary bicarbonate should be consumed during the period of egg shell formation (Balnave and Muheereza, 1997, 1998). Enhanced body weight gains can be achieved among broilers kept at high temperatures with the addition of either sodium bicarbonate or ammonium chloride, the latter (at 10 g/kg of diet) resulting in a 25 percent increase in growth rate over the controls (Teeter *et al*., 1985). Sodium salts reduces the alkalotic pH and enhanced the blood sodium content, which ultimately improves the blood electrolyte balance and overall performance of heat-stressed chickens. Supplementing chicken diet with sodium salt improves the live performance of heat-stressed chickens and better productive performance observed with NaHCO3 than other sodium supplements (Ahmad *et al*., 2006).

Energy Source

The metabolizable energy (ME) system does not provide a sufficiently accurate description of the feed energy available to the animal, because it does not take account of the efficiency with which different sources of dietary energy are used for different anabolic processes. Emmans (1994) has proposed an effective energy (EE) system that is more accurate in both these aspects. A major advantage of this system of energy evaluation is that it predicts accurately the amount of heat that will be produced by a given animal given a particular feed and housed in a given environment. This information is particularly important when determining how much the animal can lose in that environment. This explains the advantageous effects of providing feeds at high temperature containing highly digestible nutrients, minimal excesses of protein, and a high proportion of the carbohydrate energy replaced with digestible fat energy.

Glucose

Exposure of chickens to elevated environmental temperature markedly reduce food intake and the associated lower growth rate is accompanied by increased plasma glucose levels (McCormick *et al*., 1979) and increased *in vivo* uptake of galactose and methionine when measured on a tissue dry weight basis (Mitchell and Carlisle, 1992). This apparently enhanced absorption capacity was confirmed in *in vitro* studies in which enterocytes from chronically heat-adapted birds showed a 50% increase in galactose accumulation ratio compared with cells from control chickens (Mitchell *et al*., 1995). Heat stress increased microvillous length in chickens, indicating that the surface brush-border membrane is increased even in a metabolic situation characterized by a general reduction in protein synthesis (Geraert *et al*., 1996). This effect on apical surface, together with increased activity of sodium-dependent glucose transporter 1, enhances the capacity to absorb glucose and can, therefore, be interpreted as physiological adaptations of the chicken jejunum to guarantee energy supply (Garriga *et al*., 2006). Thus, supplemental glucose intake by chickens on exposure to high temperature alleviates the influence of heat stress and prolongs the survival time. When chickens are exposed to high temperature, rectal temperature enhances quickly in birds given tap water, while slow increases are found in birds offered glucose water with subsequently higher plasma glucose levels (Iwasaki *et al*., 1998). Oral administration of glucose prevents decrease in feed intake and growth rate, normalizes physiological and immunological responses, and alleviates the influence of heat stress on whole blood viscosity and plasma osmolality in heat-stress-exposed chickens (Zhou *et al*., 1998; Takahashi and Akiba, 2002).

Feed Form and Feeding Time

Offering pelleted feed to broilers can result in a 67% reduction in the energy required for eating. Whereas the ME of the feed is the same whether pelleted or not, the energy sparing effects of pellets is about 6% as a result of the reduced activity (McKinney and Teeter, 2003). Because the physical nature of the pellets allows the birds to consume their feed with less wasted energy, the quality and durability of the pellets is particularly important. A change of 10% in fines may result in a change of 0.01 in feed conversion ratio. At high temperatures there should be an advantage in providing broilers with high quality pellets, with the minimum amount of fines, thereby reducing the proportion of heat expended in acquiring food (see the review Gous and Morris, 2005).

Heat production by broilers can be reduced by withholding feed prior to, and dur-

ing, a limited period of high temperature stress. Survival is increased if food is withdrawn at least four to six hours before the period of heat stress (Smith and Teeter, 1988; Francis *et al*., 1991; Boulahsen *et al.*, 1993; Hiramoto *et al*., 1995). Withdrawing food during day, and replacing it at night once the temperature has declined, would appear to be a sensible approach to deal with uncomfortably high temperatures. The mechanism responsible for this beneficial effect is that the heat increment associated with feeding is reduced during the hottest part of the day. Because food remains in the intestine for up to 6 hours, withdrawal of the food must take place about six hours before the high temperature is experienced if the full impact if this practices to be realized.

New Concepts for Nutritional Strategies to Reducing Oxidative Stress and Damage

Hyperthermia can induce the metabolic changes that are involved in the induction of oxidative stress, and heat stress is responsible for stimulating reactive oxygen species (ROS) production. There is direct evidence of mitochondrial superoxide generation using both electron spin resonance (ESR) spectroscopy, with 5,5-dimethyl-1-pyrroline N-oxide as a spin trap agent, and lucigenin-derived chemiluminescence (LDCL) in skeletal muscle of acute heat-stressed birds (Mujahid *et al*., 2005). Additionally, in chickens the liver is more susceptible to oxidative stress than heart during acute heat exposure (Lin *et al*., 2006). Acute heat stress causes oxidative damage to mitochondrial proteins and lipids in skeletal muscle of chickens (Mujahid *et al*., 2007b). Heat stress also causes higher serum malondialdehyde levels (Mujahid *et al*., 2007b) that depends on duration of exposure (Pamok *et al.*, 2009). Under heat stress conditions, down-regulation of avian uncoupling protein (avUCP) and mRNA expression are accompanied by increased mitochondrial superoxide production (Mujahid *et al*., 2006), and these effects occur in a time-dependent manner (Mujahid *et al*., 2007a). It is well-known that ROS production can be decreased by mild uncoupling of mitochondrial respiration (Brand *et al*., 2004; Skulachev, 1998). UCPs are specialized members of the mitochondrial transporter family that allow passive proton transport through the mitochondrial inner membrane. This transport activity leads to uncoupling of mitochondrial respiration and to energy waste, which is well documented with UCP1 in brown adipose tissue. The uncoupling activity of more recently discovered UCPs (post-1997), such as UCP2 and UCP3 in mammals or avUCP in birds, is more difficult to characterize. However, recent extensive data support the idea that the newly discovered UCPs are involved in the control of ROS generation rather than thermogenesis (Negre-Salvayre, 1997; Abe *et al*., 2006). This fits with the hypothesis that mild uncoupling caused by the UCPs decrease ROS production. Therefore, it can be assumed that avUCP, expressed appropriately, may play a role in the alleviation of mitochondrial ROS production and an antioxidant role under conditions of acute heat stress.

Up-regulation of avUCP could attenuate oxidative damage caused by acute heat stress. In recent study chickens were fed either a control diet or an olive oil-supplemented diet (6.7%), which has been shown to increase the expression of UCP3 in mammals, for 8 days and then exposed either to heat stress (34°C, 12 h) or kept at a thermoneutral temperature (25°C). Heat stress increased mitochondrial ROS production and malondialdehyde levels, and decreased amount of avUCP in skeletal muscle mitochondria. Feeding chickens an olive oil-supplemented diet increased the expression of avUCP in skeletal muscle mitochondria, and decreased ROS production and oxidative damage. A subsequent study on mitochondrial function showed that heat stress increased oxygen consumption in state 4 and membrane potential in state 3 and state 4, which were abolished by feeding chickens with olive oil supplemented diet (Mujahid *et al*., 2009a). These reports, suggest that feeding olive oil under heat stress reduce mitochondrial ROS production in chickens due to changes in skeletal muscle mitochondrial avUCP contents as well as in mitochondrial respiration and membrane potential thus alleviating the effect of heat stress by changing the redox status and improving production performance.

A variety of ROS react readily with methionine residues in proteins to form methionine sulfoxide, thus scavenging the reactive species. Most cells contain methionine sul-

foxide reductases, which catalyze a thioredoxin-dependent reduction of methionine sulfoxide back to methionine. Thus, methionine residues may act as catalytic antioxidants, protecting both the protein where they are located and other macromolecules acting as an endogenous antioxidant in cells (Luo and Levine, 2009). The administration of methionine reduces the process of lipid peroxidation (a decreased in the concentration of MDA) with best antioxidative properties demonstrated by methionine in rat liver (Błaszczyk *et al*., 2009a). Methionine administration also increases the activity of antioxidative enzymes in rat kidneys, with significant effect on the activities of glutathione peroxidase, glutathione reductase and glutathione transferase (Błaszczyk *et al*., 2009b). Such antioxidant effect of dietary methionine on lipid peroxidation and increased antioxidative enzymes in chickens still need to be confirmed and is the interesting area for future research.

CONCLUSION

High environmental temperature exposure is of major concern for poultry industry especially in the hot region of the world. Different nutritional strategies have been used to alleviate stress in high temperature-exposed chickens. The nutritional strategies are designed after considering the factors such as type of birds, age of birds, stage of production, duration and intensity of heat exposure, and health of the birds. The nutritionists can base their strategy on less heat-production, increased nutrient intake, decreased energy wastage, and reduction in heat-induced oxidative stress and damage in birds to overcome the deleterious effects of high temperatures on metabolism, physiology, feed efficiency, production performance and health. This can be accomplished by traditional nutritional strategies to reduce heat stress by feeding good quality feed with high digestibility and nutrient density, adding fat as an energy source, balancing and provision of additional amino acids, and supplementing with vitamins, minerals and glucose. Recently, there are new concepts for nutritional strategies to focus on redox status of the chickens and to decrease the oxidative stress and damage on exposure to high environmental temperature. None of these strategies are effective alone in terms of growth, feed efficiency, livability or meat quality, therefore, a combination of the nutritional strategies may help to alleviate the negative effects of heat stress and improve the chicken performance under high environmental temperature.

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Anticipating the Outbreak of Zoonotic Infectious Diseases Related to Animal Industry

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ABSTRACT

Keeping and using animals and animal products is an age-old recognition. There are many reasons to own animals and some are as follows:

1. The Animal products are rich sources of essential protein needed by human being so they are farmed for meat or other animal products.
2. The animals which have economic values and the potential for making profits are reliable sources of income to the owner,i.e working animals.
3. The animals kept primarily for human companionship and pleasure.
4. The animals are useful for the government missions ,i.e sniffing dogs.
5. The animals that are free-living and captive animals or species lives in the wild without human intervention, that now under the regulations and guidelines that apply to the movement and trade of certain wild animal species.
6. The use of animals in experimentation or animal use for the purpose of research, testing and education.

Health experts from around the world representing human health and animal health i.e WHO, FAO, CDCP, IUCN and others that participating in an infectious diseases symposium on September 29, 2004 in USA known as 'One World One Health'event and followed by another meeting in December 2007 in India recommended that the international community draw on experiences with some infectious diseases such as HPAI to address the spread Emerging Infectious Diseases (EID) means infectious diseases that emerge (or re-emerge) from the interfaces between animals and humans and the ecosystems in which they live .

The experts agreed that the spreading of the diseases is as a result of several trends including exponential growth in human dan livestock population, rapid urbanization, rapidly changing farming systems, closer integration between livestock and wildlife, forest encroachment, changes in ecosystems and globalization of trade in animal and animal products.

The expert panelists described priorities for an international interdisciplinary approach for combating threats to the health of life on earth. The product called "Manhattan Priciples" or the One World One Health (OWOH) approach that currently recognized all over the world.

New Development of Animal Production in Indonesia

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Indonesia indicates low consumption of animal originated products including milk, eggs and meat. Although the consumption per capita of animal protein is low, the importation of live animal and animal produces increase. High importation indicates the limitation of production level which is associated with low population and low productivity of animal. An abundant research in various aspects of animal productions has been conducted with various results. Genetic potential and genetic related disorders of local and imported animals has been studied. Both local and imported animals have been adapted to the local environmental conditions, but a conventional breeding system may have been resulted in reduction in animal performance. Some genetic related disorders associated with low productive and reproductive performance have been identified. Feed shortage during dry season and their low quality have been constrains in improving productive and reproductive performance especially of ruminants for many decades. Improvement of forage cultivation and processing technique of many grass and legumes species has been conducted to improve their yield and quality. Herbs and plant components have been developed to reduce production problems and losses associated with gastrointestinal nematodes and microbe infection in animals. Supplementation of mineral and other nutrient have been studied. Although the results are vary among researchers, but most of the results almost consistent to improve animal performance. Processing of animal product is a main concern of Indonesian government and researchers, since it has been a critical factor for farmers to make a profitable farm business. Animal waste utilization for biogas and fertilizer has been evaluated to minimize the negative impact of animal industry activities on environment. However, most researches have been conducted at small scale at laboratory level. Therefore scaling up at field level is necessary to make the result available for farmers and industries.

Herbs and Herbals in Animal Nutrition

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ABSTRACT

Herbs and herbal extracts have been used for thousand of years in curing and treating diseases in both human and animals. Records have shown that animals were fed herbs and spices to cure certain diseases more than 5000 years ago. The Indian ayurvedic and Chinese medicine used herbs and spices to enhance health and also treat diseases. Common spices such as tumeric, ginger and garlic have been indicated to contain active compounds that apart from giving flavour to our otherwise mundane cooked food, also contain compounds with properties that can improve health and increase immunity. Despite the widespread use of herbs as medicine, healing aids and health foods little work has been done to support the claims scientifically. Only in recent years that scientists have isolated and identified the active compounds in herbs in relation to their functions. Is is known that many herbs contain one or more active ingredients and that combination of herbs tend to be more effective than the use of single herbs. However, the combinations have not been established and a lot more research is needed so that we can understand the functions of herbs. Herbs can be classified according to their functions or actions such as those that have antioxidants properties, antibiotic properties, immune system enhancer etc. Caution should be taken in using herbal preparations as many of these herbs have not been tested out fully and their safety as far as human health are concerned are still much to be understood. While it is natural for the public to assume that herbs are organic and safe, there has been incidences of toxicity, abuse and side effects produced by these herbs. Special care must be given when giving herbs as medication when the animal is suffering from kidney, heart or lung ailments.

Key words: herbals, antioxidants, natural cures, herbs in nutrition

INTRODUCTION

Under natural conditions animals instinctively look for and consume herbs to cure certain diseases. It has been observed that animals such as cats and dogs, rabbits and horses search for special herbs when they are sick. Traditional herbal medicine, whether Ayurvedic medicine, Indian herbs, Chinese herbs, Western herbs or African herbs are generally holistic in therapy and relies upon the whole plant, roots, seeds or leaves that has been established to be more effective. Traditionally the selected herb or parts of the herbs has not been presumed to contain a single pharmacologically-active ingredient.

Modern herbal medicine is going towards pharmacognosy, the science of defining 'active' ingredients, then extracting and purifying them and using them in isolation. This is not a holistic approach as individual active compounds may act differently and cause different effects. When herbs are used as a whole plant or leaves etc combined with other herbs these active ingredients work in synergy and in harmony hence give the desired effects naturally. However, this is not the case with regards to modern medicine as more and more herbs have been identified and the active ingredients isolated.

Classifications of Herbs

The Cherokee herb medicine advocated by (Winston D, 1992) divide herbs into three classes, namely, (a) the "food herbs" which are gentle in action, have very low toxicity, and are unlikely to cause an adverse response (e.g. Lemon Balm, Peppermint, Marshmallow, Ginger, Garlic, Chamomile, Hawthorn, Rose hips, Nettles, Dandelion Root and Leaf, and fresh Oat extract) and can be utilized in substantial quantities over long periods of time without any acute or chronic toxicity, (b) the "medicine herbs" which are stronger acting and used for specific purposes at proper dosage and may have some adverse

effects if wrongly used (examples are Andrographis, Blue Cohosh, Cascara Sagrada, Celandine, Ephedra, Goldenseal, Senna and Oregon Grape Root, and (c) "poison herbs", which are potentially toxic and need to approved by medical doctors under specific conditions (examples, Belladonna, Bryonia, Datura, Gelsemium, Henbane, Male Fern, Phytolacca, Podophyllum, and Veratrum).

Herbs contain a variety of pharmacologically-active ingredients and each herb has its own unique combination and properties. In modern herbal medicine they are classified according to their action. Many herbs contain ingredients with actions such as anthelmintic, anti-catarrhal, anti-emetic, anti-inflammatory, antibacterial, antifungal, laxative, aromatic, diuretic, stimulant, etc. Herbal medicines can be classified according to the type of constituents in their composition, such as acids, alcohols, alkaloids, anthraquinones, bitters, cardiac glycosides, coumarins, flavones, flavonoid glycosides, phenols, saponins, tannins and volatile oils.

Why Use Herbs

The increase in research on the use of herbs in animal nutrition was spurred by the global effort in reducing the use of antibiotic growth promoters in poultry and livestock feeds. A lot of work has been directed towards replacing antibiotics with probiotics, prebiotics and also herbs while maintaining growth performance. Herbs are natural growth promotants and are safe for human consumption as they do not leave residues in animal products. The progressively reduction in the use of antibiotics will ensure that less and less antibiotic residues are in animal products. Herbs are also cheaper in the long run and may lead to lower feed costs. Ghalyanchi *et al*., (2008) compared the use of antibiotic, probiotics and two herbal preparation showed that the herbal preparations were effective in replacing virginiamycin as a growth promoter in broiler chicks. Earlier Demir *et al.* (2003) showed that replacing antibiotic with essential oils are effective in supporting growth performance of broiler chicks. These studies suggested that antibiotics can be replaced with herbs and essential oils of herbs without much affecting their growth performance.

Active Ingredients in Herbs

There is a logic in the notion that herbs indigenous to the patient's country should be used in preference to 'exotic' herbs, although Chinese and Ayurvedic herbs have become fashionable in the UK, at present. There follow some simplified examples of Western herbs, classified according to pharmacological activity: Herbal medicine includes such amazingly effective agents as willow bark (providing salicylate, which is an Aspirin-like and effective pain killer, at much lower doses than one might expect, when compared to Aspirin itself), Digitalis or foxglove (a remarkably effective heart drug, having action on all aspects of cardiac function), dandelion (an effective diuretic,

Table 1. Some common and traditional cures using herbs*

	Herbs	Uses
1	Hops *Humulus lupulus*	Young shoots given to colts as a tonic to condition them. Flowers fed in fodder to quiet restless animals.
2	Conmmon Ivy *Glechoma hederacea*	Good for internal cleansing after birth, treating retained afterbirth.
3	Ladies mantle *Alchemilla vulgaris*	Cure for stones and a tonic after treating colic.
4	Common nettle *Urtica dioica*	Rich in minerals, calcium, sodium, iron, chlorine and a naturally good in protein value. Nettle juice is used to wash the coat, to give a beautiful shine for show.
5	Black poplar *Populus nigra*	Buds crushed in milk or honey make good tonic for horses. Make ointment for sores, wounds, ulcers.
6	Wild strawberry *Fragaria vesca*	For cleansing and antiseptic. A brew of the root and herb thickened with borax, removes old sores and dandruff.
7	Indian tea *Thea sinensis*	Good for sun stroke, sunburn, fine burns. To given as a drink.

* from various sources

Table 2. Shows some herbs and their function in wound healing

	Herbs	Uses and actions
1	Alfalfa	extremely rich in vitamins and minerals including iron, calcium, magnesium, phosphorus, sulfur, chlorine, sodium, potassium, silicon, and trace elements. It is a good source of carotene (Vit. A) and Vit. K, the blood clotting vitamin, prevent tooth decay.
2	*Aloe vera*	excellent for burns. It can be used by nursing mothers for sore nipples. Can be used as an eye drop to improve circulation and eyesight. It stimulates the circulation in wounded areas, which also promotes healing.
3	Calendula Flowers	Anti fungal inflammation fighter, soothing to the skin, healing properties, use as lip balm, or on cuts, burns, abrasions, and even sprains, anti fungal action is an aid for candida albicanas, athlete's foot.
4	Cayenne, capsicum	builds up the body's resistance, high in Vitamin C, general stimulants which is the key to healing, improves the entire circulatory system,
5	Comfrey	an infection fighter and blood cleanser, a contact healer (relieves pain and starts healing on contact). It is cell proliferate (helps grow new flesh and bone) and accelerates the healing process. The cell proliferate and active ingredient in comfrey is called allantoin. It helps with pain, repairs and heals, excellent for wounds, burns, cuts and abrasions and broken bones.
6	Echinacea	excellent infection fighter and is used as a natural antibiotic. It is especially good in glandular infections and problems. A good cleanser for glands and the lymphatic system.
7	Tea tree oil	has antiseptic and antibiotic qualities. It can be used as a topical antiseptic and unlike the other topical antiseptics (iodine, mercurochrome, etc.) it does not damage healthy tissue as well as kill bacteria so it does not interfere or slow the process of healing.

providing copious potassium, which modern diuretics tend to drain from the body! - French name *pis en lit*) and periwinkle or Vinca (a predecessor of the potent cancer drug Vincristine).

Herbs and Immune Response

A large number of studies have shown that *Echinacea* destroys many types of viruses and bacteria. It is popular in America, Europe and China as an immune enhancer. Siberian ginseng is another herb and its use is widespread in Soviet Union and it helps the body adapt and improve immunity under stressed conditions. Several medicinal herbs are used to enhance the immune system or bring it back up to normal levels folllowing an illness. Shitake mushrooms has been shown to exert positive effects on the immune system. An antiviral compound called lentinan in shitake mushroom stimulates the immune system. It appears that lentinan increases interferon activity. Peruvian rainforest herb cat's claw can be used to treat disorders related to the immune system, including rheumatoid arthritis, gastric ulcers, colitis and Crohn's disease, by increasing the amount of disease-fighting immune cells in their blood.

Herbs and Parasites

A number of herbs for cooking can be used to kill intestinal parasites such as ginger and essential oil can kill roundworms. Some studies have shown that ginger can be more effective than piperazine citrate.

Herbs and Meat Quality

The use of artificial antioxidants such as BHT, santoquin, and TBHQ to increase meat product shelf life is common practice. Although these have been claimed safe the long term effect on humans if taken continuously is not known. It is felt that natural antioxidants are more effective in retaining the quality of meat products and also their shelf life. Grape seed extract, a by-product of grape fermentation has been shown to be an effective antioxidant due to its phenolic contents. Jang *et al*. (2009) evaluated the antioxidative potential and quality of the breast meat of broiler chickens fed a dietary medicinal herb extract mix (MHEM, consisting of mulberry leaf, Japanese honeysuckle, and goldthread at a ratio of 48.5:48.5:3.0). They showed that

MHEM did not affect proximate composition of the breast meat. Phenols content of the breast meats in treated T2 diets was twice that of the control. The 2-TBA in the treated diets were lower than the control and did not increase during storage. They concluded that this herbal mixture increased the antioxidative potential and overall preference of breast meat during cold storage. A study was conducted to evaluate efficacy of herbal liver tonic and growth promoter (Superliv and Xlivpro) on the overall growth, performance and carcass quality of broilers (Sharma *et al*., 2004). There were significant improvement in growth performance in the in treated groups. The results also showed improvement in livability, carcass yield and carcass quality. It appears that polyherbal liver tonic formulations enhanced nutrient utilization. Meat from lambs raised on mountain pastures without any supplementary feeding or treatment is often considered to be of superior quality. This was because lambs on mountain pastutures had access to herbs and wild shrubs and the meat were often tasty. Adney *et al*. (2004) compared the carcass characteristics and meat quality of lambs grazed on lowland and mountain pastutures and showed that there were significant differences between the groups were found in grading, fat content and fatty acid composition, meat colour, and meat flavour. At UPM Karami (pers. comm.) compared goats fed supplements of Andrographis paniculata, tumeric and vitamin E to compare their antioxidant contents in meat. Meat of goats fed A. paniculata and tumeric had higher antioxidant activities and better sensory qualities.

Herbs and Reproduction

There are quite a number of research on the use of herbs in reproduction. In early humans civilization the problem of infertility has been treated using herbs and other traditional ways. The effect of herbs on the reproductive activity in animals are not exactly defined. A number of articles written in the internet showed that herbs are commonly used in horses at various stages to improve reproduction (Wheeler and Wait, 1992). Recently, natural herbs have been investigated to look into their potential as reproduction enhancers. Work by Allan and Bilkei (2004) showed that Oregano (Origanum vulgare) enriched with essential of oregano reduced morality and increased farrowing rate in sows. Sows fed oregano had lower annual sow mortality rate , lower sow culling rate during lactation, increased farrowing rate, increased number of liveborn piglets per litter (10.49 ± 1.5 versus 9.95 ± 1.22, $P<0.05$), and decreased stillbirth rate. In the yak (Poephagus grunniens L.) using prepared Indian herbs (Prajana) Mohanty *et al* ---- showed improved estrus cycle by more than 40 %. On the other hand, Oyeyemi *et al*. (2008) examined the effect of extracts of Veronia antygdalina on the spermatozoa of Wistar rats. Rats supplemented with 500 and 250 mg of the extracts showed lower spermatozoa livability and mobility. They concluded that the uncontrolled use ol Vernonia amygdalina have an adverse effect on the spermiogram and spermatozoa/morphology of the intact rats. In humans there are a number of herbs that can be used as improving the fertility of women. The table below shows some of the known herbs that are commonly used in herbal preparations to improve fertility.

Table 3. Examples of some herbs that improve fertility in animals.*

	Herbs	Actions
1	*Centella asiatica* (gotu cola)	enhance male fertility, maintain healthy blood vessels including sperm-producing seminiferous tubules
2	Siberian ginseng	Effective aphrodisiac, promotes sexual functions, regulatory effect on mentrous cycle
3	Black cohosh	phytoestrogenic effect on the female body, naturally stimulating the ovulation process
4	Horny goat weed Epimedium grandiflorum	improves male potency and acts as an aphrodisiac. It also increases a man's sperm quantity and quality
5	Chasteberry:	hormonal balancer that helps the pituitary gland to function properly, regulates the ovulation process and maintains a healthy hormonal level within the body

* from various sources.

Herbs and Egg Quality

Studies conducted at UPM (Al kirshi, pers. comm) indicated that mulberry leaves when supplemented in layer diets shows that the yolk colour was enhanced and its antioxidant properties increased. This suggests that mulberry leaves can be useful in egg quality and increase their shelf life. In his study the antioxidant content of egg yolk was also increased due to mulberry leaf supplementation.

CONCLUSION

Herbs and herbal extracts are potentially useful as growth promotants in diets and also as therapeutic agents to treat certain diseases and disorders. They can replace antibiotics, have immune enhancers and fight bacteria and viral infections. Although there are many herbs and herbal preparation in the market and their claims of healing and curing diseases it must be noted that there have been few scientific research and testing on the safety to animals and man. The dosage indicated by the manufacturers is generally based on common practice and traditional notes. Side effects are not normally stated and users are advised that they should follow strictly to the instructions given. Selecting what herbal preparation to use and when depends on various factors such as the species of animals, age and physiological status. Care must be taken not to abuse the use of these herbs by understanding their functions and what they can do.

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Broiler Chicken Welfare: What Do They Want and What Do We Want?

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INTRODUCTION

The broiler chicken industry today is a growing part of global agribusiness and the production level has achieved outstanding biological and economic performance and thus contributing powerfully to cheap, abundant food and improved quality of life. Broiler chickens are probably the most numerous farmed species: worldwide, some 44 billion are produced each year worldwide and this figure is likely to rise in the coming years (Morton, 2004). The chicken meat industry is highly automated, integrated and intensified of the animal production industries. Intensive farming refers to the number of animals per production unit, the degree of crowding to which they are kept, and the artificiality of the environment in which they are kept (Duncan, 2001). The vast changes in modern poultry production systems have had a positive impact not only on global food security but also animal welfare. Indoor confinement provides better protection against thermal extremes and predators, and improvement in disease prevention. However, the poultry industry incessant drive for efficiency and reduction of costs has resulted in numerous social and ethical concerns that diminished its exceptional achievement. The vast majority of studies have emphasised on the welfare laying hens in battery cages. Welfare concerns pertinent to broiler production have received less attention. The over intensification of broiler chicken farming, whereby large number of birds may be forced to grow super fast in overcrowded conditions, not only have serious impacts on the environment and public health but also causes scientifically proven suffering to the birds.

What is "Chicken Welfare"?

A great deal of controversy has arisen over the definition of animal welfare because it often represents an assortment of vague notions. Concerns for animal welfare seems to be determined significantly by people's perception of animal suffering and how they interpret what they see or measure in terms of animal behaviour or changes in physiological processes. Like "sin" and "love", 'animal welfare' means different things to different people. Some people believe that human and animals have equal rights and thus, killing an animal for food is preposterous. They consider animals have the right not to be used for any purpose by humans. A different extreme is represented by the belief that humans have no obligation at all to consider the welfare of animals in agriculture. All that matters is productivity because animals exist specifically for humans to exploit and have no intrinsic value or internal purpose. Somewhere in the middle are those who do not oppose human use of animals but believe adequate well being of animals while they are under human care is necessary. Many people may feel that the slaughter of animals is necessary and acceptable (although they may recognise that 'some' animal suffering will be involved even with 'humane' methods) because they see no other alternative if they are to continue to eat meat. The 'welfare' of an animal refers to its quality of life, and this involves many different elements such as health, happiness, and longevity to which different people attach different degree of importance (Fraser, 1995). In the *Dictionary of Farm Animal Behaviour* (Hurnik *et al*., 1995), 'animal welfare' was defined as "A state of harmony between animal and its environment, characterised by optimal physical and psychological functioning and high quality of animals' life."

Can a Broiler Chicken Suffer?

Welfare refers to the particular kind of moral concern we have for animals as a result of their capacity for subjective feelings, particularly the unpleasant subjective feelings of suffering and pain (Dawkins, 1988). Thus, to the animals, welfare is all that matters. The idea that welfare concerns the feelings of animals needs some development because 'feelings' may mean either just sensations such as touch and sight or more complex processes such as pain and emotions. This raises a problem because all animals have at least some of the five senses, and it is less clear

that all animals are capable of suffering or experiencing pleasure. Thus, the relevant question is can a chicken suffer or feel pleasure.

"It is generally accepted that welfare is a term which cannot be applied sensibly to the lower animals or to plants but only to sentient animals".

(Duncan, 1996)

'Sentient' has the same spread of meanings as 'feelings'. Leahy (1991) states

"….to be sentient is to have the power of sense-perception; to see, hear, smell, taste or touch".

In this sense all animals are sentient. However, the term may be used to mean the 'capacity for suffering or enjoyment'. Poultry is definitely considered as sentient animals. Although chickens respond to stimuli that we call painful, but neither their mental nor their physical responses are necessarily the same as those of humans (Appleby, 1999). Suffering is affected by thinking, and types of thinking vary between species. A particular animal species will therefore be able to suffer in certain ways but not others: chickens probably feel pain but not grief. It can be concluded that all animals are sentient, but to varying degrees.

Selection for Rapid Growth and Welfare Issues

Genetic selection has increased production levels of broiler chickens considerably. Since feed costs are economically the most important costs, the breeding goal in poultry is to create a population with high economic production efficiency, i.e. high production with relatively low feed intake. Through intense genetic selection during the last five decades for economic traits, and improvements in management, nutrition, and disease control, there has been a consistent decrease in the age at which slaughter weight (approximately 2 kg) is reached, by 1 day per year, as well as large relative increases in breast muscle size (Griffin and Goddard, 1994). The time required for a meat-type chicken to reach 2.0 kg live weight has been reduced from 14 weeks in 1950 to 5 weeks in 2005. It is anticipated that genetic changes resulting in enhanced performance will continue (Albers and Groot, 1998). Such selection practices have resulted in birds both genetically distant and with reduced genetic diversity compared with their red jungle fowl ancestors (Siegel *et al.*, 1992). Apart from the desired effects of genetic improvements in economic traits, broiler chickens are at risk for behavioural, physiological and immunological problems.

Lameness is considered the one the most serious welfare issues in broiler chicken production. Because such problems are rare among slow-growing birds, it clearly suggests that these disorders in modern broilers strains are associated with rapid growth. Julian (2004) indicated that the tendons and bones of broilers are not strong enough to support their weight and may lead to painful conditions such as spondylolisthesis, ruptured gastrocnemius tendon and separation of the femoral epiphysis, backward bending of the proximal tibia in bones weakened by dyschondroplasia, epiphysolitis, and pressure-induced microfractures at the diaphysis of the proximal tibia. Kestin *et al.* (1992) showed that 90% of commercially raised broilers chickens had detectable gait abnormalities at market age while 26% had serious gait abnormalities, resulting in impaired locomotor abilities. These birds are unable to walk and thus unable to get to feed and water. Because birds with leg problems are likely to spend more time lying down on corrosive soiled litter, they are susceptible to potentially painful skin problems such as breast blisters, footpad dermatitis and hock burns (Berg, 2004). Apart from welfare concern, lameness is also costly to the industry. Studies in the United States indicated that leg problems were responsible for 1.1% of broiler mortality and 2.1% of carcass condemnation and downgrades annually, and cost the industry billions of dollars each year (Morris, 1993).

There are numerous reports of an increasing incidence of cardiovascular diseases such as ascites, pulmonary hypertension, and sudden death syndrome (SDS) in fast growing broiler chickens (Julian, 2004). Ascites is a condition that occurs when a fast growing bird has insufficient heart-lung capacity to supply all of the soft tissues with oxygenated blood. This leads to an increase in blood pressure, dilation and hypertrophy of the right ventricle, and leakage of serous fluids into the body cavity (Julian, 1998). In the

case of SDS, well-fleshed and healthy broiler chickens die suddenly while standing, walking or feeding. They die with a short terminal wing-beating convulsion and are often found on their back (Julian, 1986). SDS has been associated with to high carbohydrate intake, rapid metabolic rate, defective cell membrane, defective cell membrane integrity, and intracellular electrolyte imbalance. In good flocks, 2-4% of males may die from SDS.

Intensive Farming and Broiler Chicken Welfare

Julian (1995) defined intensive farming as conditions where animals are confined in close proximity, where freedom or movement is restricted, where all feed is supplied and the quantity and quality regulated, where the environment is controlled (to some extent) and where the greatest number of animals are cared for by the minimum number of attendants. Intensive farming is competitive and the methods adopted are those that cut costs and increase production. Many of the conditions in which poultry live and the procedures to which they are subjected may compromise their welfare.

i. Space requirement

Birds require space to accommodate normal body functions and to access the utilities that support the function intended for a production system or simply for maintaining normal behaviour repertoires such as grooming and social behaviour (Albentosa and Cooper, 2004). Intensive animal farming emphasizes on optimising cost per unit of output from such systems. Such emphasis increases the likelihood that individual animal welfare will be compromised in favour of enterprise efficiency. The perception of acceptable floor space for both broiler chickens varies widely according to country, region, and organisation concerned, and there is no evidence that the existing recommendations are based on scientific evidence. Limited floor space is a major welfare issue in both chicken meat production. Crowding has been shown to adversely affect growth rate, feed efficiency, survivability, feather condition, comfort behaviour, aggression, underlying fearfulness, and incidence of lameness and skin injuries in poultry (Bessei, 2006). Crowding may also affect environmental parameters such as ventilation, temperature and humidity. Even though higher stocking density is widely known to be detrimental to productivity, economic studies showed that such practices may increase the net profit of meat production. This leads to an obvious conflict between animal welfare and profitability.

Many studies have examined the effect of bird density on growth and slaughter quality of broiler chickens. It is now recognised that stocking densities of 500 cm^2 per bird or less or 30 kg /m^2 are detrimental to both productivity and welfare of broiler chickens (Bessei, 2004). There is a significant reduction on feed intake and reduced growth rate when stocking density exceeds 30 kg / m^2 under deep litter conditions. The detrimental effect of stocking density on growth rate was partially compensated by increased ventilation rates (Grashorn and Kutritz, 1991). These results lead to the assumption that problems of dissipating metabolic heat may be the causal factor for depression of growth rate. High stocking density impedes heat transfer from the litter surface to the ventilated room.

Hock burns, breast blisters and foot pad lesions, which may be summarised under the expression contact dermatitis, are common in broiler flocks during the last decades (Berg, 2004). It is evident, that the influence of stocking density on contact dermatitis acts through its influence on litter and air quality. High moisture content of the litter enhances microbial activity, which in turn leads to increase of temperature and ammonia in broiler houses, and thus, high incidence of contact dermatitis, breast blisters, leg weakness and soiled plumage (Weaver and Meijerhof, 1991). In a larger scale experiment with commercial farms using different breed, management systems and stocking densities, Dawkins *et al*. (2004) concluded that the management conditions (litter quality, temperature and humidity) and stockmanship were more important than stocking density in determining the welfare of broiler chickens.

ii. Feed restriction

The meat-type chickens used for breeding stock have the same voracious appetite as their progenies and thus have to be feed restricted severely to control obesity. Fertility, immunity and survivability will be adversely affected in obese birds (Siegel and Wolford, 2003). The demand for feed and water is inelastic in most animals (Dawkins, 1990). There is mounting evidence that restricting the intake of feed, water or both can result in physiological stress responses, boredom, stereotypies, aggression, and other abnormal behaviours in poultry (Zulkifli, 1999; Zulkifli *et al*., 1993; 1995a; 2006). The modern broiler breeder industry is caught in a welfare dilemma, since on one hand breeding stock appears to be chronically hungry, while on the other hand *ad libitum* feeding or less severe food restriction leads to reproductive and health problems (Savory *et al*., 1993). Evidence, however, is accumulating to show that chickens readily habituate to fasts of moderate duration (Zulkifli, 1999; Zulkifli *et al*., 1993; 2006).

iii. Handling and transportation

There is a growing concern regarding the welfare problems associated with harvesting and transport of broiler chickens. Prior to slaughter, birds are exposed to an array of factors that may evoke both stress and fear reactions (Figure 12) (Zulkifli *et al*., 2000a;b; 2009; Zulkifli, 2003; Al-Aqil and Zulkifli, 2009). These factors include feed and water deprivation, physical contact with human, social disruption, noise, overcrowding, motion and vibration, and thermal extremes (Mench, 1992). The handling method adopted may have implications for the bird welfare. Zulkifli *et al*. (2000b) reported that rough inverted handling, as practised in commercial settings, augmented both stress and fear reactions. Improper handling of chickens may result in physical injuries, pain, and mortality. Farsaie *et al*. (1983) estimated that one in four broilers processed in the USA sustained bruising of the legs, breast or wings during catching and transport. Birds that have died between catching and the moment of slaughter are termed 'dead on arrival' (DOA). Published mean DOA percentages ranged from 0.05 to 0.57% (Bayliss and Hunton, 1990). Factors that influence DOA percentage are catching crew (Bayliss and Hinton, 1990), transport time (Wariss *et al*., 1992), lairage time (Bayliss and Hinton, 1990), type of transport crates (Stuart, 1985), time of day of catching and transport (Bayliss and Hinton, 1990), stocking density per crate (Bayliss and Hinton, 1990), age and sex of the birds (Bayliss and Hinton, 1990).

Zulkifli *et al*. (2000a) compared stress and fear reactions to handling and crating; and handling, crating and transport in broiler chickens under hot climate. The authors concluded that the transport component was more traumatic. The magnitude of stress, as measured by HLR and CORT, attributed to transportation increased with transit time and this could be due to longer exposure to noxious and stressful stimuli (Al-Aqil and Zulkifli, 2009). The author showed that raising chickens in conventional open-sided houses with cyclic ambient temperatures improved heat shock protein hsp 70 expression and may lead to better ability to cope with the stresses associated with road transportation in hot, humid climate than those under environmentally controlled closed house. Mitchell and Kettlewell (1998) suggested that high ambient temperature is a major factor in the elicitation of physiological stress responses during transit. With the growing importance of broiler production among livestock industries in hot regions of the world, heat-stress related predicaments during transit are of major concern.

How Can We Improve Broiler Chicken Welfare?

In discussing the welfare of poultry in modern production systems, Mench (1992) posed a simple but very critical question of whether we should change the bird to suit the environment or we should change the environment to suit the bird.

i. Early age stimulation

Stressful experiences may perturb homeostasis, but there can be long-term benefits in improving resistance to other

forms of insults. For example, early age feed restriction improved tolerance to high ambient temperature (Zulkifli *et al*., 1994a;b; 2000c) and transportation (Zulkifli, 2003) stresses, and disease resistance (Liew *et al*., 2003) in chickens. Work by Zulkifli *et al*. (1994b; 1995b) suggested that transient perturbations of homeostasis during the neonatal stage without current increases in the synthesis and release of corticosterone may not aid an animal in responding to subsequent stressors. Thus, stress-elicited elevations in adrenal corticoid concentrations may be crucial in preparing the body in responding to subsequent stressors. Acquired enhanced heat tolerance resulting from early age feed restriction in broiler chickens could be attributed to improved hsp 70 response (Zulkifli *et al*., 2002a; 2003). Liew *et al*. (2004) indicated that hsp 70 expression appears to be beneficial in enhancing resistance to infectious bursal disease in heat-stressed chickens. The hsp 70 play a profound role in regulating protein folding and in coping with proteins affected by heat and other stresses.

ii. Human contact

The quality of human-animal interaction can have a profound impact on many facets of an animal's physiology and behaviour. Work in poultry suggest that regular positive human contact can reduce flightiness (Hughes and Black, 1976), feather pecking and cannibalism (Zulkifli, 2008), stress and fear reactions to capture and transportation (Zulkifli *et al*., 2002b; Zulkifli and Siti Nor Azah, 2004; Al-Aqil, 2009), and improve antibody production and disease resistance (Gross and Siegel, 1982; Zulkifli *et al*., 2002b). Gross and Siegel (1982) suggested that positive human-animal relationship reduced the resources otherwise required by the birds to respond to their human associates and that resources can be utilised to compensate for environmental insults. Recent work in our laboratory demonstrated that pleasant physical contact with human beings augmented hsp 70 expression following transportation (Al-Aqil, 2009). As measured by heterophil to lymphocyte ratios and plasma corticosterone concentration. it appears that hsp 70 played a key role in improving tolerance to transportation stress by pleasant human contact. Despite the numerous desirable effects of physical interaction on poultry, regular handling of every bird is obviously not feasible and practical to commercial flocks. Zulkifli *et al*. (2002b) reported that simply allowing chicks to see experimenter was as effective as physical contact in reducing underlying fearfulness and physiological stress responses to catching and crating. The authors showed that there was a circumscribed sensitive period for the induction of the visual contact phenomenon in the domestic fowl.

iii. ***Genetic selection***

Although genetic selection for economic traits may compromise the well-being of poultry, selective breeding could be used to improve welfare. Although there is a strong correlation between leg weakness and growth rate (Sørensen *et al*., 1999), it has been shown that progress can be made in reducing and eliminating leg disorders by genetic means. Previous studies (Wong-Valle *et al*., 1993) showed that the incidence of tibial dyschondroplasia can be effectively reduced through genetic selection.

Three of the major behavioural problems facing the poultry industry are fear, feather pecking and social stress. Mench (1992) suggested that:

"Genetic selection may prove to be a powerful tool for decreasing the incidence of behaviours associated with welfare problems."

Earlier studies have shown that these behavioural traits respond readily to genetic selection (Faure and Jones, 2004). Gross and Colmano (1971) reported the divergent selection of two line of chickens selected for adrenocortical response to social stress. Faure (1975) demonstrated that domestic chicks of the lines selected for high activity in the open field were not only less fearful but they also showed lower resting and stress-induced plasma corticosterone levels than their inactive counterparts (Faure, 1975).

In discussing selective breeding and animal welfare, Mills *et al*. (1997) concluded that:

"The major problems associated with such genetic improvement programmes are the need to develop measures of welfare which are compatible with selection on a commercial scale, and potential competition between selection for production and welfare-related traits."

iv. Environmental manipulation

Intensively raised chickens are often housed in barren, visually restricted and monotonous environment. This may lead to frustration, boredom and harmful abnormal behaviour (Petherick and Rushen, 1997). Even when their basic needs are met, animals will seek moderate novelty, or environmental challenge (Jones, 1996). Environmental complexities can be considered as an enrichment or extra stimulation in the house environment that may enhance behavioural repertoire, increase ability to cope with challenge, decrease fearfulness, aggression and depression, and improve health and productivity (Jones, 1996). Jones (1982) and Jones *et al*. (1991) reported that chicks and quail reared with a range of novel objects and stimuli were less fearful and more able to tolerate stress later in life than those raised in barren environment. A greater complexity of rearing conditions may attenuate reactions to subsequent environmental challenges. Environmental complexities can be considered as an enrichment or extra stimulation in the house environment of poultry that may enhance ability to cope with novelties and stressful situations (Fraser and Broom, 1997). The vast majority of enrichment studies in poultry have focused on underlying fearfulness. Birds housed in different systems have been reported to exhibit different levels of underlying fearfulness (Kujiyat *et al*., 1983). Al-Aqil and Zulkifli (2009) compared physiological stress reactions of broilers raised in open-sided and windowless houses (varied in the level of environmental stimulation) to road transportation. Birds raised in open-sided house which had experienced a greater variety of visual and auditory stimuli than those in windowless house had greater hsp 70 expression, and lower CORT and HLR responses. The visual and auditory stimuli can be considered as a form of environmental enrichment. Previous studies indicated that chickens exposed to outdoor environment (Scott *et al*., 1998) or even video stimulation (Jones, 1996) were less fearful than those deprived of extra stimulation in the home environment. Fear is a potent stress-elicitor in poultry (Jones, 1996). According to Wemesfelder and Birke (1997) environmental challenge may be considered as an integral part of behavioral development and well-being. Successful enrichment may increase the behavioral repertoire, reduce the occurrence of abnormal and undesirable behaviors, and enable animals to cope with challenges in a normal fashion (Chamove and Anderson, 1989).

Although environmental enrichment can improve poultry welfare and productivity Jones (1996) suggested that:

"..it is important to avoid applying too much environmental enrichment at any one time or to suddenly translocate an animal from a barren home environment into an unfamiliar enriched one."

CONCLUSION

Our relation with chickens is symbiotic and we have an obligation to take care of their interest as well as our own. Chickens are sentient animals, not machines or mere mechanical factors for production. Hence, suffering of chickens must be reduced and welfare must be seriously addressed. The vast scale on which chickens are raised may have desensitized all parties associated with poultry. Humans may also find it easier to relate to and recognize sign of pain in the larger animals, especially if these animals are our companions, such as cats and dogs. There is also a possibility that because chickens appear so different from us and other mammals that we find it difficult to empathise with their suffering. These arguments are obviously not strong enough for us to treat chickens differently from other animals. Poultry producers in the European Union (EU) are presently facing the task of ensuring higher welfare standards to comply with the

regulations laid down in the EU Directive. The majority of concerns relating to the poultry industry in Malaysia and other developing nations are to do with producing cheap, plentiful food in a profitable way. However, we have to recognize that the priorities of developed, developing and underdeveloped nations differ. Countries that have difficulty feeding their people understandably put more emphasis on food security rather than animal welfare. Hence, the most difficult challenge to modern agriculture is to make intensive production systems 'animal friendly' and yet economically viable. In order to resolve the welfare problems of poultry and other farm animals, the industry must first see them as problems in animal agriculture. Major advances in understanding poultry welfare will come through scientific research and development. Scientists, and educators can provide reliable information to the society, and it is the society who ultimately dictate the direction and standards of poultry welfare. The decision is in our hands!

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The Global Market of Organik Animal Product – Chances and Risks

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ABSTRACT

Organic farming has a high reputation of sustainable food production chain. The global market has enchroached to over 40 billion US-Dollar. Main consumption is located in Europe and the USA but in about 135 countries of the world organic production is practices. The global market for organic livestock products is less developed than crop products. Sanitary trade restrictions of importing countries, lack of organic infrastructure (processing facilities, tracebility, inspection and certifiction, training and education, public support) and comparative cost disadvantages (mass production versus premium production) and production efficiency. It is difficult to meet the requirements of the international market for organic meat, milk and egg products and it will remain on low quantities as niche production. Nevertheless, some livestock products like aquaculture can become relevance for countries like Indonesia.

INTRODUCTION

In the last decade organic farming has left its niche and is spreading worldwide. Organic farming is a world wide harmonized concept (IFOAM standards 2005, FAO/WHO codex alimentarius 2008) to ensure the environmentally sound and socially fair production and consumption of agricultural products. Organic production is practiced in more than 135 countries (of a total of 197 countries) on 30 million hectares of land (0.7 % of total agricultural land use) by more than 718,000 farms (of a total of 700 million farms) (Table 1).

The products are mainly consumed in developed western countries – the market has a value of about 40 billion US-$ and is growing by more than 15 % annually (Organic Monitor 2008). The global organic market is attractive for developing countries to sell premium products to the developed countries.

The EU and the US are the biggest markets (97 % of the world market) with an annual growth of 10 to 20%. Tropical fruits, vegetables, coffee, tea, cotton etc. are important products exported from tropical like Indonesia to these importing countries.

The quantities of organic livestock products on the national and international markets are not known. The monetary share can be estimated be about 25%. Milk, beef and eggs are the main animal products. Pork, lamb and poultry meat have only little share on the main organic markets. Honey and fish, prawn, scrimps and molluscs can be considered as livestock products as well. 20 Mio. Ha permanent grassland is certified organic and used by ruminants. A share of the arable crops is used as well for livestock (poultry, pigs, milk production). About 4.222 ha ponts are used for aquaculture. Only aquaculture can be seen as important livestock export products from Asia (Taiwan, Thailand etc.). Other livestock products (beef, eggs, poultry meat, livestock non-food products like wool, feather and leather) are exported as well but on a very little level. High international sanitary product quality demands particularlt for arganic products are

Table 1. Organic farming in the world (2006)

	No of countries with organic farming (%)	Certified Organic Farmland in ha (% total farm land)	Certified Organic Farms No.	Consumption US-Dollar
Africa	30 (55%)	417,059 (0.05%)	175,266	0.1 billion
Asia	30 (61%)	3,090,924 (0,17%)	97,020	0.8 billion
Australia/Oceania	8 (61%)	7,389,085 (1.62%)	203,523	0.3 billion
Europe	42 (93%)	4,915,643 (0.68%)	223,277	20.0 billion
Latin America	23 (70%)	2,224,755 (0.57%)	12,064	0.1 billion
North America	2 (100%)	12,380,796 (2.70%)	7,594	17.3 billion
Total	135 (69%)	30,418,261 (0.65%)	718,744	38.6 billion

Source: Willer *et al.* 2008.

Table 2. Organic land use (ha in 2006)

Main use	Africa	Asia	Europe	Latin America	North America	Oceania
Arable land total	34,190	93,873	3,061,840	306,454	958,338	n.a.
Permanent crops	163,447	66,126	701,103	494,692	45,321	100
Permanent grass-land total	50,305	11,452	3,171,533	3,792,234	991,024	11,925,461

Source: Willer *et al*. 2008

difficult to fulfil in many countries (comparativedisadvantages). Little quantities of livestock product face high transaction costs (particularly if permanent cooling is obligatory). Australia (beef), New Zealand (lamb, milk), Argentina (beef, lamb) can be seen as main organic livestock export orientated countries. They have the relevant infrastructure and the standards demanded. USA and Japan – and increasingly Arabian countries – are the main importing countries, while the EU is self sufficient in livestock products.

The chances for countries like Indonesia for export orientated organic livestock production are limited but not zero. Aquaculture products like scrimps etc. are on increasing demand. Nevertheless, the risks of the highly sophisticated organic niche markets have to be considered.

Standards of Organic Livestock Production

Organic farming[1] is based on the idea of practices that are environmentally friendly, animal welfare oriented and geared toward improving the living conditions of farmers. To "strive for close-to-nature farming" is a central piece of the farmers' own concept.[2] The first organic standards have been defined in the mid of the last century by farmer associations (mainly in Europe) and have been harmonized troughout the organic world in 1980 in the first IFOAM basic standards (Huber et al. 2006).[3] In the mid of the 80s, Austria and France were the first countries with legal national organic standards. In 1991, the EU created the first international legal organic standard with the regulation 2092/91. Not just agriculture but even processing, inspection and labelling was considered.

Many countries followed the EU in the 90s and the FAO defined organic plant production in 1999 in the codex alimentarius. In the last 10 years many developing countries have followed to gain from the premium export opportunities. About 70 countries have own legal organic standards and more than 20 are preparing for them.

The EU, USA and Japan dominate the international organic trade (about 90 % of the international trade is done into these countries) and therefore they dominate the international organic standards.[4] Most of the organic standards are harmonized between the countries but still there are some important differences. Inspection and certification guarantees the standards of the importing countries. Worldwide, more than 460 organisations are active in organic inspection and certification. Most of them are located in the EU (37 %), Asia (31 %) and North America (18 %) (Rundgren, 2008; www.organic-world.net). Many of them are active all over the world to guarantee organic standards in international organic trade.

The EU has reformed her organic standards and replaced in 2009 the old regulation 2092/91 with regulation 834/2007 and the implementation regulation 889/2008. These regulations integrate goals for organic agriculture (834/2007 article 3). Organic production shall pursue the general objectives of establishing sustainable manage systems for agriculture, that respects nature's systems and cycles and enhances the health of soil, water, plants and animal, and the ba-

[1] The term „organic farming" is imprecise since it is used for both the production of food as well as off-farm processes (farm inputs, processing, trade, consumption).

[2] Defined by the International Federation of Organic Agriculture Movements (www.ifoam.org).

[3] A comparison of the serveral international organic standards are found under http://organicrules.org/.

[4] EU: regulation 834/2007 (be found under: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri= OJ:L:2007:189:0001:01:EN:HTML, USA: NOP (www.ams.usda.gov/nop/indexIE.htm), Japan: JAS (www.maff.go.jp/soshiki/syokuhin/hinshitu/e_label/specificJAS-organic.htm)

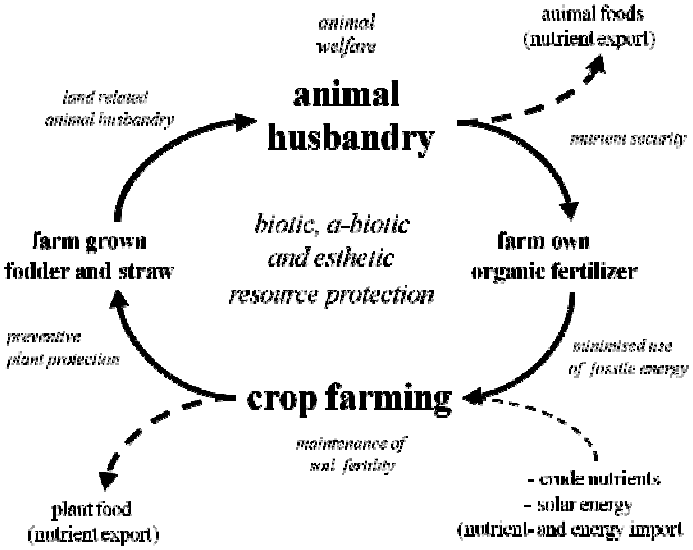

Figure 1. The Organic Farming Model

lance between them. A high level of agricultural and natural biological diversity is also target as responsible use of energy, water, soil, organic matter and air. Animal welfare is of major importance. The products shall have high process and product qualities.

The overall principles (834/2007, article 4) of organic production shall follow an appropiate design and management of biological processes and ecological systems using natural resources. Livestock has to be land-related and integrated into a crop system

Table 3. Differences between conventional and organic anima

	Conventional	Organic (834/2007)
Breeds, origin	Highly performing special breeds and cross-breeds according to product aimed for	Only animals reared on organic farms, diversity of breeds, sometimes rare breeds of working animals
Keeping (buildings and free runs)	Animal protection laws (requirements for keeping of animals according to species)	Special requirements for keeping of animals orientated towards animal welfare (occupation-density, size of buildings, keeping tied inside the stable forbidden, etc.)
Feeding	According to current food stuffs legislation (permitted food additives such as enzymes, synthetic amino acids, etc.)	Food stuffs produced as much as possible on site, feeding rations according to animal welfare (e.g., minimum use/parts of roughage) only specifically permitted additives, no synthetic amino acids, no genetically modified organisms
Management and treatment	Managed breeding, if necessary stable-wide prophylaxis, legally required waiting periods according to drug prescription law	No prophylaxis (exception: legally required inoculations), only two allopathical treatments per year, double the waiting period after use of drugs, minimum 48 h. Restricted interfering with the animals´ integrity (removal of horns, shortening of beaks, shortening of teeth, docking of tails etc.)
Transportation	Animal-transport regulation	Animal- transport regulation, short transport ways aimed for

(see figure 1). GMOs and products produces by GMOs are not allowed on any stage and purpose (except veterinary products). Therefore organic principles base on preventive measures and risk assessment. External inputs are limited and shall not harm the environment.

Further on, in 834/2007 in article 14 the livestock production rules are defined more detailed. Organic livestock shall be born and raised in the organic system. Only organic feed is allowed and shall come primarily from the farm. All warm blood livestock shall have access to pasture or roughage. Conversion periods as well as housing and out-door space as well as stocking densities are defined for all livestock species. Organic livestock can not be kept with conventional livestock together. Tethering and isolation of livestock is prohibited, duration of transport shall minimized. Mutilation and suffering of livestock is not allowed during the entire live (including slaughter). Veterinary treatments and drugs as well as desinfection methods are restricted and natural measures are favored, cloning and embryo transfer are not allowed. Special standards are made for beekeeping and aquaculture animals (article 15).

Organic Trade

Long time, organic products have been only sold locally or in closed communities. This changed in the 90s, as organic products became protected labels and started to offered in supermarkets and other ordinary market places. National and international trade of organic products started and increased significantly in the last 20 years. Some countries are mainly exporters (developing countries, Australia, etc.).

Some countries are mainly exporters (developing countries, Australia, etc.), others are the countries of consumption (USA, EU). In the EU many farmers produce organically and can deliver a big share of the organic demand. This is different in the USA and Japan, where domestic products have a smaller share of the national organic market. Approximately 75 % of the international organic trade goes into the USA and Japan, 20 % into the EU and 5 % somewhere else (e.g. Arabic countries) (own estimations). Clear statistics of organic sales are available from the EU and USA markets, but not about the origin of the products.[5] There are no clear statistics about organic world trade and animal products trade. The International Trade Centre (ITC) tries to get better overview.[6] Annually ITC presents the latest figures at the Biofach.[7] Nevertheless, the chances and risks of the international trade of organic animal products can can be qualified.

Milk Products

Milk is a high valuable product. In Europe the farmers get about 0.14 Euro more than conventional farmers. With the low conventional milk prices (0.20 Euro/kg) this is significant. The production impact going organic is about 20% less milk than in comparable conventional farms. Therefore milk production is profitable as long as organic feed is available. Therefore dairy production is found in adjacent areas of organic crop production and close to the consumers (northern Europe, south west USA). Milk products are used for fresh milk, cheese and other processed products. Certified organic dairy plants are mainly found in countries of consumption. Even New Zealand has little organic milk because of the market distance (mainly milk powder is relevant for New Zealands dairy exports, this needs mass production). Milk has very high hygenic standards and is difficult to fulfil in areas with mainly small scaled farming systems and less developed infrastructures.

Eggs Products

In Europe the ban of cage keeping of chicken has an impact on egg production.

[5] For an worldwide organic overview see http://www.fao.org/organicag/en/ and under http://www.organic-world.net. For the EU you can find information under http://ec.europa.eu/agriculture/organic/home_en. An overview of the US market is found under http://www.ota.com/organic/mt.html. A wide selection of more than 10.000 organic publications can be found under http://orgprints.org/.

[6] ITC is a joint technical cooperation agency of the United Nations Conference on Trade and Development (UNCTAD) and the World Trade Organization (WTO). All statistics and publications of ITC are free available and can be downloaded from www.intracen.org/dbms/organics/index.asp.

[7] Biofach ist the world biggest organic trade fair with more than 2,700 exhibitors from all over the world, held every February in Nuernberg, Germany. Satellite fairs are held irregularly in China, Japan, India, Brazil and USA (www.biofach.de/en/).

The big producer see an option in organic fresh egg production and the production does increase significant in the last years. Organic eggs get about 50 % (0.18 Euro) more than conventional eggs, but the production costs are 50 % higher as well. The variable production costs are high (organic feed). Large units (10,000 – 100,000 layers, splitt into 3,000 layer units) become more populare to reduce the fix costs (stable, labor, logistic). High sanitary restriction (SARS, Salmonella, etc.) and difficult organic standards as well as availability of organic feed hinder international trade. As long as industrial-intensiv conventional egg production is not restricted, the pomparative production advantage is much higher than organic production.

Honey Products

Honey is an excellent product for global trade. The production costs are relatively low, the problem of environmental pollution (Europe, China) and GMO-crop areas (Northern and Latin America) limit the honey production in Europe. Non-polluted – mainly remote areas in Africa, Southern America, Asia) have production advantages. If special qualities (tastes, fair trade, environment protection, pollination) can be labeled, the market potential is high.

Meat Products

Beef and lamb have the main importance in organic meat production. Grassland areas like Australia, New Zealand and Argentina have cheap natural resources to feed beef cattle and sheep and can fulfil organic standards without big changes and differences compared to conventional production. The added value on the market is about 20%. As long as stables are not necessary (semi-arid, arid environments) and water not limited, production costs are low. In areas with organic milk production, beef is a co-product and mainly done in Europe. If stable are necessary the production cost are much higer than beef production in out-door grazing areas. Organic feed and more in-door space for livestock are the main costs. This is the reason of the expensive production of pork and poultry meat, the market share is very low. Compared with conventional pig and poultry systems the production costs are much higher and the added value must be 100% - this is difficult to get on the market. Organic meat production demands certified processing

Table 4. Estimation of chances and risks of international organic trade

Product	Important export countries	Important import countries	International trade chances	International trade risks
Cash crops	Developed countries	USA, EU, Japan	+++	--
• herbs, fruits, vegetables, coffee, tea, cocoa	Developing countries (Africa, Asia)	EU, USA, Japan	++++	-
• grain, potatos, oil fruits, pulses	Developed countries, Latin America	USA, Japan	++	---
• Non-food products (e.g. fibre, wool, wood)	Developing countries	EU, USA	+	--
• Processed crops (e.g. wine, cosmetics, spices, dried fruits)	Developed countries, Africa	USA; EU	++++	-
Livestock food products	Developed countries	USA, EU, Japan, Arabic countries	++	----
• Dairy	New Zealand, EU	USA, Japan		----
• Meat	Australia, Argentina, Brazil, Mexico	USA, Japan	+	--
• Eggs	EU	USA	+	----
• Honey	Latin America, Africa, Asia	EU, USA	++++	-
• Aquaculture	Asia, Latin America	EU, Japan, USA	+++	--
• Non-food (e.g. wool)	Oceania, Argentina	EU	++	-

Note: +/- = low, ++/-- = fair, +++/--- = high, ++++/---- = very high.

facilities (e.g. abbatoirs). International sanitary standards are difficult and have to be certified for organic production as well. Newcomers on the global markets need large quantities and high qualities. This is difficult for small scaled farming systems.

Aquaculture Animals Products

Aquaculture will be a big chance for countries like Indonesia and other countries in Asia, which have high skills in fresh water aquaculture. Recently, the global market for organic scrimps, mollusce, crustacee, fresh water fish is relatively small but growing fast. The environmental conditions of fresh water ponts are important for production. Research and development is necessary to develop efficient aquaculture with high organic standards.

Non-food Products

International trade in non-food livestock products is very little. Nevetheless, organic fibre (wool, cashmere, mohair), fir, leather, organic fertilizer (manure, horn, feather), silk, earth worms (improvement of compost and vegetable production), insects (protein feed), cosmetic ingredients (linolenic, fat etc.) are getting more importance. These markets can be developed by countries with special production advantages.

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Future of Domestic Ducks in Rice Field

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ABSTRACT

According to FAO statistics Asia is the most duck meat production area of the world, whereas Southeast Asia stands at the 2nd rank after China. The major form of duck raising in the area is free ranging type with very low investment; requiring no feeding cost, no growth promoter, no specific medication and no complicated management system. Duck feeds are channeled apple snails (*Pomacea canaliculata*) and left-over rice after harvesting in the rice field. A few serious health problem of those ducks are duck plaque and fowl cholera, which could be prevented by conventional vaccination. Before Avian Influenza epizootic occured in 2004, rice farmers were beneficial to such duck raising system, because ducks helped reducing snails damaging their rice production. Such ducks and production system reflect long genetic development of the animal and wisdom of local people. After H5N1 had broken out in Asia from industrial chicken together with ineffective regulatory control, domestic ducks were sometimes interpreted as natural reservoirs of AI virus. The future of those free ranging ducks in rice field is being shaken, as long as no proper disease control guideline for such ducks is available.


Oral
Presentation

Effect of Mung bean as Local Feed Ingredients to Substitute Soybean Meal in the Diet on the Performance of Broilers

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ABSTRACT

In response to feed antibiotics ban and feed security, a research was carried out to study the effect of local feed ingredients in the diet based on corn or sorghum in combination with soybean meal or mung beans on broilers performance. A completely randomized design with 4 treatments and 5 replicates was employed. Each replicates consisted of twelve birds. Two hundreds and forty day old broiler chicks with initial body weight of 45. 85 ± 3.01 gr were randomly assigned into four treatment diets i.e. D-1 (diet based on corn and soybean meal), D-2 (diet based on corn and mung bean), D-3 (diet based on sorghum and soybean meal), D-4 (diet based on sorghum and mung bean). Is energy and is protein diet and water were given *ad labium*. Body weight, feed consumption, feed conversion ratio, apparent metabolizable energy (AME), protein digestibility, and protein efficiency ratio were measured and determined on day 35. All data were analyzed by ANOVA, and Duncan's multiple range test was conducted when means were significantly different ($p<0.05$). The results showed that local feed ingredients in the diet affected significantly all performance parameters of broilers except for protein digestibility. Diet-1 had the highest body weight and AME. Diet 2 and 4 had similar AME and lower than Diet 1 and 3. Diet 1, 2, and 4 had similar feed conversion ratio. Diet-3 had the lowest consumption and feed conversion ratio and the highest protein efficiency ratio. However, considering the ability of mung bean to substitute imported soybean meal, it can be concluded that Diet-2 or Diet-4 with similar feed conversion to Diet-1 can be used as local feed ingredients to substitute imported soybean meal.

Key words: local feed, sorghum, mung beans, soybean meal, broiler, performance

INTRODUCTION

Feed ingredients accounts for approximately 70% of total cost in broilers production. Corn and soybean meals are the most common feed ingredients used in broilers diet worldwide including in Indonesia, and such diet creates global dependency and demand on "single" feed ingredients. Alternatives to corn and soybean meal have been suggested, encouraging diversity and flexibility in feed formulation and feed resources to sustain local poultry production (Utomo, 2009; Leary, 2009; Sodiq, 2009).

One of locally available vegetable protein is mung bean which are abundantly available in Central Java during the harvest season. Protein content of mung bean varies from 22-25% and it can be used as feed ingredients (El Khimsay *et al.*, 1998; Indriani and Murwani, 2005). Other locally available grain is sorghum which has been well studied to substitute for corn and contains a potent antioxidants poliphenolic tannin (Awika and Rooney,2004; Awika *et al.*, 2000; 2003; Hikosaka *et al.*, 2006).The use of locally available feed grains such as corn, sorghum, or mung beans provide not only macro- and micronutrients but also other bioactive nutrients such as carotenoids in corn and mung beans, and poliphenols in sorghum and mung beans.

The antioxidant and immune-modulating properties of carotenoids and poliphenols have been well demonstrated. They can affect immune response by protecting against oxidative stress and lipid peroxidation, improving humeral and cellular immune response indicated by increase in B and T cell proliferation (Bendich, 2004; Chew and Park, 2004; Scalbert *et al.*, 2005; Hikosaka *et al.*, 2006). Such naturally occurring bioactive nutrients in feed ingredients such as mung bean and sorghum

in the diet conceivably could also exert immuno-modulating function *in vivo*. Several studies using such local feed ingredients have been shown to improve antibody titers against NDV vaccination (Murwani, 2008a; 2008b; Murwani and Murtini, 2009) and provide relevance approach in response to in-feed antibiotic ban. However such application pose a new challenge to obtain optimal or best performance.

The following study was carried out to investigate local feed ingredients i.e. corn or sorghum in combination with mung bean to substitute soybean meal in the diet on the performance of broilers. The results of this study added weight in the importance of optimizing broilers diet based on local feed ingredients to substitute competing protein source such as soybean meal, MBM, PMM, and CGM in broilers diet and may contribute to local feed empowerment.

MATERIALS AND METHODS

Birds and diets

All feed ingredients were obtained from local feed producers except for soybean meal which was obtained from commercial feed producers. Corn, sorghum and mung bean were obtained in grain form with moisture content around 10 to 11%. These feed ingredients were ground separately and stored in clean water tight plastic drum until mix. They were also checked for the presence of mycotoxin under UV light and no mycotoxin was detected.

A total of 240 Ross CP 707 one day old unsexed broilers with body weight 45.58 ±3.01 g were used in this experiment. They were given free access to sugar containing water at their arrival. The experimental chicks were then selected randomly and assigned into 4 large groups in floor pen (in a warm brooder) with 50 birds in each group so that each group had similar average body weight. The groups were given the following treatment diets : D1 (diet based on corn and soybean meal), D2 (diet based on corn and mung bean), D3 (diet based on sorghum and soybean meal), D4 (diet based on sorghum and mung bean). Mung beans were used to replace soybean meal (Table 1). Local fish meal and rice meal were added to complete and formulate the diets so it meets nutrient requirement for broilers. Chicks were given *ad libitum* access to the diet and drinking water. Antibiotic-free vitamin and mineral mixture were given through drinking water and diet respectively to meet micronutrients needs (Murwani, 2008a; 2008b). On day 7, the birds from each large group were further allocated randomly into 5 replicates with 12 chickens in each replicate. Birds were vaccinated with commercial NDV La Sota vaccine (Medion) on day-4 via eye drop and on day-21 intramuscularly (inactive vaccine). The dose and vehicle of vaccine were used according to instruction sheet. Intramuscular route was given by technical personnel from Local Veterinary

Table 1. Composition and Nutrient Contents of Experimental Diets

Ingredients (%)	Diet 1 (D1)	Diet 2 (D2)	Diet 3 (D3)	Diet 4 (D4)
Corn	43	12	-	-
Sorghum	-	-	44	11.5
Soybean meal	21.5	-	22	-
Mung-beans	-	50	-	50.5
Rice meal	20	20	13.5	19
Fish meal	15.5	18	20	19
Coconut oil			0.5	
Nutrient contents:				
Crude protein[3] (%)	20.10	20.06	20.04	20.04
Crude lipid[3](%)	4.92	4.07	4.39	3.85
Crude fiber[3] (%)	2.91	3.27	2.81	3.26
Metabolic energy (Kkal/kg)[4]	2980.1	2980.8	2967.9	2966.9

[1] Vitamin contents per kg vitamin mix (Medion) : 6000000 IU vitamin A, 1200000 IU vitamin D3, 2.5 IU vitamin E, 3 g vitamin K, 2 g vitamin B1, 3 g vitamin B2, 1 g vitamin B6, 2 mg vitamin B12, 20 g vitamin C, 15 g Nicotinate acid, 5 g Ca-D Panto-thenate, 750 g Na, Ca, K and Mg electrolyte. This mix was used at 1g/l drinking water according to recommended instruction.

[2] Mineral contents per kg mineral mix (Medion): 32.5% Ca, 10% P, 6 g Fe, 4 g Mn, 0.075 g I, 0.3 g Cu, 3.75 g Zn, 0.5 g vitamin B12, 50000 IU vitamin D3. This mix was used at 20 g/kg diet according to recommended instruction.

[3] Based on feed composition table (Hartadi *et al*., 1986) and verified by proximate analysis.

[4] Based on feed composition table (Hartadi *et al*., 1986).

Health Office with automatic injector so that each bird received the same amount of NDV vaccine. The experiments were performed in an open broiler-house at the Faculty facilities with similar condition as that used by most small to medium scale broiler chicken producers in the region (Murwani and Bayuardhi, 2007).

Determination of Broilers Performance

Body weight, Feed Consumption, and Feed Conversion. Birds from each replicates were weighed weekly on 5 kg scale. Feed consumption was recorded everyday by subtracting the amount of feed given per day (*ad libitum*) to each replicate birds with the amount remained. Feed conversion ratio was calculated from feed consumption divided by final body weight.

Metabolic Energy

Apparent Metabolizable Energy (AME) values of each treatment diets were determined in a classical energy balance study involving measurements of total feed intake, total excreta output during feeding, and subsequent measurement of gross energy values of feed and excreta by bomb calorimeter. As the treatment diets were fed from day one, the excreta for AME values were collected for 24 hr. Prior to excreta collectio birds were fasted to void the excreta fron previous feeding. Gross energy values o dried excreta and feeds were measured with a Parr bomb calorimeter (Parr Instrument Company, USA). The AME of treatment diets were calculated by subtracting the total energy intake from feed with the energy of excreta.

Protein Digestibility

Protein digestibility was calculated from the following equation : (N in feed – (N in excreta- N-endogenous))/ (N in feed) x 100%. N in feed and excreta was analyzed by Kjeldahl method.

Protein Efficiency Ratio (PER)

PER was calculated by dividing body weight to protein consumption. Protein consumption was obtained from feed consumption X protein content in feed.

Statistical Analysis

A completely randomized design with 4 treatments and 5 replicates was employed. All data were analyzed by ANOVA, and Duncan's multiple range test was conducted when means were significantly difference ($p<0.05$).

RESULTS AND DISCUSSION

Figure 1 showed that treatment diets affected significantly feed consumption ($p<0.05$). Broilers with corn-soybean meal base diet (D1) had the highest average feed consumption followed by corn-mung bean (D2), sorghum-mung bean (D3), and the lowest one was sorghum-soybean (D4). However corn-soybean meal base diet was not significantly different to corn-mung bean, but significantly higher than sorghum-mung bean and sorghum- soybean base diet ($p<0.05$). This data indicated that corn base diet either combined with soybean or mung bean had no effect on feed consumption. However sorghum base diet combined either with soybean or mung bean significantly reduced feed consumption. Reduced consumption in sorghum base diet (D3 and D4) led to the effect of tannin containing sorghum.

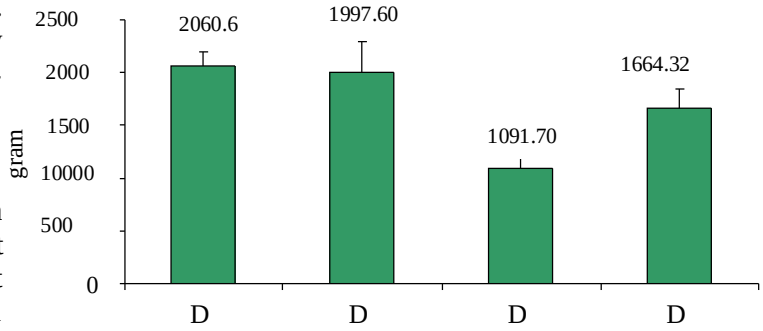

Figure 1. Feed consumption of broilers

Tannin sorghum has been well known to reduce feed consumption and body weight gain, reduce intestinal absorption of sugar and amino acids, and increase liver and protein catabolism (Marzo *et al.*, 2002). Tannin sorghum in the diet at 1% or 2.5% equivalent to tannic acid resulted in reduce body weight gain and liver protein synthesis and significant increase in liver proteolysis activity (Fuller *et al.*, 1967; Badawy, *et al.*, 1969; Marzo *et al.*, 2002). On the other hand raw red sorghum could be used up to 33% (0.475% tannin) in the diet replacing 50% corn with no significant difference in body weight gain and feed intake (Mandal *et al.*, 2005, Ku-

mar *et al*., 2005, 2007). Tannin contents in sorghum-soybean base diet (D3) and sorghum-mung bean (D4) in our experiments were approximately 0.43% and 0.13% respectively. These level of tannin were lower than previous findings, however it had affected significantly on feed consumption. The lowest consumption was found in sorghum- soybean based diet in which tannin content from sorghum was highest i.e. 0.43%. Tannin can interact with salivary protein leading to astringency which resulted in low palatability and hence low consumption (Nyachoti *et al.*, 1996; Bennick, 2002). Furthermore, sorghum base diet can increased the requirement for essential amino acids such as lysine and methionine. In high tannin sorghum, lysine was the first and methionine was the second limiting amino acid showing decrease availability of essential amino acids when tannin content in sorghum increased (Ebadi *et al*., 2005). There was also an increase of endogenous loss of these essential amino acids by tannic acid (Mansoori and Acamovic, 2006). Our results support this possibility as we did not add essential amino acids i.e. lysine and methionine in our experimental diets which could led to essential amino acid imbalance.

The combination of sorghum-mung bean or sorghum-soybean meal may also increase the requirement for micronutrients such as calcium. Dietary tannins have been shown to transiently alter apparent calcium absorption (Chang *et al*., 1994). Mung bean was known to contain antinutrients such as phytic acid and other phenolic substance which can bind to minerals such as calcium, leading to reduce availability of this mineral absorption (Chitra *et al*., 1996, Mubarak, 2005). Such reduce availability of calcium in mung bean and sorghum or their combination in broilers diet could increase mineral requirement which could not be met by the mineral mixture added in the diet. In overall, reduce feed consumption is a consequences of anti nutrient content, low availability of essential micronutrients, as well as nutrients imbalance.

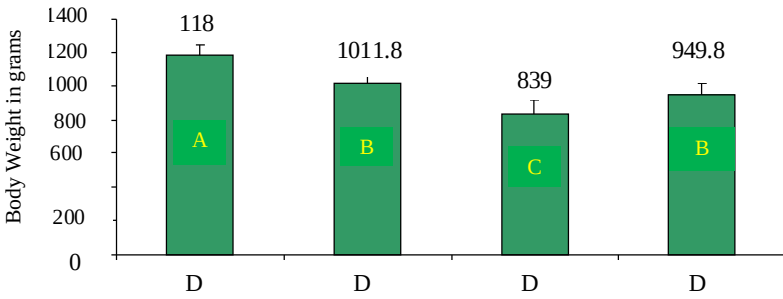

Figure 2. Body weight of broilers at day 35

Reduce feed consumption in broilers fed sorghum base diets i.e. D3 and D4 was followed by similar pattern of reduce body weight (Figure 2). Reduce body weight therefore was a consequence of reduce feed intake, as body weight was a reflection of muscle or protein synthesis in broilers. All factors affecting reduced feed consumption and apply for reduce body weight. The amount of major nutrients, low availability and provision of limiting amino acids and calcium were important for protein accretion led to decrease in body weight.

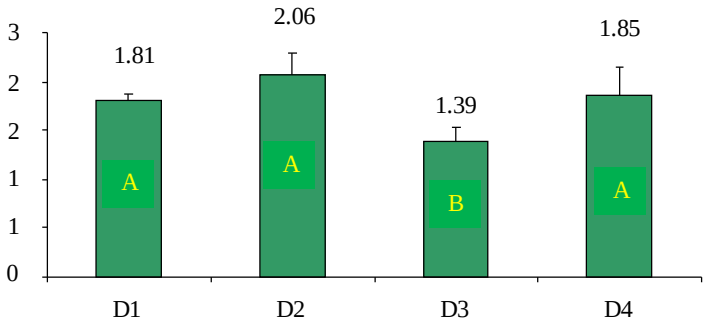

Figure 3. Feed Conversion of broilers until day 35

Interestingly, broilers fed sorghum-soybean base diet (D3) had the lowest or best feed conversion ratio, followed by the other three treatment diets which were not significantly different to one another (Figure 3). This FCR may reflect birds attempted to compensate nutrient imbalance by lowering feed intake and effectively use it for growth. Therefore the lowest feed consumption in D3 divided by final weight which was also lowest of all treatment produced the lowest FCR.

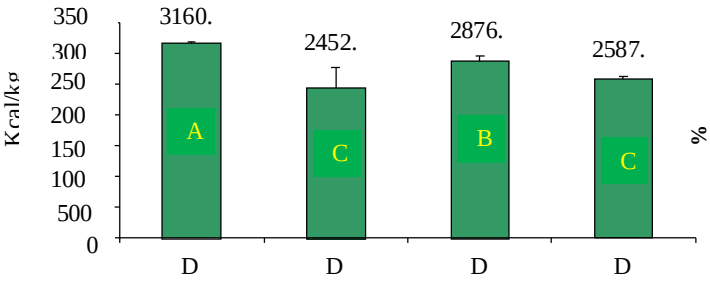

Figure 4. Metabolic Energy in broilers at day 35

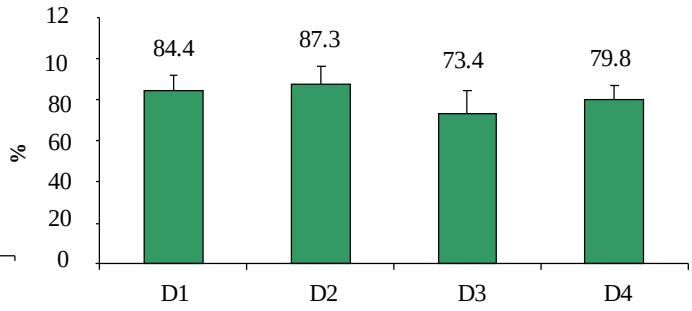

Figure 5. Protein Digestibility in Broilers until day 35

AME was significantly affected by treatment diets, with corn-soybean meal base diet (D1) having the highest value followed by sorghum-soybean meal, and the lowest value in corn-mung bean and sorghum-mung bean base diet. These results indicated that the use of mung bean to substitute soybean meal in broilers diet resulted in lower AME value. As AME value represents energy derived from feed that can be used for broilers need, this result indicated that the energy derived from mung bean containing diets (D2, D4) were lower than soybean containing diets. Lower AME from former diets could be due to lower digestibility of nutrients in mung bean associated with disturbance of gastro-intestinal enzymatic activities. This disturbance could be due to antinutritional contents in mung bean such as antitrypsin, phytic acids and poliphenolic compounds which can interfere with the action of digestive enzymes. However, such anti nutritional compounds were also found in sorghum, but AME value in sorghum-soybean base diet (D-3) was higher compare to sorghum-mung bean base diet, inspite of the fact that the percentage of sorghum in D-3 was higher than D-4. This led to the possibility that there was indigestible constituents in mung bean which can not be digested by the host digestive enzymes. This possibility is supported by several studies which showed that mung bean contained indigestible carbohydrates which could certainly lower its digestibility (Machaiah *et al.*, 2005; Mubarak, 2005; Aman, 2006).

Protein digestibility determination showed that there was no significant difference among treatments. This result indicated that from overall protein content in each diet which came from vegetable protein either soybean meal or mung bean together with animal protein i.e. fish meal provided similar digestibility. However, it should be noted that the numerical value of digestibility in sorghum-soybean meal based diet was lowest followed by sorghum-mung bean based diet, although not statistically different. This must be born in mind when future optimize is to be made.

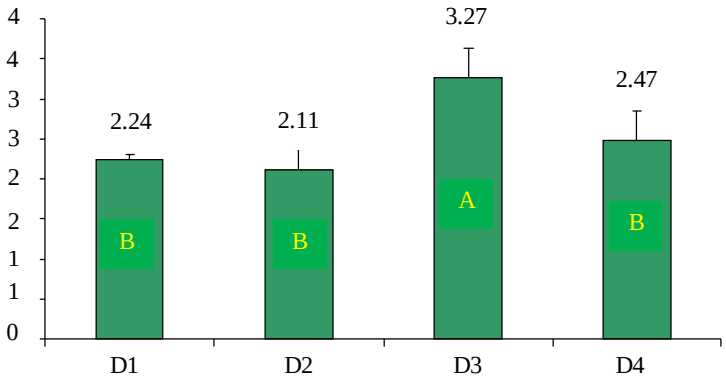

Figure 6. Protein Efficiency Ratio of broilers at day 35

Protein efficiency ratio determination showed a similar pattern as FCR. This again indicated that broilers had made attempts to efficiently use protein intake into muscle protein accretion in spite of antinutrient effect of sorghum in diet D3 which is higher than D4. From all the data described and discussed above we can conclude that mung bean can be used to replace soybean meal in corn-mung bean or sorghum-mung bean base diet with important note. As final body weight and AME of corn-mung bean or sorghum-mung bean base diet are still lower than corn-soybean meal base diet, this problem must be solved and the possible

source of this shortcoming must be found. On the basis of this shortcoming, our recent attempt to optimize mung bean as substitute for soybean meal have made a good progress where body weight gain of corn-mung bean base diet was nearly the same as the body weight of broilers fed commercial diets (Murwani, 2009).

ACKNOWLEDGEMENT

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Cattle Integration in Oil Palm Plantation through Systematic Management

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ABSTRACT

The oil palm industry in Malaysia has expanded rapidly from 60,000 ha in 1964 to 4.49 million ha in 2008. More than 80% of the matured areas may provide vast opportunity for integration with livestock. This is to maximize the utilization of such production resources as feeds, land and workforce. Cattle integration in oil palm plantation offers one of the best options to increase local beef and dairy supply. Studies and observations on cattle-oil palm integration have shown promising benefits in terms of savings in weeding and labor costs, as well as improved biological and agro-ecosystem impact. A case study on systematic management for the integration of cattle into oil palm was conducted at Sawit Kinabalu Sdn Bhd plantations. The objective of the study is to evaluate the effect of systematic management of cattle integration in oil palm plantation on labor requirement and weeding cost. The study comprised of data collection from participating plantations with regards to maintenance, labor cost, chemical/herbicides usage and yield. The results showed that the integration of cattle into oil palm through systematic management is sustainable. The results also indicated that cost savings in maintenance, labor requirement and labor cost can be achieved.

Key words: cattle integration, oil palm industry, weeds, systematic management

INTRODUCTION

Sustainability of Palm Oil Industry

The world's palm oil production was 36.85 million metric tons (USDA Report, 2007) and palm oil is the main commodity for Malaysia. It has been the most significant agriculture sector that generates around 30% of the Malaysian economy. Both Malaysia and Indonesia are the two leading palm oil producers in the world with an estimated planted area of 4.48 million hectares in Malaysia (MPOB, 2008) and 6.07 million hectares in Indonesia (USDA Report, 2007).

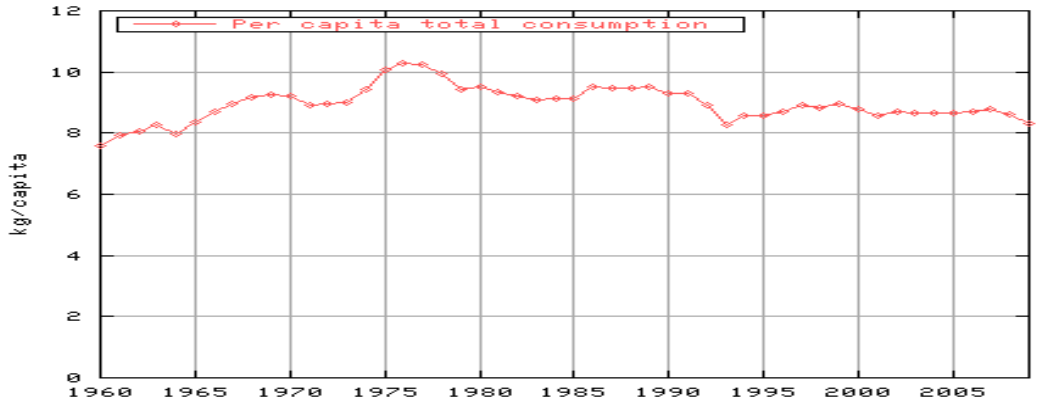
The year 2000 has witnessed the most difficult and challenging year for the industry when surplus stock had caused the commodity price spiraling down below production cost. Further speculation and the environmental issues of global warming have serious negative impact to the industry sustainability. Several measures and initiatives have been taken by Malaysian government to stabilize the situation through Malaysian Palm Oil Board, Malaysian Palm Oil Council and the plantation sector. Among others is the replanting directive to lower down the stock and to diversify its utilization such as bio-fuel.

The raid expansion of oil palm plantation has leads to single or mono cropping land utilization. This will predispose the commodity to biological and economic risk. Alternative approaches must be offered to minimize the risk and simultaneously utilize the plantation area towards resource maximization. One of which is through systematic integration of livestock into the oil palm plantation area.

Beef Supply

The increasing world population has affected food self sufficiency level including red meat. Figure 1 below shows the world's per capita consumption from 1960 to 2005 at the range level 7.8kg to 8.5kg (USDA, 2007).

In Malaysia the per capita consumption of beef has increased from 4.3 kg in 1995 to 6.7 kg in 2005, and projected to further increase to 8.4 kg by 2010 (Table 1). Even though the self sufficiency level in steadily improved from 19.2% (1995) to 22.5% (2005), the imported beef into Malaysia from Brazil, Australia, India, New Zealand, Thailand and China has continually increased from 85,277mt to 102,304mt in 2007 (Malaysia Veterinary Department, 2008).



Source: USDA (2007).

Figure 1. Per capita total consumption of beef

Ruminant Industry in Southeast Asia

Large ruminant particularly cattle and buffalo is an essential historical component of the agricultural sector in South East Asia. They have been providing draught power for agriculture activities, mean of transport and source of meat. Increasing meat consumption requires bigger ruminant population, hence higher forages requirement to feed them. Remenyi and Mc William (1986) suggested the need for doubling of forage supply for the livestock, and one obvious source of naturally occurring forage and of land for improvement of forage supply is the area under plantation crops. The presence of a range of perennial tree crops in many of the countries of South East Asia provides a common platform for development of integrated system involving ruminants. Production system integrated with perennial tree crops like coconut, rubber, oil palm and fruits as well as the use of available agro-industry by-product.

Cattle Integration in the Oil Palm Plantation

The integration of cattle in oil palm is a form of mixed farming where the combinations of the two commodities can be synergized in order to optimally utilize the same piece of land. The two commodities, when properly integrated can contribute towards sustainable food production system. They are 60 to 70 species of undergrowth under oil palm plantation consisting of planted leguminous cover crop, naturally growing grasses, broad leaves and ferns. These are considered as weeds that need to be controlled periodically with either chemical spray or manual slashing. These are potential feeds source as the yields, palatability and nutritive values are adequate for cattle. Under appropriate conditions and systematic management, cattle can be effectively used for weed control. The use of cattle as a biological weed control mechanism in oil palm plantation allows the establishments of a harmonious relationship between cattle, the undergrowth and oil palm. Reduced herbicides usages are environmentally healthy, and simultaneously help to reduce total weeding cost through lower volume of chemical use and reduced and extra labor. Reduced herbicides usage means reduced maintenance cost and less environmental contamination and pollution (Azid, 2004).

In Malaysia, 39.2 million hectares of the

Table 1. Projected and self-sufficient level of beef in Malaysia (1995 – 2010)

	1995	2000	2005	2010	Average annual growth rates (%)			
					1995-2000	2000-2005	2005-2010	1995-2010
Projected total demand	88	122.5	172.3	240	6.8	7.1	6.9	6.9
Per capita consumption (kg)	4.3	5.3	6.7	8.4	4.3	4.8	4.6	4.6
Self-sufficiency level (%)	19.2	20.8	22.5	24.4	1.6	1.6	1.6	1.6
Forecast production ('000 tones)	16.9	20.3	23.9	28	3.7	3.3	3.2	3.4

Source: Ministry of Agriculture (2006).

oil palm plantation areas are suitable for integration (MPOB, 2009). Samsudin (2002) and Harun (2003) as sighted by Azid (2004) reported that by implementing the integrated system, the estates manage to save between **30 to 60%** of the maintenance cost especially on herbicides. Rosli (2000) concluded that the systems will also improved productivity per unit area of land and it may also contribute positively to local beef production.

Systematic Management

Systematic management is the philosophy of management that evolved in response to new needs, later to be labeled systematic management, promoted rational and impersonal systems in preference to personal and idiosyncratic leadership for maintaining efficiency in a firm's operation (Yates, 1989).

Systematic management of cattle integration in oil palm plantation defined as a management of cattle that integrated with oil palm where the objectives are to maximize the land used through the optimal use of resource and also to control the weeds through biological control with the cattle integration system. This involved the strategic insertion in the activities of cattle integration without disturbing the estate operation such as harvesting, manuring maintenance work and other related estate operations.

The integration system with the major crops was not a new concept and has been practiced when the coconut was introduced in Malaysia (Sani *et al*., 1993). Cattle in the oil palm have existed since the establishment of plantation crop in the country. Traditionally cattle production was managed through open grazing system. The cut and carry system using coconut by product, tapioca waste, banana waste and *Imprerata cylindrica* and weeds has been practiced earlier.

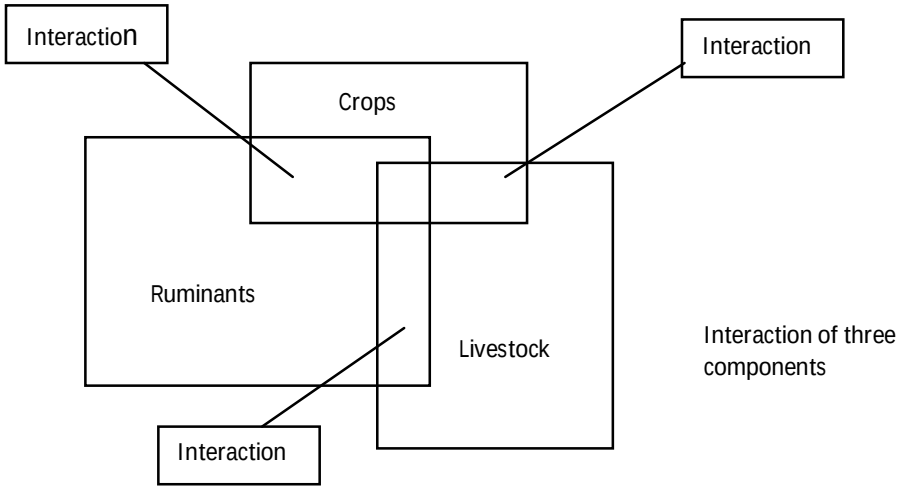
Types of Ruminants-Oil Palm Interaction

There are many benefit of crop-animal-soil interaction (Devandra and Thomas, 2002). The following interactions are common, almost all of which result in tangible benefit:

i. Benefit effect of shade and available feeds on livestock.
ii. Draught animal power on land preparations and effect on crops growth.
iii. Beneficial effect of dung and urine on soil fertility and crops growth
iv. Used of crop residue and agro-industrial by product (AIBP) from tree in situ.
v. Use of native vegetation and effects on cost of weed control, crops management and growth.
vi. Type of animal production system

Table 2. Comparison between non-systematic and systematic management of cattle integration in oil palm plantation

No.	Non-systematic management	Systematic management
1.	The cow owner and estate worker left the cow graze in the oil palm grazing area without realized the cattle received insufficient ruminants (Hadi, 1998)	The estate management will ensure the cattle will get sufficient ruminants supply by using the portable electric fence (Rosli, 2000)
2.	The cow owner has sent the cattle to the same place of grazing which could cause the soil compaction.	Through the "cattle smart system", cattle only graze at one piece of land four times in a year and minimal compaction (Zainuddin, 2008)
3.	The cattle were exposed with the disease such as mainly brucellosis in the oil palm plantation.	The systematic management will carry out the health management system to ensure free from disease such as brucellosis, tuberculosis and leptospirosis (Rosli, 2000).
4.	The cattle was no permanent identification	The cattle have the permanent identification
5.	The inbreeding was always occurred in non-systematic cattle rearing.	The management will always do the selection after grazing rotation completed. The green looting will be carried out to avoid inbreeding.
6.	Normally the owners or the farmers reared the local breed.	The estate management will rear the imported breed.



Source: Rosli (1998)

Figure 2. Illustration concept of crop-livestock integration

The interactions can be positive or negative depending on types of livestock and trees, age of trees and management system. Cattle are well suit to integration with tree crops such as coconut and oil palm. However sheep, cattle and buffaloes are not suitable with rubbers as they caused damage to tree bark and disturb the latex collection cups.

Forage Supply in Oil Palm Plantation

Under oil palm plantation, light penetration allows forages or 'weeds' to grow and there are more than 50 species identified (Wong and Chin, 1998). The common weeds as *Ottochlora nodosa*, *Axonopus compresses*, *Mikania micranta* and *Asystapia intrusa*. The Table 3. below shows their nutrient values.

Objective of Study

The objective is to evaluate the effect of systematic management of cattle integration in oil palm plantation on labor requirement and weeding cost.

Data and Methodology

This study is based on analysis of secondary data. Discussion with Sawit Kinabalu Farm Products Sdn Bhd was held to get more clearer picture of the model and the operation of integrating cattle under oil palm. The

Table 3. Nutrient value contain from the natural forage in Malaysia

Location	Species	Nutrient Contain	
		Raw protein	Raw Fiber
Rubber plantation	Weed	11.4	33.1
	Fern	13.4	27.2
Oil palm	P. conjugatum	15.8	-
	A. compressus	13.0	-
	O. nodosa	16.8	-
	I. cylindrical	8.7	-
	N. biserrata	18.2	-
Open shade	A. compressus	7.5	30.0
	P. conjugatum (4 weeks)	13.6	26.3
	Guinea (4 weeks)	12.4	33.8
	I. cylindrical	11.7	32.0
	Asystasia intrusa	15.8	35.8
	P. phaseoloides	22.8	33.5
	C. pubescens	25.4	35.7

Source: Mustapa (1983) dan Chin (1991).

any adverse effect to existing palm oil production (Azid, 2008). The integration model comprises of pilot project and feasibility study which incorporate two important strategic mechanisms: ***strategic insertion*** and ***strategic rotational grazing management***.

RESULTS AND DISCUSSION

Cost Saving in Weeding and Labour

The cattle integration in the oil palm plantation can reduce the cost of weeding and labor. Weeding is still carried out as not all the weeds grazed by the cattle such as *Clidemia hirta Melastoma malabatricum*, *Pennisetum spp*. and other brushes. Chong (2001) observed significant 60% reduction in workers requirement to perform weeding with the introduction of cattle. He reported that with 144 head of grazing cattle, only two (2) workers are required to perform weeding in 400ha mature oil palm instead of six (6) under normal circumstances.

The savings from weeding varies according to the stages of integration. In the first two years the saving can be as much as 30%, and increased further to more than 70% when the number of cattle is at optimum with palatable undergrowth (Harun, 2003). Samsudin (2002) reported significant saving on labor requirement and weeding cost. The weeding area coverage per labor tremendously increased by almost 600% from 137ha to 956ha (Table 4), which lead to total weeding cost saving of 52.85% (Table 4). The fact that grazing cattle is contributing positively towards weed control is undeniable. As circle and spot spray of herbicides normally cover about 25 per cent of planted area, the weeds are 'cleared' naturally by rearing of sheep, cattle, goats, or deer under the palm (Anon, 2006). Combined together with biological pest control, both will help to reduce the use of herbicides and pesticides in oil palm estate.

Cattle Integration Projects in Sawit Kinabalu Sdn Bhd

Two pilot projects were conducted successfully in two 'experimental' estates which indicate that cattle had survived and multiplied. The cattle population had increased by 65 per cent within two years with no significant negative impact on overall estate management (Table 6). The common agronomic practices such as harvesting, manuring, maintenance were carried out as planned. It was also observed that the cost of weeding in Mensuli estate was reduced by 21 per cent after cattle was introduced (Table 7).

Table 4. Difference of labor cost with cattle integration (1912 hectares) and without grazing area

No.		Manual	Grazing	± Difference
1.	Total mandays	14	2	-12
2.	Cost/ manday	RM 15.00	RM 30.00	- 15
3.	No. of spraying rounds (*Imperata*, circle spraying and woodies)	1 ½	1 ½	
4.	Labor force/Hectare/year	1:137	1:956	819 ha

Source: Samsuddin (2002).

Table 5. Comparison cost summary cattle integration project

No.	Types	Before cattle project			After cattle project			± Difference	Saving percentage
		Total expenditure for 5 years (RM) (1991– 1995)	Area completed (Hectare)	Cost per hectare	Total expenditure for 7 years (RM) (1996-2002)	Hectare completed	Cost per hectare		
1	Weeding	771,398.26	9,536.85	80.89	512,419.91	13,380.99	38.29	-42.60	52.66
2	Lallang	168501.23	9536.85	17.67	109386.14	13380.99	8.17	-9.50	53.76
.	Total	939899.49	9536.85	98.55	621806.05	13380.99	46.47	-52.08	52.85

Source: Samsuddin (2002).

Table 6. Cattle population growth in Mensuli and Sandau Estates

Estates	July 2002	July 2003	December 2003	December 2004
Mensuli	197	-	348	407
Sandau		224	268	287
Total	421		616	694
% Increase			**46**	**65**

Source: Azid (2008).

Table 7. Weeding cost at Mansuli estate

Year	2001	2002	2003	2004
Weeding cost (RM/ha/yr)	79.40	63.30	60.80	63.50

Source: Azid (2008).

Feasibility Study

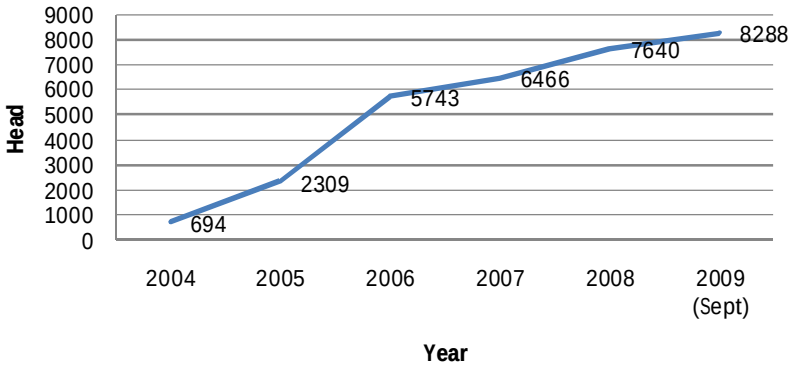
In his feasibility study, Azid (2004) reported that 36,028 ha was suitable for integration, and should be able to accommodate up to 20 000 heads of cattle. The integration model was then adopted to handle three thousand heads of cattle "breeders" and 97 head of stud bulls that were brought in from Australia in three consignments over two years in 2005 and 2006 (Table 8).

Table 8. Cattle breeder important for oil palm-cattle integration system

Year	Breeder	Stud Bulls
2005	1004	50
2006	1041	47
2006	1054	0
Total	3099	97

Source: Azid (1998).

The introduced cattle had settled down and multiplied as they adapted to the new environment as illustrated in Figure 3. As of September 2009 there are 8,288 heads of cattle integrated under oil palm and distributed in 14 estates across the region of Lahad Datu (59%), Tawau (30%) and Sandakan (11%); details of which are shown in Table 9. The total grazing area has reached 22,224 ha and stocking rate varies between 1.9 and 3.7 ha per head depending on forage availability



Source: Sawit Kinabalu Farm Products Sdn. Bhd Data (2009)

Figure 3. Cattle population in Sawit Kinabalu Sdn. Bhd

Table 9. Cattle distribution and grazing area under oil palm estates

Region	Estate	Grazing area (ha)	Cattle Population (head)	Stocking rate (ha/head)
Tawau	Sg Balung	1,689	566	3.0
	Madai	2,394	716	3.3
	Sg Kawa	1,077	405	2.7
	Bongalio	2,549	766	3.3
	Matamba	0	0	0
	Sebrang	1,639	650	2.5
	Mensuli	1,370	373	3.7
Lahad	Sandau	2,005	715	2.8
Datu	Boonrich	1,590	661	2.4
	Bagahak 1	1,370	719	1.9
	Bagahak 2	1,919	1027	1.9
	Bagahak 3	2,360	761	3.1
Sandakan	Gomantong/Green	974	379	2.6
	Menanggol	1,288	550	2.3
Total		22,224	8,288	2.68

Source: Azid (2009).

under the palm and on-going replanting program at the respective estate.

Strategic Insertion

In the strategic insertion approach, the introduction of the cattle into an estate operation is carefully planned by studying its existing operation, mainly harvesting, manuring, and up keep or maintenance. This is basically the 'fact-finding mission' to determine the suitability of the estate in terms of individual estate manager's understanding and commitment, availability and types of weeds of vegetation, herd size and timing of introduction (Azid, 2007). "Dry-weight-rank' (DWR) method described by Aminah and Chee (1999) as cited by Azid (2004) can be a very useful estimation tool to determine the population size. Sufficient fund has to be allocated for stock purchase as well as basic infrastructure as described by Rosli (2000). Cattle shall be introduced during the time when operational activity is minimal.

Rosli (2000) and Azid (2004) had concluded that commitment of estate management is the perquisite for successful cattle-oil palm integration. The strong commitment and understanding will be translated into supportive approach toward it implementation, and potential operational matters shall be constructively handled and adjusted to accommodate the 'additional' activity within the same premise. The SKSB top management team has been the driving force toward the successful adoption of the model as its provides confidence to them that the core business of producing palm oil will never be compromised along the way (Azid, 2007).

The newly introduced cattle need to be 'conditioned' to adjust themselves with new environment as well as new feeding regime that are physiologically stressful especially for imported cattle. The cattle should be exposed gradually to new feedstuff comprising of common 'weed' under oil palm as described by Aminah and Chee (1999), with the rotational grazing management using electrical fencing. Addressing these factors in strategic insertion had allowed successful management of the 'stressed period' to both people and the animals, hence contributed positively to cattle performance.

Grazing Management

The application of strategic rotational grazing management through the use of mobile electrical fencing is a dynamic process in which cattle grazing will be adjusted to suit the operational requirement of the estate. Movement of cattle within the estate will be synchronized with such estate common agronomic practices as harvesting round, fertilizer application, weeding round, and other important activities. Ideally, grazing cattle should be ahead of the other estate operational activities. 'Collision' will occasionally happen when grazing cattle and estate activity arrive at the same spot. The strategic rotational grazing management will require constant adjustment and modification according to prevailing estate operation to minimize such 'collision' and to reap the optimum benefit from grazing cattle as 'biological lawnmower' (Azid, 2007). This explains the different level of saving in weeding cost achievement (15 to 40%) among the participating estates.

The most significant highlight of the model of integration adopted by SKSB is the fact that the primary operation of its estate to produce fresh fruit bunch (FFB) was not affected by the introduction of cattle. Azid (2008) had sighted Kok (2008) observation that the presence of cattle in the participating estates had never been identified as important factor affecting yield as compared to other such more prominent factors as rainfall, nutritional status and operational efficiency. He further noted that the notion of soil compaction due to grazing cattle was unfounded as he observed that grass regeneration was rapid, luxuriant and complete after each grazing round. Similar observations had also been reported elsewhere by Rosli (2000) and Samsudin (2002). Further research is very much required to possibly quantify the relationship.

CONCLUTION

The cattle integration in oil palm can be successful venture through systematic approach and implementation. The holistic approach of cattle-oil palm integration encompasses Good Agriculture Practices (GAP) elements. It is environmentally friendly as it reduce herbicides use by offering biological weeds control, reduce labor requirement, palm productivity not being compromised

and optimized available resources (land and feedstuff). It's synergistic, cost effective and augurs well toward sustainable agriculture.

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Pig Species Identification in Meatballs Using Polymerase Chain Reaction Restriction Fragment Length Polymorphism

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ABSTRACT

The information given to consumers is essential for them to choose one food product over another. The falsification of food contents on product labels is a widespread problem, especially with products related with pig or others prohibited food in Islam. Proving conclusively that fraud has occurred requires the detection and quantification of food constituents. Falsifications of meat or food are often biochemically similar to the materials they replace, consequently the identification and measurement extremely difficult. The DNA based methods have now been successfully adapted for detection of food substitution. In this research, Polymerase Chain Reaction (PCR) products of cytochrome b mitochondrial DNA gene were applied to identify the existence of pig in meatball product. Genomic DNA of pig, bovine, and chicken were isolated and subjected to PCR amplification targeting the mitochondrial cytochrome b gene. Pig species differentiation was determined by digestion of obtained 359 bp amplified product with BseDI restriction enzymes, which generated pig species electrophoresis pattern. PCR-Restriction Fragment Length Polymorphism (RFLP) revealed the presence of the pig meat in meatball product and distinguished between bovine, chicken, and pig sample. Pig mitochondrial cytochrome DNA gene was cleaved into 228 bp and 131 bp fragments but the bovine, and chicken cytochrome b gene were not digested by BseDI enzyme. The digestion was conducted at 55^{o}C for 3 h and visualization of the digest product was performed in 2% agarose gel. PCR-RFLP technique using BseDI restriction enzymes is reliable for the detection of the pig meat in meatball for the *Halal* authentication.

Key words: pig species, identification, PCR-RFLP, halal authentication

INTRODUCTION

Indonesian traditional meatballs is one of the comminuted meat products and its popularity in all classes of Indonesian society. The products are served in hot soup with others stuff such as tofu, noodle, cabbage and chili or tomato sauce and the popular name in Indonesia is called bakso. Meat are processed to make bakso originally from beef but nowadays some others such as chicken, fish, and pork commonly also been mixed in some meatball products. The wide variety of meatball products availabe on the market in Indonesia seems favourable but leads to several fears, where almost population are moslem who prohibited to consume pork. This is an important challenge for the people in charge of the official control of food, that have to verify the species of meat ingridients that are not always easily identifiable.

Strategies utilized to detect an adulterated products have traditionally relied on wet chemistry to determine the amount of a marker compound or compounds in a test material followed by a comparison of the value(s). Obtained with those previously documented for authentic material of the same type. This approach is often time-consuming and therefore expensive; it also has the shortcoming that food adulterers are becoming increasingly sophisticated at masking their efforts and the range of analytes which must be quanticed to ensure authenticity is continuously increasing (Downey, 1998).

Many various methods based on DNA techniques have developed such as multiplex PCR assay (Matsunaga *et al*., 1999), *PCR-based finger printing* (Saez *et al*., 2004). Colgan *et al*. (2001) analyzed meat bone meal using real time PCR to investigate the meat source origin and to verify the quantity of meat in DNA mixture complex. Lopes-

Andreo *et al*. (2005) was also able to identify the meat species using the same methods.

Processed meat products such as sausage, meatball, chicken nugget and cornet were exposed with the high temperature during meat processing. Some of the heating of meat may will degrade the DNA then the isolation of DNA will be difficult. The adulterated of porcine in mixture of processed meat products can be identified with the method which is based on DNA. Although high temperature treatment affects the quality DNA, did not make the constraint in identifying of porcine material at processed meat.

However the identification of meat species in Indonesia was very rare and there was a little publication on this field. This study reported PCR-RFLP method for meat species identification in Indonesian meatball using DNA mitochondrial as universal primer in PCR reaction. The digestion of the PCR amplicon products to determined meat species was established by BseDI restriction enzyme.

MATERIALS AND METHODS

Sample Preparation and DNA Extraction

Authentic muscle samples of beef, pork and chicken were obtained from the traditional market in Yogyakarta, Indonesia. Meatball was prepared in laboratory scale with separate equipment to prevent unexpected cross contamination. Test meatballs were prepared with pork meat added to the final concentration of pork were 0; 1; 2, 5; 5; 10 and 25 percent to beef or chicken meatballs.

DNA was extracted from meatball samples using the *High Pure PCR Template* Protocol for animal tissue provided with the *High Pure PCR Template Kit* (Roche, Germany). Approximately 50-100 mg of meat samples was blended using a blender, placed in a 1.5 ml microcentrifuge tube. Three hundred microlitres *tissue buffer* and 40 µl *Proteinase* K were added and mixed by vortexing. The mixture was incubated at 55°C in a water bath to disperse the sample overnight until the tissue was completely lysed. The following day, 200 µl of *binding buffer* was added and incubated for 10 min at 70°C. The mixture was mixed by vortexing for 15 s. One hundred microlitres isopropanol was added to the sample, mixed thoroughly by vortexing. Put into the *High Filter Tube* in *collection tube*, and then pour sample in it. Placed all tube in table top centrifuge and spun at 8,000 g for 1 min. The flow-through and *collection tube* was discarded and the *High Filter Tube* was placed in a new 2 ml *collection tube*. Five hundred microlitres *wash buffer* was added and spun at 8,000g for 1 min. The flow-through and collection tube was discarded and the *High Filter Tube* was placed in another 2 ml *collection tube*. After throw out solution, then spun at full speed for 10 s to dry the *High Filter Tube* and the flow-through and *collection tube* was discarded. The *High Filter Tube* was placed in a clean 1.5 ml micro centrifuge tube. Two hundred microlitres *pre-warmed elution buffer* was added and spun at 8,000g for 1 min to elute. The DNA solution was stored at 4°C.

PCR Amplification of a Conserved Cytochrome 2b of Mitochondrial Gene Fragment

The set of primers used for amplification consisted of Cyt b-FW and Cyt b-REV oligonucleotides:

CYT b FW 5'-CCA TCC AAC ATC TCA GCA TGA TGA AA-3'
CYTb REV 5'-GCC CCT CAG AAT GAT ATT TGT CCT CA-3'

Amplification of the mt cyt b gene was performed in a final volume of 25 µl containing 250 ng of extracted DNA, mega-mix royal (optimized mixture of *Taq* polymerase, anti-*Taq* polymerase monoclonal antibodies in 2 X reaction buffer (6 mM $MgCl_2$ with 400 µM dNTPs, stabilizer and blue loading dye) (Microzone Ltd, West Sussex, UK), and 20 pmol of each primer. Amplification was performed with a thermal cycler according to the following PCR step-cycle program: predenaturation of 94°C for 2 min to completely denature the DNA template, followed by 35 cycles of denaturation at 95°C for 36 s, annealing at 5°C for 73 s, and extension at 72°C for 84 s. Final extension at 72°C for 3 min followed the final cycle for complete synthesis of elongated DNA molecules. Two microlitres of PCR products were electrophoresed at constant voltage (50 V) on 2% agarose gel (Promega, Madison, USA) for about an hour in 1x TBE buffer, pH 8.0 and stained by ethidium bromide. A 100 bp DNA ladder (Promega, Madison, USA) was used as size

reference. The gel photo was taken using the Syngene gel documentation system.

Restriction Fragment Length Polymorphism

Two units/µl of RE *Bse*DI (Fermentas) were applied to 10 µl of amplified DNA in a final volume of 20 µl digestion mixture [containing 1x reaction buffer (10 mM Tris-HCl, 100 mM KCl, 1 mM EDTA, 0.2 mg/ml BSA, 1 mM DTT and 50% glycerol)] and were incubated at 55°C for 3 h for optimal result. Five microlitres of the digested samples were electrophoresed at constant voltage (50 V) on 2% agarose gel (Promega, Madison, USA) for about an hour in 1x TBE buffer, pH 8.0 and stained by ethidium bromide. A 100 bp (Promega, Madison, USA) was used as size reference. The gel photo was taken using the Syngene gel documentation system.

RESULTS AND DISCUSSION

This research was aimed to develop a specific and sensitive method to identify pork meat species. PCR-RFLP based on the sequence of the mitochondrial cytochrome *b* gene by using a pair primer was used as an analytical tool for pork meat identification. The PCR amplicon then digested using BseDI restriction enzyme to distuingesed of chicken, beef and pork.

DNA was extracted from meatball in various meat ingridients using the High Pure PCR Template Kit (Roche). DNA extraction was done using the proteinase K 1 mg/ml. Genomic DNA isolation from the meatball can extracted with this kit but it is ascribed to the fact that thermal strongly accelerates DNA degradation from the meatball samples (Figure 1). This result coincided to Arslan *et al*. (2004) and Tanabe *et al*. (2007) that heated process by various treatment did not significantly affected to DNA fragment detection. Matsunaga *et al*. (1999) has also studied of DNA isolation in meat which was processed with high temperature around 100 and 120°C for 30 minutes of various meat flesh as cattle, goat, chicken, sheep, horse and pig, while Tanabe *et al*. (2007) provided similar to process of pork at various cooked. According to Martinez and Yman (1998) and Saez *et al*. (2003) reported that heat treatments which mainly affected the quality DNA causing degradation into small size fragment.

Genomic DNA was used as a template for the amplification of PCR with the universal primer. Gene of cytochrome *b* used for the amplification of PCR resulting fragment of approximately 360 bp (Figure 2.). This result indicated that isolate DNA of mixture meatball was enough for amplification on PCR reaction. The same result of amplification has also been reported yet, according to Kocher *et al.* (1989), Aida *et al*. (2005) and Erwanto *et al*. (2007).

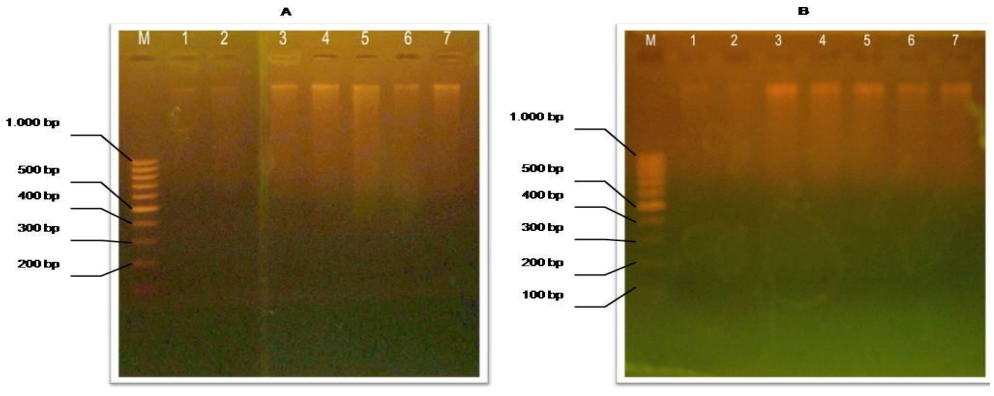

Figure 1. Total genomic DNA extracted from beef-pork meatball and chicken-pork meatball. (A) M: marker 100 bp DNA ladder (Invitrogen), 1: pork (100%), 2: (beef 75% : pork 25%) 3: (Beef 90% : Pork 10%), 4: (Beef 95% : Pork 5%)5: (Beef 97% : Pork 3%), 6: (Beef 99% : Pork 1%), 7: (Beef 100 %). (B): M: marker 100 bp DNA ladder (Invitrogen), 1: pork (100%), 2: (chicken 75% : pork 25%) 3: (chicken 90% : Pork 10%), 4: (Chicken 95% : Pork 5%)5: (Chicken 97% : Pork 3%), 6: (Chicken 99% : Pork 1%), 7: (Beef 100 %)

Selection of target gene and primer design very influenced sensitivity and specification of method of detection. PCR method was very sensitive when primer target represent a gene multicopy of like gene mitochondrial. This research used the area mitochondrial DNA of the cytochrome *b* as target for detection of porcine.

The PCR reaction allowed fragments of the expected length to be obtained in all meatball samples both beef and chicken mixed with pork, although with variable efficiency. PCR has the potential sensitivity and specificity required to achieve detection of a target sequence from template DNA. The mitochondrial cytochrome b gene has been selected in this study as template for DNA amplification because it has an acceptable length and an adequate grade of mutation and there are numerous sequences available in the databases (Kocher *et al*., 1989). The mitochondrial primers Cyt b-FW and Cyt b-REV used in the PCR technique developed in this work successfully amplified a conserved 359 bp region from the cytochrome b gene of all chicken, beef and pork individuals analyzed.

Sequence DNA of cytochrome b gene cattle, goat, chicken and pig obtained from database NCBI, then employed by alignment using the software CLC sequencer. The result average of alignment mitochondrial of cytochrome b gene among beef, mutton, chicken and pork is 86.64%.

As a result of the preliminary computerized analysis for the detection of specific restriction sites on pig sequence, a site recognized by BseDI enzyme was cleaved into two fragment 131 bp and 228 bp long were consequently expected. A clear band with a length between 100 and 150 bp and thus referable to the 131 bp fragment can be observed in Fig 3 (lane 1). In the same lane a thicker band can be traced back to the 228 bp fragment.

The results obtained suggest that, compared with BsaJI endonuclease profiles, the DNA restriction patterns obtained after digestion of the amplicons with *BseD*I enzymes consisted of same patterns. The difference between BsaJI and BseDI restriction enzyme is the incubation time for the digestion. Using BseDI needed 3 h for digestion but digestion using BsaJI have to incubated for more than 12 h.

Based on the laboratory analysis RFLP method can preliminary observed the DNA fragmentation using CLC sequencer software. The result of cytochrome b alignment using CLC sequencer softwer showed that pig intra species have the same restriction sites and their homology was 98.2%.

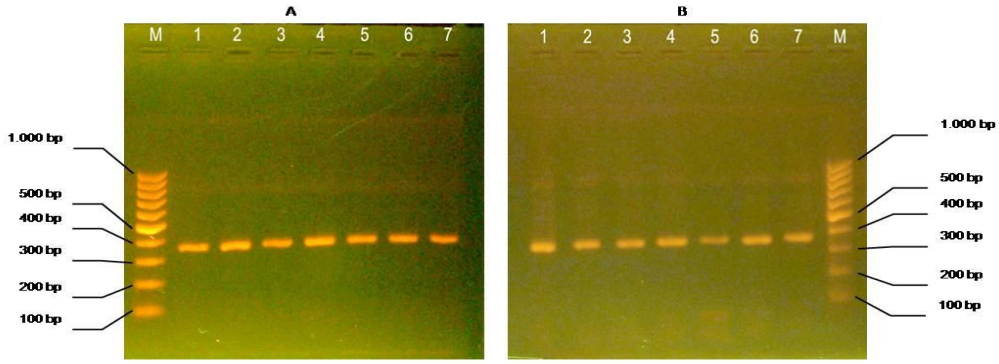

Figure 2. PCR products of cytochrome b gene fragments 359 bp long of samples from different meatball products separated by 2% high-resolution agarose gel electrophoresis. PCR amplification using cyt b universal primer. (A) M: marker 100 bp DNA ladder (Invitrogen), 1: pork (100%), 2: (beef 75% : pork 25%) 3: (Beef 90% : Pork 10%), 4: (Beef 95% : Pork 5%) 5: (Beef 97% : Pork 3%), 6: (Beef 99% : Pork 1%), 7: (Beef 100 %). (B): M: marker 100 bp DNA ladder (Invitrogen), 1: pork (100%), 2: (chicken 75%: pork 25%) 3: (chicken 90% : Pork 10%), 4: (Chicken 95% : Pork 5%) 5: (Chicken 97%: Pork 3%), 6: (Chicken 99% : Pork 1%), 7: (Beef 100 %)

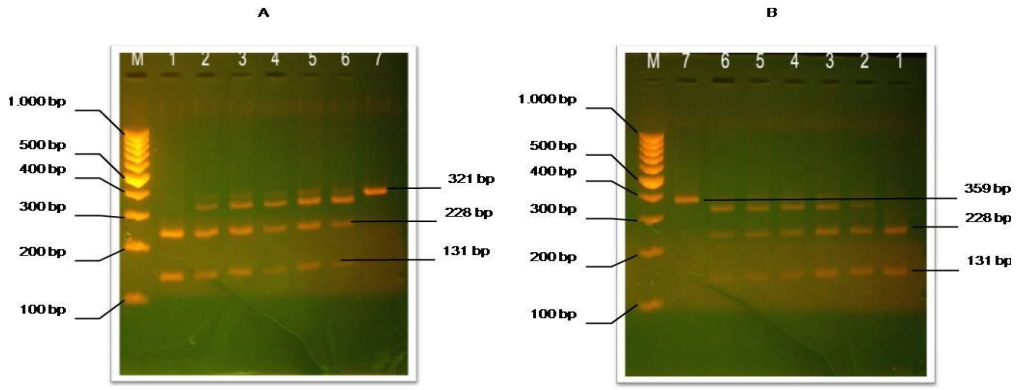

Figure 3. Restriction fragment produced by BseDI restriction enzyme on 359 bp amplicons of cytochrome b gene from different meatball products separated by 2% high-resolution agarose gel electrophoresis. PCR amplification using cyt b universal primer. (A) M: marker 100 bp DNA ladder (Invitrogen), 1: pork (100%), 2: (beef 75% : pork 25%) 3: (Beef 90% : Pork 10%), 4: (Beef 95% : Pork 5%) 5: (Beef 97% : Pork 3%), 6: (Beef 99% : Pork 1%), 7: (Beef 100 %). (B): M: marker 100 bp DNA ladder (Invitrogen), 1: pork (100%), 2: (chicken 75% : pork 25%) 3: (chicken 90% : Pork 10%), 4: (Chicken 95% : Pork 5%) 5: (Chicken 97% : Pork 3%), 6: (Chicken 99% : Pork 1%), 7: (Beef 100 %)

CONCLUSION

PCR-RFLP of the mitochondrial Cytochrome b gene is a suitable alternative that can be applied to the detection of pig species present in commercialized products such as meatballs.

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The Chemical Composition and Nutritive Value of Mulberry Leaf as a Protein Source in Poultry Diets

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ABSTRACT

A study was conducted to determine the chemical composition of mulberry (*Morus alba*) leaf meal (MLM) and its nutritive value as a feed ingredient in diets of broiler and layer chickens. The crude dry matter, protein, ash, fat, crude fibre, NDF, ADF, Ca, P and gross energy contents were 89.3%, 29.8%, 11.8%, 11.1%, 32.3%, 22.8%, 0.28% 2.7% and 4220kcal/kg respectively. The amino acids composition of MLM indicates it is a good source of essential amino acids especially lysine 1.88% and leucine 2.55%. The overall ileal true amino acids availability was similar between layers and broilers. The digestibility coefficients of individual amino acids for aspartic acid, glutamic acid, tyrosine, histidine, argnine, glycine and tryptophan.was significantly ($p<0.05$) higher for layers compared to those for broilers. However, the digestibility of methionine was higher ($P<0.05$) in broilers than layers.

Key words: mulberry; leaf meal, digestibility; amino acids, layers; broilers

INTRODUCTION

Mulberry grows well in the tropics and subtropics, and is reported to have excellent nutritional value. It is grown extensively for leaves, which are used for raising silkworms in the sericulture industry. Mulberry leaves are very rich in protein (15-35%), minerals (2.42-4.71% Ca, 0.23-0.97% P, 1130-2240 kcal/kg metabolisable energy and absence of anti-nutritional factors (Omar *et al*., 1999; Sanchez, 2002, 2000; Saddul *et al*., 2003; Sarita *et al*., 2006). Its protein quality is comparable to those of soybean meal (Machii, 1989). Excellent results have been obtained with mulberry leaves as ruminant feed (Rojas & Benavides, 1994; Oviedo *et al*., 1994; Esquivel *et al*., 1996; Gonzalez, 1996). Published values on the digestibility of amino acids and chemical components for chickens are lacking. Thus the objectives of this study were to determine the nutrient composition and amino acid availability to chicks.

MATERIALS AND METHODS

Birds and Housing

Fifteen layer (Isa-brown strain) and fifteen male broiler (cobb strain) 17 weeks and 42 days old respectively were used in the experiment to determine amino acids availability of MLM. The birds were assigned to individual cages, during which time they were fed on a commercial layer and broiler finisher diet.

Diets

A basal diet (adaptation diet) which contained MLM as the sole source of dietary protein and DL- methionine was added at 0.12% in the basal diet. This diet was formulated to contain 16 % CP, 2850 kcal ME/kg. Assay diet was the same with basal diet except for the elimination of synthetic methionine. A protein-free diet was also formulated specifically to allow the determination of endogenous flows of amino acids. All diets were fed in mash form. Chromic oxide was included in all diets as an indigestible marker. The composition of the basal, assay and protein-free diet and its amino acid contents are shown in Table 1 and Table 2 respectively.

Feeding Trial

The chickens were offered the basal diet *ad libitum* for three days adaptation period, which is considered sufficient to eliminate the carry-over effect between the two different diets (Kadim *et al*., 2002). Following diet adaptation period, the birds were fasted for 24 hr. The birds were then allowed to consume the respective diets for a one hour period (Kadim and Moughan, 1997). Four

Table 1. Composition of the diets used in AA determination

Ingredient	Basal Diet	Assay Diet	Protein-free Diet
Mulberry	54.00	54.12	-
Dextrose	37.38	37.38	94.50
Corn oil	5.00	5.00	-
Salt	0.40	0.40	0.40
Dicalcium phosphate	1.50	1.50	2.50
Limestone	0.50	0.50	1.50
Choline choloride	0.30	0.30	0.30
Mineral-vitamine premix	0.50	0.50	0.50
DL-Methionine	0.12	0	0
Chromic oxide	0.30	0.30	0.30
Calculated analysis			
ME (kcal/kg)	2850	2850	3460
Crude protein%	16	16.1	0
Crude fibre%	6	6.1	0
Calcium%	2.00	2.00	1.17
Phosphorus%	0.40	0.40	0.45

*Supplied per kg diet: Fe, 35mg; Mn, 70mg; Cu, 8mg; Zn, 70mg; I, 1mg; Se, 0.25mg ; Co, 0.2mg; calcium-D-pantothenate, 8mg; folic acid, 0.5mg; D-biotin, 0.045 mg; vitamin C, 50mg; vitamin A, 8000 IU; vitamin D, 1000 IU; vitamin E, 30 IU; vitamin K3, 2.5mg; vitamin B1, 2mg; ; vitamin B2, 5mg; vitamin B6, 2mg; vitamin B12, 0.01 mg; and niacin, 30mg.

Table 2. Dry matter, crude protein and amino acid contents (%) in diets and mulberry leaf meal used in metabolic study

	Basal diet	Assay diet	MLM
Dry matter	93.11	93.11	89.30
Crude protein	16.00	16.10	29.80
Aspartic acid	1.50	1.50	3.06
Glutamic acid	1.70	1.70	3.33
Serine	0.70	0.70	1.22
Glycine	0.80	0.80	1.57
Histidine	0.44	0.44	0.69
Arginine	1.30	1.30	1.80
Threonine	0.79	0.79	1.31
Alanine	0.70	0.70	1.54
Proline	0.60	0.60	1.30
Tyrosine	0.30	0.30	0.82
Valine	0.96	0.96	1.76
Methionine	0.38	0.25	0.52
Cyctine	0.15	0.15	0.30
Isoleucine	1.10	1.10	1.43
Leucine	1.80	1.80	2.58
Phenylalanine	1.20	1.20	1.94
Lysine	1.10	1.10	1.88
tryptophan	0.15	0.15	0.27

hours after initial meal, the birds were killed by an intravenous injection of 1ml sodium pentobarbitonein the wing vein. The use of pentobarbiotone is to minimize peristaltic movements and mucosal shedding of the gastrointestinal tract, which may occur with other methods of killing. When the birds were completely immobilized, the body cavity was opened, the ileum removed from the location of Meckel's diverticulum to a point 5 cm proximal to the ileo-caecal junction and digesta gently flushed by using a syringe into a plastic container. Digesta samples were immediately stored at -20°C. The samples were subsequently freeze-dried, finely ground and stored at -20°C for further chemical analysis.

Chemical Analysis

Amino acid concentrations for ileal digesta were determined by HPLC as described by Cohen and Strydon (1994) following pre-column derivatisation with AQC reagent (6-aminoquinolyl-N-hydroxysuccinimdyl carbamate, waters, USA). Cystine and methionine were analysed as cystic acid and methionine sulfone by oxidation with performic acid for 16h at 0ºC and neutralization with hydrobromic acid before hydrolysis. Tryptophan contents were determined following alkaline hydrolysis of sample with 4.3N. $LiOH.H_2O$ for 16h at 120°C and neutralization with 6N HCl. Rest of the AA were hydrolyzed by 5ml 6N HCl for 22 hours at 110°C. Chromium was determined according to Saha and Gilbreath (1991).

Calculations

Apparent digestibility of the assay diet was calculated using the following equation (Kadim and Moughan, 1997):

Apparent AA digestibility (AID) (%) =

AA concentration in diet -

$$\frac{\text{AA output in ileum}}{\text{AA concentration in diet}} x100$$

True AA digestibility of the assay diet was calculated using the following equation (Kadim and Moughan, 1997):

True AA digestibility (%) =

$$\frac{\text{AID} + \text{Endogenous AA output}}{\text{AA concentration in diet}} x100$$

Where:
AID: Apparent amino acid digestibility

Statistical Analysis

Data were subjected to analysis of variance (ANOVA) using SPSS (2005). Duncan's multiple range test was used to compare the means.

RESULTS AND DISCUSSION

The chemical and amino acid composition of the MLM are presented in Table 3. The amino acids content of mulberry leaves was comparable to those reported by Machii (1989) and Coto (1996). Table 4 shows a comparison of the composition of amino acids from mulberry leaves with the amino acid requirements of chicks as given by the NRC (1994). It was found that mulberry leaves have a good essential amino acid profile relative to meet the chicks requirements. However, cystine is deficient in mulberry leaves. True AA digestibility of mulberry leaf meal is shown in Table 5. The amino acid digestibility of MLM ranged from 65.54% for lysine in broilers to 81.99% for tryptophan in layers.

Overall amino acids digestibility for layers and broilers fed MLM was 74.23% and 74.53% respectively. There was no significant difference in overall amino acids digestibility between the two classes of birds. However layers fed MLM significantly ($p<0.05$) digested more aspartic acid, glutamic acid, tyrosine, histidine, argnine, glycine and tryptophan. Whereas the digestibility of methionine is significantly higher ($p<0.05$) in broiler than layer. The class of bird influenced the digestibility coefficients of amino acids in mulberry leaf meal. However, the average overall digestibility of amino acids was similar between layers and broilers. Effects on the digestibility of individual amino acids were higher in layers. The digestibility of glycine, histidine, arginine, aspartic, glutamic, tyrosine and tryptophan was higher in layers compare to broilers, while that of methionine, was higher in broilers. The digestibility of other amino acids was similar between the two classes of birds. The poor individual amino acid digestibility in broilers feeding mulberry leaves attributed to the high level of NDF in mulberry leaves. This is in agreement with findings that the nutrients in diets containing high fibre levels are poorly digested in broiler (Annison *et al.*, 1997).

Table 3. Chemical composition of mulberry leaves (%DM)

Nutrients	Content
Dry matter, %	89.30
Crude protein, %	29.80
Ether extract, %	5.57
Crude fiber	11.10
Gross energy, kcal/kg	4220
Ash, %	11.8
Neutral detergent fiber, %	35.80
Acid detergent fiber, %	28.00
Hemicellulose, %*	7.80
Calcium, %	2.73
Phosphorus, %	0.28
Amino acids	
Aspartic acid	3.06
Glutamic acid	3.33
Serine	1.22
Glycine	1.57
Histidine	0.69
Arginine	1.80
Threonine	1.36
Alanine	1.54
Proline	1.31
Tyrosine	0.82
Valine	1.77
Methionine	0.52
Cyctine	0.30
Isoleucine	1.43
Leucine	2.58
Phenylalanine	1.94
Lysine	1.88
Tryptophan	0.27

* Neutral detergent fibre-acid detergent fibre.

Table 4. Composition of amino acids from mulberry leaf meal (DM basis) in relation to the requirements for growth of chicks

Amino acid	N.R.C. requirement (%)	Mulberry leaf meal (%)
Argnine	1.44	1.80
Lysine	1.20	1.80
Histidine	0.35	0.69
Phenylalanine	0.72	1.94
Tyrosine	0.62	0.82
Methionine	0.50	0.52
Leucine	1.35	2.58
Isolecine	0.80	1.43
Valine	0.82	1.76
Threonine	0.80	1.31
Tryptophan	0.23	0.27
Cystine	0.43	0.30

Table 5. True amino acid digestibility (%) of mulberry leaf meal of layer and broiler chickens

Amino acids	Layers	Broilers	S.E.M
Aspartic acid	75.85[a]	71.69[b]	0.37
Glutamic acid	75.95[a]	73.78[b]	0.38
Serine	77.60	80.85	0.40
Glycine	78.30[a]	72.71[b]	0.35
Histidine	68.80[a]	66.55[b]	0.67
Arginine	79.15[a]	75.33[b]	0.55
Threonine	72.73	73.16	0.45
Alanine	73.21	72.99	0.56
Proline	73.79	73.25	0.20
Tyrosine	75.83[a]	69.47[b]	0.79
Valine	73.82	74.03	0.41
Methionine	76.21[b]	79.72[a]	0.85
Cyctine	74.67	74.36	1.14
Isoleucine	79.38	79.72	0.37
Leucine	76.62	77.02	0.71
Phenylalanine	73.75	75.18	0.86
Lysine	66.74	65.54	0.83
Tryptophan	81.99[a]	79.10[b]	0.92
Over all mean	74.23	74.53	0.69

CONCLUSION

Mulberry (*Morus alba*) leaf meal can be used as a potential source of protein in poultry production. The true ileal digestibility of amino acids in MLM is influenced by the class of birds. This need further research in order to confirm this findings.

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Effects of Polyethylene Glycol (PEG) on *In Vitro* Dry Matter and Nitrogen Digestibility of *Leucaena* Species and Signal Grass (*Brachiaria decumbens*)

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ABSTRACT

The tropical legume *Leucaena* contains condensed tannin (CT) that binds protein and other components of feed. The fact that Leucaena reduces digestibility of nutrients and depending on tannin content and astringency. Polyethylene glycol (PEG) has ability to neutralize CT by displacing protein-tannin complexes, as a consequence of CTs interact more strongly with PEG than they do with protein. *In vitro* studies were conducted to investigate the effect of polyethylene glycol (PEG) on *in vitro* digestibility of *Leucaena* species and grass. *In vitro* studies were conducted in two stages as described by Tilley and Terry (1963) and Jones *et al.* (1998).The results indicated that the digestibility vary depending on the nutrients content of forages and its CT content. Low tannin content of forages had a high digestibility of dry matter and nitrogen, and the rate of ammonia-N production. PEG consistently improved the digestibility of nitrogen and to some extent of dry matter, and the rate of ammonia-N production of tannin-containing forages but not non-tannin-containing of grass. The maximum values were 60.6%, 71.2% and 93.6 mg/d for corrected dry matter digestibility, nitrogen digestibility and the rate of ammonia-N production respectively, when PEG was included at rate of 200 mg/g samples of forages. High tannin content of forages required more PEG to neutralize the effect of tannin to the same extent of low tannin content forages. PEG, on the other hand, had no effect on digestibility and the rate of ammonia-N production on non tannin-containing grass.

Key words: in vitro, digestibility, condensed tannin, polyethylene glycol

INTRODUCTION

The tropical legume *Leucaena* contains condensed tannin (CT) concentrations which vary widely with species. Of the 26 species which have been studied, Leucaena *collinsii* has the lowest CT content while *Leucaena pallida* has the highest (Dalzell *et al*., 1998; McNeill *et al*., 1998). Tannins are known to affect the availability of nutrients by formation of soluble and insoluble complexes and their effects on the digestibility of nutrients will vary depending on tannin content and astringency (McNeill *et al*., 1998). *In vitro* studies by Makkar *et al.* (1995) have shown that CT influenced nutrient digestibility, to a great extent as measured by reduced gas production (fermentative activity). These researchers also noted that even at the same levels in feed, different tannins had different degrees of effect.

The use of polyethylene glycol (PEG) to neutralize CT has proved useful in further elucidating the specific nutritional consequences of dietary CT as PEG displaces protein-tannin complexes, as a consequence of CTs interact more strongly with PEG than they do with protein (Mangan, 1988). Palmer and Jones (2000) have shown that PEG improved the *in vitro* digestibility of nitrogen in *Calliandra* and most other legumes containing tannins. The objective of the present study was to investigate the effect of the level of PEG on the *in vitro* digestibility of a wide range of *Leucaena* species and a representative grass (*Brachiaria decumbens*) using the two stages digestion technique of Tilley and Terry (1963) and *in vitro* technique using PEG described by Jones *et al*. (1998b).

MATERIALS AND METHODS

Actively growing *Leucaena* species (*Leucaena pallida* K748, *Leucaena leucocephala* cv. Tarramba K636 and KX2 F1 hybrid of *Leucaena pallida* and *Leucaena leucocephala*) and signal grass (*Brachiaria decumbens)* were used as plant sources. Leaf and edible stem materials were collected from these plants, and immediately frozen with dry-ice in an insulated container. These samples were kept frozen until freeze drying.

A mixture of rumen fluid and buffer (1: 3 v/v) was used as an inoculant for incubation. Rumen fluid was collected from permanently rumen fistulated cattle that had been grazing signal grass pastures for ten days. The buffer solution used was based on that described by McDougall (1948). *In vitro* studies were conducted in two stages as described by Tilley and Terry (1963) and Jones *et al*. (1998). The rate of PEG application was 0, 50, 100, 150, 200 and 250 mg/g of sample and the samples size were 5 gram. The *in vitro* digestibility of dry matter (IVDMD) and *in vitro* digestibility of nitrogen (IVND) were estimated by subtracting the DM and N content of the samples before and after incubation, while the rate of ammonia-N production was estimated by the distillation method. The neutral detergent fiber (NDF) fraction of residues was also determined. It is assumed that the NDF differences from one level to the next level of PEG is due to the complexation of PEG and tannin that remained as insoluble fractions to contribute an additional DM fraction of residues after incubation. Subtracting the percentage of increase in NDF on IVDMD values will give a corrected value of IVDMD (CIVDMD).

Chemical Analysis

Dry matter (DM) was calculated as the residue remaining after the samples were dried at 65^{o}C for 48 h, and organic matter (OM) as the loss of sample DM weight after incineration at 550^{o}C for 5 h. The N content of the samples was determined by using a Leco CNS-2000 Combustion Analyzer (Leco Corporation, USA). The rate of ammonia-N production after the first stage of incubation was also analyzed by using the steam distillation and titration method. Neutral detergent fiber (NDF) determinations were based on the method of Van Soest (Van Soest and Wine, 1967), by using the Filter Bag Technique (FBT) in an ANKOM^{220} fiber analyzer (ANKOM Technology Corporation, New York, USA). Separation of CT into free, protein bound and fiber bound CT was done as described by Perez-Maldonado (1994). CT was estimated with Butanol-HCl by the method of Dalzell and Kerven (1998).

Statistical Analysis

Data collected for *in vitro* digestibility and the rate of ammonia-N production were analyzed using analysis of variance to test for the effect of treatments by using GLM of SAS (SAS, 1998). The model used was 4 (forage types) x 6 (levels of PEG applied) factorial design with 4 replicates per plot. The extent of the digestibility was regressed on the level of PEG. The further analysis for the mean values used LSD for the comparison between treatments.

RESULTS AND DISCUSSION

It is clear that the nutritive values of forages differed within the species of legumes and grass. Legumes had a high nitrogen and low fiber content (NDF) and were generally of higher nutritive value than signal grass (Table 1). The nitrogen content of tropical legumes, however, is not the only determinant of their nutritive value. Protein degradability in the rumen and digestibility of bypass proteins in the small intestine are also important and are related to the tannin type and content in the plant material (Kaitho *et al*., 1998). The tannin content of legumes studied in the current experiment was higher

Table 1. Chemical composition (g/kg DM) of selected edible fractions of *Leucaena* species (*L. pallida*, KX2 and *L. leucocephala*) and signal grass (*Bracharia decumbens*)

	L. pallid	KX2	*L. leucocephala*	Signal grass
OM	945.0	935.7	945.2	896.3
NDF	243.5	278.8	173.1	586.5
N	29.9	32.5	34.2	5.9
CT:				
Free	225.7	169.7	90.1	ND*
Fiber bound	3.7	2.9	2.0	ND
Protein bound	8.2	7.8	5.6	ND
Total	237.6	180.3	97.8	ND

*) ND not detected.

than that suggested by Barry *et al*. (1986) as optimal for ruminants (30-40 g/kg DM).

Effect of Treatments on Dry Matter Digestibility

The reaction between PEG and tannins in the leaf samples reduces the overall *in vitro* digestibility of DM (Table 2), because tannin and PEG form indigestible complexes that cannot be degraded by rumen microorganisms. It is possible that such complexes are also not soluble in the acid-pepsin (Makkar *et al.*, 1995). Hence, the inclusion of PEG in incubation mixtures containing tanniniferous forages is likely to result in an underestimation of *in vitro* DMD (McSweeney *et al*., 1999; Palmer and Jones, 2000).

Palmer and Jones (2000) suggested that the amount of PEG have been bound by tanniniferous forage has to be corrected to get the real value of *in vitro* DMD. The present study also found that the NDF content of the residues was increased (Figure 1) when PEG was included in the incubation. *L. pallida* samples resulted in the greatest increase in NDF with increasing PEG and this was presumably due to the fact that *L. pallida* forage had the highest tannin content (Table 1). Such evidence agrees with the previous studies (Makkar *et al.*, 1995; McSweeney *et al*., 1999). Makkar *et al*. (1995) reported that the apparent and true digestibility of tanniniferous feeds was slightly lower when PEG was included, as tannin-PEG complexation increased "apparent" NDF content of the residue. The present study found a similar trend to that observed by others (Makkar *et al*., 1995; Palmer and Jones, 2000), that is, there are only small to negligible effects of PEG on the DMD of high tannin forages *e.g Pallida*. This result differs to other research which found that the inclusion of PEG or PVP increased *in vitro* gas production of tannin-containing feeds (Makkar *et al.*, 1995; Khazaal *et al.*, 1996; Getachew *et al*., 2002). However, the true effects of PEG on digestibility in the present study were masked by the formation of insoluble PEG-tannin complexes which are recorded as indigestible NDF residues (Figure 1). When DM digestibilities were corrected for these experimental artifacts, it was found, as shown by others, that the addition of PEG did have a significant and real effect on DM digestibility (Figure 2 B). In the absence of PEG, each forage species differed significantly in *in vitro* digestibility of DM, with signal grass having the highest value, and L. *pallida*, KX2 or *L. leucocephala* having the lowest values (Table 3). This observation may be explained by the different levels of tannin in the forages. Forages with high in tannin content is of lower digestibility, supporting the convention that the digestibility of organic matter, protein and cell walls is inversely related to tannin concentrations in the plant material (Silanikove *et al*., 2001).

Interestingly, at the same level of PEG in the solution, each forages responded differently to PEG effect on IVDMD. The high tannin forages appeared to require more PEG to reduce the negative effects to the same extent as that in low-tannin forages. Signal grass, however, did not show any response in the DMD to the level of PEG applied (Figure

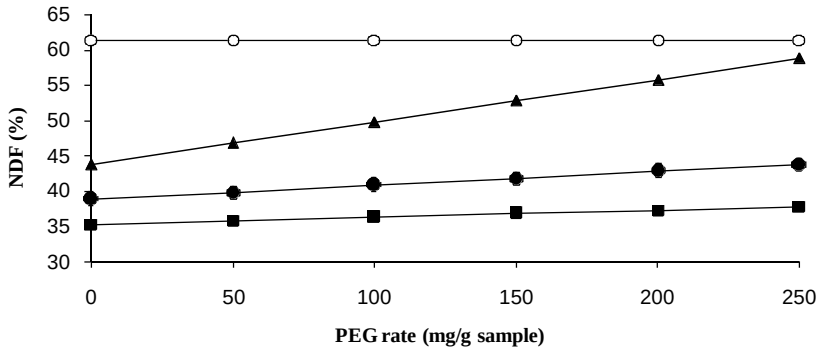

Figure 1. Relationship between neutral detergent fiber (NDF) fraction in the residues of either *L. pallida* (▲), KX2 (●), *L. leucocephala* (■) or signal grass (○) and inclusion rate of polyethylene glycol (PEG) in the mixtures

Table 2. Mean values for the main effects of increasing levels of PEG on the in *vitro* digestibility of dry matter (IVDMD), corrected *in vitro* digestibility of dry matter (CIVDMD), *in vitro* digestibility of nitrogen (IVND) and rate of ammonia-N production from the incubation of different forage types

PEG mg/g sample	IVDMD %	CIVDMD %	IVND %	Ammonia-Nmg/d
0	49.3^{A}	49.3^{A}	30.7^{A}	52.3^{A}
50	52.8^{B}	53.9^{B}	53.8^{B}	78.2^{B}
100	54.9^{C}	57.2^{C}	67.0^{C}	89.1^{C}
150	55.7^{D}	59.1^{D}	68.0^{D}	91.3^{CD}
200	56.1^{D}	60.6^{E}	71.2^{E}	93.3^{D}
250	56.1^{D}	61.7^{F}	69.5^{F}	93.6^{D}
SEM	0.18	0.18	0.24	0.97

Note: Values within the columns followed by different superscript letters are significantly different (P<0.001).

2A, B, C, D) and is consistent with the previous result from *in vitro* studies with non-tanniniferous forages (Makkar *et al*., 1995; Jones and Palmer, 2000). Similarly, PEG had no significant effects on either the *in situ* degradability (Silanikove *et al*., 1996a) or *in vivo* digestibility of wheat straw (Silanikove *et al*., 1996b). There was a small to negligible improvement in DMD from *L. pallida* when PEG concentrations were increased in the solution. This agrees with the previous studies (Jones *et al*., 2000; Jones and Palmer, 2000). The reduction of DMD is simply because the high CT tannin of *L. pallida* formed insoluble tannin-PEG complexes which were increased as the level of PEG increased. These complexes remained in the solution even after digestion in acid-pepsin and contributed to the indigestible components of dry matter.

Effects of Treatments on Nitrogen Digestibility and Rate of Ammonia-N Production

Increasing levels of PEG steadily increased the IVND of forages (Table 2). The increase of nitrogen digestion was accompanied by an increase in the rate of ammonia-N production, supporting the view of Palmer and Jones (2000) that this technique (*in vitro* N digestibility) provided a better evaluation of the effects of tannins on the nutritive value of tannin-containing feeds and forages. The significant correlation between the level of PEG and digestibility of N and rate of ammonia-N production in tannin-containing forage indicated that the presence of tannins depressed the digestibility of nitrogen and further reduced the ammonia level in the rumen. The linear interrelationship between the level of PEG and improvement of digestibility supports the concept that PEG may replace protein in pre-existing tannin-protein complexes, releasing proteins for further digestion (Mangan, 1988).

The inclusion rates of PEG in the present study were lower (0-250 mg PEG/g sample) than those used by Makkar *et al*. (1995) (2 g PEG/g sample) or Palmer and Jones (2000) (0-1000 mg PEG/g sample), but the levels used here were still higher than the recommended optimum level of PEG (160 mg/g sample) for binding *Leuceana* tannins (Palmer and Jones, 2000). None of these authors actually measured the tannin content of their samples, and the large differences in apparent PEG requirements for tannin neutralisation may simply be related to differences in the tannin contents of the materials assayed. It is therefore not possible to directly compare the efficacy of PEG in the present

Table 3. Mean values for the main effects of forage types on the *in vitro* digestibility of dry matter (IVDMD), corrected *in vitro* digestibility of dry matter (CIVDMD) *in vitro* digestibility of nitrogen (IVND) and rate of ammonia-N production from the incubation of forage types at a common level of polyethylene glycol (PEG) inclusion

Forages	IVDMD (%)	CIVDMD (%)	IVND (%)	Ammonia-N (mg/d)
L. pallid	41.0^{A}	48.5^{A}	58.4^{A}	95.5^{A}
KX2	51.8^{B}	54.3^{B}	64.6^{B}	98.9^{B}
L. leucocephala	60.0^{C}	61.2^{C}	72.9^{C}	92.4^{C}
Signal grass	63.9^{D}	63.8^{D}	44.2^{D}	45.7^{D}
SEM	0.15	0.15	0.19	0.80

Note: Values within the column followed by different superscript letters are significantly different (P<0.001).

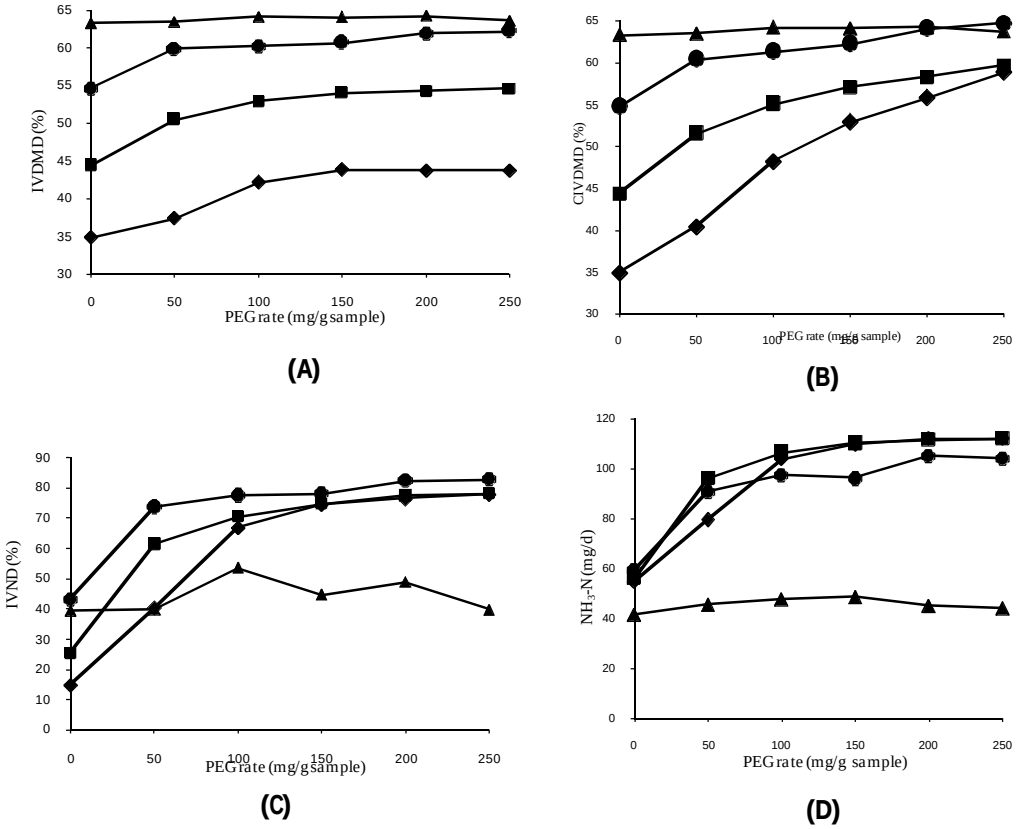

Figure 2. The interaction between inclusion rate of polyethylene glycol (PEG) and types of forages on IVDMD (A), CIVDMD (B), IVND (C) and rate of ammonia-N production (D). The type forages include *Leucaena pallida* (◆), KX2 (■), *Leucaena leucocephala* (●) and signal grass (▲)

experiment with their results. In the present case, at the "optimum" level of PEG applied (to achieve about 77% of N digestibility), 1.06, 1.05 and 1.02 mg PEG/mg tannin were required to approximately neutralize tannins from *L. pallida*, KX2 and *L. leucocephala* respectively.

In general, *L. leucocephala* produced the highest value of *in vitro* digestibility of nitrogen, followed by KX2 and *L. pallida* (Table 3). The superiority of *L. leucocephala* in nitrogen digestibility indicates that *L. leucocephala* would have potentially high nutritive values for animals, with *L. pallida* having the lowest nutritive value, with KX2 being intermediate. Interestingly, the highest value of nitrogen digestibility of *L. leucocephala* did not produce a higher rate of ammonia-N production (Table 3), suggesting that significant amounts of ammonia N were being incorporated into microbial cells during the period of incubation. However without direct measurements of microbial protein synthesis this explanation must remain speculative.

The superiority of *L. leucocephala* as compared to the others legumes in terms of IVND is related to its low tannin levels, particularly in the free form (Dalzell *et al*., 1998). The form in which the tannins exist in the plant material are an important determinant of the extent to which protein digestibility and microbial protein synthesis are affected by plant tannin content. For example, high levels of free tannins are most likely to directly affect protein (nitrogen) digestibility, because free tannins can readily bind to soluble proteins rendering them indigestible (Fondevila *et al*., 2002). The consequences of complexes between tannin and protein (protein bound) or carbohydrate (fiber bound) and decreased digestibility, the microbial population is denied access to essential amino acids and decreased N availability which may lead to restricted growth and depressed fermentative activity (Longland *et al*., 1995). Therefore, the *in vitro* digestibility of *L. leucocephala* in the current study was significantly higher than that of *L. pallida* or KX2

(Table 3). Nevertheless, the level of tannins in a feed alone cannot be used to determine the value of a legume as a protein supplement since McSweeney *et al.* (1999) found a poor correlation between total tannin content and digestibility of dry matter and nitrogen. Factors such as reactivity, structure, molecular weight and interactions of different secondary compounds in the plant are also important (Barry *et al*., 1986; Waghorn *et al*., 1994). For instance, studies by Kaitho *et al*. (1998) showed that the rumen degradability of protein was 22.9 and 37.7% for *L. pallida* and *L. leucocephala* respectively and the intestinal digestibility of *Leucaena* proteins was 45.2 and 46.0% for *L. pallida* and *L. leucocephala*, respectively even though the total soluble tannin of *L. leucocephala* was higher than that of *L. pallida* (Kaitho *et al*., 1998; Garcia *et al*., 1996). The latter effect could be linked with the ability of tannins to bind with feed protein and enzymes, thus reducing their digestibility.

The digestibility of nitrogen of the *Leucaena* species studied improved as the level of PEG addition was increased (Table 2). A similar trend was also recorded for the rate production of ammonia-N. These observations suggest that the CT of *Leucaena* caused a significant depression on the digestion of nitrogen, diminishing its value as a feed for animals consuming such legumes. However, the extent of improvement in *in vitro* digestibility at the same rate of PEG application varied among the legumes, with some legumes requiring more or less PEG to counteract the effects of tannins. For instance, additional PEG at the rate of 50 mg/g sample resulted in 73.6% IVND for *L. leucocephala* compared with 40.3% of IVND for *L. pallida*. If these results were to be translated into practical recommendations, then animals consuming different tannin containing leguminous feeds will require different levels of PEG supplementation to overcome the varying negative effects of the different tannins in each species.

CONCLUSION

The *in vitro* digestibility of *Leucaena* species varied according to their nutrient and CT content. *Leuceana* with a lower CT tended to have higher IVDMD, CIVDMD and IVND. Inclusion of PEG increased IVND and to some extent the IVDMD or CIVDMD. PEG, however, did not have any effect on the dry matter digestibility of signal grass. There was an interaction between the level of PEG and *Leucaena* species; *Leucaena species* with a high CT content required more PEG to neutralize tannins than did species with low tannin content.

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The Effect of Work on Reproductive Performance of Bali Cattle Under the Oil Palm Plantation in Bengkulu

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ABSTRACT

The integration of cattle rearing under the oil palm plantation would beneficially support both livestock and plantation sector. Farmer would benefit the integration of livestock-oil palm plantation through its better income, optimum land use, labor efficiency, and better environment. In Bengkulu, the use of Bali cattle as Draught Animal Power (DAP) for carrying fresh fruit bunch (FFB) has proven to benefit both to the plant and livestock. The objective of this study was to determine the effect of work on reproductive performance in Bali cow. A total of 40 Bali cows within the range of body condition scored (BCS) 6-7 are divided into two groups, namely *Working* animals and *No-Working*. The *Working* group was subjected to three kinds of day-to-day work; these were *Work-1* (pulling cart from home to oil plantation), *Work-2* (pulling cart with FFB from plantation to collection site) and *Work-3* (pulling cart from plantation to home). Parameters measured were working regime (distance, speed, duration, and load) and reproductive performance (S/C, length of gestation, days open, birth weight and calving interval). Results showed that the average of distance, speed and load of *Work-1* and *Work-3* were significantly lower ($P<0.05$) than those of *Work-2*. For the respective *Work-1*, *Work-2* and *Work-3*, it was known that the distance measured were 1.287, 0.407, and 1.287 km; the load were 138.75, 582.5 and 89.09 kg; and the speed were 2.082, 0.856, and 2.055 km/hr respectively. Result also showed that there was no significant difference on reproductive performance between *Working* and *No-Working* animals, as presented by S/C (1.50 vs. 1.41), gestation length (284.2 vs. 281.6 d), days open (82.5 vs. 80.53 d), calf birth weight (14.6 vs. 16.25 kg), and calving interval (375.9 vs. 370.9 d). In conclusion, there was no working effect on reproductive performance for Bali cows.

Key words: Bali cattle, working, oil palm plantation, integration, reproductive performance

INTRODUCTION

Population and production of cattle in Indonesia has decreased in the last two decades, the major constraint to increased livestock production is the difficulty in providing feed of sufficient quantity and with adequate nutrient composition throughout the year .and decreasing land for livestock production mainly due to increasing land used for plantation such as oil palm, rubber, etc. There is considerable chance to optimizing land use through integration between crops and livestock, palm oil plantation have a huge potential to increase livestock population through the use of its by products for feed. The inter row areas of these crop are usually covered with vegetation comprising legume, grasses, broadleaf species and fern which usually considered as weeds that can be utilized as source of for ruminant feed (Dwatmadji, 2005; Wahab, 2002). According to Jalaludin (1996) the cost of weeding control is quite significant and can be easily eliminated if the vegetation in the inter-rows is utilized for animal nutrition. Integrating animals in the plantation can also reduce fertilizer application since the nutrients returned to the soil from the animals are quite substantial. Reducing chemical fertilizers in the long-run will not only reduce production costs but, more importantly, will also minimize further deterioration in soil fertility.

In Bengkulu, the importance of cattle and oil palm integration can play an important role for weeding control, providing manure compost, producing calves, as life-saving, and for draft purposes. As a draft animal in oil palm system, Bali cattle can be used for transporting Fresh Fruit Bunch (FFB) from the harvesting area to collection site (main road). It is generally accepted that working animal requirement for energy-yielding substrates increases during working, therefore Zerbini *et al*. (1993) found that the

incidence of ovulation without estrus was higher in working than in non-working cows. Reducing ovarian activity was also reported in working buffaloes (Teleni *et al*., 1989), it is unlikely that the cessation of cyclic activity in working animals was result of direct competition for nutrient between the ovary and other tissues. According to Zerbini *et al*. (1999), the primary need of the working animal is to increase feed and metabolic energy intakes to meet energy requirements for work and avoid deleterious body weight losses. This becomes more critical in working cows requiring extra energy for lactation and reproduction, and where the main feed source is roughage.

MATERIALS AND METHODS

The research was conducted in oil palm plantation PT. Agricinal located in Muko-Muko District, 140 km north of Bengkulu. Forty well trained Bali cows 5-7 year of age within the range of body condition scare (BCS) 6-7 on scale of 1-9 (1 = emaciated to 9 = obese) (see Teleni *et al*., 1993) were used in this research and then subjected into two groups, *Working* and *No-Working*. The working cows were assigned to three kinds of work: *Work-1* (pulling cart from home to oil plantation), *Work-2* (pulling cart with Fruit Fresh Bunch from plantation to collection site) and *Work-3* (pulling cart from plantation to home). The working cows were grazed on the available native pastures available between oil palm inter row and based on the prevailing system of 8 hour day-grazing (06.00 - 14.00). Parameters measured were working regime (distance, speed, duration and load), physiological (respiration rate, pulse rate and temperature), and reproductive performance (service per conception, length of gestation, birth weight, estrus post partum, calving interval, calf weight). Data were tabulated and analyzed using ANOVA (Daniels, 1991).

RESULTS AND DISCUSSION

Working Regime

Most parameters on working regime measured (distance, speed, and load), except duration load), indicated that *Work-1* (pulling cart from home to oil plantation) and *Work-3* (pulling cart from plantation to home) were significantly different with *Work-2* (pulling cart with Fruit Fresh Bunch from plantation to collection site) (see Table 1). Work-3 had the highest load among the other two.

Based on the parameters measured, the nature working regime employed for carrying FFB in this experiment can be categorized as light work. This due to that working regime of current experiment was below the reported working regime measured by other researchers (see Pearson *et al*., 1989; Goe and McDowell, 1980; Dwatmadji, 2000).

In response to the working regime, physiological changes were measured before cows started working (*Pre*) and just finished working (*Post*) (see Table 2). *Pre* and *Post* parameters were measured to crosscheck the fatigue condition (see Upadhyay and Madan, 1985). Based on these workers the physiological responses measured were not under fatigue condition, which was adjacent to the working regime employed in the current experiment. During work contracting muscle produces heat as a by-product of metabolism. Some of this energy is used by contractile proteins while the rest is liberated as heat energy which needs to be eliminated by various thermoregulatory processes in order to maintain normal body temperature, thereby sustaining work (Moran, 1973; Nangia *et al*., 1980; Mathers *et al*., 1984; Pieterson and Ffoulkes, 1988). An increase in body temperature, measured as an increased in rectal

Table 1. Mean ± standard deviation of working regime (distance, duration, speed, and load) of Bali cows during *Work-1*, *Work-2*, and *Work-3*.

Parameters	Work-1	Work-2	Work-3
Distance (km/day)	1.29±0.155[a]	0.41±0.028[b]	1.29±0.155[a]
Duration (hour/day)	0.65±0.084[a]	0.52±0.045[a]	0.67±0.085[a]
Speed (km/hour)	2.09±0.118[a]	0.86±0.085[b]	2.05±0.214[a]
Load (kg)	138.7±15,26[a]	582.5±56.49[b]	89.1±10.71[a]

Note: [a, b] means within rows bearing different letters in superscripts differ significantly (P<0.05).

Table 2. Mean ± standard deviation of respiration rate, pulse rate and rectal temperature of *Working* during pre and post working periods during *Work-1, Work-2,* and *Work-3*

Parameter	Work-1		Work-2		Work-3	
	Pre	Post	Pre	Post	Pre	Post
Respiration (breaths/minute)	22.9±1.07[a]	43.9±2.40[k]	29.7±0.95[b]	57.0±2.44[l]	34.2±1.61[b]	57.0±2.53[l]
Pulse rate (beats/minute)	60.7±0.78[a]	80.4±2.46[k]	65.5±0.96[b]	87.8±2.46[k]	69.1±1.31[b]	86.9±2.13[k]
Temperature (°C)	36.6±0.06[a]	37.2±0.06[k]	37.1±0.02[b]	37.9±0.07[l]	37.2±0.05[b]	37.8+0.07[l]

Note: [a,b] means within *Pre* rows bearing different letters in superscripts differ significantly (P<0.05);
[k,l] means within *Post* rows bearing different letters in superscripts differ significantly (P<0.05).

temperature (RT), of 2.5°C above normal resting value is regarded as intolerable to ruminant animals (Upadhyay and Madan, 1985). These research workers found that cattle were unable to work when RT increased by more than 2.5°C above resting value.

Reproductive Performance

Result shows that average number of services per conception was 1.5 ± 0.16 ranging from 1 to 3 in *Working* cows and 1.41 ± 0.12 varying from 1 to 2 in *Non-Working* cows. Statistically, there was no difference between working and non working control (Table 3). While Zerbini and Larsen (1996) found that the average services per conception for *Working* and *Non-Working* cows were 2.1 and 1.9, respectively. Findings of the present study are supported by the results of Ahmad *et al*. (2007) that average number of services per first conception was 1.5 ± 0.152 ranging from 1 to 6. Some other workers like Murdia and Tripathi (1990) who reported 1.58 services per conception, while Singh and Mishra (1980) have also found almost similar results (2.0 ± 1.15). Sekerden (1996) reported comparatively large number of services per conception (3.3 ± 0.17). The average number of services required for each conception was 1.8 for supplemented Bali Cows and 2.0 for non-supplemented Bali cows was reported by Oka (2002). Successful service or insemination depends on many factors as quality of semen, skill of the inseminator, proper time of insemination and cows to be inseminated themselves; management, nutrition and climate conditions may also affect the success of service or insemination.

Average gestation length of *Working* and *Non-Working* control cows was presented in Table 3. It was found that average gestation length for *Working* group was 284.18± 2.520 days, for *Non-Working* cows was 281.65±1.930 days. Gestation length of Bali cows under farm and urban conditions were studied by Fordyce *et al*. (2002), and found that the mean gestations of Bali cows were between 280-290 days.

The time taken for first estrus *post partum* in *Working* cows was 82.50±1.98 days, and it was longer than estrus *post partum* in *Non-Working* of 80.53 ± 1.770 days, but the difference was not significant. Our findings are in fair confirmation with Sinha *et al*. (1998) who observed the postpartum fertile estrus interval in prostaglandin treated cows was shorter (86.43 ± 4.01 days) than that of untreated control (144.50 ± 5.23 days).

The average birth weight was 14.63 ± 1.026 kg (*Working* cows) and 16.25 ± 0.984 kg (*Non Working*). In general, birth weight was not affected working. Our result is in line with Billi *et al*. (2000) who found that

Table 3. Mean ± standard deviation reproductive performance of *Working* and *Non-Working* cows

Parameter	Working	Non-Working	P
Service per conception	1.50±0.160	1.41±0.120	0.236
Length of gestation (day)	284.2±2.52	281.6±1.93	0.238
Birth weight (kg)	14.6±1.03	16.27±0.984	0.988
Estrus post partum (day)	82.5±1.98	80.5±1.77	0.753
Calving interval (day)	375. 9±4.45	370.9±3.54	0.675

Bali calves have birth weight varying from 11.4 to 21.5 kg with male calves were significantly ($P<0.05$) heavier than female calves. In addition, Bamualim and Wirdahayati (2002) found that Bali calves birth weight varying from 11.9-14.9 kg, Bamualim and Wirhayati (2002) also reported that supplemented cow 3 months before calving had no effect on calves' birth weight.

The mean values for calving interval found for *Working* cows was 375.94 ± 4.45 days and *Non-Working* cows 370.94 ± 3.54 days, our result in the present study are shorter than the results of Zerbini and Larsen (1996) in which calving intervals for working and non working cows were 525 and 495 days, respectively. Wirdahayati *et al*. (2000) found that calving interval for smallholder Bali cows in Nusa Tenggara region was 510 days (non-supplemented) and 481days (supplemented). Moreover, Bamualim and Wirhayati (2002) also reported that supplemented cow 3 months before calving had shorter the calving interval than those of unsupplemented cows. According to Martojo (2002) the lengths of calving interval of Bali cows depend on management and environment conditions. Martojo (2002) found that calving interval of Bali cows depend on the management of each region, *e.g.* calving interval of Bali cow was found 15.4 months (NTT), 16 (NTB) and 15.7 months (South Sulawesi). Our results indicate that there were no differences between *Working* and *Non-Working* on reproductive performance. Agyemang *et al.* (1991) reported that the reproductive and productive performances of draft and non-draft cows were similar when the work load was light.

CONCLUSION

There were no differences between *Working* and *Non-Working* on reproductive performance of Bali cattle.

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The Effect of Ration with Antibiotics (Virginiamycin) and Temulawak (Curcuma Xanthorriza Roxb.) to Broiler Performances

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ABSTRACT

The purpose of this research is to learn about the effect of Virginiamycin and Temulawak addition to performance . The research used 100 broiler chickens from Cobb CP 707 strain. The research used Completely Randomized Design (RAL-Rancangan Acak Lengkap) with four treatments. R0: Control,; R1: ration with 15 ppm Virginiamycin; R2: ration with 9% Temulawak; R3: ration with 15 ppm Virginiamycin and 9% Temulawak. Duncan's Multiple Range Test is used to indicate the difference between treatments. Variable being observed is performance, and income over feed cost. The results of the research showed that temulawak and virginiamycin addition does not provide significant difference on final body weight and carcass weight.

Key words: Virginiamycin, temulawak, carcass and final body weight, broilers

INTRODUCTION

The preventive deseases and enhancement of growth, feed intake and feed efficiency are importance factor. Most antibiotic as a feed additive requires alternative ways to stabilize the health and growth performance as antibiotic as a feed additive Virginiamycin as antibiotic used to growth promoter. Antibiotic is feed additive

Used virginiamycin in true composition, effected to restraining pathogen bacteria population in intestinal and decreasing negative effect of normal flora population that over in the gastrointestinal.

Combinations of vitamin and amino acid which is be able do together to prevent micro nutrient deficiency, increasing growing and not caused if used at all times. Despitefully of antibiotic, utilizing herbal be also a choice as feed additive.

Utilizing herbal contribute to increasing appetite and also growing. Herbal used occasionally is *curcuma xanthorhiza* (temulawak). Temulawak as far back as medicine concoction matter. Contents of temulawak consist of c*urcumin*, colagoga, atsiri oil (volatile oil).

Curcumin is substance that protected of oxidasi on eritrocite and haemoglobin by nitrite compounds. *Curcumin* can be increasing hepathoglobin and haemopexin protein synthesis in the liver, so lead which is bonding with haemoglobin be able to destruction in the liver. Kolagoga is substance that to increasing role of gall and decreasing body fat, so we get low fat meat and high meat composition.

Volatile oil also called essential oils, that formed at reticulum endoplasmic in the plan cells, and providable by distillation. Volatile oil not effected on microorganism population, but positive effected on digestion enzymes. Now, volatile oils getting popular in the agriculture and animal livestock sector, because this oils used as digestion and metabolism promoters, and not giving rise to resistance in animal.

Based on description above, so need to do research The Effect of Ration with Antibiotics (Virginiamycin) and Temulawak (Curcuma xanthorriza roxb.) to Broiler Performance.

MATERIALS AND METHODS

The experiment was carried out in the Experimental poultry unit of the Faculty of Animal Husbandry, Padjadaran University. One hundred , day old chick, Cobb broiler were chosen. The research used Completely Randomized Design (CRD). They were consisting four different treatments, with four replications in one treatment. Chiken were fed ad libitum with four ration. The composition of the ration were :

R_0 = Control
R_1 = Ration with 15 *ppm virginiamycin*
R_2 = Ration with 9 % temulawak

R_3 = Ration with 15 *ppm virginiamycin* and 9% temulawak

The analysis of the treatment were body weight, and carcass weight,

RESULT AND DISCUSSION

The Effect of Treatment on Final Body Weight in Broiler

In Table 1. are presented the results of the treatment, the data were followed with statistic analysis.

Tabel 1. Average final body weight on each treatment

Replication	Treatment			
	R0	R1	R2	R3
	(g)			
1	1320	1300	1180	1300
2	1320	1280	1270	1120
3	1500	1300	1420	1300
4	1160	1320	1580	1400
5	1200	1250	1250	1540
Total	6500	6450	6700	6660
Average	1300	1290	1340	1332

Results indicated that all treatments have no significantcy on the final body weight. 9% **Curcuma xanthorriza Roxb/** temulawak) combined with 15 ppm virginiamycin, have no significancy, even the. Virginiamycin effectively as anti bacteria and content cystein and lysine (Komisi obat Hewan, Departemen Pertanian, 2006). According to Rofiq, 2003, using 15 ppp virginiamycin will affected the gastrointestinal tract, and the ration added with antibiotic for a long term even in low dosage will disturb the balance of acid-base in small intestine and intestinal damage, also l affected the nutrient absorbtion at least will results the lower of final body weight. .. According to Lesson and Summers, 2001), virginiamicyn will change the instestinal microflora, and the ceca will be oedema and filled with humid excrete and the epithelium will lysis.

Antimicrobial growth promoters (virginiamycin) are antibiotics added to the feed of food animals to enhance their growth rate and production performance. The mechanism by which virginiamycin work is not clear. Virginiamycin reduce normal intestinal flora and harmful gut bacteria. The effect on growth may be due to a combination of both fewer normal intestinal flora and fewer harmful bacteria.

The combination between virginiamycin and temulawak has advantages, because the anti inflammatory effect, reduce the diarrhea effect and to improvethe villi and mucosa structure, and the inflammation of intestine.

The influence of the treatment on the broiler carcass weight

In Table 2. results indicated that 9% virginiamycin in ration (R1), have the lowest average than other treatment (R2 and R3) although have no significant on the final body weight.

Tabel 2. The influence of the treatment on the broiler carcass weight

Replication	Treatment			
	R0	R1	R2	R3
	(g)			
1	850	900	700	900
2	900	770	700	650
3	1000	750	825	870
4	700	700	1000	900
5	750	700	1200	1000
Total	4200	3820	4425	4320
Average	840	764	885	864

The function of colagoga in temulawak in ration (R2), is to change the lipid became energy and increased the muscle, at least could increased the carcass weight.

The choleretic properties and cinarina colagoga, could stimulates the production of bile in the liver and facilitates the clearing later in the gallbladder, which helps digestion of fats. Bile, made up of bile salts and cholesterol, is secreted by hepatocytes and is stored in the gallbladder. It is excreted after ingestion of food to metabolize and digest. By action of bile, fats from oily foods and fried foods are emulsified (broken up into small molecules), transformed into droplets that are degraded by pancreatic and intestinal lipases, being apt to be degraded by enzymes secreted by lipases pancreas. The bile, prepared digestion of fatty substances and later digestion occurs through the pancreatic juice, the only way to proceed with the breakdown of fats. Digestive enzymes are secreted by the pancreas and comprise lipases, colesterolasas, glucidasas and proteases (Jakobsen, dkk, 2009). The use of choleretic and Bile means in case of non-ulcer dyspepsia and there are heavy and slow digestion.

CONCLUSION

Temulawak and virginiamycin has positive response to body weight, and carcass weight. R2 (9% temulawak) and R3 (9% temulawak and 15pp virginiamycin), was better than R0 (control) and R1 (virginiamycin 15ppm)

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Growth, Production and Nutritive Value of *Brachiaria mulato* as Affected by Levels of Urea Fertilization

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ABSTRACT

This experiment was aimed at investigating growth, production, and nutritive value of Mulato grass (*Brachiaria mulato*) as affected by levels of urea fertilization. The experiment was designed with a 3 x 6 completely randomized design to test three application levels of urea fertilizer (0, 150, or 300 kg/ha); each of which was replicated six times. The grass was planted, with a planting size of 60 x 75 cm, on 18 plots having an individual size of 3 x 3 m. All the plants were cut at week three after planting and urea was applied on week four. Parameters measured were plant height, tillage number, leaf width, biomass production and nutrient contents (crude protein [N x 6.25], neutral detergent fibers, and crude fat) of plant dry matter, and measurements of (or sample collection for) these parameters were done on week seven after cutting. Between treatment differences were statistically analyzed with an analysis of variance. Results indicated that the levels of urea fertilizer significantly ($P<0.05$) affected the plant tillage number, dry matter production and crude protein and neutral detergent fiber contents. Applying 150 kg/ha urea increased tillage number, dry matter production and crude protein content of the plant while reducing the neutral detergent fiber content of the plant compared to unfertilized plants. Increasing the urea application level to 300 kg/ha resulted in further increase in tillage number, dry matter production and crude protein content of the plant and further reduced the neutral detergent fiber content. The level of urea application up to 300 kg/ha did not affect the height, leaf width, dry matter, organic matter or crude fat contents of the Mulato grass.

Key words: mulato, urea, growth, production and nutritive value

INTRODUCTION

Indonesian beef cattle production system is to a large extent managed traditionally by small scale farmers who maintain a limited number of cattle and apply a low level of production technologies. Under the system, the cattle herd is primarily dependent upon feeds available from road sides and communal grasslands, which is provided to the animals through the cut and carry practice or by allowing the animals to graze themselves. Cattle productivity is generally low. Under such systems, Wiryosuhanto (1999) reported the following cattle performances: calving rates 22%, calves mortality rate 18%, calving intervals 15-17 months, and cow mortality rate 2.7%. Some studies (Doho, 1989; Marsetyo *et al*., 2006; ACIAR, 2008) have indicated that native grasses are not able to meet the nutrient requirements of growing calves and the animals will exhibit low growth rates when the native grass is used as the only source of nutrients. This is not surprising because native grasses contain low levels of nutrient; ACIAR (2008) reported a typical nitrogen content of native grasses between 5 and 8%. Availability and nutritional qualities of native grasses are also reduced during dry seasons with a direct impact on animal performances. Wirdahayati *et al*. (1998) and Damry *et al*. (2008) reported that the growth rates of Bali cattle grazing in native pastures during rainy seasons were 0.25-0.50 kg/day, but the animals lost 20% of their body weight during dry seasons.

Introduction of new grass species is one approach that may overcome the low nutritional contents and seasonal availability of grasses for beef cattle production system. The introduced grass has to be tropical in origin or highly adaptable to tropical environments and has appreciably high production rates and nutrient contents. This effort will ensure a continuous supply of feed to beef cattle throughout the year.

One of the tropical grasses seems to be promising is *Brachiaria mulato*. Currently there are two Mulato cultivars available: Mulato I (*Brachiaria* hybrid CIAT 36061) and Mulato II (*Bra*

chiaria hybrid CIAT 36087). Mulato I (resulted from crossing *Brachiaria ruziziensis* clone 44-6 and *Brachiaria brizantha* CIAT 6297) is the first product of hybridization program conducted by the International Center for Tropical Agriculture (CIAT) in Columbia (CIAT, 2001). Although Mulato I requires soil condition of medium to high fertility, but it has a high tolerance to drought, fast recovery after grazing, high plant vigor and very good forage quality (Argel *et al*., 2007). Mulato II is the second hybrid, released in 2005 and resulted from three cycles of hybridization and screening by the Center involving *Brachiaria ruziziensis*, *B. decumbens*, and *B. bizantha*. In addition to having the outstanding characteristics of the first cultivar, Mulato II has an ability to adapt well to a broad range of local condition including those with acid soils of low fertility, moderate moisture saturation, and is resistant to spittlebug (Argel *et al*., 2007).

Although the Mulato grass has been introduced into some Indonesian areas, there have been no published works reporting the production parameters of the grass under Indonesian climatic conditions. Therefore, this study reported the effects of nitrogen fertilizer on the growth, production and nutrient contents of the Mulato grass.

MATERIALS AND METHODS

Experimental Site, Design and Treatment

This study was carried out in the village of Taipa, the Municipality of Palu, Central Sulawesi. The experiment was done on a land with a total area of 15 x 25 m which was divided into 18 small plots having an individual plot size of 3 x 3 m. Ditches (50 cm in wide) were made between experimental plots for drainage to avoid water logging. The experiment employed a 3 x 6 completely randomized design, and each of the tested urea fertilization levels (0, 150, or 300 kg/ha) was replicated six times. These levels of urea application corresponded to 0, 72, and 144 kg N/ha, respectively.

Experimental Procedures

Prior to the experiment, the planting area was prepared, clear from unwanted materials and fenced. The area was thoroughly ploughed using hoes. The experiment was commenced by planting *Brachiaria mulato* grass using pols on the experimental plots with a planting size of 75 x 60 cm. The planting was commenced in the morning and completed in one day. Watering was done every two days by draining water to the plantation area. After three weeks of plantation, all the plants were cut at about five cm from the ground to eliminate growth variation of the plant. Urea then was applied on each plot according to the treatment. The urea fertilizer was placed in small holes at distance of about five cm from the plant. Measurements of plant height, number of tillage, leaf width and production were facilitated using 1 x 1 m sized quadrant at week seven after cutting. The plant was cut at about 5 cm from the ground and brought to laboratory for analysis.

Chemical Analysis

Samples of Mulato grass were ground using a refrigerated blender before passing them through a 1mm screen. The samples were analyzed for dry matter and ash (AOAC, 1984) and ash-free neutral detergent fiber (NDF) (Goering and Van Soest, 1970). Feed samples were analyzed for nitrogen with the Kjeldahl method (AOAC, 1984), and ether extracts with the soxhlet method (Woodman, 1941).

Statistical Analysis

Data obtained were analyzed using one way analysis of variance on the Minitab statistical program. Differences in parameter measured among the treatments were analyzed with Least Significant Differences (Steel and Torrie, 1989).

RESULTS AND DISCUSSION

Agronomic parameters and nutritional contents of *Brachiaria mulato* as affected by the three levels of urea fertilizer application are presented in Table 1. Level of urea fertilization increased ($P<0.05$) the Mulato tillage number and dry matter production, crude protein and neutral detergent fiber contents. Applying a urea level of 300 kg/ha consistently exhibited the highest values for all of the affected parameters. Urea Results of this study confirm those of the previous one (CIAT, 2007) that *Brachiaria mulato* is highly responsive to nitrogen fertilizer. In the current study, providing 300 kg urea/ha corresponding to a nitrogen quantity of about 144 kg/ha to the soil increased the grass dry

Table 1. Effect of urea fertilization on growth, production and nutritive value of *Brachiaria mulato*

Parameters	Levels of urea fertilization (kg/ha)		
	0	150	300
Agronomic parameters			
Plant height (cm)	61.6± 2.3[a]	64.08±4.2[a]	62.34±4.7[a]
Tillage number (m^2)	22.2±2.9 [a]	36.52±3.9[b]	39.47±3.7[c]
Leaf width (cm)	2.42±0.13[a]	2.59±0.15[a]	2.56±0.16[a]
Dry matter production (ton/ha)	4.3±0.13[a]	7.92±0.15[b]	10.76±0.10[c]
Nutritive value			
Dry matter, DM (g/100 air dry)	29.02± 2.2[a]	28.58±2.1[a]	26.96±2.3[a]
Organic matter (g/100g DM)	87.12± 3.6[a]	88.75±3.5[a]	89.42±3.3[a]
Crude protein (g/100g DM)	9.37±0.12[a]	11.63±0.08[b]	13.78±0.09[c]
Neutral detergent fiber (g/100g DM)	65.31±3.2[a]	63.28±2.7[b]	60.74±2.8[c]
Fat (g/100g DM)	1.64±0.03[a]	1.73±0.02[a]	1.80±0.02[a]

Note: means with different superscripts in same column are significantly different (P<0.05).

matter production by about 150 times, its crude protein contents by about 47 times and thus its crude protein production by approximately 115 times compared to unfertilized soil. The Mulato dry matter productions obtained in the present study were significantly higher than those obtained by studies reported in CIAT (2007) with a nitrogen application rate of 30 kg/ha producing 2.4 kg dry matter/ha. Mulato grass appears to efficiently absorb the increased nitrogen provided in the soil and use it to form new plants as indicated by increased tillage number associated with urea fertilizer and at the same time increased synthesis rate of nitrogenous substances of the plant tissues, either as protein or non protein nitrogen. This occurs in the expense of fiber synthesis as shown by reduced neutral detergent fiber contents. Growing the Mulato grass will therefore require a regular application of nitrogen fertilizer at rates depending on the existing soil fertility to maintain optimum forage productivity.

Mulato grass is a high quality forage source which is indicated by its high crude protein contents. The crude protein contents of Mulato grass in this study were found to be higher than those of native or elephant grass (*Pennisetum purpureum*) with crude protein contents between 5-9% on dry matter basis (ACIAR, 2008). The grass as animals nutrient source is therefore expected to result in better animal performances than native or other previously introduced grasses such as *Pennisetum purpureum, Pennisetum purpureophoides, Panicum maximum*, etc. Marsetyo *et al*. (2009) reported that the daily weight gain of young Bali cattle given native grass as the sole feed was 193 g/day which was improved to 366 g/day when the native grass was replaced by the Mulato grass. The low animal performances associated with native or introduced grasses are also found in other reports (e.g. Marsetyo *et al*., 2006; Damry *et al*., 2008; ACIAR, 2008).

The increased animal performances resulted by the Mulato grass over native grass reported by Marsetyo *et al*. (2009) was in line with increased digestible dry matter due to better forage digestibility for Mulato grass than for native grass, without affecting intakes of the forages by the animals. This improved digestibility may due to increased nitrogen contents of Mulato grass had meet the nitrogen requirements of rumen microbes for breaking down the plant biomass. Further studies are needed to study the mechanism of the Mulato grass that exhibits better animal performances compared to other feeds.

CONCLUSION

Fertilization of Mulato grass with urea increased the plant tillage number, dry matter production, protein content and reduced the neutral detergent fiber content.

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Coprase Produced by *Aspergillus niger* and *Trichoderma spp* Improved Broiler Performance Fed Copra Meal Based Diets

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ABSTRACT

Despite the fact that copra meal is rich in protein (up to 25%), and can meet the crude protein demands for young chicks, it is poor in quality. An experiment was carried out to determine the effect of coprase (an enzyme produced by solid state fermentation that suit to copra meal) on performance of birds fed different levels of copra meal and different enzyme treatments. A total of 108 day old unsexed Cobb were obtained from local company and randomly allocated to brooder cages. The birds were fed with starter diet from days 1 to 21 and grower diets from days 21 to 42. Feed and water were available at all times. The experimental design was a two way factorial with two basal diets (10% and 30% copra meal in the diets), three enzyme treatments (nil, 2.0% coprase produced by *Aspergillus niger* and 2.0% coprase produced by *Trichoderma spp*) and three replicate cages. Analysis of variance indicated that the inclusion of 0.05% coprase produced by *Aspergillus niger* significantly increased body weight gain of birds kept for 42 days from 1494 gram to 1686 gram. Feed conversion ratio was also significantly affected by coprase from *Aspergillus niger*. No interaction between diets and enzyme treatments was found. Among enzyme treatments, enzyme produced by *Aspergillus niger* was more effective in improving body weight gain and decreasing feed conversion ratio.

Key words: coprase, copra meal, broiler and solid state fermentation

INTRODUCTION

Problems of using copra meal in poultry diets have been well reviewed by Sundu *et al*. (2009). Despite the vast amount of previous research conducted into the improvement of the quality of copra meal, there have been few studies of the inclusion of enzymes for increasing its feeding values. Sundu *et al*. (2005) found that the inclusion of commercial enzymes did not improve growth rate of birds to the same level of the growth of birds fed corn-soy diet, particularly in the birds fed the copra meal based mash diets during the starter period. It seems likely that the enzymes used did not entirely suit copra meal as the major ingredients in the diet. This is possibly because most of enzymes available in the market were designed for soybean and wheat diets.

Attempts to improve copra meal quality through the use of specifically designed enzyme have not been reported in data base. The term "coprase" in this project refers to enzyme produced that is suitable for copra meal. Of two methods of producing enzyme, production of enzyme by solid state fermentation method has potential advantages in animal nutrition applications (Filler, 2001). Filamentous fungi such as *Aspergilus niger* is one of the best microorganisms producing enzyme in solid state fermentation method (McCleary, 1988), due to their hyphal growth, which have the capability to not only grow on the surface of the substrate particles but also penetrate through them.

This study was designed to examine the effect of enzyme "coprase" produced by *Aspergillus niger* and *Trichoderma spp* through the solid state fermentation method on birds fed high level of copra meal.

MATERIALS AND METHODS

Solid state fermentation Copra meal was used as solid substrate for fermentation. A total of 500 gram of substrate was placed in a plastic tray and moistenned with 250 ml distelled water. The medium was sterilized by steaming it for 1 hour. The substrate was then incubated with 1 gram fungi (either *Aspergillus niger or Trichoderma spp*). Those fungi were purchased from Laboratory of plant disease at Agriculture Faculty, University of Tadulako. The substrate was placed in a cabin for 5 days at room temperature for fermentation.

Enzyme Extraction

A method of Jacob and Prema (2006)

was used in this study to extract enzyme. A 100 gram of fermented copra meal was throughly mixed with 900 gram distilled water. The mixture was shaked for 1 hour at 200 rpm then was filtered through muslin cloth. The filtrate was centrifuge at 10,000 rpm for 20 minutes. The supernatant was collected as crude enzyme of coprase.

Location and Animals Used in Study

The study was conducted in the animal house at The University of Tadulako, Palu, Indonesia. A total of 108 day-old unsexed Cobb chicks were available for use as experimental animals. They were placed in a brooder pen from days 1 to 21 and given a starter diet. After the 21 day, birds were transferred into floor pen and were offered a grower diet.

Feed and Feeding

Diets were formulated to meet the nutrient requirements of starter broilers (see Table 1), using the UFFF computer program version 1.11 (Pesti *et al.*, 1986). The six diets imposed are described in Table 2. Four hundred ml of a solution of "coprase" was sprayed onto 20 kg feed using a small pressure sprayer and then mixed. The diets were then sun-dried for a day prior to feeding. Feeds were offered *ad libitum* twice a day at 08.00 and 16.30 hours, and water was available at all times. Feed intake and body weight were measured on day 1 and day 42.

Statistical Analysis

The experimental design was a two way factorial with two different levels of copra meal, three different enzyme treatments and three replicate cages. Data was analysed by analysis of variance using the SAS 6.2 statistical program (SAS Institute, 1990). The significance of difference between pairs of treatment means within any overall treatment effects, found significant by analysis of variance, was tested by Duncan's Multiple Range Test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Data of live weight gain, feed intake, feed conversion Ratio (FCR) and growth uniformity are shown in table 3 and 4. The inclusion of 30% CM significantly decreased

Table 1. Ingredient and nutrient composition of the experimental diets (%)

Dietary components	10% CM diet		30% CM diet	
	Starter	Grower	Starter	Grower
Copra Meal	10.0	10.0	30.0	30.0
Maize	44.2	48.0	31.2	32.9
Soybean	24.0	23.0	25.5	25.0
Fish Meal	12.5	9.5	8.0	6.0
Rice bran	7.5	8.0	0.2	0.1
Vegetable oil	0.0	0.0	2.5	3.5
Dicalcium Phosphate	1.1	1.0	1.8	1.8
Premix	0.3	0.3	0.3	0.3
DL-Methionine	0.3	0.1	0.3	0.2
L-Lysine	0.1	0.1	0.2	0.2
Calculated composition; ME (kcal/kg)	3086	3113	3032	3105
Protein	22.0	20.2	22.2	20.9
Methionine	0.7	0.5	0.7	0.6
Lysine	1.3	1.2	1.3	1.2
Calcium	1.1	0.9	1.0	0.9
Available phosphorus	0.8	0.6	0.8	0.6

CM: Copra meal; ME: Metabolizable energy; Kcal: Kilo kalori.

Table 2. Details of the experimental treatments

Diet	Enzymes	Treatments
Diet 1; 10% copra meal based diet (D1)	- without enzymes (E1)	- D1E1
	- with enzyme from *Aspergillus niger* (E2)	- D1E2
	- with from Trichoderma spp (E3)	- D1E3
Diet 2; 30% copra meal based diet (D2)	- without enzymes (E1)	- D2E1
	- with enzyme from *Aspergillus niger* (E2)	- D2E2
	- with from Trichoderma spp (E3)	- D2E3

(P<0.05) the body weight gain of birds fed from 1 to 42 days. Inclusion of enzymes significantly (P<0.05) affected body weight gain and feed conversion rasio. No interaction between levels of copra meal in the diet and enzyme treatments was found for body weight, feed intake and FCR (Table 5).

Meat chickens fed the 10% copra meal based diet (D1) tended to consume less feed (but not significant) than those fed the 30% CM diet. This finding is contrary to the earlier finding of Sundu *et al*. (2004), who found that chicks fed a higher level of CM based diet had decreased feed intake. The reduction in body weight gain due to increased level of copra meal in the diet may indicate that copra meal impaired the quality of diets. According to Sundu *et al*. (2006), there was a very strong negative correlation between the percentage of copra meal in the diet and dry matter digestibility of diet. This is may be the reason of a decreased body weight gain of birds from 1671 gram to 1534 gram.

It has long been believed that enzymes can improve feed intake through hydrolysis of components of the diet. The faster the food components are hydrolyzed the faster the gastro-intestinal tract empties. Studies by Farrell and Martin (1993), Choct *et al*. (1995) and Pluske *et al*. (1997) examining the effect of enzymes on feed intake did not give consistent results for feed consumption. Our current data on the effect of enzymes "coprase" on feed intake also adds to this inconsistency.

Improvement of live weight gain following the addition of enzymes to many different feedstuffs has been reviewed by Teves *et al*. (1988). Data of this current study also gave the same results. Addition of coprase produced by *Trichoderma spp* improved body weight gain by about 9%, compared to those birds fed the diet without coprase supplementation. When coprase produced by *Aspergillus niger* was added to copra meal based diets, the body weight gain was 13% more than birds fed unsupplemented enzyme. It appears that *Aspergillus niger* tended to be more effective in producing enzyme which is suitable for copra meal. The reason why *Aspergillus niger* was slightly more effective in enzyme production in this current study is unclear. Possibly, this filamentous fungi *(Aspergillus niger)* could penetrate their filaments inside the copra meal particle and thus could broken down the nutrient of copra meal into smaller size. Another reason, method of enzyme production in this study may be suitable for *Aspergillus niger* rather than for *Trichoderma spp.* Further experiment is needed to prove this rationale.

Table 3. The effect of level of copra meal in the diets on broilers performance from day 1 to 42

Diet	Feed intake (g)	Weight gain (g)	FCR	Uniformity (% CV)
10% copra meal diet	2837	1671 [a]	1.70	5.4
30% copra meal diet	2883	1534 [b]	1.90	8.3

Note: values with the same superscript within a column are not significantly different (P<0.05).

Table 4. The effect of coprase supplementation on broilers from day 1 to 42

Enzymes treatment	Feed intake (g)	Weight gain (g)	FCR	Uniformity (% CV)
Nil	3017	1494 [b]	2.04 [b]	8.4
Enzyme from *Aspergillus niger*	2650	1686 [a]	1.58 [a]	6.8
Enzyme from *Trichoderma spp*	2913	1627 [ab]	1.78 [ab]	5.4

Note: values with the same superscript within a column are not significantly different (P<0.05).

Table 5. The effect of enzyme treatments in two different diets on broilers performance from day 1 to 42

Diet	Enzyme treatments	Feed intake (g)	Weight gain (g)	FCR	Uniformity (% CV)
10%	Nil	2950	1568	1.88	2.1
CM	Aspergillus niger	2417	1733	1.40	6.9
	Trichoderma spp	3143	1714	1.82	7.2
30%	Nil	3083	1421	2.20	14.7
CM	Aspergillus niger	2883	1641	1.77	6.8
	Trichoderma spp	2683	1540	1.74	3.6

Note: CM: copra meal; CV: cofficient variation.

Since the pattern of weight gain of broilers fed different enzyme treatments did not correlate with their intake, this may indicate that the factor which is mostly responsible for weight gain is feed digestibility. Sundu *et al*. (2006) found that dry matter, protein and lipid digestibilities were improved due to enzyme supplementation in copra meal based diets. In this current study, broilers fed diet supplemented with coprase produced by Aspergillus niger ate less feed but produce higher body weight. Therefore, their feed conversion ratio was much lower than those feed without any coprase suppmentation (1.58 vs 2.04).

CONCLUSION

Addition of 30% copra meal in the diet deteriorate feed quality and thus impairs the performance of birds. Enzyme "coprase" produced by *Aspergillus niger* made a large improvement in body weight gain of birds and reduced feed conversion ratio.

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Effect of Protein Level and Energy-Protein Ratio on the Broodstock Growth Performance of Senggaringan Fish (*Mystus Nigriceps*)

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ABSTRACT

This research was conducted to evaluate the effect of protein level and energy protein ratio on the growth performance broodstock of Senggaringan fish (*Mystus nigriceps*). Three experimental diets and five replications were used in this experiment. Diet A containing 25% protein with energy-protein ratio 18.0 kcal/g protein (25%;18.0), B containing 30% protein with energy-protein ratio13.9 kcal/g protein (30%;13.9), C containing 35% protein with energy-protein ratio 12.0 kcal/g protein (35%;12.0). Fish was fed on experimental diets two times a day at libitum for 35 days. Based on body weigth biomass of the fish, A treatment has a growth from 43.06g to 44.25g, treatment B growth from 43.31g to 46.36g and C treatment growth from 47.40g to 47.70 g. The result found that diets B produced the highest growth performance.

Key words: senggaringan, protein level, protein-energy ratio, growth

INTRODUCTION

Senggaringan fish (*Mystus nigriceps*) represents important and potential fishery source to be developed in Purbalingga Regency. The case proven by utilization for consumption by the society because it has delicious taste. Demand of Senggaringan fish tends to increase, however until now, the supply still depends on natural catch. Therefore, domestication technology needs to be developed to support its continues production.

Success of domestication was highly determined by several aspects, one of it was nutrition (Slamet *et al.*, 1999; Laining and Rachmansyah, 2002; Suwirya *et al*., 2002). To date the information about nutrients demand for senggaringan fish in every level is not available. One of aspect nutrition approach that can be carried out was by estimating protein requirement and energy protein ratio. The fish can be grown when fish consume diet. The growth only happens when energy requirement for maintaining live processes and other functions are fulfilled.

Several important information that can support general waters management and cultivation are continuously collected and studied by researchers, such as ecology and reproduction (Sulistyo and Setijanto, 2002), reproduction biology (Rukayah *et al*., 2003), morphoanatomy index of female senggaringan fish (Sulistyo *et al*., 2007), diet and feeding behavior (Setyanto, 2007) and initial study of life cycle (Pramono and Marnani, 2006). However, research information about nutrients requirement of main parent candidate protein and energy-protein ratio is still very limited. The information is very important in determining success effort of future feeding management.

Protein is important nutrients in fish ration for somatic or gonadic development (Hammer *et al*., 2006; Rodriquez- Gonzalez *et al.,* 2006) or feed cost (Thompson *et al*., 2005; Lee *et al*., 2006). Protein represents the most abundance nutrient in fish body, therefore protein diet should be utilized as efficient as possible for fish growth. In order to utilize feed protein efficient, by the protein must be compensated by non protein energy, such as fats and carbohydrates that have role as sparing effect of the protein (Shiau and Huang, 1990; Perez and Teles, 1999). Majority of protein should be utilized as growth, not being converted into energy (NRC, 1993). Requirement of protein and energy-protein ratio in senggaringan fish need to be studied to obtain information of optimum demand, because the requirement was highly influenced by fish species, age, fish size, diet protein quality, feed digestibility and environment conditions. Knowledge about optimum protein demand is one step that can be conducted to guarantee the success of domestication effort of senggaringan fish.

MATERIALS AND METHODS

Location and Time of Research

The research was conducted during 35 days located in Aquaculture Laboratory of Fishery and Marine Department, Science and Technique Faculty, Jenderal Soedirman University. While analysis of proximate was conducted in the Laboratory of Cattle Feed and Nutrition, Animal Husbandry Faculty, Unsoed.

Experimental Diet

Experimental diet used for growth observation was commercial diet that has different protein content and protein-energy ratio. Diet protein content comprised of three protein level namely, A (25%; 18), B (30%; 13.9) and C (35%; 12). Diet was made in pellet form. Composition of experimental diet was illustrated in Table 1. The gross energy of pellet diet was measured in Animal Physiology Laboratory of Biology Faculty Unsoed and proximate analysis was conducted in Laboratory of Cattle Nutrition and Feed, Animal Husbandry Faculty, Unsoed.

Fish Rearing and Data Collection

Experimental fish is senggaringan fish obtained from Klawing River with assistance of fishermans. Before feeding with experimental diet, fish was adapted for 20 days in rearing container, with the size of measure 60 x 40 x 40 cm. The research was carried out in three treatments and five replications. During rearing period, 50% water was replaced every morning before fish fed. Experimental diet was conducted for 35 days.

Water quality parameters observed were dissolved oxygen (titimetry method) pH and water temperature. Observation on oxygen was conducted in initial and at the end of research while pH and water temperature was conducted once a week during sampling of biomass weight. Biomass weigh was measured once a week during rearing to observe weight and length of fish. Feed was given ad libitum two times per day at 08.00 and 15.00 hours.

Proximate Analysis

Proximate analysis comprised of crude protein, crude fat, ash, crude fiber, nitrogen-free extract and water content of each material such as; fish meat and experimental diet. Proximate analysis of ingredients and experimental diet was conducted at the beginning of research while analysis of fish body was conducted at the beginning and the end of study.

Samples of experimental diet and experimental fish meat were chemically analyzed based on standard procedure (Takeuchi, 1988). For crude protein by Kjeldahl method, crude fat with extraction method by Sochlet equipment. Ash by sample heating in tenure at temperature of 600°C, crude fiber with sample destruction in boiling acid and strong base and water content by heating method in

Table 1. Composition of experimental diet with different protein and protein-energy ratio (g / 100g diet)

Ingredient	Diet (% protein; protein-energy ratio)		
	A (25;18)	B (30;13.9)	C (35;12)
Fish Meal	18.37	22.04	25.72
Soy Meal	13.08	20.28	31.35
Pollard	43.80	38.93	28.04
Wheat Flour	7.00	9.00	5.00
Tapioc meal	3.00	3.00	3.00
Fish Oil	7.37	1.23	1.37
Soy oil	1.86	0.00	0.00
BHT	0.01	0.01	0.01
Mineral Mix	3.00	3.00	3.00
Vit. Mix	1.50	1.50	1.50
Vit. C	0.01	0.01	0.01
C. Clorida	0.50	0.50	0.50
Atractan	0.50	0.50	0.50

Note: protein content (dry weight): fish meal 68.05%, soybean residue meal 41.01%, pollard 14.23%, wheat flour 12.45% and tapioca meal 0.91%

oven at temperature of 105°C. Measured parameter was increase of biomass weight which is discussed descriptively.

RESULTS AND DISCUSSION

Experimental Diet

Experimental diet produced has certain protein level and different energy ratio, analysis result of chemical composition presented in Table 2. Result of proximate analysis showed that diet material used for experimental diet production was meet requirement, so that obtain result that according with Table 1. This caused by in experimental diet formulation, each raw material selected understood it nutrition value. Nutrient content of each diet material understood from laboratory assessment so that commercial diet formulated contains nutrient as expected.

Result of feeding study with different energy and protein content in diet was can influence growth of senggaringan fish. Change of fish biomass was presenting in Figure 1. Based on Figure 1, each treatment increased fish biomass. Average fish biomass was increased at the end of rearing that was A = 44.25g, B = 46.36 g, C = 47.7 g. Growth was indicated by change of length size, weight and volume. Growth of fish was closely related with protein availability in the diet. This can be understood by considering that almost 65-75% of fish flesh dry weight comprise of protein (Watanabe, 1988). Protein represents nutrient that is highly required by fish for growth. Number and quality of protein would influence fish growth (Harper, 1988).

The increase of fish biomass was limited by level of protein content and protein energy ratio (or total energy) of diet. After 35 days treatment it showed that there was biomass change in each treatment (Figure 1). This was caused by energy content in diet consumed by fish was higher than demand for maintenance energy, including for respiration, metabolite transportation, body temperature regulation and other physical activities as stated by Lovell (1988). It means that energy requirement for maintenance should meet first

Table 2. Result of Experimental Diet Proximate Analysis (% dry weight)

Nutrient	Proximate result (%DM)		
	A (25;18)	B (30;13.9)	C (35;12)
Crude Protein	24.98	30.25	35.46
Crude Fat	10.3	2.08	2.12
Ash	8.52	9.2	10.68
Fiber	8.77	6.7	5.4
NFE	47.38	51.6	46.29
Total Energy (KJ/g)*	430.97	400.512	417.69

Note: NFE = Nitrogen-Free Extract.

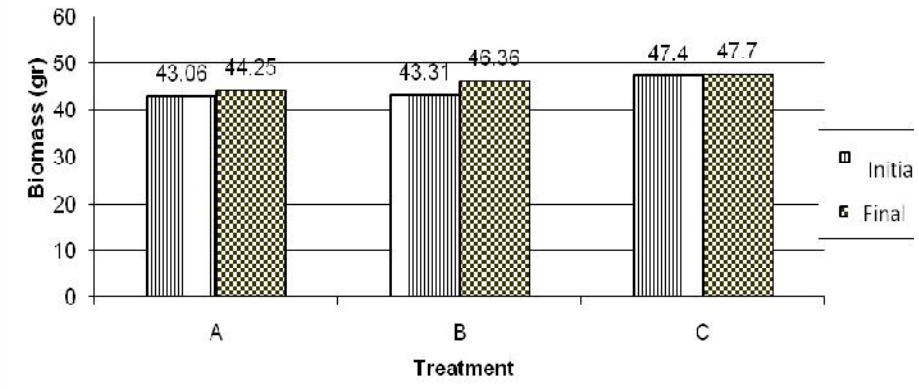

Figure 1. Change of fish biomass influenced by protein and energy level

and the remaining will be used for growth. This proves that usage protein number of diet by fish in treatment was different, because there was difference between protein in diet and non protein energy of diet in each treatment.

From growth data of fish biomass showed that diet B yielded highest growth than diet A and C. Diet B comprised of protein 30%, while diet A 25% and C 35% while fat content relatively same and carbohydrate level of diet B higher than diet C, means that protein ratio of B diet higher than diet C (Table 2).

Generally, this study showed that senggaringan fish also require non protein energy, either from fat or carbohydrate from diet. Actually senggaringan fish is able to utilize carbohydrate as energy source, although protein level of diet B lower than C. However diet B can store diet protein into body protein the same as diet C. This means that the majority energy for fish activities is expected comes from non protein nutrient (fat and carbohydrate). When energy contribution from non protein was low then protein will be degraded as used for energy, so that protein deposition as body tissue is decreased.

Balance of energy and protein in diet was highly responsible to support fish growth. Treatment A has protein content of 25% with energy compensation in diet (430.97kcal GE/g) could not fulfill protein requirement for senggaringan fish. Low protein retention that occurred in protein level of 25% means that protein provided was still low for fish growth, although it was provided high total energy. According to NRC (1993), the optimum energy level in diet is very important because energy shortage will result in decrease of growth rate. Further more, Chow and Watanabe (1988) also stated that young animals generally need higher energy per bodyweight unit for maintenance function than adult animals, although reproduction process increased energy demand of adult animals.

Fish survival during study was relative by the same between treatments. For dissolved oxygen content in each treatment ranged from 8-9 ppm, temperature ranged from 21-25C and pH level ranged from 6-7. This indicated that amount or type of diet provided already enough to meet maintenance requirement of fish and even provide growth for fish.

CONCLUSION

Diet B protein of 30% that compensated by energy protein ratio of 13.9 kcal GE/g of protein, providing highest growth rate of biomass weight in broodstock of senggaringan fish (*Mystus nigriceps*).

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Profil Of Milk Industry In The Province Of Central Java (Study Of Milk Cooperatives Profitability)

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ABSTRACT

This research was conducted to study of profitability performances of milk cooperatives in area which are as centre of milk production in the Province of Central Java. The cooperatives generally have some kind of business to support their main function in supplying fresh milk to milk processor industries, such as feed industry, farm industry and as milk collector. This research was done in 2008 at eleven milk cooperatives in north and east of Mount Merbabu area (Milk cooperatives: Gotong Royong, Mekar, Rukun, Pabelan, Getasan, Andhini Luhur in District of Semarang and Mojosongo, Musuk, Cepogo, Ampel-Ganesha and Kota at the District of Boyolali). The method used in this research was survey with interviews to cooperative's chairman and collected the annually reports of cooperative's RAT (annual cooperative membermeeting from 2004-2006 (reported in 2007). The variable observed were its financial ratio on profitability, and financial efficiency. The financial ratio's on profitability consist of net cooperatives income from operations, Return on Assets (ROA), Return on Equity (ROE), operating profit margin ratio, while for financial efficiency is the measure of Assets Turn-Over (ATO). The research results have indicated that average net cooperatives income from operations between IDR 20-75 millions annually in the District of Semarang and between IDR 25-172.7 millions rupiahs in the District of Boyolali. Its value on ROA and ROE were varied and fluctuated around 1.07-1.44 % and 2.46- 3.58 %, in the District of Semarang respectively, and ranged between 0.15-3.8 % and 1.06-4, 44 % in the District of Boyolali. It's value on operating profit margins have reduced sharply from average 17 to 8.64 % between 2004-2006, while it's assets turn over were around 18.93 to 23.91 %, in the District of Semarang,and fluctuated around 10.7-16.8% and 12.2-15.4 % for profit margin and assets turn over in the District of Boyolali. The low performance of milk cooperatives, therefore by regarding it's profitability and it's value on assets turn over indicated that the managerial skill of cooperatives top management were still too weak lead to uncertainly milk supply sustainability to milk processors. The milk industries in Central Java were worst.

Key words: *Central of Java, Milk Cooperatives, Profitability, Financial Efficiency*

INTRODUCTION

Milk farming was first introduced in Indonesia on the island of Java during the Dutch colonial era, when small herds of Holstein-Friesian cattle were kept close to the cities of Jakarta and Surabaya and in the highlands where the climate suited this temperate breed. After independence, the herds were broken up and smallholder dairies emerged. Each farmer owned one or two cows and raw milk was sold in urban areas through a system of private collectors who acted as middlemen; the farmers were paid about 25 percent of the retail price. A serious attempt to develop the milk industry along modern lines was made by the government, commencing with the first Five-Year Development Plan (1969-1974). A separate Department of Cooperatives, which had a strong influence on rural development including milking, was formed at this time. During the next Five-Year Development Plan (1974-1979), attention given to milk development was intensified. Milk consumption was increasing yet local production was still below 20 percent of the national requirements. In 1979 the National Union of Milk Cooperatives of Indonesia (GKSI) was established. This was to be a significant event as it brought under one umbrella all the existing milk cooperatives in the rural areas. The union provided assistance to small farmers by way of imported cattle (with help from the Department of Livestock Services), credit for the purchase of cattle

and equipment for milk collection and milk chilling, as well as vehicles for transport.

During the subsequent Five-Year Development Plan (1979-1984), higher priority was given to milk development with the objective of improving the living standards of smallholder farmer families through creating new rural job opportunities as well as reducing import subsidies. Marked development in the milk sector attributable to substantial government inputs and strong leadership occurred during this period. As a result of the increased volume of milk produced locally, the government had to strengthen facilities for milk handling and chilling, efficient milk collection, road transport and marketing. Foreign assistance and expertise were obtained by the government to assist in these activities. The primary-level milk cooperatives, the National Union of Milk Cooperatives and the private-sector milk-processing industries participated jointly in the measures taken to absorb the steadily increasing quantities of milk. The milk-processing plants were directed to accept these increasing amounts on a quota system to be reviewed every six months. The quota established was the ratio of the quantity of locally produced milk to be purchased against milk powder imported by the processing factories in liquid equivalent. The real renaissance of the milk cooperatives resulted from Presidential Decree No. 2 of 1978, which enhanced the role of the multipurpose village unit of cooperative society or Koperasi Unit Desa (KUD), giving members greater participation in rural economic development. This enhanced self-reliance and increased participation of the rural population led the way to fuller development of all cooperatives including those in which milking was the dominant activity. Indeed, the President of Indonesia remarked at that time: "The primary task of development is to uplift the common people from the abyss of poverty and the principal solution for raising the weak from poverty and destitution is through cooperatives." (Tambunan, 2008).

Milk farming in Indonesia still remains a small farmers' operation with an average of 3-6 cows per farmer, who traditionally has also kept cattle for manure. More than 85 percent of the milk farmers are members of milk cooperatives that handle the collection, chilling and distribution of milk to milk-processing plants. It is therefore, talking about milk industry in Indonesia should consider the performance of milk cooperatives: their roles and tasks'. Farmers' livelihoods depend largely on the strength of milk cooperatives. The milk cooperatives strength, it self depend, on skill of managers who carry out milk cooperatives.

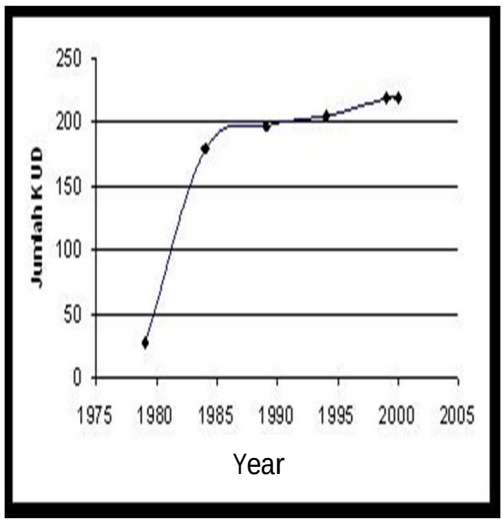

Figure. 1. The number of milk cooperatives in Indonesia and their role in milk Industry

In general the function of milk cooperatives are as : marketing which transport of milk to market, purchasing which purchase and sell of milk, bargaining which give services (credit, insurance, payment of telephone and electricity) as well as negotiation and product processor (Wissman, 2005; Williamson, 1998).. During recent years, the agribusiness sector has more and more been confronted with the challenges

Table 1. The number of milk cooperatives in Indonesia and thei role in milk Industry

Year	No of coop*	Dairy cows (head)		Milk Production (ton)	
		National	Kept by coop	National	Kept by coop
1977	2	91,000	1,184	60	1.6
1982	80	140,000	84,891	117.6	72
1987	190	233,000	169,795	234.9	194.6
1992	201	312,226	289,384	367.2	332.9
1997	212	353,119	338,354	446.4	421.4

of internationalizing food markets. This development has also seized milk cooperatives which are forced to become more competitive (Ebneth and Theuvsen, 2002). Milk Cooperative managers seemingly have main objectives in their quest for maintaining a viable milk farm business included profitability, and financial efficiency

Profitability, meaning the ability of the milk cooperatives to cover all costs over time and accumulate wealth in order to survive over time (Trannel, 2002). Profitability of milk cooperatives which have a function as bargaining cooperative or milk manufacturing provide information about the success and failure of business activities.(Henehan, 2002). While, financial efficiency measures the intensity a business uses it assets to generate gross revenues and the effectiveness of production, pricing, financing, and marketing (Ling, 2006)

DATA COLLECTION AND ANALYSIS

Data Collection

Primary and secondary data were collected by survey from Milk Cooperatives in District of Semarang and Salatiga as well as in Boyolali.

The primary data were collected from person interview from manager which carries out milk cooperatives, while the secondary data were from the annual report of cooperatives (RAT, annual cooperative member meeting) 2004-2006, collected at the early of 2008.

Data Analisis

Data obtained were analyzed descriptively of its value on Return on Equity, Return on Investment, Operating Profit Margins Friesian Flag as well as Indolakto in Jakarta , Citra National in Salatiga and Sari Husada in Yogyakarta.

Net Cooperatives Income from Operations (NCIFO)

This is the measures of cooperatives revenues from any operations- cost of production known as SHU (Sisa hasil usaha), as in Table 2. In general, the values of NCIFO at the District of Boyolali were greater than those in Semarang- Salatiga District. Trend of NCIFO value at the District of Semarang-Salatiga during 2004-2006were increase, except for KUD Getasan. Values NCIFO of of KUD Mekar and Andhini Luhur were fluctuated indicating the change of managerial style or profit opportunities. While in Boyolali District these values were decrease except for Mojosongo. In general, the value of Net Cooperatives Income from Operation of Milk Cooperatives was less and less in Boyolali District, while there relatively stable in Semarang District.

and Assets Turn Over. As compared to those of the ideal value as Tranel (2002)

a. Profitability
   1. Net Cooperatives Income From Operations (NCIFO) = Total Revenue – Total Cost
   2. ROA = $\frac{\text{NCIFO}}{\text{Total Asset}}$
      Target: more than Bank interests
   3. ROE = $\frac{\text{NCIFO}}{\text{Total Modal}}$
      Target: more than Bank interests
   4. *Operating Profit Margin Ratio*
      = $\frac{\text{NCIFO}}{\text{Gross revenue}}$
      Target: more than 25%

b. Financial Efficiency

$$\textit{Asset Turnover Ratio} = \frac{\text{Gross revenue}}{\text{total asset}}$$

Target more than 35%

RESULTS AND DISCUSSION

Milk Production Area

At the District of Semarang and Salatiga as well as in the District of Boyolali, milk farmers are associated with village milk cooperatives at sub District level. The village units of cooperatives implicated at this study were KUD Mekar Ungaran, KUD Rukun Salatiga, KUD Gotong Royong Tengaran, KUD Getasan, KUD Sumber Karya Pabelan, KSU Andini Luhur Tengaran. While at the District of Boyolali were KUD Mojosongo, KUD Kota, KUD Ampel, KUD Cepogo and KUD Musuk. These village unit of cooperatives have a function in bargaining and milk marketing, generally to milk factories, i.e.:

Return on Assets

Return on Assets (ROA) eliminates the influence of different capital structure . Thus, it is more comparable to judge cooperative enterprise effeciency.. All of village unit cooperatives (milk cooperatives) in Central

Table 2. Net Cooperatives Income from Operations of Milk Cooperatives at Central Java

No	Milk Cooperatives	NCIFO (Rupiah)		
		2004	2005	2006
1	KUD Gotong Royong	9,091,527.50	18,668,600.87	20,081,415.00
2	KUD Mekar	82,421,925.14	44,837,717.84	52,217,440.68
3	KUD Rukun	52,472,531.67	60,610,444.15	75,498,136.84
4	KUD Pabelan	28,661,547.56	36,043,671.17	37,491,818.96
5	KUD Getasan	54,955,714.55	55,254,134.08	35,066,723.41
6	KSU Andini Luhur	32,904,731.95	21,710,600.00	55,634,950.00
	Avg District of Semarang-Salatiga	**43,417,996.40**	**39,520,861,35**	**45,998,414.15**
7	KUD Mojosongo	161,589,153	164,118,210	172,402,914
8	KUD Musuk	143,035,592	130,882,545	120,397,234
9	KUD Ganesha-Ampel	54,408,202	45,463,704	6,375,390
10	KUD Cepogo	71,085,543	45,367,065	25,244,830
11	KUD Kota Boyolali	137,903,629	132,139,142	127,573,766
	Avg District of Boyolali	**113,604,423.8**	**103,594,133.2**	**90,398,826.8**

Java have presented their values on ROA lower than 5 %.

The value of ROA were still lower than the interest of conventional bank at the moment or the value of profits sharing of Islamic or Syariah Banks, 5 %. Even in general , trend of ROA were decrease. In USA, the value of ROA of milk industry was between 10-14 % (Bolland *et al*., 2000).

Return on Equity **(ROE)**

Return on Equity (ROE) reflects a company potential to realize profit and income . Such as their value on ROA, the values of ROE of milk cooperatives in Central Java were also less than 5 % of interest or profit , far below the value of ROE of milk industries in USA (Bolland *et al*., 2000), and lower and lower during consecutive years.

Operating Profit Margins (OPM)

The value of operating profit margins of most milk cooperatives in Central java were far below 25 % of ideal value. Milk cooperatives KSU Andhini Luhur have presented the the ideal value. Even though, it is considered as pseudo-cooperatives since, it was not clear the kind of cooperatives members which provide milk.

Table 3. Return on Assets of Milk Cooperatives at Central Java

No	Milk Cooperatives	ROA (%)		
		2004	2005	2006
1	KUD Gotong Royong	0.24	0.49	2.15
2	KUD Mekar	2.71	1.44	1.45
3	KUD Rukun	1.71	2.01	2.54
4	KUD Pabelan	0.64	0.75	0.75
5	KUD Getasan	0.73	0.73	0.44
6	KSU Andini Luhur	2.60	1.02	2.04
	Avg. District of Semarang-Salatiga	**1.44**	**1.07**	**1.23**
7	KUD Mojosongo	2.32	2.29	2.23
8	KUD Musuk	1.58	1.51	1.35
9	KUD Ganesha Ampel	1.37	1.1	0.15
10	KUD Cepogo	0.57	0.36	0.2
11	KUD Kota Boyolali	3.38	2.84	2.84
	Avg District of Boyolali	**1.84**	**1.62**	**1.35**

Figure. 1. Map of milk area production in the province of Central Java

The OPM measure profit per unit of output. Since milk cooperatives in Central Java are in the form of village unit cooperatives which their main businesses are not only milk and milk products, it was therefore not clear whether the most profits among the village unit cooperatives come from milk business.

Asset Turnover Ratio

Assets turnover ratio reflects financial efficiency of milk cooperatives as presented in Table 6.There were only villages –unit cooperatives of KUD Rukun and Mojosongo which have received the ideal ratio, 35 % (Tranel, 2002). The value of financial efficiency will measure the intensity a business uses it assets to generate gross revenues and the effectiveness of production, pricing, financing and marketing (Ling, 2006). It also indicates that milk cooperatives managers do their business in best way or not.

Table 5. Value of OPM of Milk Cooperatives at Central Java

No .	Koperasi	Operating Profit Margin Ratio (%)		
		2004	2005	2006
1	KUD Gotong Royong	5.67	11.19	9.22
2	KUD Mekar	9.19	4.45	5.22
3	KUD Rukun	4.08	5.96	6.10
4	KUD Pabelan	3.90	4.07	3.21
5	KUD Getasan	3.90	3.38	2.37
6	KSU Andini Luhur	76.99	33.99	25.69
	Avg District of Semarang-Salatiga	**17.29**	**10.52**	**8.64**
7	KUD Mojosongo	0.07	0.06	0.06
8	KUD Musuk	0.08	0.07	0.08
9	KUD Ganesha Ampel	0.11	0.08	0.01
10	KUD Cepogo	2.47	0.36	0.18
11	KUD Kota Boyolali	0.25	0.26	0.23
	Avg District of Boyolali	**0.60**	**0.17**	**0.11**

Table 6. Value of Assets Turn Over (ATO) of Milk Cooperatives in Central Java

No	Milk Cooperatives	Asset Turnover Ratio(%)		
		2004	2005	2006
1	KUD Gotong Royong	4.26	4.40	23.35
2	KUD Mekar	29.55	32.31	28.55
3	KUD Rukun	41.92	33.68	41.62
4	KUD Pabelan	16.48	18.49	23.37
5	KUD Getasan	18.67	21.71	18.62
6	KSU Andini Luhur	2.68	3.08	7.94
	Avg District of Semarang-Salatiga	**18.93**	**18.95**	**23.91**
7	KUD Mojosongo	33	33	35
8	KUD Musuk	1.6	19	16
9	KUD Ganesha Ampel	12	12	12
10	KUD Cepogo	0.23	1	1
11	KUD Kota Boyolali	14	11	11
	Avg District of Boyolali	**12.17**	**15.2**	**15**

GENERAL DISCUSSION

In general, the performances of milk cooperatives in the Province of Java Central were low. In District of Semarang-Salatiga were better than those in District of Boyolali. The low performance of profitability and financial efficiency of milk cooperatives in Central Java were generally due to low level of education of farmers, milk cooperatives managers and also low productivity of milk cows kept by farmers. This situation has weighted by low land ownership which generally less than 0.5 Ha per farmers with 4-6 head of cows. It was also found that milk cooperatives are still engaged in a ruinous predatory competition against each others, rather than to choose a merger strategy. One of bad attitude of milk cooperatives in these provinces was to receive and collects milk from farmer's members of other milk cooperatives. In the past the village unit cooperatives or primary cooperatives which were joint to secondary cooperative called as Association of Milk Cooperatives or GKSI has produced milk products by their own brand and managed by themselves. But now the brands of Indomurni and Indolakto have been sold to PT Indolakto, and becoming new private industry named PT Indolakto. This was an indication of weak position of milk cooperatives in market. In the other hand, the privately owned milk industry often own strong brands and have comparatively strong market position, even in low price mass market articles like milk powder, pasteurized milk and butter as well as sterilized milk. It was a worst situation for milk cooperatives considered lead to bankruptcy due its value of OPM far below 25 %. Two ways to increase profitability are to increase profit per unit of output or to increase volume of output , while maintaining profit per unit.

Increasing profits per unit of output need to reduce cost of milk production at the farm level by modify fixed cost or variable cost and reduce handling cost of milk at the milk cooperatives level. With the handling cost of milk around IDR. 560- 700 for the milk price IDR. 2.800 per liter or 25 %, it was considered as so expensive by farmer-members, lead to incapability of farmers to improve the management of milk cattle and milk quality. While for increasing the volume of output are limited by the low productivity of milk cows. Majority of milk cows kept by farmers are Friesians Holstein breed imported from temperate region. High temperature and humidity in Indonesia are making heat stress for milk cows, lead to sharp reduction of milk production at least 30 %. Gradual acclimatization or placing the cows in the hill with sufficient water supply will keep the productivity of this breed more than 20 liter per days. It is a case of the District of Semarang but not District of Boyolali which suffers from the lack of water, especially during dry seasons..

But, improving farmer's skill and knowledge to formulate feed for their milk cows are regarded as the best way in order to reduce cost of production and increase the milk production without add the number of cows in limited land surface and will maximize the profits. Addition the number of heads wills risqué to reduce fodder base support.

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Physiological Status, Blood Profile and Body Composition of Sheep Fed With Ca-Saponified Lemuru Oil Coated by Herbs

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ABSTRACT

Indonesia is one of humid-tropical country with average daily temperature range from 22 - 35^{o}C with relative humidity around 85%. In this condition, animal has problem with body thermoregulation and utilization of energy budget. To solve the problem, nutritionist tries to reduce *heat increment* trough feeding management. This study was aimed to evaluate physiological status, blood profile and body composition of sheep fed with ca-saponified lemuru oil coated by herbs. Twenty thick tail sheep (av. BW 23 kg) were used in this experiment. The average room temperature during experiment was 22–33^{o}C with 83–92% relative humidity. All animals fed with concentrate contained 3% ca-saponified lemuru oil and king grass (1:1) *ad libitum*. Treatments were control diet without ca-saponified lemuru oil (R1); ca-saponified lemuru oil coated by curcumae (R2); ca-saponified lemuru oil coated by ginger (R3); ca-saponified lemuru oil coated by *Eugenia polyantha* (R4) and ca-saponified lemuru oil coated by *Pluchea indica* Less (R5). Design of this experiment was completely randomized design with four replications of each. The parameters observed were respiration rate, heart rate and body temperature, while from the blood profile were erythrocyte, haemoglobin, PCV, leucocytes and it differentiations. Using Urea space technique the body composition (body water, protein and fat) were calculated. Result showed that thick tail sheep reared in high temperature room fed with ca-saponified lemuru oil coated by herbs had not significance difference on body temperature, heart rate and respiration rate in all treatments. There was tendency to increase heart rate and respiration rate in the afternoon caused by environment temperature and the activity. The percentage of body composition was same in all treatments, but for total body fat and energy retained in treatments were lower than control. The parameters of blood profile showed that total leucocytes, netrophil and lymphocytes were significantly increased in herb treatments compared to control. It is concluded that supplementation of 3% ca-saponified lemuru oil coated by herb (curcuma, ginger, *Eugenia polyantha* and *Pluchea indica*) in thick tail sheep had better immune respond (higher leucocytes, lymphocytes and netrophil) and lower total body fat and energy retained.

Key words: Ca-saponified, lemuru oil, herbs, curcuma and leucocytes

INTRODUCTION

Lamb meat has high cholesterol which caused metabolic syndrome disease (atherosclerosis). This situation reduces demand of this product. On the other hand, sheep is one of favorite animal and usually reared as a saving animal. It is important to manage system in order to get good quality of meat without reduce the productivity. Feeding management can solve the problem through manipulation rumen fermentation. It was reported that meat cholesterol in sheep (80 mg%) was higher than in beef (74 mg%) and chicken broiler (73 mg%). Composition of lamb meat contained 18.6% protein, 12.94% of fat and 0.07% of cholesterol (Nutrition Glossary, 2005). Ruminant meat contain high saturated fatty acids (laurate, myristate, and palmitate) that caused high cholesterol in the plasma (Grande, 1975).

Polyunsaturated fatty acids (PUFA) omega-3, in the fish oil can reduce risk of atherosclerosis (Iger, 2003). Substitution polysaturated fatty acid with polyunsaturated fatty acid could reduce total cholesterol, include LDL-cholesterol (Marsic and Yodice, 1992). Source PUFA, which high omega-3 is lemuru oil from lemuru fish.

Fat has higher energy content compared to carbohydrate and protein. As a feedstuff, fat resulted low heat increment in metabolism, so it is good add in animal ration of the tropical country to reduce heat stress. Specific problem in tropical country is high humidity and environment temperature which caused decrease feed intake, difficulties to

evaporate heat production and increase respiration rate. On the other hand, high forages intake caused high heat increment (Sudarman and Ito, 2000). In order to reduce the negative effect of high environment temperature, it is suggested to give fat in the ration. Fat addition in the ration will reduce heat increment but increase energy intake. Wachira *et al*. (2002) reported that fish oil could reduce dry matter intake in sheep. Palmquist and Jenkins (1980) reported that fatty acid in the rumen can reduce by supplement calcium, so the fat could not coupled rumen microorganism. Through the by pass rumen technique, fat can add until 5% without decreased digestibility fiber.

Sudarman *et al*. (2006) reported that utilization of 1.5% ca-saponified lemuru oil in ration could reduce meat cholesterol-LDL on sheep until 32 % and increased feed efficiency until 36%. Problem was the ration had bad smelt and caused decrease feed intake.

Herbs have good effect to palatability. *Curcumae domestica, Zingiber officinale* Rosc., *Eugenia polyantha and Pluchea indica* Less are some herbs that has specific smelt and good taste. The secondary compound of *Eugenia polyantha* is eugenol, citral and methylchavicol, while secondary compound of *Zingiber officinale* is fellandren, kamfer, borneol, zingiberin, zingiberol, gigerol, zingeron, vitamin A, B1, C and the secondary compound of *Curcumin domestica* is turmeron, zingiberen; turmerol, fellandren, (Asiamaya, 2006). The secondary compound of *Pluchea indica* is some amino acids such as leusin, isoleusin, triptofan, treonin, vitamin A and C (Asiamaya, 2003). In order to increase feed intake, ca-saponified lemuru oil has coated with those herbs. Percentage of herbs on lemuru oil should be determined for reducing side effect of the herbs.

This research was aimed to evaluate the effect of supplementation kind of herbs to ca-saponified lemuru oil (*Curcumae, ginger, Eugenia polyantha and Pluchea indica* Less) on physiological status, blood profile and body composition using urea space method in thick tail growing sheep.

MATERIALS AND METHODS

Twenty male thick tail growing sheep (av. 25 kg BW) were used in this experiment. Animal were fed concentrate contained Ca-saponified lemuru oil coated by kinds of herbs and water served *ad libitum* in all time. Ration was consist of concentrate and native grass 1:1. Concentrate (87.5% DM) supplemented with 3% of ca-saponified lemuru oil coated by herb. Ca-saponified lemuru oil was made by double decomposition method (Jenkin dan Palmquist, 1984). Before the ingredient mix with other feedstuff, the herbs were added around 10% into ca-saponified lemuru to coat the fat in order to reduce the strong smelt. The formulation ration consisted of palm kernel meal, coconut meal, cassava meal, pollard, molasses, urea, soybean meal and trace minerals. Composition of proximate analysis was 16% crude protein, 5% of extract ether, 10% crude fiber and totally has 70% TDN. Using a completely randomized design, four treatments with five replications were arrange as :

R1 = control ration without lemuru oil
R2 = ration supplemented with 3% ca-saponified lemuru oil coated by curcumae
R3 = ration supplemented with 3% ca-saponified lemuru oil coated by ginger
R4 = ration supplemented with 3% ca-saponified lemuru oil coated by *Eugenia*
R5 = ration supplemented with 3% ca-saponified lemuru oil coated by *Pluchea*

During two months experiment the physiology parameters like daily heart rates, respiration and body temperature were evaluated morning, at noon and afternoon.

Prior to and the last week of the experimental period, each sheep was sampled the blood in order to evaluate the profile of blood (erythrocyte, haemoglobin, PCV, leucocytes and its differentiation) and continued injected with 130 mg urea/kg metabolic body size (MBS) dissolved in sterile saline (200 $g.ltr^{-1}$) through jugular vein within one minute, and times was recorded at the beginning and the end of injection. Jugular vein was flush with 3 ml of heparinized saline after urea injection. The actual quantity of urea injected was determined gravimetrically by weighing syringes before and after injection. Blood samples were obtained before injection and 12 minutes after the mean injection time. The zero time samples were analyzed for hematology parameters while the rest was

separated by centrifugation of blood at 10.000 x g for 10 minutes to get plasma for urea-N by KIT method. Urea space was calculated by dividing the dose of urea N injected with the change in plasma urea –N before and after 12 minutes injection, following the equation as described by Bartle *et al*. (1983), while body protein and fat were calculated following Panaretto and Till (1963), and body water according to Rule *et al*. (1986). All formulas were expressed as following:

Urea Space (%) =

$$\frac{\text{Dose of urea–N urea injected (mg)}}{\text{Change in plasma urea–N (mg\%)x 10 x Live Weight (LW)}}$$

Body Water (%) = 59,1 + 0,22 X US (%) – 0.04 LW
Body Protein(%) = 0,265 X BW (%) -0,47
Body Fat (%) = 98 – 1,32 X BW (%)

The hematology parameters were measured as mention by Sastradipradja *et al*. (1991). The significance of difference between means were compared using Duncan Multiple range test after ANOVA using program Minitab/SPSS release 6.1 version.

RESULTS AND DISCUSSION

The data on physiological status are presented in Table 1. There was no significance difference of body temperature, respiration rate and pulse in all treatments. Supplemtation of 3% herbs in the ca-saponified lemuru oil resulted increasing of respiration and heart rate, especially at noon and afternoon. Increasing of pulse and respiration rates at that time had correlation with daily activities and environmental temperature. This data were similar with body temperature, 38.5^{o}C, in the morning and 39.30^{o}C in the afternoon that reported previously (Astuti and Sastradipradja, 2000).

Blood profile data of erithrocytes, hemoglobines and PCV were same in all treatments and in the range of normal condition. The normal value of hemoglobine is 10 – 12 mg%, PCV is 28 – 32% and erithrocyte is 6-9 x 10^6/mm3 (Guyton dan Hall, 1997). Total erythrocyte will decrease following the age. Data of erithrocyte in this experiment was higher than data reported previously (erithrocyte was 4 x 10^6/mm3 and emoglobine was 6.7 mg%) in local sheep under the forest area with low quality diet (Astuti *et al.*, 2009). Leucocytes, lymphocytes and netrophyl were significantly difference caused by treatments ($P<0.05$). Herbs (dominantly curcumae) on ca-saponified lemuru oil increased number of lecocytes, lymphocytes and netrophyls. It is known that leucocyte and lymphocytes has imune effect on body condition.

There was no significance difference in body water using urea space technique, the same situation holds for body protein and boby fat. This experiment has data of body water around 57%, while body fat and body protein were 21.8% and 14.9% , respectively. The value of percentage body water and body protein in this study were lower than data reported previously in growing priangan sheep (Astuti and Sastradipradja, 1999). The body water and body protein of growing

Table 1. Physiologis status of sheep fed with ca-saponified lemuru oil coated by herbs

Parameters	R1	R2	R3	R4	R5
Body temperature (^{o}C)					
Morning	38.7	38.7	38.4	38.5	38.6
Noon	38.9	38.8	38.8	38.7	38.9
Afternoon	39.1	39	39	39	39
Heart rate: beat/min					
Morning	97	94	90	97	96
Noon	102	100	91	98	96
Afternoon	115	105	100	113	103
Respiration: /min					
Morning	29	30	32	30	31
Noon	49	63	61	60	58
Afternoon	52	53	54	43	48

Note: R1, R2, R3, R4 and R5 were control, curcumae, ginger, *Eugenia and Pluchea indica Pluchea indica*, respectively.

Table 2. Blood profile of sheep fed with ca-saponified lemuru oil coated by herbs

Parameters	R1	R2	R3	R4	R5
Erythrocyte (10^6/mm3)	10.6±1.01	10.4±0.70	10.7±099	9.1±1.1	9.7±0.37
Haemoglobine	9.1±0.70	8.67±0.58	8.7±0.51	8.5±0.40	7± 0.90
PCV	26±2	29±4	29±2.6	28.5±2.5	28±1.5
Leucocyte (10^3/mm3)	8±0.3^b	9.5±0.09^a	8.4±0.01^{ab}	8.9±0.37^a	9±0.22^a
Lymphocyte	41.75±15^b	52.75±13^a	37±11^b	39.5±7^b	35.25±5^b
Netrophyls	45±15^b	49.5±9.7^a	56±11.5^a	50±5.85^a	53±15^a

Note: R1, R2, R3, R4 and R5 were control, curcumae, ginger, Eugenia *and Pluchea indica*, respectively. Superscript with different notation in th same colom was significance (P<0.05).

Table 3. Body composition of sheep fed with ca-saponified lemuru oil coated by herbs

Parameters	R1	R2	R3	R4	R5
Body water (%)	57.7	57.8	57.8	57.9	57.8
Body fat (%)	21.8	21.7	21.7	21.6	21.7
Total body fat (kg)	7.62^a	7.34^b	7.35^b	6.90^b	7.32^b
Body protein (%)	14.8	14.8	14.9	14.9	14.9
Total body protein (kg)	5.18	5.03	5.03	4.76	5.01
Energy retained (kJ)	424.21^a	410.04^b	409.83^b	385.68^b	408.44^b

Note: R1, R2, R3, R4 and R5 were control, curcumae, ginger, Eugenia *and Pluchea indica*, respectively. Superscript with different notation in the same colom was significance (P<0.05).

priangan sheep were around 68% and 16.80%, while body fat was 9.78%. Local sheep with high fat and low body water and protein showed that these animal were older than growing priangan sheep. There is close correlation between age and body composition. Fat percentage will increase following the age, while body water and protein will decrease in older animal.

Total body fat (kg) and energy retained (kJ) in herb treatments were lower than those of control (P<0.05). Herbs with secondary compound tended to decrease fat deposition on body composition. It was similar with previously data that curcumae could reduce fat-cholesterol (Sudarman, 2006).

CONCLUSION

Supplementation of 3% ca-saponified lemuru oil coated by herb (curcumae, ginger, *Eugenia polyantha* and *Pluchea indica*) in thick tail sheep had better immune respon (higher leucocytes, lymphocytes and netrophyls) and lower total body fat and energy retained.

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The Performance of Bali Cattle Fed With Ratio Containing *Pleurotus osteorus* Fermented and Urea-Ammoniated Sago Waste

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ABSTRACT

Sago waste is an agricultural by product with low nutritive value. The present experiment was designed to study the effects of using treated sago meal to substitute grasses on Bali cattle productivity. Fifteen young male Bali cattle, approximately 18-month of age were allocated to experimental treatments according to the average weight of the animal in a randomized complete block design. Native grass was used in the experiment. The dietary treatments were (1) 60% grasses and 40% concentrate, as control, (2) substituting 15% of grasses with fermented sago waste, (3) substituting 30% of grasses with fermented sago waste, (4) substituting 15% of grasses with ammoniated sago waste, and (5) substituting 30% of grasses with ammoniated sago waste. The results showed that substituting 50% of the grass with treated sago waste gave the greatest daily weight gain (0.66kg) as compared to the other treatments ($P<0.05$). Intake and digestibility of feed dry matter, organic matter and fiber components were not significantly different among treatments. However, the digestibility of ADF and cellulose were greater when 15% and 30% of the grass were substituted with fermented sago waste. The feed to gain ratio was most efficient in the animal receiving 50% bio-processed sago waste as substitute of native grass. The rumen characteristics showed that concentration of NH_3 and VFAs were not significantly different among treatments. It was concluded that bio-fermentation of sago waste using *Pleurotus ostreatus* and ammoniation with urea improved its nutritive value and could be used to substitute native grass for cattle.

Key words: sago waste, biofermentation, Pleurotus ostreatus, ammoniation, beef cattle

INTRODUCTION

Lately, the demand of beef in Indonesia increase rapidly, therefore efforts to accelerate the beef production should be done. Bali cattle an alternative breed to be developed to fill the gap between the supply and demand of meat. Beef consumption increased from 330,300 ton in the year 2002 to 389,300 ton in 2006. Unless an appropriate move in accelerating beef production being taken, the beef cattle population will decrease significantly. Fortunately, the cattle population tend to increase from 10,532,889 in 2004 to 11,869,158 in 2008 (DITJENAK, 2008).

The primary constraint to cattle is productivity that the requirement of nutrient for a better production is not satisfied by the existing feed condition, especially protein so that the livestock do not perform their maximum genetic capacity. The grasses in tropical countries are mostly of low quality and usually fed to the animal at mature stage in which advanced lignifications of the structural polysaccharide components have developed. Meanwhile, planting superior grasses such as elephant grass and guinea grass have a constraint of limited area.

Utilization of by products of agro industries, such as sago waste is one alternative to overcome this problem. However, it is realized that the utilization of the sago waste need preliminary treatment due to the high in fiber and low in protein content. Toharmat (2002) stated that feed with high fiber content will be slowly digested in the rumen and ends approximately after 72 hours inside the rumen.

Biological processing of sago waste that has been done was bio-fermentation using oyster mushroom. This mushroom is grouped in class of *basidiomycetes* which could be cultivated on different kinds of agricultural wastes and could also be consumed by human being. Rai and Saxena (1990) stated that cultivation is not only cheap, but also able to improve the quality of feed. Okano *el al*. (2005) stated that the fermentation of *Japanese red cedar* with white rot *basidiomycetes* (oyster mushroom) could increase the dry

matter digestibility. Ammonia treatment could also to increase the quality of feed where the processing technology with urea will increase the crude protein content (Broudiscou *et al*., 2003). The *in vitro* nutrient digestibility of amoniated rice straw resulted from ammoniation was higher than rice straw which was not ammoniated (Eun *et al*., 2006). Based on these informations, the sago waste which has been processed through fermentation with oyster mushroom and ammoniation with urea was tried to feed to cattle replacing native grasses.

The objective of the present experiment was to study the influence of sago waste based ration on the digestibility and fermentability of feed and the productive performance of Bali cattle. It is hoped that the sago waste can be used as feedstuff to substitute the grasses during the dry season, in addition to preventing the environmental pollution especially in the area of sago powder producer.

MATERIALS AND METHODS

Fifteen young male Bali cattle with an approximate age of 18 months and an average body weight of 130 ± 6.25 kg were used in the experiment. Cattle were grouped based on body weight and put in the stall based on respective dietary treatment. Sago waste has been fermented with oyster mushroom. Sago waste were fermented by inoculating around 10 g of mushroom per kg of substrates and incubated until first and second harvest (50-70 days after planting). Whereas ammoniation of sago waste were based on the results of Kardaya (2006) using urea at 3% and incubated for 14 days. The ration consisted of 40% concentrate and 60% of native grass based on dry matter, which will be substituted gradually by the product of sago waste processing (Table 1).

The rations were given 3 times a day, i.e., early morning (07:00), 12:00 and 17:00. Drinking water was available at any time. The parameters observed were: 1). The change of body weight, which were measured by weighing the cattle bi-weekly, 2) Feed intake and feed efficiency, 3) Income over feed cost, which were estimated based on the prices of feed and the value of cattle as if being sold based on the live weight, 4). Digestibility of dry matter, organic matter, protein, NDF, ADF, and cellulose using total

Table 1. Ingredient composition and nutrient content of rations

Component of ration	Treatment of ration				
	R0	R1	R2	R3	R4
	……%……				
Ingredient					
Native grass	60	45	30	45	30
Fermented-sago waste [a)]	0	15	30	0	0
Ammoniated-sago waste	0	0	0	15	30
Concentrate	40	40	40	40	40
Total	100	100	100	100	100
Nutrient composition	……%……				
Proximate Analysis[*)]					
Dry matter	88.1	88.3	88.4	88.1	87.9
Crude protein	12.8	12.1	11.5	12.2	11.6
Fat	4.6	4.5	4.4	4.5	4.4
Crude fiber	27.4	23.5	19.6	24.2	20.9
Ash	8.6	9.3	9.9	8.7	8.7
NFE	46.6	50.6	54.6	50.5	54.4
Goering &Van Soest Analysis [**)]					
NDF	43.9	42.8	41.8	44.4	44.9
ADF	30.4	29.3	28.1	31.2	31.9
Cellulose	22.4	21.2	20.0	22.5	22.6
Lignin	4.2	4.1	4.0	4.6	4.9
Silica	1.4	2.2	2.9	2.3	3.3
TDN[***)]	51.3	55.9	59.2	55.0	58.7

Note: NFE = Nitrogen free extract; NDF = *Neutral Detergent fiber*; ADF = *Acid detergent fiber*; TDN= *Total Digestible Nutrients*
[*)] Analysis of Animal Biology Laboratorium; [**)] Indonesian Research Inst. Animal Production, Ciawi-Bogor; [***)] Calculated;
[a)] Fermented sago waste from field for biological test, fermented for 50-60 days.

collection methods for 7 days, 5) NH_3 concentration which were measure by spectrophotometer, 6) VFA (total and partial com pound) which were measured using GC (gas chromatography).

The experiment was carried out in a randomized block design, with 5 treatments and 3 groups of cattle based on body weight. The differences between the treatment means were analyzed using Duncan test (Steel and Torrie 1991). The treatments were as follows:

R0 = 60% native grass + 40% concentrate
R1 = 45% native grass + 15% fermented sago waste + 40% concentrate
R2 = 30% native grass + 30% fermented sago waste + 40% concentrate
R3 = 45% native grass + 15% ammoniated sago waste + 40% concentrate
R4 = 30% native grass + 30% ammoniated sago waste + 40% concentrate

RESULTS AND DISCUSSION

Feed Intake, Body Weight Gain and Feed Conversion

Intake of dry matter ranges between 2.58-3.06% of body weight. These results are higher than those observed by Anggraeny and Umiyasih (2004) in beef cattle which were 2.0-2.1%. Cattle receiving rations containing fermented sago waste at 30% (R2) consumed higher ($P<0.05$) feed compared to cattle fed with R0, R1 and R3 but there was not different from R4 (Table 2).

Average intake of organic matter by cattle receiving R2 treatment was higher ($P<0.05$) than the cattle at R0; however the organic matter intake of cattle at R1 and R3 were equal to R4. The lack of difference in intake of dry matter and organic matter between R2 and R4 treatment showed that the use of fermented sago waste or ammoniated sago waste to replace 50% grass in the ration did not bother the palatability and other factors which affect consumption. This may be caused by physical and chemical properties of processed sago waste which resulted in a better quality so that it is preferred by the animal. Parakkasi (1999) stated that such factors influencing the consumption of feed materials include physical and chemical properties of the feed.

The product of fermentation has a higher nutritional value than the original material because the microbes have degraded the more complex material into simpler substances, making them easier to digest. Fermentation process will also cause changes in chemical composition such as fat, carbohydrates, amino acids, minerals and vitamins as a result of the activities and proliferation of microorganisms during the fermentation process (Winarno, 1992). The use of fermentation in the sago waste tends to increase the rations NFE, lower crude fiber, NDF, ADF, cellulose, hemicellulose and lignin thereby increasing the ration consumption.

Ammonia treatment can increase the consumption of dry matter and organic matter (Weiss and Underwood, 2002). The increased consumption due to the ammonia treatment was related to the damage of the chemical bonds between the lignin and the cellulose or hemicellulose. The fact that lignin inhibits fiber digestion therefore damaging the bond causes hemicellulose and cellulose to be more digestible. The high cosumption of dry matter and organic matter can also

Table 2. The average of dry matter, organic matter consumption, body weight, body weight gain and rations conversion

Items	Treatment of rations				
	R0	R1	R2	R3	R4
Consumption					
Dry matter (% BW)	2.58±0.1^{a}	2.69±0.3^{ab}	3.06±0.1^{c}	2.67±0.1^{ab}	2.85±0.3^{bc}
Organic matter (%BW)	2.42±0.1^{a}	2.45±0.0^{a}	2.68±0.1^{b}	2.44±0.0^{a}	2.64±0.1^{b}
Body weight					
Initial (kg/head)	142±2.0	133±2.3	132±2.1	136±2.0	124±2.5
Final (kg/head)	17±1.8	162±5.1	171±2.0	163±4.7	161±3.6
Total gain (kg/head)	29.2±1.8	29.7±5.1	39.3±1.5	27.3±3.5	36.7±5.1
Daily gain (kg/head/day)	0.48±0.1^{ab}	0.49±0.1^{ab}	0.65±0.1^{c}	0.45±0.1^{a}	0.61±0.1^{bc}
Ration conversion	7.96±0.4^{b}	7.47±1.4^{ab}	5.96±0.3^{a}	8.05±0.9^{b}	6.07±1.1^{a}

Note: ^{a,b,c}Within rows, means followed by different superscript differ ($P<0.05$);R0=control ration; R1= 45% grass + 15% fermented sago waste + 40% concentrate; R2=30% grass + 30% fermented sago waste + 40% concentrate; R3= 45% grass + 15% ammoniated sago waste + 40% concentrate; R4= 30% grass + 30% ammoniated sago waste + 40% concentrate; BW= body weight.

be affected by the treatment of fermentation and ammoniation by creating a more conducive environment of rumen conditions for microbial activity which is indicated by the normal range of total VFAs. These conditions encourage the growth of microbes and the movement of food in the digestive tract become aroused quickly and animal to consume more. Leng (1991) suggested that the level of consumption is strongly influenced by the coefficient of indigestion, the quality of rations, fermentation in the rumen and the physiological status of animal.

Beef cattle production which is reflected in the increase of body weight is the main goal in testing a ration, where the change in body weight is the result of such treatment. "Body weight gain" of cattle receiving dietary treatments in the present experiment ranged from 0.456 - 0.655 kg/head/day. These values were higher than the results of experiment reported by Mathius *et al*. (2005), using Bali cattle fed with palm kernel meal fermentation, which increase body weight gain ranging from 0.310 - 0.582 kg/head/day. The increased body weight was significantly different between cattle treated with R2 ($P<0.05$) as compared to R0, R1 and R3 but not different from R4, and R0 was not different from R1 and R3. The higher body weight gain in cattle fed rations containing 30% fermented and ammoniated sago waste, showed that there was a positive response of the cattle to the rations. Body weight gain is the accumulative response of animal to the feed intake, digested nutrients, fermentation, metabolism and absorption of nutrients. The higher increase in the body weight of cattle fed with 30% treated sago waste was caused by higher consumption of dry matter and organic matter.

The lowest "Feed Conversion ratio" (FCR) was observed in cattle receiving fermented sago waste at 30% (R2) i.e., 5.96 and the highest was for the cattle treated at 15% ammoniated sago waste i.e., 8.05. Research conducted by Adamovic *et al*. (1998) for beef cattle fed rice straw biodegraded by oyster mushrooms showed higher FCR, i.e., 7.41 - 9.14. Table 2. shows that the feed conversion ratio of cattle fed R2 was significantly lower ($P<0.05$) as compared to R0 and R3, but equal to R1 and R4.

The results showed that the substitution of grass by the fermented and ammoniated sago waste until 50% tended to improve the feed conversion ratio significantly. These were indicated by the higher body weight gain in R2 and R4 than those in R0, R1 and R3. This proves that the treatment using product fermentation and ammoniation of sago waste at 30% were able to improve the changes of feed dry matter into live body weight , which for the formation of 1 kg of live weight only 5.96 and 6.07 kg feed dry matter were needed.

"Analysis of the cattle business income" is necessary to know the advantages of a livestock business. Income over Feed Cost (IOFC) as illustrated in Table 3 is a simple economic analysis used to see the benefits of beef cattle business. The income over feed cost is influenced by the amount of feed consumption, prices of feed ingredients and the amount of the daily weight increase produced. Income over Feed Cost of cattle in R2 was substantially higher ($P<0.01$) compared to R3 and substantially higher ($P<0.05$) from R0, R1, R4, while R0 equal to R1, R3 and R4.

IOFC difference is due to the additional cost of the oyster mushroom fermented sago waste. Income over Feed Cost was higher with increasing use of fermentation in the sago waste in the rations. This shows that the rations containing fermented sago waste is more efficient and profitable. The use of ammoniated sago waste will provide greater benefits than cattle fed the control treatment when used up to 30% in the ration (Table 3).

Nutrient Digestibility

The ration digestibility was influenced by feed intake, nutrient content and digesting process in the rumen and the post-rumen compartment (Beever and Mould, 2000). Dry matter, organic matter, protein and NDF digestibility of Bali cattle were not influenced by ration type. Results of this experiment showed that the use of processed sago waste for feeding Bali cattle to substitute the grass gave the same effect with the control (R0). The fiber source of R0 treatment was native grass with good quality which was comparable to sago waste. Based on the data, of Table 4, there is an indication that fermentation and

Table 3. IOFC (Income Based on Value of Body Weight Gain)

Items	Treatment of rations				
	R0	R1	R2	R3	R4
Feed cost (Rp/head/day)	5,759.7	7,377.5	10,133.4	5,779.7	6,099.0
Value of BW (Rp/head/day)	10,685.7	10,057.1	14,457.1	9,219.0	13,409.5
Value of mush. (Rp/head/day)	0.0	4,033.2	8,738.6	0.0	0.0
IOFC (Rp/head/day)	4,926.1^{ab}	6,712.8^{ab}	13,062.4^{c}	3,439.4^{a}	7,310.6^{b}

Note:^{a,b,c}Within rows, means followed by different superscript differ (P<0.05);R0=control ration; R1= 45% grass + 15% fermented sago waste + 40% concentrate; R2=30% grass + 30% fermented sago waste + 40% concentrate; R3= 45% grass + 15% ammoniated sago waste + 40% concentrate; R4= 30% grass + 30% ammoniated sago waste + 40% concentrate; BW= body weight; mush= mushroom; IOFC= income over feed cost.

ammoniation treatment could be applied to increase the quality of sago waste resulting in similar digestion responses.

Dissolution of crystalline cellulose-lignin bonds (in the form of an ester bond) with koniferil, sinapil and *p*-kumaril alcohol in fermentation treatment facilitate the penetration of cellulases produced by rumen microbes. Dissolution of the bond was marked by increasing the solubility of each fibrous component (hemicellulose, cellulose, lignin). This decreases the percentage content of fiber components so that *in vivo* digestion of feed fiber components increased (Maynard *et al*., 1980).

The average digestibility of dry matter in this study is higher than studies conducted by Li *et al*. (2001) in cattle using cotton seed hull fermented by oyster mushroom which was 52.3%. An experiment conducted by Prasetiyono *et al*. (2007) in beef cattle fed rice straw with the addition of CASREA (Cassava urea) also showed a lower result, i.e., 50.42%.

There were significantly different ADF digestibilities between treatments. The use of ammoniated sago waste at 30% in the ration resulted in the same effect on ADF digestibility as in control, but decreased significantly as compared to the use of ammoniated sago waste at 15%, and fermented sago waste at 15% and 30%. This difference is probably due to the ADF content of the rations. Table 1 shows that the level of ADF tended to increase with increasing use of ammoniated sago waste in rations (0.8 - 1.5%).

Digestibilities of nutrients in all treatments were greater than 50% (Table 4.) indicating that the ration treatment and the condition of the cattle rumen bacteria support an optimal activity. One way to measure the rumen microbial activity is the value of nutrients in digested ration components. Increased rumen microbial activity was stimulated by the availability of ammonia and VFA in the rumen, where the concentrations of these two compounds are in the normal range to support the growth and rumen microbial activity.

Characteristics of Rumen and the Microbial Fermentation

Ammonia

The results showed that the characteristics of microbial fermentation in the rumen were not affected by treatment (Table 5).

Table 4. Average Nutrient digestibility of Bali cattle

Items	Treatment of rations				
	R0	R1	R2	R3	R4
	---------- % ----------				
Dry matter	76.5±0.1	70.1±4.9	72.6±0.7	74.4±0.7	72.6±2.5
Organic matter	73.4±2.6	69.5±3.8	70.3±6.6	72.4±1.1	71.7±1.7
Protein	79.8±2.3	75.4±7.8	75.3±0.7	79.5±1.0	75.7±3.8
NDF	58.9±1.1	55.1±7.8	56.7±0.9	59.1±2.3	58.5±2.7
ADF	54.4±6.1^{ab}	60.4±3.7^{b}	60.3±1.5^{b}	59.9±3.3^{b}	51.8±2.3^{a}
Cellulose	66.4±2.2	63.4± 9.8	67.9±10.4	69.5±2.8	58.3±1.2

Note: ^{a,b,c} Within rows, means followed by different superscript differ (P<0.05);R0=control ration; R1= 45% grass + 15% fermented sago waste + 40% concentrate; R2=30% grass + 30% fermented sago waste + 40% concentrate; R3= 45% grass + 15% ammoniated sago waste + 40% concentrate; R4= 30% grass + 30% ammoniated sago waste + 40% concentrate; NDF= Neutral detergent fiber; ADF= Acid detergent fiber.

No differences between the treatments on ammonia concentrations showed that all treatments provide similar level of ammonia into the rumen, although the type of bacteria that use ammonia are different.

The main product of protein degradation by rumen microbes is ammonia, which most of the microbes use it for *de novo* protein synthesis. Therefore, the ability to provide sufficient ammonia in the rumen is often used as a benchmark in the evaluation of feed protein for ruminant livestock. Ammonia levels ranged from 10.10-13.67 mM (Table 5), and the highest values obtained in R4. Preston and Leng (1987) stated that the concentrations of ammonia in the rumen for optimal microbial growth were 4 - 14 mM. The results of this study showed that fermentation technology and ammoniation of sago waste were still able to ensure adequate supply of ammonia in the rumen.

Volatile Fatty Acids (VFAs)

Carbohydrates are fermented in the rumen by microbes into fatty acids or VFAs. VFAs will be absorbed and used as source of energy for animal and a source of carbon skeleton for the bacteria. Concentration of volatile fatty acids in the fermentation media described the effectiveness of the fermentation process. In general, the higher concentration of VFA indicates fermentation process to be more effective; however, when the VFA concentration is too high, it will disrupt the balance of rumen microbial population.

Higher concentration of VFA in rumen fluid of cattle given R2 indicates that rumen bacterial growth in this treatment is better than the other treatments. These results indicate that overall carbohydrate rations degradation was higher in R2, although not significantly different. R2 ration containing fermented sago waste at 30% allows the easy availability of carbohydrates being degraded, because the fermentation process has disrupted the ligno-cellulose bonds causes fiber-degrading bacteria to work better. Total VFA productions in this study were within the normal range of 106.8 - 144 mM (Table 5). The results of this study are not much different from what was obtained by Deborah *et al*. (2005) on the use of probiotics in the Bali cattle, i.e., 130.33 - 158.67 mM.

Fermentability of feedstuff carbohydrate as measured from the VFA results are supported by the availability of sufficient ammonia in the rumen. Most of rumen microbes use ammonia to proliferate especially for cell protein synthesis (Suryahadi & Amrullah, 1984), whereas the origin of feed carbohydrates will be hydrolyzed to VFA as an energy source. This shows the relations between the two compounds (ammonia and VFA) in supporting optimum growth for the rumen microbes.

Partial VFA production were still fall within the normal proportions. This experiment produced higher proportion of acetic acid, i.e., 63.8-68.8%, followed by propionic acid 19.0-22.2%, butyric acid 9.4-10.9%, isobutiric acid 1.2-1.7%, valeric acid 0.0-0.5% and 1.1-1.7% isovaleric (Table 6). Nagaraja *et al*. (1997) concluded that the partial VFA production was influenced by the balance between roughage and concentrates in the ration, or the higher fiber content of the feeds will result in relatively higher

Table 5. The average of ammonia concentration, VFA total and partial of Bali cattle rumen fluid

Items	Treatment of rations				
	R0	R1	R2	R3	R4
	..... mM				
Ammonia	12.2±2.7	10.1± 1.9	11.0±2.1	11.3±3.6	13.7±2.1
Total VFA	114±5.0	120.3±10.0	144±9.4	106.8±27.8	113.2±31
Acetic acid	93.4±3.1	74.4±7.1	82.9±7.8	68.5±16.9	71.4±17
Propionic acid	30.4±3.3	23.8±2.7	22.8±2.1	22.7±6.1	25.6±9.9
Butyric acid	15.8±2.3	12.8±2.3	11.3±0.5	11.5±3.3	11.5±4.4
Isobutyric acid	1.8±0.2	1.3±1.5	1.8±0.6	1.6±0.6	2.6±1.2
Valeric acid	0.5±0.4	0.5±0.4	0.2±0.3	0.6±0.7	0±0.0
Isovaleric acid	2.2±0.4	1.8±0.4	1.3±0.1	1.9± 1.1	2.0±1.0

Note: R0=control ration; R1= 45% grass + 15% fermented sago waste + 40% concentrate; R2=30% grass + 30% fermented sago waste + 40% concentrate; R3= 45% grass + 15% ammoniated sago waste + 40% concentrate; R4= 30% grass + 30% ammoniated sago waste + 40% concentrate; VFA= volatile fatty acid.

Table 6. The average of VFA proportion in rumen fluid of Bali cattle

Items	Treatment of rations				
	R0	R1	R2	R3	R4
	……..mM……				
Acetic acid	64.8±3.1	65.3±2.4	68.8±1.2	64.2±0.9	63.8±5.6
Propionic acid	21.1±1.7	20.9±2.2	19.0±0.4	21.2±1.4	22.2±3.4
Butyric acid	10.9±1.6	11.2±1.1	9.4±0.5	10.8±2.2	10.0±1.3
Isobutyric acid	1.2±0.2	1.2±0.1	1.5±0.7	1.5±0.3	1.7±0.3
Valeric acid	0.3±0.3	0.4±0.3	0.2±0.3	0.5±0.5	0±0.0
Isovaleric acid	1.6±0.3	1.1±0.2	1.1±0.1	1.7±1.5	1.7±0.4

Note: R0=control ration; R1= 45% grass + 15% fermented sago waste + 40% concentrate; R2=30% grass + 30% fermented sago waste + 40% concentrate; R3= 45% grass + 15% ammoniated sago waste + 40% concentrate; R4= 30% grass + 30% ammoniated sago waste + 40% concentrate.

proportions of acetic than propionic, and butyric. On the other hand, if concentrate portion was higher in the ration, then the proportion of propionic acid was higher. Preston and Leng (1987) reported that VFA production in the rumen was influenced by various factors including: kind of animal, kind of feed, quantity and quality of feed. No differences in VFA concentrations between the treatments may also be due to similar feed quality between treatments. This study shows that the use of sago waste, after being fermented or ammoniated, in the ration did not affect rumen characteristics. This means that the residue of sago waste processing can be used up to 30% in cattle rations, because they do not significantly interfere the rumen microbial activity.

CONCLUSION

Sago waste, after being fermented or ammoniated can be used until 30% in the rations or substituting 50% of grass and significantly increased the dry matter and organic matter intake, body weight gain and feed conversion ratio, but did not influence the nutrient digestibility and rumen characteristic of Bali cattle.

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The Effect Of Fermentation Ragi Tape Products In Diets On Nutrients Digestibility And Growth Performance Of Bali Drake

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ABSTRACT

This research was carried out to study the effect of fermentation ragi tape products (rice bran, pollard, soybean-hull, and cocoa-pod fermented) in diets on nutrients digestibility and growth performance of Bali drake aged 4-10 weeks. The design of experiment used a completely randomized design (CRD) with nine treatments and six replications. There were 10 birds in each replication with relative homogenuous body weight (359±12,75 g). The diets were formulated to 17% crude protein and 2900 kcal ME/kg as a control diet (A), diets with 15% rice bran (B), 15% rice bran fermented by 0,30% yeast culture (C); 15% pollard (D), 15% pollard fermented by 0,30% yeast culture (E), 15% soybean-hull (F), 15% soybean-hull fermented by 0,30% yeast culture (G), 15% cocoa-pod (H), and 15% cocoa-pod fermented by 0,30% yeast culture (I), repectively. Diets and drinking water were provided *ad libitum*. Variables were observed in this experiment are nutrient digestibility (protein, crude fibre, and energy), feed consumption, live weight gains (LWG)s, and feed conversion ratio (FCR). The results of this experiment showed that fermented rice bran, pollard, and soybean hull in diets, resvectively did not effect on feed consumption, but increasing LWGs compared with unfermented feeds. The nutrient digestibility (protein, crude fibre, and metabolizable energy) and FCR, however decreased. Birds offered fermentation ragi tape products (treatment C, E, G, and I) had higher (P<0,05) nutrient digestibility and growth performance compared with those of control and unfermented products. It is concluded that inclusion of fermentation ragi tape products (rice bran, pollard, soybean-hull, and cocoa-pod fermented) in diets could increased nutrient digestibility and growth performance of Bali drake aged 4-10 weeks.

Key words: Yeast culture, dietary fiber, digestibility, performance of drake

INTRODUCTION

Aspergillus oryseae (AO) and *yeasts*, particularly *Saccharomyces cereviseae*, have been used as probiotics by many workers (Piao *et al*., l999). Both *Aspergillus spp*. and *Saccharomyces* belong to the *Ascomycoyina* subdivision and have many industrial applications in the brewing, destilling, ang baking industries (Han *et al.*, 2001).

Yeast culture product, which have some fermentation ability consist of yeast (*S. cerevisae*) and the media which the yeast grew on (Bidura *et al.*, 2008a). Piao *et al*. (l999) showed that 0,10% yeast added to a diet could reduce animal wastes, and similar results were reported by Park *et al*. (l994). But, Piao *et al*. (l999) reported no significant improvement in weight gain, feed intake, and feed efficiency with 0,10% yeast culture. Feeding live yeast to broiler breeder reduced colonization of salmonela in their ceca and improved posphorus utilization in growing chickens.

The potensial of forages by-products as energy sources for poultry depends considerably on such factors as cell wall content, degree of microbial fermentation in the large intestine, and extent of absorption and utilization of the volatile acids produced (Kahlique *et al*., 2003). Agro-industry by-product is one such product abundantly and cheaply available during the season. These toxic factors are trypsin inhibitor, lectin (hemagglutinin), phytic acid as phytate, and crude fiber. These anti-nutritive factors have been re-

ported to reduce feed intake and depress performance of poultry.

Knudsen (2001) reported that dietary fiber (DF) has been defined as the complex macromoleculer substances in food plants that are not degraded by mammalian digestive enzymes. With the exception of lignin, all of the materials called DF are carbohydrates in nature. DF is thought to mediate protective effects on the colonic epithelium through their fermentation products and fecal bulking capacity (Wang *et al.*, 2003).

Ration high fiber resulted in a lowered rate of lipogenesis and tendency of an increased capacity to utilize acetyl-CoA in pigs (Zhu *et al.*, 2003). Non starch polysaccharide (NSP) are the carbohydrate components of DF and predominant substrates for anaerobic fermentation. Non starch polysaccharide can be broken down by microflora permanently colonizing the gastrointestinal tract and their breakdown in all nonruminant mainly occurs in the hindgut by microbial fermentation (Weng *et al*., 2003).

Among the cell wall polysaccharides known as nonstarch polysaccharides (NSP) are celluloses, pectins, and oligosaccharides can not be degraded enzymitically in the digestive systems of the birds due to the lacking of enzymes degrading the NSP in their digestive systems (Choct, 2002). Beta-glucans and pentosans decrease digestion and absorbtion of nutrient due to their effects on the intestinal viscosity (Ikegami *et al*., 1990). Most of the recent studies focus on the effect of the bacterial and fungal enzymes used in cereal based diets.

More than 50% of phosphorus in other plants seeds in the phytate form, which is poorly available in digestive tract of monogastric animal (Ilyas *et al*., l995). Phytic acid found in vegetable feed sources affect protein and amino acid digestibilities negatively by preventing activities of the proteolytic enzymes such as pepsin/trypsin. Futhermore, phytic acid has a higher P content and chelating ability and phytate form of phytic acid diminishes the availability of Ca and P (Pointillart, 1991). Monogastric animal can not use of phytin phosphorus due to lacking of phytase enzyme in their digestive systems and consequently phytin posphorus is mostly excreted in the faeces.

Therefore, it is sugested that fermentation of feedstuff by yeast can be used in order to alleviate negative effect of phytic acid. Gut microfloral enzymes are beneficial to the nutrition of the host because they increase the digestion of nutrients, especially in the lower intestine. Previous experiments showed that the inclusion of microorganisms in diets improved feed conversion efficiency and digestibility (Chen *et al.*,2005).

The objective of this study was to determine effect of fermentation ragi tape products (rice bran, pollard, soybean-hull, and cocoa-pod fermented) in diets on nutrients digestibility and growth performance of Bali drake.

METERIALS AND METHODS

Animal Experiment

Five hundred fourty of Bali drake 4-wk-old were randomly allotted to colony wire-floored cages, 10 birds per cages. A 500 ml plastic muge/bottle equipped was placed of each cage. Experimental diets and drinking water were provided *ad libitum* during the entire experimental period (for a 6-week periods). Body weight and feed intake were recorded weekly.

Ration Experiment and Drinking Water

The nine experimental diets (Table 1) based on corn-soybean meal were as follows : a control diet (A); diet with 15% rice bran (B); 15% fermented pollard by 0.30% yeast culture (C); with 15% pollard (D), 15% fermented pollard by 0.30% yeast (E), 15% soybean hull (F), 15% fermented soybean hull by 0.30% yeast (G), 15% cocoa-pod (H), and 15% fermented cocoa-pod by 0.30% yeast cultur (I), respectively. The basal diets (Table1) were formulated based on meet or exceed nutrient requirement (NRC, 1994). All diets were iso-energy (2900 kcal ME/kg) and iso-protein (CP:17%). Through all the experimental period, birds were allowed *ad libitum* acces to feed and water. The composition of ration compiler substances and nutrient which is used in diets can be seen in Table 1.

Saccharomyces cereviseae

Ragi tape is brand name of *Saccharomyces cereviseae* culture produced locally by fermenting rice brand with *S. cereviseae*. *Saccharomyces cerevisieae* from ragi tape

Table 1. Formula and chemical composition of diets of growing Bali drake aged 4-10 weeks (as-fed basis)

Ingredient (%)	Treatment Diets[1)]								
	Control	B	C	D	E	F	G	H	I
Yellow corn	68.70	63.20	63.20	67.50	67.50	60.00	60.00	58.10	58.10
Soybean meal	19.70	1.60	1.60	11.30	11.30	1.50	1.50	1.90	1.90
Pollard	11.30	5.90	5,90	15.00	**15.00***	3.36	3.36	6,20	6.20
Fish meal	0	14.00	14.00	6.10	6.10	13.9	13.9	14.60	14.60
Rice bran	0	15.00	**15.00***	0	0	3.30	3.30	3.00	3.00
Soybean hull		0	0	0	0	15.00	**15.00***	0	0
Cocoa-pod	0	0	0	0	0	0	0	15.00	**15.00***
Palm oil	0	0	0	0	0	2.64	2.64	0,90	0.90
NaCl	0.1	0.10	0.10	0.10	0.10	0.10	0.10	0,10	0.10
Mineral mix	0.2	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Total	100	100	100	100	100	100	100	100	100
Chemical composition[2)]									
ME (kcal/kg)	2903	2903	2903	2904	2904	2901	2901	2903	2903
Crude protein (%)	17	17	17	17	17	17	17	17	17
Eter extract (%)	6.43	7.46	7.46	8,07	8.07	7.10	7.10	6.32	6.32
Crude fiber (%)	4.04	8.02	8.02	7.25	7.25	7.93	7.93	8.06	8.06
Calsium (%)	1.57	1.26	1.26	1.55	1.55	1.55	1.55	1.55	1.55
P-available %)	0.63	0.58	0.58	0.64	0.64	0,64	0.64	0.64	0.64
Argynine (%)	1.28	1.20	1.20	1.20	1.20	1.20	1.20	1.20	1.20
Lysine (%)	1.21	1.16	1.16	1.21	1.22	1.21	1.22	1.22	1.22
Metionine (%)	0.42	0.37	0.37	0.39	0.40	0.39	0.40	0.40	0.40
Triptophan %)	0.19	0.18	0.18	0.18	0.18	0.18	0.18	0.18	0.18

Note : *) : Feeding ingredient fermented by 0.30% yeast culture before using in diets; [1]The ration without rice bran, pollard, soybean hull, cocoa-pod, and yeast as a control (A), ration with 15% rice bran (B); 15% fermented rice bran by 0.30% yeast culture (C); with 15% pollard (D), 15% fermented pollard by 0.30% yeast (E), 15% soybean hull (F), 15% fermented soybean hull by 0.30% yeast (G), 15% cocoa-pod (H), and 15% fermented cocoa-pod by 0.30% yeast cultur (I), respectively; [2]According to the calculation of Scott *et al.* (l982).

which used is common yeast used in "tape" making title "Na Kok Liong", ensiled in number 26895.

Fermentation of Feedstuff

Fermentation of rice bran, polard, soybean hull, and cocoa-pod ingredient were prepared from the same batch approximately 0.3% yeast culture ("ragi tape") were added to each feeding. Following the fermentation, water was added to bring the product to 50% content and fermented for 6 days. After fermentation, fermented of rice bran, polard, soybean hull, and cocoa-pod was dried at 45^0C for 6 h.

Retention and Excretion of Nutrients

After three weeks of feeding trial, 6 birds from each treatment were randomly assigned to individual metabolic cages to determine retention and excretion of dietary nutrients. Excreta was collected for 6 days. Foreign substances (feathers, scurf, etc) mixed in the collected excreta were removed before drying at 60^0C for 48 h and subse quent grinding. Ration and feces were analyzed by AOAC (l994) procedures for proximate components. The retention of nutrients was calculated by dividing the amount of retained nutrient (ingested nutrient minus excreted nutrient) by the amount of ingested. To determine the concentration of Ca and P in feeds and excreta, samples were dry ashed (AOAC, 1994) and assayed at the specific wave lengths for each element.

Analysis of Nutrients

Dry matter (DM), organic matter (OM), CP and CF were done according to the Assocciation of Official Analytical Chemists (l994). The CP content of the diets was determined using the Kjeldahl procedure (AOAC, 1994). Crude fibre of the feeds were determined using procedure of Van Soest *et al.* (l991).

Statistical Analysis

All data were analyzed by a one-way analysis of variance test (Steel and Torrie, 1989). Statistical significances among treat-

ment means were determined by method of New Multiple Range Test of Duncan when the F value was significant at 5 % level.

RESULTS AND DISCUSSION

Nutrient digestibility and metabolizable energy of unfermented feeding (UFF) and fermented feeding (FF) ingredient are showed on Table 2. Digestibility of crude protein, and crude fiber were slightly increased significantly different (P<0.05) by the fermentation.

The metabolizable energy on birds offered FF was increased than metabolizable energy on birds were offered UFF (Table 2). Fermented of feeding ingredient were caused increased of crude protein and crude fibre digestibilities, resvectively than UFF ingredients.

The result indicated that all of nutrient digestibility of fermented feeding (rice bran, pollard, soybean hull, cocoa pod, and poultry feather meal) by yeast culture were increased significantly (P<0.05) different than UFF.

The average of final body weight and LWGs during six weeks observation at birds which having the ration control (A) are 1165 g/birds and 715 g/birds/6 weeks, respectivelly (Table 3). The average of final body weight and LWGs of the birds having ration with UFF ingredients e.g. rations with 15% rice bran (B); with 15% pollard (D), 15% soybean hull (F), and 15% cocoa-pod (H), respectevely were not affect significantly different (P>0.05) both on final body weight and body weight gains. But, the bird were offered FF ingredient e.g. 15% fermented rice bran by 0.30% yeast culture (C); 15% fermented pollard by 0.30% yeast (E), and 15% fermented soybean hull by 0.30% yeast (G),respectively were increased significantly different (P<0.05) than birds were offered UFF and control diets.

At the end of the experiment (at 42 days of age) the body weight gain was significantly (P<0,05) increased in the group FF (fermented fed) diet compared to groups that received control and UFF (unfermented fed). In general, the body weight gains were tended to decrease with used of cocoa-pod (treatment H and I) which is probably due to high theobromin, tannin, and high fiber contents on the cocoa-pod used in experiment.

Feed consumption was not affected by fermentation of feed (FF) in diets. The averages of feed consumption between in treatments B, C, D, E, F, G, H, and I were not significantly different (P>0.05) than control groups.

The average of feed conversion ratio (feed : gains) during six weeks observation at birds which having the ration control (A) is

Table 2. The effect of fermented rice bran, pollard, soybean hull, and cocoa-pod by Yeast culture on nutrient digestibilities (% dry matter), respectively

Feeding Ingredient	Digestibility (%)			ME
	DM	Crude Protein	Crude Fibre	(kcal/kg)
Rice bran				
• Control (UFF)	58.03 [b]	64.59 [b]	43.70 [b]	1460 [b]
• Yeast culture (FF))	62.06 [a]	88.62 [a]	57.06 [a]	1897 [a]
Pollard				
• Control (UFF)	66.69 [b]	65.97 [b]	35.09 [b]	2305 [b]
• Yeast culture (FF)	72.40 [a]	84.93 [a]	60.85 [a]	2517 [a]
Soybean hull				
• Control (UFF)	63.71 [b]	61.09 [b]	48.96 [b]	2061 [b]
• Yeast culture (FF)	73.63 [a]	82.83 [a]	66.79 [a]	2567 [a]
Cocoa-pod				
• Control (UFF)	52.32 [b]	47.31 [b]	44.37 [b]	1656 [b]
• Yeast culture (FF)	67.93 [a]	60.99 [a]	63.07 [a]	2258 [a]
Poultry feather meal				
• Control (UFF)	46.29 [b]	42.10 [b]	13.32 [b]	1760 [b]
• Yeast culture (FF)	61.41 [a]	74.39 [a]	35.07 [a]	2341 [a]

Note: The different superscript at the same column in the feedstuff, respectivelly is significantly different (P<0,05) ; UFF = Unfermented feeding; FF = Fermented feeding by 0.30% yeast culture

Table 3. The effect of fermentation ragi tape products (rice bran, pollard, soybean hull, and cocoa-pod, respectively) in diets on performance of Bali drake eged 4-10 weeks

Variabel	Treatments[1)]									SEM[2]
	A	B	C	D	E	F	G	H	I	
Final body weight (g/birds)	1065[c3)]	1046[c]	1225[b]	1058[c]	1306[a]	104[c]	1295[a]	1018[c]	1035[c]	26.79
Body weight gains (g/birds/6 weeks)	715[c]	694[c]	873[b]	708[c]	953[a]	690[c]	943[a]	666[c]	682[c]	25.08
Feed consumption (g/d/birds)	4022[c]	3945[a]	4240[a]	4036[a]	4152[a]	3914[a]	4112[a]	3784[a]	3572[a]	278.4
FCR (feed/gains)	5.64[a]	5.69[a]	4.88[c]	5.70[a]	4.36[d]	5.68[a]	4.36[d]	5.68[a]	5.24[b]	0.137

Note : [1] The ration without rice bran, pollard, soybean hull, cocoa-pod, and yeast as a control (A), ration with 15% rice bran (B); 15% fermented rice bran by 0.30% yeast culture (C); with 15% pollard (D), 15% fermented pollard by 0.30% yeast (E), 15% soybean hull (F), 15% fermented soybean hull by 0.30% yeast (G), 15% cocoa-pod (H), and 15% fermented cocoa-pod by 0.30% yeast cultur (I), respectively; [2]*Standard Error of The Treatment Means*; [3]The different superscript at the same row is significantly different (P<0.05)

5.64/birds (Table 3). The average of feed-conversion ratio (feed : gains) of the birds having ration FF were decreased significantly (P<0.05), both than control and UFF groups. Feed conversion ratio on treatment A,B, D, F, and H groups were higher than other groups.

Table 2 showed chemical composition of unfermented feeding (UFF) and fermented feeding (FF) ingredient. Crude protein, crude fiber, and ME content were slightly increased by the fermentation, on the other hand, the content of GE was decreased by fermentation. These results indicated that carbohydrates other than fibres were used for microbial growth and the reduction of nitrogen free extract resulted in increased concentration of the other components. Yi *et al*. (l996) reported that supplementation of microbial in diets improved N retention in broiler chickens and *in vitro* digestibility of vegetable protein. Also, Chen *et al*. (2005) reported that addition of 0.20% complex probiotic (*L. acidophilus* and *S. cerivisae*) in basal diets were increased digestibities of DM and N.

According to Ilyas *et al*. (l995), when the fermented product was suplemented in formula feeds, phytase in the fermented product might partly degrade the phytate in order ingredient in the digestive tract. The fermented product is possibly used as a phytate-free protein source of feed, which contains high available phosphorus. It's was reported that fermentation of soybean meal by *Aspergillus usami* reduced phytate phosphorus levels.

Ilyas *et al.* (l995) reported if the enzyme effectively degrades phytase in the digestive tract, phytase in fermented soybean meal can be degraded phytate from other ingredients of ration. The reasons for the reduction both of excreta protein and energy by the feeding fermentations may be related to the fact that fermentation process may improve dietary protein and energy digestibility (Chiang dan Hsieh, 1995). Dilaporkan juga bahwa a dosage of 0,25g of probiotics/kg of diet is needed to maximize growth performance during both the starter and finisher periods. A higher dosage (approximate 0.5 g/kg diets) is needed to minimize litter ammonia production.

Ration with high dietary fiber caused a decrease metabolizable energy. It's couse that the passage time of digesta was shortened and the fecal excretion was increased in broiler. Cao (2001) reported that 1.5-3.5% dietary cellulose enhaced growth and metabolizable energy retention of 7-15 d old chicks, but the levels of more than 5% suppressed the growth and metabolizable energy. Denbow *et al*. (1995) reported that supplementary microbial phytase improves the bioavailability of dietary.

The use of 15% rice bran, pollard, soybean hull, and cocoa-pod ingredient in diets, respectively were not effect on final body weight, body weight gains, feed consumption, and feed conversion ratio compared than control diets. Fermentation feedstuff by yeast culture before using in ration were increased final body weight, body weight gains, feed consumption, and feed

conversion ratio. Fermentation process by ragi which contains *Saccharomyces cerevisiae*, according to Wallace dan Newbold (1993), *Saccharomyces cerevisiae* can improve crude fibre digestibility on the ceca of birds to become volatile fatty acid (acetate, provionate, and butirate acid). Volatile Fatty Acid (VFA), according to Sutardi (1997) are energy sourches both to birds and ceca microorganism. Piao *et al*. (l999) reported that used of 0,10% *yeast* (*Saccharomyces cerevisae*) in diets were increased body weight gains, feed efficiency, and absorption of nutrient in broiler, and were decreased N and P excretion in manure. Park *et al.* (l994) suggested that body weight gain and feed efficiencies were significantly improve by the addition of 0,10% *yeast culture* in diets of broiler. Bidura (2008) reported that birds were offered fermented diets coaused body weight, carcass weight, and performance (Warmadewi *et al*., 2008) of drake were increased.

Biofermentation of rice bran, pollard, soybean hull, and cocoa-pod fed ingredient by ragi (yeast culture) and it's addition to the diet had better nutrient digestibilities, because *Saccharomyces cerevisae* in the gastrointestinal tract can part of an probiotics sourches. *Saccharomyces cerevisae* as part of an probiotics were increased retention of mineral Calsium, Posphor, and Mangane (Nahashon *et al*., l994; and Piao *et al*., l999). Also, Piao *et al*. (l999); Sibbald and Wolynetz (l986), suggested that probiotics in the gastro intestinal tract can improve protein and energy retention on the body of birds. Most of the recent studies focus on the effect of the bacterial and fungal enzymes used in cereal based diets. These fungal are effective in degrading of the complex compounds such as b-glucans and arabinoxylans (Bedford and Classen, 1992).

Chen *et al*. (2005) reported that dietary supplementation of complex probiotic increased the body weight gain and decreased fecal NH_3-N concentration, and slightly improved digestibility of nutrients. Fermented feed product to the rations coused numerical increases in the body weight gain. This study is consistent with some studies which indicated that fermented diets effect performance positively (Bidura *et al*., 2008b; Warmadewi *et al*., 2008)). This case can be attributed to the positive effects of fermented feed product on phytates and protein. Wu *et al*. (2005) reported that supplementation of *Aspergillus xlanase* can improve the performance of the broilers fed the wheat-based diet.

The final body weight, body weight gains, feed consumption, and feed conversion ratio in the birds were offered 15% rice bran, pollard, soybean hull, and cocoa-pod fed ingredients were not different than control. But, there were indicate decreased than control groups. It's coused that the content of crude fibre from that's feed was difficulty to digestibilities by gastro intestinal enzymes. According to Siri *et al*. (1992), for increasing of crude fibre in diet to become decrease energy digestibilities and lipida absorption. Cao *et al*. (2003) reported that body weight gain, nitrogen utilization and retention time of the diet in the digestive tract decreased significantly while the total microflora count in the caecal contents increased significantly in the group fed 10% dietary cellulose compared to group fed 3.5% dietary cellulose. The inclusion of fiber sources reduced the maintenance energy requirement and the fecal energy excretion was increased with the increase of crude fibre in diets. According to Wang *et al*. (2004), feeding dietary fiber caused an increased daily amount of methane and thus an additional energy loss from methane production. The pig fed on diets with the inclusion of either dietary fiber or resistant starch had a higher maintenance requirement. A higher daily DM intake resulted in a higher energy loss from feces. The decreased ileal and fecal digestibility of energy were found in pigs fed on diets with the inclusion of dietary fiber.

Ration which high crude fibre can couse rate of passage diets in the gastro intestinal tract were increased rather than ration which low crude fibre content. It's couse nutrient in diets was unabsorbed and there were exit to feces (Suhendra, l992). Also, Linder (l985), suggested that fraction of crude fibre i.a. pectin can chelate bile salt and lipida to throw away in excreta. There are some experiment were support this data. Reported by Bakhit *et al*. (l994) that used of soybean hull in diets were decreased performance of broiler and carcass weight (Bidura *et al*., 2008b). Cao *et al*. (2003) reported that chickens fed 10% dietary cellulose had significantly increased counts of uric-acid degradative bac-

teria . It has been reported that passage time of digesta was shortened and the fecal excretion was increased in broiler, and the caecal flora was increased in turkey dietary cellulose. These studies indicated that gastrointestinal transit time of digesta might be increase and gastrointestinal flora would be rather hard to stay there, when without undigestible material in the intestine.

CONCLUSION

It was concluded that used of fermentation ragi tape products (rice bran, pollard, soybean-hull, and cocoa-pod fermented) in diets could increased nutrient digestibility and growth performance of Bali drake aged 4-10 weeks. Further researches also needed to determine the optimum addition level of the *Shaccaromyces cerevisae* as probiotics sourches in this study for both in basal diets and low quality diets on Bali drake.

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Importance of Phosphorous Supplementation in Improving Fermentability, Microbial Protein Synthesis and Degradability of Ammoniated Rice Straw

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ABSTRACT

Rumen microbes need minerals for supporting its growth and activity in fermenting and digesting feeds. However, the supply of minerals should match the amount of energy that is available for microbial growth and protein synthesis, and for fermentation. An experiment is carried out to study the importance of phosphorous (P) supplementation in rations containing ammoniated rice straw (RS) and concentrate on its fermentability, microbial protein synthesis and degradability. The *in vitro* experiment was carried out following the first stage of Tilley and Terry method. The treatments consisting of four diets were A = 50% ammoniated rice straw (RS) + 50% concentrate (control), B = A + P supplement at 0.2%, C = A + P supplement at 0.4%, and D = A + P supplement at 0.6%. Completely randomized design was used as the experimental design with differences among treatment means were examined using Duncan multiple range test. Variables measured were ammonia (NH_3) and volatile fatty acid (VFA) concentrations, total bacterial and cellulolytic bacterial population, cellulolytic enzyme activity, as fermentability indicators and microbial protein synthesis, as well as degradability indicators including dry matter (DM), organic matter (OM), neutral detergent fibre (NDF), acid detergent fibre (ADF) and cellulose. The results indicate that P supplementation at the levels of 0.2, 0.4 and 0.6% reduced ammonia concentration ($P<0.05$) and increased VFA concentration ($P<0.05$), but did not affect other variables. Degradabilities of DM, OM, NDF, ADF and cellulose were increased by P supplementation ($P< 0.05$). It is concluded that P supplementation is important for improving fermentability and degradability of rations containing ammoniated RS and concentrate. The improvement occurred through the increase in cellulolytic bacterial population, cellulolytic enzyme activity, total VFA concentration, and degradabilities of DM, OM, and fibrous fraction. The best level of P supplementation is 0.4% (DM basis).

Key words: ammoniated rice straw, phosphorous, fermentability, microbial protein synthesis and degradability

INTRODUCTION

The presence of microbes in the rumen has enabled ruminants to eat fibrous diets such as grasses, agricultural and agriculture industrial byproducts. Rice straw (RS) is one of fibrous feed that is usually used to substitute or to replace grasses; the use of RS becomes important when the availability of grasses is limited during summer. However, the animal production has not reached its optimum level when RS is given to the animals. This is due to limitations in nutrient content and digestibility of RS.

The use of RS can be improved by treatments such as physical (grinding), chemical (alkali or ammoniation) and biological (treatment with microbes producing fibrous degrading enzymes) treatments. Among these treatments, ammoniation with urea is the best chemical treatment. This is because the treatment was simple and easy with low cost, the treatment has also enabled to loose the lignocellulose bonds which increased fibre degradation by rumen microbes (Leng, 1981). The use of urea-ammoniated RS as animal feed has increased body weight gain and milk production, feed intake, dry matter (DM) and organic matter (OM) digestibilities (Promma *et al*., 1985; van Soest, 2006; Sundstol, 1991). However, the use of urea-ammoniated RS could not be used up to 100% level as this level has slowed down animal growth rate (Zain *et al*., 2000; Zain and Jamarun, 2001). To increase the use of

urea-ammoniated RS, the important strategy is stimulating growth of rumen microbes. This is because of rumen microbes are important organisms that are capable of degrading or digesting fibrous feeds. The other reason is rumen microbial cells are important nitrogen sources for ruminants.

Rumen microbes need minerals for supporting its growth and activity in fermenting and digesting feeds, other than energy, protein, lipid, vitamin, etc. However, the supply of minerals should match the amount of energy that is available for microbial growth and protein synthesis, and for fermentation. Mineral supplementation is necessary, especially when high fibrous diets were given to the animals; these minerals are required for supporting microbial growth (Preston and Leng, 1987; Komisarczuk and Durand, 1991; Little, 1986). One of the minerals is phosphor (P). P is important as part of microbial cells, supporting microbial growth and enzyme activities, having roles in nutrient metabolism in animal, low in its content and availability in feed (Bravo *et al*., 2003; Rodehutscord *et al*., 2000; Little, 1986). P supplementation has increased growth and enzyme activity of cellulolytic bacteria which subsequently increased degradation fibrous fraction of feeds (Komisarczuk *et al*., 1987; Kennedy *et al*., 2000). This P mineral, then, must be used as supplement to improve utilization of ammoniated RS.

Therefore, this experiment is carried out to study the importance of phosphorous (P) supplementation in rations containing ammoniated rice straw (RS) and concentrate on its fermentability, microbial protein synthesis and degradability.

MATERIALS AND METHODS

Materials

Materials consisted of ammoniated rice straw (RS), concentrate, P_2O_5, rumen fluid, McDougall buffer solution, H_2SO_4 15% solution, HCl solution, $HgCl_2$ saturated solution, Na_2CO_3 saturated solution, boric acid solution, NaOH solution, solid brain heart infusion (BHI) medium, and dilution solution.

The experimental diet composed of 50% ammoniated RS and 50% concentrate, and this diet was used as a control diet (A). The rice straw was previously treated with 1.5% urea. Crude protein of diet was 10.16%. P_2O_5 was used as P source and added in diet with the levels were 0, 0.2, 0.4 and 0.6% (DM basis).

Experiment in Nutrient *In Vitro* Fermentability and Degradability

In vitro fermentability and degradability of nutrient were conducted in a batch culture system which followed the first stage of Tilley and Terry procedure (1969). Feed samples (5 g) were placed into a 100 ml polyethylene tube which was then mixed with anaerobic McDougall buffer solution (pH 6.8; 40 ml) and rumen fluids (10 ml) from a rumen cannulated steer. The tubes were then incubated in a shaker water bath at 39^{o}C for 48 h. Two fermentation tubes without sample diets were also incubated and used as blanks. Sample was taken from each fermentor for bacterial counting at the end of fermentation time. After 48 h, fermentation was terminated by injecting the tubes with $HgCl_2$ (1 ml). Tubes were then centrifuged at 2000 x g for 15 min and the supernatant was removed. Supernatants were used to analyse NH_3 concentration (microdifusion Conway method), total VFA concentration (Gas chromatography) and rumen fluid pH. Total and cellulolytic bacterial population was determined by methods described in Suryahadi (1990), cellulase enzyme activity and microbial protein synthesis was, respectively, determined by methods described in Widyastuti (2004) and (Gopar, 1981). The residues were dried at 60 ^{o}C for 48 h and weighed and the data were used for degradability determination. These residues were also analyzed for its DM, OM and N based on proximate analysis, the NDF, ADF, and cellulose of residues were determined by Goering and van Soest (1970) procedure.

Experimental Design and Data Analysis

The experimental design was a completely randomized design consisting of four treatments was used with four replications. Variables measured were fermentability indicators (total and cellulolytic bacterial population, cellulolytic enzyme activity, ammonia and total VFA concentration), synthesized microbial protein and degradability of dry matter (DM), organic matter (OM), and fibrous fractions (NDF, ADF and cellulose). ANOVA using the GLM procedure was used

to analyse the data (Steel and Torrie, 1981). Differences between the control treatment and P supplementation treatment were determined by Duncan multiple range test (DMRT) (Steel and Torrie, 1981).

RESULTS AND DISCUSSION

In Vitro Fermentability and Microbial Protein Synthesis Study

Treatment effects were significant on ammonia and total VFA concentrations ($P<0.05$), but did not influence total and cellulolytic bacterial population, cellulolytic enzyme activity and microbial protein synthesis (Table 1). Ammonia concentration was decreased with the increase levels of P supplement. However, the ammonia concentration was still in the normal range for microbial protein synthesis, i.e. 4-12 mM (Erwanto *et al*., 1993). The reduction in ammonia concentration was, presumably, due to the utilization of ammonia by the rumen microbes. The rumen microbes used the ammonia as nitrogen (N) source for stimulating its growths (Preston and Leng, 1987); however, the data in microbial protein synthesis was not in a line with the results in ammonia concentration. This indicates that P supplementation may affect growth of rumen microbes that degrade protein.

The increase in the levels of P supplementation had produced positive effects by increasing total VFA concentration. This result was in agreement with the results of Komisarczuk *et al*. (1987) indicating that VFA concentration could be increased by P supplementation. VFA is a product of feed fermentation in the rumen which is used by the ruminants as source of energy (McDonald *et al*., 2002). This experiment indicated that the availability of P in ammoniated rice straw (RS) based diet was not sufficient for supporting rumen microbes to ferment the ammoniated RS. P supplementation at the levels of 0.2 up to 0.6% was capable of increasing VFA concentration meaning that P is important mineral required by the rumen microbes for fermenting ammoniated RS. Therefore, P mineral needs to be added in ammoniated RS based diet.

The importance of P supplementation into ammoniated RS based diet can also be shown by the results in total and cellulolytic bacterial population although the effects were not significant. P supplementation at a level of 0.6% tended to increase total bacterial population. A linear increase in cellulolytic bacterial population was also observed with the increase in the levels of P supplement. Cellulolytic enzyme activity in rations containing ammoniated RS with P supplementations tended to be greater than that in control diet. These results indicate that P supplementation has stimulated the growth of rumen bacteria, especially the cellulolytic bacteria. The increase in cellulolytic bacteria was followed by the increase in cellulolytic enzyme activity. This caused an increased in fermentability of ammoniated RS based diet which was indicated by the increased in VFA concentrations.

The increase in VFA concentrations as a result of P supplementation did not increase microbial protein synthesis. This is because of the increase in VFA concentrations was not followed by the increase in ammonia concentrations. This indicates that P supplementation may support cellulolytic bacterial growth, but may depress proteolytic bacterial growth. As a consequence, there was a shift

Table 1.Effect of phosphorous supplementation on total and cellulolytic bacterial population and fermentation in the rumen (mean values)

Variables	Treatment[1]			
	A	B	C	D
N-NH_3 concentration (mM)	11.09[a]	10.02[b]	9.25[c]	8.80[d]
Total VFA cocnentration (mM)	88.75[c]	98.12[b]	106.87[a]	111.87[a]
Total bacterial population (x 10^7colony/ml)	29.67	13.50	26.50	39.19
Cellulolytic bacterial population (x 10^7 colony/ml)	18.83	21.67	24.30	31.67
Cellulolytic enzyme activity (unit/ml)	1.42	2.17	1.72	1.64
Microbial protein synthesis (%/g)	0.19	0.18	0.21	0.13

Note: [1]A = (ammoniated RS + concentrate 50%:50%) ration + P supplement 0%, B = A + P supplement 0.2%, C = A + P supplement 0.4% and D = A + P supplement 0.6%; P = phosphorous; Values within the same rows differ significantly at ($P< 0.05$).

in the ratio of cellulolytic and proteolytic bacterial populations although there was no change in total bacterial population. This means that the ratio between energy source and N source was not in optimum level for microbial protein synthesis. As a result, a readily N source, such as urea, is still needed when P is added as supplement to ammoniated RS basal diet. Other possibility is in relation with the requirement of rumen microbes for minerals other than P, such as sulphur (S). The availability of minerals other than P may be limited to stimulate rumen microbial growth and protein synthesis in the present experiment. As a result, P supplementation needs to be combined with other minerals.

In Vitro Degradability

Table 2 indicates that P supplementations produced significant effects on degradabilities of dry matter (DM), organic matter (OM) and fribous components (NDF, ADF and cellulose). There was a linear increase in all nutrient degradability with an increase in the levels of P supplementation. P supplementation has increased cellulolytic bacterial populations and cellulolytic enzyme activities (Table 1). These have improved fermentability of ammoniated RS based diet by increasing total VFA concentration, and improved DM, OM and fibrous fraction degradabilities. The present result is in agreement with the result of Kennedy *et al*. (2000) who indicated P supplementation increased digestability of baggase (sugarcane byproduct) by 44%.

Although there was a linear increased in fermentability and degradability with the increase of P levels, there were no significant differences between P level at 0.4% and 0.6%. This means that P level at 0.4% (DM basis) is sufficient for improving fermentability and degradability of ammoniated RS based diet.

CONCLUSION

P supplementation is important for improving fermentability and degradability of rations containing ammoniated RS and concentrate. The improvement occurred through the increase in cellulolytic bacterial population, cellulolytic enzyme activity, total VFA concentration, and degradabilities of DM, OM, and fibrous fraction. The best level of P supplementation is 0.4% (DM basis). Further study is required to determine effects of N source or other minerals supplementation in combination with P supplementation for stimulating rumen microbial growth protein synthesis.

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Table 2. Effect of phosphorous supplementation on *in vitro* degradability of ammoniated rice straw (mean values)

Variables	Treatment[1]			
	A	B	C	D
Dry matter degradability (%)	52.91^{c}	54.85^{bc}	57.66^{ab}	60.79^{a}
Organic matter degradability (%)	54.69^{c}	58.43^{b}	60.18^{ab}	62.69^{a}
NDF degradability (%)[2]	39.31^{b}	41.58^{b}	43.94^{ab}	50.91^{a}
ADF degradability (%)[2]	27.99^{c}	32.78^{bc}	37.59^{ab}	40.30^{a}
Cellulose degradability (%)[2]	29.47^{b}	33.04^{b}	38.74^{a}	41.61^{a}

Note: [1] A = (ammoniated RS + concentrate 50:50%) ration + P supplement 0%, B = A + P supplement 0.2%, C = A + P supplement 0.4% and D = A + P supplement 0.6%; P = phosphorous; Values within the same rows differ significantly at ($P < 0.05$).

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Effectivity of *Jatropha curcas* Seed Meal Fermented with Various Moulds as Protein Source for Male Mice (*Mus musculus*)

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ABSTRACT

Jatropha curcas L seed meal (JCSM) is a waste product of seed processing to produce oil as biofuel. This JCSM contains high crude protein (24.3%) leading to its use as protein source for animals. However, the utilization of JCSM is limited by the presence of antinutritional factors, such as phorbolester, curcin, trypsine inhibitor, phytate, saponin, tannin and lectin. Detoxification is needed to improve its utilization. Fermentation with various moulds as biological treatment was applied to reduce phorbolester and curcin in this experiment. This experiment was carried out to evaluate the effectivity of JCSM fermented with various moulds as protein source for mice. JCSM was treated with *Aspergillus niger*, *Rhizopus oryzae*, *Rhizopus oligosporus*, *Trichoderma viride*, or *Trichoderma reesei*. Treatments applied in this experiment were R0 (control diet without JCSM), R1 (95% R0 + 5% JCSM treated with *A. niger*), R2 (95% R0 + 5% JCSM treated with R. oryzae), R3 (95% R0 + 5% JCSM treated with *R. oligosporus*), R4 (95% R0 + 5% JCSM treated with *T. viride*), and R5 (95% R0 + 5% JCSM treated with *T. reesei*). Treatments were allocated in a completely randomized design with five replications and applied to thirty five sexual matured of male mice. Variables measured were feed intake, body weight gain, feed efficiency, nutrient digestibility and mortality. The data were analyzed with descriptive analysis. The results showed that the use of fermented JCSM as protein sources reduced feed intake and feed efficiency. The mice had drastic reduction in body weight. Treatment JCSM with *A. niger* increased nutrient digestibilities such as dry matter, organic matter, protein and energy. Mice consuming JCSM treated with various moulds still had high mortality rate. The use of JCSM treated with *R. oligosporus* was better than that treated with *R. oryzae*. It is concluded that the use of fermented JCSM with various moulds at 5% in ration has not been effective as protein sources for male mice.

Key words: Jatropha curcas L., *Aspergillus niger*, *Rhizopus oryzae*, *Rhizopus oligosporus*, *Trichoderma viride*, *Trichoderma reesei*., mice (*Mus musculus*)

INTRODUCTION

Jatropha curcas L seed meal (JCSM) is a waste product of seed processing to produce oil as biofuel. This JCSM contains high crude protein (24.3% DM basis) leading to its use as protein source for animals (Tjakradidjaja *et al*., 2007). JCSM also contains ether extract in high concentration (15.99% DM basis) that causes high energy content. These protein and other nutrient contents are affected by the presence of seed husk during oil extraction. Exclusion of seed husk increased significantly protein (37.56% DM basis) and lipid (35.02% DM basis) contents, and reduced crude fibre and nitrogen free extract (Tjakradidjaja *et al*., 2007). However, the utilization of JCSM is limited by the presence of antinutritional factors, such as phorbolester, curcin, trypsin inhibitor, phytate, saponin, tannin and lectin (Makkar and Becker, 1999; Aregheore *et al*., 2003).

JCSM had been used as protein supplements that substituted control diets (Siagian *et al*., 2007; Wardoyo, 2007). The maximum use of JCSM was 5%. The use of greater levels of JCSM (7.5-15%) caused weakness, lost of appetite, reduction in feed intake, decrease in body weight gain, presence of yellow colour liquid in anus (rectum), and high mortality rate. These are caused by curcin and phorbolester as the main antinutrients/toxins (Stirpe *et al*., 1976; Aderibigbe *et al*., 1997; Evans, 1986; Brodjonegoro *et al*., 2005). Basically, curcin has similar protein structure to that of ricin which is present in *Ricinus communis*; curcin and ricin are lectin type toxins (Aderibigbe *et al*., 1997; Aregheore *et al*., 1998; Aregheore *et al*., 2003). Curcin is capable of inactivating enzyme that synthesise proteins (Hadi, 2008).

Phorbolester is an ester that dissolves in organic solvent. This analogoue of diacylglycerol (DAG) is capable of inactivating protein kinase C enzyme which changes the normal physiological function of cells (Rug *et al*., 2006; Asaoka *et al*., 1992; Makkar dan Becker, 1997). Concentrations of curcin and phorbolester varied among varieties of JC (Makkar and Becker, 2004; Francis *et al*., 2006).

Detoxification is needed to reduce phorbolester and curcin and to improve its utilization. Fermentation with various moulds is one biological method that can be applied. It is expected that the enzyme activities present in various moulds and production of ethanol can degrade the toxins from JCSM during fermentation. The previous result indicates that fermentation with various moulds reduced protein and lipid concentrations, and increased ash and crude fibre contents although curcin and phorbolester contents were not detected (Tjakradidjaja *et al*., 2007). The fermented products of JCSM need to be evaluated as protein source in ration for animals. Therefore, the present experiment is an evaluation of the effectivity of JCSM fermented with various moulds as protein source for mice (*Mus musculus*).

MATERIALS AND METHODS

Materials

This experiment used 35 heads of male mice (*M. musculus*) aged at about 28 days with average body weight was 22.77 $\pm$ 4.95 g/head. The mice were kept in individual cages (36 cm x 28 cm x 12 cm) for 7 weeks (28 days). Each individual cage was provided with feed through and drinking bottle, rice husks were used to cover the basis of each individual cage. Moulds used for fermenting JCSM were *Aspergillus niger*, *Rhizopus oligosporus*, *Rhizopus oryzae*, *Trichoderma viride*, and *Trichoderma reesei*. Comercial broiler starter (BR 1 CP 511, PT Charoen Pokphand) was used as a control diet.

Treatments

The control diet was a commercial diet, and about 5% of control diet was substituted with fermented JCSM. All diets were given in pelleted form. Treatment diets (6 treatments) were :

R0 : Control ration
R1 : 95% R0 + 5% unfermented JCSM
R2 : 95% R0 + 5% JCSM fermented with *A. niger*
R3 : 95% R0 + 5% JCSM fermented with *R. oryzae*
R4 : 95% R0 + 5% JCSM fermented with *R. oligosporus*
R5 : 95% R0 + 5% JCSM fermented with *T. viride*
R6 : 95% R0 + 5% JCSM fermented with *T. reesei*

Experimental Design and Data Analysis

Treatments were allocated in a completely randomized design with five replications; each replication consisted of 1 male mice. Variables measured in this experiment were feed and nutrient intakes, nutrient digestibility, body weight gain, feed efficiency, and mortality rate. The data were analysed with descriptive analysis. The descriptive analysis was conducted due to high mortality rates of mice during experiment (Steel and Torrie, 1981).

Procedures

Medium and Inoculum Preparation

Bean sprout extract medium was used as basal medium for growing the moulds. The medium was prepared following the method of Lestari (2006). Bean sprout (250 g) was mixed with distilled water (1000 ml) and boiled by keeping the volume at 1000 ml. The mixture was then filtered with muslin cloth. The filtrate (100 ml) was then mixed with Bacto agar (2 g) and boiled (100^{o}C) until the medium become clear. The medium was then divided into 10 tubes (3ml/tube). The tube was covered with cotton and aluminum foil, and was sterilized with autoclaving (121^{o}C 15 min). The tubes were then cooled.

Inoculum was prepared by adding sterillised distilled water (10 ml) into a tube containing culture stock of each mould. Each tube was then homogenised with sterilised glass loope. This mixture was then inoculated on to bean sprout extract medium. The cultures were then incubated for 3 days at 30^{o}C (Lestari, 2006).

JCSM Fermentation with Various Moulds

JCSM was put into a plastic bag which was added with water up to 60% moisture. This mixture was then homogenised and autoclaved (121^{o}C 15 min). Each incubated cultures were diluted with sterill water (6 ml)

and homogenised. The sterille JCSM was then cooled at room temperature. The cooled JCSM was then placed and spread in a plastic tray. This JCSM was then inoculated with each mould inoculate (3 ml inoculum/50 g sterille JCSM) and covered with plastic wrap. Holes were made in plastic wrap as fermentation was conducted in aerobic condition at room temperature (30^{o}C) for 6 days.

Diet Pelleting, Diet and Fecal Analysis and Body Weight Measurement

Fermented JCSM was dried under the sun which was then dried in an oven (60^{o}C 24 h) and ground. Commercial diet was ground; the ground commercial diet (95%) was mixed with each of dried ground fermented JCSM (5%). Each mixture was homogenised using a mixer, and was then placed in pelleting machine. The pellet diets were then dried.

The pelleted diet were given to the animals based on the treatment applied. Diets and water were given *ad libitum*. The amount of diets given and the residual diets from the through or from the base cage were collected and recorded every week. Fecal collection was carried out for a week. The animals were kept for 7 weeks consisting of 1 week of preliminary period and 6 weeks of experimental period. Diet and fecal samples were dried under the sun and in an oven (60^{o}C 24 h) which were then analysed with proximate analysis for nutrient contents.

Body weights were determined with a scale at the beginning of experiment and every weeks until the end of experiment. Body weight gain (g/day) was calculated by substracting body weight at a week with body weight at a week before which was then divided by a period of measurement.

RESULTS AND DISCUSSION

Nutrient Content of Treatment Diets

Results of proximate analysis of treatment diets showed variations in nutrient content among the diets (Table 1). The commercial diet (R0) contained 91.98% DM, and replacing R0 with 5% untreated JCSM slightly reduced DM content (R1). Reductions in DM contents were also observed in R5 and R6 (*Trichoderma* spp.), but increases in DM contents were found in R2 and R3 (*A. niger* and *R. oligosporus*). The highest DM content was in R4 (*R. oryzae*). Differences in DM contents were due to addition of water before fermentation process and due to heat and drying treatment during pelleting.

Variations in ash contents caused variations in OM contents. Ash and OM contents in R0 were 6.20% and 93.80%. The ash content was reduced in R1, R3, R4, R5 and R6 causing increases in its OM contents. Reverse results were observed in R2. There were no significant variations in crude protein contents among treatment diets. Ether extract contents in all diets containing JCSM (R2-R6) were lower than that of control diet (R0). On the other hand, there were

Tabel 1. Nutrient content of treatment diets

Nutrient contents	Treatment diets[1]						
	R0	R1	R2	R3	R4	R5	R6
Dry matter (%)	91.98	90.08	92.24	92.92	95.42	88.90	87.68
Ash (%DM)	6.20	5.63	6.50	6.11	5.31	4.68	5.86
Organic matter (%DM)	93.80	94.37	93.50	93.89	94.69	95.32	94.14
Crude protein (%DM)	24.53	24.40	25.80	24.21	24.67	24.51	25.01
Ether extract (%DM)	8.39	7.13	7.94	6.52	6.46	7.21	6.91
Crude fibre (%DM)	2.34	5.06	4.37	4.72	5.43	4.45	5.66
Nitrogen free extract (%DM)	58.55	57.78	55.39	58.43	58.13	59.15	56.56
Ca (%DM)	0.04	0.04	0.05	0.05	0.05	0.06	0.08
P (%DM)	0.10	0.09	0.11	0.08	0.08	0.08	0.05
Gross energy (cal/gDM)	4408.57	4601.47	4185.82	4100.30	4211.91	4466.82	4639.60

Note: [1]R0 : control diet; R1 : R0 (95%) + untreated JCSM (5%); R2 : R0 (95%) + *A. niger* fermented JCSM (5%); R3 : R0 (95%) + *R. oryzae* fermented JCSM (5%); R4 : R0 (95%) + *R. oligosporus* fermented JCSM (5%); R5 : R0 (95%) + *T. viride* fermented JCSM (5%); R6 : R0 (95%) + *T. reseei* fermented JCSM (5%).

significant increases in crude fibre contents in all JCSM containing diets compared to that of control diet (R0). Variations were found in nitrogen free extract (NFE), Ca, P and gross energy contents among treatment diets.

Variations in nutrient contents among treatment diets were affected by nutrient contents of JCSM fermented with various moulds (Fardiaz, 1989; Winarno, 2002; Tjakradidjaja *et al*., 2007). Reductions in ether extract may indicate that there were degradations of lipid by the enzyme produced by the moulds, especially by *Rhizopus spp.* (Nuraida *et al.*, 2000; Salleh, 1993). Reductions in ether extract may also indicate the reductions in phorbolester contents as this antinutrient is an ester compound. On the other hand, the increases in crude fibre contents may demonstrate that not all the moulds degrade crude fibre for their growth (Rahma, 1996; Yuniah, 1996). However, all nutrient contents in all treatment diets were still in the ranges that are recommended by Smith and Mangkoewidjojo (1988) for mice, except for crude fibre contents.

Feed and Nutrient Intakes

Due to high mortality rates occurred in this experiment, data in feed and nutrient intakes are presented according to the number of mice and the time of mice life or the time of experimental period (Table 2). Feed intake for R0 was 3.91 g/head/d. Replacing control diet (R0) with untreated (R1) and fermented JCSM (R2-R6) reduced feed intake. The same trends were also found for intakes of DM and other nutrients. Mice consuming R4 (95%R0 + 5% *R. oligosporus* fermented JCSM), R5 (95%R0+5% *T. viride* fermented JCSM) and R2 (95%R0 + 5% *A. niger* fermented JCSM) tended to have greater feed and nutrient intakes.

Results in feed intakes were comparable to those obtained by Siagian *et al*. (2007) (1.68-3.04 g/head/day), but were lower than that recommended by Smith and Mangkoewidjojo (1988) (7g/head/day) and Tjakradidjaja *et al*. (2009) (4.26 g/head/day). The results also confirmed the other experimental results in which using untreated and treated JCSM reduced feed and nutrient intakes (Siagian *et al*., 2007; Wardoyo, 2007; Tjakradidjaja *et al*., 2009). Low feed intakes in untreated JCSM diets were due to the presence of curcin and phorbolester as antinutrients and crude fibre (Becker and Makkar, 1998; Aregheore *et al*., 2003; Wardoyo, 2007). Fermentations JCSM with various moulds have not yet produced significant effects in increasing feed and nutrient intakes although fermentation with *R. oligosporus* showed a better result. This could be due to differences in enzyme activity among the moulds (Shurtleff and Aoyagi, 1971; Sutopo, 1987; Hardjo *et al*., 1989), the nutrient content of JCSM fermented with various moulds (Table 1, Tjakradidjaja *et al.*, 2007), and nutrient utilization in the alimentary tract of mice (Makkar and Becker, 1999).

Nutrient Digestability

Digestibilities of dry matter, organic matter, crude protein and energy of R0, repectively, were 91.68, 94.60, 92.30 and 93.62%. Using untreated JCSM in R1 tended to reduce DM digestibility, but slightly

Table 2. Feed and Nutrition Intakes

Intakes (g/head/day)	Treatment diets[1]						
	R0	R1	R2	R3	R4	R5	R6
Fresh (feed)	3.91±0.70	1.48±0.29	2.03±0.30	1.60±1.08	2.62±0.95	2.31±1.08	1.8±0.54
Dry matter	3.60±0.64	1.34±0.26	1.87±0.28	1.49±1.01	2.50±0.91	2.05±0.96	1.64±0.47
Organic matter	3.38±0.60	1.26±0.24	1.75±0.26	1.40±0.94	2.37±0.86	1.96±0.91	1.54±0.45
Crude protein	0.88±0.02	0.33±0.06	0.48±0.07	0.36±0.24	0.62±0.22	0.50±0.23	0.41±0.12
Ether extract	0.30±0.05	0.10±0.02	0.15±0.02	0.10±0.07	0.16±0.06	0.15±0.07	0.11±0.03
Crude fibre	0.08±0.02	0.07±0.01	0.08±0.01	0.07±0.05	0.14±0.05	0.09±0.04	0.09±0.03
Nitrogen free extract	2.11±0.38	0.77±0.15	1.04±0.16	0.87±0.59	1.46±0.53	1.21±0.57	0.93±0.27
Energy (cal/head/day)	158.72±28.40	61.46±11.92	78.36±11.75	60.94±41.26	105.40±38.29	91.62±42.80	75.94±21.94
n (head)	5	1	1	1	1	3	1
Weeks	6	5	6	6	6	3	6

Note: [1] R0 : control diet; R1 : R0 (95%) + untreated JCSM (5%); R2 : R0 (95%) + *A. niger* fermented JCSM (5%); R3 : R0 (95%) + *R. oryzae* fermented JCSM (5%); R4 : R0 (95%) + *R. oligosporus* fermented JCSM (5%); R5 : R0 (95%) + *T. viride* fermented JCSM (5%); R6 : R0 (95%) + *T. reseei* fermented JCSM (5%).

Table 3. Nutrient Digestability

Nutrient digestibility (%)	Treatement diets[1]						
	R0	R1	R2	R3	R4	R5	R6
Dry matter	91.68±1.24	89.56±1.44	93.34±1.70	70.72±45.84	89.04±2.19	80.53±22.85	82.28±5.90
Organic matter	94.60±0.80	95.38±0.64	97.20±0.71	87.60±19.41	94.07±1.19	92.50± 8.81	90.78±3.07
Crude protein	92.30±1.14	93.57±0.88	95.92±1.04	80.19±31.01	91.63±1.68	89.16±12.72	88.04±3.98
Energy	93.62±0.95	94.61±0.74	96.36±0.93	82.79±26.95	92.31±1.54	90.60±11.03	89.81±3.40
n (head)	5	1	1	1	1	3	1
weeks	6	5	6	6	6	3	6

Note: [1]R0 : control diet; R1 : R0 (95%) + untreated JCSM (5%); R2 : R0 (95%) + *A. niger* fermented JCSM (5%); R3 : R0 (95%) + *R. oryzae* fermented JCSM (5%); R4 : R0 (95%) + *R. oligosporus* fermented JCSM (5%); R5 : R0 (95%) + *T. viride* fermented JCSM (5%); R6 : R0 (95%) + *T. reseei* fermented JCSM (5%).

increase other nutrient digestability. This may be due to high crude fibre content and the presence of antinutrients/toxins. Treatment JCSM with *A. niger* increased all nutrient digestibilities. This was because of crude fibre content was low with high crude protein and ether extract contents in R2.

Fermenting JCSM with *Rhizopus* spp. (R3 and R4) and *Trichoderma* spp. (R5 and R6) did not produce similar effects to that of *A. niger*. The use of JCSM treated with *R. oligosporus* was slightly better than that treated with *R. oryzae* in nutrient digestibility. This could be due to differences in type and activity of the enzyme secreted by the two species of *Rhizopus* (Shurtleff and Aoyagi, 1971; Nuraida *et al*., 2000; Salleh, 1993). No significant differences in nutrient digestibilities of JCSM treated with both *Trichoderma* spp. Feed intake data (Table 2) indicate that mice given R4 and R5 tended to be greater than those of R3 and R6. This means that higher feed intakes may cause less feed retention in the gastrointestinal tract and less feed contact with digestion enzyme; this made feed were less digested and reduced nutrient availability in gastrointestinal tract (McDonald *et al*., 2002).

Body Weight Gain and Feed Efficiency

With DM intake was 3.60 g/head/day, mice consuming R0 had the highest body weight gain and feed efficiency. All diets containing untreated and treated JCSM caused negative body weight gain and feed efficiency (Table 4). However, treatment JCSM with *A. niger* produced the lowest body weight lost with the highest feed efficiency among treatment JCSM with various moulds. These negative effects were due to low feed intakes in relation to high content of crude fibre, the presence of antinutrients/toxins and low nutrient digestibility and availability (Fajariah, 2007; Asaoka *et al*., 1992, Wardoyo, 2007; McDonald *et al*., 2002). These results showed that treatment JCSM with various moulds has not yet improved performance of mice.

Mortality Rate

Mortality rate was zero for R0 (Table 5). Given untreated and treated JCSM caused high mortality rates. 100% mortality rates were found in mice consuming R1 (untreated JCSM), R2 (*A. niger* fermented JCSM), and R5 (*T. viride* fermented JCSM). A lower mortality rate (80%) was found in mice

Table 4. Body weight gain and feed efficiency

Variables	Treatment diets[1]						
	R0	R1	R2	R3	R4	R5	R6
Dry matter intake g/head/day)	3.60±0.64	1.34±0.26	1.87±0.28	1.49±1.01	2.50±0.91	2.05±0.96	1.64±0.47
Body weight gain (g/head/day)	0.19±0.19	-0.47±0.17	-0.43±0.21	-0.45±0.30	-0.44±0.45	-0.48±0.29	-0.54±0.26
Feed efficiency (%)	4.68±4.27	-35.58±12.25	-22.58±8.93	-38.16±31.65	-32.00±52.64	-25.10±13.00	-35.26±16.55
n (head)	5	1	1	1	1	3	1
weeks	6	5	6	6	6	3	6

[1] R0 : control diet; R1 : R0 (95%) + untreated JCSM (5%); R2 : R0 (95%) + *A. niger* fermented JCSM (5%); R3 : R0 (95%) + *R. oryzae* fermented JCSM (5%); R4 : R0 (95%) + *R. oligosporus* fermented JCSM (5%); R5 : R0 (95%) + *T. viride* fermented JCSM (5%); R6 : R0 (95%) + *T. reseei* fermented JCSM (5%)

Table 5. Mortality Rates

Treatment diets[1]	Mortality per week (%)						Total (%)
	1	2	3	4	5	6	
R0	0	0	0	0	0	0	0
R1	0	0	40	40	20	0	100
R2	0	0	40	20	20	20	100
R3	40	20	20	0	0	0	80
R4	20	0	0	60	0	0	80
R5	0	40	60	0	0	0	100
R6	20	40	20	0	0	0	80

[1] R0 : control diet; R1 : R0 (95%) + untreated JCSM (5%); R2 : R0 (95%) + *A. niger* fermented JCSM (5%); R3 : R0 (95%) + *R. oryzae* fermented JCSM (5%); R4 : R0 (95%) + *R. oligosporus* fermented JCSM (5%); R5 : R0 (95%) + *T. viride* fermented JCSM (5%); R6 : R0 (95%) + *T. reseei* fermented JCSM (5%).

eating R3 (*R. oryzae* fermented JCSM), R4 (*R. oligosporus* fermented JCSM) and R6 (*T. reseei* fermented JCSM). For mice consuming R1 and R2, high mortality rates occurred at the 3rd week up to the 5th and 6th weeks. Given R3, R4, and R6 caused death at the 1st week,death occurred at the 2nd week for mice fed with R5. These differences in mortality rate and the occurrence of death among mice may indicate differences among treatment diets producing negative effects on mice, and mice had different response or tolerance to the presence of curcin or phorbolester. These antinutrients/toxins reduced feed intake and nutrient digestions in gastrointestinal tract, and the metabolites released damaged other organs such as intestinal organ, liver, renal, and lungs (Adam, 1974; Makkar and Becker, 1998[a]; Makkar and Becker, 1998[b]). The death mice showed the same characteristics as those found by Wardoyo (2007), Fachrudin (2007) and Fajariah (2007).

Since the use of JCSM fermented with various moulds at 5% has not yet produced positive effects on mice performance, it is necessary to carry out other treatments such as combined fermentation with two or more species of mould, or combined treatment between physical, chemical or biological treatments. Such experiment had been done by Hadriyanah (2008) in which JCSM treated with methanol or 4% NaOH combined with drying and autoclaving (121^{o}C 15 min 15 psi). The use of this treated JCSM at 5% in a diet has produced good results in body weight gain of mice. Therefore, it is warrant to carry out combined treatments to detoxify antinutrients.

CONCLUSION

The use of fermented JCSM with various moulds at 5% in ration has not been effective as protein sources for male mice. Combined treatments should be applied to improve JCSM utilization.

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Early Growth of *Panicum sarmentosum* Roxb. – A Promising Grass in Livestock - Coconut Integration System

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ABSTRACT

Coconut plantations have been long widely used as growing area. In Central Sulawesi this integrated system traditionally plays important roles in small scale farmers. In Central Sulawesi, the system is suitable and productive forage genotypes are important aspect for the production systems. *Panicum sarmentosum* and *Panicum maximum* were compared in a plot trial under coconut plantation at Lalombi of Lembasada village, South Banawa, district of Donggala. The treatments: *P. sarmentosum, P. maximum, P. sarmentosum* with *Desmanthus virgatus*, and *P. maximum* with *D. virgatus* were replicated 5 times. The 20 experimental units were arranged in completely block randomized design. This paper reports plant height, number of tiller, and herbage production at the early growth, 8 weeks after planting, of the grasses. The study has shown that *P. sarmentosum* grew better than *P. maximum*, both on grass monoculture, and on mixed-with desmanthus. *P. sarmentosum* and *P. maximum* did not differ statistically in plant heights, neither without (149.3 cm vs. 141.7 cm), nor with desmanthus (138.7 cm vs. 133.9 cm). Nevertheless, the number of tillers and dry forage yields of *P.sarmentosum* was significantly higher than those of *P. maximum*, both without and with desmanthus, i.e.; 145 vs. 81 and 124 vs. 75, and 425.6 vs. 235.1 kg/ha and 316.5 vs. 141.2 kg/ha, respectively. The correlation of these two attributes is also significantly high ($R^2 = 0.9132$). This result has suggested that *P. sarmentosum* grows better than *P. maximum* that well adapts under shade. It is concluded, therefore, *P. sarmentosum* is another promising grass for use in shaded niches.

Key words: *Panicum sarmentosum; Integrated farming; shaded niches*

INTRODUCTION

Steady growth of the human population leads to an increased demand for agricultural products. These stuffs essentially depend on agricultural land availability, while this sort of land is continuously shrinking resulting from land use convertion (Singh and Ghosh, 1993; Sukmana, *et al*., 1994), which is another imfact of the population increase and needs. In addition, the number of farm animals has increased, resulting in severe competition for land use between crops and livestock, therefore, there is an urgent need for increased productivity per-unit area from forage plants to help redress the problem (Blair, 1991; Dzowela and Kwesiga, 1994). Incorporation of forage plants onto plantation lands is an alternative solution to provide herbage. Morover rising livestock under plantation crops has long been practised, such as on coconut lands in Central Sulawesi, though none or very limited forage improvements have been done by the farmers.

Overall integrated farming is suggested as the largest category of livestock systems in the world in terms of animal numbers, productivity and the number of people it services (Thornton *et al.*, 2002). They maintain that, over the last decade, meat production from these systems has grown at a rate of about 2% per year, and about two-thirds of the rural small-scale farmers rely on mixed crop-livestock systems for their livelihoods. Moreover, given the demand increases for livestock products forecast for the coming decades, mixed systems are going to have to provide a disproportionate part of this increase, especially in developing countries – so they will become even more important in the future (ILRI, 2000).

Coconuts plantation lands have long been widely used in rising livestock in most of tropical countries. This integrated land-use system plays important roles for small scale farmers. In Central Sulawesi, however, the system is still practiced by farmer traditionally with limited forage improvements. Since it is relized that integrated farming systems are

very helpful in forage supply, significant attentions have been paid placed by researchers and agronomists on the use of plantation for the multi-purposes land-use systems. Finding suitable and more productive forage genotypes is, therefore, one particular aspect that attracts researches' attentions. This experiments aimed to compare the agronomical performances of a new promising grass species *Panicum sarmentosum* Roxb. to *Panicum maximum*. Grown under coconut canopy the later species was chosen for comparison, since it has already been well known for its suitability under shade environments (see for instances, Lowry and Jones, 1988; Reynolds, 1995; Ibrahim, 1998), and as a member of *Panicoideae* (genus *Panicum*). Available information on *P. sarmentosum* is very scarce, particularly on forage cultivation, but Clayton *et al*. (2008) have explained the grass description. This grass is considered as a weed; however, few studies have indicated the grass potential as forage crop (Amar *et al*., 2005; Tarsono and Amar, 2007).

MATERIALS AND METHODS

The current was conducted on farmer's coconut-plantation, started on May 2009, at Lalombi of Lembasada village, sub-district South Banawa, district of Donggala, Central Sulawesi. The study site was invested by various weeds, mainly ferns, shrubby and some herbaceous species. Soil of the site has quite low fertility with moderate total nitrogen content (0.29% N), and very low phosphorus (2.70 ppm P_2O_5, Bray I).

Two studied grasses were *Panicum sarmentosum* and *Panicum maximum* that compared as monoculture (either grass alone), as well as, in mixture with legume *Desmanthus virgatus*. Therefore, there were 4 compared treatment-combinations, i.e.:

1) PsL0 = *Panicum sarmentosum* in monoculture (without legume)
2) PmL0 = *Panicum maximum* in monoculture (without legume)
3) PsL1 = *Panicum sarmentosum* with legume desmanthus
4) PmL1 = *Panicum maximum* with legume desmanthus

The four treatment-combinations were replicated 5 times, resulting 20 experimental units. Each of these 20 experimental units was placed in a 15 m^2 plot (5 m x 3 m). The experimental units were arranged by completely randomized block design. One replication of the experimental units was illustrated in Figure 1.

Each of the grasses was planted in 3 rows of 125 cm apart, and 75 cm distance in row. There were 21 grass planting holes in each plot. Two vegetative planting materials (tillers) were planted per-hole. At the grass-desmanthus mixture, Two 20cm-wide legume planting rows were formed, each row was placed in between grass rows (Figure 1). Two hundred and twenty nine seeds of desmanthus were sown at each row, or 458 seeds/plot (equivalent to planting rate of 2 kg/ha). Seeds were buried at approximately 0.5 cm depth. *Desmanthus* was planted 28 days before the grasses' to allow the legume formed vigorous seedling prior to the grass planting.

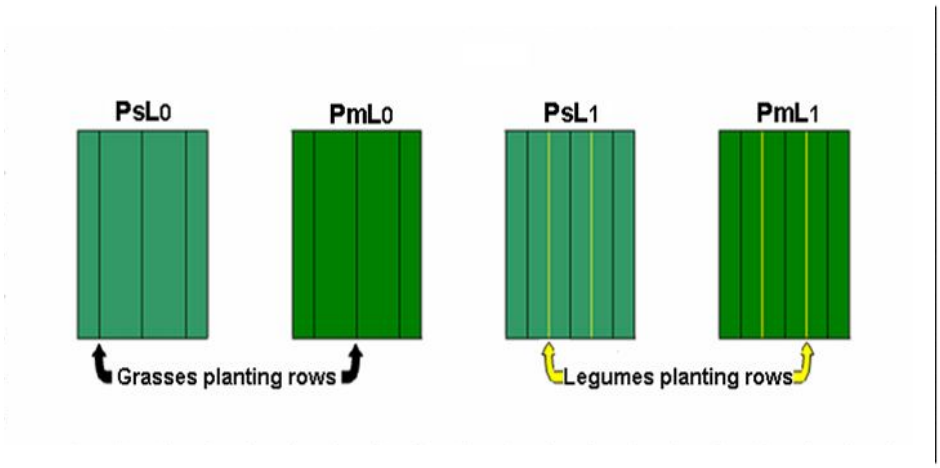

Figure 1. Illustration of the plot trial (replicated 5 times)

Early growth of the 2 grass genotypes was compared through 3 plant parameters were; plant height, number of tillers/planting hole, and dry-matter of herbage yields. These parameters were collected at 8 weeks after planting (56 DAP). Vertical plant heights were measured from the base (at soil surface) to the top of the grasses. Numbers of tillers were counted prior to herbage harvesting. Herbage was harvested by cutting, all the clumped grasses in each plot, at 15 cm from soil surface. Fresh harvested herbage at each plot was weighed, and sampled approximately of 400 gram for determination of dry-matter content, and to be used in calculation of herbage production in dry-matter basis. The samples were dried in a forced-drought oven at 70^{o}C for 3 days.

All parameter data were analyzed by '*analysis of variance*' (ANOVA) using statistical package (*Statistix 4.1 windows version*). Parameters that statistically affected by experimental treatment were compared by '*the least significant differences*' (LSD) at 95% confident level (P=0.05).

RESULTS AND DISCUSSION

The study has suggested that *P. sarmentosum* grew better than *P. maximum*, both on grass monoculture, and on mixed-planting with desmanthus. The plant heights did not differ statistically (Table 1) between *P. sarmentosum* and *P. maximum*, neither without (149.3 cm vs. 141.7 cm), nor with desmanthus (138.7 cm vs. 133.9 cm). However, the number of tillers and forage dry-matter yields of *P. sarmentosum* were significantly higher than yield of *P. maximum*, both without and with desmanthus, i.e.; 145 vs. 81 and 124 vs. 75, and 425.6 vs. 235.1 kg/ha and 316.5 vs. 141.2 kg/ha, for number of tillers and forage yields, respectively (Table 1).

Grass of *P. sarmentosum* produced significantly more number of tillers (shoots), both in monoculture and in grass-legume mix, over the grass of *P. maximum*. Farther, the first grass species yielded higher herbage than the latter. These two superior characteristics of *P. sarmentosum* over *P. maximum* were also approved by highly significant correlation of the number of tillers and herbage production (R^2 = 0.9132; P<0.01). This suggested that the more the tillers produced the higher herbage yielded. Though herbage production positively correlated (R^2 = 0.8624; P<0.01) with plant height, it is not as meaningful as the first correlation for 3 reasons. Firstly, the first correlation (R^2 = 0.9132) is stronger than the second (R^2 = 0.8624). Secondly, it is suggested that plant height dependent to stem elongation. Lastly, the heights of these 2 grasses were not differing significantly. Therefore, it is reasonable to assume that herbage of *P. sarmentosum* produced higher leaf: stem ratio than *P. maximum*. However, this hypothetical assumption needs to be approved by further study. This is important to be found out, due to leafy forage crops are considered to be better than the less leafy species. It was also noted that morphologically, white hair at the plant base are softer on *P. sarmentosum* compared to those on *P. maximum*. This plant characteristic may influence forage palatability, but confirmed studies are needed.

Nevertheless, this early growth study has showed that *P. sarmentosum* Roxb. promises for use as forage under plantation environment. It produced more tillers and herbage than *P. maximum*. While, the grass of *P. maximum* has widely been recognized

Table 1. Comparison of studied grasses in plant height, number of tillers, and dried-herbage yields at 56 days after planting (n = 5, P = 0.05)

Compared parameters	Treatments			
	Grass alone		Grass-legume mixture	
	P. sarmentosum	*P. maximum*	*P. sarmentosum*	*P. maximum*
1) Plants height (cm)	149.3 [a]	141.7[a]	138.7 [a]	133.9 [a]
2) Number of tillers	145.0 [a]	81.0[b]	124.0 [a]	75.0 [b]
3) Dried-herbage yields (kg/ha)	425.6 [a]	235.1 [b]	316.5 [b]	141.2 [c]

Note: values of a parameter followed by different superscript letters differ statistically at 0.95 confident levels.

Amar, 2003). In addition, the above results might also indicate some better environmental adaptation of *P. sarmentosum* over *P. maximum*, particularly, on such low soil's fertility of the study site. However, in this particular experimental site, more studies are necessary to allow firm conclusion and recommendation.

CONCLUSION

P. sarmentosum is another promising grass for use in shaded niches. It is also recommended father more detail studies on other environmental adaptation of this promising grass genotype, as well as the forage's quality and palatability to confirm the potential used by livestock.

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Carcass Composition of Broilers Fed Diets Based on Total and Digestible Amino Acid Formulation

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ABSTRACT

The objective of this research was to determine the difference in carcass composition between male and female birds and between birds of two different genotypes fed four diets differed in formulation, i.e: 1) based on total amino acids, 2) based on digestible amino acids (published book values), 3) based on digestible amino acids values determined, 4) based on digestible amino acids formulated commercially. This study demonstrated that sex and strain of broilers affect the requirement of amino acids. Male broilers had higher dietary amino acid requirements than females. With regard to genotype, the more rapid the growth of the birds, the higher requirement of amino acids. Birds given diets based on digestible amino acid formulation had a higher proportion of body protein and a low proportion of body fat ($P<0.05$) than birds fed diets based on total amino acid formulation.

Key words: amino acid, broiler, carcass, diet formulation, digestible

INTRODUCTION

The efficiency of protein utilization depends to a large extent on the amino acid composition of the diet. Within commercial poultry nutrition, there has been an increasing interest in the concept of using digestible amino acids to formulate cost-effective diets which more accurately meet the nutrient requirements of the bird. The concept of digestible amino acids is based on the realization that amino acids in most ingredients are not completely digested and diets based on total amino acid concentrations may not provide an appropriate balance of amino acids to meet the birds' requirements. However, many feed companies still use total amino acids to specify poultry diets and there is a need for a better-feed formulation system than one based on total amino acids.

For reliable application of digestibility in practice, it is necessary to quantify the effect of genotype and sex. Growth rates of male and female broilers were differ and it is suggested that they have different nutritional requirements (Ten Doeschate *et al.*, 1992). In regard to genotype, Leclerq (1983) stated that genetically lean chickens use dietary protein more efficiently than fat birds to make their own body protein. Pym *et al.* (2004), however found that the growth rate of genetically lean chickens was much more severely depressed at low dietary levels of protein than that of genetically fat birds. Strain and sex differences in digestibility of feed need further investigation and have implications for mixed sex versus single sex rearing.

MATERIALS AND METHODS

One hundred and sixty male and female chickens were obtained from two commercial hatcheries. The experiment used a factorial design with 2 genotypes, 2 sexes, and 4 diets. From day 21, the birds were given 4 experimental diets (iso-energetic and iso-nitrogenous). The diets were formulated based on: 1) total amino acids; 2) digestible amino acids refferring to publish book values (Ravindran *et al*., 1998); 3) digestible amino acids values determined on the same ingredients as used in diet 1; and 4) digestible amino acids formulated commercially.

Birds were wing-banded for identification. Feed and water were given *ad libitum*. Birds were weighed at 21 days of age and again at 42 days of age. Feed intake was recorded weekly and feed conversion ratio was calculated. On day 42, 10 males and 10 females of both genotypes per dietary treatment were randomly selected for carcass analysis. Birds were killed by cervical dislocation to avoid loss of blood following a 12h fast. They were then placed in individual plastic bags and frozen at -20^{o}C. The whole body of the chickens were minced and dried and then re-ground prior to determination of fat and protein. Carcass composition (fat and protein in the whole body of chicken) was determined and calculated as follow:

Breast meat yield (%)

$$= \frac{\text{Breast meat weight without bone (g)}}{\text{Body weight}} \times 100$$

Abdominal fat content (%) =

$$\frac{\text{Abdominal fat weight}}{\text{Body weight}} \times 100$$

Statistical Methods

The experiment used a factorial design with 2 genotypes, 2 sexes, 4 diets and 10 individual bird replicates per genotype X sex X diet sub group. Main and interaction effects were determined by analysis of variance. The statistical methods for body composition included the effects of strain, sex, diet and all possible interaction. The data was analysed using SAS (SAS/STAT 6.04, 1987; SAS Institute Inc., Cary, North California). Superscripts were used in tables to indicate statistical differences between means. The significant level was set at P<0.05 and if F-ratio indicated significance, the differences between the means were separated using the Least Significance Difference test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

As shown in Table 1 there was a significant effect of diet on body fat (P=0.004). Body fat content of birds fed diet 1 (based on total amino acid formulation) was significantly higher than that in birds fed diet 2 (based on digestible amino acids formulation). However, body fat content in birds given diet 2, 3 and 4 was not significantly different.

There was a significant effect of genotype on body protein (P<0.01), but not on body fat. Strain B had significantly higher body protein content than strain A. There was also a significant effect of sex (P<0.001) on body fat. Females had a significantly higher percentage of body fat than males. This difference is most likely due to the developmental characteristics of these two genotypes. It seems logical that male broiler would require higher levels of amino acids than females, because male chicks contain more protein and less fat.

There also was a significant interaction between genotype and sex for both body protein (P=0.001) and fat (P=0.02). The effect for protein was due to significantly higher levels of protein in the males birds of strain B than in the males of strain A. The effect for fat was due to slightly higher levels in the strain B than strain A males, but the reverse for the females. In strain A, the sex difference in body fat was much greater than in strain B. This study confirmed previous studies (Acar *et al.*, 1991) that breast muscle and fat development is affected by sex and strain.

There was a significant effect of diet on both fat (P=0.004). Body fat content in strain B males fed diet 1 was significantly higher than birds fed diets 2 based on digestible amino acids formulation. However, body fat content in birds given diet 2, 3 and 4 was not significantly different.

It is of interest that in the females of both genotypes, the commercially formulated diet produced birds with the highest fat content, whilst in the males highest fat levels were found in birds received diet 1, formulated on total amino acid levels. It may be

Table 1. Protein and fat composition (%) of female and male broilers from two strains fed diets formulated on total and digestible amino acids

Parameter	Diet	Strain A			Strain B			Overall
		Females	Males	**Mean**	Females	Males	**Mean**	**means**
Protein	1	17.5±0.63^{d}	17.4±0.56	17.45±0.6	18.4±0.67	19.2±0.56^{b}	18.8±0.6	18.13
	2	18.2±0.72^{bcd}	17.5±0.59	17.85±0.6	18.5±0.67	19.3±0.63^{ab}	18.9±0.6	18.38
	3	17.6±0.59^{cd}	18.2±0.59	17.90±0.6	19.3±0.63	21.0±0.72^{a}	20.15±0.6	19.03
	4	18.2±0.59^{bcd}	17.4±0.59	17.80±0.6	18.2±0.67	19.1±0.56^{ab}	18.65±0.6	18.23
	Mean	**17.88±0.31**	**17.63±0.35**	**17.75±0.31** ^{b}	**18.6±0.31**	**19.65±0.30**^{a}	**19.13±0.32** ^{a}	
Fat	1	16.1±0.69^{a}	12.4±0.62^{defg}	14.25±0.74	14.4±0.74^{abcd}	14.2±0.62^{abc}	14.3±0.6^{a}	14.28 ^{a}
	2	14.2±0.80^{bcde}	11.5±0.65^{efg}	12.85±0.74	13.2±0.74^{cdef}	11.3±0.69^{efg}	12.2±0.6^{b}	12.55 ^{b}
	3	13.5±0.62^{cdef}	11.3±0.65^{fg}	12.40±0.69	14.1±0.69^{abc}	12.4±0.80^{defg}	13.2±0.6^{ab}	12.83 ^{ab}
	4	16.4±0.65^{a}	11.4±0.65^{g}	13.90±0.74	15.5±0.74^{ab}	12.5±0.62^{defg}	14.0±0.6 ^{ab}	13.95 ^{ab}
	Mean	**14.70±0.35** ^{a}	**11.40±0.30** ^{b}	**13.05±0.31**	**14.27±0.35** ^{a}	**12.07±0.35** ^{b}	**13.17±0.32**	

Note: Values are means and standard deviation of 20 individual birds;
Values with different superscripts are significant difference (P<0.05).

that amino acids excess ofrequirement for protein metabolism by the females were deaminated, and used for energy. This is included by the higher levels of total lysine and methionine in the diet 4 than in the two diets formulated on digestible amino acids. The fact that it did not happen in the males suggest that they not only have higher overall amino acid requirements, but possibly a different for a specific amino acids in the two sexes.

The greater proportion of both fat and protein in the strain B than in the strain A males, suggest that the latter genotype birds have a significantly greater proportion of water in their bodies. This is reflected in the lower FCR of the strain A than strain B males, and suggests a greater emphasis on selection for improved feed efficiency in strain A. Pym and Solvyns (1979) reported positive phenotypic correlations between FCR and carcass fat in chickens. It is interesting that the relationship between fat, protein and water have been altered by selection in the two lines. Normally, an increase in body fat is accompanied by a reduced in both water and protein. The sexes responded differently suggesting some influence of differential hormonal control on the process.

CONCLUSION

Birds given diets formulated on digestible amino acid basis had a higher proportion of body protein and a low proportion of body fat than birds fed diets formulated on a total amino acid basis. This study indicated that genotype and sex differences in body composition should be taken into account in implementing formulation diets to maximize performance of broiler chickens.

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Performance of Grade-1 Kids as A Result of Grading-Up Between Local Goat and Boer Goat

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ABSTRACT

This research was conducted to get information on performances of grade-1 kid as a result of grading-up between Etawa Grade goat or Kacang Goat and Boer Goat. Some variables observed were litter size, sex, birth type, and birth weight. Does used in this research were 80 heads, consisted of Etawa grade does (40 heads) and Kacang does (40 heads). They were reared by the farmers intensively in Batu Kramat Village, Kotaagung Timur District, Tanggamus Regency. Artificial Insemination was applied for the mating. Twenty Etawa grade does and twenty Kacang does were mated to Boer buck (BP and BK), while twenty Etawa grade does and twenty Kacang does were mated to Etawa buck and Kacang buck respectively (PP and KK). The result showed that litter size in all mating (BP, BK, PP, and KK) was not significantly different ($P>0.05$). Sex of KK male kid was the highest (60%), while the lowest was BK grade 1 (33%). BK female kid was the highest (67%), while the lowest was KK kid; the birth type (twin and triplet) of PP was higher (70%) than BP (45%); birth weight of BP was higher ($P<0.05$) than PP, and birth weight of BK were also higher ($P<0.05$) than KK.

Key words: grading-up, grade 1, Boerawa and Boercang goat

INTRODUCTION

Lampung province has a potential chance in supporting the development of animal sector. Lampung province has the ability to hold ruminant 1.41 milion animal unit, but only 33.2% was used (Supriyono, 2005). So the chance for increasing animal production is still open. Beside of that, Handiman and Tawaf (2005) stated that Lampung province was the fourth rank in goat population (815,667=7%) in Indonesia after Central Java (22%), East Java (18%), and West Java (10%). The potency and chance were taken by Lampung Government by developing beef cattle and Boerawa goat. The efforts was supported by the Indonesia Government (General Directorate of Husbandry) that in 2004 decided "Lampung as centre of Boerawa goat development".

Productivity of local goat (Eatawa grade and Kacang goat) is still low. Performance of Etawa grade goats that is also dairy type affected low productivity in meat. While productivity of Kacang goat performance is very low because of its small body, so meat production of Kacang goat is low. To improve local goat productivity, it is important to introduce temperate or tropical exotic breeds, for example Boer goats. Boerawa goat (Filial 1 or Grade 1) is crossbreed between Boer buck and Ettawa grade does. In Lampung Boerawa goat was crossed by Boer buck continuously, so the progeny was named grade-1, grade-2, etc.

Boerawa goat development in Lampung was pioneered since 2001 by importing two Boer buck from Australia and two Boerawa buck from South Sulawesi (Hadi, 2006). By nowdays there was no information on performance of this crossbreed (Boerawa grade-1 kid).

The objective of this study was to get information on performance of Boerawa kid (Boer buck x Etawa grade does) and Boercang kid (Boer buck x Kacang does).

MATERIALS AND METHODS

Fourty Etawa grade does and fourty Kacang does were used in this study. Twenty Etawa grade does and twenty Kacang does were crossed by Boer buck, while twenty Etawa grade does and twenty Kacang does were crossed by Etawa grade Buck and Kacang Buck respectively. They were inseminated artficially using semen of Boer buck and Etawa grade buck, unless twenty Kacang does that were mated naturally. All does were reared intensively by farmers in Batu Kera-

mat Village, Kota Agung Timur District, Tanggamus Regency, Lampung Province.

Boer semen was gotten from Frozen Semen Production Instalation Terbanggi Besar, Dinas Peternakan dan Kesehatan Hewan Lamapung Province, while Etawa grade semen was gotten from Balai Inseminasi Buatan Ungaran, Dinas Peternakan Central Java Province. All does were synchronized using Prostaglandin Noroprost hormon (N105424).

Parameters measured in this study were litter size, sex, birth type, and birth weight. Litter size and birth weight parameters were analyzed using ANOVA with completely randomized design, while sex and birth type were analyzed using descriptive analysis (Steel and Torrie, 1993).

RESULTS AND DISCUSSION

Performance of kid grade 1 consisted of litter size, sex, birth type, and birth weight are presented in Table 1. Table 1 showed that litter size of Boerawa, Etawa grade, Boercang, and Kacang goat were 1.5, 1.7, 1.3, and 1.3 respectively. The result indicated that kind of crossing did not affect litter size (P>0.05). This result was appropiate with the result of Sulastri and Dakhlan (2006) that litter size of Boerawa (1.71 ± 0,37) did not differ from that of Etawa grade (1.57 ± 0,28). Nevertheles this present study was lower than the result of Browning et al. (2004) that litter size of Boer was 1.92 ± 0,12.

Table 1 showed that sex ratio of Boerawa, Etawa grade, Boercang, and Kacang goat were 15 male (50%): 15 female (50%), 16 male (47%): 18 female (53%), 4 male (33%): 8 female (67%), and 9 male (60%): 6 female (40%) respectively. The result indicated that the highest male was Kacang (60%) whereas the lowest male was Boercang (33%). The highest female was Boercang (67%) whereas the lowest was Kacang. This result was different from the result of Suharyati (1999) that sex ratio of Ettawa grade kid was 72.72% (male): 27.28% (female). Sex ratio is affected by genetic and environment factor (Nalbandov, 1990).

Table 1 showed that birth type of Boerawa was 55% single, 40% twin, and 5% triplet. Birth type of Etawa grade, Boercang, and Kacang were 30% single and 70% twin, single 67% and twin 33%, and single 70% and twin 30% respectively. The result indicated that twin and triplet birth types was higher in PP (70%) than in BP (45%). Twin and triplet birth types was higher than single birth types in PP, but it was lower than single birth type in BP. The result indicated also that twin birth types in both BK (33%) and KK (30%) was relatively the same and it was lower than single birth type.

Birth weight of BP, PP, BK, and KK kids were 2.91±0.47; 2.36±0.36; 2.43±0.20, and 1.87±0.64 kg/kid respectively. The result indicated that birth weight in BP was higher (P<0.05) than in PP. Birth weight in BK was higher (P<0.05) than KK. This result indicated that kid grade-1 as a result of grading up between Boer goat and local goat was higher than local goat. Birth weight of BP (2.91 kg) in the present study was higher than that of crossbreed between Boer and Haimen goat (2.5 kg) reported by Yonghong (1999) and was within the range (2.51-3.33 kg) of the result of crossbreed between Boer goat and seven native goat in China reported by Jiabi *et al*. (2009). Birth weight of BK (2.43 kg) in the present study was higher than that of crossbreed between Boer and Kacang goat (2.35 kg) reported by Elieser *et al*. (2006). While birth weight of local goat (PP and KK) was within the range of the result reported by Supriyono (2005) that birth weight of PP and KK were 2.4—2.6 kg and 1.6—2.0 kg respectively.

Table 1. Performance of kid grade 1 as a result of grading up between local goat and Boer goat

Performance		BP	PP	BK	KK
Litter size		1.5[a]	1.7[a]	1.3[a]	1.3[a]
sex	Male	15 (50%)	16 (47%)	4 (33%)	9 (60%)
	Female	15 (50%)	18 (53%)	8 (67%)	6 (40%)
Birth type	Single	11 (55%)	6 (30%)	6 (67%)	7 (70%)
	Twin	8 (40%)	14 (70%)	3 (33%)	3 (30%)
	Triplet	1 (5%)	0 (0%)	0 (0%)	0 (0%)
Birth weight (kg)		2.91[c]	2.36[b]	2.43[b]	1.87[a]

Note: the different superscript in the same row in litter size and birth weight indicated significant effect (P<0.05);
BP: Boerawa; PP: Etawa grade, BK: Boercang; KK: Kacang

CONCLUSION

Performance of kid grade-1 as the result of grading up between Boer goat and local goat is better than local goat.

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Detection of *Enterobacter sakazakii* and other *Enterobacter sp* from Dairy Cow's Milk in Boyolali and Sleman

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ABSTRACT

Enterobacter sakazakii is considered as an opportunistic pathogen that has been implicated in severe forms of necrotizing colitis and meningitis especially in neonates with a mortality rare varying from 40%-80%. The natural habitat *E.sakazakii* is not well understood and have been reported as frequency isolated from different environments including soil, rats, flies, milk powder factories, chocolate factories and households. A total of 100 samples were obtained from dairy cow's milk were studied. The presence of *E.sakazakii* and *Enterobacter sp* was detected using the Holt *et al*., 1994; Guillaume *et al.*, 2005 and Turner *et al*., 2000 procedure on TSBA medium. *E.sakazakii* was not isolated from both district Sleman and Boyolali. However, *E.cloacae* was found in 33 of 75 isolates (44%) of samples from Sleman. Meanwhile 12.7% *E.cloacae* and 5.4% *E.gergoviae* was found of samples from Boyolali.

Key words: *Enterobacter sakazakii, Enterobacter sp*, dairy cow's milk

INTRODUCTION

Enterobacter sakazakii and other Enterobacter species have caused foodborne illness through consumtion f a variety of foods. *Enterobacter sakazakii* is a Gram negative, facultative, rod-shaped bacterium. *Enterobacter sakazakii* is a Gram negative, facultative, rod-shaped, non-spore forming bacterium. The organism was called "yellow-pigmented Enterobacter cloacae". It belongs to the family *Enterobacteriaceae* and genus *Enterobacter* that contains a number of species including *E.agglomerans, E.cloaceae, E.aerogene*s and *E.gergoviae*. The differentiation among these species is based on biochemical reactions, and serological and molecular techniques (Hoffman and Roggenkamp, 2003; Iversen *et al*., 2004).

E.sakazakii, E agglomerans, and *E.cloacae* are considered the main species of this genus that are frequently isolated from clinical samples and food products (Farmer *et al*., 1980). *E.sakazakii* and *Enterobacter species* have been reported as frequently isolated from different environments including soil, rats,flies, milk powder factories, chocolate factories and households (Kandhai *et al*., 2004). *E.sakazakii* has been also isolated from a wide range of foods including ultra high-temperature treated milk (UHT milk), cheese, meat, vegetables, grains,sorghum seeds, rice seeds, herbs, spices, fermented bread, fermented beverage, tofu, and sour tea (Iversen&Forsythe, 2004; Leclercq *et al*., 2002).

E. sakazakii is considered an opportunistic pathogen that has been implicated in severe forms of necrotizing colitis (Van Acker et al., 2001) and meningitis (Bar-Oz *et al*., 2001) especially in neonates with a mortality rate varying from 40% to 80% (Muytjens *et al*., 1988).

The US Food and Drug Administration (FDA,2002) has issued an alert to health care professionals about the risk associated with *E.sakazakii* infections among neonates fed with milk-based infant formula. The alert stated that a major contribution to the avoidance of *E.sakaz*akii infection in premature babies and neonates is the prevention of contamination of infant milk formula during production and bottle preparation. However, knowledge of etiological and ecological characteristic of *E.sakazakii* is sparse and its occurrence in factories that produce infant formulas and in hospital kitchens has not been studied in depth.

The natural habitat *E.sakazakii* is not well understood and have been reported as frequency isolated from different environments including soil, rats, flies, milk powder factories, chocolate factories and households. The organism is known to tolerate extremely dry condition. In 2003-2004 announced find-

ing that 22 % of formula milk for infants on the market in Indonesia was infected with the *E.sakazaakii.* In a survey of infant formula products from 11 countries, was isolated *E.sakazakii 1*3.5%.

Enterobacter cloacae and *E. aerogenes* are opportunistic pathogens. The most common infection they produce is bovine mastitis. *Enterobacter sakazakii* is known occasionally to cause meningitis and sepsis in human neonates (Carter and Wise, 2004). *Enterobacter cloacae* causes occasional bacteremia in human and *E.aerogenes* can be associated with mastitis. *Enterobacter .sakazakii* is the name given to the yellow-pigmented variant of *E. cloacae*; it is isolated from food but only rarely from human clinical specimens

The objective of this study was to investigate the prevalence *of E.sakazakii* and *Enterobacter sp* in dairy cow's milk in Sleman and Boyolali.

MATERIALS AND METHODS

All media materials used in the study were obatained from Oxoid.

Dairy cow's milk samples

A total of 100 samples were obtained by using bottles, 50 samples from daity cow's milk. from Sleman and 50 samples from dairy's cow from Boyolali. These samples were collected from udder when a time for milking.

Detection, isolation and identification of Enterobacter sp.

The procedure for detection, isolation and identification of *E. sakazkaii* and other *Enterobacter sp* by the Holt et al, 1994; Guillaume *et al*., 2005 and Turner *et al*., 2000 procedure on TSBA medium. Milk on bottles were homogenate by centrifugation. A loopful of the suspension was streak on TSBA (Enrichment culture). The plates were incubated for 18-24 h at 36 ^{0}C. Five colonies of the yellow colonies were then tested by Motility, Sitrat, MR and VP for examine genus *Enterobacter*. *Enterobacter sp*. appeared under microscope as short-rods in shape and was Gram negative. For determine *E.sakazakii* and other *Enterobacter* sp that had tested of Sitrat (+), MR (-), VP (+) and Motil were then tested Urea, KCN and Lisin D (Holt *et al*., 1994)

RESULTS AND DISCUSSION

Detection by isolation *E.Sakazakii* and other *Enterobacter* from dairy cow's milk are given in Table 1. The positive *E.sakazakii, E.cloacae,* and *E.gergoviae* formed yellow colonies on TSBA after 18-24 h on incubation at 37 ^{0}C (Figure 1). Gram stain of *Enterobacter sp*. given in Figure 2. *E.sakazakii* was not isolated from both district Sleman and Boyolali. However, *E.cloacae* was found in 33 of 75 isolates (44%) of samples from Sleman. Meanwhile 12.7% *E.cloacae* and 5.4% *E.gergoviae* was found of samples from Boyolali. These result is likely the result of Budiarsa (2007) none of *E.sakazakii* were isolated from manure, farm environment and raw milk in Yogyakarta. *Enterbacter cloacae* and *E. gergoviae* are categoty in B, have been associated with neonatal infections which include necrotizing enterocolitis, which is the most common important gastrointestinal illness in the new born (FAO/WHO, 2004; Iversen&Forstyte , 2004b. FAO/WHO have stated "Other Enterobacteriaceae are in category"B" because they are well-established causes of ollness in infants (e.g. systemic infection, NEC and severe diarrhea) and have been found in powdered infant formula, but contaminated powdered infant formula has not been convincingly shown, either epidemiologically or microbiologically, to be the vehicle and source of infection in infants. These organisms include,: *Enterobacter agglomerans, Hafnia alvei, Klebsiella pneumonia, citrobacter koseri, C freundi, Klebsiella oxytoca and Enterobacter cloace* (Lehner and Stepan, 2004). Shaker *et al*., 2007 reported *E.sakazakii* was isolated from (2/15) infant food formula (2/8) infant milk formula and (1/8) cereal products (semolina) but none of the powder milk (full cream) cereal product (starch, ground rica, bread crumbs, oat, flour, mixed spices, fine sugar) and environmental samples. Moreover *E.cloacae* was isolated in infant formula, cereal product factory.

More research is needed to determine the sources of *E.sakazakii* for example : the raw material and particular the heat sensitive nutrien added after pasteurization, the processing environment i.e equipment and processing lines. Also studies of condition affecting biofilm formation by *E.sakazakii* in food factories.

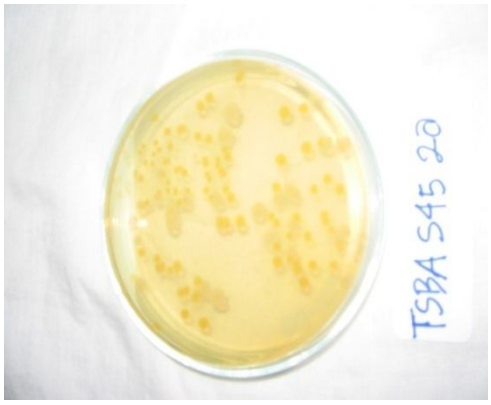

Figure 1. *Enterobacter sp* in TSBA

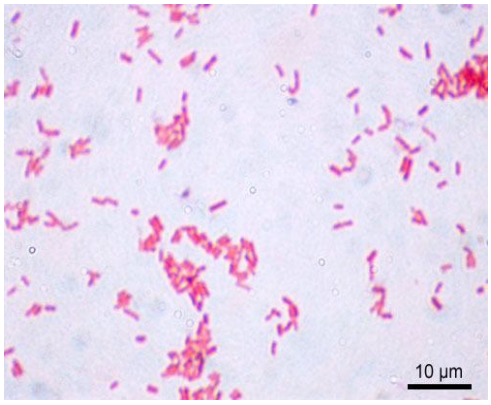

Figure 2. Gram stain of *Enterobacter sp*

Table 1. Prosentase *Enterobcter sp*. were Isolated from Dairy Cow's Milk in Sleman and Boyolali

Dairy cow's milk Farm location	Number of samples	*Enterobacter sp*		
		E. sakazakii	*E. cloaceae*	*E. gergoviae*
Boyolali	50	-	19 (12.7%)	1 (0.7%)
Sleman	50	-	33 (44%)	-

CONCLUSIONS

The result of this study show that *E.sakazakii* was not isolated from both district Sleman and Boyolali dairy cow's milk farm. However, *E.cloacae* was found in 33 of 75 isolates (44%) of samples from Sleman, meanwhile 12.7% *E.cloacae* and 5.4% *E.gergoviae* was found of samples from Boyolali.

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Market Structure and Marketing Efficiency of Beef Cattle inNTT (Case in Kupang Regency)

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ABSTRACT

The aims of this research were to : 1) analyze potency of national cattle market to develop cattle in NTT, 2) analyze supply channel management of beef cattle in NTT and its efficiency, and 3) provide local government's role to develop cattle farm. Primary data based on questioners collected from farmers, trader, and companies. Secondary data collected from legal documents and publications. Potential and marketing opportunities analysis based on propensity models. Cattle market distribution channels were analyzed on scheme of marketing channels were carried out from farmers and traders. Marketing efficiency was analyzed by calculation of cost, and analysis of marketing margin. The research result indicated that, 1) beef cattle in NTT is a tradable commodity, and is the fifth position nationally, 2) beef cattle marketing in NTT is efficient in terms of the price. 3) Local government policy worked to optimize the beef cattle farm in order to optimize the livestock potency for society prosperity, domestic earnings, economic growth and change of economy structure and its sustainability.

Key words: beef, cattle, market structure, efficiency, NTT

INTRODUCTION

Economics of East Nusa Tenggara (NTT) province is predominated by agricultural sector. In Second Quarter of 2008, approximately 41.15% of Gross Regional Domestic Product (GDP) of NTT comes from agricultural sector. When observed more specifically, the leading of NTT agricultural sector are food crops and livestock. Livestock sub-sector growth in the Second Quarter of 2008 reached 15.35%, the highest among the other agricultural sub-sector.

NTT is a national livestock supplier region. Realization of livestock inter-insular trade in 2007, is exceeding the target with details of 63,036 cattles (156.61%), 7,745 buffalos (77.45%), and 7,881 horses (135.35%), while target of 2008 approximately 58,750 head. Cattles from NTT was distributed to Capital City of Jakarta, West Java, Batam, South Celebes, and other regions to stabilize national meat prices, especially during religious special day, in which approximately 90% of cattle for those three regions come from NTT.

Livestock Directorate General recorded balance of national beef production in 2008 is only reached 64.9% of projected consumption needs or there is a shortage of 135,110 tons (35.1%) of the total demand of the meat. With population of 11.26 million heads, the national meat production is estimated up to 249,925 tons, while consumption of meat is estimated up to 385,035 tons. Indonesian Chamber of Commerce and Industry (Kadin) noted that Indonesia require 350,000-400,000 tons of beef per year (equivalent to 1,7-2 million beef cattle), which is the beef import is 30% of those were imported. Import policies carried out in order to support domestic production shortage *martinsihombing@bisnis.co.id*).

Market potential of beef cattle in Indonesian has a wide excess demand and NTT province can be a potential region of beef production. Statistic Biro of NTT (2006) provided that NTT cattle are mostly located at Kupang Regency (Kupang) and South Central of Timor (TTS). From a number of 544,482 beef cattle, about 260,406 heads were rear in these two districts. Thus, the study focused on those two regions in order to accelerate the economic development through beef cattle development. Local government development policies related to beef cattle productions discussed.

The aims of this research were: 1) to analyze and present the potency of the domestic beef cattle market as an opportunity to develop beef cattle business in NTT, especially in Kupang; 2) to analyze the distribution/marketing channel of beef cattle from East Nusa Tenggara (and Kupang in particular), and its efficiency. 3) to present the role

of local government in supporting beef cattle development.

MATERIALS AND METHODS

The research was carried out in Kupang Regency by consideration of accessibility. District and village sample were taken purposively and with consideration of cattle population, developed cattle marketing, and distribution of supporting infrastructure of cattle production. Respondent of farmers were choose purposively from each village by high patterns of homogeneity consideration.

Primary data were collected by interviewed with farmers, livestock traders, and head of the institutions concerning with beef cattle industry. Primary data was collected refer to questionnaire. Secondary data was collected from various official documents and related publications.

Potential and marketing opportunities analysis based on propensity models. Cattle market distribution channels were analyzed on scheme of marketing channels were carried out from farmers and traders. Marketing efficiency was analyzed by calculation of cost, and analysis of marketing margin.

RESULTS AND DISCUSSION

Overview of Regional Economic of NTT and Kupang Regency

NTT GDP based on constant price in 2006 showed a significant increase from Rp12,877,107,214,- (2004) to Rp16,729,571,469,- (2006). In 2006, the agricultural sector is still dominant, which livestock subsector contributed about Rp1,936,536,193,- (approximately 28.08%) of the total agricultural sector about Rp6,895,959,564,-.

Economy sectoral linkages of NTT indicated by IO analysis in 2006 (BPS of NTT, 2007). The agricultural sector has a value of forward linkages and backward which is not dominant compared to other sectors. The total index of agriculture forward linkages was in the third rank, of 2.009 (of which 0.581 was dirrect of and 1.428 was indirrect forward linkage). While the backward linkages was in the sixth rank, of 1.416 (of which 0.278 was directly and indirectly backward was 1.383),

Kupang Regency GRDP in 2006 base on constant prices in 2000 were about Rp690,443,204,000,-, while in 2004 were about Rp632,789,351,000,-. Agricultural sector contributed about 45.86%, was the largest compared to other sectors. Livestock sector became the largest contributor in agricultural sector, i.e 18.24%.

National Market Potential of Beef Cattle and Possible Utilization

Data from Directorate General of Livestock, Department of Agriculture (2007), the exported of NTT beef cattle increased from 35,061 (2003) to 61,279 heads (2006) and was ranked 5th. The number of cattle exported reached 11.25% of the total population of 544,482 heads. NTT is one of the province as a potential and a regular contributor to the national beef needs, while West Java and Lampung is not continuous as a contributor to the expense of livestock (although always ranked first and second).

Exported of beef cattle from Kupang with destination of Capital City of Jakarta, West Java and several other provinces fluctuated, but tended to increase from 10,760 heads (2000) to 33,570 heads (2007) with an average of 24,126 heads per year or growth of 26.37%. Meanwhile, exported of beef cattle from NTT in 2006 was 61,279 heads of which 33,453 heads (54.59%) came from Kupang. In 2007, exported of beef cattle from Kupang was 33,570 heads or 22.68% of population 148,044 heads (Talib, 2008).

Domestic meat production during 2003 to 2006 was increased from 1,872,600 tons to 2,063,900 tons. Beef meat contributed about 369,700 tons (19.74% of total national meat production) in 2003 and about 395,800 tons (19.19% of total national meat production) in 2006. In 2006, the largest contributor to beef production is West Java about 77,759 tons, while NTT produce 7,269 tons (1.83%).

In 2006, import of cattles breed were about 6,200 heads (equivalent to US $2,545,500,-) and 265,700 heads (equivalent to US$ 108,596,700,-), while beef imports was about 25,949.2 tons in 2006 (equivalent to US$ 49,077,200,-). The big number of commodity imports and tend to increase is a challenge and opportunity to improve the national beef production, especially in potential areas.

According to distance and duration to transport cattle from the area and the same destination (Jakarta and West Java) is relatively short (4-5 days) for beef cattle from East Nusa Tenggara, West Nusa Tenggara and Bali and other regions, compared to beef imports from Australia and New Zealand. Another advantage, beef cattle from NTT and other provinces are relatively younger with taste and quality of meat is more desirable.

Beef Cattle Marketing in NTT and in Kupang Regency

Marketing Transaction System for Beef Cattle in Community Level

In Kupang Regency, the most dominant marketing agencies directly related to the breeder (*farmer*, F) are the collectors (*local assemblers*, LA). LA position determines the level of success of cattle business (especially farmers), because the LA was dominant in determined the level of sale price of cattle. Location of transactions are take place at the home of farmers, cooperatives/NGOs, village markets, slaughter (*butchers*, B), or stock-yards owned by inter-island traders (*inter-insular traders*, IT). Ranchers would prefer to trade at home, because it is cheaper or no cost and avoid the risk of marketing (stress, accidents, regardless, or death).

At farmer level, generally base of price formation of cattle is weight, body condition, and age of cattle, where the formation of prices based on weight (weighed) began to dominate. Prices are usually refers to a benchmark price of quality standard price (body weight), but in practice the farmer's bargaining position was not strong enough, because the pricing is still dominated by LA and IT. This allegation comes as the market beef products in this area is still oligopsony, so it possibly of a closed cartel formation created among IT.

However, the trend of cash payment that widespread at the level of breeder can minimize the delay in payment or loss of breeders. The involvement of farmer groups, cooperative and NGOs is very important to strengthen the bargaining position of farmers, so that marketing process becomes fairer.

At the level of LA and even IT, a common problem is the availability of working capital or business capital. Reality shows that almost all of LA is depend much on capital from IT. Similarly, almost all highly depend on IT capital of the merchant buyer (*wholesale receiver*, WSR) in Jakarta.

Bad consequences of dependence lead to unstable purchases capacity by LA and IT, stagnating purchase, and local merchants difficult to be independent. The problems reflects that the bargaining position of WSR (and owners of capital) in Jakarta is very powerful in determining the price. The concentration of capital resulted in the level of the price situation is highly dependent on cattle price situation in Jakarta. In such conditions, then as a trader (LA and IT) always maintain and even increase business profits, in whatever of change in price situation in Jakarta. This will directly lead to depressed cattle prices at farmer level that have implications farmer's share are getting lower.

Marketing Channel of Local and Inter-insular Life Beef Cattle

Parties along the beef cattle marketing channels (i.e. F, LA, IT, WSR, and B) and beef marketing channels (i.e. butchers (B), intermediate consumers (IC), and final consumers (FC) is a marketing channel itself. Shorter or longer the market channel depends on economic conditions and regional characteristics.

Several patterns of local marketing channels operating simultaneously are:

a. F → LA → IT → WSR → B → IC → FC (Jkt)
b. F → IT → WSR → B → IC → FC (Jkt)
c. F → IT → B → IC → FC (Local)
d. F → LA → B → IC → FC (Local)
e. F → B → FC (Local)
f. F → FC (Local)

Figure 1 shows the marketing channel of cattle from Kupang to the end consumer. The marketing channel is traditional route running since a long time ago. There are some old actors survive, in addition to new players. This shows that this business still promising profits, even though under the influence and market pressures, both direct and indirect.

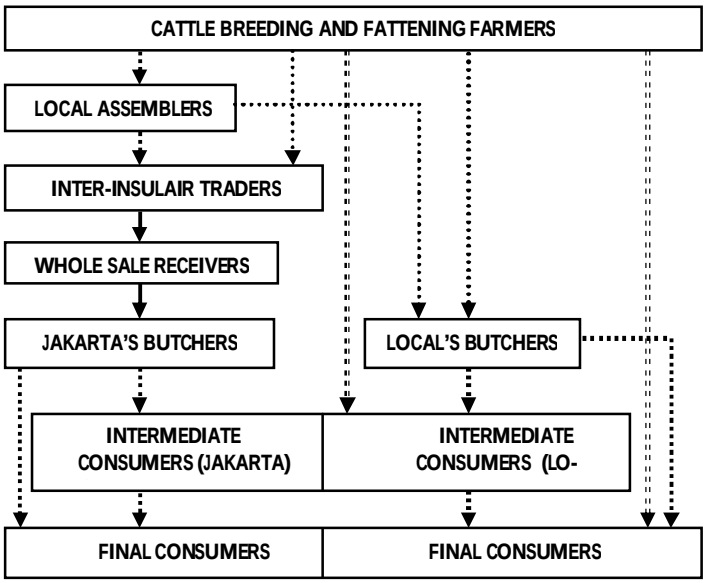

Figure 1. Marketing Channels of Beef Cattle and Meat from Kupang

Efficiency of Beef Cattle Marketing

Price forming is a central phenomenon in a market economy/free. Sell price formation is the accumulation and/or interaction of the size of the base price, availability of capital, marketing costs, and profits. In the beef cattle marketing, price formation occurs in every level of marketing channel (see Table 1).

Price analysis and marketing margins in beef marketing channel in Kupang Regency showed in Table 2. Table 2 shows the price variations of beef cattle in farmer level (i.e. the price at the level of F vs LA, F vs IT, or F vs B) is greater than the price variation in other levels (such as the LA vs IT, LA vs B, IT vs WSR, or WSR vs B). This is because farmers can sell their cattle to a different level of channel in unofficial price. In addition, prices at the farmer level was determined by estimated or weighed, which in general pricing breeders are still under pressure from a strong bargaining position of the trader.

On the other hand, at level of traders (LA, IT, WSR, and B) the price of cattle has a relatively slight variations, because all of the cattle is weighted and the price is entirely based on the price-table agreed by market participants.

Table 3 shows the selling price from one marketing level to the next was increase, because the trader had some marketing function such as transporting, exchange/barter, and financing. The highest marketing costs is in IT level, because the IT hold some major marketing functions, particularly the transport functions (include shipment) from the collector trader in production area, inter-island transport, and distribute to the consumer area. Other costs were SP3, clinical test, quarantine, feed and water, livestock guard force (kleder), insurance, and unloading.

Table 1. Point of price making in beef cattle marketing channels of Kupang regency

Point of Making Price			Inisial		
Farmer	*vs*	Local Assembler	F	*vs*	LA
Farmer	*vs*	Inter-insular trader	F	*vs*	IT
Farmer	*vs*	Butchery	F	*vs*	B
Local Assembler	*vs*	Butchery	LA	*vs*	B
Local Assembler	*vs*	Interinsular trader	LA	*vs*	IT
Inter-insular trader	*vs*	Whole Sale Receiver	IT	*vs*	WSR
Whole Sale Receiver	*vs*	Butchery	WSR	*vs*	B

Table 2. Price analysis and marketing margin of cattle fattening in regency of Kupang

Marketing Channels		Price (Rp)				MC		MP		Share (%)
		Pessimistic	Moderate	Optimistic	Average	Rp	%	Rp	%	
I	F	3,555,325	3,999,600	4,454,825	4,003,250	-	-	-	-	61.5
	LA	4,043,556	4,541,213	5,065,075	4,549,948	130,000	2.00	546,698	8.4	69.9
	IT	5,170,244	5,791,088	6,422,881	5,794,738	700,000	10.76	1,244,790	19.1	89.1
	WSR	5,558,325	6,221,600	6,895,825	6,225,250	75,000	1.15	430,513	6.	95.73
	B Jkt	5,808,700	6,499,350	7,200,950	6,503,000	50,000	0.77	277,750	4.2	100.00
	C Jkt	-	-	-	-	-	-	-	-	-
II	F	3,805,700	4,277,350	4,759,950	4,281,000	-	-	-	-	65.83
	LA	5,170,244	5,791,088	6,422,881	5,794,738	700,000	10.76	1,513,738	23.28	89.11
	IT	5,558,325	6,221,600	6,895,825	6,225,250	75,000	1.15	430,513	6.62	95.73
	WSR	5,808,700	6,499,350	7,200,950	6,503,000	50,000	0.77	277,750	4.27	100.00
	B Jkt	-	-	-	-	-	-	-	-	-
III	F	3,555,325	3,999,600	4,454,825	4,003,250	-	-	-	-	85.22
	LA	3,805,700	4,277,350	4,759,950	4,281,000	130,000	2.77	277,750	5.91	91.13
	B Kpg	4,181,263	4,693,975	5,217,638	4,697,625	50,000	1.06	416,625	8.87	100.00
	C Kpg	-		-	-	-	-	-	-	-
IV	F	3,680,513	4,138,475	4,607,388	4,142,125	-	-	-	-	88.17
	B Kpg	4,181,263	4,693,975	5,217,638	4,697,625	75,000	1.60	555,500	11.83	100.00
	C Kpg	-	-	-	-	-	-	-	-	-

Source: Primary and secondary data, 2008.

Table 3. The Average Cost, Profit and Marketing Margin of Beef Cattle in Regency of Kupang (2008)

Marketing Channel		Price (Rp)	Share (Rp)	Marketing Margin					
				Cost		Profit		Margin	
				Rp	%	Rp	%	Rp	%
I	F	4,003,250	61.56	-	-	-	-	-	-
	LA	4,549,948	69.97	130,000	2.00%	416,698	6.41%	546,698	8.41%
	IT	5,794,738	89.11	700,000	10.76%	544,790	8.38%	1,244,790	19.14%
	WSR	6,225,250	95.73	75,000	1.15%	355,513	5.47%	430,513	6.62%
	B Jkt	6,503,000	100.00	50,000	0.77%	227,750	3.50%	277,750	4.27%
	C Jkt	-	-	-	-	-	-	-	-
II	F	4,281,000	65.83	-	-	-	-	-	-
	LA	5,794,738	89.11	700,000	10.76%	813,738	12.51%	1,513,738	23.28%
	IT	6,225,250	95.73	75,000	1.15%	355,513	5.47%	430,513	6.62%
	WSR	6,503,000	100.00	50,000	0.77%	227,750	3.50%	277,750	4.27%
	B Jkt	-	-	-	-	-	-	-	-
III	F	4,003,250	85.22	-	-	-	-	-	-
	LA	4,281,000	91.13	130,000	2.77%	147,750	3.15%	277,750	5.91%
	B Kpg	4,697,625	100.00	50,000	1.06%	366,625	7.80%	416,625	8.87%
	C Kpg	-	-	-	-	-	-	-	-
IV	F	4,142,125	88.17	-	-	-	-	-	-
	B Kpg	4,697,625	100.00	75,000	1.60%	480,500	10.23%	555,500	11.83%
	C Kpg	-	-	-	-	-	-	-	-

Source: Primary and secondary data, 2008

Table 3 indicate the benefit obtained by IT was Rp572,025.- (16.96%) on Channel I and Rp544,250.- (16.72%) on Channel II. This differences was caused by the selling price of IT to the WSR is higher and able suppressed the purchase price at the level of IT and F. Smallest gains obtained by LA in the amount on Channel I of Rp267,725,-

(2.30%) and Channel III registration Rp295,500,- (3.15%). This is because the LA had a relatively small price difference between the price at the level of F and IT, in addition to the cost of components such as transportation costs, charges and village cooperatives, and labor.

Results of analysis showed that the marketing of beef cattle in Kupang has been running efficiently, reflected from the aspect of the small efficiency financing. Cost efficiency in Channel I of 31.13%, Channel II of 26.35%, Channel III of 14.78%, and Channel IV of 11.83%. From the four beef cattle marketing channel, Channel IV is the most efficient, because the level of cost efficiency is lowest (1.60%) and the share received by farmers is very high (88.17%).

The indicator of marketing efficiency was the share obtained by farmers, compared to consumer price. The higher farmer share was the more efficient marketing. The value in Table 3 concluded that beef cattle marketing system in Kupang is good enough. An efficient mechanism for marketing farm products is a positive driving energy for the effort of intensification, extensification and diversification of livestock sub-sector development, especially in the context of beef self-sufficiency in Indonesia.

The Role of NTT and Kupang Local Government Policies

Livestock development in NTT, and Kupang Regency directed to improve the welfare of farmers through the optimization of farm resources utilization. Therefore, local government policies related to livestock development (mainly cattle), was focused on efforts to optimize the potency utilization for farmer welfare, increase revenue, increase economic growth and accelerating changes in economic structure and maintain its sustainability. The programs implemented were: 1) Improvement Breeder Production and Productivity, 2) Economic Institutional Strengthening of Breeder, and 3) Management, Supervision and Settings Livestock Rearing and Commerce.

The first program was aimed to increase production and productivity of livestock at farmer level as food security, strengthened of agro-industry development, and increase farmer income and local district revenues. The second program was aimed to: 1) strengthen the economic institutional of breeders to support the production, processing, marketing and provision of production inputs, and 2) functioning of economic institutions as a farmers learning media in developing business and technical skills. The third program was aimed: 1) Encourage to increase the cattle population and good management of animal farm, and 2) to ensure a justice trade between farmers, traders and governments.

Another aspect that requires more accurate treatment is related to the development efforts of local butchery industryes. This is intended to beef cattle marketing activities in the form of live cattle for meat fulfillment of national interests that had been continue can be changed in structure. Thus, delivery of live cattle shipment is substituted by fresh meat (frozen meat). The advantage of this policy development is to suppress leakage areas (regional leakages) through increased absorption of added value in the area of NTT and / or the Kupang regency in particular. It also will increase employment and to encourage increased production of beef cattle are more significant.

CONCLUSION

Beef cattle commodity in NTT and Regency of Kupang is potential commodity to be trade out of the region, where NTT occupies fifth position as supplier of cattle livestock. Up to 2006, the amount of cattle that released/exported from NTT were 61.279 heads which was 54.59% from Kupang. Livestock and beef import is big opportunity for NTT to improve potencial region.

In general, cattle marketing system in Regency of Kupang was efficient. This was indicated from its cost that was fair. Other Indication is shares marketing for farmer was high compared to to product price that paid by final consumer. Efficient marketing mechanism is impeller energy for intensification effort, extensification, and diversified development of livestock subsector

Local government programs included production, productivity, and marketing management is to optimize livestock potency to increase income, society prosperity, economic grow and economic structure. The changing of marketing structure of livestock

commodity and beef cattle is people desire in this region in order to obtain better quality.

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Identification of Growth Hormone Releasing Hormone Gene in Local Buffalo Buffalo (*Bubalus bubalis*) Using PCR-RFLP

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ABSTRACT

GHRH is a hypothalamic hormone which stimulates growth hormone secretion in the pituitary gland. The objective of this study was to identify polymorphisms Growth Hormone Releasing Hormone (GHRH) gene of Indonesian buffalo's. A total of 320 blood samples from Indonesian buffaloes were used to determined polymorphism using PCR-RFLP method. The polymorphism of GHRH gene that spanned within exon 2 and exon 3 was amplified, and their mutation was detected using endonuclease *Hae*III. In this study, there were three GHRH/*Hae*III genotype (AA genotype 0%, AB genotype 36% and BB genotype 64%) determined by two alleles, A (18%) and B (82%). The frequency of A allele was found 15% for Semarang population, 19% for Mataram population, 2% for Medan population and 40% for Banten population. The frequency of B allele was found 85% for Semarang population, 81% for Mataram population, 98% for Medan population and 60% for Banten population. The observed heterozygosis value were different among populations. The highest heterozygosis ($\hat{h}$) 0,485 for Banten population and the lowest was 0.037 for Medan population and the average heterozygosis for all populations ($\hat{H}$) detected was 0.270. Index fixation value of GHRH gene showed there was not fixed into one gene type ($F_{ski} \neq 0$). The smallest genetic distance value of GHRH gene was found between Semarang and Mataram population (0.001) and the highest between Medan and Banten population (0.202).

Key words: buffallo, GHRH gene, PCR-RFLP

INTRODUCTION

Local buffaloes has great potential to be developed as a meat-producing animals because it is easy to adjust, has a relative carcass weights higher than the local cows and always maintained in rural areas (Hasinah and Handiwirawan, 2006). Generally local buffaloes not used to meat-producing livestock, although in terms of body weight potential. Genetic quality improvement is still far behind the buffalo from the other cattle. Improvement by considering the genetic markers can be used as an alternative in conducting the selection. One selection method that is currently developing a method of MAS (Marker Assisted Selection) is selected on the basis of markers DNA controlling economic traits.

Growth Hormone Releasing Hormone (GHRH) is one of the role of growth factors stimulate the synthesis and secretion of Growth Hormone in an additive effect on growth. Therefore, GHRH gene is a genetic marker which can be used as one basis for selecting cattle. This study aims to identify the gene diversity of Growth Hormone Releasing Hormone (GHRH) on the local buffalo in Indonesia.

GHRH is a hypothalamic hormone which stimulates growth hormone secretion in the pituitary gland. GHRH stimulates both synthesis and secretion of pituitary growth hormone (GH) binds to specific receptors on somatotrophs (Frohman *et al.*, 1992). Growth-hormone-releasing hormone *(GHRH),* also known as growth-hormone-releasing factor (GRF or GHRF) or somatocrinin, is a 44-amino acid peptide hormone produced in the arcuate nucleus of the hypothalamus (Connor *et al*., 2005). Other studies showed that somatotropin, somatoliberin and their synthetic equivalents increased milk production in both dairy cows (Bonneau dan Laarveld, 1999) and in meat cows (Achtung *et al.*, 2001) as well as improved cattle growth rate thereby reducing the time necessary to reach the slaughter weight. Cheong *et al*. (2006) suggest that polymorphism in GHRH might be one of the important genetic factors that influence carcass yield in Korean

native cattle (Hanwoo). Bovine GHRH gene was linked to CSSM30 on chromosome 13 (Barendse *et al*., 1994) consists of five exons separated by four introns (Zhou *et al*., 2000).

Moody et al. (1995) reported the existence of GHRH gene diversity in cattle by PCR-RFLP method using GHRH forward primer 5'-GTA AGG ATG GCT CTG CCA GGT3 'and GHRH reverse 5'-TGC ATG ATG CTG TCC CTC TGG A-3' and restriction enzymes *Hae*III which produces two alleles of 317, 83, 55 bp (allele A) and 196, 121, 83, 55 bp (allele B). Polymorphism sites for GHRH / *Hae*III in cattle Polish Black and White by Dybus and Grzesiak (2006) it covers a part of exon 2, the entire intron 2, and a part of exon 3; the analysed polymorphic site is located in intron 2 (AF242855 – GenBank 2000).

MATERIALS AND METHODS

Sample Collection and DNA Isolation

A total of 320 blood sample from Indonesian buffaloes collected from 4 populations; 75 from Semarang (Central Java), 103 from Mataram (West Nusa Tenggara), 65 from Siborong-borong (North Sumatera), and 77 from Banten. DNA isolation was performed using minikit DNA *Genaid*.

PCR-RFLP Analysis

PCR-RFLP method was applied to determine individual genetic variants of the analyzed gene fragment. A 451 bp fragment of the GHRH gene was amplified using a pair of primer Moody *et al*. (1995) with the following nucleotide sequences : *forward* 5'-GTA AGG ATG CCA GCT CTG GGT3' and GHRH *reverse* 5'-TGC CTG CTC ATG ATG TCC TGG A-3' (2 µl), 2 µl sample DNA, 0.75 unit *Taq polymerase* enzyme, 0.24 mM dNTP, 2 mM $MgCl_2$, 10x *buffer* 2.5 µl and 17.85 µl destilata water. The condition of thermal cycling began with an initial cycle of *pradenaturation* 94°C for 5 min followed by 30 cycles of *denaturation* 94°C for 1 min, *annealing* 60°C for 1 min and *final extention* 72°C for 5 min. Afterwards, the amplification product was digested with endonuclease *Hae*III, which recognizes the sequence GG│CC. Then silver staining Tegelstrom (1992) used to visualize the bands.

Statistical Analysis

Alleles frequency for each buffaloes population was calculated in the form type of GHRH gene according to Nei (1987) :

$$X_i = \frac{2\,nii + \sum nij}{2n}$$

Where

X_i = allele frequency of -i

n_{ii} = number of individu with genotipe ii

n_{ij} = number of individu with genotipe ij

n = total individu sample

Degree of heterozygosity ($\hat{h}$) is calculated based on allele frequencies at each locus DNA using the formula of Nei (1987):

$$\hat{h} = \frac{2n(1-\sum x_{i^2})}{2n-1}$$

Where

$\hat{H}$ = heterozygosis locus

X_i = allele frequency of GHRH gene type-*i*

N = total of individu sample

Variance of heterozygosity in each population can be calculated by the following formula:

$$V_{s1}(\hat{h}) = \frac{2nii + \sum nij}{2n}\left\{2(2n-2)\left(\sum x_{i^3} - \left(\sum x_{i^2}\right)^2\right) + \sum x_{i^2} - (x_{i^2})^2\right\}$$

Average heterozygosity ($\hat{H}$) is calculated by the following formula:

$$\hat{H} = \sum_{j=1}^{r} \hat{h}_i / r$$

where

$\hat{h}_j$= degrees of heterozygosity for the locus of -j

r = number of loci tested

$\hat{H}$ = average heterozygosity

Fixation index in each population derived from the equation:

$$F_{ISki} = \frac{X_{kii} - X^2{}_{ki}}{X_{ki}(1-X_{ki})}$$

where

X_{kii} = Frekuensi genotipe homozigot alel i pada populasi ke-*k*

X_{ki} = Frekuensi alel i

Genetic distance (D) calculated using the formula:

$$I = \sum_{i=1}^{m} (P_{ix} \times P_{iy}) \Big/ \left[\left(\sum_{i=1}^{m} P_{ix}^2 \right) \left(\sum_{i=1}^{m} P_{iy}^2 \right) \right]$$

$$D = -\ln I$$

Where
D = Genetic distance
P_{ix} = i allele to the population X
P_{iy} = Frequency of allele i in population Y

RESULTS AND DISCUSSION

A 451 bp of GHRH gene fragment was successfully amplified using polymerase chain reaction technique (Figure 1). The polymorphism GHRH gene were found by PCR-RFLP analysis of exon 2 and exon 3 (Figure 2).

GHRH gene segments was amplified in this study located in a part of exon 2, intron 2 and part of exon 3; the analysed polymorphic site is located in intron 2. Fragment length GHRH gene amplification results in cattle according to Moody *et al*. (1995) was 455 bp, according to the target in the yak Ou *et al*. (2002) was located at 450 bp in exon 2, intron 2 and exon 3. Franco *et al*. (2005) reported the GHRH gene fragment length amplification results in pigs is 455 bp located in exon 3.

As a result of digestion with restriction enzyme *Hae*III, three genotypes were identified in this study, their restriction fragments being as follows: 312, 94 and 45 bp- $GHRH^A$ $GHRH^A$ genotype; 312, 194, 118, 94 and 45 bp- $GHRH^A$ $GHRH^B$ genotype; and

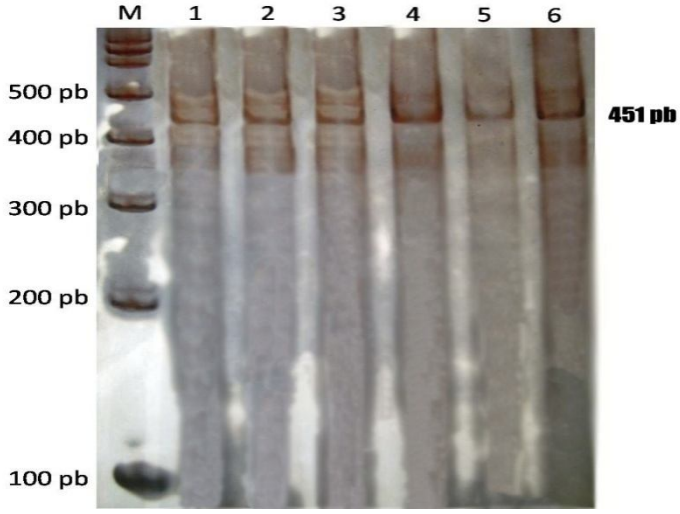

Figure 1. Results GHRH Gene Amplification Using PCR Method at 6% Poliacrilamida Gel on the GHRH gene sequences in cow cattle (GenBank Access No. AF242855) (Zhou *et al*., 2000).

```
4321 cctgtctgtc atttcccagg taccagcaca ggggtgaagg atgctgctct gggtgttctt
                                          ──Primer Forward──►
4381 cctcgtgacc ctcaccctca gcagcggctc ccacggttcc ctgccttccc agcctctcag
4441 gtaagcagtt ctgagaagag aagcaagaga gg|ccctttga ggatgcgact cgagctggtc
4501 cccagctggg tcctcaggca gcctcccttg ctcatctctg ggagggtggc agactgagcc
4561 ccagagaggt caccacccag ccctggttcc agccctctct ggggacgagc agggcaagag
4621 gcgacagaaa gacctcacag agaccaagtg agcacagtcc cctgg|cctc ccacccccacc
4681 ctttgacctc tgactccttc tactaggatt ccacggtacg cagatgccat cttcactaac
4741 agctaccgga aggttctgg|ccagctgtct gcccgcaagc tactccagga tatcatgaac
                                          ◄── Primer Reverse
4801 aggcagcagg ggtgagccgg cgttctcgtg acttctccct gcaccctcgg ttcatcatga
```

Figure 2. Primary attachment position and the site *Hae*III Restriction Enzyme based

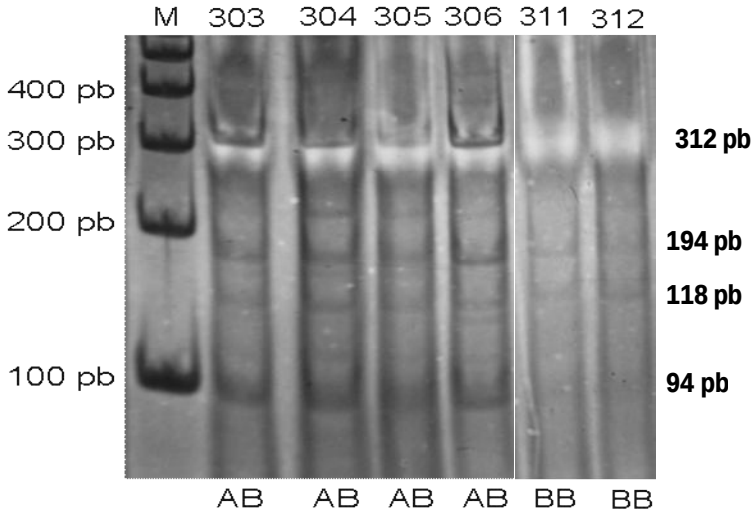

Figure 3. GHRH gene fragment using PCR-RFLP method with *Hae*III enzyme.

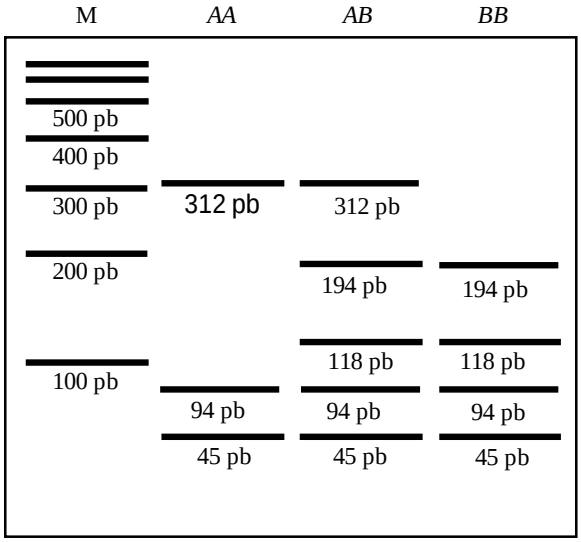

Figure 4. Zymogram of electrophoretic pattern showing genotipe AA,AB and BB of GHRH gene.

194, 118, 94 and 45 bp-GHRH^{B}GHRH^{B} genotype. DNA bands with size of 45 bp can not be displayed because the concentration of acrylamide with 6% less appropriate is used to separate long DNA with less than 60 bp (Muladno, 2002).

The results of this study indicate that the GHRH gene in the local buffalo are polymorphic (various) in all populations from four regions in Indonesia. The percentage of successful detection with GHRH gene diversity of PCR-RFLP method in this study for 76.56%, ie from 320 samples can be identified GHRH gene diversity as much as 245 samples. Genotype frequency and allele frequency of the local buffalo GHRH gene is presented in Table 1. Heterozygosity value of the local buffalo in this study ranged from 0.037 to 0.485 (Table 2). The results of the analysis of genetic distances based on GHRH gene between the four local buffalo population in Indonesia is presented in Table 4.

Genetic distance can be used to create a dendogram (tree phylogeny) that can be used to show the relationship between population kinship. According to Nei (1987) kinship between populations can be identified by using the simplest method of average - the average genetic distance UPGMA (Unweighted Pair-Group Methode with Arithmetic mean). Kinship relations between the four local buffalo populations are shown in Figure 5.

Table 1. Frequencies of GHRH/*Hae*III genotypes and alleles in local buffaloes based on location

Location	Total Sample (n)	Genotype	Genotype Frequencies	Allele Frequencies
Semarang	61	AA (0)	0.000	A = 0.147
		AB (18)	0.705	B = 0.853
		BB (43)	0.295	
Mataram	86	AA (0)	0.000	A = 0.186
		AB (32)	0.372	B = 0.814
		BB (54)	0.628	
Medan	53	AA (0)	0.000	A = 0.019
		AB (2)	0.038	B = 0.981
		BB (51)	0.962	
Banten	45	AA (0)	0.000	A = 0.400
		AB (36)	0.800	B = 0.600
		BB (9)	0.200	
Total	245	AA (0)	0.000	A = 0.180
		AB (88)	0.360	B = 0.820
		BB (157)	0.640	

Table 2. Heterozygosity values ($\hat{h}$) and average heterozygosity ($\hat{H}$) GHRH gene in Local Buffaloes

Location	$\hat{h} \pm SE$	$\hat{H}$
Semarang	0.252 ± 0.045	0.270 ± 0.024
Mataram	0.305 ± 0.003	
Medan	0.037 ± 0.026	
Banten	0.485 ± 0.022	
Total	0.461 ± 0.022	

Index fixation value of GHRH gene showed there was not fixed into one gene type ($F_{ski} \neq 0$).

Table 3. Fixation index values of Local Buffaloes GHRH gene

Location	Allele	F_{Iski}
Semarang	A	-0.175
	B	-3.450
Mataram	A	-0.229
	B	-0.228
Medan	A	-0.019
	B	-0.019
Banten	A	-0.666
	B	-0.666
Total	A	-0.218
	B	-0.197

Table 4. Genetic distance values of GHRH Gene in Local Buffaloes

Location	Location			
	Semarang	Mataram	Medan	Banten
Semarang	0.000			
Mataram	0.001	0.000		
Medan	0.012	0.023	0.000	
Banten	0.089	0.064	0.171	0.000

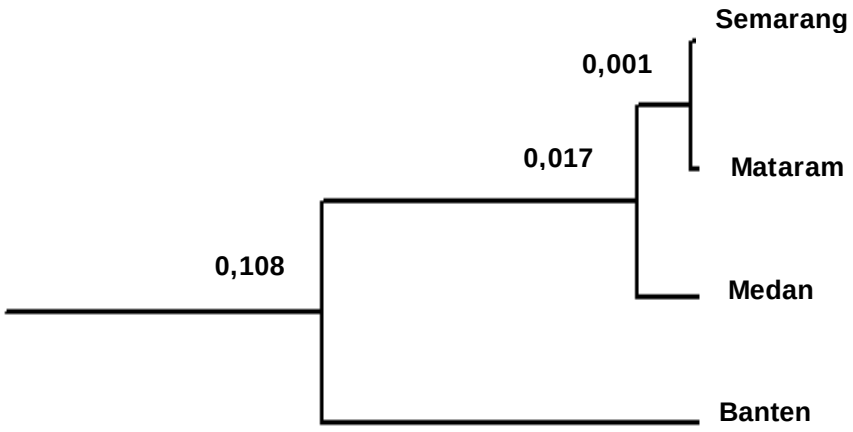

Figure 5. Genetic Tree Dendogram Based on GHRH Gene Local Buffaloes Population.

CONCLUSION

GHRH gene in Indonesian local buffaloes were polymorphic, there were determined by two alleles; A and B. The frequency of B type was higher than A type in this study. Based on locations, buffalo's from Banten population has highest frequency of A allele (40%) and the lowest from Medan population (2%). Conversely, for frequency of B allele. The observed heterozygosis value were different among populations. The highest heterozygosis ($\hat{h}$) 0,485 for Banten population and the lowest was 0.037 for Medan population. Index fixation value of GHRH gene showed there was not fixed into one gene type ($F_{ski} \neq 0$). The highest genetic distance value of GHRH gene was found between Medan and Banten population (0.202) and the smallest between Semarang and Mataram population (0.001).

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Birth Type and Ewe Age on Milk Yield of Local Sheep at Up3 Jonggol (Jonggol Animal Science Teaching and Research Unit)

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ABSTRACT

The objective of this research is to study the effect of age and litter size on milk yield. The sheep used in the research were local. Lamb suckling weight differences were used to calculate milk yield of ewe. I3 gave the highest milk yield (443.43±102.62 g/ewe/6h), while I1 the lowest (254.53±49.67 g/ewe/6h). I3 reached its maturity stage faster than (I1 and I2). The litter size in twin ewe gave the highest milk yield (344.95 ± 66,22 g/ewe/6h), while the lowest in single (413.99 ± 128,62 g/ewe/6h). The highest milk yield in twin ewe was because the increased mammary secretory cell which caused nutrient requirement.

Key words: sheep, local, milk, yield, age, birth type

INTRODUCTION

Many researches have been conducted to study milk yield on dairy sheep, and mostly conducted in Australian sheep, Awassi and Assaf from Palestine, East Friesian from the Mediterranean and Sarda from Italy. However, fewer research has been focused on Local Sheep. Therefore, the research could become a data base of Local Sheep (Jonggol Sheep) and increase sheep production.

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Pollot and Gootwine (2004), Pullina and Nudda (2004), Snowder and Glimp (1991) said that birth type and age could effect milk yield in dairy sheep. So that, we want to know what a level of in a local sheep which is non-dairy sheep (meat production). In such a way that, we would be know the pattern of age and birth type related to milk yield in local sheep.

MATERIALS AND METHODS

Materials

All experiments were conducted at Jonggol Animal Science Teaching and Research Unit and Feed Laboratory, Faculty of Animal Science, Bogor Agricultural University.

The Local Sheep from Thin Tail Javanese Sheep species was used in this experiment. Ewe and males have chosen for natural matted in the paddocks. The total number of stem is used for research is 92 head which 78 single births (I_1 = 9 heads, I_2 = 18 heads, I_3 = 16 heads, I_4 = 35 heads) and 14 twins (I_1 = 3 heads, I_2 = 3 heads, I_3 = 1 head, I_4 = 7 heads.

The animals were located in colony cages. Enclosure ewe and lamb are separated during yield counting, but they are still in eye contact.

Ewe and lamb were reared on the pasture with additional *Brachiaria humidicola* while in the cage. The pasture consist of green grass (*Themeda sp*), weed (*Eupatorium sp, Melastoma sp, Cantana sp*), and legumes.

Statistical Method

Experimental design is randomize block design with factorial 2 x 4, as a factor are the type of birth (the level of a single type of birth and type of twinning), and age of the ewe (with the age level I_1, I_2, I_3, and I_4). Tukey test was conducted if further analysis shows the variety of significantly different results.

Variables observed in this research were: (1) Milk yield base on difference body weight of lamb before and after suckling; (2) Type of birth of ewe (single or twin);.(3) Age of ewe.

Procedure

Ewe samples was taken which birth between June 16th until August 31th, 2007. The lamb were left with ewe who giving birth for 4-5 days. This was done to improve the sensor senses of loving ewe to her lamb. In addition, on 4-5 day, ewe left free to give colostrums as antibodies and nutrients that are important for lamb (Owen, 1976). The calculation of the amount of milk yield by the method of the ewe obtained the difference in lamb's body weight (Suckling lamb weight differential technique). This method is done by considering the difference in lambs weight before and after suckling. Lamb who want to calculate the milk yield were fasting for six hours before and then do the weight calculation of the lamb's body weight after suckling. Interval milk calculations were performed on milk yield at 05.00 am, at 11.00 am, 05.00 pm, and 11.00 pm. Calculation of milk yield were conducted twice each week. The method was usually done for the sheep instead of dairy or non-dairy ewe (Doney et al., 1979; Owen, 1976).

After having milk yield data and analyzing those the variables were named: average milk yield (g/ewe/6h), and the shrinkage rate (g/6h), then performed the processing of statistical data based on the design formula that written previously (Pollott and Gootwine , 2004).

RESULTS AND DISCUSSION

Study Site

The research was conducted at the Unit of Education and Research Animal Jonggol (UP3 Jonggol) Faculty of Animal Science, Bogor Agricultural University. UP3 Jonggol area approximately 169 ha consisting of the animal cages and pastures. Pasture conditions is wet damp situation on June, July, and August with a peak in late July and early August. The grass condition is dry (temperature is 33^{o}C, rainfall 8.50-15.90 mm) and water supplies are not adequate. While at the end of August and September had entered rainy (rainfall of 25.20 mm) so that the grass is a lot and grow fast. While the humidity 89.40% -91.70% described the conditions damp shaded land or wet air.

Shepherd the sheep in a meadow in the ranch-ranch overgrown *Brachiaria humidicola*, *Brachiaria decumbens*, *Pennisetum purpuroides*, weed (Eupatorium sp, Melastoma sp, Cantana sp), and legume. Rotation is conducted if the supplies of grass or water were empty. Overgrown area Brachiaria humidicola ± 55 ha, Brachiaria decumbens ±19 ha and Pennisetum purpuroides ± 2 ha. During the research, sheep grazing at 09.00 - 16.00 WIB to avoid bloat or tympanic. It is done because the water content before at 09.00 am and was still very high in the tropics or the equator that can cause bloat or tympanic disease and intestinal worms.

Sheep Milk Yield

Local sheep is a non-dairy sheep (non-dairy ewe) so it has lower than the dairy sheep. In Table 1 shown that the average of milk yield of local sheep was 355.29 ± 72.43 g/animal/day. The milk yield from, local sheep was lower than from the East Friesian dairy sheep (EF) which a minimum of 1,420 ± 0.04 g/animal/day (McKusick *et al*., 2001) and from Priangan Sheep 671.98 ± 4.56 g/animal/day (Pulina and Nudda, 2004; Sumaryadi, 1997). Lower milk yield in local sheep is caused by environmental factors, feed, and the limitation of feed during the dry season. That causes a significant effect on the development secretory cell of glandulla mammae. According to Sumaryadi (1997) and Inounu (1996) the development of secretory cells of the udder glandular accour during pregnant or pre-lactation of ewe, thus the nutrition indispensable enough to produce milk.

Tabel 1. Local sheep milk yield at UP3J on 60 days lactation

Matter	Local Sheep (n = 92 ewe)
Litter size	1.15
Avarage of daily milk yield (g/ewe/days)	355.29 ± 72.43
Declining rate (%)	4.89

Breed differences is one of the effect. The proportion of milk yield on sheep are different in each breeds, which caused by breed differences or genetics (Pulina and Nudda, 2004; Sumaryadi, 1997).

The low production of local sheep's at UP3 Jonggol is caused by the high quality of single births and a lack of superior ewe. Single births will produce less milk than the birth of twins.

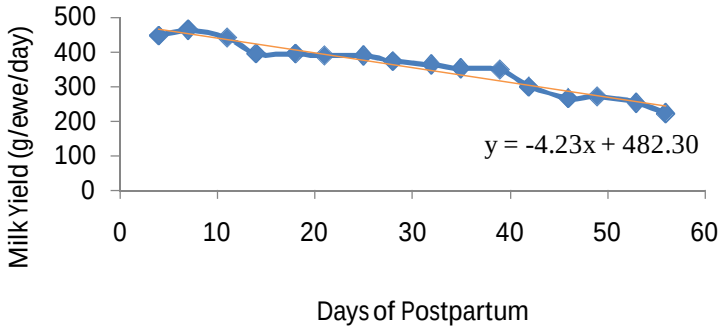


Figure 1. Milk yield curve of Local sheep at UP3 Jonggol.

Twinning will respond by producing enough milk for twins by sekretori cells and hormonal functions, thus twinning milk production will be higher compared to single birth type (Capuco *et al.*, 2003).

Local sheep is relatively higher for 355.29 ± 72.43 g/ewe/day compared with the research conducted by Adriani (1998) using sheep Priangan with oxytocin injection method of producing milk 0.3 µU of 355.13 ± 130.52 g/ewe/day.

Figure 1 showed that milk production likely to decline during the 56 days after birth or postpartum, The declining of milk was 4.89 g/day. This was caused by shrinkage of cells in the lymph sekretori, decreasing laktogenic hormones and increase of connective tissue and the high concentration of collagen (Adriani, 1998; Capuco *et al.*, 2003). This condition can occur by increasing age of the ewe in the lactation period.

Declining milk yield in postpartum was also caused by hormonal functions, namely: decreased levels of the hormone prolactin (Rensis *et al*., 1993). While before the lamb was born the role of hormones such as LH, follicular development, steroid genesis, and FSH (Rensis *et al*., 1993).

FSH and LH contributes indirectly to the yield of milk. FSH and LH are produced gonadrotropin by gonadal cells located in the pituitary gland. The function of FSH is to stimulate follicular maturation, whereas LH help release the ovary from the follicle cells. The relationship of this hormone on milk production is indirect, means of FSH and LH relating to children born. FSH and LH hormone which will produce twins that will increase milk production. FSH and LH levels appropriate to produce more than one child will produce milk higher than single births (Rensis *et al.*, 1993).

Milk production was peak in the first week or at 7th day (464.01 g/day). The highest differences or peak lactation occurs in early lactation (Cardelino and Benson, 2002). In addition, milk yield curve has a linear equation y = 482.30 - 4.23x, while y is milk yield (grams) and x is the time of lactation or suckling (day).

Type of Birth on Milk Yield

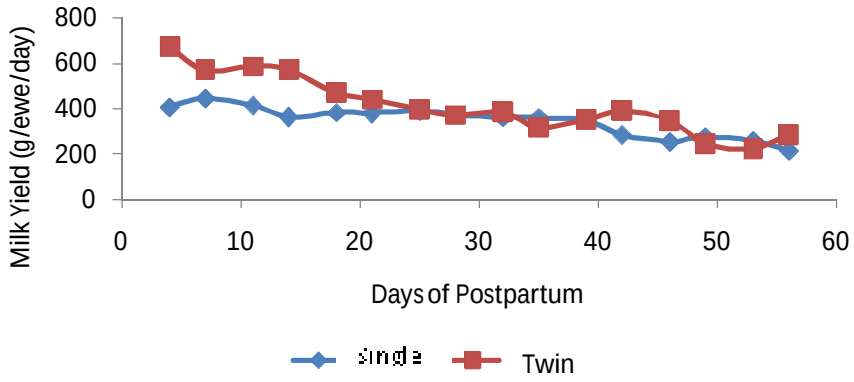
The litter size of local sheep on the UP3 Jonggol was 1.15 where 78 heads were a single type and 14 heads were twins. Sheep with two types of twinning was greater on milk production compared with a single type (16.67%). Average daily milk production of single type was significantly difference with the two types of multiple births (P<0.05). This correspond with Cardelino and Benson (2002), Gonzalo *et al*. (2002), and Adriani (1998) that a single type of birth between the twins affects milk yield.

Physiologically ewe which twins would result higher on milk yield than a single. This is due to the adequacy of food provided for lamb. Thus the growth and development of the secretory cell of udder glands must also higher in the order to provide food for lamb.

Table 2. Milk yield base on type of birth

Birth Type	Avarage of Daily Milk Yield (g/ewe/day)	Sampel (ewe)	CV (%)
Single	344.95 ± 66.22^{a}	78	19.20
Twin	413.99 ± 128.62^{b}	14	31.07

Note: ^{a,b} superscript later show difference (P<0,05).



Thus the growth and development of the secretory cells of the udder glands must also higher in order to provide food for their children (Capuco *et al*, 2003).

By implication, the parent of twin birth rate of depreciation will have a large udder glands so that the end of lactation milk production tend to be lower than single births (Adriani, 1998). It can be seen in Figure 2, on 35 day the twins significantly decreased firstly. Decreasing on milk yield is declining in the last day of lactation (Cardelino and Benson, 2002).

The factors described above in accordance with the opinion of Gonzalo (2002), which states factors that influence litter size of the parent age, time of marriage, weight and condition of the mother, and a genetic influence. The average body weight of the parent between single and twins did not differ significantly (24.36 kg for single and 23.79 kg twin for twin).There is not a factor that directly affects the dominance of a single type of birth and milk production.

The age factor in sheep was no influence on milk production (P <0.05). This correspond with Cardelino and Benson (2002), Gonzalo (2002), and Adriani (1998) that the age of the ewe had effect on milk yield.

Each age class is different and has its own pattern on milk yield. Table 3. and Figure 3, showed that age I_3 has highest milk yield, in contrast the age of I_1 has the lowest milk production (P<0.05). Milk production between the ages of age I_1 and I_2 to I_4 age I_2 did not differ significantly for the total and the average daily milk yield (P<0.05). Even though the age of I_1 to I_3 and I_4 age and I_2 to I_3 significantly different from the average daily

Tabel 3. Milk yield base on age of ewe

Age	Avarage of daily milk yield (g/ewe/day)	Sample (ewe)	CV (%)
I_1	254.53 ± 49.67^{a}	12	19.51
I_2	324.22 ± 55.16^{ab}	21	17.01
I_3	443.43 ± 102.62^{c}	17	23.14
I_4	366.02 ± 85.75^{b}	42	23.43

Note: ^{a,b} Superscript letter shows difference (P<0,05).

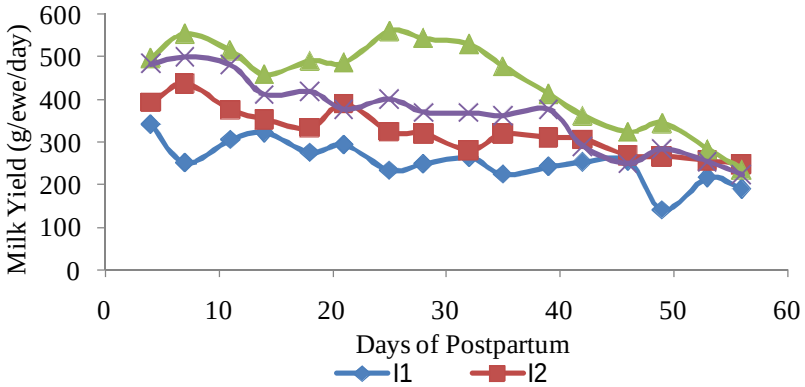


Figure 3. Milk Yield base on age of ewe at UP3 Jonggol

milk yield (P<0.05). Even though the age of I_1 to I_3 and I_4 age and I_2 to I_3 significantly different from the average daily milk yield (P<0.05).

In the Figure 3 showed that in the first week the milk yield was decreased. The reluctance of ewe breastfeed to their lamb is one of causes. The young ewe show less motherhood response. Due to, less experience in caring for and milking. In addition, the maturity of reproductive function has not occurred up. As a result, the milk yield curve looks not so ideal and maximum occurred on the fourth day.

I_2 age group had third ranks on milk yield curve with an average 324.22±55.16 g/ewe/day. Peak yield had occurred in the first week 438.67 g/ewe/day and decreasing after. I_2 looks quite ideal curve, although not too perfect. Increasing in maturity of reproductive function in I_2 compared with age I_1 have appeared in Figure 3.

I_3 age group had been the highest milk among the others 443.43 ± 102.62 g/ewe/day. Peak yield occurred on 25th day 561.46 g/ewe/day. However, I_3 curve have had previous local peak maximum that occurred on the seventh day. After that, the yield decreased drastically. I_3 gradient is the greatest. one because the response of high milk yield before the depreciation of peak rate which make a large causing gland Udder after. Shrinkage is affecting the decline in milk production. Implications of the high yield is a decline in production due to the depreciation rate of large udders glands (Adriani, 1998).

I_4 age group had a second rank after I_3 366.02±85.75 g/ewe/day. Peak yield occurred on the seventh day or the first week of 500.12 g/ewe/day. Then, followed by a decreasing on milk yield. The declining on milk yield is not higher, I_4 slope over a relatively small.

Ewe age factor on milk yield was noted to correlate with reproductive maturity. I_3 have mature on glandula mammae, on other hand the lowest yield of I_1 explaining that the udder gland not really mature during the first year. Ewe who has a maturity of reproductive function is sufficient to produce a healthy child with adequate milk production for their lamb (Capuco et al., 2003; Owen, 1976).

Table 3. explained that type of birth on each level in single births and twins would be a real effect on the age level I_1 and I_4 (P <0.05), whereas no obvious effect on the age level of I_2.

CONCLUSION

Age of ewe and birth types have a real effect on milk yield. The highest yield occurred at the age of I_3 and the lowest at the age I_1. Milk yield on age level had increased overall. Milk yield on the type of twinning is higher than a single type.

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The Level of Mass Media Usage in Cattle Extension Communication Network

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ABSTRACT

This research aim were: (1) to recognize the communication behavior of cattle farmers in searching information, (2) to analyze the level of farmer participation in cattle farmer communication network, (3) to explore the relationship between individual characteristics, (4) to explore interpersonal communication behavior and mass media utilization in cattle farmer communication network. The results showed that: (1) there was a significant difference of communication behavior between the advanced cattle farmers group and the less advanced one. This result informed the distinction of mass media used by cattle farmers in searching information i.e. interpersonal communication relationship in receiving and diffusing information and impersonal communication (media communication) behavior, particularly from television, broadcast and newspapers. (2) There was a significant relationship between formal education characteristic and impersonal communication behavior (television and radio and newspaper). There were significant relationship between economic class and newspaper impact behavior, between mass media ownership and television impact behavior, between education level and mass media ownership with the information search behavior. Individual characteristics of advanced farmers group have negative correlation with the information distribution behavior. Advanced farmer group were characterized by: well educated, higher economic class, more variety of mass media ownership, have more capability to select information according to their needs, profit oriented, risk taker, cosmopolites, have a communication pattern and good relationship among cattle farmer group. All of those characteristics caused un-proper of information diffusion.

Key words: mass media usage, extension communication, cattle farmer

INTRODUCTION

This article deals with the cattle agribusiness extension activities, which were supposed to be a changing in communication structure. The communication pattern usually in the form of "oil droplets"(an effort in extension to diffuse innovation speedily and broadly) extension processes. The extension activity was scheduled from top down, or relying on the visiting and training (so call LAKU). LAKU is a dynamic pattern, integrate of top down and bottom up interest by interpersonal or group communication approach. The intensity of providing extension, the improvement of knowledge and the more experiences from "farmer as partner", will create synergism between advance technology and local traditional technology application. Farmer communication pattern in cattle extension suppose to be fully dependently on interpersonal communication.

Puspadi research (2002) exposed that there were a changing in information requirement according to farming business phases (from less to more commercial). This research intended in changing of communications channel, and the model of extension communications. Furthermore, this research to support Slamet (1995) statement was farmers have changed clearly. Higher level of the farmer education, will be more progressive in farm business, better skill, and better in impersonal communication.

The objectives of this research were (1) to explore communication behavior of cattle farmers in pursuing information, (2) to analyze the relationship between individual characteristics of cattle farmer, interpersonal communication behavior and employing mass media, (3) to develop the communication extension model.

MATERIALS AND METHODS

The respondent consist of 125 cattle farmers come from two developed cattle farmers groups (in Gedangsari District Gunung kidul Regency Yogyakarta, and Polokarto District Sukohardjo Regency Central Java) and two less develop cattle farmers groups

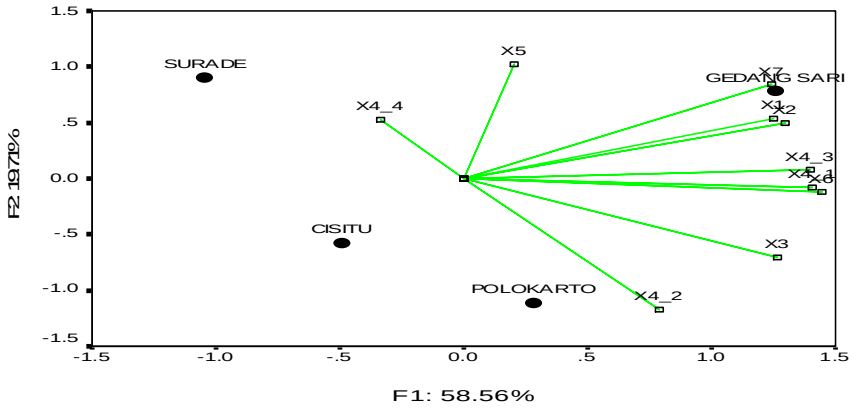
(in Cisitu and Surade District Sukabumi Regency West Java). Data was analyzed descriptively, correlation test of biplot and discriminant function (canonical) analysis (Scheaffer *et al.*, 1992).

RESULTS

Figure 1, explained that in developing a farmer group, the behaviors of searching information, clarification of new information obtained, selective exposure of television and newspaper information were better compare to less developed farmer group. Individual characteristic of farmer from developed group (level of education, economic class and access to mass media) were also better the less developed one. Among the individual characteristics, there was a positive correlation between one characteristic to the other, with high variance.

Performance of farmer group member in Gedangsari (i.e. individual characteristic, behavior for looking for information, behavior for clarify of new information, selective exposure of newspaper, television program, and radio broadcast) were mostly above the average of farmer sample. Performance of farmer group member in Polokarto tends to variety. The behavior for looking for information, and the access to mass media were above the average of farmer sample. While farmer performance in Cisitu was lower. Farmers performance in Surade, although they left behind the farmer from Gedangsari, but their behavior in propagating information were relatively high.

Analysis biplot in Figure 1 informed that 78.27 % of variance can be explained by explanatory data. Interpersonal communication behavior of cattle farmer in propagating information has moderate correlation with behavior to select radio broadcast exposure. The farmer behavior in propagating information, were almost stagnant, either in cattle farmer group of Cisitu and also Surade. Scuttle angle (means distributed scattered in Figure 1) indicated that the value of behavior to select radio broadcast exposure and behavior of propagating the information were above the sample average, with low variance. Farmers in Surade, more intensive in disseminating information of cattle technology compared to other farmer group. Level of radio utilizing and behavior to clarify information of advance cattle farmer group in Gedangsari were higher than farmer group in both Surade and Cisitu. Behavior of radio exploitation by cattle farmer group in Polokarto was below the value of sample average (include Gedangsari, Cisitu and Surade. While behavior of communications to disseminate information of farmer in Gedangsari were lower compared to sample averages. Figure 1, show that mostly (cattle owner) farmer were lower intensity in reading newspaper. It was concluded that reading newspaper was less related to cattle ownership.



Notes:

X1 = formal education	X4_3 = information clarified behavior
X2 = economic class	X4_4 = information distribution behavior
X3 = mass media ownership	X5 = radio impact behavior
X4_1 = information accept behavior	X6 = television impact behavior
X4_2 = information search behavior	X7 = newspaper impact behavior

Figure 1. SPSS Output of bi-plot analysis between personal characteristics relationship, interpersonal communication behavior and the usage of mass media at cattle farmer

Behavior of interpersonal communication in receiving cattle rearing information and behavior in disseminating information in Figure 1, explain that behavior of informal interpersonal communications of less advance farmer group is higher than the advance one. Farmer behavior in obtaining information has a strong correlation with behavior to clarify information or discuss it in the group and with behavior of television exposure.

While behavior of interpersonal communication in searching information and clarify/discuss information of advance cattle farmer usually done with people from outside the country or the group. According to Rogers (2003), this behavior is using cosmopolitan channel.

The discriminate function coefficient (canonical) of 10 observation variables indicate the differences characteristic between advance farmers group and less advance farmer group. In advance group, there are positive correlation between high education (X_1) to activity in searching the cattle rearing information (X_{4_2}), and between level of economic class (X_2) to behavior of selective television exposure (X_6) and newspaper (X_7). The discriminate function canonical be $Y = 0.617\ X_1 + 0.581\ X_{4\_2} + 0.502\ X_2 + 0.440\ X_6 + 0.372\ X_7$.

This relationship explains that spreading information of technical cattle rearing required both “interpersonal" and "impersonal," extension communications in order to "fulfill the farmer requirement of technical cattle rearing information”.

Above phenomenon indicates that there were behavior frictions from personal communications to impersonal communication or media communication. More advance of farmer group, will be wider in both television and newspaper media exposure. While behavior of listening radio, less advance farmer group will be more intensive than the advance one. For most rural community, radio is a popular media for entertainment amusement and information sources about development news. In recent year, there are many radio stations, operate by local government, private, NGO and also by college. According to Schramm (Depari and MacAndrews, 1998), almost all societies (rich or poor societies) in developing countries, such as Asia (including Indonesia), African and Latin America, have a radio as an information source. The difference is the quality of radio. Rich man has a set sophisticated stereo radio, while the poor has a small transistor radio. Now day "internet radio" becomes a popular media for pubic communication.

Those difference communications behavior between advance farmer group and the less advanced group means that "there is a friction in exploiting level of mass media by cattle farmer in searching the information". Especially friction of communication pattern, from interpersonal communications (in receiving and propagates information) to impersonal communications (mostly television and newspaper). Cattle farmer more interest in selective exposure such as news, entertainment and infotainment (sport, film/series-film). There is no media provide information needed by farmers, including information of cattle rearing technology. Higher level of mass media usage by farmer will increase farmer knowledge, curiosity and awareness.

The research results also prove that there were significant relationships between: (1) formal education level with television and radio impact behavior, (2) formal education level with newspaper impact behavior, (3) economic class with newspaper impact behavior, (4) mass media ownership with television impact behavior, (5) education level and mass media ownership with behavior for searching information. In the advanced farmers group, individual characteristics have negative correlation with the information distribution behavior. The advanced farmer group characteristics were: well educated, higher economic class, more variety in mass media ownership, more capable to select information according to their needs, profit oriented, risk taker, cosmopolites, Importantly, they have a common friendly communication pattern. Some characteristics caused the information distributed stagnantly. In globalization era, as supporting facilities, the role of mass media also for educating farmer, beside as entertainment amusement and information sources (Mulyana, 2005; McQuail, 2006, Jahi, 1993). Previously, mass media are exploited only for entertainment, then for both entertainment and news. Actually mass media also can be use as supporting facilities for education (such as agricultural extension), but it was not. Therefore, cattle farmer search information from other source, including in-

terpersonal communication network. Those sources were: farmer organization, informal leader, and farm supplier agencies.

To solve the problems of extension workers and operation cost, need to entangle local institution (social capital) in implementation of agricultural extension communications program. This particular program should be continued.

By integrating social capital and management of extension communications could more productive in national development as well as in rural area, to solve their problems.

On the other side, farmer experience and communications skill, could support the dynamic communication process, individually as well as group. This situation created a model to facilitate mutual understanding of extension message (Schramm & Roberts 1974). Furtherly,Rogers and Kincaid (1981) described as a convergence model of communication.

Sumardjo research (1999) concluded that to increase farmer ability, dialogue (dyadic) and convergent extension approaches are more effective than centralized/top down linear communications model.

Research results offered several suggestion for developing communication strategy in cattle farming extension: (i) farmer individual characteristics, (ii) messages distortion and unavailability of information (including marketing, price, appropriate technology needs, farmer capacity, and access to capital), (iii) bureaucratic involved (such as: social institution as well as extension, technology producer and capital accessibility), (iv) involving opinion leader and others pertinent information source in delivering information. Extension communication techniques to be considered are (i) extension campaign continuously, (ii) utilizing traditional media and social learning through mass media interactive and multi directions; increase communication group network through enhance cattle farmer institutions. (iii) Securing participation based on local social culture.

CONCLUSION

There was a significant distinction in the communication behavior between the advanced cattle farmers group and the less advanced group. This difference indicates the communication behavior of farmer in usage of mass media for searching the information. The least level was interpersonal communication for receiving and diffusing information. The advance level was through media communication, particularly television broadcast and newspapers. The communication behavior of two cattle farmers group members have changed from interpersonal communication to impersonal communication (through radio and television). However, the farmer reason in using of mass media (listening the radio or watching television) dominantly for explores news and entertainment. For technical information, farmer still rely on communication network. Thus to get mutual understanding of extension message, the relevant communications models are dyadic and convergence.

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Feeding Fermented *Jatropha curcas L.* Meal Supplemented with Cellulase and Phytase to Kampong Chicken

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ABSTRACT

Fermented *Jatropha curcas* meal using *Rhizopus oryzae* could decrease the fat content in the meal (5.8% Vs 0.39) and eliminated trypsin inhibitor up to 67.95 %. The decreasing of fat content indicated the elimination of the main toxic substance contained in the meal, i.e. *phorbolesters.* Most of the *phorbolesters* could be extracted with the oil fraction of the *Jatropha curcas* meal. Hopefully, this treatment could destroy the toxic jatropha curcas meal to a high quality meal as poultry feed. However, the fiber and phytate content in the meal were still high. This experiment was conducted to study the effects of using fermented *Jatropha curcas* meal treated with cellulase and phytase in the kampong chicken diet as to increase the growth and decrease the mortality rate. Two hundred kampong chickens were used in this experiment and reared from day old up to 10 weeks of age. The data analyzed with a Completely Randomized Design with 5 treatment diets and 4 replications, with 10 birds in each replicate. The experimental diets were: T0 (the control diet, without *Jatropha curcas* meal), T1 (the diet contained 5% untreated *Jatropha curcas* meal), T2 (the diet contained 5% fermented *Jatropha curcas* meal + cellulase 200 ml/ton of feed), T3 (the diet contained 5% fermented *Jatropha curcas* meal + 1000 FTU phytase), and T4 (the diet contained 5% fermented *Jatropha curcas* meal + cellulase 200 ml/ton + 1000 FTU phytase). The parameters observed were feed consumption, body weight gain, final body weight, feed conversion ratio, and mortality rate. The results showed that there were no significant difference on the parameters observed due to the treatments. However, feeding untreated *Jatropha curcas* meal in the diets (T1) decreased the body weight gain approximately 10.52% and the final body weight approximately 10.13% as compared to that of the control (T0). Feeding fermented Jatropha curcas meal supplemented with cellulase + phytase(T4) yielded the final body weight and feed conversion ratio similar to those the control (T0) diet. The final body weight of the chickens fed T0, T1, T2, T3 and T4 were 955.08 g/bird, 858.33 g/bird, 872 g/bird, 935 g/bird, and 951.25 g/bird, respectively. The feed conversion ratio of the chickens fed T0, T1, T2, T3 and T4 were 2.93, 3.51, 3.49, 3.20, and 2.89, respectively. The the feed consumption per bird during 10 weeks period of experiment 2567.53 g, 2663.76 g, 2752.32 g, 2685.05g, and 2520.5 g, for chickens fed T0, T1, T2, T3 and T4 respectively. There was no mortality observed in all treatments.

Key words: fermented Jatropha curcas meal, growth, mortality, kampong chicken

INTRODUCTION

Jatropha curcas (physic nut or purging nut) is a drought-resistant shrub or tree belonging to the Family *Euphorbiaceae*, which is cultivated in Central and South America, South-East Asia, India and Africa (Schmook and Seralta-Peraza, 1997). The seeds of physic nut are a good source of oil, which can be used as a diesel substitute (Becker and Makkar, 1998). The increasing of *Jatropha curcas* cultivation as raw material of biodiesel in Indonesia leads to increase *Jatropha curcas* meal as byproduct. Besides being a source of oil, *Jatropha curcas* also provides a meal which may serve as a highly nutritious protein suplement in animal feed if the toxins and antinutrients present in the meal are removed. The meal has high trypsin inhibitor and lectin activities, which could be inactivated by heat treatment. In addition, high concentration of antimetabolic, metal-chelating and heat-stable factor, phytic acid, has been reported in *Jatropha curcas* meal (Makkar *et al*., 1998). 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 (Su-

miati *et al*., 2007). Feeding *Jatropha curcas* meal at the level of 5% in the diet to the broilers reduced feed consumption, caused 100% mortality at the age of 22 days and it damaged the liver as well as kidney (Sumiati *et al*., 2007)

Martinez-Herrera *et al*. (2006) used different treatments to decrease or neutralize the antinutrients present in the meal. Trypsin inhibitors were easily inactivated with moist heating at 121^{o}C for 25 min. Extraction with ethanol, followed by treatment with 0.07%$NaHCO_3$ considerably decreased lectin activity. The same treatment also decreased the phorbolester content by 97.9% in seeds. Sumiati *et al*. (2007) conducted various treatments (physical, combination of chemical + physical, and biological) to detoxify Indonesian *Jatropha curcas* meal as poultry feed. The treatments used in this experiment were: (1) heat treatment using autoclave at 121^{o}C during 30 min.; (2) adding NaOH 4%, followed by autoclaving at 121^{o}C during 30 min.; (3) fermentation using *Rhizopus oligosporus*. The results of this experiment showed that all treatments decreased the curcin or lectin activities, Increased protein utilization efficiency, retention of calcium and phosphorus, and increased metabolizable energy values of meal. 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.

Sumiati *et al*. (2008) fermented Indonesian *Jatropha curcas* meal using *Rhizopus oryzae* and it could decrease the fat content in the meal (5.8% Vs 0.39) and eliminated trypsin inhibitors up to 67.95 %. The decreasing of fat content indicated the eliminating of the main toxic substance content in the meal, i.e. *phorbolesters*. Most of the *phorbolesters* could be extracted with the oil fraction of the *Jatropha curcas* meal. Hopefully, this treatment could destroy the toxic jatropha curcas meal to a high quality meal as poultry feed. However, the fiber and phytic acid content in 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. Pyitic acid is also known to form complexes with protein and proteolytic enzymes(pepsin and trypsin). Because of the lack of endogenous phytase enzymes that hydrolyze phytic acid: phosphorus, calcium, protein and other phytic acid bound nutrients are less available to poultry. This experiment was conducted to study the effects of using fermented *Jatropha curcas* meal using *Rhizopus oryzae* supplemented with cellulase and phytase in the kampong chicken diets on the growth and mortality rate.

MATERIALS AND METHODS

Jatropha curcas Meal Sample

Jatropha curcas meal sample was obtained from Surfactant and Bioenergy Research Center, Bogor Agricultural University. Chemical composition of the sample was analyzed at the Faculty of Animal Science, Bogor Agricultural University (Table 1).

Table 1. Chemical composition of untreated and fermented *Jatropha curcas* meal*

Component	Untreated *J. curcas*	Fermented *J. curcas*
Dry matter, %	84.99	94,01
Ash, %	5.63	5,95
CP, %	24.71	22,39
EE, %	5.8	0,39
CF, %	32.58	44,22
NFE, %	16.27	21,06
Ca, %	1.00	0,68
P, %	0.99	0,35
GE, kcal/kg	3893	3984
Pytic acid, %**	10.18	7,45

*The nutrients were analyzed at the Laboratory of Feed Science and Technology, Faculty of Animal Science, Bogor Agricultural University;
** Phytic acid was analyzed at the Animal Research Institute, Bogor, Indonesia.

Fermentation Procedures

In this experiment, the culture that usually used to ferment soybean in Indonesia to make a food called tempe, was used as source of *Rhizopus oryzae*. This culture was used to ferment *Jatropha curcas* meal. The procedure of *Jatropha curcas* meal fermentation can be seen on Figure 1.

Feeding Trial Using Kampong Chickens

Two hundred kampong chickens were used in this experiment and reared from day old up to 10 weeks of age. A completely Randomized design with 5 treatment diets

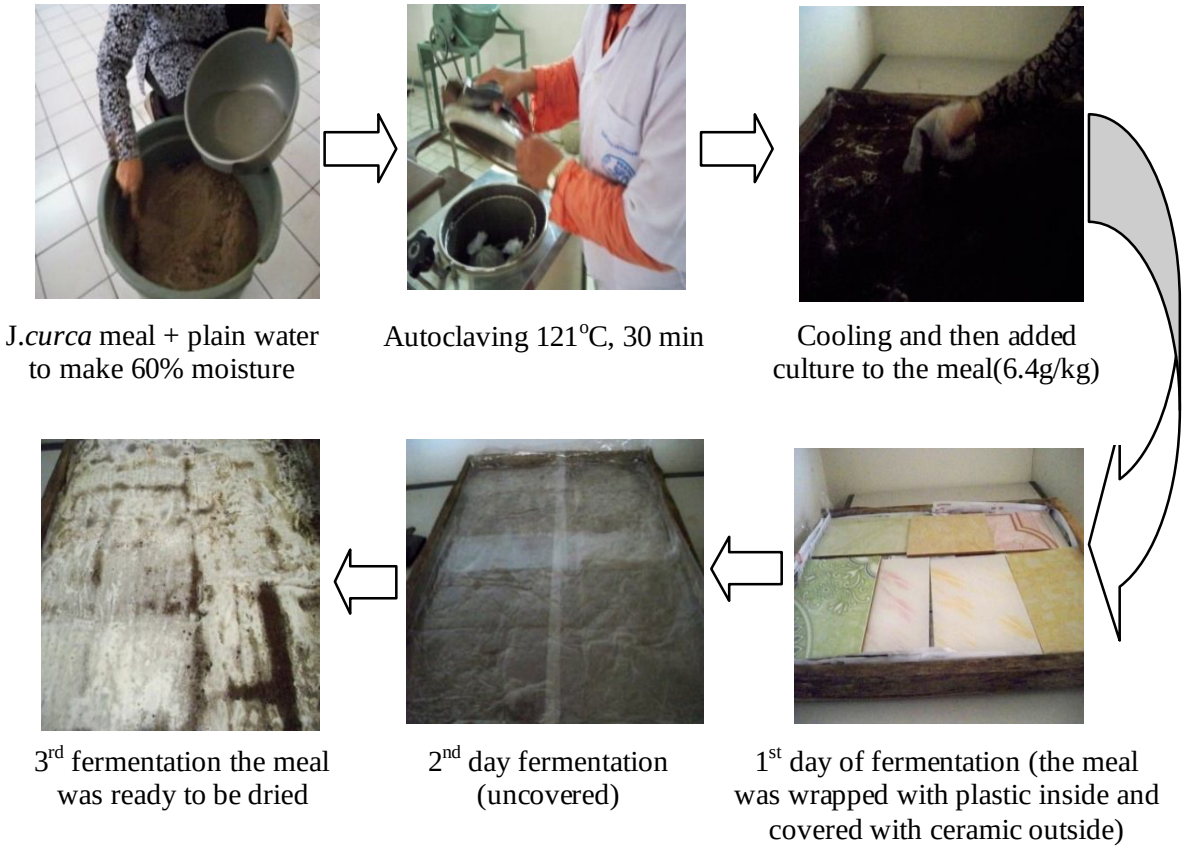

Figure 1. The procedure of *Jatropha curcas* meal fermentation

and 4 replications, with 10 birds in each replicate was used in this experiment.

The experimental diets were: T0 (the control diet, without *Jatropha curcas* meal), T1 (the diet contained 5% untreated *Jatropha curcas* meal), T2 (the diet contained 5% fermented *Jatropha curcas* meal + cellulase 200 ml/ton of feed), T3 (the diet contained 5% fermented *Jatropha curcas* meal + 1000 FTU phytase), and T4 (the diet contained 5% fermented *Jatropha curcas* meal + cellulase 200 ml/ton + 1000 FTU phytase). The composition of experimental diets is presented on Table 2. The experimental diets were fed to 2 weeks old up to 10 weeks old in order to minimize the mortality. During the two weeks of the experiment (0- 2 weeks of age), the chicks were fed commercial diets.

The parameters observed were feed consumption, body weight gain, final body weight, feed conversion ratio, and mortality rate. The data were analyzed using analyses of variance according to Steel and Torrie (1995).

RESULTS AND DISCUSSION

The Effect of Treatments on Feed Consumption

The average of feed consumption of kampong chickens in this experiment is presented on Table 3. Feeding diets contained *J.curcas* (T0, T1, T2, T3, T4) did not affect the feed consumption. It showed that feeding 5% untreated as well as fermented *J.curcas* did not influence the feed consumption, and thus it indicated that the meal used in this experiment was from *J.curcas* seed contained low phorbolesters. Generally, the presence of phorbolesters in feed has significant effect on its acceptance (Aregheore *et al*., 2003). Sumiati *et al*. (2007) reported that feeding 5% untreated *J.curcas* meal highly significantly ($P<0.01$) reduced feed consumption of broilers. 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.

Table 2. The composition of the experimental diets

Ingredient	Treatment				
	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	5.00	0	0	0
Fermented *J.curcas* meal	0	0	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
Cellulase, ml/ton			200	0	200
Phytase, FTU/kg[1)]			0	100	100
Calculated composition[2)]					
ME, kcal/kg	2855.64	2862.71	2865.11	2865.11	2865.11
CP, %	18.23	18.39	18.26	18.26	18,20
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).

Table 3. The average feed consumption of kampong chicken during 10 weeks of experiment (0-10 weeks of age) (g/bird)

Replication	Treatment				
	T0	T1	T2	T3	T4
1	2760.31	2430.12	2769.88	2396.72	2522.78
2	2693.89	2791.88	2769.88	2801.55	2513.04
3	2411.07	2.695.62	2.743.38	2792.74	2264.2
4	2404.83	2737.42	2726.13	2749.19	2781.99
Average	2567.53	2663.76	2752.32	2685.05	2520.50
SD	186.26	160.67	21.47	193.58	211.45

The Effect of Treatments on Body Weight Gain

Table 4. The average body weight gain of kampong chicken during 10 weeks of experiment (0 - 10 weeks of age) (g/bird)

Replication	Treatment				
	T0	T1	T2	T3	T4
1	877.80	920.33	802.97	651.93	942.47
2	984.10	613.47	812.77	1001.87	918.53
3	973.43	855.20	944.50	1016.10	930.60
4	842.10	906.53	791.70	934.83	875.40
Average	919.36	823.88	837.99	901.18	916.75
SD	70.26	143.05	71.53	169.90	29,25

These results indicated that the supplementation of cellulase in the diet containing *J.curcas* meal had a little effect on the growth of kampong chicken. It could be due to high fiber and lignin content in the meal, and thus the cellulase with concentration of 200 ml/ton feed was not effective to break down the fiber. Sumiati *et al*. (2008) reported that fermented J.*curcas* meal used in this experiment contained 44.22 % fiber and 25.8% lignin.

Phytase supplementation in the fermented *J.curcas* meal diet seemed to be effective in degrading the phytate contained in the meal. Phytase is an enzyme which hydrolyses phytic acid to inositol and inorganic phosphorus, leading to improve phosphorus

utilization and overall performance of broilers (Singh *et al*., 2003b). Supplementation of cellulase and phytase in the fermented J.*curcas* meal diet (T4) gave more body weight gain as compared to that of a single enzyme supplementation (T2 and T3).

The Effect of Treatments on Feed Conversion Ratio

The average of feed conversion ratio of kampong chickens in this experiment is presented in Table 5. Feeding 5% untreated *J.curcas* meal (T1) reduced the feed efficiency with the value of 19.8% as compare to that of the control diet (T0/without *J.curcas* meal in the diet). Supplementation of cellulase did not seem to be effective in increasing feed efficiency. However, phytase supplementation in the diet (T3) increased feed efficiency 8.83%, while the supplementation of cellulase and phytase in the diet (T4) yielded the highest feed efficiency, i.e. 17.66%. These results showed that the supplementation of cellulose and phytase enzymes gave higher effect on feed efficiency as compared to that of a single enzyme supplementation.

The Effect of Treatments on Final Body Weight

Feeding 5% untreated J.*curcas* meal (T1) decrease the final body weight 10.14% as compared to that of the control diet (T0/without J.*curcas* in the diet). The supplementation of the enzymes to the diets contained fermented J.*curcas* meal tended to raise final body weight of kampong chicken. Supplementation cellulose and phytase in the diet (T4) yielded the final body weight similar to that of the control diet (T0). This data indicated that phytase was effective to degrade phytic acid content in the meal. There were several studies which indicated that microbial phytase supplementation increases body weight gain, feed intake and feed efficiency in broiler chikhens (Singh and Khatta, 2002; Singh *et al*., 2003a). A significant improvement in the growth performance of broiler chickens, as a result of phytase supplementation, were reported by karim (2006), Pillai *et al*. (2006), Singh and Sikka (2006) and Selle *et al*. (2007).

The Effect of Treatments on Mortality Rate

There was no mortality due to the treatments found in this experiment, although feeding untreated J.*curcas* meal (T1) retarded the growth 10.13% as compared to that of the control diet (T0). These results indicated that using 5% J.*curcas* meal in the diet was not toxic to the kampong chickens, and phorbolester found in the J.*curcas* meal used in this experiment was low.

CONCLUSION

Feeding 5% untreated as well as fermented *Jatropha curcas* meal in the diets were safe to the kampong chickens. Supplementation of cocktail enzymes (cellulose 200

Table 5. The average feed conversion ratio of kampong chicken during 10 weeks of experiment (0-10 weeks of age) (g/bird)

Replication	Treatment				
	T0	T1	T2	T3	T4
1	3.29	2.76	3.64	3.92	2.81
2	2.86	4.88	3.68	2.93	2.88
3	2.59	3.30	3.01	2.87	2.54
4	2.99	3.12	3.63	3.09	3.33
Average	2.93[a]	3.51[a]	3.49[a]	3.2[a]	2.89[a]
SD	0.29	0.93	0.32	0.48	0.32

Table 6. The average final body weight of kampong chicken at 10 weeks of age (g/bird)

Replication	Treatment				
	T0	T1	T2	T3	T4
1	914.00	953.33	953.33	688.33	976.67
2	1018.00	646.67	846.67	1036.67	953.33
3	1008.33	890.00	980.00	1050.00	965.00
4	880.00	943.33	826.00	968.33	910.00
Average	955.08[a]	858.33[a]	872.34[a]	935.83[a]	951.25[a]
SD	68.6	143.82	72.27	168.84	29.10

ml/ton+ phytase 1000 FTU/kg) yielded the best performances of growth and feed efficiency of kampong chickens.

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Productivity of *Brachiaria humidicola* as Results of Different Nutrient Source Application

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ABSTRACT

In many tropical pastures invasive weed like *Chromolaena odorata* becomes a serious species with no redeeming feature and causes poor and low calving rates of local cattle. Utilization of *C. odorata* biomass as organic nutrient source may be an alternative management to eliminate the distribution of the weed and improve pasture productivity. A field study in mini pastures was conducted to recognize annual forage production (AFP), carrying capacity, N and P Uptake, and protein production of *Brachiaria humidicola* (signal grass) grown on soil amended with *C. odorata* biomass and feces as organic nutrient source. Block Randomized Design consisting of: no treatment (blank control = P0); 7.2 kg plot^{-1} of *C. odorata* (PC); 21 kg plot^{-1} of manure (PF); combination of *C. odorata* (3.6 kg plot^{-1}) and manure (10,5 kg plot^{-1}) (PCF) and inorganic fertilizer (573.3 g urea plot^{-1} and 217 g super phosphate plot^{-1} (positive control=PA), with 4 replications. Carrying capacity was calculated according to simulation of accumulate grass production throughout the year. Dried herbage was use to determine forage production, N and P uptake. Protein production was calculated from N concentration multiplied by 6.25. The results showed that PC improved ($p<0.01$) AFP about 225% and 110% as compared to P0 and PF, respectively. PC and PF are able to substitute inorganic fertilizer about 60% and 50%, respectively in resulting similar AFP as compared with those of PA. PC and both PF and PCF increased ($p<0.05$) carrying capacity of the pasture up to 1.7 and 1.3 Animal Unit, respectively as compared with P0. PC, PF and PCF produced higher protein production ($p<0.05$) than P0, and substituted to inorganic fertilizer by 46%, 40% and 49%, respectively.

Key words: Brachiaria humidicola, Chromolaena odorata, manure

INTRODUCTION

High intensity of pasture use due to intensive animal rearing for replacement stock leads to soil and nutrient degradation. In many pasture area in Indonesia, most of degraded pasturelands are invaded by invasive weed species like *Chromolaena odorata*. It is a perennial species and has other name *Eupatorium odoratum* L., *E. Conyzoides* Vahl and *Osmia odorata* (L.) Schultz-Bip (Hanum and Maesen, 1997). In Indonesia it is known with name Kirinyu or Babanjaran (Tjitrosoedirdjo *et al*., 2002 and Sipayung *et al*., 2002). *Chromolaena* grows very aggressive and has ability to intensive sprouting that can change of botanical composition, reduce of pasture quality and cause toxic to animals. Our previous study records that reduction of pasture area due to *Chromolaena* invasion ranged 8-15% a year depending on grazing intensity. It becomes a serious species with no redeeming feature and causes poor and low calving rates of local cattle.

To maintan pastureland and eradicate *Chromolaena*, an alternative management has been studied in this experiment to utilize its biomass as mulch material, rather than eradicating with chemical agent (because it is harmful to animals). From chemical composition view point of *Chromolaena* indicated high quality of mulch material, because rate of decomposition and nutrient mineralization is affected by both nutrient content and chemical composition of the plant material (Abdullah, 2001; Bossuyt *et al.* (2001); Breland (1997). *Chromolaena* has a relatively high quality as compared to other weed species. It's leaves *have* a lower C/N ratio (25.8%) and C/P ratio (395), lower lignin (13.1%), ADF (53.3%) and cellulose (40.2%) content than common pastoral weed species (Abdullah, 2001).

Base on its chemical composition, it is expected that amendment of *Chromolaena* as mulch material (organic nutrient) can supply nutrient into soil slowly, through decomposi-

tion process and mineralization. The presence of fungi and bacteria in soil cause the major chemical transformations in decomposition, e.g. the degradation of polysaccharide complexes of plant litter to carbon dioxide, and mineralization of protein to ammonium and nitrogen and organic phosphorus to inorganic P. The soil microorganism activities in soil during decomposition are strongly influenced by soil moisture content (Taylor *et al*., 1999), litter type and fertilizer (Donnison *et al*., 2000), and temperature (Grisi *et al*., 1998)

The objective of the experiment were to recognize the effect of *Chromolaena* biomass compared with conventional fertilizer (manure and inorganic fertilizer) on *Brachiaria humidicola* (signal grass) growth, dry matter production and calculated carrying capacity, and to investigate the contribution of *Chromolaena* amendment ability to compensate inorganic fertilizer.

MATERIALS AND METHODS

Time and Location

The field experiment was conducted in 2004 in research station of Grassland Science Division, Faculty of Animal Science, Bogor Agricultural University (IPB). The site has two seasons, the rainy season with seven to eight consecutive wet months and the dry season up to four consecutive dry months. The average monthly rainfall during experiment was 364 mm month^{-1} (4375 mm year^{-1}). The highest rainfall intensity was on April with 639 mm (25 days) and the lowest rainfall intensity was on June (169 mm, 8 days) as reported by Abdullah (2009).

Signal Grass Plots

Experimental plots were formerly used by Abdullah (2009) for one year before current experiment. There was no agronomical treatment before the plots used in the experiment. Signal grass had well established and produced 594.9 g/plot/harvest or 2092 g/plot/year (3487 kg/ha/year). The plots were made by marking each border of plots, and separated with bare area (50 cm). Before application of fertilizer, signal grass was trimmed to avoid physiological effect of grass individual.

Mulch Material and Manure Preparation

Chromolaena biomass (before flowering stage) collection was according to Abdullah (2009), originated from pasture area that invaded grazing area of university grassland. The collected biomass was chopped with 10 cm length before application. The amount of biomass as mulch material (consisting of all above ground biomass) applied on each plot was 12 ton fresh weight/ha (3 folds of potential biomass production of *Chromolaena*. This amount of applied biomass contributed to 398 kg N/ha and 186 kg P/ha. According to laboratory analysis, nitrogen and phosphorus content of *Chromolaena* biomass were 3.32% and 0.16%, respectively. Based on those calculations, the amount of biomass applied on each plot was 7.2 kg fresh weight/plot

Nitrogen and phosphorus content of manure were analyzed before application. The N and P content of the manure were 1.13% and 0.37% (Abdullah, 2009). Manure was collected from university farm. The manure originated from manure produced by 1.5 animal units (this animal numbers was accordingly the carrying capacity of signal grass pasture each ha/year). The amount of applied manure was 4 folds of manure production (54,750 kg fresh weight/ha or 21 kg fresh weight/ha). This amount of manure contributed to about 618 kg N/ha and 130 kg P/ha. Based on this calculation, for combination treatment, the amount of both *Chromolaena* and manure were a half of single treatment dosage 3.6 kg/plot and *Chromolaena* 10.5 kg/plot.

The Inorganic fertilizers used in the experiment were urea (45% N) and SP-36 (36% P_2O_5). The dosage of applied urea and SP-36 was based on N and P supply contributed from both *Chromolaena* and manure i.e.: 895.6 kg urea/ha and 361.1 kg SP-36/ha (537.3 g/plot and 217 g/plot, respectively).

Mulch and Fertilizer Application

Mulch and manure were applied (5 cm thick) directly on the top of trimmed signal grass according to experimental design. Urea and SP-36 were applied using broadcast method on trimmed grass surface. A half dosage was applied at the beginning of experiment and other half dosage was applied prior to dry season.

Parameters Observations

During growing, signal grass was taken care. Some parameters including number and length of stolon (primer and secondary stolon), tiller number and tillering rate were

measured every two weeks. To enable measuring stolon length and counting tiller numbers at certain grass individual, observed individual of grasses were marked by using different color of pennant. Primer stolons were main stolon that grew directly from main crown and produced tillers (daughter tiller) at their nodes. Secondary stolons were branches of stolon that grew from daughter tillers and produced grand daughter tillers from their nodes Tiller numbers consisting of daughter and grand daughter tillers were calculated from plots every two weeks. Tillering rate was ratio of grand daughter tiller number to daughter tiller number resulted within a week.

Dry matter production was investigated every harvesting time. Sample of dry forage was analyzed to recognize the N and P content of the forage. Forage was harvest every 60 days, and dry matter production was accumulated according to seasons (dry season and rainy season).

Experimental Design

The fertilizer sources, which were used as treatments, originated from *Chromolaena* biomass, manure (cow manure) and inorganic N and P fertilizer (Urea and SP-36). The experimental design used in this study was block randomized design, consisting of : PO = blank control, P*C* = *Chromolaena* biomass (7,2 kg fresh weight/plot), PF = manure (21 kg fresh weight/plot), P*C*+F = combination of *Chromolaena* biomass (3,6 kg fresh weight/plot) and manure (10,5 kg fresh weight/plot), Pi = inorganic fertilizer (urea 537,3 g/plot and SP-36 217 g/plot) as an positive control. Each treatment was repeated 4 times, so that the number of experimental plots was 20 plots. Collected data were analyzed using analyses of variance, and significant differences of average data on each treatment were tested with orthogonal contrast (Steel and Torrie, 1991), and compared with blank control and positive control.

RESULTS AND DISCUSSION

Effect of Fertilizer on Stolon and Till Growth

Application of *Chromolaena* as mulch material and manure as single treatment and their combinations significantly increased primer stolon number ($P<0,01$), secondary stolon number, daughter tiller and length of stolon, length rate of primer stolon ($p<0.05$) as compared with blank control (Table 1). However fertilizer application did not significantly affect grand daughter tiller number, tillering rate and length of secondary stolon. Application of either *Chromolaena* or manure as single treatment and their combination significantly doubled primer stolon comparable to blank control. However, combination of both *Chromolaena* and manure resulted in lower stolon number than those of plot applied with *Chromolaena* or manure as single nutrient source. Different from primer stolon, application of *Chromolaena* and manure did not affect secondary stolon number, but application of inorganic fertilizer resulted in highest stolon number.

Application of *Chromolaena* or manure,

Table 1. Effect of different nutrient sources on stolon and tiller growth of signal grass

Parameters	Fertilizer application				
	PO	P*C*	PF	P*C*+F	Pi
Primer stolon number (stolon/plot)	2.3^{d}	5.5^{b}	5.2^{b}	4.9^{c}	13.7^{a}
Secondary stolon number (stolon/plot)	0^{b}	1^{b}	0.8^{b}	0.3^{b}	3^{a}
Daughter tiller number (tillers/plot)	0.75^{b}	8.62^{a}	$9,28^{a}$	8.97^{a}	7.59^{a}
Grand daughter tiller no. (tillers/plot)	0	1.75	0.77	0.25	0.74
Tillering Rate (no./week)	0	0.18	0.07	0.02	0.22
Length of stolon (cm/week)	5.8^{c}	20.3^{a}	17.2^{a}	14.7^{b}	20.3^{a}
Length rate of primer stolon (cm/week)	0^{c}	7.5^{a}	3.2^{b}	3.3^{b}	6.9^{a}
Length rate of secondary stolon (cm/week)	2.07	2.53	2.61	2.71	2.70

Note: PO = blank control, PC = *Chromolaena* amendment, PF = manure application, PC+F = combination of Chromolaena and manure, Pi = inorganic fertilizer.

And their combination resulted in significant higher tiller number of daughter (8-9 folds)and length of stolon as compared with control, but has no different tiller number of daughter as compared with inorganic fertilizer (Table 1). However combination of *Chromolaena* and manure resulted in lower length of stolon than those of other three fertilizer application. *Chromolaena* and manure application was able to substitute 35%-39% the use of inorganic fertilizer to result in a same number of grass stolon.

Forage Production

As depicted in Table 2, amendment of *Chromolaena*, manure and their combination as nutrient sources for the grass improved respectively about 139% and 100% of average DM production during rainy season. Application of inorganic fertilizer resulted in the highest average DM production. It is shown in Table 2, that *Chromolaena* application resulted in higher DM production than those of either manure or combined fertilizer.

During dry season, grass production reduced drastically, in particular grasses that grew on the plots fertilized with *Chromolaena* biomass and inorganic fertilizer. It may be caused that *Chromolaena* and inorganic fertilizer can supply faster inorganic nutrient than manure and combined fertilizer. This was approved by research results of our previous study finding that *Chromolaena* was the fastest degradable materials and very fast recovery of mineralization, that indicate fast release and fast supply of organic nutrients.

Application of *Chromolaena* resulted in higher DM production ($p<0.05$), as well as combination of Chromolaena and manure. Application of manure as single fertilizer led to produce higher grass production than those of *Chromolaena* or combined nutrient sources. Application of inorganic nutrient resulted in the highest grass production.

Cumulative grass production was significantly influenced ($p<0.01$) by *Chromolaena* amendment, manure application and inorganic fertilizer. Application of *Chromolaena* doubled cumulative grass production, and manure and combined fertilizer application as well. Application of inorganic fertilizer resulted in the highest cumulative DM production of signal grass. Application of organic nutrient sources can only reach a half of grass production comparable to inorganic nutrient sources.

Carrying capacity is the capability of area that can supply forage for animals throughout a year without causing destruction of the pasture area. The carrying capacity was calculated and based on cumulative dry matter production of the grass and converted to one ha. It is assumed that animal consumes 6.29 kg DM of grass/day/head (Indonesian condition). Application of organic nutrient sources originating from *Chromolaena*, manure and their combination significantly increased 1.3-1.7 animal units. Application of inorganic fertilizer increased carrying capacity about 3.7 animal units.

Amendment of *Chromolaena* biomass into soil improves nutrient supply 32 days after application (Abdullah, 2002). Our results confirmed this finding, and showed that amendment of *Chromolaena* biomass at the beginning of application led to immobilization of released nutrient by soil microorganisms. This was indicated by yellow leaves of signal grass at the beginning of application, but then the grass grew better and leaves showed green.

Increased tiller number and dry matter production was associated with increase of stolon number and stolon length. Application of fertilizer gave more chance to grass to extent their solons. There was a tendency that application of combine organic fertilizers

Table 2. Effect of different nutrient sources on dry matter production of signal grass and ruminant carrying capacity

Parameters	Fertilizer application				
	PO	PC	PF	PC+F	Pi
Average DM production in rainy season (g/plot)	512[d]	1224[b]	1024[c]	1046[c]	2034[a]
Average DM production in dry season (g/plot)	282[d]	422[c]	495[b]	443[c]	738[a]
Cumulative DM production (kg/ha/year)*	3043[d]	6857[b]	5973[c]	5997[c]	11457[a]
Calculated animal carrying capacity (ST/ha/year)*	1.3[c]	3.0[b]	2.6[b]	2.6[b]	5.0[a]

Note: PO = blank control, PC = *Chromolaena* amendment, PF = manure application, PC+F = combination of Chromolaena and manure, Pi = inorganic fertilizer.

(*Chromolaena* and manure) led to slower growth than application of them as single treatment. In general, it can be mentioned that application of *Chromolaena* as mulch material resulted in better growth performance of signal grass.

CONCLUSION

Amendment of biomass originated from *Chromolaena* and manure and their combination improved signal grass growth, production and carrying capacity and could substitute about 50% of chemical fertilizer (urea and SP-36). Use of *Chromolaena* may be an alternative pasture management to sustain quality and production of signal grass pasture.

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Identification of Growth Hormone (Gh) Gene Mspi and Alui Loci Polymorphism in Beef Cattles

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ABSTRACT

The research was conducted in order to identify the polymorphism of MspI and AluI locus of the growth hormone (GH) gene in Bali, Limousine and Simmental cattle. Total blood samples of 87 cattle were taken from population of Bali cattle were collected from Balai Pembibitan Ternak Unggul Sapi Bali in Bali island, whereas Limousine and Simmental cattle blood samples were collected from Balai Inseminasi Buatan (BIB) Singosari-Malang, West Java. PCR-RFLP and sequencing methods used to detect the polymorphism and mutation at MspI and AluI loci of GH gene. The results showed that at MspI locus, the Bali, Limousine and Simmental cattle had one genotype (-/-), three genotypes(+/+, +/-, -/-) and two genotypes (+/+, +/-), respectively whereas for AluI locus, the Bali, Limousine and Simmental cattles had one genotype (LL), two genotypes (LL, LV) and three genotypes (LL, LV, VV), respectively. The allele frequencies of + and – alleles in Bali, Limousine and Simmental cattle were 0.000 and 1.000; 0.636 and 0.364; 0.889 and 0.111 respectively, whereas the frequencies of L and V alleles in Bali, Limousine and Simmental cattle were 0.000 and 0.000; 0.818 and 0.182; 0.694 and 0.306 respectively. Based on polymorphic informative content (PIC) value, it can be concluded that MspI and AluI loci in Bali cattle are monomorphic, while in Limousine and Simmental cattle is polymorphic. Based on the sequencing results, the MspI (+/+ and -/- genotypes) and AluI (LL and VV genotypes) loci showed a occurrences of nucleotide base mutation from cytosine (C) to thymine (T) and cytosine (C) to guanine (G), respectively.

Key words: Indonesian cattle, growth hormone gene, PCR-RFLP and sequence, polymorphism

INTRODUCTION

Indonesia has some animal genetic resources that need more attentions to be utilized and developed sustainable. The Bali breed is one of the four existing indigenous cattle breeds (Aceh, Pesisir, Madura and Bali) in Indonesia. 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, 2003).

Some molecular genetic studies have been reported in Indonesian cattle breeds using microsatellite DNA (nuclear genome) and mitochondrial genome markers (Handiwirawan *et al*., 2003; Nijman *et al*., 2003; Abdullah, 2008; Uggla 2008; Mohamad *et al*., 2009). However, molecular genetic marker based on coding sequence or candidate gene approach is limited and still needs more in depth study of its existence in Indonesian cattle breeds.

Molecular genetic markers in animal breeding programs could make selection more precise and efficient. Some of these markers are called candidate genes, e.g. the growth hormone genes, which are usually selected because of their biological significance on the quantitative traits of interest. Growth hormone has wide physiological activities, which include the regulation of growth, lactation and mammary gland development, gluconeogenesis, the activation of lipolysis, and the enhancement of amino acid incorporation into muscle protein (Burton *et al*., 1994). There is also evidence that growth hormone may be involved in the pubertal development and testicular function (Lin, 1996). Because of these important relationships, GH is a candidate gene for marker-assisted selection programs in cattle.

The GH gene is considered as an attractive candidate gene to be used as a marker due to its role in galactopoietic metabolism and the growth process. Growth hormone gene is localized in chromosome 19 (Hediger

et al., 1990), and consists of five exons separated by interval introns (Gordon *et al*., 1983). Several polymorphisms were identified in the *GH* gene. Cowan *et al*. (1989) and Hilbert *et al*. (1989) detected a polymorphic site for *Msp*I restriction endonuclease, the polymorphism being localized in the intron 3 of the *GH* gene in the position 1547 (Zhang *et al*., 1993), while a polymorphic site for AluI restriction endonuclease, has a genetic variant characterized by the substitution of one amino acid (leucine) for another (valine) at position 127, localized in the exon 5 in GH gene (Lucy *et al*., 1991).

The study of GH gene MspI and AluI loci have been reported in Bavarian Simmental cattle (Schlee *et al*., 1994), Hereford and Composite cattle (Sutarno *et al*., 1996; Sutarno 1998), Ongole Grade (PO) cattle (Sutarno *et al*., 2005), Brahman cattle (Beauchemin *et al*., 2006), Angus and Shorthorn cattle (Barendse et al. 2006), Iranian cattle (Zakezadeh *et al*., 2006), Indian Zebu cattle (Shodi *et al*., 2007) and West Sumatra Pesisir cattle (Jakaria *et al*., 2007).

The aim of this research was conducted in order to identify the polymorphism of growth hormone (GH) gene of MspI and AluI loci in Bali, Limousine and Simmental cattle breeds.

MATERIALS AND METHODS

Blood Sample and DNA Extraction

The total number of blood samples were taken from 87 samples consisting of Bali cattle 47 from Balai Pembibitan Ternak Unggul Sapi Bali in Bali island, whereas Limousine and Simmental cattle 40 were collected from Balai Inseminasi Buatan (BIB) Singosari-Malang, West Java. Blood sampling were performed by veterinarians from the Faculty of Veterinary Medicine IPB Bogor. Bali, Limousin and Simmental cattle blood samples were taken via jugular vein contain 5 ml by venoject tube, and then preserved in ethanol absolute.

Total genome was extracted from blood samples by the phenol/chloroform method followed by ethanol precipitation (Sambrook *et al*., *1*989) and dissolved in TE solution. The quality of the total genome was analyzed using by 1% agarose gel electrophoresis.

Amplification of GH Gene *Msp*I and *Alu*I Loci and Genotyping

The GH gene *Msp*I and AluI loci were analyzed by using the Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method (Table 1). A 329 bp fragment of intron 3 and 211 bp fragment of exon 5 in *GH* gene was amplified by PCR using forward and reverse primers. The PCR products of GH gene MspI and AluI loci were digested at 37°C for overnight by *Msp*I and AluI enzyme respectively. The digestion products were separated by horizontal electrophoresis (85 volts, 50 min) in 2% agarose gels in 1 × TBE and 10% ethidium bromide.

Sequences of GH *Msp*I and *Alu*I Fragment

Sequences of GH MspI and AluI fragment was done by sequencer machine of ABI Prims 3100-Avant Genetic Analyzer to find nucleotide mutation in both fragments. The sequences of GH MspI and AluI fragment carry out in individual homozygot (+/+ and -/-) and (LL and VV) respectively. The sequences material used PCR product of GH MspI and AluI fragment, primer *forward*, QIA-Quick PCR Purification Kit-Qiagen, 125 mM EDTA, ethanol absolute, 70% ethanol and Hi-Di *Formamide*.

Data Analysis

PCR-RFLP data was analyzed by allele frequency (Nei, 1987). The allele frequency was calculated by counting method as :

$$p = \frac{2(AA) + (Aa)}{2N}, q = \frac{2(aa) + (Aa)}{2N}$$

Table 1. Primer sequences were used in amplificates of GH *Msp*I and *Alu*I loci

Locus	Primer sequence	Annealing
GH *Msp*I[1)]	F 5'-CCC ACG GGC AAG AAT GAG GC-3' R 5'-TGA GGA ACT GCA GGG GCC CA-3'	53°C
GH *Alu*I[2)]	F 5'-GCT GCT CCT GAG GGC CTT C-3' R 5'-CAT GAC CCT CAG GTA CGT CTC CG-3'	55°C

Note : F = *Forward*, R = *Reverse*. [1)] Mitra *et al.* (1995), [2)] Reis *et al.* (2001).

Where, p is the (+) or (L) allele frequencies, q is the (-) or (V) allele frequencies and N is the total number of cattle tested.

Polymorphic Informative Content (PIC) value was estimated by calculated (Hildebrand et al. 1992) as :

$$\int PIC = 1 - \sum_{i=1}^{n} p_i^{\,2} - \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} 2 p_i^2 p_j^2$$

Where, p_i is the population frequency of the i^{th} allele and n is the number of alleles per marker.

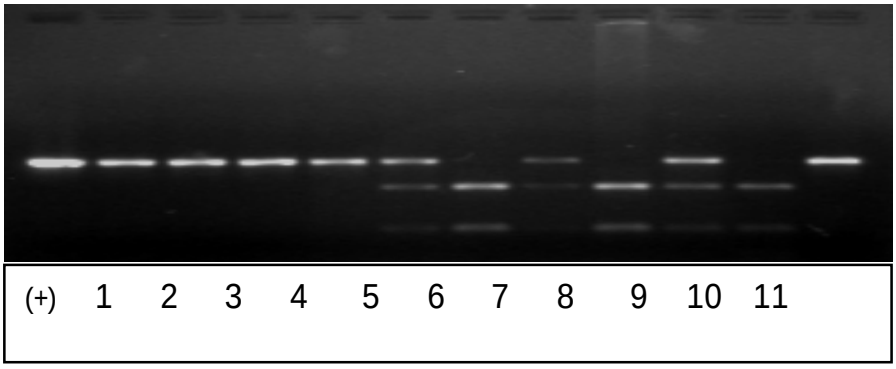
Sequences result were analyzed by *Molecular Evolutionary Genetic Analysis* (MEGA4) packed program with *alignment explorer/clustal* method (Kumar and Tamura, 2006).

RESULTS AND DISCUSSION

Allele Frequencies of GH Gene MspI and *Alu*I Loci

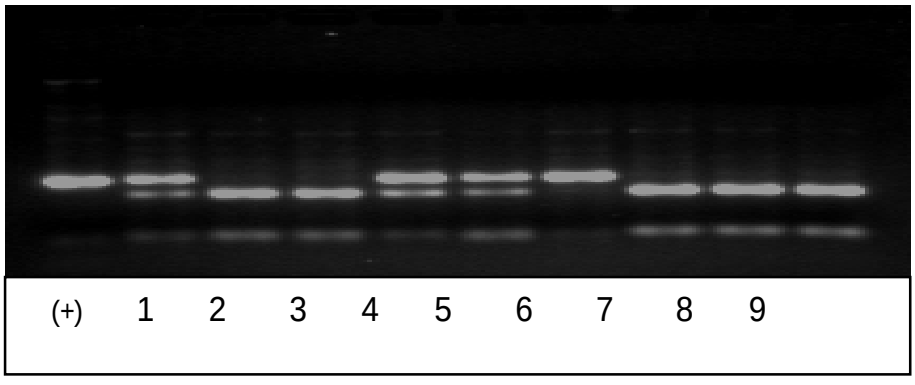
The following DNA restriction fragments result was obtained three genotype for the GH gene MspI and AluI loci repectively. Based on genotyping data of GH MspI locus showed 222 bp and 105 bp for +/+ genotype, 327 bp, 222 bp and 05 bp for +/- genotype and 327 bp (no digestion) for -/- genotype (Fig. 1). Whereas genotyping data of GH AluI locus showed 160 bp and 51 bp for the LL genotype, 211, 160 and 51 bp for the LV genotype and 211 bp (no digestion) for the VV genotype (Fig. 2).

The genotype and allele frequencies of GH MspI and AluI loci for Bali, Limousine and Simmental cattles were presented in Table 1 and 2. The (-) allele with a high frequency in Bali cattle and low frequencies in Limousine and Simmental cattle breeds while (+) allele for GH-Mspl locus was high frequencies in Limousine and Simmental cattle breeds and low frequency in Bali cattle. The allele frequency of the GH-alul locus ranged



+) ~ positive control (PCR product); lanes 1, 2, 3, 4, 11 ~ -/- genotype; lanes 5, 7, 9 ~ +/- genotype; lane 6, 8, 10 ~ +/+ genotype.

Figure 1. Genotyping results of *GH-Msp*I locus detected by agarose gel electrophoresis.



(+) was positive control (PCR product); lanes 2, 3, 7, 8, 9 were LL genotype; lanes 1, 4, 5 were LV genotype; lane 6 was VV genotype.

Figure 2. Genotyping results of *GH-Alu*I locus detected by agarose gel electrophoresis.

Table 2. Genotype and allele frequencies of GH MspI loci in Bali, Limousin and Simmental cattle breeds

No.	Breed	N	Genotype			Allele	
			+/+	+/-	-/-	(+)	(-)
1.	Bali	47	0.000	0.000	1.000	0.000	1.000
2.	Limousine	22	0.409	0.454	0.136	0.636	0.364
3.	Simmental	18	0.773	0.222	0.000	0.889	0.111

Note: n = individual number.

from 0.694 to 1.000. The highest L allele frequency was observed in Bali cattle breed herd (1.000).

Study of the distribution of the GH gene MspI locus in several regions of the world's cattle reported that the frequency of (-) allele was higher in *Bos indicus* (hump) cattle group and lower in the Bos taurus (humpless) cattle group (Lagziel *et al*., 2000; Sodhi *et al.*, 2007) (Table 3). The same research results also obtained that the frequency of (-) allele was high in West Sumatra Pesisir cattle (Jakaria *et al*., 2007) and Ongole Grade (PO) (Sutarno *et al*., 2005).

Based on the research results reported that the L allele frequency of GH-AluI locus was higher in hump (*Bos indicus*) cattle than humpless (*Bos taurus*) cattle (Table 4). There is a tendency that the L allele frequency higher than the V allele in Bos indicus cattle groups and including Indonesia native cattle. The low frequency of GH-AluI L allele of cattle population studied can be due to low number of samples, low actual allele frequency or the effect of severse natural selection at this locus.

P olymorphic Informative Content (PIC) Value

Based on the analyzed result showed that PIC values for GH-MspI and GH-AluI loci in Bali, Limousine and Simmental cattles were presented in Table 5.

Based on the estimation of PIC value could be concluded that the GH-Mspl and GH AluI Loci were less (no) imformative (monomorphic) in Bali cattle breed, on the

Table 3. Genotype and allele frequencies of GH AluI loci in Bali. Limousin and Simmental cattle breeds

No.	Breed	n	Genotype			Allele	
			LL	LV	VV	L	V
1.	Bali	47	1.000	0.000	0.000	1.000	0.000
2.	Limousine	22	0.636	0.364	0.000	0.818	0.182
3.	Simmental	18	0.500	0.389	0.111	0.694	0.306

Note: n = individual number.

Table 4. Distribution of the (-) allele frequency of GH-*Msp*I locus in humpless and hump cattle breeds

Cattle breeds	n	Breed types	Allele frequency (-)
Angus	65	Humpless	0.40
Charolais	7	Humpless	0.22
Gelbveih	15	Humpless	0.06
Limousine	18	Humpless	0.39
Simmental	23	Humpless	0.04
Brahman	23	Hump	0.65
Nellore	20	Hump	0.82
Siri	9	Hump	0.45
Grade Ongole (PO)*	114	Hump	0.26
West Sumatra Pesisir**	133	Hump	0.80
Indian zebu (17 breeds)***	750	Hump	0.67-0.94

Sources : Lagziel *et al.* (2000), *) Sutarno *et al.* (2005), **) Jakaria *et al.* (2007), ***) Shodi *et al.* (2007).

Table 5. Distribution allele frequency of GH-*Alu*I locus in humpless and hump cattle breeds

Cattle breed	n	Allele frequencies		Autors
		L	V	
Mazandrani	97	0.910	0.090	Zakezadeh *et al*. (2006)
Beef cattle Portugis	195	0.759	0.241	Reis *et al*. (2001)
Angus	527	0.770	0.230	Barendse *et al*. (2006)
Shorthorn	500	0.760	0.240	Barendse *et al*. (2006)
Brahman	324	1.000	0.000	Beauchemin *et al*. (2006)
Nellore	79	1.000	0.000	Curi *et al*. (2006)
West Sumatra Pesisir	133	0.993	0.007	Jakaria *et al*. (2007)

other hand is more informative (polymorphic) in Limousine and Simmental cattle breeds.

Sequensing of GH-MspI and AluI Loci

Sequencing results showed that there are changes in nucleotide bases (mutation) from cytosine to thymine and cytosine to guanine for GH-MspI and AluI loci respectively (Table 6).

Based on obtained result showed that same results reported by Zhang *et al*. (1993) and Lucy *et al*. (1993), GH gene MspI and AluI loci involved changes of nucleotide base between cytosine (C) to thymine (T) and cytosine (C) to guanine (G) respectively.

CONCLUSION

Based on our research results can be concluded that the frequency of (-) allele is high in Bali cattle, while low in Limousine and Simmental cattle breeds of GH-MspI locus. We also founded high frequency of L allele on all cattle breeds tested for GH-AluI locus. GH gene MspI and AluI loci founded monomorphic in Bali cattle, while polymorphic in Limousine and Simmental cattle breeds. The mutations occurred between cytosine (C) to thymine and cytosine (C) to guanine (G) for GH gene MspI and AluI loci respectively.

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Tabel 6. Estimation of polymorphic informative content (PIC) value in Bali, Limousin and Simmental cattle breeds

Cattle breed	n	PIC Value	
		GH *Msp*I locus	GH *Alu*I Locus
Bali	47	0.0000	0.0000
Limousine	22	0.2760	0.2598
Simmental	18	0.1779	0.3343

Note: n = individual number.

Table 7. Mutation of nucleotide basa in GH *Msp*I dan *Alu*I loci

Locus	Enzyme restriction site	Mutation	Position
GH-*Msp*I	C*CGG	Cytosine to Thymine	(1547)*
GH- *Alu*I	AG*CT	Cytosine to Guanine	(2141)*

*) based on sequences of Gordon *et al* (1983).

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Production, Characterization and Purification of Xylanase from *Staphylococcus aureus* MBXi-K4

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ABSTRACT

Pollard is a by-product from dry milling wheat into flour and contains 16,49% of crude fiber. In order to increase nutritional value of pollard a xylanolitic enzyme is added to catalyze hydrolysis of xylan components. The addition of xylanase in wheat-pollard diet is necessary to reduce viscosity of digest. Thus could be easily absorbed in intestinal gut. The objectives of this research were to produce xylanase in batch system bioreactor, and to characterize and purify xylanase from *Staphylococcus aureus* MBXi-K4. Maximum enzyme production was reached after 72 hours of cultivation with specific enzyme activity of 10,5 U/mg protein. Biomass specific growth rate (μ) was 0,107 per hour, yield of product was 2,255 (g product/g substrate) and yield of biomass was 0,004 (g biomass/g substrate. The optimum temperature and pH was 70^{o}C and 6 respectively. The xylanase maintained its stability for 30 minutes at 70^{o}C and over pH range 4 – 8. The Km and Vmax value at 70^{o}C on oatspelt xylan was 1,086 (mg/ml) and 3,195 (μmol xylose/min.ml) respectively. Xylanase was purified from the culture supernatant of *S.aureus* MBXi-K4. The purity of xylanase increased 11,69 fold than those of the crude enzyme. The specific activity after purification was 383,9 U/mg. Three kinds of xylanase activities were visualized by zymogram technique with estimated molecular weights of 45,6 kDa, 28,1 kDa and 21,6 kDa. The purified xylanase had one band protein with molecular weight of 47,9 kDa as shown on SDS-PAGE. It is concluded that xylanase from *S.aureus* MBXi-K4 was a moderate thermo stable enzyme and a good candidate as feed additive on feed industry with an improvement on its productivity and thermo stability.

Key words: xylanase, S.aureus, production, characterization, purification

INTRODUCTION

Poultry production in Indonesia has fulfilled more than 50 percent of meat demand of the Indonesian community including chickens and ducks (Statistik Peternakan, 2007). In order to improve food security, the government still continues to improve availability of meat from chickens and ducks in sufficient quantity, high quality and affordable by the public. The feed is a major component and contributes about 60% - 70% of the total production costs in animal husbandry. Therefore it is very important to provide supply of cheap, easy and sustainable feed raw material without competing with human needs. Fine wheat bran (pollard) is one by-product of wheat processing that is available throughout the year in the country with a stable quality. Production of wheat processing industry in Indonesia reached 3.3 million tons per year (Aptindo, 2004). Pollard utilization as monogastrics diet is limited by high crude fiber content (16.49%), Neutral Detergent Fiber/NDF (38.4%) (Pantaya, 2003) and low energy content (1300 kcal EM / kg) (NRC, 1994). The use of pollard in poultry rations is generally not more than 30%.

Consumption of high crude fiber by the chicken broilers can increase the viscosity of the contents of small intestine (digest), eventually interfere the absorption of energy and protein of rations (Adam, 2000) and thereby reducing the growth of the animals. To improve the nutritional value of diets containing high crude fiber ingredients, one of the methods is utilization of enzyme as feed supplement to hydrolyze crude fiber components into simpler products, which can be absorbed directly by livestock. The addition of xylanase enzymes into diets based on wheat bran (pollard) can decrease the viscosity of digest and increased body weight of broiler age 6 weeks to 14.72% and 2.6% (Chiang *et al*., 2005). Xylanase can reduce viscosity of digest by hydrolyze arabinoxylan into arabi-

nose and xylose, so can easily be utilized by poultry.

This research is a continuation of previous studies by Setyowati (2006), who did isolate and characterize microbes isolated from corn cob which produce xylanase. Isolate obtained (MBXi-K4) and the xylanase produced grew optimally at temperature of 37^{o}C and pH 7 (mesophilik), whereas the xylanase has an optimum temperature of 70^{o}C and stable at wide pH range (4 - 10) with optimum pH of 6.

The objectives of this research were to obtain pure enzyme from indigenous isolate *Staphylococcus aureus* MBXi-K4 and obtain information about the characters of xylanase produced including information of growth kinetics such as the specific growth rate of biomass (μ), the yield of product formation (Yp/s) and the yield of use of the substrate (Yx/s).

MATERIALS AND METHODS

Chemicals. *Oat spelt xylan* (Sigma), pollard xylan, corn-cob xylan, yeast extract, K_2HPO_4, NaCl, $MgSO_4.7H_2O$, Na_2HPO_4, NH_4Cl, bacto agar, Tris-HCl buffer, *3,5-dinitro salycilic acid* (DNS), NaOH, Na-K tartarate, xylosa, and aqua destilated. Analysis Protein of xylanase used reagent of Bradford, bovine serum albumin (BSA) as protein standard , sephadex G-100 (Sigma), Ammonium Sulphate, Sodium Dodesil Sulphate (SDS), Poliacrilamide, bis acrilamide, N,N,N,N-Tetrametiletilendiamina (TEMED), glycin, protein marker with Low Molecular Weight, silver staining ($AgNO_3$), Na_2CO_3, and ethanol 95%.

Medium. Media preparation and regeneration of the media to grow bacteria thermophilic refers Richana *et al* (2000). Substrate used was 0.7% pollard, which mixed with growth media and media production. *S.aureus* MBXi-K4 regenerated in the LA medium (Luria Agar) with the composition of tripton 10g / L, yeast extract 5 g / L, NaCl 10g / L and bacto to 15G / L) and then grown in medium containing 0.7% oatspelt xylan with the same composition as the growth media. Inoculum grown at optimal pH and temperature was taken as many as 10% (v / v) and added to the media production with media composition of 0.2% yeast extract, K_2HPO_4 1.5%, NaCl 0.25%, 0.025% $MgSO_4.7H_2O$, Na_2HPO_4 0.5%, 0.5% NH_4Cl with substrate of 0.7% oatspelt xylan and pollard xylan (Dung *et al*, 1993). Propagation of the cell culture was carried out in 250 ml erlenmeyer with 100 ml work volume and production of xylanase to study of growth kinetics in Bioreactor was carried out in 2 liters of volume and work volume of 1500 ml. Cultivation was done at optimum temperature and pH, agitation of 160 rpm and aeration 1 vvm, for 96 hours. Growth kinetics were measured including the value of specific growth rate (μ), the yield of use of the substrate and the yield of the product formation. Xylanase activity was observed by the method of DNS (Miller, 1959) and specific enzyme activity was measured by comparison of enzyme activity and protein concentration. Protein measurements performed by the method of Bradford (1976) using BSA (Bovine Serum Albumin) as standard protein.

Enzyme characterization. The characteristics of crude extract enzyme (supernatant) observed were optimum temperature and pH, and also thermo stability of enzyme. Analysis of kinetics of enzymatic reaction was done by determining the value of Km and Vmax. These values were obtained by plotted of substrate concentration and reaction rate into lineweaver-Burk equation. Enzyme thermo stability was determined by incubation of filtrate enzyme at temperature of 70^{o}C, 80^{o}C and 90^{o}C at its optimum pH without the addition of substrate and xylanase activity was measured every 15 minutes during 120 minutes. (Chaplin and Bucke, 1990). One unit of enzyme activity (U) was the number of enzymes that can produce 1 μmol xilose / min / ml in certain conditions.

Purification of Xylanase. Supernatant as crude extracts of xylanase was obtained by centrifugation of culture at 10,000 rpm for 10 minutes at a temperature of 4^{o}C. Purification was carried out by ammonium sulfate precipitation followed by centrifugation. The supernatant was dialysed in a membrane dialysis with Molecular Weight Cut-off (MWCO) 12kDa in 0.1 M Tris-HCl buffer pH 7.5 overnight. The supernatant was then applied to gel filtration chromatography using matrix of Sephadex G- 100 equilibrate with 0.1 M Tris-HCl buffer pH 7.5 with the volume 3 times of volume of the column. Protein concentration obtained by measuring the value of

absorbance using UV spectrophotometer at 280 nm wavelength.

Gel Electrophoresis / SDS-PAGE (Laemmli, 1970). SDS-PAGE method was used to predict the molecule weight of the protein, determine the number of protein components in the sample and determine the distribution of protein fraction in the sample and for the purification of proteins. Samples of 100 µl was taken and mixed with loading buffer of 10 µl. The mixture was heated at a temperature of 95^{o} C for 5 minutes, to break the peptide bond, and denatured the proteins. Electrophoresis was done at 50mA power and voltage of 100 volts for 2 hours in cold temperatures. Gel staining was done by using the Silver Staining methods.

Zimmogram. Xylanase activity in the gel was determined through zimogram analysis, by adding 1% (w / v) oatspelt xylan as substrate into poliacrilamide gel mixture before polymerization. After electrophoresis, the proteins in gel were denaturized by immersion in 2.5% (w / v) Tritone X-100 for an hour. and then gel incubated in 0.1 M Tris-HCl buffer at optimum pH and temperature of enzyme overnight. After that it was colored with 0.1% (w / v) of Congo-red dye for 30 minutes and washed (destaining) with 1 M NaCl solution, for 30 - 60 minutes until the clear zone appear.

RESULTS AND DISCUSSION

Enzyme Production

The maximum enzyme activity was 2.26 U/ml at 72 hours of fermentation and specific activity was 10.5 U / mg protein. The rate of biomass, the use of substrate and product formation were presented in the Figure 1.

From the data, *S.aureus* MBXi-K4 growth in pollard xylan substrate concentration value of 0.7% had Xmaks = 4.44 g / l. Data cell growth in exponential phase were plotted with the logistic model based on Monod equation, obtained form a linear ship with the equation ln (X) = 0.107x+0.134. The slope of the line was the value of specific growth rate (µ) of 0.107 / hour. Product yield (Yp/s) was obtained by plotting the value (P-Po) which the data from xylanase enzyme activity (U/ml) against the use of substrate (So-S). The slope of the line obtained was the value of Yp/s, in the amount of 2.255 (U/mg substrate). This value showed that during the cultivation process, efficiency of each milligram of substrate (xylose) sumed produced 2.255 Unit xylanase. Xylanase yield produced in the cultivation process was influenced by various combinations of factors, include accessibility to the substrate, the rate and number of xylooligosaccharide and xylose released. Important role of lose, xilobiosa, xylooligosaccharide and rodisaccharide of xylose and glucose was as inducer for the regulation of xylanase biosynthesis. Biomass yield (Yx/s) was obtained by plotting the value (X-Xo) against the use of substrate (So-S). The slope of the line was the value of Yx / s, ie for 0.004 (g biomass / g substrate), it mean in each gram of substrate consumed obtain 4 mg of biomass.

Characteristics of Xylanase

Xylanase was resistance against temperature and the pH. Xylanase response to temperature and Stability of xylanase against pH are shown in Figure 2 A and B. At temperature of 70^{o}C, initial activity of the enzyme was 1.7 U / ml.

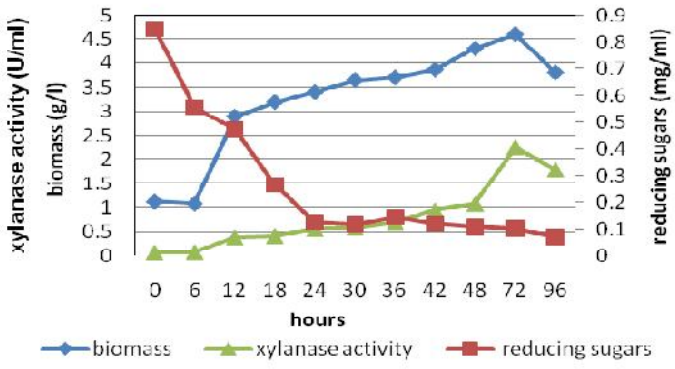

Figure 1. Rate of biomass, xylanase activity and reducing sugar formation

A

B

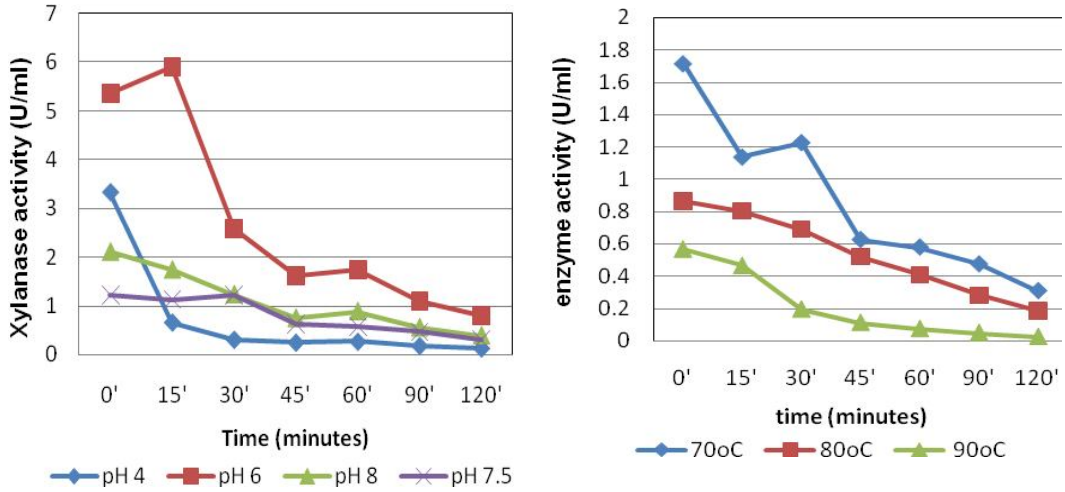

Figure 2. A.Stability of xylanase against temperature. B. Stability of xylanase against pH

Relative activity of Xylanase decreased in the 15th minute which was 1.1 U / ml which means that the remaining activities 66.2% of the initial activity. Xylanase activity increased at minute 30, in the amount of 71.5% and back down to the minute 45 – 120. At temperatures 80°C and 90°C xylanase activity tends to decline sharply. The resistant of xylanase against temperature of 70°C – 80°C for 30 minutes has a chance to be applied in the manufacture of feed pellets. Pelleting process is processing of the feed with the addition of heat through steam ranged over 20 to 255 seconds depending on the amount of feed processed. The addition of this steam increased temperature of feed about 5% with end temperature of 70°C – 90° C (Moran, 1989). The xylanase estimated able to maintain its activities since heat treatment is quite brief. At temperature of 80°C showed xylanase activity decline smaller than at the temperature of 70°C and 90°C, although from a lower enzyme activity than the activity at a temperature of 70°C. This is presumably because in the crude enzyme was not only consist of one type of xylanase. Zimogram results showed three clear zones on the gel with different molecular weight.

Xylanase from *Bacillus thermoleovorans* K-3d and *Bacillus flavothermus* LB3A reportedly remain stable after incubated at 70°C for 2 hours. At a temperature of 80°C xylanase from isolate K-3d and LB3A shows a half-life 18 and 10 minutes (Sunna *et al*. 1997). Xylanase from cloning gene XynB of *Thermotoga maritima* MSB8 on *E. coli* and *Pichia pastoris* had an optimum temperature at 90°C and after incubation at temperature of 100°C for 30 minutes still retain 70% of enzyme activity (Yang *et al*, 2005).

All enzymatic reaction is strongly influenced by pH, therefore to make the enzymatic reaction run at its optimum rate required buffer with appropriate pH. Stability of the xylanase against pH can be seen in Figure 2B. In the graph shows that the enzyme resistant to the pH range from 4 to 8, with the highest enzyme activity at pH 6. At pH 6 showed that the enzyme activity reached 5.8 U / ml in the incubation for 15 minutes. The enzyme was maintain its relative activity more than 90% during the first 15 minutes and then decreased to 44% at minute-30. The decline in enzyme activity due to changes in pH caused by the changing state of enzymes ions and also the substrate ions. These changes can occur at amino acid residue which has catalytic function on substrate binding or at the amino acid residues that functions to maintain the tertiary and quaternary structure of active enzyme. Decreased of enzyme activity could be recovered by changing the reaction conditions at pH optimum of enzyme. At a certain pH, changes in the ionic charge of the ionizable side chain from amino acid residues of enzyme becomes too large, which causes denaturation of enzymes and loss of enzyme catalytic activity. Tertiary structural changes can cause direct contact of hydrophobic groups with water so that the enzyme solubility reduced. Reduced of solubility caused a decline in enzyme activity (Meryandini *et al*. 2008; Palmer 1981).

Soluble proteins are a condition that make proteins interact with the solvent easily, so that when the pH changes above or below

the optimum pH, it will be directly in contact with the active side, as consequence it will be a decline in the enzyme activity rapidly (Scopes 1987). Optimum pH of xylanase from *Bacillus sp* strain K-1 is 5.5 and its activity was stable at pH 5.0 to 9.0. At pH 12 still having relative activity of xylanase of 88% (Ratanakhanokchai *et al.* 1999). Xylanase from *Bacillus stearothermophillus* T-6 has a high activity at pH range 5 - 11 with optimum pH 7.0 and 6.5 and still maintain the activeness of 60% at pH 10. At pH 9 and temperature of 65^{o}C , half-life time of enzyme about 6 hours (Khasin *et al.* 1993). Xylanase from *Staphylococcus sp* SG-13 has an optimum pH of 7.5 and 9.2 (Gupta *et al*. 2000).

Kinetics Parameters of Enzymatic Reaction

Observations of kinetics parameters were done by measurements of Km and Vmax values. Kinetics of enzymatic reaction were determined by measuring of xylose concentration as a result of oatspelt xilan hydrolysis at concentration of 0.05% - 0.2%. To calculate the Km and Vmax parameters used linear transformation of Michaelis-Menten equation, by making a linear plot of the value of 1/V and 1/S on Lineweaver-Burk equation. The value of Vmax = 3.195 (µmol xylose / menit.ml) and the value of Km = 1.086 (mg / ml). It can be said that the maximum reaction velocity of xylanase from *S.aureus* MBXi-K4 could produce xylose of 3.195 µmol / min. ml. Km value (Michaelis-Menten constant) is a constant value which is not affected by the concentration of enzyme, whereas the Vmax value affected by the concentration of enzyme. The smaller the value of Km the higher enzyme activity and enzyme affinity for substrate. Vmax value is defined as the enzyme reaction velocity when it has been saturated by the substrate (Suhartono, 1989).

Km and Vmax values of xylanase from *Staphylococcus sp* SG-13 on the substrate of oat spelt xilan were 7 mg / ml and 55 µmol xylose / min / mg respectively (Gupta *et al*. 2000). Xylanase from recombinant bacteria *Geobacillus sp*.MT-1 had a Km value of 1.579 mg / ml and Vmax of 289 µmol / min.mg (Wu *et al*. 2006).

Enzyme purification

Purification technique using ammonium sulfate precipitation was selected because easy to do, quite cheap and get good enough precipitation. Addition of ammonium sulfate into the protein caused binding of the water molecules by molecules of ammonium sulfate, so that protein-protein interactions become more dominant than protein-water interactions. This caused the aggregation of protein that would precipitate the protein. Environmental temperature were kept low (approximately 4^{o}C) , therefore it did not cause a denaturation of proteins, but still can be dissolved again in its buffer.

Precipitation of crude extract xylanase of *S.aureus* MBXi-K4 by using ammonium sulfate has been done on concentration of 40% - 60%, with an optimum concentrations of 40% and the enzyme activity obtained of 1.8 U/ml. The more hydrophilic amino acids on the enzyme molecule or protein, the higher concentration of ammonium sulfate necessary to precipitated. Dialysis is a process of selective diffusion through cellophane membrane. This process aims to eliminate the salt ammonium sulfate and dissolved substances other. During dialysis the water enter the dialysis bag by osmotic pressure. Dialysis is done at cold temperatures to prevent damage of the purified protein. The molecules are smaller than 12 kDa would get out of the dialysis bag. Enzyme activity after dialysis was 1.76 U / ml. Decrease of xylanase activity after dialysis allegedly due the elusion of small proteins that may play a role in enzyme activity. This can happen because of some microorganisms which produce xylanase had xylanosome, multienzyme and multifunctional complex that located on the cell surface and plays an important role in the hydrolysis of hemicellulose (Sunna *et al*, 1997).

Purification of Xylanase by Gel Chromatography

Xylanase purification results using matrix of Sephadex G-100 showed the three peaks of protein and the highest was the fraction of no 4 - 10. Another peak was smaller in fraction number 54-55, and the fraction numbers 68-69 (Fig. 4). Highest activity of xylanase was obtained from the fraction number 6 in the amount of 1.56 U/ml with specific activity of 383.9 U/mg.

Profile of gel filtration elusion shows the levels of protein and enzyme activity had the same tendency. Eluen with high protein con-

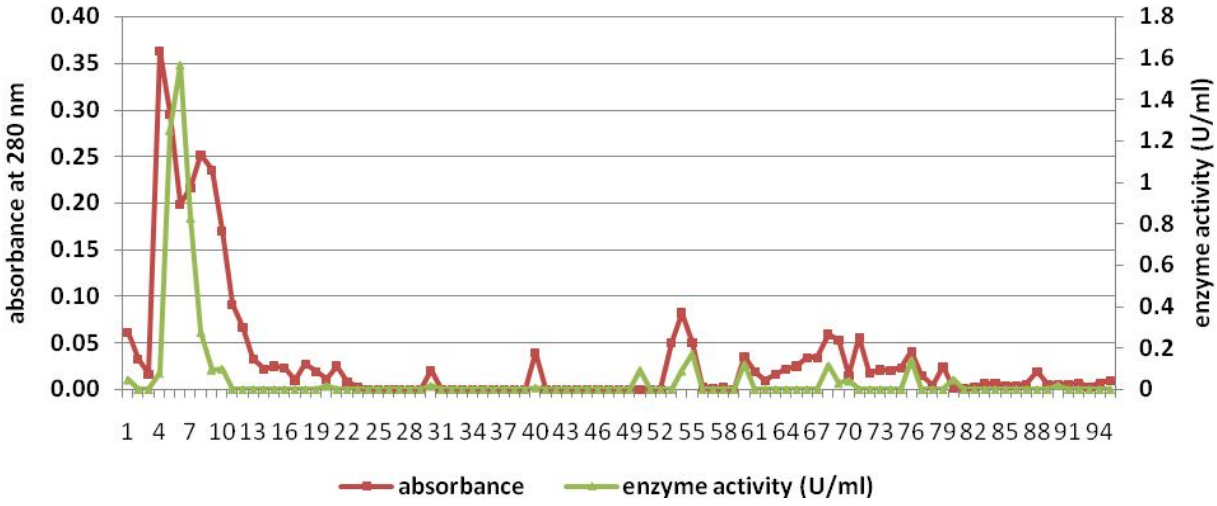

Figure 4. Profile of proteins elusion on Sephadex G-100 gel chromatography

tent has a high enzyme activity as well. It shows that the protein derived largely desired enzyme. This is confirmed by the results of SDS-PAGE electrophoresis and zimogram. Xylanase purification step in this study used only one column chromatography of gel filtration. This process was shorter with a purity level not much different when compared with xylanase from *Staphylococcus sp* SG13 which is purified in two steps of chromatography columns with a purity level of 12 times of crude extracts, but the value of the enzyme specific activity is much lower ie 2.74 U / mg (Gupta *et al*. 2000). The purification process are summarized in Table 1.

Xylanase purity level obtained was higher than the purity of β-xylanase II from *Aspergillus fumigatus* Fresenius (Silva *et al*. 1999), *Aspergillus tereus* UL 4209 (Chidi *et al*. 2008) and *Thermomyces lanuginosus* SSBP (Lin *et al*. 1999). The activity were 0.74, 1.2 and 4.6 times than that of crude extracts and the specific activities for consecutive 4.67 U / mg, 12 U / mg and 3209 U / mg respectively. These results were lower compared with the purification of xylanase from *Streptomyces sp* (Meryandini *et al*. 2008), *Bacillus pumillus* PS 213 (Degrassi *et al*. 1998), *Cellulomonas flavigena* (Martinez-Trujillo *et al*. 2003) with consecutive increase of 12 , 97, 179.2 and 30.6 times of crude extract enzymes.

SDS PAGE and Zimogram Profile

Results from each stage of purification were visualized in SDS-PAGE profile and zimogram in order to estimate the molecular weight and see the level of enzyme purity. Xylanase was visualized by Coomasie Brilliant Blue staining, showed a single band at the well number 2, 3 and 4. They are the results of purification with Sephadex G-100 fraction number 4 to 6, with an estimated molecular weight of 47.9 kDa. This indicated that the enzyme can be purified effectively with gel filtration chromatography technique. The size of molecular weight was estimated by comparison marker LMW (Low Molecular Weight) of Pharmacia composing of fosforilase (97 kDa), albumin (66 kDa),

Table 1. Purification of xylanase from *S.aureus* MBXi-K4

Step	Volume (ml)	Total Protein (mg)	Total Xylanase activity (U)	Enzyme spesific activity (U/mg)	Recovery (%)	Fold
Crude Extract	81	3.32	109.01	32.82	100	1
Amm.sulfate precipitation	10	0.48	18.06	37.39	16.57	1.14
Dialysis	5	8.81	8.81	32.59	8.08	0.87
Sephadex G-100	3	0.012	4.69	383.90	4.30	11.69

ovalbumin (45 kDa), carbonic anhydrase (30 kDa), trypsin inhibitor (20.1 kDa) and lysozyme (14.4 kDa).

In zimogram profile can be seen the three bands of clear zones in wells No. 2-6, respectively xylanase crude extracts, precipitation with ammonium sulfate and dialysis results, with an estimated size of the molecular weight of 45.6 kDa, 28.1 kDa and 21, 6 kDa. In the well number 7 and 8 which are the result of purification by Sephadex G-100 obtained a clear zone with xylanase activities high enough and the molecular size of about 21.63 kDa. Markers which are used is LMW Fermentas consisting of ß-galactosidase (116 kDa), BSA (66.2 kDa), ovalbumin (45 kDa), lactate dehydrogenase (35 kDa), RE-Bsp981 ase (25 kDa), ß-laktoglobulin (18.4 kDa) and lysozyme (14.4 kDa). SDS-PAGE profile and zimogram presented in Figure 5 A and B. The ability of microbial species in producing more than one type of xylanase.has been reported. The complexity of molecular structures of xylan found in nature so that the necessary work of some kind xylanase synergistically to completely degrade substrates into its sugar components (Beg *et al*. 2001). The size of molecular weight of pure xylanase on SDS-PAGE profile was greater than that seen in zimogram. It was assumed to have termination of enzyme subunit or the release of the cofactor during the purification process caused by the interaction between proteins with polysaccharides in the matrix gel (Lin *et al*. 1999). Some of the xylanase from *Streptomyces viridosporus* T7A missing in the purification process using gel filtration chromatography with Sephadex G-matrix 75 according to Magnuson and Crawford (1997) caused by an interaction of xylanase with spinal polysaccharide on Sephadex matrix. It can not be avoided despite the addition of 0.5 M NaCl in buffer elusion.

Another possibility is in the SDS-PAGE profile, protein bands of xylanase with small molecular weight did not appear. This is presumably because the protein concentration on gel filtration elusion were too low, but has a high activity that does not appear on the gel elektoforesis, but able to form clear zones on zimogram profile.

Oat spelt xylan containing 52.5% xylose, 22.3% arabinose, 15.7% glucose and 9.5% galactose (Li *et al*. 2000). Therefore bacteria capable of producing more than one type of xylanase if grown on media containing oat spelt xylan. *Streptomyces Sp*.B-12-2 produced five types of endoxylanase when grown on oatspelt xylan media, while *Aspergillus niger* can produce 15 types of xylanase. Post-translational modifications such as glycosylation, proteolysis, or even both may also lead to the formation of several types xylanase on one type of microorganisms (Subramaniyan and Prema 2002).

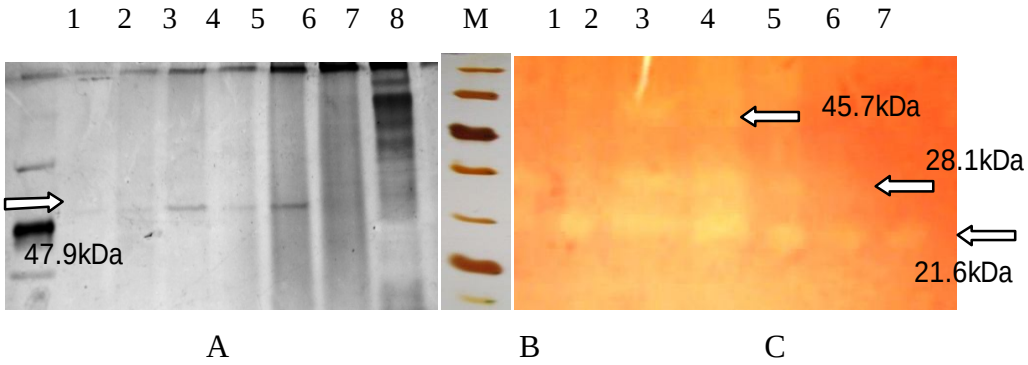

Figure 5. A. Profile of SDS-PAGE, well no. 1 : Marker of LMW Pharmacia, 2: Sephadex G-100 fraction no6; 3: fraction no 5; 4: fraction no 4; 5: dialysis; 6: ammonium sulfate precipitation 1; 7: ammonium sulfate precipitation 2; 8: crude extract enzyme.
B. Marker of LMW Fermentas
C.Zimogram profile of purified xylanase. Well no.1: crude extract enzyme, 2: ammonium sulfate precipitation on enzyme, 3: freeze dried of crude extract enzyme, 4: freeze dried of ammonium sulfate precipitation on enzyme, 5: xylanase after dialysis, 6: Sephadex G-100 fraction no 4,5 and 6, 7: fraction no 6.

CONCLUSION

There were three types of xylanase obtained with an estimated molecular weight of 45.6 kDa, 28.1 kDa and 21.63 kDa in zimogram profile. Xylanase from *Staphylococcus aureus* MBXi-K4 was classified as moderate thermostable where its maximum activities at 70^{o}C and still be maintained its activity more than 70% for 30 minutes. This enzyme worked at a pH range from 4 to 8 with optimum pH value of 6 and optimum temperature of 70^{o}C. Based on the characteristics, the xylanase could be applied to the feed industry with some improvements, especially in its productivity.

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Growth Selection by Evaluation of Exterior Parameter and Nutritional Approach on Local Meat Chicken

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ABSTRACT

Related research efforts exploring the genetic potential of local chickens *(Gallus gallus domesticus)* in order to get the parent of superior local meat chicken (ALPU) through the development of selection methods was applied. Economical and practical approach to nutritional requirement especially protein requirement has been done since July, 2008 as well. The research was located at the Field Laboratory of Poultry Science and Technology, Department of Animal Science, Faculty of Agriculture, Syiah Kuala University, Darussalam, Banda Aceh. The research was divided into 2 groups: the female and male parents selection for 2 months, and the selection and enlargement of the derivative (F1) for 4 months. The selection process by using the parameters, was developed starting with the selection of the DOC of local chicken which has potential as meat chicken, was divided into 2 groups: medium and heavy types. Selection was continued on the derivative chicken which was mated by artificial insemination method (IB). Beranger (2007) developed an exterior selection method which has a positive correlation with genetic potency: broad skull, wide back, long back, the depth of the body, thigh circumference, carcass weight, chest width and proportions of the chest muscles and thigh muscles (Yaman *et al.*, 2000). Ration treatment aimed to obtain optimal nutrition standards to stimulate the optimum production. Protein content in ration was 17, 19, and 21%, respectively. Selection of the exterior parameter has been able to classify the candidate of local meat chicken into 2 type specific growth models: heavy and medium types. Based on the parameters that were developed in this study, the local meat chickens have a huge potential as superior meat chickens. Growth patterns of male and female of selected chicken were significantly different for final body weight and exterior size. The male chicken achieved commercial weight at the age of 3-4 months (<1.0 kg), while females have a tendency to continue to grow after 4 months of age. Male chicken fed with 19% of protein resulted better performance, while the female chicken growth was in line with the increasing of protein in the ration. In conclusion, the exterior parameters and nutritional approach have an important role to evaluate the growth process on local meat chicken.

Key words: local chicken, selection, exterior and nutrition

INTRODUCTION

In local animal, the genetic improvement program is often done by crossbreeding with non-local animal. This technique requires a large cost, long time, and must be done judiciously and focused because it can threaten the purity of indigenous animal (local) that basically has its own advantages as compared to non-local animal. It can be overcome by selection programs aiming to change the frequency of genes from a population that expressed in the production ability. Most of animal selection methods were focused on the determination of the potential nature of phenotype or genotype. This will be more difficult with the local livestock as well as local chicken genetic trait in which variation is very high because of the history of origin. The selection program to increase genetic potential of local animal should consider the aspects of economic value, cost, and time to achieve the target. This selection program resulted in economic value property such as the increase in fertility, vitality, weight gain, color or body performance (Le-Bihan *et al.*, 2001:2008).

Recent parameter selection method, based on the existing exterior performance to determine the genetic potential of chicken, needs to be developed with the exterior parameters selection process which is useful as a standard reference to obtain local meat chicken. The basic selection method of the exterior parameters in meat chicken to get parent with higher productivity ability has been developed by the American Livestock

Breeds Conservancy Pilot Project (Beranger, 2007). The principle of this method indicated that the chicken body performance could be genetically determined by the size of the parent performance. This method resulted in a faster growing meat chicken. The exterior selection parameters used were broad skull, wide and long back, the depth of the body, thigh circumference, and carcass weight. In fact, the genetic diversity of the local chicken is highly influenced by the interaction between genetic and environmental factors. Nutritional adequacy plays a large part to produce maximum growth. Many failures performance of optimum genetic potential of animal was reported due to the problem in nutritional balance, especially protein and energy requirements. Yaman *et al*. (2000) reported that nutritional conditions are corresponded with the breast and thigh muscles of the chickens. These muscles are the most dominant muscles in meat chicken which are influenced by genetic and nutritional factors. These parameters are considered as important in the selection of local meat chicken.

In present study, the combination of exterior parameter and protein requirement in ration were implemented during selection program to determine the genetic potential of local meat chicken. This study focused on the adjustment of both parameters to produce selection methods which is practical, valid, and applicable for breeding program of local meat chicken.

MATERIALS AND METHODS

The present study was conducted in Field Laboratory for Poultry Research Development and Teaching Laboratory of Poultry Science and Technology, Department of Animal Science, Faculty of Agriculture, Syiah Kuala University, Darussalam, Banda Aceh since June 10, 2008. This research used 400 selected local chicks (unsexed) and reared for 5 months and used artificial insemination (AI) for breeding purposes. Organic materials used in the ration were sago flour, rice bran, soybean meal, palm meal, DCP, mineral mix, vitamin mix and the ISP (isolated soybean protein). The selection program was started from egg to pre-laying period. At the age of 2 months, the male and female chickens were divided into 2 groups: medium and heavy types which were based on the established criteria, while the rest was eliminated (killed). All chicks were nutritionally treated and the evaluated parameters were observed until the age of 4 months (16 weeks). Individual selected chicken for both males and females of each type was maintained and artificial inseminated for breeding purpose in 2 times/week of each group (Yaman and Sari, 2004). All hatching eggs (F1) were incubated by automatic egg incubator. Produced DOC was continuously selected based on the body performance achievement. Applied selection parameters of the parent and the derivative of local chicken were growth, weight gain, final body weight, and parameters of the body exterior.

The referenced weight growth at the age of 3 months for prospective parents were 1,2-1,4 kg (males) and 0,8-1,0 kg (females); and 1,2-1,4 kg (males) and more than 1,0 kg (females) for medium type and heavy type,

Table 1. Diet Composition of Local Meat Chicken

Diet	Amount
Corn	42.8 %
Soybean meal	27.1 %
Rice Brain	13.0 %
Palm Meal	6.0 %
Coconut Meal	3.0 %
Sago Meal	6.0 %
Extracted Cereal Protein	0.5-0.9 %
DCP	1.0 %
Vitamin and mineral premix	0.2 %
Total	100 %
Total Nutrient	
Crude Protein	19-21%
Energy	2910 kcal ME
Calcium	0.89%
Phosphor	0.75%

respectively (Yaman *et al*., 2002; unpublished data).

Selection criteria of the body exterior was based on methods developed by Beranger (2007): skull broad, wide back, long back, depth of body, thigh circumference, width of chest, carcass weight, chest and thigh muscles (Yaman *et al*., 2000).

The formulation of energy and protein balance (Yaman *et al*., 1998) as treatment in each group was modified by the provision of *ad libitum.*

RESULTS AND DISCUSSION

The present study proved that the selection parameters and nutritional treatments stimulated significantly the genetic potential of local chicken as a local meat chicken with high standard of production. Based on the parameter of growth development, this study showed that the selection program resulted in two types of local meat chicken: medium and heavy types. Population of selected local meat chicken was higher than population of eliminated chickens (18.20% - 22.54% of 386 chickens). In addition, during the first step of selection program, it was observed that the mortality rate was very low (3.50%). It indicated that the local chickens were better adapted to the treatment. This condition was supported by the quality of rations, the suitability of maintenance management, and vaccination program during selection program.

In this research, the change in exterior body is the main selection parameter which is correlated with the growth and development of local meat chicken. The study indicated that weight gain and exterior parameters of derivative 1 (F1) of male local meat chicken were achieving standard size over 4 months. The final body weights of male chicken were 1.47 kg (heavy type) and 1.26 kg (medium type), respectively. These results indicated that classification of chicken, based on weight grouping as external selection criteria since the early ages, affected the achievement of final weight in male selected local chicken. It showed that selection based on body size groupings based on the exterior and initial body weight correlated positively to the growth of male local chicken. This result is consistent with previous findings that the maximum weight gain could be obtained at the level of a certain age (Wartomo *et al*., 1977; Gunawan and Hetzel, 1983).

In this study, the commercial weight of local meat chicken (>1 kg) was reached at the age of 3 months (1.09 kg) and increased significantly at the age of 4 months (1.26 kg). Beranger (2007) and Berri *et al*. (2001) reported there were parts of the body on local chicken which are responsive to the increase with age. Meanwhile, the nutrients availability could affect the achievements of final weight. However, the maximal body weight was stimulated by endocrine metabolic system a factor which contributes to growth (Guernec *et al*., 2004).

Table 2. Number and Type Distribution of Selected Chicken during First Step of Selection Program until 2 Months of Age

Criteria	Total (chicks)	(%)
1. Number of DOC / chicks	400	100.00
2. Mortality / chicks	14	3.50
3. Total Population / chicks	386	96.50
4. Number of Male / chicks	151	39.12
5. Number of Female / chicks	235	60.88
Post Selection:		
Male chicken :		
-Number of Heavy Type /chickens	54	35.76
-Number of Medium Type /chickens	63	41.72
-Number of Elimination /chickens	34	22.52
Female chicken :		
-Number of Heavy type / chickens	98	41.70
-Number of Medium type / chickens	94	40.00
-Number of Elimination / chickens	43	18.30

Table 3. Body weight gain and exterior body size of male local meat chicken until 4 month of age

Sex/ Type of Chicken	Exterior parameters								Final body weight (g)
	Age (month)	Thigh circum Ference (cm)	Skull broad (cm)	Width of chest (cm)	Height of body (cm)	Depth of body (cm)	Weight of breast mus-cle (cm)	Weight of thigh mus-cle (g)	
Heavy Male	1	7.3	2.3	17.8	18.7	7.3	37.47	48.57	357
	2	8.3	2.7	22	27.5	10.6	70.77	84.94	770
	3	10.6	3.2	23.5	30.2	11.6	174	227	1 093
	4	12.5	3.5	31	31.5	12.05	285.6	546.5	1473[a]
	Total	38.7	11.7	94.3	107.9	41.55	567.84	907.01	
	Mean	9.68[a]	2.93[a]	23.58[a]	26.98[a]	10.39[a]	141.96[a]	226.75[a]	
Medium Male	1	6.7	2.1	15.2	16.2	7	34.5	39.8	302
	2	7.1	2.4	19.3	24.5	9.4	65.4	74.3	645
	3	9.4	2.9	19.4	27.7	10.5	156	201	879
	4	11.1	3.1	29.5	28.2	11.3	235	523	1259[b]
	Total	34.3	10.5	83.4	96.6	38.2[b]	490.9	838.1	
	Mean	8.58[b]	2.63[b]	20.85[b]	24.15[b]	9.55[b]	122.73[b]	209.53[b]	

Table 4. Body weight gain and exterior body size of female local meat chicken until 4 month of age

Sex/ Type of Chicken	Exterior parameters								Final body weight (g)
	Age (month)	Thigh circum Ference (cm)	Skull broad (cm)	Width of chest (cm)	Height of body (cm)	Depth of body (cm)	Weight of breast mus-cle (cm)	Weight of thigh mus-cle (g)	
Heavy Female	1	4.8	2.15	13.5	15.3	5.7	18.57	22.74	209
	2	8	2.4	20.5	24.2	9	62.81	69.15	550
	3	9.1	2.9	21.3	25.2	9.4	122	122	889
	4	9.5	2.95	29	26.3	11.1	184.6	460.4	1297[a]
	Total	31.4	10.4	84.3	91	35.2	387.98	674.29	
	Means	7.85[a]	2.6[a]	21.075[a]	22.75[a]	8.8[a]	96.99[a]	168.57[a]	
Medium Female	1	4.2	2.1	11.2	15.3	5.2	16.5	21.6	189
	2	7.3	2.3	18.5	24.2	7.6	56.2	64.2	473
	3	8.7	2.4	19.2	24	8.6	111.7	120.1	657
	3	8.7	2.4	19.2	24	8.6	111.7	120.1	757
	4	8.9	2.7	26	26.3	10.4	176.9	421	1106[b]
	Total	29.1	9.5	74.9	89.8	31.8	361.3	626.9	
	Means	7.28[b]	2.375[b]	18.73[b]	22.45[b]	7.95[b]	90.33[b]	156.73[b]	

The phenomenon of growth model on local male chicken was followed by the same pattern in female local meat chicken. The exterior parameter differed significantly between heavy type and medium type of female chicken until 4 months of age. At the age of 4 months, the female heavy type reached 1.30 kg of final body weight, while medium type was 1,11 kg. This result indicated that weight grouping as external selection criteria since the early age affected the final body achievement and exterior parameters on female local chicken.

In a series of selection method of local meat chicken were also carried out to adjust the protein requirement as a major environmental factor that influence overall gene expression and certain body parts that became the main parameters of the study. Based on previous research (Yaman and Sari, 2004) that observed the protein requirement in local chicken was different caused by differences in gender, type of chicken and growth patterns. In chicken, the lower protein content in ration stimulating the amount of consumption (Yaman *et al.*, 1998; 2000a and 2000b). The influence of differences in crude protein ration on female of local chicken significantly affected growth development and final weight until the age of 16 weeks in line with the increased availability of protein in the ration (Table 5 and 6). The female required higher than that it in male probably due to avian species require a high protein body and mature sex. In this phase, female chicken need a higher protein intake to achieve weight loss of productive and reproductive organs towards the preparation of egg-laying age.

In conclusion, the protein requirement of local meat chicken during selection program was significantly different between male and female. The female chicken still

Table 5. The effect of protein percentage in ration on final body weight of male and female local meat chicken until 5 month of age

Protein Content (%)	Final Body Weight (gram)	
	Male	Female
17.0	1543[a]	1245[a]
19.0	1804[c]	1332[a]
21.0	1753[b]	1421[b]

requires a high enough protein for growth and preparation of the reproductive organs until the age of 4 months. In contrast, the male local meat chicken fed with 19% of protein achieved the commercial weight at the age of 4 months. The genetic expression in chicken is the result of internal and external factors in which the nutritional factor plays important role on growth achievements (Kino, 1993: Kato *et al*.,1992: Gondwe *et al*., 2006: Musa *et al*., 2006).

CONCLUSION

Selection of the exterior parameter was able to classify the candidate of local meat chicken into 2 types with specific growth model: heavy and medium types. Based on the parameters that were developed in this study, the local meat chicken has a huge potential to become oriented superior meat chicken. Growth patterns of male and female of selected chicken had a significantly difference in final body weight and exterior size. The male chicken achieved commercial weight since the age of 3-4 months (<1.0 kg), while females have a tendency to continue to grow after 4 months of age. In male chicken fed 19% of protein resulted better performance, while female chicken growth in line with the increasing availability of protein in the ration. The provision of 21% protein in female chicken resulted in optimal growth until the age of 4 months. The next program of present study will focus to achieve uniformity of the derived variation with the higher production on F3.

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The Evaluation of Rumen Metabolism of Fries Holstein (Fh) Calves Fed Biofermented Cocoa Pods Using *Phanerochaete chrysosporium*

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ABSTRACT

An *in vivo* experiment was conducted to evaluate cocoa pods to substitute forages for ruminant. The experiment was carried out using latin square design on 5 head of FH calves with 5 treatments and 5 replications. Ration was designed iso-protein (17%) and iso-TDN (65%) used cocoa pod as forages (35%) and other cocoa by-product were used as concentrate (65%). The treatment consisted of concentrate plus untreated cocoa pods (R1); urea ammonia treated of cocoa pods (R2); silage of cocoa pods (R3); bio fermented of cocoa pods using rumen content (R4) ; and bio fermented of cocoa pods using *P chrysosporium* (R5). Variables measured were pH, N-NH3, VFA, Microbial Protein, Alantoin, Non Glucogenic Ratio (NGR), Ration Utilization Efficiency (EPR) , Net Protein Utilizatin (NPU) . Data were analyzed using analysis of variance and Duncan multiple range test was further used to test the significant differences. Results showed that rumen metabolism variables such as pH, N-NH3, VFA, Microbial Protein on ration consisted of cocoa pods bio fermented by *P. Chrysosporium* were increased ($P<0.01$) compared to the others. There was positive correlation between microbial protein and alantoin. Microbial protein and alantoin excreted to urine indicated that there was an increase of rumen microbe population, while NGR value had positive correlation with methane production (CH4). Ration containing cocoa pods bio fermented by *P. Chrysosporium* showed the lowest NGR as indicator for optimum ration utilization efficiency for animal growth. It was concluded that cocoa pods bio fermented by *Phanerochaete chrysosporium* Burdsall ATCC 34541 is potential to be used as forages replacing elephant grass.

Key words: cocoa Pod, bio fermentation, Phanerochaete chrysosporium Fungi, rumen metabolism

INTRODUCTION

The shortage of agricultural land and low quality of the forages and roughages, encourage us to use waste such as cocoa plantations (Theobroma cacao L.) as energy source for ruminant. In Indonesia, Cocoa Plant Area is almost 1.5 millions Ha in 2008 and it produced 75% Cocoa Pod as by product. Utilization of cocoa pods as mulch around plants can be a host for growth of fungus *Phytophthora palmivora* known as Black Pod Diseases (Awuah and Frimpong, 2002) which can disrupt the development of cocoa plants. This fungus causes late blight, leaf blight and the cancer stem in cocoa plants. Nutrient quality of cocoa pods is equal to elephant grass, with 53.3% of TDN (Aregheore, 2002). Cocoa pods is potential as forage sources for ruminant, which have energy sources such as hemicelluloses and cellulose. Cacao pods contain approximately 6.28% protein; 39.9% crude fiber; 1.61% crude fat; 82.84% NDF, 78.74% ADF and 35.27% lignin (Laboratory of Feed Science and Technology IPB, 2005). Inhibitor factor in utilizing cocoa pods as feedstuff is high water content (85%) and lignin and also contains alkaloid *theobromine* (Tequia *et al*., 2004).

Utilization cocoa pods as energy sources requires decomposition of lignin with polysaccharide bond becomes a simple product. Ration in high lignin can decrease consumption, ration digestibility and animal performances. Benefit values of cocoa pods as energy sources for animal could be improved by degradation of lignocelluloses bonds with biofermentation or ammonization (Taherzadeh, 1999). Lignin degradation could be done with bioprocesses by the ligninolytic fungi such as *Phanerochaete chrysosporium* (Amjed *et al.*, 1990) and rumen bacteria (Akin and Benner, 1988). P. *chrysosporium* fungi is one of the microorganisms White-rot fungi that can degrade lignocelluloses (Takano *et al*., 2004; Coulibaly *et al*., 2003). Lignin degradation by fungi involved ligninolyt-

ic enzyme activity such as lignin peroxidase (LiP), mangan peroxidase (MnP), and laccase (Takano *et al*., 2004). Fermentation of cocoa pods by P.*chrysosporium* is able to decrease 18.36% lignin content (Laconi, 1999). Digestibility and nutrient metabolism process depends on the amount of rumen microbe and it's enzyme activities. Volatile fatty acid (VFA) is end product of carbohydrate hydrolysis namely acetatic acid, propionic acid, and butyric acid. Energy and protein balance on ration is needed by microbes to synthesize protein microbe. This research was carried out to observe rumen metabolism variables on FH calves fed biofermentation cocoa pod using *P. chrysosporium* such as Total and Partial VFA Concentration, N-NH3 concentration, microbial protein, and urine allantoin and also to evaluate nutrition quality of ration containing biofermentation cocoa pod.

MATERIALS AND METHODS

Experimental Rations and Animals

Ration was designed iso-protein (17%) and iso-TDN (65%) used cocoa pod as forages (35%) and cocoa seed shell, cocoa powder and palm kernel meal were used as concentrate (65%). The treatments consisted of concentrate plus untreated cocoa pod (R1); 1.5% urea ammonia treated of cocoa pod (R2); silage of cocoa pod (R3); biofermented of cocoa using 3.5% rumen liquor (R4) and cocoa pod bio fermented by *P chrysosporium*. Five rations were used cocoa pod as a sources of forages (35%) and other cocoa by-product were used as concentrate fed on *in vivo* research of 5x5 latin square design five head of FH calves.Ration in pellet form and fed twice each day and *ad lib* drinking water.

Experimental Procedures

The experimental design was Latin square design on 5 head of FH calves (95-100 kg body weight) with 5 rations as treatment and 5 time period as replication. Each treatment had 20 days of preliminary and 10 days for data collecting. The variables measured were rumen metabolism variables such as pH, N-NH3 (Micro diffusion Conway Technique), VFA-Total (steam distillation technique), VFA-Partial (Gas Chromatography Technique), microbial protein synthesis (SPM) by rate of incorporation ^{32}P tracer counting (Swandyastuti, 1986), urine allantoin (AOAC, 1984), methane production by Non Glucogenic Ratio (NGR) approach and calves average daily gain (kg/day). While ration quality such as Biological Value (BV), ration utilization efficiency (EPR) and Net Protein Utilization (NPU) were calculated. Data were analyzed using analysis of variance and Duncan multiple range test was further used to test the significant differences (Steel and Torrie, 1980).

Partial VFA concentration was analized using gas chromatography techniques. Rumen liquor taken by stomach tube was filtered and 5 ml of this liquor was added 1 ml protein coagulant (metaphosphoric acid), centrifuged 10 000 rpm for 15 minutes on temperature 40^0C. Amount 1µ supernatant was injected into the gas chromatograph. The calculation of the partial VFA concentration rumen liquor using equation:

VFA-Partial (mM) = (Sample Area /Standard Area) x Fp x Standard Concentration

Analysis of allantoin urine (AOAC, 1984), using phosphortungstic acid to deproteination. Phosphortungstic acid solution (1.5 g/5ml aquadest) was added 5 ml urine sample, centrifuged at temperature of 40^0C for 90 minutes until clear. Pb-acetate was added 5 ml and centrifuged, added again 5 ml of H_2SO_4 5%, centrifuged until homogenize. Amount of 2 ml homogenized sample was inserted into the Follin-Wu tube 100 ml volume, neutralized with 100 ml 5% NaOH pH 7.0. Folin ammoniacal copper added 2 ml and water bath heating for 10 minutes, cooled, add 2 ml molibdic acid and 2.4 -- dinitro phenil hydrazine (2,4-DNPH), conducted reading by Spectrophotometer with 520 nm wave length. Allantoin standard solution created for the 1 mg compared with the standard. Calculation allantoin urine levels using equation:

Allantoin (mg/100 ml)={(allantoin standard/alantoin sample) x 1 x 100/5}

RESULT AND DISCUSSION

Cocoa pod contains lignocelluloses composed of celluloses and hemicelluloses are bound by lignin. Lignin contains potential energy, but very hard to revamped by rumen microbes, especially the aromatic ring solution. Improving the nutritional value of cocoa

pod through the application of technology 1.5% urea ammonization and bio fermentation with fungus *Phanerochaete chrysosporium* Burdsall ATCC 34,541 were significantly (P<0.01) decrease NDF, ADF and lignin and increasing crude protein and Beta-N (P<0.05) (Laconi, 1999). Lowest lignin content of the cocoa pod bio fermentation *Phanerochaete chrysosporium* ATCC Burdsall was 31.66%. Cocoa pod biofermentation *P. chrysosporium* can break and soften the fiber cell walls of cocoa pod effectively, so that micro fibril ribbons can be easily digested by rumen microbes. Digestibility of high fiber rations needs cooperation among rumen microbes; higher fibrolytic activity of rumen fungi which can penetrate cell wall fiber rations and create access for rumen bacteria. Increasing nutrient digestibility gave implication that rumen microbial population are not disrupted, this showed that rations made from cocoa waste and palm kernel waste can provide good environment for rumen microbial growth in the rumen. Delignification can reduce lignin and increase the surface area of cell wall of high-fiber rations and easier penetration process.

Rumen Metabolism and Allantoin Urine

The increasing of rations fermentation can be done by providing a source of carbohydrate and nitrogen balance and sustainable in the rumen. The influence of cocoa pod processing treatment on rumen metabolism variables such as pH, N-NH3 concentration, total VFA concentration, synthesis of protein microbe, allantoin urin and gas non glucogenic ratio (NGR) are given in Table 1.

Result showed that rumen metabolism variables (NH3, VFA, Microbial protein) on ration consisted of bio-fermented cocoa pods by P. *chrysosporium* were increased (P<0.01), but rumen liquor pH in the normal range of 6.06 to 6.38, where cellulolytic microbes can live in the rumen (Jean-Blain, 1991). Dynamics concentration of ammonia and total VFA in rumen liquor illustrates effectiveness of the fermentation process. Concentrations of ammonia ranged from 4.18 – 6.30 mM was lower than that recommended by Mc Donald *et al*. (2002). This reflects the fermentation process work better or protein in the ration difficult to be degraded in the rumen. Total VFA concentrations between treatments was significant different (P<0.01), ranged from 85.50-114.74 mM. This value is still within the range of VFA concentrations that support the optimum conditions of 60-120mM (Waldron *et al.*, 2002). Microbial protein synthesis (SPM) describes the contribution of microbial protein to the animal host. Rations with cocoa pod bio fermented by *P.chrysosporium* had the highest yield of microbial protein synthesis (SPM) values (520.44 g /d/ head) and urine allantoin 5.10 g/head. Allantoin is intermediate metabolite from rumen bacterial digestion in the small intestine. There was positive correlation between microbial protein and allantoin. Increase of microbial protein and allantoin excreted to urine as indicator that there was an

Table 1. Rumen metabolism variables on various rations

Parameters	Treatments					P Values
	R-1 Control	R-II Ammoniation	R-III Silage	RIV Silage of Rumen Content	R-V P.*Chrysosporium*	
Rumen Metabolism						
pH Rumen	6.06	6.26	6.21	6.15	6.38	NS
N-NH_3 (mM)	4.69^{b}	6.30^{b}	4.18^{b}	4.84^{b}	5.90^{a}	0.01
T- VFA (mM)	85.50^{b}	120.62^{a}	90.23^{b}	102.77^{b}	114.74^{a}	0.01
Protein Microbe (g/h) (SPM)	253.23^{b}	298.90^{b}	317.5^{b}	330.54^{b}	520.44^{a}	0.01
Allantoin (g/h)	3.32^{bc}	3.98^{b}	3.69^{bc}	2.85^{c}	5.10^{a}	0.01
Non Glucogenic Rasio (NGR)	3.23^{b}	3.15^{b}	3.69^{ab}	4.44^{a}	2.86^{b}	0.05
Blood Glucose (mg/100 ml)	68.80^{c}	91.80^{a}	67.80^{c}	68.00^{c}	78.40^{b}	0.05

Note: Different superscript in the same row indicates significantly different (P <0.05) and (P <0.01).
RI = 65% concentrate +35% Cocoa Pod; R-II = 65% concentrate +35% Cocoa Pod Urea Ammonization ; R-III = 65% concentrate +35% Silage Cocoa Pod; R-IV = 65% concentrate +35% Cocoa pod bio fermentation Rumen liquor; and RV = 65% concentrate +35% Cocoa Pod bio fermentation *P chrysosporium* Fungi.

increase of rumen microbial population. Blood sugar as the main energy source of organ function. The range of blood glucose concentration was 68.00-74.40 mg/100ml. It was still in normal category fulfilled energy sources required for normal function of animal organs. Concentration of total VFA reflects the balance of production rate and it's usage in the rumen. Partial Volatile Fatty Acid (P-VFA) concentration is influenced by the composition of the feed in the ration (Table 2).

Ration Quality

Propionic acid concentration increased in rations containing cocoa pod bio fermented by *P. chrysosporium*, whereas the C2/C3 ratio was not significantly different with the control (P>0.05). Ration fermentation system in the rumen that leads to the synthesis of propionate which use many H2 gas will influence available free H2 gas and reduce formation of methane (CH4) gas. Reduction non glucogenic ratio as an indicator decrease production of methane gas (CH4). NGR values have positive correlation with production of methane gas (CH4). Non Glucogenic Ratio (NGR) of ration with cocoa pod bio-fermented by *P. chrysosporium* was the lowest (2.26) but it is still in the range of 2.25-3.00. It was an indicators of optimum utilization efficiency of ration on growing period of FH calves. Ration with cocoa pod biofermented by *P. chrysosporium* can increase microbial protein synthesis as a contribution to the host protein and propionic acid synthesis. Propionic acid as a precursor formation of muscle meat. NGR value was the lowest (2.86) obtained in the range 2.25-3.00 as for growth and fattening cattle. Ration quality of various treatments are presented in Table 3.

The indicator of rations protein quality is reflected by biological value (BV). Application of processing cocoa pods as a forage sources did not significant affect biological value, but significantly (P<0.05) increased net protein utilization (NPU) and ration utilization efficiency (EPR). Ration contained the cocoa pod bio-fermented by *P. chrysosporium* had the highest value of NPU (53.30%) and EPR (0.29). In management of livestock production, EPR value as the basis of the decision making, greater value of the EPR

Table 2. Total and Partial Volatile Fatty Acid (P-VFA) concentration at various ration

Parameters	Treatments					P Values
	R-1 Control	R-II Ammoniation	R-III Silage	RIV Silage of Rumen Content	R-V P.*Chrysosporium*	
T- VFA (mM)	85.50^{b}	120.62^{a}	90.23^{b}	102.77^{b}	114.74^{a}	0.01
VFA Partial (mM)						
Acetate (C2)	63.31^{b}	73.86^{a}	69.71^{a}	69.51^{b}	72.50^{b}	0.05
Propionate (C3)	22.51^{b}	26.59^{a}	22.98^{b}	19.69^{b}	**29.34**^{a}	0.01
Butyrate (C4)	5.10	5.25	5.74	6.09	5.05	NS
Ratio C2/C3	2.84^{b}	2.80^{b}	3.24^{ab}	3.88^{a}	2.54^{b}	0.05

Table 3. Nitrogen retention and ration quality of various treatments

Parameters	Treatments					P Values
	R-1 Control	R-II Ammoniation	R-III Silage	R-IV Silage of Rumen Content	R-V P.*Chrysosporium*	
Nitrogen Retention (g/kg BB $^{0.75}$/h)	1.06^{b}	1.45^{a}	1.12^{b}	1.16^{b}	**1.60**^{a}	0.01
Ration Quality						
Ration utilization efficiency (EPR)	0.17^{b}	0.31^{a}	0.20^{b}	0.15^{b}	**0.29**^{a}	0.01
Biological Values ,BV (%)*	97.03	96.62	96.59	96.90	96.11	NS
Utilization Protein Net, NPU (%)**	40.58^{b}	50.14^{ab}	41.38$_{b}$	42.99^{ab}	**53.03**^{a}	0.05
Average Daily Gain (kg/h)	0.76^{b}	1.56^{a}	0.94^{b}	0.75^{b}	1.46^{a}	0.01

would be advantageous because it can reduce feed costs as the biggest cost component in production of livestock.

CONCLUSION

Cocoa pod biofermented by *Phanerochaete chrysosporium* Burdsall ATCC 34541 is potential used as forage sources replacing elephant grass and other by product of cocoa and palm kernel oil as concentrate for fed calves in growing period.

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The Relationship Between Ruminal Macro Mineral Solubility and Fermentability of Selected Tropical Legumes Tree With Mineral Absorption on Local Sheep

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ABSTRACT

A research to study relationship between macromineral solubility, fermentability of some tree legumes with *in vivo* digestibility using local sheep has been conducted. In the first experiment, five tropical legume trees such as *Pterocarpus indicus* (PI), *Sesbania gradiflora* (SG), *Gliricidia sepium* (GS), *Callyandra callotyrsus* (CC) and *Leucaena leucocephala* (LL) were used. A modified *in vitro* technique was used to determine degradation, fermentation and macromineral bioavailability of selected legume trees. The gas production was measured using Hohenheim method. The ruminal DM degradation and the cumulative gas productions were calculated using a model of $Y = a + b\ (1\text{-}e^{-ct})$ following method of Ørskov and McDonald (1979). The second experiment aimed to evaluate macro mineral abroption of the legumes. The *in vivo* digestibility was measured using eighteen male local sheep. The animals were divided into 6 groups with 3 replications (A = native grass as a control, B = Ration A + 20% PI, C = Ration A + 20% SG, D = Ration A + 20% GS, E = Ration A + 20% LL and F = Ration A + 20% CC). The result showed that biodegradation and cummulative gas production of selected legume trees were not significantly different. However, the gas production rate of SG and GS were significantly higher. No difference was observed on VFA production of the legumes, while the NH_3 production was different. Legume SG produced more NH_3 than other tree legumes. In general, the ruminal Ca solubility was higher than P and Mg. The solubility of Ca and Mg of legume LL were higher than other legumes, while the solubility of P from legume LL was the highest. The *in vivo* experiment showed that digestibility of the ration containing 20% of tropical legume trees was no difference. However, the DM and OM consumption of ration were significantly different. The DM and OM consumption of ration D was higher (398 and 347 kg head $^{-1}$day^{-1}) than other rations. The absorption of mineral Ca and P from ration E was higher than other legumes.

Key words: *tropical tree legumes, macromineral, solubility, fermentability and in vivo digestibility*

INTRODUCTION

In tropical countries, legume is normaly used as protein sources to increase the quality of grasses in ruminant ration. Legume is adaptable in a wide range climate and soil condition, even under heavy grazing (Khamseekhiew, 2001). Legume tree is more adapted in tropical climate. The mineral content of legume was higher than grass (Underwood and Suttle, 1999). Sutardi *et al*. (1994) reported that legume trees had macro mineral especially calcium and therefore the legumes tree can be used as mineral supplement.

In ruminant, minerals are required not only for the animal it's self but also for the activity of rumen microbes. The mineral are used for cellulolytic microbe activities, osmotic pressure, buffering capacity, and reduction potential in the rumen (Duran and Kawashima, 1990). Therefore, in assessing mineral requirements of ruminant, both the quantity of minerals in the feeds and their bioavailability need to be considered. A method that can be used to evaluate the mineral availability in the rumen is *in vitro* technique. A modified *in vitro* technique can measure the extent and rate of release of macrominerals (especially Ca, P. Mg and S) in the rumen where most of organic matter digested.

The aim of this research was to study DM degradability, fermentability, gas production and solubility of macro mineral (Ca, P. Mg and S) of selected legumes tree using *in vitro* and *in vivo* method.

MATERIALS AND METHODS

Materials

Five tree legumes namely *Pterocarpus indicus* (PI), *Sesbania gradiflora* (SG), *Gliri-*

cidia sepium (GS), *Callyandra callotyrsus* (CC) and *Leucaena leucocephala* (LL) were used in this experiment. The samples were cut about 10-30 cm from growing point and dried at 60°C and then ground through 2 mm screen sieve.

Dry Matter Degradation

Ruminal DM degradation was determined according to a modified method of Tilley and Terry (1963). Rumen liquid was collected from a sheep using vacuum pump. The samples (1.0 g) were placed in 50 ml fermentor tubes and suspended anaerobically with 8 ml of rumen fluid and 12 ml of phosphatecarbonate buffer. All samples were prepared in duplicates. The suspension was incubated at 39°C for 3, 6, 12, 24, 48 hours in shakerbath. Every 12 hours the samples were gassed with CO_2. After the specified incubation incubation, the samples were centrifuged at 3,000 rpm for 15 min. The supernatant was collected and the residues were filtered with filter paper and washed with boiled water. After that, the residues were dried at 60°C for 48 hours. Samples in tubes without fermentation (0 h) were washed and dried in similar manner as the above samples as a control.

Gas Production

Gas production was measured using Hohenheim Gas Method (Close and Menke, 1986). Samples (230 mg) were put in syring glasses and then added 30 ml suspension of rumen fluid mix with buffer. The samples were incubated in water bath at 39°C for 3, 6, 12, 24 and 48 hours. The total gas productions were measured. The kinetic of gas productions were calculated following method of Ørskov and McDonald (1979).

In vivo Experiment

In vivo experiment digestibility was measured using 18 male local sheeps with average body weight was 15 kg. The animals were divided into 6 groups with 3 replications. The animals were kept in individual metabolic cages for 4 weeks (2 week for adaptaion period, followed by data collection period for 2 weeks). The rations used in this experiment were:

- Ration A = 100% Native grass as a control
- Ration B = 80% Native grass + 20% *Pterocarpus indicus* (PI)
- Ration C = 80% Native grass + 20% *Sesbania gradiflora* (SG)
- Ration D = 80% Native grass+ 20% *Gliricidia sepium* (GS)
- Ration E = 80% Native grass + 20% *Leucaena leucocephala* (LL)
- Ration F = 80% Native grass + 20% *Callyandra callotyrsus* (CC)

During data collection period, diet and fecal were totally collected. The diet and fecal samples were dried under the sun and in oven (60°C) for 24 hours, then analysed for dry matter, Ca and P content. The paramenters in this *in vivo* experiment were feed consumption, DM digestibility and mineral absoption.

Chemical Analysis

The nutrient compositions of legumes trees were determined by the AOAC (1984) procedures. The DM content of all tree legumes and residue collected in the *in vitro* experiment were also determined. Then, they were digested with nitric and percloric acids using wet ashing method for determination of Ca and P. The prepare solution were analyzed for Ca, Mg and S using Atomic Absorption Spectrophotometric, and for P using a UV-Visible Spectrophotometer.

Total VFAs of supernatan were analyzed using steam distilation method, while NH_3 were analysed using Conway's micro diffusion method.

Statistical Analysis

Cumulative gas production and ruminal DM degradation rate were evaluated mathematically as a function of incubation time according to the method of Ørskov and McDonald (1979). The equation was $P = A + B (1 - e^{-ct})$, where: P is actual degradation at time t, A is water soluble fraction, B is the insoluble but potentially degradable fraction in time t, and c is degradation rate of B (% h^{-1}) and t is incubation time (h).

The data were subjected to analysis of variances using the general linear model procedure of the SPSS package program. The differences between means were tested using the contrast analysis.

RESULTS AND DISCUSSION

Chemical composition of legume trees

The chemical composition of legumes tree was shown in Table 1. The CP content of tree legumes varied from 19.97 - 24.09%,

where the CP of LL was relative the highest concentration of Ca contents ranged from 1.02% (CC) to 1.84% (LL). In the tropic, the Ca content of legumes tree was relatively higher than grasses (Serra *et al*. 1996). The P content of the legumes tree ranged from 0.27% (CC) to 0.41% (SG). The P content of legumes trees were also much close to that reported by Serra *et al*. (1996).

In Vitro

Ruminal Dry Matter Degradation

The ruminal DM degradation of selected tree legumes were shown in Table 2. The data showed that at 3 hours all legumes were degraded in the same level, while from 6 to 24 hours the DM degradation of GS and SG were significantly higher (P<0.05). However, after 48 hours the DM degradation of all legumes was not significantly different

The water soluble fraction (A), the insoluble fraction (B), the potential degradation and the degradation rate of all legumes were not significantly different. The potentially degradability of the legumes as source of CP and macro minerals were the same.

Gas Production

Gas (carbondioxide and methane) is a waste product of ruminal fermentation. In this experiment, the cumulative gas production was measured using Hohenheim Method. The cumulative gas productions of selected legumes trees were shown in Table 3. The cumulative gas production for 24 hours varied from 12.71 ml (CC) to 23.7 ml (SG). For 48 hours, legum GS produced gas relatively higher (28.2 ml) compare to CC (17.6 ml).

The gas production rate (C) of SG and GS were significantly higher than PI, LL and CC (P<0.05). The gas production rate of LL and CC was low due to content of tannin and mimmosin (Keir *et al*., 1997). However, the potential gas production (A+B) for all legumes was not significant (P>0.05). The gas productions correspond to ruminal DM degradation as described above.

Table 1. Chemical composition (% based on DM basis) of *Pterocarpus indicus* (PI), *Sesbania gradiflora* (SG), *Gliricidia sepium* (GS), *Leucaena leucocephala* (LL) and *Callyandra callotyrsus* (CC)

Parameter	Legumes ---------- % ----------				
	PI	SG	GS	LL	CC
Organic matter (OM)	92.54	90.52	91.20	91.89	94.88
Crude protein (CP)	23.95	26.18	21.46	25.50	21.50
Ether Extract (EE)	1.58	1.65	2.39	3.39	1.66
Crude fibre (CF)	27.64	27.07	22.81	20.69	22.37
NFE	39.38	35.62	44.53	42.31	49.35
Calsium (Ca)	2.01	1.14	1.68	1.50	1.18
Phosphorus (P)	0.40	0.30	0.31	0.43	0.36
Magnesium (Mg)	0.45	0.46	0.47	0.50	0.45

Table 2. Ruminal DM degradation (%) of *Pterocarpus indicus* (PI), *Sesbania gradiflora* (SG), *Gliricidia sepium* (GS), *Leucaena leucocephala* (LL) and *Callyandra callotyrsus* (CC)

Parameter	Legumes				
	PI	SG	GS	LL	CC
Ruminal DM degradability					
3 hours	16.28	17.00	17.72	18.04	17.37
6 hours	17.36^{b}	19.72^{a}	22.23^{a}	21.05^{a}	17.67^{b}
12 hours	25.70^{b}	28.36^{a}	29.01^{a}	26.50^{b}	23.85^{b}
24 hours	33.63^{a}	34.63^{a}	33.12^{a}	29.98^{b}	29.66^{b}
48 hours	39.39	42.04	41.50	40.12	37.67
DM degradability parameter (%)					
A	10.58	11.86	14.42	16.44	14.03
B	31.59	32.55	29.34	35.05	31.87
(A+B)	42.17	44.31	44.76	51.49	45.90
C (% h^{-1})	0.052	0.053	0.049	0.023	0.028

Note: Means in the same row with different superscripts are significantly different (P<0.05).

Table 3. In vitro gas production (ml) of *Pterocarpus indicus* (PI), *Sesbania gradiflora* (SG), *Gliricidia sepium* (GS), *Leucaena leucocephala* (LL) and *Callyandra callotyrsus* (CC)

Parameters	Legumes				
	PI	SG	GS	LL	CC
Cumulative Gas production (ml)					
3 hours	3.8	4.0	6.0	4.5	3.8
6 hours	7.6	9.5	10.2	6.9	5.5
12 hours	13.7	16.9	16.6	11.1	8.4
24 hours	21.4	23.7	23.6	16.7	12.71
48 hours	27.9	27.0	28.2	22.2	17.6
Gas production parameter (ml)					
A	0.45	0.00	1.46	1.53	1.60
B	26.4	27.9	28.3	24.9	34.3
(A+B)	36.9	27.9	29.8	26.4	35.9
C (% h^{-1})	0.05^{b}	0.10^{a}	0.07^{a}	0.04^{b}	0.04^{b}

Note: means in the same row with different superscripts are significantly different (P<0.05).

VFAs and NH_3 production

Total VFA production at 12 hours incubation varied from 39.98 to 96.82 mM (Table 4). According to Hungate (1966) the total VFA concentration was lower than the optimum level (11m mM). At 12 hours GS and SG produced total VFA was relatively lower than another legumes.

At 12 hours LL and CC produced total VFA relatively lower than another legumes, however after 24 hours incubation, LL and CC produced more total VFA. The data showed that rumen microbes need more time to degraded of LL and CC.

Ammonia (NH_3) is a product of protein degradation with rumen microbe. The CP content of SG was higher than another legumes. The CP content correlated with the NH_3 concentration. As shown in Table 5, the ammonia concentration after 12 hours incubation varied from 2.51 mM (CC) to 28.83 mM (SG). After incubation for 24 hours the concentration increased. The NH_3 production of SG after incubation for 12 and 24 hours was significantly highest (P<0.05). The indication correspond with Ørskov (1982), that the NH_3 production depend on protein solubility, protein content, time incubation and rumen pH. The optimum concentration for microbe growing was 6 - 21 mM (McDonald *et al*., 1995).

The correlation between degradation rate of selected legumes tree with production of VFAs and NH_3 were calcullated. The result showed that the degradation rate correlated negatively with VFAs production (r = -0.831). However, the degradation rate correlated closly with NH_3 production (r = 0.931). More ammonia was produced when the degradation rate more higher.

In Vivo

Macro Mineral Solubility

The procentage of macro mineral solubilization (Ca, P, Mg and S) were shown in Table 5. Generally the solubility of Ca was higher than P, Mg and S. The data showed that the solubilities of Ca and P at 6 and 24 hours incubation time were siginificantly different (P<0.05). The Ca solubility of LL was

Table 4. The production of total VFA and NH_3 of *Pterocarpus indicus* (PI), *Sesbania gradiflora* (SG), *Gliricidia sepium* (GS), *Leucaena leucocephala* (LL) and *Callyandra callotyrsus* (CC) after 12 and 24 hours incubation.

Parameter	Legum				
	PI	SG	GS	LL	CC
Total Volatile Fatty Acids (mM)					
12 hours	60.94	86.51	96.82	43.88	39.98
24 hours	52.75	52.94	69.89	83.65	72.20
NH_3 production (mM)					
12 hours	12.28^{b}	28.83^{a}	8.30^{c}	6.30^{d}	2.51^{d}
24 hours	19.91^{b}	39.55^{a}	13.33^{c}	11.30^{d}	3.49^{d}

Note: means in the same row with different superscripts are significantly different P(<0.05).

Table 5. The solubility of Ca, P, Mg and S of *Pterocarpus indicus* (PI), *Sesbania gradiflora* (SG), *Gliricidia sepium* (GS), *Leucaena leucocephala* (LL) and *Callyandra callotyrsus* (CC) after 6 and 24 hours incubation

Parameters	Legumes				
	PI	SG	GS	LL	CC
Ca (%)					
6 hours	80.44[c]	ND	94.87[b]	96.91[a]	95.49[b]
12 hours	85.08[d]	ND	96.12[c]	97.85[a]	97.32[b]
P (%)					
6 hours	98.44[a]	ND	76.59[b]	74.97[b]	76.55[b]
12 hours	93.93[a]	ND	81.83[b]	83.37[b]	83.45[b]
Mg (%)					
6 hours	61.36[b]	ND	57.62[b]	67.36[a]	62.48[b]
12 hours	77.61	ND	74.98	79.36	79.60
S (mg residu /g sample)*					
6 hours	1.400	ND	1.610	1.093	0.890
12 hours	1.295	ND	0.755	1.011	0.587

Note: means in the same row with different superscripts are significantly different (P<0.05); ND is not determined.

higher than CC, GS and PI. However, in contrast the P solubility of PI was the highest. The solubility of Mg of selected legumes tree was not significantly different. However, the solubilities of CC and LL tended to be higher than PI and GS. The data of S presented the ruminal insoluble S. CC had tendency less insoluble S compare to another legumes.

Feed Consumption and Macro Mineral Absorption

The dry matter and organic matter consumption of ration B, D and E significantly higher than A and C ration, while the consuption of ration F containing 20% *G. sepium* was the lowest The Ration D which containing 20% *G. sepium* has the highest palatability. However the digestibility of the rations containing 20% of legumes tree was no significant different compare to control.

Calcium and Phosphor Absorption

The consumption, excretion and absorption of Ca and P of ration containing 20% of legumes tree is shown in Table 7. The result

Table 6. Dry Matter and Organic Matter consumption of ration containing tree legumes

Ration*	Feed Consumption (g animal-1 day-1)	
	Dry Matter	Organic Matter
A	220.9[b] ± 106	190.4[b] ± 91.6
B	276.1[a] ± 29.1	242.0[a] ± 23.9
C	213.2[b] ± 72.4	184.7[b] ± 63.6
D	398.5[a] ± 19.9	347.4[a] ± 17.3
E	358.4[a] ± 62.5	313.7[a] ± 54.7
F	123.0[c] ± 58.7	107.7[c] ± 51.5

Note: A = native grass as a control, B = Ration A + 20% PI, C = Ration A + 20% SG, D = Ration A + 20% GS, E = Ration A + 20% LL and F = Ration A + 20% CC. Means in the same row with different superscripts are significantly different (P<0.05).

Table 7. The consumption, excretion and absoprtion of calcium and phosphor of ration containing tree legumes

Parameters	Ration					
	A	B	C	D	E	F
Calcium (g animal-1 day-1)						
consumption	1.44±0.69	1.81±0.19	1.39±0.47	1.90±1.23	2.34±0.41	0.93±0.38
excretion	0.98±0.18	1.33±0.30	0.92±0.16	1.98±1.42	1.61±0.24	0.81±0.43
absorption	0.46±0.33	0.38±0.65	0.47±0.32	-0.08±0.32	0.73±0.27	0.12±0.05
Phosphor (g animal-1 day-1)						
consumption	0.38±0.18	0.54±0.11	0.42±0.16	0.64±0.41	0.76±0.13	0.24±0.11
excretion	0.38±0.11	0.41±0.12	0.32±0.08	0.56±0.29	0.55±0.12	0.35±0.22
absorption	0.00±0.17	0.13±0.07	0.10±0.06	0.08±0.16	0.21±0.11	-0.11±0.11

Note: A = native grass as a control, B = Ration A + 20% PI, C = Ration A + 20% SG, D = Ration A + 20% GS, E = Ration A + 20% LL and F = Ration A + 20% CC.

showed that ration E containing 20% of *L. leucocephala* had the highest Ca and P absorption, while ration D (20% *G. sepium*) and F (*C. callotyrsus*) had low Ca and P absorption.

CONCLUSION

Ruminal DM degradation and cummulative gas production of selected legumes tree were not significantly different. The legumes tree had significatly different on macro mineral solubility. However, the highest Ca and P absorption was in ration containing 20% *Leucaena leucocephala*.

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Utilizing Potential Soil Microorganisms, Humic Acid, Grasses and Legumes Forages in Marginal and Degraded Lands in Indonesia

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ABSTRACT

Marginal and degraded lands in Indonesia are considerably extensive and include many kinds of soil, for instance acid soil and post mining soil. The efforts which can be conducted are the use of biological fertilizers, soil conditioners, grasses and legumes forages. Biological fertilizers such as arbuscular mycorrhizal fungi (AMF), phosphate solvent microorganisms (MPP), and nitrogen fixing microorganisms (MPN). The soil conditioners such as humic acid. The forages such as, *Pueraria phaseoloides*, *Centrosema pubescens*, *Panicum maximum*, and *Setaria splendida*. The objectives of this research were to obtain new formulation of biological fertilizers which constitute a consortium of AMF and MPP, MPN, humic acid which could increase its ability to supply nutrients and help to increase forage plant survival in less favourable environment; to get grasses and legumes tolerances in marginal and degraded lands. There were 6 formulas of biological fertlizers which were tested in this research, namely (a) AMF with addition of MPP isolates 1, 2 and 3; (b) AMF with addition of Azospirilum isolates 1, 2 and 3; (c) AMF with addition of Rhizobium isolates 1, 2 and 3; (d) AMF with addition of humic acid; (e) AMF with addition of MPP, Azospirilum or Rhizobium; (f) AMF with addition of MPP, Azospirillum or Rhizobium and humic acid. Legumes and grasses were used *Centrosema pubescens, Pueraria phaseoloides, Panicum maximum*, and *Setaria splendida.* The results showed that the four test plant species responded differently to the latosol and post gold mining. In general, the four kinds of plants were not supported by single bio-fertilizer, but requires a consortium of several types microorganisms and the results will be better when it was combined with humic acid. Growth response to the four types of plants in soil latosol was better compared to gold post-mining soil. At planting media from gold mine tailings many plants died, especially in the control treatment which was a treatment without the addition of bio-fertilizers.

Key words : soil potential microorganism, humic acid, legumes and grasses forages

INTRODUCTION

Marginal and degraded land in Indonesia was a lot, such as acid lands and post-mining lands. The existence of such lands in Indonesia is very high that covers 30% or 0.51 million km^2 of land area in Indonesia spread over the area of West Java, Sumatra, Kalimantan, Sulawesi and Irian Jaya. The main problems in acid soil are (1) decrease the solubility of P and Mo. (2) decrease the concentration of macro elements such as N, Mg, Ca and K. (3) increase concentrations of Al, Mn and Fe which can cause poisoning (4) inhibits root growth and water absorption, causing nutrient defficiencies, drought stress and increase nutrient leaching (Maschner, 1995). Post-mining land in addition to problems with acid conditions, as well as problems that may result is a heavy metal contamination.

The problems of acid soil can be overcome with the use of bio-fertilizers. Bio-fertilizers are the arbuscular mycorrhizal fungi (AMF), microorganisms solvent phosphate (MPP) and Nitrogen Fixing Microorganisms (MPN). Arbuscular mycorrhizal fungi can help plants to supply and absorp of elements of low P availability in acid soil and have ability to adapt to acid soil (Koslowsky and Boerner, 1989). Phosphate solvent microorganisms are soil microorganisms that can improve the provision of P in acid soil by producing organic acids so that the solubility of Al can be reduced because it is bound by organic acids (Illmer *et al*., 1995). Malate, citrate and oxalate are organic acids that have high affinity to metal having such as Al^{3+} and Fe^{3+} (Jones and Brassington, 1998; Karti, 2003). Nitrogen fixing bacteria like *Azospirillum* and *Rhizobium* are bacteria that can cause changes in root morphology, such as an increased number of hair root, root extension, and root surface area. Influence on plant morphology may be caused by the production of compounds that support plant growth pro-

duced by Azospirillum. The speed of absorption of N, P, K and the accumulation of dry weight was higher in corn, sorghum, wheat and setaria inoculated with *Azospirillum* (Okon and Kapulnik, 1986).

Humic acid is an organic material that is not degraded by microorganisms, have the ability to help provide nutrients for carboxyl and phenolic groups. Both these groups can adsorb soil cations and anions such as Al and Fe in acid soil, heavy metals and fertilizers that do not adsorb easily will be washed. Special nature and potential of other humic acid is its ability to interact with metal ions, oxide, hydroxide, and organic minerals, including toxic pollutants by forming an association.

The objective of this research was to obtain new formulation of biological fertilizers which constitute a consortium between AMF with MPP, MPN, and humic acid which could increase its ability for supplying nutrients and help to increase forage plant survival in less favorable environment. To get grasses and legumes tolerances in marginal and degraded lands.

MATERIALS AND METHODS

This research consists of 3 stages, namely: (1) potential microbial multiplication (AMF, MPP and MPN), (2) formulation of new biological fertilizers which is a consortium of microbiology with humic acid, (3) new formulations test on marginal and gold post-mining land at laboratory scale.

Stage 1: Preparation for augmentation material: a potential microbial multiplication (AMF, MPP and MPN). AMF propagated through the open pot culture with the media and the host zeolite grown sorghum. AMF propagation procedures follow the standards from Laboratory of Forests and Environment Biotechnology, which includes the inoculation technique, nutrition, maintenance and monitoring for 4 months. Propagation from MPP and MPN was performed using liquid media and with the help of shaker to obtain the desired population, and formulated in liquid carriers. The isolates have been selected in the laboratory of Forest and Environment Biotechnology. Humic acid in the form of liquid was added 1 ml for each polybag.

Stage 2: formulation of new biological fertilizer which is a consortium of microbiology with humic acid. There were 6 formulas of biological fertlizers which were tested in this research, namely (1) Control (without microorganisms), (2) AMF (M); (3) AMF with addition of Rhizobium or Azospirilum isolates 1, 2 and 3 (MR/A); (4) AMF with addition of humic acid (MH); (5) AMF with addition of MPP, Rhizobium or Azospirilum (MPR/A); (6) AMF with addition of MPP, Rhizobium or Azospirillum, and humic acid (MPR/AH).

Stage 3: New formulations test on marginal and gold post-mining land at laboratory scale. All the formulations obtained in the nursery were tested with Legum cover crop (LCC) and grasses with the condition that the marginal growth media (acid soil/latosol soil) and gold post-mining soil. Testing with the LCC and grasses should be done to determine the production of legumes and grasses. Containers were a 3-kg capacity pot soil. Tests conducted at nursery for 3 months. Legumes and grasses used *Pueraria phaseoloides*, *Centrosema pubescens*, *Panicum maximum*, and *Setaria splendida*

RESULTS AND DISCUSSION

AMF, MPP and MPN and propagule density testing methods using most probable number (MPN) and the results are presented in Table 1. MPN test can be performed to know the quality of the isolate to be used for further testing.

Table 1. Most Probable Number of four type inoculans

No	Inoculans	MPN
1.	AMF	95.68 active propagule/30 g inoculan
2.	MPP	1.7×10^{10} (per g inoculan)
3.	MPN (Azospirillum)	1.6×10^{9} (per g inoculan)
4.	MPN (Rhizobium)	5.3×10^{6} (per g inoculan)

Latosol land taken from Dramaga, Bogor district showed acid pH, low organic C content. The content of N, P, K, Ca, Mg was also low. Cation exchange capacity (CEC) is very low. The results of the analysis of Pongkor gold mine tailings, Bogor district generally had alkaline pH, organic C and nutrient elements such as N, P, K, Mg were very low. With P and K levels of high potential. High levels of Ca and Pb could affect the availability of P. Very low value of CEC may affect the exchange of positively charged nutrients,

namely micro-nutrient elements (Fe, Cu, Zn and Mn) which are essential minerals for plants.

Treatment M gave the best response for shoot dry weight for *Pueraria phaseoloides*, and *Setaria splendida* or *Panicum maximum* and the best response was MR and MA treatments. *Centrocema pubescens* did not give response (Table 2).

Treatment M gave the best response to the root dry weight in *Pueraria phaseoloides* and *Panicum maximum*, whereas treatment MP for *Centrosema pubescens*. *Setaria splendida* did not give response (Table 3).

Latosol land constraints for crop growth are acid pH, low organic C, P, N, K, Ca, Mg, but very high level in P and K. The addition of biological fertilizers such as nitrogen fixing microorganisms was expected to help provide the nitrogen through N2 fixation, so that one of the constraints on the availability of land latosol N elements can be resolved. Phosphate solvent microorganisms (MPP) will help P solubilization. Through the release of organic acids by microorganisms, it can dissolve P bound by inorganic minerals such as Al, Fe or Mn. The existence of arbuscular fungi arbuscular (AMF), available nutrient elements can be quickly absorbed through the external and internal hifa so that plants will always be provided the necessary nutrients for growth. Ensuring the availability of nutrients to the plants because of the addition of biological fertilizer will help overcome the obstacles on the latosol soil.

MR treatment and MPAH or MPA gave the best response to the shoot dry weight of *Pueraria phaseoloides* and *Panicum maximum* on a gold mine tailings, but did not gave response on *Setaria splendida* and *Centrocema pubescens* shoot dry weight (Table 4).

Table 2. The influence of treatments on shoot dry weight for *Pueraria phaseoloides, Centrosema pubescens, Setaria splendida and Panicum maximum* at latosol soil

Treatment	Shoot dry weight *Pueraria phaseoloides* (g/pot)	Shoot dry weight *Centrosema pubescens* (g/pot)	Shoot dry weight *Setaria splendida* (g/pot)	Shoot dry weight *Panicum maximum* (g/pot)
Control	7.412 ^{ab}	2.99	10.3 ^{b}	8.10 ^{ab}
M	8.016 ^{a}	3.08	11.0 ^{ab}	9.28 ^{a}
MR/MA	7.698 ^{ab}	2.78	12.2 ^{a}	9.60 ^{a}
MH	6.908 ^{ab}	3.02	10.7 ^{b}	6.56 ^{bc}
MP	7.102 ^{ab}	2.01	11.4 ^{ab}	7.50 ^{abc}
MPR/A	6.440 ^{b}	2.38	11.5 ^{ab}	5.48 ^{c}
MPRH	7.040 ^{ab}	3.00	11.4 ^{ab}	8.30 ^{ab}

Note: 1. M = Mycofer, MR/MA = Mycofer + Rhizobium/Azospirillum, MH = Mycofer + humic acid, MP = Mycofer + MPP, MPR/A = Mycofer + MPP + Rhizobium/ Azospirillum, MPR/AH = Mycofer + MPP + Rhizobium/ Azospirillum + humic acid;
2. Different superscript at the same column means significantly difference (P <0.05).

Table 3. The influence of treatments on root dry weight for *Pueraria phaseoloides, Centrosema pubescens, Setaria splendida and Panicum maximum* at latosol soil

Treatment	Root Dry weight *Pueraria phaseoloides* (g/pot)	Root Dry weight *Centrosema pubescens* (g/pot)	Root Dry weight *Setaria splendida* (g/pot)	Root Dry weight *Panicum maximum* (g/pot)
Control	2.660^{bc}	1.12^{ab}	13.94	5.08^{ab}
M	3.93 ^{a}	1.14^{ab}	12.30	6.62^{a}
MR/MA	3.484^{ab}	0.83^{bc}	16.1	4.90^{ab}
MH	2.276^{c}	0.82^{bc}	12.98	2.78^{b}
MP	2.83 ^{bc}	1.20^{a}	13.90	4.70^{ab}
MPR/A	2.454^{bc}	0.54^{c}	15.32	3.80^{ab}
MPRH	2.186^{c}	0.71^{c}	18.1	5.24^{ab}

Note: 1. M = Mycofer, MR/MA = Mycofer + Rhizobium/Azospirillum, MH = Mycofer + humic acid, MP = Mycofer + MPP, MPR/A = Mycofer + MPP + Rhizobium/ Azospirillum, MPR/AH = Mycofer + MPP + Rhizobium/ Azospirillum + humic acid;
2. Different superscript at the same column means significantly difference (P <0.05).

Table 4. The influence of treatments on dry weight for *Pueraria phaseoloides, Centrosema pubescens, Setaria splendida and Panicum maximum* at Gold Mining soil

Treatment	Shoot Dry weight *Pueraria phaseoloides* (g/pot)	Shoot Dry weight *Centrosema pubescens* (g/pot)	Shoot Dry weight *Setaria splendida* (g/pot)	Shoot Dry weight *Panicum maximum* (g/pot)
Control	0.240[b]	0.00	1.00	0.22[b]
M	0.82 [ab]	0.77	1.05	0.42[b]
MR/A	1.710[a]	0.74	1.32	0.48[b]
MH	0.476[ab]	0.84	1.08	0.70[ab]
MP	1.224[ab]	0.68	0.90	0.33[b]
MPR/A	0.874[ab]	1.18	0.88	1.04[a]
MPR/AH	0.506[ab]	0.81	1.22	1.04[a]

Note: 1. M = Mycofer, MR/MA = Mycofer + Rhizobium/Azospirillum, MH = Mycofer + humic acid, MP = Mycofer + MPP, MPR/A = Mycofer + MPP + Rhizobium/ Azospirillum, MPR/AH = Mycofer + MPP + Rhizobium/ Azospirillum + humic acid;
2. Different superscript at the same column means significantly difference (P <0.05).

MP and MH treatmens on gold post mining stimulated the best response to dry weight of roots in *Pueraria phaseoloides* and *Setaria splendida*, but it did not gave response on *Centrosema pubescens* and *Panicum maximum* root dry weight (Table 5). *Setaria splendida* grew better than other plants, because the grass can release organic acids like malate, oxalate and citrate on the soil, root and shoot (Karti, 2003).

In general the gold mine tailings needs all types of biological fertilizer for growth. Constraints for plant growth in gold mine tailings are alkaline pH, with very high Pb, and low organic C content and macro nutrients like N, P, K, Mg. However, P and K levels were high potential. Ca levels are also high, which may affect the availability of P. Very low value of CEC may affect the exchange of positively charged nutrients, namely micro-nutrient elements such as Fe, Cu, Zn and Mn which are essential minerals for plants. The addition of biological fertilizers such as nitrogen fixing and phosphate solubilizing microorganisms will help provide nutrients N and P. AMF will assist the absorption of nutrients that are available to the plants continuously, so the plants can grow better. Humic acid which is a soil conditioner will help in providing nutrients simultaneously, because the humic acid can help to adsorb the nutrients and then be easily removed through a process of exchange. Humic acids can help to bind the heavy metal such as PB which is generally high in the gold post-mining land, so the availability decreases and eventually the plants can grow normally.
Availability of nutrients will be continuously improved the process of photosynthesis. Increased photosynthesis process wll lead to an increase of shoot dry weight and root dry weight. In the post-mining lands, plant growth seems to be lower than in the latosol soil, so the addition of biological fertilizers

Table 5. The influence of treatments on root dry weight for *Pueraria phaseoloides, Centrosema pubescens, Setaria splendida and Panicum maximum* at Gold Mining soil

Treatment	Root Dry weight *Pueraria phaseoloides* (g/pot)	Root Dry weight *Centrosema pubescens* (g/pot)	Root Dry weight *Setaria splendida* (g/pot)	Root Dry weight *Panicum maximum* (g/pot)
Control	0.184[b]	0.00	2.44[b]	2.06
M	0.224[b]	0.33	2.80[b]	1.74
MR/MA	0.558[ab]	0.30	3.90[ab]	1.46
MH	0.158[b]	0.38	5.08[a]	1.88
MP	0.716[a]	0.23	2.98[b]	1.13
MPR/A	0.350[ab]	0.43	3.16[ab]	2.22
MPR/AH	0.224[b]	0.23	3.78[ab]	2.24

Note: 1. M = Mycofer, MR/MA = Mycofer + Rhizobium/Azospirillum, MH = Mycofer + humic acid, MP = Mycofer + MPP, MPR/A = Mycofer + MPP + Rhizobium/ Azospirillum, MPR/AH = Mycofer + MPP + Rhizobium/ Azospirillum + humic acid;
2. Different superscript at the same column means significantly difference (P <0.05).

with different types of microorganisms is required.

CONCLUSION

The four test plants species responded differently to the latosol and post gold mining soil. In general, the four plants were not stimulated by single bio-fertilizers, but they required a consortium of several types microorganisms and the result will be better when it is combined with the humic acid on gold post mining soil. Growth response of the four types of plants in latosol soil were better when compared to gold post-mining. At planting media from gold mine tailings many plants die, especially in the control treatment which was treatment without the addition of bio-fertilizers. *Setaria splendida* grew better than other plants.

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Increasing Local Sheep Growth Performance Through Rapid Selection at Fattening Farm

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ABSTRACT

Sheep fattening farms have recently been growing rapidly to produce better quality of sheep meat, however the bussiness could make a crucial loss of good quality of local sheep because they can be sold. It is therefore elite flocks of sheep in a fattening farm should be selected. The experiment was conducted at PT Tawakal, a sheep fattening farm located in Caringin, Bogor. One hundred and sixty nine young male sheep (less than one year old) were selected based on physical judging and their average daily gain (ADG) into two groups having the highest growth rate (above 150 g/head/day) and lowest group (leass than 65 g/head/day). Selection differential and its progress of the selected flock was also calculated. The results showed that there were 13 heads of fast growing and 11 heads of slow growing sheep with the average daily gain of 173.8 ± 26.3 g/head/day and 53.9 ± 15.7 g/head/day, respectively (P<0.01), while the ADG of their population was 98.5 ± 43.6 g/head/day. Based on selection differential calculation (75.3 g/head/day), it was found that selection progress was 7.53 gr/head/day of ADG per year and therefore it may need 6.8 years to improve sheep population to achieve ADG of 150 g/head/day, a relatively short period of a genetic improvement program. It is concluded that rapid selection approach can be recommended as among other selection methods used to increase growth performance of local sheep thus continuously in general, to develop sustainable sheep agribussiness.

Key words: selection, local sheep, growth

INTRODUCTION

Local sheep has a very good potency to be developed, as they have some advantages: prolific, well adapted, more disease resistant, quick yielding and low capital input than cattle, besides their weakness, i.e, slow growth rate compared to 'imported' breed (70-80 vs 200-250 g/head/day, respectively; Edey, 1983; Cottle, 1991).

In recent years, private sectors have been attracted to sheep agribussiness, but still on sheep fattening bussines, because it is less in capital and land needed and also fast in return. The fattening bussines is raising, fattening period of 2-4 months period and post weaned lamb of 6-9 months under intensive and good management practices could stimulate optimal growth of the lambs. But with this fattening bussiness, the best quality lambs can be sold and slaughtered, as previous study showed that the average daily gain (ADG) of local lambs in a fattneing farm had a very large range from 30 g/head/day to 250 g/head/day (Yamin *et al*., 2002; Yamin *et al*., 2003). Similar condition may occur in small sheep farms, the loss for good quality lambs tend to be high because the fattening animal will have better price. These conditions will endanger sheep production and population in Indonesia, because it will decrease the genetic quality of local sheep.

It is therefore, selection of the best sheep in the population of fattening farms was proposed, aiming at obtaining elite flock as genetic sources for sheep breeding improvement. Selection methods used was low cost, simple technique, output oriented, easy to do and the results will be more obvious and sustainable.

This study was conducted (a) to develop group of sheep farmers as initial step to establish fast growing local sheep that adapted to local condition, socially and culturally; (b) to identify selection criteria for sheep flock in Bogor.

MATERIALS AND METHODS

Locations and Time of Experiment

This experiment was conducted in the

Lab of Small Ruminant Production , Lab of Animal Breeding and Genetics, Faculty of Animal Science, Bogor Agricultural University and Sheep commercial farm "Tawakal Farm" Cimande village, Bogor. This research was conducted for 3 months (July-September 2009).

Experimental Animals

Local sheep used in this experiment were 169 heads from sheep fattening farm 'Tawakal' Cimande Bogor. The sheep were selected from the total number of 1071 heads in the farm, based on average daily gain (ADG) and morphometric parameters (girth, body length and body height). Two groups of fast and slow growing sheep (FG and SG) were determined based on ADG. The two groups of selected sheep had ADG of above 150 g/head/day and less than 50 g/head/day, respectedly for FG and SG. The sheep were lambs (I_0), with the age between 6-12 months of age. The reason of using lambs within the of 6-12 months old, because the growth rate within this ages was is in its peak period for effective growing, as reported by Otoikhian *et al*. (2008).

Data Analysis

Analysis of variance

Data were analysed by T-test, with design model as follows:

$$t = \frac{\bar{d} - \mu_d}{\frac{s_d}{\sqrt{n}}} \text{ or if } \mu_d = 0 \text{ then } t = \frac{\bar{d}}{\frac{s_d}{\sqrt{n}}}$$

where degrees of freedom (df) = n-1

Notes :

D = difference between individual or object paired

μ_d = value of difference mean **d population from whole data pairs, usually** 0

$\bar{d}$ = mean of **d**

S_d = standard deviation of **d**

n = number of data pairs

Analysis of genetic parameters

Selection Differential = $((X_S - X_B)$

Selection Progress/year = h^2 x (SD)/GI

Where,

h^2 = Heritability of ADG

X_S = ADG of selected sheep

X_B = ADG of population

GI = Generation interval: mean age of dams (year) when giving births in their life time

RESULTS AND DISCUSSION

Effects of Selection on Sheep Growth Performance

Selection of 169 heads of lambs resulted in 11 heads of FG and 10 heads SG sheep. There was significant difference of growth rate between sheep of 04-12 month old and 25-36 month old. Sheep of 4-12 month old had faster growth rate which was decreased with the increasing age. Male sheep had fast growth rate and higher final weight than female sheep. This is also in accord to work done by Villarroel *et al*. (2008), showing sex has significant effects (P<0,05) on growth rate and final weight of sheep. This may relate to effect of sexual hormone on animal growth influencing body dimension, fat, meat and bone compostions.

The results showed that the average body weight of male lambs population was 28,06±5,20 kg, while their ADG was 98.48±43.62 g/head/day. Previous study by (Villarroel *et al*.,2008) found that male lambs had higher body weigh and ADG than female lambs; (for body weight, 20.70±0.7 for males and 17.60±0.5 kg for females), while, (for ADG of males and females, was 77 gr/head/day and 55 g/head/day, respectively). Higher body weight and ADG in current study might associated with: (i) the sheep used was from fattening farm that conducted selection on animals bought from farmers or traditional market; (ii) intensive management system applied in fattening program.

The results also show significant differences (P<0.01) between body weight of FG and SG sheep, i.e 34.57±3.98 kg and 26.58±5.62 kg, respectively for the two groups of sheep. In terms of ADG, FG sheep had significantly higher (P<0.01) (P<0.01) ADG (173.78±26.34) than SG sheep (53.85±15.71) (Table 1. and Picture 1).

Application of Selection Approach Sheep Breeding Program

The results show that the percentage of selected sheep was only 14 head (8.3%) from its of population (169 heads), indicating the growth rate performance of the population was still vary, so a continuous selection program might be needed.

Table 1. The Average body weight and ADG of selected sheep

Sheep groups	Average Body weight (Mean ± SD) (kg)	ADG (Mean ± SD) (g/head/day)
FG (Fast growing)	34.57^{A}±3.98	173.78^{A}±26.34
SG (Slow growing)	26.58^{B}±5.62	53.85^{B}±15.71

Note: Different superscript capitals in the same column shows very sinificant difference (P<0,01).

a. Fast growing sheep b.Slow growing sheep

Picture 1. Photographs of Fast growing (a) and Slow Growing Sheep (b)

Based on the data of ADG of selected FG sheep (173.78±26.34 g/head/day) and ADG population (98.48±43.62) g/head/day), selection differential was 75.3 g/head/day. By assuming heritability of ADG is 0.25 (Noor, 2008) and generation interval of sheep is 2,5 years, selection progress of sheep population in the farm could become (0.25x75.3)/2.5 or 7.53 gr/head/day of ADG per year. This indicated that, to improve ADG of 150 /head/day, within sheep population, needs aproximately (150- 98.48)/7.53 = 6.8 years, which is considered as a relatively short period of time required to obtain a long term better results. Implementation this selection approach is recommended to improve local sheep genetic quality especially for growth variable, as this method is relatively simple, cheap and sustainable.

CONCLUSION

Rapid selection method had been succesfully selected 8,3% fast growing sheep from its population in the fattening farm, resulted in significantly higher mean body weight (34.57±3.98 kg) and ADG (173.78±26.34 g/head/day, as compared to those in the population (28.06±5.20 kg and 98.48±43.62 g/head/day).

Selection progress was 7.53 gr/head/day of ADG per year, needs only 6,8 years of selection process to improve ADG of 150 g/head/day in sheep population.

The founding recommended a continous selection program carried out in village breeding centre to achieve a sustainably best quality of local sheep.

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Poster Presentation

The Effect of Concentrate Supplementation Made From Palm Oil Sludge and Several Local Feed Resources to Production Performance of Bali Calves

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ABSTRACT

This experiment was aimed to investigate the production performance of Bali calves male supplemented by concentrate diet made from palm oil sludge (solid decanter) and several local feed resources (rice bran, cassava flour, cassava leaf flour, and blood meal). This experiment was arranged in Latin Square Design with 4 treatments and 4 replications. Four weaned Bali calves ± 9 months old were used. The treatment was the ratio of concentrate and forage ration in the diet consisted of P1=80% concentrate (C) : 20% Forage (F), P2= 60%C:40%F, P3 = 40%C: 60%F, P4= 20%C: 80%F. Data were analyzed by using analysis of variance (ANOVA) and any significant difference were further tested with Duncan Multiple Range Test. The result showed that the treatment had no a significant effect on average daily gain, crude protein consumption and feed conversion value, but significantly decreased dry matter consumption. The results indicated that concentrate diet made from palm oil sludge and local feed resources had positive effect on Bali calves performance ranged about 0.167kg/day-0.271kg/day respectively.

Key words: palm oil sludge, concentrate diet, production performance, Bali calves

INTRODUCTION

Good production performance of livestock can be reached by supplying good quality and quantity of feed. The problem is the availability of forage that is difficult to get it and the concentrates price now is getting expensive. Because of that it is urgent to create the innovation by using local feed resources which are much available, cheap, good nutritive values, safe and not compete with human needs. One of this is palm oil sludge (POS) or solid decanter.

Palm oil sludge (POS) is one of by product resulted from the first stage of palm fresh fruit processing by mechanical pressure to get palm oil. POS is produced 2% from crude palm oil produced (Devendra, 1977). Until now POS has not been used yet by the factory, but it is still a waste that can pollute the environment.

POS can function as feed ingredient mixture. In dry matter base, it contains crude protein 13% that is close to crude protein of rice bran 13.3%. Its TDN value is 74% higher than rice bran which has 70% (Agustin, 1991). The use of POS as feed significantly enhanced the weight gain of male Ongole cattle which was applied POS 1.5% dry matter from live weight *adlib* as long as three months got average daily gain 440 and 770 gram/head/day. While for control (without solid) got 200 gram/head/day (Utomo and Widjaja, 2004).

Gohl (1981) and Aritonang (1986) said that without any treatment, LMS can be applied in ruminant diet up to 50% from total concentrate. Hidayat *et al*. (2002) reported that fresh POS (without treatment) can be used for feed ingredient up to as much as 24.96% from total diet or about 49.82% from total concentrate.

The main constraint in POS utilization is that POS is too easy to become decay (rancid flavor) so it needs special handling for long lasting endurance in storage processing. The way to preserve POS is by pelleting or blocking it. Through this way, POS have long lasting preparation, have more complete nutrient value because several other ingredients can be added. POS has a character to become harden after drying so it can be used as glue in pelleting or blocking process. Several advantages from feed pellet form are it is easy to handling the feed, efficient enough because it did not need much space. The feed does not produce many wastes, dusty and very suitable with feedlot system. The pellet form made weight per volume of feed enhanced 7-8 times before ground so it can enhance the nutrient density and decreased the activity of depraved microbial. The pellet form can be long lasting preparation (Rockey *et al*., 2008).

This research used several local feed resources such as rice bran, cassava meal, cassava leaf meal, and blood meal. Cassava

meal contains high crude protein ranged between 19.5% and 22.6% of dry matter based (Kartiarso *et al*., 1991 and Granum *et al*., 2007). Bakrie *et al*. (1996) reported that PO cattle which was supplemented with cassava leaves meal had weight gain significantly higher than the group that supplemented by soybean cake. Blood meal derived from abattoar waste blood containing high crude protein (80%). Close *et al*. (1986) said that the bood protein has low biological value especially due to lower concentration of isoleucine and methionine amino acid. Blood meal is difficult to be degraded in rumen. However It is expected to be bypass protein source which can be used post rumen by animal

Cassava meal is one of concentrate material which has energy sources (12.9MJ/kg) and it is classified as readily available carbohydrate (Sommart, 2000), while rice bran is protein source (13-15% crude protein) and energy source (65-67%TDN). Ibrahim (1986) reported that the addition of 0.5 kg rice bran meet the basic need of cattle with 100-150 kg live weight fed urea ammoniated rice straw, increased live weight 100 gram per day.

The objective of this research was to evaluate the production performance of Bali calves supplemented with concentrate diet made from palm oil sludge and several local feed resources (rice bran, cassava meal, cassava leaf meal and blood meal).

MATERIALS AND METHODS

Feed Preparation

The diet used in this research consisted of natural field grasses (mainly *Phragmites* sp) and concentrate served with several level of feedstuff (Table 1.). The production of concentrate diet was conducted by mixing the feed sources that has little quantities, then with feed sources that has larger quantities. Lastly, all of the concentrate materials were mixed with palm oil sludge (solid decanter) homogenously, then pellet was made. The compositions of concentrate diet (Table 2) contains crude protein ± 15% and TDN ± 60%. This value met nutrient standard requirement of cattle (Kearl, 1982). Result of Laboratorium nutrient analysis was presented is Table 3.

Blood meal was made through gradually drying method of blood taken from abattoir. The blood meal was cooked by using mild fire flame of kerosene stove. Blood were then dried under sun radiation and then it was milled. Cassava leaf meal derived from the old cassava leaf hay.

Table 1. The Proportion of forages and concentrate pellet diets in the feeding trial

Treatments	Forage (%)	Concentrate (%)
1	20	80
2	40	60
3	60	40
4	80	20

The use of feed trial was twice a day at 09.00 am and 17.00 pm. All the diet treatments were given to four wean Bali calves male with live weight of 80±10 kg and aged of ± 9months old.

Each period spent as three weeks that consisted of one week preliminary period and two weeks collection period. Variables observed were:

a. Consumption of dry matter and crude protein
b. Daily weight gain
c. Conversion of diet

Table 2. Composition of concentrate ingredients

Feed Sources	(%)
Solid decanter /POS	50
Rice bran	15
Cassava meal	15
Cassava leaf meal	5
Blood meal	4
Mineral mix	4
Limes	3
Salt	3
Urea	1
Total	100

Table 3. Nutrient composition of forages and concentrate pellet based palm oil sludge

Feed sources	Dry matter (%)	Crude protein (%)	Crude fiber (%)	Gross energy (%)
Natural field grass	86.42	11.68	22.18	3553
Pellet concentrate	85.82	17.07	19.76	3259

Source: Nutrition and Feed Technology Lab, Dept of Nutrition and Feed Technology, Animal Sci. Faculty. Bogor agricultural University (2009)

Location and Time

This research was conducted at farmer farm Sidodadi Village, Muara Bangkahulu district in Bengkulu town, for 4 months from May to August 2009.

Data Analysis

Data were tabulated and analized by using analysis of variance (ANOVA) and for significance test data were further analized by using Duncan Multiple Range Test (DMRT) (Lenten and Bishop, 1986).

RESULT AND DISCUSSION

Average Daily Gain (ADG) of Bali calves is one of indicator for the production performance value. ADG of cattle which supplemented with concentrate diet can be viewed on Table 4. The results showed that the treatment had no significant effect on average daily gain. The average daily gain value that can be reached was 0.167 kg/day up to 0.271 kg/day. The production performance of Bali calves in this research was higher than the results of Pamungkas *et al*. (2009) that the ADG was 0.102kg/day, 0.192 kg/day and 0.122 kg/day in Bali calves receiving the diet kinggrass, kinggrass + *Leucaena*, and natural field grass respectively. Other research was conducted by Pamungkas *et al.*(2009) and showed that ADG of weaned Bali cattle of nine months old got diet 100% Primafeed (Commercial complete feed in East Java made from agro industrial by product), 65% Primafeed + 35% *Leucaena*, 35% Primafeed + 65% *Leucaena* and 100% *Leucaena* respectively were -0.039, 0.161, 0.191 and 0.243 kg/day. This values are less than the results of the current work. This data showed that the application of concentrate made from palm oil sludge up to 80% level (diet 1) can produce a good growth.

The results of the current research showed that ADG can be reached in 80% forages + 20%concentrate treatment (diet 4) with average daily gain was 0.271 kg/head/day. The optimal supplementation of concentrate can be done on diet 3 (60% forages and 40% concentrate) because it had still a good acceptability with good average daily gain value (0.219 kg/day). Krisnan *et al*. (2008) reported that the optimal value using solid decanter as the single supplement could be reached by treatment of 55% forages+45% concentrate with the highest average daily gain on goat.

The avarage daily gain of the Bali calves in this experiment is in accordance to the dry matter consumption rate and protein consumption in the diet. The higher consumption of dry matter and protein in the diet gave the higher response to weight gain. The dry matter consumption in this research was 3.25-4.17 % and this value in was enough to made the weight gain of 200 gram/head/day. The consumption 2.25-3.0% from the live weight for cattle that has the live weight under 100 kg (National Research Council, 1984). This data showed that the utilization of concentrate pellet diet had good enough response to dry matter consumption. Dry matter consumption is one criteria in judging the palatability of diet that needed to ascertain the quality of diet (Parakkasi, 1999). Analysis of variance showed that the treatment had a significant effect on dry matter consumption, where the higher ratio of concentrate the consumption of dry matter decreased. The dry matter consumption of diet 3 and diet 4 was not significantly different but significantly higher than diet 1 and diet 2.

The results showed that the treatments had no significant effect on crude protein consumption. The value of crude protein consumption in this research was 0.397-0.438 kg/head/day. This value met protein requirement of calves that was 0.333-0.379 kg/head/day (NRC, 1984).

The treatment had no significant effect to the diet efficiency value. This indicated that the application of concentrate diet made from palm oil sludge and several local feed resources up to 80% is technically efficient.

Table 4. Production performance, consumption and feed conversion

Variables	Treatment				SE
	1	2	3	4	
Avarage daily gain(AVG)(kg)	0.167[a]	0.175[a]	0.219[a]	0.271[a]	0.085
Dry matter(DM) consumption (kg/day)	2.705[a]	2.985 [a]	3.36 [b]	3.470[b]	0.200
Crude Protein consumption (kg/day)	0.397[a]	0.414[a]	0. 438[a]	0.428[a]	0.100
Diet Efficiency (Kg DM/kg AVG)	16.198[a]	17.057[a]	15.365[a]	12.804[a]	1.037

Note: The different letter on the same row mean significant different (P<0.05).

CONCLUSION

The results showed that the application of concentrate diet made from palm oil sludge and several local feed resources gave positive response to Bali calves performance. The average daily gain of Bali calves in this experiment ranged between 0.167 and 0.271 kg/head/day. The optimal applicattion of concentrate diet was on treatment 3 (60% forages and 40% concentrate). It can be seen from average daily gain 0.219 kg/day.

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Reproductive Indices in Determining Regular Calving of Holstein-Fresian Cows Under Intensive and Semi-Intensive Managements in Central Java

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ABSTRACT

Reproductive efficiency of dairy cows can be determined by various reproductive indices (RIs) as essential components of regular calving period. The aims of this research were to assess some RIs, correlate among those RIs and describe possible causes of their variation for Holstein-Friesian (HF) cows maintained under two different conditions in Banyumas district, Central Java. Data of reproduction of HF cows were collected for 458 records from a dairy breeding station (BS), 1992 - 2002, and for 417 records from a number of small dairy holders (SDH), 1996 - 2002. A number of RIs studied were intervals from calving to first service (CFS), first service to conception (FSC), days open (DO) and calving interval (CI). Pearson correlation method was used to correlate (r) among RIs. Simple regression up to the third levels was used to investigate the effects of individual RIs (CFS and FSC) on DO and (CFS, FSC and DO) on CI. Means of each RI of HF cows between the BS and SDH were compared by the least square technique of GLM analysis by considering location, age-, season- and year of calving as dependent variables. Location resulted in very significant effect ($P<0.01$) on CFS, DO and CI, at a range of 1.58 - 21.56 %. Adjusted means of CFS, FSC, DO and CI in the BS were 78, 22, 134 and 410 d respectively; and those in SDH were 91, 24, 156 and 427 d respectively. For both locations, FSC was a more major factor in affecting DO compared to CFS with the r value of FSC-DO almost twice the strength against CFS-DO (BS= 0.84 vs. 0.48; SDH= 0.82 vs. 0.44 %). Further, DO had the highest effect on CI compared to individual FSC or CFS, r value = 0.98 (the BS) and 0.97 (SDH. Lengthening each day of DO resulted in linearly increased CI of 0.99 d (the BS) and 0.98 (SDH). Major differences in RIs of HF cows in the current study compared to those in temperate and other tropical regions require definite researches in assessing various physiological and environmental factors affecting reproductive performance of HF cows under specific tropical region of Central Java.

Key words: Reproductive indices, days open, Holstein-Friesian cows, tropical region

INTRODUCTION

Reproduction plays a key role to achieve profitability in a dairy production as an inefficient reproduction results in many kinds of adverse indicators such as less milk and fewer calves per cow per year, increased culling rates, slow genetic improvement, increased replacement cost, increased breeding cost or AI services and low net returns (Dekkers *et al*., 1998). It is desirable that cows have a good fertility because the more frequently a dairy cow calves, the greater is the amount of milk produced in her lifetime. According to Plaizier *et al.* (1997) inferior reproduction in dairy cattle causes a severe disadvantage in dairy production operation by reducing the amount of milk produced per cow per day of herd life, increasing breeding costs, intensifying the rates of voluntary and involuntary culling and slowing the rate of genetic progress for the traits of economic importance in a dairy herd.

Factors governing reduced reproductive performance in dairy cattle are numerous and often difficult to diagnose. Even under optimal conditions, the reproductive process is less than perfect because of the multiple factors which contribute to produce a live calf (Stevenson, 2001). Overall reproductive performance at a herd level is directly influenced by the reproductive activity of individual cows. A dairy cow is clearly fertile if she has the ability to conceive and maintain pregnancy after service at the appropriate time in relation to ovulation (Darwash *et al.*, 1997). Any condition leading to failure in establishing a pregnancy following completion of uterine involution, on the other hand, results in sub-fertile or infertile cows. Failure to establish a pregnancy at the expected time after the peri-parturient period may reflect a num-

ber of abnormalities including failure to ovulate, failure to show oestrus, inappropriate pattern of ovarian cyclicity and loss of pregnancy that might be a reflection of dysfunction at the hypothalamic, pituitary, ovarian or uterine level, or in conceptus development (Royal *et al*., 2000). Challenges maintaining efficient reproduction in dairy cattle apparently include a complex process involving various factors of genetic, nutritional, physiological, management and environmental (Stevenson, 2001; Masama *et al*., 2003b; Shiferaw *et al*., 2005). An understanding this multitude of interrelated factors which can result in cows successfully becoming pregnant, therefore, is essential.

Calving interval has been considered as a practical and useful parameter to indicate the reproductive status of individual cows and the reproductive efficiency of a dairy herd. It is supposed that by maintaining optimal calving interval ensures efficient dairy reproductive management. Reproductive efficiency of dairy cows can be determined by calculating the periods of various reproductive indices (RIs) as essential components of the calving interval (Stevenson, 2001). These are mainly for the intervals from calving to first service, from first service to conception, days open and gestation length, besides the calving interval itself. Calculation of the duration of these reproductive parameters allows identification of various limiting factors associated with reproductive disorders which subsequently allows relevant adjustments to be undertaken for the improvement.

The aims of this research were to assess some RIs, correlate among those RIs and describe possible causes of their variation for Holstein-Friesian (HF) cows maintained under two different conditions of the tropical Indonesian climate in Banyumas district, Central Java.

MATERIALS AND METHODS

Materials

This dairy-field research was carried out in one government dairy breeding station (the BS) and a number of small dairy holders (SDHs) in Banyumas district, Central Java. The total area of Banyumas is 5,364 ha (4.04 %) of the total area of 132,759 ha of Central Java located between longitude 108° 39'- 109° 27' east and latitude 7° 15'- 7° 37' south. Almost half of Banyumas district is in the lowland, spreading out from the centre to the south and from the west to the east of the district. Banyumas can be classified into three regions according to its elevation, namely, the altitudes of 25 - 100 m (31.77 %), 100 - 500 m (30.42%) and the remainder over 500 m (37.71 %) asl. The dairy breeding station (the BS) was set up on a highland at the slope of Slamet Mountain over 1,500 m asl with an area of approximately 13.5 ha in Baturraden sub-district. Small dairy holders, which were developed under the supervision of the BS, have been located in five sub-districts of the overall 27 sub-districts in Banyumas district. Small dairy husbandries have been entirely developed within 25 villages from the five dairy sub-districts of Pekuncen, Cilongok, Karang Lewas, Baturraden and Sumbang for a total area of 32,960.5 ha, 24.83 % of the area of Banyumas. The development of these dairy villages has mostly been located on the relatively higher regions (above 140 m asl.) rather than those of sub-districts without dairy establishment. Table 1. describes the general management under the two research locations.

Investigation into various aspects of reproduction of dairy HF heifers and cows in the present study was conducted in each location of the BS, SDH and Overall combining between two locations. Reproduction records used for the study were for HF cows kept during the period of 1992 - 2002 in the BS and during the period of 1996 - 2002 in SDH. Data were collected from the reproductive database which was recorded under the Breeding and Recording Sub-division of the BS. All these reproductive field data were recorded in daily book records and then entered into the computerized database using the recording packet program of Cow Search by the breeders and recorders under the supervision of the BS. The reproduction data of individual cows mainly consisted of the dates of birth, calving, service (or insemination) and conception as well as the number of services. Knowledge of these dates allowed a range of reproductive indices (RIs) to be calculated as presented in Table 2. There were no data collected on the reproductive physiology of the animals during the current research.

The unavailability of physiological reproduction data resulted in constraints making impossible more specific investigation

Table 1. General management under the two locations of the BS and SDH

Description	BS	SDH
Dairy breed	Pure Holstein-Friesian	Pure Holstein-Friesian.
Management	Large scale and intensive	Small scale and semi intensive (Group or individual dairy farmers)
Feeding	Improved forages and recommended concentrate	Mixture of forages and variable concentrate
Reproduction	Visual and recording heat detection, regular heat induction and palpating conception detection	Visual heat detection, limited heat induction and visual detection on conception
Calf rearing	Artificially rearing	Artificially rearing
Heifer and cow Rearing	Different houses and feeding based on physiological status	In similar stall and feeding as available
Mating	Insemination using HF and Holstein frozen semen from AI institution in central Java	Insemination using HF and Holstein frozen semen of AI institution in Central Java
Milking	Machine and hand - morning and afternoon	Hand – morning and afternoon

Table 2. Various reproductive indices considered in the current study and their definition

Reproduct. indices (d)	Abbrev.	Definition
Age at first calving (mo)	AFC	Number of days from birth to first calving of animals
Calving - 1st service	CFS	Number of days from calving to first service
1st service – conception	FSC	Number of days from first service to subsequent conception (calculated only for cows that were confirmed pregnant).
Days open	DO	Number of days between parturition and subsequent conception (calculated only for cows that were confirmed pregnant).
Gestation length	-	Number of days from the last conception to subsequent calving
Calving interval	CI	Interval between two consecutive calving.
Services per conception	S/C	Number of services or inseminations required for conception (calculated only for cows that were confirmed pregnant).

and discussion on the reproductive performance of HF cows in the current study. However it is confidently expected that by utilizing the available service and calving dates, will still yield essential information on the reproduction of HFs in both locations.

Methods

A number of reproductive indices (RIs) as contributing factors to regular calving interval were studied. These were: the intervals of calving to first service (CFS), first service to conception (FSC), days open (DO), gestation length and calving interval (CI) (Table 2). The final observations analyzed for individual RIs of CFS, FSC, DO, gestation and CI are presented in Table 3. They were obtained by extracting data on individual cows that had complete RIs. Outlier data identified through box plot distributions were omitted from the final analyses.

Various correlations between RIs of CFS, FSC, DO, gestation and CI of HF cows within location were calculated by Pearson correlation method. A number of simple regressions, up to the third levels of equation, were analyzed to investigate the effects of individual RIs (CFS and FSC) on DO and (CFS, FSC, DO and pregnancy) on CI for HF cows in each location.

To compare means of each RIs of HF cows in the BS and SDH, individual RIs was transformed into a normal distribution then analyzed by the least square technique of GLM analysis for unbalanced data with location, age-, season- and year of calving determined as dependent variables.

RESULTS AND DISCUSSION

Description on Reproductive Indices

Data on six reproductive indices (RIs) of HF heifers and cows providing CFS, FSC, DO, gestation length, CI and S/C in the BS, SDH and Overall are presented in Table 3. All these estimated reproductive parameters were calculated from the available information on the dates of services and calving of animals from the computerized reproductive data base in the BS. Following calving, the gravid uterus has to return to a non-gravid

Table 3. Reproductive performance (months) of Holstein-Friesian heifers and cows by location

Reproductive performance (days)	N	Mean	Median	SD	Min.	Max.
			Location			
			BS			
Calving - 1st service	458	86	80	39	25	190
1st service – conception	458	49	24	62	0	264
Calving – conception	458	136	120	70	31	334
Gestation	340	275	275	5	262	291
Calving interval	458	408	393	69	393	609
Services per conception*	527	1.84	2.0	1.0	1	6
			SDH			
Calving - 1st service	417	102	95	43	25	215
1st service – conception	417	49	19	66	0	279
Calving – conception	417	150	139	73	25	361
Gestation	253	273	274	6	256	292
Calving interval	417	418	404	74	280	653
Services per conception*	517	1.93	2.0	1.1	1	6
			Overall			
Calving - 1st service	878	94	86	41	25	215
1st service – conception	878	49	22	64	0	279
Calving – conception	878	143	129	72	25	361
Gestation	593	274	274	5	256	292
Calving interval	878	412	398	71	398	653
Services per conception*	1044	1.88	2.0	1.1	1	6

* in score

state and sexual cycle must be resumed to achieve conception. The efficiency of these two critical reproductive processes, along with the efficiency of artificial insemination, is reflected in estimates of various components of inter-calving interval. The description of each RI, which contributes to calving interval, is essential in quantifying general reproductive performance of Holstein cows within and between locations.

Each RI of CFS, FSC, DO and CI had the same observational number for 417 records. This was because the final data for analyses were obtained by including only animals possessing complete information on all the RIs which are determinant components of CI in order to achieve consistent values. This decision risked under estimation of the respective RIs due to the exclusion of the reproduction data of culled cows. The exclusion of data on incomplete RIs could mean the exclusion of animals which had inherent low fertility and failed to conceive or animals which were culled due to management reasons such as poor milk yield, diseases etc. Excluding animals with low fertility from the analysis is equivalent to truncating/censoring the data. By contrast, the final observations on gestation length of HF cows in both locations were obtained after omitting the identified outlier data of the previous RI completing CI (417 records). The exclusion of the identified outlier gestational data decreased the number of observations, by respectively 25.8 % in the BS (from 458), 39.3 % in SDH (from 417) and 32.5 % in Overall (from 878).

As presented in Table 3, means for CFS, DO and CI of HF cows in the BS (86, 136 and 408 d) were shorter compared to those of HF cows in SDH (102, 150 and 418 d) and the ranges of the respective RIs were also narrower in the BS (25 – 190 d, 31 – 334 and 393 – 609 d) than those in SDH (25 - 215, 25 - 361 and 280 - 653 d). All these RIs of HF cows in both locations had wide ranges indicating they were highly variable. Means of FSC in both locations were similar (49 d), but the range was narrower in the BS (0 – 264 d) compared to SDH (0 – 279 d). Mean of gestation length of HF cows in SDH was slightly shorter than that in the BS (273 vs. 275 d), but its range was longer in SDH than in the BS (256 – 292 d vs. 262 – 291 d). The values for mean and median for the gestation length were the same in the BS (275 d) and similar in SDH (273 vs. 274 d). This indicates that the distribution of gestation of HFs in the current study followed a normal distribution.

Frequencies of the distribution for individual RIs at each location are not given here.

However, there was generally a positive skewed pattern of most RIs both in BS and SDH. The typically positive skewed distribution which normally exists for reproduction traits is clearly identified for CFS, DO and CI in both locations. These were marked by the higher values of the mean to the median. Nevertheless, different skewed positive distributions existed for FSC distribution compared to the former with the highest frequency, as would be expected, occurred at the FSC 0 d showing the majority of heifers conceive at the first insemination. The results revealed that reproductive performance of HF cows in the BS was better than that in SDH meaning that generally HF cows in the BS were better in expressing reproductive performance than HF cows in SDH. By combining the two sets of reproduction data the mean and the median of each RI in Overall was in between values for the BS and SDH.

Comparison among Reproductive Indices

Compared to the means of CFS of HFs and other temperate dairy breeds, the mean CFS of HF cows in the BS (86 d) was longer than the range of 70 – 86 d HFs and other temperate breeds maintained under temperate regions of USA (Moore *et al*., 1990; Simerl *et al*., 1991), The Netherlands (Ouweltjes *et al*., 1996) and Sweden (Avel and Örnsro, 2001). This FCS was also longer than the range of 76 – 80 d for HF cows kept under tropical regions of India (Dhaliwal *et al*., 1996), but it was comparable to the CFS range of 63 – 92 d of Friesian kept in temperate regions of Italy (Bagnato and Oltenacu *et al*., 1994). The mean CFS of HF cows in SDH (102 d) was shorter than the range of 106 – 115 of the CFS of various exotic crosses kept in tropical regions of Bangladesh (Islam *et al*., 2002) and Philippines (Alejandrino *et al*., 1999). These results indicate that the CFS of the HFs maintained under intensive management in the BS was comparable to the CFS of HFs raised in some temperate and many tropical regions and the CFS of HF cows kept under SDH were comparable to those of Holsteins maintained in some tropical regions.

Some studies have reported that the period from first service to subsequent conception of HFs and some temperate breeds to be within a range of 22 – 45 d in USA (Simerl *et al*., 1992) and The Netherlands (Ouweltjes *et al*., 1996). A similar range of the FSC of 26 – 49 d was observed for HFs in tropical region of India (Dhaliwal *et al*., 1996). The mean of the FSC of 49 d for HF cows in the present study are within the range of FSC of HFs in India (Dhaliwal *et al*., 1996), but longer than the range for FSC of some temperate breeds in USA (Simerl *et al*., 1992) and The Netherlands (Ouweltjes *et al*., 1996). The median values of FSC for HF cows in the present study were considerably shorter than their means both in the BS (24 vs. 49 d) and in SDH (19 vs. 49 d). As presented in Table 2, this was because the highest frequency of HF cows conceived at the first time they were inseminated in both locations (BS = 45 %, SDH = 45 %).

The means from calving to conception or DO, as the summation of the two periods of CFS and FSC, were shorter in the BS (136 d) than the SDH (150 d). The ranges for DO of HF cows in both locations varied considerably, 31 – 334 d in the BS and 25 – 361 d in SDH. Large variation of DO in both locations was definitely a result of high variation in the two determining indices of CFS and FSC. This was similar for CI in which the range was narrower in the BS than in SDH (BS = 393 - 609 d, SDH = 280 - 653 d). It is likely that the wide ranges in CI were predominantly determined by the large variation in DO rather than gestation length. A previous study pointed out the duration of gestation is fairly constant and can not be shortened significantly without adversely affecting the health or viability of the new born (Bazer and First, 1980). The mean DO of HF cows in the BS was longer compared to those within a range of 72 - 128 d of HFs, temperate breeds and crossbreeds maintained both in temperate and tropical regions in USA (Moore *et al*., 1990; Luna-Dominguez *et al*., 2000; and Simerl *et al*., 1994), Sweden (Avel and Örnsro, 2001), India (Dhaliwal *et al*., 1996), Israel (Arbel *et al*., 2001), Tanzania (Msanga and Bryant, 2003), Turkey (Türkyilmaz, 2005) and Tunisia (Salem *et al*., 2006). The mean DO for HF cows in both locations of the BS and SDH were comparable to the values of 134 - 159 d for Holsteins in USA (Oseni *et al*., 2003) and 123 – 154 d in Turkey (Kaya *et al*., 2003). Other studies reported longer DO within a range of 186 - 284 d for HFs and crossbreds in tropical regions of Philippines (Alejandrino *et al*., 1999) and Pakistan (Niazi

and Aleem, 2003). These results show that the DO HF cows in both locations were comparable to DO of HFs and crossbreds in some temperate and tropical regions. However, to achieve the recommendation of 90 days open, attention should continue to be focused on reducing both CFS and FSC.

A number of previous studies on temperate dairy breeds either in temperate and tropical regions have reported the mean gestation length in a range of 278 – 282 d. The means of gestation period of HF cows in the present study (BS = 275 d, SDH= 273 d) were lower than this range. Referring to Table 3, various studies have reported CI of both temperate breeds and their crossbreds maintained in temperate regions were between 335 – 445 d, but the CI tended to be longer (436 – 734 d) for temperate dairy breeds and their crossbreds maintained under tropical regions such as in Philippines (Alejandrino *et al*., 1999), Sudan (Ageeb and Hayes, 2000), Pakistan (Niazi and Aleem, 2003), Ethiophia (Shiferaw *et al*., 2005) and Zimbabwe (Ngongoni *et al*., 2006). The mean CI of HF cows in the BS (408 d) and SDH (418 d) were comparable to the HFs and other temperate dairy breeds kept in some temperate and tropical regions.

The number of services required for HFs conceiving either in the BS (mean = 1.84, median = 2.0) and SDH (mean = 1.93, median = 2.0) were high compared to the range of 1.58 – 1.68 for HFs and temperate dairy breeds maintained in temperate regions of USA (Moore *et al*., 1990) and The Netherlands (Ouweltjes *et al*., 1996). The S/C of HFs in both locations were also higher than the means of 1.27 – 1.65 of HFs and exotic breeds reared in some tropical regions of Philippines (Alejandrino *et al*., 1999) and Malawi (Chagunda *et al*., 2004); however they were still comparable to some previous studies in India (Dhaliwal *et al*., 1996), Pakistan (Niazi and Aleem, 2003), Turkey (Türkyilmaz, 2005) and Tunisia (Salem *et al*., 2006).

Phenotypic Correlation and Regression among Ris

Table 4. presents coefficients (R) of the phenotypic correlation between various RIs as determinant factors of regular calving of HF cows for all locations. CFS was correlated negatively to FSC at a low level (BS = -0.09, SDH = -0.15, overall = -0.12), but the correlations were significant ($P<0.05$) both in SDH and Overall. As expected both CFS and FSC were correlated positively to DO with a higher R value resulting from correlating DO to FSC than to CFS across locations. The R value of the FSC and DO correlation resulted in almost twice the strength when compared to that of the CFS and DO correlation for HF cows in each location of the BS (0.84 vs. 0.48 %), SDH (0.82 vs. 0.44 %) and Overall (0.82 vs. 0.47 %). This implies FSC is a more dominant factor in differentiating DO compared to CFS.

Consistently, a similar situation occurred for CI, the two factors of CFS and FSC positively correlated to CI, with the R value for the FSC and CI correlation was higher than the CFS and CI correlation in the BS (0.79 vs. 0.50 %), SDH (0.80 vs. 0.44 %) and Overall (0.80 vs. 0.47%). In contrast, correlating gestation length to CI resulted in only small R values across locations varying from 0.04 to 0.14, however, the correlation was statistically significant in SDH ($P<0.05$). These results support the previous finding that the two factors of CFS and FSC are the dominant factors affecting CI with FSC being the more important. Gestation had a relatively small effect on CFS and FSC on CI, but it significantly affected on CI of HF cows in SDH ($P<0.05$). As was expected, the DO and CI correlation resulted in the highest R value because DO was resultant from the factors of CFS and FSC. Some possible factors might result in the differentiation on the periods from CFS, FSC and gestation length as the three most important periods in determining the length of calving interval of HF cows in this study.

A number of simple regressions up to the third levels of equation were analysed to investigate the effects of individual RIs (CFS and FSC) on DO and (CFS, FSC, DO and pregnancy) on CI of HF cows for each location of the BS, SDH and Overall. Coefficient regressions from various simple linear regressions developed to describe the effects of individual RIs (CFS and FSC) on DO and (CFS, FSC and DO) on CI are presented in Table 5. As both a quadratic and a cubic expression of each corresponding RI resulted in no significant effect on either DO or CI, these

Table 4. Phenotypic correlation among reproductive indices of Holstein-Friesian cows by location

Reproductive indices (d)	BS			SDH			Overall		
	1st M-C	DO	CI	1st M-C	DO	CI	1st M-C	DO	CI
Calving – 1st service	-0.09^{ns}	0.48**	0.50**	-0.15**	0.44**	0.44**	-0.12*	0.47**	0.47**
1st service – conception		0.84**	0.79**		0.82**	0.80**		0.82**	0.80**
Days open			0.98**			0.97**			0.98**
Pregnancy			0.04^{ns}			0.14*			0.07^{ns}

Table 5. Coefficients of the linear regressions to predict days open and calving interval from various reproductive indices of Holstein-Friesian cows by location

Estimator	Predictor	BS			SDH			Overall		
		a	b	R^2 (%)	a	b	R^2 (%)	a	b	R^2 (%)
DO	CFS	61.54	0.858	22.4	72.74	0.762	19.4	66.50	0.812	21.7
	FSC	89.13	0.944	69.6	106.4	0.902	66.8	97.51	0.923	67.4
CI	CFS	333.5	0.857	24.5	341.3	0.783	18.9	336.7	0.825	22.3
	FSC	365.8	0.924	63.0	373.1	0.893	63.9	369.0	0.910	63.5
	DO	277.1	0.988	96.0	272.8	0.981	94.3	275.8	0.981	95.1

equations are not presented here. Based on coefficient determination (R^2) of various simple linear regressions obtained, the effect of the linear regression of FSC, compared to that of CFS, resulted in higher R^2 values in describing the changes on DO and CI. For the respective locations of the BS and SDH, the linear regression of DO on CFS resulted in R^2 values (22.4 and 19.4) which were lower than those of the linear regression of DO on FSC (69.6 and 66.8). Certainly, the highest R^2 values were obtained, for the BS and SDH, by regressing CI on DO (0.99 and 0.98) rather than on CFS (24.5, 18.9) or on FSC (63.0, 63.9). This was, as previously described, due to the fact that DO is the summation of the periods of CFS and FSC.

Various coefficient regressions (b), obtained to describe the changing DO by lengthening of CFS and FSC or to describe the changes in CI by delaying CFS, FSC and DO for the three locations are also presented in Table 5. Based on the coefficient regressions obtained successively for the BS, SDH and Overall, it can be predicted that for each day delay in CFS of HF cows resulted in prolonged DO of 0.86, 0.76 and 0.81 d respectively; whereas for each day delay FSC of HF cows resulted in a prolonged DO of 0.94, 0.90 and 0.92 d respectively. Further for the respective locations, a delay of one day in CFS caused a lengthened CI of 0.86, 0.78 and 0.83; while a one day delay in FSC resulted in a prolonged CI of 0.92, 0.89 and 0.91 d respectively. In the case of DO as a predictor of CI, for each day prolonged DO it can be estimated to lengthen CI by 0.99 d in the BS, 0.98 d in SDH and 0.98 d in Overall.

The Effect of Location on Ris of Holstein-Friesian

Least square analyses for Overall resulted in location was a major factor in affecting CFS, DO, gestation and CI ($P<0.001$) with the exception of FSC which was not significantly influenced by location ($P>0.05$). The contribution of location to the variation in individual RIs, presented by the coefficient of variations or R^2 values (R^2 = variable SS / total SS %), were significant. The R^2 values for the effect of location on CFS, DO, gestation and CI respectively were 3.41, 2.06, 7.16 and 1.50 %. In contrast, a small non-significant location effect resulted for FCS (0.01 %). Adjusted means of CFS, FSC, DO and CI in the BS were 78, 22, 134 and 410 d respectively; and those in SDH were 91, 24, 156 and 427 d respectively.

All these statistics support the previous results that there was a considerable difference in the reproductive performance of HF cows reared under the two dairy production systems of an intensive management in the BS against mainly semi intensive management in SDH. Some factors that may cause the inferior reproduction of HF heifers and cows in the SDH are described. The general inferiority in feeding, management, raising animals, insemination services, health services and treatment, and housing of animals in SDH might be major factors. Daily heat stress was possibly another factor resulting in low reproduction of HF cows in SDH. All

these factors might cause cows in SDH to be more susceptible to many kinds of reproductive disorders, such as failed oestrus and ovulation, reduced conception rate, decreased pregnancy rates and increased reproductive diseases. As well as the limited technical aspects, constraints in social factors such as the skill and knowledge of small dairy farmers to take daily decisions about their farms and animals might also be important in reducing both reproduction and production of the animals.

CONCLUSION

Reproductive performance of HF heifers and cows in the current study, represented by individual RIs of CFS, FSC, DO and CI, was mostly in a wide range and higher than the recommended values indicating that cows showed delayed calving regularly in each year. FSC was a more important factor in affecting both DO and CI compared to CFS. DO, as the summation of CFS and FSC, had the highest effect on CI compared to individual FSC or CFS, while gestation length had only a minor effect on CI. Any favorable factors of genetics, nutrition, physiology, management and environment could result in reduced individual RIs, whilst unfavorable conditions caused prolonged individual RIs.

Reproductive performance of HF cows in the BS was superior to that in SDH which might be due to higher genetic potential of the animal and better factors of feeding, management, health treatment and services, reproductive treatment and preventive diseases in the BS. More favourable climate and housing management in reducing heat stress could be another factor resulting in better reproductive performance of HF cows in the BS to SDH.

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The Influence of Beef Submersion with Various Concentration of Coconut Shell Liquid Smoke Against Total Bacterial Count, Shelf Life and Acceptability

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ABSTRACT

The research on the influence of beef submersion used various concentration of smoke liquid coconut shell toward total bacteria count, shelf life and acceptability was to know the level of concentration of coconut shell liquid smoke of beef thus gained the lowest total bacteria count, the longest shelf life and acceptability of (color, taste, smell and total acceptance) the most favor. This research did based on Complete Randomized Design, with five type of repetitions of submersion coconut shell liquid smoke with concentrations those were 0%, 2.5%, 5% and 10% (v/v), with repetition as many as 4 times. The research result showed that the beef submersion used smoke liquid coconut shell influenced to the decreasing total bacteria, increasing shelf life, taste and total acceptance of beef but did not influence to color and smell of beef ($p<0.05$). Liquid smoke can be used until to the concentration 10% resulting total bacteria count as many as 11.73×10^6 CFU/g, shelf life as long as 1341 minutes and acceptability (color, taste, smell and total acceptance) with hedonic scale from rather favor to very favor.

Key words: beef, liquid smoke, coconut shell, shelf life

INTRODUCTION

After cuts of beef will experience changes in chemical, physical and microbial. Changes in chemistry began after the cut, which stops the blood circulation which causes the blood functions as an oxygen carrier halted all, as a result the process of oxidation - reduction come to a halt. Then there is a solution of anaerobic glycolysis of glycogen to lactic acid so that the pH of the meat becomes fall and cause the enzyme to be active catepsin. Protein split into peptone and amino acids, these products are used by bacteria to grow and form enzymes, which bacteria break down proteins and produce more products that stank (Muchtadi T and Sugiono, 1992). Microorganisms can grow in the range pH 5 to 8, a common bacterium grows optimally at pH 7.0, while for yeast and molds grow at pH 3 to 8.5. pH value of beef ranged from 6.2 to 6.4 so that bacteria, yeasts and molds can grow well in it (Frazier WC and Westhoff *et al*, 1998). The total number of bacteria that is conditioned upon the Indonesian National Standard (2000) maximum limit microbial contamination on fresh meat is 10^4 CFU / ml. Preserving meat has a goal to secure the meat from the damage or decay by microorganisms and to extend the shelf life (Soeparno, 2005). Liquid smoke can be used as a meat preservative because it has a degree of acidity (pH) of liquid smoke that reached 2.0 which causes stunted growth of harmful bacteria. Liquid smoke proved to suppress the growth of spoilage bacteria and pathogens such as *Escherichia coli*, *Bacillus subtilis*, *Pseudomonas* and *Salmonella* groups. Pyrolysis of coconut shell to produce liquid smoke containing phenol compounds by 4.13%, carbon 11.3% and 10.2% organic acids (Darmadji P, 2006). The result showed a coconut shell liquid smoke contains higher antimicrobial compounds and has a lower pH than most other liquid smoke (Darmadji P, 1997). Liquid smoke is a mixture of wood smoke dispersion in water is made with liquid smoke pyrolysis results. Results of pyrolysis liquid smoke is dependent on raw material and pyrolysis temperature. The main compounds in liquid smoke consists of phenols and organic acids (acetic acid, propionic, butyric and valerat), which can effectively control microbial growth (Darmadji P, 1997). The use of liquid smoke for preservation mackerel (*Rastrelliger neglectus*) Fresh conducted with 5-10% concentration of liquid smoke for 30 minutes to maintain freshness of the fish up to 24 hours. Results of research on dumbo spiced filet marinated in liquid smoke concentration of 10% for 1 minute to produce the most desirable organoleptic qualities include taste, odor and color. Results of research on tuna (*Euthynus affinis*) are

soaked in liquid smoke concentration of 5% for 30 minutes produces organoleptic quality of the most preferred (Maydina S, 2004).

MATERIALS AND METHODS

The materials used for this study is beef. The stages are carried out in meat preservation using liquid smoke following a coconut shell, meat weighed, then washed with running water and then drained for 5 minutes, then soaked in liquid smoke concentration of coconut shell with 0%, 2.5 %, 5%, 7.5%, 10% for 30 minutes. Then the meat that had been soaked with liquid smoke first drained for 5 minutes. Packaging performed by using sterile plastic. Then do a test on the total number of bacteria using the method of Total Plate Count (TPC) (Ministry of Health, 1991). Durable power is determined by initial testing decay (Puntodewo HS, 1998) and the acceptability of this test using a scale hedonic/preference level (Soekarto ST, 1985). This research conducted experiments in the laboratory. Experimental design used is Complete Random Design (RAL) with 5 treatments of various levels of concentration of liquid smoke coconut shell 0% (A0), 2.5% (A1), 5% (A2), 7.5% (A3), 10% (A4) and 4 times repeated, so that 20 units of the experiment. The total amount of data obtained bacteria transformed with logarithm transformation (log x), while the organoleptic test data to the color, taste, smell and the total revenue that will be transformed with the transformation. The data obtained were analyzed using Varian Analysis and to know the difference between the treatment performed Tukey test (Honestly Significant Difference / HSD) (Gasperz, 1991).

RESULTS AND DISCUSSION

The ability to suppress the total number of bacteria increases with increasing concentration of liquid smoke. This is caused by the phenol compounds contained in the liquid smoke. Phenol is an acidic alcohol, so called carbolat acid. Acidic conditions by the presence of phenol can affect the total number of bacteria. Growth of bacterial cells can be disrupted by the component phenols, phenol has the ability to damage proteins and cell membrane (Rahayu PW, 2000). Phenol binds to the protein through hydrogen bonds resulting in protein structures become corrupted. Most of the cell wall structure and bacterial cytoplasmic membrane protein and fat. Instability in the cell wall and cytoplasm membrane of bacteria causing selective permeability function, the function of active transport, control of protein structure from bacterial cells become disrupted. Cytoplasm integrity disruption resulted in the escape of macromolecules and ions from the cell. Bacterial cell to lose its shape, and there was lysis. fenolat compounds are bacteriostatic or depending on the concentration of liquid smoke (Pelczar and Chan, 1988). Bacterial cell death means the loss of the ability of bacteria to permanently reproduce (grow and multiplicate). Phenol compounds can also be combined with organic acids that work synergistically to prevent and control the growth of bacteria (Astuti, 2000).

Table 1. Difference between Total Number of Treatment Against Bacteria

Treatment	The average number of total bacteria (X 10^6 CFU / g)	Significance 0.05
A_1	76,05	a
A_2	64,37	ab
A_3	55,31	ab
A_4	43,60	b
A_5	11,73	c

Description: The same small letters to the column indicates not significantly different at α 0.05 .

A1: to-1 treatment (without soaking liquid smoke)
A2: 2nd treatment (concentration of coconut shell liquid smoke 2.5%)
A3: treatment to 3 (the concentration of liquid smoke coconut shell 5%)
A4: 4th treatment (concentration of coconut shell liquid smoke 7.5%)
A5: 5th treatment (concentration of coconut shell liquid smoke 10%)

Shelf Life of Beef Meat

Durable power of increasing in accordance with the increased concentration of liquid smoke. Symptoms of bacterial growth due to decomposition of them is formed slime (mucus) on the surface of the meat, loss of pigment color of the meat, there is gas production, the smell is less tasty and defects, and decomposition of fat. The formation of mucus on the surface of the meat caused by the growth of bacteria *L. viridens*. Slime-forming bacteria that is green *Thermospacta Enterococcus* and *Bacillus*. The formation of acid by the bacteria *Lactobacillus, Clostridium* and *Enterococci.*

Table 2. Difference between Treatment Against Average Shelf Live of Beef Meat

Treatment	Average of Shelf Live (minutes)	Signifikansi 0.05
A_1	595,67	a
A_2	800,33	b
A_3	1018,67	c
A_4	1204,33	d
A_5	1341,00	e

Discoloration caused by the meat pigment (myoglobin) change into brown metmyoglobin, a yellow or green is caused by bacteria forming sufmyoglobin (Lawrie, 2003). Change the color can also be caused by the formation of pigment by the microbes themselves (Frazier, 1988). The higher concentration of liquid smoke as a preservative coconut meat to produce the total number of bacteria that the lower and increases durable power of meat, this is in accordance with the purpose of reducing the amount of curing early microbial cells and slow the logarithmic growth phase microbes (Nurwantoro and Djariyah, 1997).

Acceptability

Test acceptability of the color of beef after the meat is marinated with various concentrations of liquid smoke has a coconut shell with boiled meat temperature 80°C for 30 minutes, prior to boiling the flesh color differences occur at each concentration. But after soaking boiled beef with various concentrations of liquid smoke coconut shell did not provide tangible effect on meat color. No significant influence on any given concentration of myoglobin caused by substances found in meat that has denatured so that the meat warming will change color from purplish red to brown (Winarno, 1992), so that each concentration of submergence showed a brown color not too different. Carbonyl compounds in the smoke has a role in coloring and flavor products. Type of carbonyl compounds contained in liquid smoke include vanillin and siringaldehide. Soaking meat by using coconut shell liquid smoke concentrations from 0 to 7.5% did not produce significant differences from each other due to the interval between the concentration of liquid smoke that is given is not much different. Soaking beef using various concentrations of liquid smoke coconut shell does not provide a real impact on odor. This is because the smell is very subjective and difficult to measure, resulting in different opinions in assessing the quality, the sensitivity difference in the feel and smell in addition one of the characteristics of liquid smoke is to make the product smell consistent (Pseszola, 1995) and panelists just kissed the surface meat which gives relatively the same smell. Soaking meat using various concentrations of coconut shell liquid smoke to the total revenue of beef provide a real or significant influence, it is because the power received on a food is determined by the stimulation that comes with food through the five senses of sight, smell, tasting, and hearing. However, the main factors that ultimately affect the total revenue of the food is flavor stimuli generated by food (Soekarto ST, 1985).

CONCLUSION

Liquid smoke can be used up to 10% concentration to produce the lowest number of total bacteria for 11.73 x 10^6 CFU/g, most long lasting power for 1341 minutes and acceptability (color, taste, smell, and the total acceptance) with the scale a bit like hedonic until very like.

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Table 3. The influence of treatment Acceptability Against Meat

Variables	Treatments				
	A_1	A_2	A_3	A_4	A_5
Acceptability					
- Colour	3,50	3,45	3,45	3,50	3,85
- Taste	$3,30^{a}$	$3,90^{ab}$	$3,30^{ab}$	$3,65^{ab}$	$4,30^{b}$
- Odor	3,60	4,05	3,50	3,95	4,15
- Total Acceptance	$3,55^{a}$	$3,60^{a}$	$3,65^{a}$	$4,15^{a}$	$4,30^{b}$

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Increasing The Egg Weight Of Burgo Chicken Offspring Through Cross-Mating Between Burgo Chicken With Native Chicken

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ABSTRACT

The research was aimed to increse the egg weight of burgo chicken offspring. The research used 300 hens, offspring from four mating types between burgo chicken and native chicken. These offspring were resulted from the succesive four mating groups of: a. Native cock with native hen (KK); b. Native cock with burgo hen (KB); c. Burgo cock with native hen (BK); d. Burgo cock with burgo hen (BB). The offspring in each mating group consisted of three postal coops, and each postal coop was filled by 25 hens. Variables collected from the hen offspring included for egg weight and egg production. Result showed that the egg weights of the cross-mating offspring of burgo chicken with native chicken were higher than those of mating offspring within BB. The increased egg weight of the KB cross-mating offspring was 30.79%, while that for the BK cross-mating offspring was 62.79%. However, the average egg production of the cross-mating offspring within Burgo chicken (BB) was the highest compared to the others (KB, BK, and KK). The egg production of the KB cross-mating offspring decreased 12.95%, while that for the BK cross-mating offspring decreased 19.77%. It can be concluded that the cross-mating offspring of both KB and BK mating types could be sonsidered for the purpose to produce relatively small egg production with the increased egg weight.

Key word: burgo chicken, cross-mating offspring, egg weight and egg production

INTRODUCTION

In Indonesia, there are many kind of native chicken that each of it had its own characteristic and some of them can be developed to broiler, layer and exotic chicken (Rasyaf, 1994). Native chicken including Burgo chicken has its potential to be both meat and egg production. This potential has not been used well yet, this fact was based on the management of Native chicken which is still very simple/traditional. Its life depends on its natural environment (Kingstone, 1979).

Burgo chicken was wide-spread in Bengkulu. It showed the variety of fowl in Bengkulu that could be a native asset of Bengkulu's Indigenous (Setianto *et al.*, 2009). Burgo chicken was the result of cross-mating type between Red Jungle Cock (*Gallus gallus*) with native hen (*Gallus domestica*) (Warnoto, 2000). This cross-mating type had produced new species that had superiority. Superiority that Burgo chicken have are the resistance of many kinds of disease, high egg production, attractive feather color and specific hi-pitched crow.

Warnoto (2001) clarified that Burgo hen had high egg production that approximately laid around 16-18 eggs a period and the interval between egg production was relative short, approximately around 7-10 days compared to native hen that usually laid 10 eggs a period with the interval between egg production approximately around 14-30 days. However, with this big amount of eggs produced, the egg weight was produced was light with an average egg weight that was approximately around 30 grams each from the interval of 25-35 grams. Low egg weight was correlated to the average weight of hen that was average around 750 grams a hen from the interval of 600 – 1500 grams/hen. Another characteristic that is beneficial as local layer, the sexual maturity of Burgo chicken was around 4-5 month, shorter than native chicken that was around of 5-7 month.

Burgo chicken developing efforts still have many obstacles. It's caused by less information and knowledge about Burgo chicken. In order to make Burgo chicken as superior commodity, it needs more scientific investigation towards Burgo chicken that will increase the potential of Burgo chicken.

From the previous research, it is known that Burgo hen had a potential to be layer (Warnoto, 2002; Warnoto and Setianto, 2009). The total of annual egg production doesn't have much difference with the total

annual egg production of native chicken if it compared. However, this high production total amount is not get along with the egg weight. The egg weight is lighter than the weight of native chicken's Egg. That's why, it is needed to find an alternative to produce a better egg, especially on its weight. One of the ways to do it is increasing the genetic quality by cross-mating method.

The research was aimed to obtain to increase the egg weight of Burgo chicken offspring through its cross-mating result.

MATERIALS AND METHODS

The research used 300 hens, offspring from four mating types between Burgo chicken and native chicken. These offspring were resulted from the succesive four mating groups of :

P1 = Native cock with Native hen (KK)
P2 = Native cock with Burgo hen (KB)
P3 = Burgo cock with Native hen (BK)
P4 = Burgo cock with Burgo hen (BB)

The offspring in each mating group consisted of three postal coops, and each postal coop was fileed by 25 hens.

The hens were raised intensively by *ad-libitum* feeding. Feeds that were given are corn, mixture of rice and bran, and concentrate with the composition of 40:30:30. The hen was raised for 2 month of its first production. The eggs withdrawn from each postal coop every day and it were identified by its offspring of mating group. Each egg was scaled.

Variables collected from the hen offspring included for egg weight and egg production. All of the variables that were observed were analyzed with Random System Program. If there are real differences at the variant analysis, than it will be continued by the different average test with Duncan's Multiple Range Test (DMRT).

RESULT AND DISCUSSION

Egg's weight average and egg production of the eggs subscribe in figure 1 and figure 2. According to the Figure 1, it was shown that the average egg weight result that was produced by the cross-mating offspring of Burgo cock with Burgo hen (BB) was significantly the lightest among others. The average egg weight of the cross-mating offspring of Native cock with Native hen (KK) was significantly the heaviest among others.

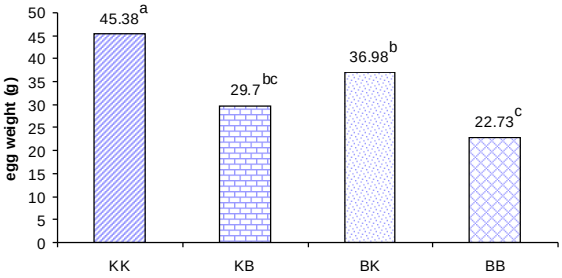

Figure 1. The average egg weight (g/egg) of the cross-mating offspring.

Explanation: Differences of Superscript were significant difference (P<0,05)
KK = Native cock with Native hen
KB = Native cock with Burgo hen
BK = Burgo cock with Native hen
BB = Burgo cock with Burgo hen

The most interesting thing was the egg weight of the cross-mating offspring of Burgo chicken with the other showed the increasing of the egg weight than the cross-mating offspring among Burgo cock with Burgo hen (BB). It can be seen that, there was an increasing of the average egg weight of 30.79% towards the cross-mating offspring of Native cock with Burgo hen (KB) and another increasing of 62.79% towards the cross-mating offspring of Burgo cock with Native hen (BK). However, the increasing of the average egg weight was still not getting the equality towards the average egg weight of the cross-mating offspring of Native chicken with Native hen (KK).

Different with the average egg weigh that was increased upon the cross-mating of Burgo chicken, the average egg production of Burgo chicken was decreased (Figure 2).

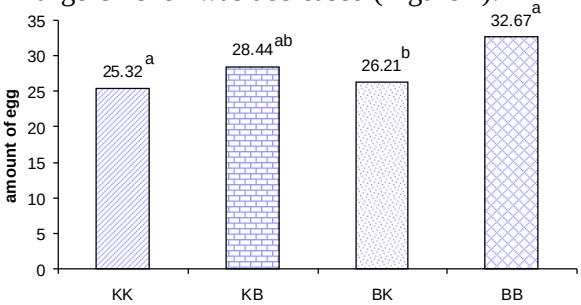

Figure 2. The average egg production of the cross-mating offspring in 2 month of first production

Explanation: Differences of Superscript were significant difference (P<0,05)

KK = Native cock with Native hen
KB = Native cock with Burgo hen
BK = Burgo cock with Native hen
BB = Burgo cock with Burgo hen

The average egg production of the cross-mating offspring among Burgo Chickens was the highest among the others (KB, BK, and KK). There was a decreasing of egg production among the cross-mating offspring of Burgo chicken with Native chicken (KB and BK) if it's compared to the cross-mating offspring of Burgo cock with Burgo hen (BB). Upon the cross-mating offspring of Native cock with Burgo hen (KB) there was a decreasing of 12.95% and a decreasing of 19.77% upon the cross-mating offspring of Burgo cock with Native hen (BK). Even though the decreasing was happened, the egg production of the cross-mating offspring among Burgo chickens, the egg production of the cross-mating offspring of native cock with Burgo hen (KB) and the cross-mating offspring of Burgo cock with native hen were still higher than the the egg production of the cross-mating offspring among Native chickens.

Changes that resulted to the offspring from the cross-mating method are the heterocyst effect from additive gene that resulting the average characteristic from both cross-mated parents. However, there were some cross-mating that could resulted better certain characteristic upon the offspring from both of its parents. This changes happened because of the effect of the dominant gene works or the over dominant gene (Warwick *et al*., 1984).

CONCLUSION

According to the observation result, it can be concluded that with the cross-mating method between Burgo chickens could increase the average egg weight. The cross-mating offspring of Native cock with Burgo hen (KB) and cross-mating offspring of Burgo cock with Native hen (BK) could be use for the purpose of the relatively small egg production with the increasing of the average egg weight.

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Some Physico-Chemical Properties of Surimi-Like Material from Beef Meat as Affected by Sucrose Level

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ABSTRACT

Recently, study of surimi-like material appeal to be studied. Surimi is a Japanese term for intermediate product made from ground meat fish deboned mechanically and washed by using chiling water repeatedly. One of the procedures of surimi processing is supplementation of cryoprotectant such as sucrose, sorbitol and phosphate to prevent from protein denaturation during processing and frozen storage of surimi. This study was evaluated the effect of sucrose level on the physico-chemical properties of beef surimi. The muscle tissue of round meat of beef was separated from fat and connective tissue manually and then was cut into 3 cm size of meat for mincing by using meat mincer. Then, the minced meat was washed three times by using chilling water (5-10^{o}C). The final wash used chilling 0.5% NaCl solution. The ratio of water to minced meat in washing was 3:1. The final step was dewatering by pressing washed minced meat in the screen of linen mesh manually. Finally, raw surimi was stirred with sucrose 3% (P1), 4% (P2) and 5% (P3) and added sodium tripolyphosphate 0.2% for each treatment. Both pH and WHC were significantly decrease from P2 to P3 ($P<0.05$), whereas the gel strength was no different. The decline of WHC was followed by the decrease of water and crude protein content ($P<0.05$). However, sucrose could not affect ash and fat content as well as salt-soluble protein . Sucrose supplementation at 3% in beef surimi is better than other level added.

Key words: surimi, physico-chemical properties, sucrose

INTRODUCTION

Surimi is a Japanese term for intermediate product made from ground meat fish deboned mechanically and washed by using chilling water repeatedly. Washing procedure is to remove fat and undesirable matters, such as blood, pigments, and odorous substances, and to increase the concentration of myofibrillar, thereby improving gel strength and elasticity, essential properties for surimi-based products (Lee, 1984). This product is light in color, bland in odor, low in fat, high in myofibrillar protein, and extremely functional due to the unique gelling properties of the myofibrillar protein (Jin *et al*., 2008).

Frozen surimi is used as a starting material in the factory due to the advantages of it rather than whole fish (Suzuki, 1981). Unfortunately, frozen storage decreases the functional properties, mainly gel-forming ability of surimi (Lee, 1984). The loss of this property is due to the denaturation of protein. The freezing increases solute concentration and favors dehydration, both of which contribute to protein denaturation (McDonald and Lanier, 1991). To prevent protein from denaturation during frozen storage, utilization of cryoprotectant, such as sucrose, sorbitol and phosphate is applied (Nowsad et al., 2000). At first, cryoprotectant applied was sucrose 8%, but it caused the surimi taste too sweet and turned the finished product a brownish color. To reduce the sweetness of surimi, cryoprotectant used was sucrose 4% and sorbitol 4%. The effectiveness of this sugar effect was markedly enhanced by adding phosphate 0.2% (Lee, 1984). Even though the formulation of cryoprotectant could not protect the gel strength, deformation was slightly improved, and water retention properties, elasticity and cohesiveness of gel were protected (Nowsad et al., 2000). Moreover, sorbitol utilization cause the surimi-based product texture is harder than the one with sucrose (Suzuki, 1981).

The characteristics mentioned above are affected by meat protein, mainly myofibril. The ability of meat protein (myofibril) binds water is important to evaluate the characteristics of meat and meat product (Aberle *et al*., 2001). The damage of meat protein will de-

crease some physico-chemical properties of meat and meat product.

Due to the negative effect of sucrose 8% and sorbitol, this study investigated the effects of sucrose level under 8% as a single agent of cryioprotectant on the physico-chemical properties of surimi-like from beef meat.

MATERIALS AND METHODS

Surimi Preparation

The *round* meat of beef was obtained from traditional market in Bengkulu. The muscle tissue was separated from fat and connective tissue manually and then was cut into 3 cm size of meat for mincing by using meat mincer. Then, the minced meat was washed three times by using chilling water (5-10^{o}C) which the final washing used chilling 0.5% NaCl solution. The ratio of water to minced meat in washing was 3:1. The final step of surimi preparation was dewatering by pressing washed minced meat in the screen of linen mesh manually. Finally, raw surimi was stirred with sucrose 3% (P1), 4% (P2) and 5% (P3) and added sodium tripolyphosphate 0.2% for each treatment. Each treatment was replicated three times.

pH and Water Holding Capacity (WHC)

Surimi pH was measured by using pH-meter (TOA HM-11p). At first, the electrode of pH-meter was calibrated to pH 4 and 7. After calibrating, the electrode of pH-meter was inserted into sample and the pH indicator rose on the monitor of pH-meter. WHC was determined by using Hamm method (Soeparno, 2005). The steps of WHC determination are below:

a) A 0.3 g sample was placed on filter paper Whatman 41 and pressed at 3,000 psi for 3 minutes by using Carver Press.
b) Two distinct areas are produced: a meat area and a water area and measured by using plain mater. The area between water and meat area is wet area (mm^2). The weight of water (mg) is counted by using formula:

$$\frac{wet\ area}{0.0948} - 8$$

c) Converting weight of free water into percentage of sample: ratio of weight of free water to weight of sample (known as % free water).
d) To determine WHC, the percentage of free water in the moisture of sample is counted by using formula:

$$\frac{100}{moisture} \times (\%\ free\ water)$$

e) Finally, WHC (%) is counted by the formula: (100 – percent free water in the moisture).

Gel Strength

Gel strength was determined according to method described by Tan et al., (1988). Surimi was mixed with 3% smooth salt and 30% chilling water by using food processor until sticky surimi formed. Then, sticky surimi was cased and was heated with double step heating: 40°C in 20 minutes and 90°C in 20 minutes. This surimi's gel strength was measured by using anvil instron 1140 and expressed as gf/cm^2.

Chemical Composition

The procedures used to determine proximate composition was similar to that of Apriyantono *et al*. (1989). Moisture was determined through oven drying method at 110°C for 24 h; crude protein was determined by using Kjeldhal method; crude fat was evaluated by using the soxhlet method; and ash content was measured by ashing the sample in a muffle furnace at 600°C. Using a modified method used by Park *et al*. (1996), Salt-Soluble Protein was measured after it was homogenized by using 20 ml salt solution for a minute in an ice bath. Homogenate was centrifuged for 10 minutes at 3020x g and the filtrate was separated. Filtrate was centrifuged for 10 minutes at 3020x g and supernatant was decanted. A ml of supernatant was used to determined Salt-Soluble Protein by using Kjehdahl method.

Statistical Analysis

One-way ANOVA was used to compare the treatments effects. Duncan's Multiple Range Test was set to determined significant differences among mean values. The level of significance was $P<0.05$.

RESULTS AND DISCUSSION

Physical Characteristics

The physical characteristics results are presented in Tabel 1. Value of pH and WHC obtained tended to be lower from 3% to 5% sucrose supplementation ($P<0.05$). Honikel (1987) reported that pH has a profound effect

on the physical properties such as WHC, tenderness and color in meat. Ini this study, the decrement of pH was followed by the decrement of WHC and it was parallel to Kristinsson and Hultin (2003). They reported that an increment of surimi gel pH led to a considerable increment of WHC. Various researchers have found that decrement of pH significantly correlated with the loss of textural qualities such as gel strength (Nowsad *et al*., 2000). Medina and Garrote (2002) reported that cryoprotectant could not prevent gel strength from decrease. This research showed that there was no different with gel strength value.

In this study, cryoprotectant had influenced pH and WHC of beef surimi and had not influenced on gel strength. The pH of P1 (4.85) was no different from P2 (4.73) but significantly different from P3 (4.63), whereas, P2 was no significant value. The pattern of pH decline was not followed by WHC's pattern which the WHC at P1 (54.44) was marked different from P2 (45.99) and P3 (43.32), and between P2 and P3 was no different statistically. Nowsad *et al*. (2000) reported that cryoprotectant could not protect the gel strength or breaking strength, but it could protect water retention of surimi. The cryoprotectant used by Nowsad *et al*. (2000) was combination of sucrose, sorbitol and Natripolyphospate. The difference result of these was probably affected by different cryoprotectant used. This study used sucrose as a single agent of cryoprotectant so that the effect of the cryoprotectant was different from Nowsad's study. The highest value of the physical variable was 3% sucrose added to the surimi material.

Chemical Characteristics

The result of the effect of sucrose level on the chemical characteristics is presented in Table 2. The moisture of surimi was significantly increased. Contrary, crude protein tended to decrease markedly. However, sucrose could not affect ash and fat content as well as salt-soluble protein.

Moisture expressed the water content of surimi. Water content of material is not parallel with the WHC value. In various cases, the higher of water content the lower of WHC value. In this study, WHC of the surimi was decreased (Table 1), while the moisture of surimi was increased significantly (Tabel 2). The average of the moisture of the study was matching to the normal moisture of fresh meat hich contains 68-80% (Aberle *et al*., 2001). Ash content and crude fat were no significant response.

The interesting here was that crude protein and salt-soluble protein of surimi. The crude protein was decline distinctly between P1 (16.16%) and P3 (13.39%), while P2 (14.63 was no different, but the salt-soluble protein response was no different. This fact indicated that the increment of sucrose added could not protect protein content of the surimi although it was no change the salt soluble content of the surimi. The decline of the crude protein corresponded to the decrement of the WHC. One of the factor affecting WHC is protein content which the protein molecules bind the water molecule (Aberle *et*

Tabel 1. The pH, Water holding Capacity (WHC) and Gel Strength of beef surimi

Variabels	Sucrose level		
	3 % (P1)	4 % (P2)	5 % (P3)
pH	4.85 ± 0.03^{a}	4.73 ± 0.07^{ab}	4.63 ± 0.04^{b}
WHC (%)	54.44 ± 0.38^{a}	45.99 ± 0.71^{bc}	43.32 ± 0.52^{c}
Gel Strength (gf/cm^2)	421.62 ± 1.72	361.52 ± 1.29	411.63 ± 1.47

Note: a-c different superscript within a row are significantly different (P<0.05).

Tabel 2. Proximate Composition and Salt-Soluble Protein of beef surimi

Variabels	Sucrose level		
	3 % (P1)	4 % (P2)	5 % (P3)
Moisture (%)	77.2 ± 0.64^{a}	79.87 ± 0.40^{b}	78.57 ± 0.27^{b}
Ash content (%)	0.66 ± 0,02	0.45 ± 0.01	0.57 ± 0.02
Crude fat (%)	3.30 ± 0.09	3.16 ± 0.12	3.76 ± 0.26
Crude protein (%)	16.16 ± 0.86^{a}	14.63 ± 1.3^{ab}	13.39 ± 0.77^{b}
Salt-Soluble Protein (%)	1.8 ± 0.03	1.85 ± 0.04	1.79 ± 0.04

Note: $^{a-c}$ different superscript within a row are significantly different (P<0.05).

al., 2001). This study resulted that 3% This study resulted that 3% sucrose added had the highest value of the variables.

CONCLUSION

The increase of sucrose level added to beef surimi decreased pH, WHC and crude protein, but it could not change the responses of gel strength, ash content, and crude fat and salt-soluble protein. The suitable sucrose level for the best characteristic of beef surimi was 3% sucrose as a single agent of cryoprotectant.

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Does Productivity Index of Boerawa Does and Etawa Grade Does Fed by Traditional and Rational Foodstuff

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ABSTRACT

Boerawa goats is crossbreed between Boer buck and Ettawa Grade does. This research was conducted to investigate: (a) interaction between goat breed and kind of foodstuff to does productivity index of Boerawa does and Etawa Grade does, (b) the effect of goat breed on does productivity index of Boerawa does and Etawa Grade does, (c) the effect of kind of foodstuff on does productivity index of Boerawa does and Etawa Grade does based on their kid weaning weight. This research was conducted with experimental method using 20 Boerawa does and 20 Etawa grade does having two-three times of kidding period. Ten Boerawa does and 10 Etawa grade does got rational foodstuff (60% forage and 40% concentrate), and 10 Boerawa does and 10 Etawa grade does got traditional foodstuff (100% forage). Factorial (2x2) of completely randomized design with ten replications was used in this study. The result showed that there was no interaction between goat breed and kind of foodstuff to does productivity index of Boerawa does and Etawa Grade does. The result indicated also that does productivity index (40,900 kg) of Boerawa does higher (P<0,01) than does productivity index (30,996 kg) of Etawa Grade does. The result indicated also that does productivity index (41,298 kg) of goat got rational foodstuff higher (P<0,01) than does productivity index (30,598 kg) of goat got traditional foodstuff. It could be concluded that Boerawa does were more productive than Etawa Grade does.

Key word: *weaning weight, Boerawa does and Etawa Grade does, does productivity index*

INTRODUCTION

Genetic improvement is an integral part of many goat development programmes in the tropics, where breeding policies mostly aim to upgrade local goats by crossbreeding with, either temperate or tropical exotic breeds. The Boer goat is a famous meat goat breed in the world. It is well-known for fast growth, high reproductive, strong adaptability, and excellent meat-purpose body conformation. Since 2001, the Lampung Provincial Government has introduced some Boer goats from Australia. The Goats were reared to be collected their semen. The semen then be processed to be frozen semen that will be inseminated to local goat of farmers in some villages, one of them was Campang Village, Gisting District, Tanggamus Regency, Lampung. In the village, the farmers had united in some groups.

Crossbreed between Boer buck and Etawa grade does was namely Boerawa goat. Based on our observation, Boerawa crossbreed has meat type characteristic. Population of Boerawa goat increased significantly in Lampung, although the farmers fed the goats with conventional ration i.e. forage without concentrate ration. The farmers like very much to the performance of Boerawa goat. Boerawa goat grew faster than Etawa grade goat. Service per conception of both goat were not different significantly, it was about two. The first kidding of Both Boerawa and Etawa grade goat were single type, but for the following kidding it would be twin or triplet. Besides that, there is not unpleasant odor of Boerawa goat body like in Kacang or Etawa grade goat, thus farmers like Boerawa goat very much.

Population of Boerawa goat increased significantly in Lampung, although the farmers fed the goats with conventional ration i.e. forage without concentrate ration. Nevertheles, until now there is no information on evaluation of this crossbreed. Crossbreed evaluation on its productivity is very important to decide the development of the crossbreed (Basuki *et al*., 1998). Crossbreed productivity can be evaluated by computing the value of does productivity index (DPI). This value describes kid body weight produc-

tion produced by a doe or group of does per year by calculating kidding interval, litter size, and kid body weight at certain age.

Development of both Boerawa and Etawa grade does was good enough although they got the same treatment by the farmers. Actually based on their original Boer and Etawa goat have some differences. Boer goat that is from South Africa and developed in Australia has mating season, but Etawa goat that is from India does not has mating season. Besides that, Boer goat is meat type, while Etawa goat is dual-purpose. Boerawa and Etawa grade does was applied by feeding the same treatment i.e forage only. It is estimated that their productivity are not optimal. It is important to optimalize by adding rational foodstuff i.e forage and concentrate.

This research was conducted to evaluate productivity of Boerawa and Etawa grade does fed by traditional (forage only) and rational foodstuff (forage and concentrate) in order to know weather both goat give the same or different respon to different foodstuff quality. In detail the objecteive of this research were to investigate: (a) interaction between goat breed and kind of foodstuff to does productivity index of Boerawa does and Etawa Grade does, (b) the effect of goat breed on does productivity index of Boerawa does and Etawa Grade does, (c) the effect of kind of foodstuff on does productivity index of Boerawa does and Etawa Grade does based on their kid weaning weight.

MATERIALS AND METHODS

This research was done in February-Nopember 2007, in Campang Village, Gisting District, Tanggamus Regency. This research was conducted with experimental method using 20 Boerawa does and 20 Etawa grade does having two-three times of kidding period. Ten Boerawa does and 10 Etawa grade does got rational foodstuff (60% forage and 40% concentrate), and 10 Boerawa does and 10 Etawa grade does got traditional foodstuff (100% forage). Factorial (2x2) of completely randomized design with ten replications was used in this study. The first factor (A) was breed of goat, i.e: Boerawa and Etawa grade does. The second factor (B) was kind of foodstuff, i.e: rational and traditional foodstuff. Parameters investigated in this study were weaning weight, kidding interval, birth type, does age, and mating recording. These parameters were used to compute does productivity index of Boerawa does and Etawa Grade does based on their kid weaning weight. Data were analyzed statistically using ANOVA and continued with Duncan's Multiple Range Test (Steel and Torrie, 1991).

RESULTS AND DISCUSSION

Weaning Weight

Weaning weight is body weight at the time of weaning in 4 months of age. Weaning weight is one of criterion considered in goat selection because weaning weight reflected a does in producing milk and growing its kids. The average of weaning weight of Boerawa and Etawa grade goat fed by traditional and rational ration are presented in Table 1.

Table 1 showed that weaning weight average of Boerawa goat fed by traditional and rational foodstuff were 18.402±0.535 kg and 22.949±4.722 kg respectively, while in Etawa grade goat were 16.813±0.885 kg and 18.063±1.475 kg respectively. The result indicated that there was interaction between goat breed and kind of foodstuff to weaning weight average. The result indicated also that weaning weight of Boerawa goat was higher ($P<0.01$) than that of Etawa grade goat weather fed by traditional or rational foodstuff. The high weaning weight of Boerawa goat was caused by its genetic. Boerawa goat grew faster than Etawa grade goat at the same age. This genetic of growing faster is inherited by Boer goat. Hass (1978) reported that average daily gain before and after

Table 1. Weaning weight (kg) of Boerawa and Etawa grade goat fed by traditional and rational foodstuff

Goat breed	Foodstuff		Average
	Traditional	Rational	
Boerawa	18.402[b]	22.949[c]	20.675
Etawa grade	16.813[a]	18.063[b]	17.438
Average	17.608	20.506	19.057

Note: The different superscript indicated significant effect ($P<0.05$).

weaning of boer crossbreed were 114.0 g and 65.0 g respectively, while in Etawa grade were 103.90±4.0 g and 65.60±2.0 g respectively (Sulastri, 2001).

Inheritance potential of Boer goat to its progeny was prooved if Boer goat crossed by other breed goat. Das *et al*. (2005) reported that body weight of Blended goat (Kamorai 55%, Boer 30%, and local goat 15%) could achieve 11.14±0.15 kg at 16 weeks of age with ADG before weaning 80.0±1.0 g. Body weight of Blended goat at 150 days was 19.7 kg that was higher than Little East Africa (LEA) goat (14.9 kg) with ADG preweaning of Blended and LEA were 84.0 g and 11.0 g respectively. Thus, Boer goat has good combining ability if it is crossed by other breed included with Etawa grade goat indicated by high potential growing of crossbreed.

The result showed that weaning weight of Boerawa goat was higher than that of Etawa grade goat. This was caused by the higher milk production of Boerawa does than of Etawa grade does. Barry and Godke (2005) reported that milk production of Boerawa does were 2.5 liter/head/day, while Etawa grade does produce 1.5 liter/head/day milk (Yusnandar, 2004).

The high weaning weight of Boerawa goat was caused by the high birth weight (2.875±0.155 kg), while birth weight of Etawa grade goat was 2.201±0.453 kg. Birth weight positively correlated to weaning weight. Sulastri (2001) reported that genetic correlation between birth weight and weaning weight estimated by parent-offspring correlation and by halfsib correlation were 0.54±0.29 and 0.29±0.9 respectively.

The result indicated that weaning weight of goat fed by rational foodstuff was higher ($P<0.05$) than that of goat fed by traditional foodstuff whether in Boerawa or Etawa grade goat. This indicated that improving foodstuff quality could increase milk production of does and finally could increase kid weaning weight.

Litter Size

The result on litter size are presented in Table 2. Table 2 showed that litter size of Boerawa does fed by traditional dan rational foodstuff were 1.767±0.37 and 1.833±0.408 respectively, while in Etawa grade does were 1.600±0.225 and 1.667±0.283 respectively. The result indicated that there was no interaction between goat breed and kind of foodstuff to litter size. Litter size between Boerawa and Etawa grade does did not differ significantly ($P>0.05$). Litter size of goat fed by traditional and rational foodstuff did not differ ($P>0.05$) either. This result was relatively the same as the result reported by Pamungkas *et al*. (2005) in crossbreed Boer x Kacang goat (1.6). The result of present study was within the range of the result reported by Barry and Godke (2005) in Boer goat (1.6-2.1), but relatively higher than the result reported by Subandriyo *et al*. (1995) in Etawa grade does (1.3-1.6).

Kidding Interval

Kidding interval is period between two sequence kidding that consist of mating period (from kidding to conception period) and pregnant period (Devendra and Burns, 1994). Kidding interval is very important factor that decide high and low production of kid resulted by doe per year (Abdulgani, 1981). Kidding interval of Boerawa and Etawa grade does fed by traditional and rational foodstuff are presented in Table 3.

Table 2. Litter size of Boerawa and Etawa grade does fed by traditional and rational foodstuff

Goat breed	Foodstuff		Average
	Traditional	Rational	
Boerawa	1.767	1.833	1.800
PE	1.600	1.667	1.633
Average	1.683	1.750	1.717

Table 3. Kidding interval of Boerawa and Etawa grade does fed by traditional and rational foodstuff (month)

Goat breed	Foodstuff		Average
	Traditional	Rational	
Boerawa	11.750	10.450	11.100
PE	11.550	10.650	11.100
Average	11.650	10.550	11.100

Table 3 showed that kidding interval of Boerawa does fed by traditional and rational foodstuff were 11.75±0.486 and 10.450±1.442 month respectively, while in Etawa grade does were 11.55±0.497 and 10.650±1.055 month respectively. The result indicated that there was no interaction (P>0.05) between goat breed and kind of foodstuff to kidding interval. Kidding interval of Boerawa and Etawa grade does did not differ significantly (P>0.05). This result was longer than the result reported by Devendra and Burns (1997) i.e 327 days (10,9 month) and that was reported by Setiadi *et al*. (1995) i.e 10 month.

Does Productivity Indeks (DPI)

Does Productivity Indeks (DPI) is does ability description in taking care of their kids up to weaning age and growing up their kids to achieve certain weight in certain age in one year (Sumadi, 1993). Does productivity index of Boerawa and Etawa grade does based on weaning weight are presented in Table 4.

Table 4 showed that DPI of Boerawa does fed by traditional and rational foodstuff were 33.22±7.068 kg and 48.579±12.969 kg respectively, while in Etawa grade does were 27.974±4.192 kg and 34.018±5.907 kg respectively. The result indicated that there was not interaction (P>0.05) between goat breed and kind of foodstuff to DPI. The result indicated also that DPI of Boerawa does was higher (P<0.01) than that of Etawa grade does. Goats fed by rational foodstuff were higher (P<0.01) than those fed by traditional foodstuff. The higher DPI of Boerawa does was caused by the higher weaning weight of their kids (18.402±0.535 kg) compared with weaning weight of Etawa grade kid (16.813±0.885 kg), although kidding interval and litter size of both breed were not difference significantly.

The higher weaning weight of Boerawa kids was caused by the higher preweaning ADG of Boerawa kids (0.099±0.007 g) compared with preweaning ADG of Etawa grade kid (0.085±0,005 g). This higher preweaning ADG of Boerawa kid was caused by genetic potential inherited by Boer buck as meat type, while the lower preweaning ADG was caused by genetic potential inherited by Etawa goat as dual purpose type.

CONCLUSION

Boerawa does were more productive than Etawa Grade does that it was prooved by the higher weaning weight of their kids compared with those of Etawa grade kid, although litter size and kidding interval of both breed were relatively the same.

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Table 4. Does productivity index of Boerawa and Etawa grade does fed by traditional and rational foodstuff

Goat breed	Foodstuff		Average
	Traditional	Rational	
Boerawa	33.221	48.579	40.900b
PE	27.974	34.018	30.996a
Average	30.598a	41.298b	35.498

Note: The different superscript in the same row or column indicated significant effect (P<0.05).

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Application of Fermented Palm Kernel Cake and Cassava Byproduct Mixture in Broiler

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ABSTRACT

This experiment was conducted to evaluate the effect of feeding PKC-cassava byproduct mixture fermented by *Aspergillus niger* in ration on broiler performance. This research used 96 DOC broiler. The birds were reared in litter floor pen and were fed 0 (P0), 10 (P1), 20 (P2), and 30% (P3) of the fermented PKC-cassava byproduct mixture in the ration. Feed and water were given *ad libitum* and reared for 6 weeks. The experiment used Completely Randomized Design with 4 ration treatments and 4 replications and each replication consisted of 6 birds. Data were analyzed statistically using ANOVA and continued with Duncan's Multiple Range Test. The result showed that diet treatments did not affect ration consumption, gain, ration conversion, carcass percentage, meat crude fat, and blood cholesterol, but affected abdominal fat percentage, and IOFC. Utilization of fermented PKC-cassava byproduct mixture untill 30% in the ration was comparable with the control diet.

Key words: palm kernel cake, cassava waste, fermentation, broiler

INTRODUCTION

The usage of fermentation product in broiler ration is predicted to increase the ration palatability and final weight, and it can decrease body fat. Evaluation on nutrient ingredient of many composition of palm kernel cake (PKC)-cassava byproduct mixture fermented by *Aspergillus niger* was investigated by Nurhayati (2007). The result indicated that composition of PKC 60% and cassava byproduct 40% was the best mixture in nutrition value compared with the other composition (100% PKC, 80% PKC : 20% cassava byproduct, 60% PKC : 40% cassava byproduct, 40% PKC:60% cassava byproduct, 20% PKC:80% cassava byproduct, and 100% cassava byproduct). It could be proved by increasing of crude protein from 10,75% to 18,61% an by decreasing crude fiber and extract ether i.e. from 16,92% to 8,07% and from 10,13% to 2,39% respectively.

It is important to apply this fermentation product in broiler to investigate boilogical effect. The objective of this research was to evaluate the effect of feeding PKC-cassava byproduct mixture (60% PKC and 40% cassava byproduct) fermented by *Aspergillus niger* in ration on broiler performance.

MATERIALS AND METHODS

Ninety six Day Old Chick of broiler were used in this research. Ration formulation used and its nutrient content were presented in Table 1 and Table 2.

Table 1. Trial ration formulation for starter and finisher period

Feedstuff	Starter (%)				Finisher (%)			
	P0	P1	P2	P3	P0	P1	P2	P3
Consentrate***)**	43	40	37	34	35	32	29	26
Corn mill[1)]	57	50	43	36	52.5	45.5	38.5	31.5
Rice bran[1)]	0	0	0	0	10	10	10	10
Fermentation product	0	10	20	30	0	10	20	30
Palm oil	0	0	0	0	2.5	2.5	2.5	2.5
Total	100	100	100	100	100	100	100	100

Note: P0, P1, P2, and P3 = Diet with 0%, 10%, 20%, and 30% of fermentation product in the ration;
*) Based on nutrient analysis of animal feedstuff factory (CP 40%; ME 2,974 ccal; EE 3%; CF 5%; Ca 2.5%; P 1.2%); 1) Wahyu (1992).

Table 2. Nutrient content in each treatment*)

Treatment	Nutrient content of starter diet						Nutrient content of finisher diet					
	CP	ME	EE	CF	Ca	P	CP	ME	EE	CF	Ca	P
P0	22.49	3,243.04	3.40	3.72	1.02	0.65	20.02	3,28.05	4.19	3.95	0.77	0.67
P1	22.50	3,200.64	3.29	4.21	1.04	0.69	20.03	3,185.65	4.08	4.44	0.80	0.70
P2	22.51	3,158.24	3.18	4.69	1.07	0.73	20.04	3,143.25	3.97	4.92	0.82	0.74
P3	22.52	3,115.84	3.07	5.18	1.09	0.77	20.06	3,100.85	3.86	5.41	0.85	0.78

*) Based on calculation result.

Birds were reared in litter floor pen that was separated 16 plots with size of 1x1.2 m^2 for each plot. Birds were divided into 16 groups (4 treatments and x 4 replications consisted 6 birds of four diet treatments i.e. P0 (diet containing 0% fermentation product), P1 (diet containing 10% fermentation product , P2 (diet containing 20% fermentation product, and P3 (diet containing 30% fermentation product).

Feed and water were given *ad libitum* and birds were reared for 6 weeks. Feed given were designed for two period: starter for birds aged 0-3 weeks and finisher for birds aged 4-6 weeks. At the end of sixth weeks, 3 samples unit of each replication of each treatment were slaughtered. Parameters measured were: (a) ration consumption (g), (b) ADG (g), (c) ration convertion, (d) income over feed and chick cost (IOFC), (e) carcass percentage (%), (f) abdominal fat percentage (%), (g) meat fat content (%), and (h) blood cholesterol content (mg/100ml).

The experiment used Completely Randomized Design with 4 ration treatments and 4 replications and each replication consisted of 6 birds. Data were analyzed statistically using ANOVA and continued with Duncan's Multiple Range Test (Steels and Torrie, 1980).

RESULTS AND DISCUSSION

The result indicated that ration consumption of broiler from the highest to the lowest were P3 (5,102.59 g), P1 (5,053.28 g), P2 (4,986.24 g), and P0 (4,926.08 g) respectively. The treatments did not affect ration consumption (P>0.05). It does mean that the fermentation did not increase feed consumption. In contrast, Saono (1976) stated that fermentation product could increase ration palatability.

Table 3 showed that gain in treatment P1, P0, P2, and P0 were 2.397,86 g, 2,393.23 g, 2,363.78 g, and 2,322.25 g respectively. The result indicated also that treatment did not affect birds gain (P>0.05). It's mean that ration containing fermentation product until 30% did not disturb gain of the birds.

Many factors responsible for the growth of birds, one of them is fibre content in the ration. Birds ration contained high crude fiber would cause difficulty in metabolizing the ration so that it would disturb nutrient metabolizing and it would go out with feces (Hough and Basett, 1975; Borgman and Wardlow, 1975) cited by Latif *et al*. (1999), and as a consequence the nutrient will not be used for birds growth. The fibre content of the diets used in this research were 3.72 – 5.18 % (Table 2), still match with NRC recommendation.

Table 3. Performance, carcass percentage and blood cholesterol of broiler fed diets containing fermentation products

Variabel	Diet treatments			
	P0	P1	P2	P3
Ration consumption (g)	4,926.08 ± 68.91	5,053.28 ± 78.02	4,986.24 ± 126.25	5,102.59 ± 163.39
Gain (g)	2,393.23 ± 48.40	2,397.86	2,363.78	2,322.25
Ration conversion	2.06 ± 0.04	2.11	2.11	2.20
IOFCC (Rp)	2,733.16 ± 1,303.54[a]	3,415.77[a]	5,098.07[b]	6,254.06[c]
Carcass percentage (%)	66.19 ± 1.47	67.56	67.82	65.17
Abdominal fat percentage (%)	2.20 ± 0.10[b]	2.07[a]	2.05[a]	1.99[a]
Meat fat content (%)	8.46 ± 0.66	8.20	7.99	8.05
Blood cholesterol (mg/100g)	127.73	117.26	115.18	112.52

Note: Different superscript in the same row indicated significant effect (P<0.05);
P0, P1, P2, and P3 = Diet with 0%, 10%, 20%, and 30% of fermentation product in the ration.

The treatments did not affect ration conversion (P>0.05). The ration conversion of birds fed P0, P1, P2, and P3 were 2.06, 2.11, 2.11, and 2.20 respectively. The result indicated that products fermentation made of cassava and palm kernel cake can be used until 30% in broiler ration.

The treatment did not affect carcass percentage (P>0.05) but significantly affected abdominal fat percentage. Carcass percentage of broiler fed P2, P1, P0, and P3 were 67.82%, 67.56%, 66.19%, and 65.17% respectively. Carcass percentage in the present study ranged from 65.17% to 67.82%. This result was fit to the standard range stated by Siregar *et al*. (2003) that carcass percentage of broiler range from 65% to 75% of body weight.

Abdominal fat percentage of birds fed P0, P1, P2, and P3 were 2.20%, 2.07%, 2.05%, and 1.99% respectively. Abdominal fat of birds fed diet P0 was higher than those birds fed P1, P2, and P3 (P<0.05), but no significant was found among abdominal fat of birds fed P1, P2 and P3. Abdominal fat percentage in the present study was still normal. Mountney (1976) cited by Yotolembah (2003) stated that abdominal fat percentage of broiler ranged from 1.3% to 7.3%. Abdominal fat percentage was affected by fermentation adding in the ration. The more fermentation product in the ration the lower abdominal fat percentage. It is supposed that the fibre present in the diet was responsible for the abdominal fat content. In this study, the fibre content increased as increasing utilization of fermentation product in the ration (Table 2). Crude fiber in the digestion tract will bind bile acid. This condition caused gall function to help in absorbing lipid impeded. Furthermore bile acid bound by crude fiber will be sent out together with crude fiber in the form of feces. If this condition was running continually, it would imped the formation of abdominal fat (Hough and Basett, 1975; Borgman and Wardlow, 1975) cited by Latif et.al (1999).

Income Over Feed and Chick Cost (IOFC) of P3, P2, P1, and P0 were Rp6,254.06, Rp5,098.07, Rp3,415.77, and Rp2,733.16 respectively. The treatments affected IOFC (P<0,05), where IOFC of P3 was higher than that of P2, P1, or P0 (P<0.05). IOFC of P2 was higher than that of P1 and P0 (P<0.05), while IOFC of P1 and P0 did not differ significantly (P>0.05). The higher IOFC of P3 was caused by the lower price of the ration per kg than that of P0, P1 or P2. The lower price of P3 diet was related to the lower use of concentrate and corn mill in the ration, where price of corn mill and concentrate was more expensive compared with that of fermentation product.

The treatment did not affect blood cholesterol and meat fat content. The blood cholesterol content P0, P1, P2, and P3 were 127,73 mg/100g, 117.26 mg/100g, 115.18 mg/100g, and 112.52 mg/100g respectively. The meat fat content of broiler fed P0, P1, P2, and P3 were 8.46%, 8.20%, 7.99%, and 8.05% respectively. The blood cholesterol and meat fat content were correlated with fibre content of the diet, where the higher fibre content in the diet, the lower blood cholesterol and meat fat content. The fibre content of the diet in this study (Table 2), however, was not enough to decrease blood cholesterol and meat fat content.

CONCLUSION

Diet treatments did not affect ration consumption, gain, ration conversion, carcass percentage, meat crude fat, and blood cholesterol, but affected abdominal fat percentage, and IOFC. Utilization of fermented PKC-cassava byproduct mixture untill 30% in the ration was comparable with the control diet.

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The Effect of Hatching Media on Hatching Capacity and Stadium Nymph in Cricket *Gryllus mitratus*

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ABSTRACT

Cricket is one of potential animals that can be used as food souces, such as human food and feed of domestic animal because of its hight protein contain. Cricket can be produced by laying eggs after the process of fertilization. Cricket do not incubate it's own eggs, but the incubation can take place in looses soil, sand, sock, cotton, and newspaper with the humidity of 70%-80%. The aim of this study was to know the effect of hatching media on hatchind capacity and stadium nymph I in cricket *Gryllus mitratus.* The study was conducted in a room of the Pandu house, Beringin Raya Village, Muara Bangkahulu Subdistrict, Bengkulu District from August until September, 2005. A number of the eggs of cricket *Gryllus mitratus* used in the study were 320 eggs with 4 replications of which each replication consisted of 20 eggs. Parameter measured were the first hatching body weight (mg), hatching ability (%), hatching weight (mg), hatching period (day), nymph period (day), the first nymp weight (mg), temperature and humidity of the chamber. The results showed that the hatchery of cricket eggs by using some haching media were significant different ($P<0.05$) on hatching ability and nymp I period. The average of hatching ability by the sand (70.00%) was not significantly different to that by the loose soil (73.75%) and combined treatments (71.25%), but it was significantly different ($P<0.05$) to that by the sock (86.25). The nymp I period by the sock was longer than the others. In conclution the sock was better to be used as hatching media of cricket eggs but it was not used for growing offspring of cricket.

Key words: cricket eggs, hatching media, hatching ability, and stadium nymph I.

INTRODUCTION

The cricket is category as a the pest of agriculture plant, because it is destroyof plant especially young leaf not only in farm but also rice cultivation (Rahman, 2002). Until now, there are 123 species of cricket is cultivated and it was difficult to differentiate between species because of almost the same (Paimin *et al.*, 1999).

Widyaningrum (2001), In natural, the cricket can be mating with many times (multi mating) with different male in species class. And then, fertility of female can increase in different male. The cricket is reproduction with lay eggs, it was became after mating. In observation, there are many technical for incubation of cricket egg. In tradition livestock Kebun Tebeng, Bengkulu District, the incubation of cricket eggs with paper as a media incubation. Besides this, livestock in Simpang Skip, Bengkulu District used cotton and paper for incubation.

The cricket is not incubation a lone, but it's need loose soil with humidity 70% - 80%. Widyaningrum (2001), state that male of cricket look for place of web and loose for lay eggs until many time. In generally, the male of cricket lay eggs in loose within 5 -15 mm (Kumala, 1999).

Base on the this information, It is important to research the effect of hatching media on hatching capacity and stadium nymph I in cricket *Gryllus mitratus*. The purpose of this research was to observe to influence hatchery media; sand, soil, combined sand and soil and web sock.

MATERIALS AND METHODS

The study was conducted in room of the Pandu house, Beringin Raya Village, Muara Bangkahulu Subdistrict, Bengkulu District from August until September, 2005.

The materials used in the study were box as a incubation, hand sprayer, hygrometer, soil tester, analytic scales, lux and filter. The eggs of cricket *Gryllus Mitratus* used in the study were 320 eggs, sand, soil, looses soil, sock.

Stage of Study

1. The collective of eggs; This eggs came from of the livestock of cricket in Bengkulu District. This eggs was selected good eggs, fresh, and clean.

2. The supplying of materials and incubation. The sand used of this research was river sand. This sand was washing before using. After drying, it was set in tray with thick of 2 cm. The soil was used of black color in surface of soil. This soil was washing before using. After drying, it was set in tray with thick of 2 cm. For mixture of sand and soil, in ratio 1:1, it was set in tray with thick of 2 cm. Beside this, The sock was also set in tray with thick of 2 cm. This humidity was incubation to endure about 60% - 80%. The tray was set in plywood with measure 10 x 15 cm with high of tray 8 cm.

Variable that Measure

a. First eggs weight; it was obtain from the weighing oh eggs before incubation.
b. Hatching capacity; it was calculated the total of hatch egg divide total of sample eggs and time of 100%.
c. Hatching weight; The first weight of hatching.
d. Hatching period; It was calculate from the lay eggs until hatching.
e. Nymph periode (day); It was calculate from hatching until it was change of skin.
f. The first nymph weight; It was calculate with the first weight after it was change skin.
g. Temperature and humidity; it was obtain from temperature (^{0}C) and humidity (%) in the morning and afternoon.

Experiment Design

The Experiment design used Completely Randomized Design (CRD) with four treatments and four replications. Each the replication consists of 20 eggs. The treatment were T1; sand, T2; soil, T3 mixture sand and soil, and T4; web sock The data were be obtain to test with statistic. It was differences that was result of analysis of variance, and then it was the test with Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

The First Egg Weight of Cricket *Gryllus mitratus*

The average first eggs weight *Gryllus mitratus* is describe in Table 1. The result of analysis of variance indicate that first eggs weight of crickets *Gryllus mitratus* were not significant different (P>0.05).

Table 1. The Average of The First Eggs Weight of Cricket Eggs *Gryllus mitratus*

Replication	First weight of cricket eggs (mg)			
	T1	T2	T3	T4
1	0.9510	0.9480	0.9480	0.9435
2	0.9445	0.9585	0.9520	0.9475
3	0.9480	0.9465	0.9435	0.9470
4	0.9520	0.9490	0.9495	0.9515
Totally	3.7965	3.8020	3.7930	3.7893
Average	0.9491^{ns}	0.9505^{ns}	0.9483^{ns}	0.9474^{ns}
SD	0.0030	0.0054	0.0036	0.0033

Note: Supersrkip is different in the same row of indicate the very significant different (P<0.01). TI= sand, T2= soil. T3=mixture sand and soil. T4=web sock.

The result of this research indicated that the average of first eggs weight of cricket eggs *Gryllus mitratus* 0.9474 mg - 0.9505 mg. Widiyaningrum (2001), the average of first eggs weight of cricket 0.6 mg - 0.8 mg. It was more high, because this egg was selected such as this measure; good and large.

The Effect of Hatching Media to Hatching Capacity Cricket *Gryllus mitratus*

The hatching capacity to hatching media; sand, soil, mixture sand and soil, and web sock is describe in Table 2. The result of analysis of variance indicate that the incubation of eggs of crickets *Gryllus mitratus* used media; sand, soil, mixture sand and soil, web sock were significant different (P<0.05) to hatching capacity cricket *Gryllus mitratus*. The average of hatching capacity used media; sand, soil, mixture sand and soil, web sock were 70.00% - 86.25%.

Table 2. The Average of Hatching Capacity of Cricket Eggs *Gryllus mitratus*

Replication	Hatching Capacity (%)			
	T1	T2	T3	T4
1	75	70	75	90
2	65	80	65	80
3	70	75	70	90
4	70	70	75	85
Totally	280	295	285	345
Average	70.00^{b}	73.75^{b}	71.25^{b}	86.25^{a}
SD	4.08	4.79	4.79	4.79

Note: Supersrkip is different in the same row of indicate the very significant different (P<0.01). TI= sand, T2= soil. T3= mixture sand and soil. T4=web sock.

Result of the test with Duncan's Multiple Range Test (DMRT) indicate that the average of hatching capacity with sand (70.00%) was not significant different (P<0.05) with soil (73.75), mixture soil and sand (71.25%), but it was significant differ-

ent (P<0.05) with web sock (86.25%). The web sock was high of hatching capacity, because it was high humidity; 70.00%.Althought, the less hatching capacity was sand, it was have less humidity (68.82%). Soenanto (1999), state that percentage of hatching is influence humidity, temperature in media, hatching box, male quality, and technical of incubation.

The Effect of Hatching Media to Hatching Weight

The average hatching weight *Gryllus mitratus* to media; sand, soil, mixture sand and soil, and web sock is describe in Table 3 below. The result of analysis of variance indicate that hatching weight of crickets *Gryllus mitratus* were significant different (P>0.05). This research indicated that the average hatching weight *Gryllus mitratus* to different media was 0.7478 mg – 0.7605mg.

Table 3. The Average of the Hatchery Weight of Cricket Eggs *Gryllus mitratus*

Replication	Hatching Weight (mg)			
	T1	T2	T3	T4
1	0.7646	0.7600	0.7573	0.7422
2	0.7538	0.7606	0.7471	0.7488
3	0.7621	0.7553	0.7507	0.7537
4	0.7600	0.7664	0.7600	0.7465
Totally	3.0405	3,0423	3.0151	2.9915
Average	0.7601^{a}	0.7605^{a}	0.7537^{ab}	0.7478^{b}
SD	0.0046	0.0046	0.0059	0.0048

Note: Superscrip is different in the same row of indicate the very significant different (P<0.01). TI=sand, T2= soil. T3= mixture sand and soil. T4=web sock.

Result of the test with Duncan's Multiple Range Test (DMRT) indicate that the average of hatching weight with sand (0.7601 mg) was not significant different (P<0.05) with soil (0.7605 mg), but it was significant different (P<0.05) with web sock (0.7478 mg). The average of hatching weight with mixture sand and soil (0.7537 mg) was not significant different (P<0.05) with web sock (0.7478 mg). It was observation that the high of hatching weight was sand (0.7605 mg). It is means that sock media decrease hatching weight.

Hatching Period of cricket eggs *Gryllus mitratus*

The Figure 1 in it was the first appearance the hatch of eggs in 5th day for all of treatments. In 5th day, web sock media was more hatch 6 eggs In 7th days the high hatch; sand 23 eggs, soil 22 eggs, mixture sand and soil 24 eggs, web sock 16 eggs. In 8th day, the hatching was decrease in each treatment. In 9th days, the hatch eggs almost the same for each treatment. In 10 th days, the last hatch in the soil was 2 eggs. In conclusion, the eggs was start in hatch 5th days until 10th day.

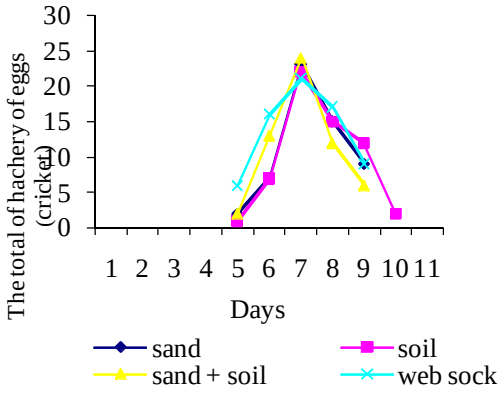

Figure 1. Hatching Period of Eggs Cricket *Gryllus* mitratus

Arko (1990), state that the eggs start hatch in 7 th day days until 10 th day for *Testaceus.* It is not the same for each eggs for hatch, the eggs will be hatch in web soft cloth or cotton.

The Effect of Hatching Media to Nymph Period

Figure 2 is describe that the nymph I stadium period of cricket *Gryllus mitratus* started in 5th day each treatments. In 5th day the offspring cricket became nymph of variation; the sand 7 offspring, soil 8 offspring, mixture sand and soil 7 offspring, and web sock 11 offspring. In 6th day was peak of offspring became nymph I; sand 26 offspring, soil 27 offspring. In 7th day was peak of offspring became nymph I in mixture sand and soil. In 7th day; sand, soil, and web sock, the development of offspring became nymph start decrease such as; sand 19 offspring, soil 16 offspring, web sock 20 offspring, mixture sand and soil 23 offspring. In 8 th day, The last of 8 days was became to stadium nymph I in sand, , mixture sand and soil, however in the web sock was 9 th day. The range of time became nymph I was 5 day until 9 and 6 th day was peak nymph that growth.

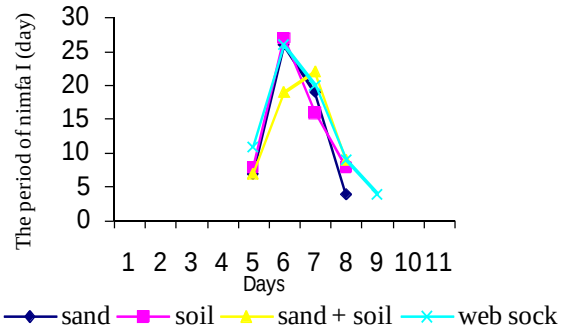

Figure 2. Period of Nimfa I Cricket *Gryllus mitratus*

Paimin *et al*., (1999) state that Development of nymph started the hatch eggs. In sand, soil, and mixture media more fast became compare with web sock, because this media was suitable with original habitat. Sridadi and Rachmanto (1999), state that The offspring of cricket of new hatch until day 20 was more sensitive to environment. If it was development, the housing cultivation must suitable with original habitat (Anonim, 1999).

Arko (1999), state that the nymph of cricket is metamorphosis change of skin (molting) until mature 5 - 6 times until the old about 7 days (is called nymph) and the cricket to change of skin is category vase nymph II (Paimin *et al*., 1999).

The Effect of Hatching Media to The First Weight of Nymph I *Gryllus mitratus*

The average of the first weight of nymph I *Gryllus mitratus* used be hatching media of sand, soil, mixture sand and soil, web sock in presented in Table 4.

Table 4. The Average of The First Weight of Nimfa I *Gryllus mitratus*

Replication	Weight of Nimfa I (mg)			
	T1	T2	T3	T4
1	1.2500	1.2575	1.2525	1.2650
2	1.2517	1.2636	1.2653	1.2536
3	1.2700	1.2671	1.2707	1.2600
4	1.2553	1.2507	1.2606	1.2557
Totally	5.0227	5.0389	5.0492	5.0343
Average	1.2568^{ns}	1.2597^{ns}	1.2623^{ns}	1.2586^{ns}
SD	0.0091	0.0072	0.0077	0.0050

Note: Superscrip is different in the same row of indicate the very significant different (P<0.01) TI= sand, T2= soil. T3= Mixture sand and soil. T4=Web sock.

The result of analysis of variance indicate that hatching media; sand, soil, mixture sand and soil were not significant different (P>0.05) to first weight of nymph I weight of crickets *Gryllus mitratus.*

The result of this research indicated that the average of weight of nimfa I 1.2568 mg-1.2623 mg. Widiyaningrum (2001), state that the average of the first weight species *Gryllus bimaculatus* about 1.36 mg^{-1} cricket, *Gryllus testaceaus* dan *Gryllus mitratus* between 1.24 mg – 1.36 mg per cricket. In this research, hatching media was not influence to weight of nymph I cricket. The weight of nymph I almost the same each treatment, because the first nymph I give the same feeding until the last nymph. Widiyaningrum (2001), The body weight growth of cricket was influence feeding and species.

The Temperature and Humidity of Chamber

The average temperature and humidity for each day is describe in Table 5. In Table 5, temperature in chamber for research was 26.00°C - 28.00°C, and humidity was 71% - 83%. Sukarno (1999), state that in Indonesia, local cricket is live in temperature between 20°C – 32°C with humidity 65% - 83%.It is to concert the temperature and humidity of chamber, because it is influence to hatching.

Table. 5 The Average of Temperature and Humidity in the Chamber

	Day temperature (°C)	Maximum temperature (°C)	Minimum temperature (°C)	Humidity (%)
Range	26.00-28.00	27-29	25-27	75-83
Average	27.55	28.11	26.11	76.22

The Temperature and Humidity of Hatching Media

The research of temperature and humidity of hatching media in describe in Table 6. In Table 6 was observed that the temperature and humidity of hatching media; 26.18°C – 26.39°C and 68.82% and 69.04%, respectelly. The Result of the temperature and humidity indicated that the temperature and humidity almost the same each treatments, because the watering relative the same ; three times per days. In humidity, the web sock relative of high (69.04%) compare with sand (68.82%), soil (68.99%), and mixture sand and soil (68.91%). The average of media temperature in this research was 26.26°C, with range 26.18°C – 26.39°C.

Table. 6 The Average of Humidity and Temperature of Hatchery Media

Replication	Humidity and temperature of hatchery media			
	T1	T2	T3	T4
1	69.00/26.05	68.69/26.93	68.76/26.24	69.43/26.10
2	68.78/26.32	68.94/26.13	68.86/26.00	68.81/26.11
3	68.95/26.34	69.42/26.36	68.90/26.15	68.81/26.28
4	68.56/26.14	68.94/28.52	68.14/26.34	69.10/26.52
Totally	275.291/104.85	275.981/107.94	275.645/104.73	276.142/105.05
Rataan	68.823/26.21	68.995/26.39	68.911/26.18	69.036/26.26
SD	0.196/0.14	0.305/1.08	0.161/0.14	0.294/0.19

Note: TI=sand, T2=soil, T3=mixture sand and soil.T4=web sock.

Sridadi and Rachmanto (1999), state that the humidity and temperature must be constant with humidity 65%-80% and temperature 26^0C in order to good hatching. Beside this, The temperature in chamber of cultivation of cricket must be suitable, and then it was the same with original habitat; 23°C – 26°C (Kumala L., 1999).

CONCLUSION

The using of many hatching media; sand, soil, mixture sand and soil, and sock can influence hatching capacity of eggs cricket *Gryllus mitratus*. The high of hatching media was web sock (86.25%). In the interval of time of stadium nymph I can be used in web sock media (9 day).

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The Effect of Vegetables on Growing Rate in Cricket *Gryllus mitratus* 10-50 Day Olds

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ABSTRACT

Cricket is one of potential animals that can be used as food sources, such as human food and feed of domestic animal because of its height protein contain. In Indonesian, there are 123 species of cricket. However, the species is cultivated*; Gryllus mitratus* and *Gryllus testaceus*. The cricket is consumption of many vegetables, such as , mustard green**,** mustard bitter, squash, cabbage. The aim of this research was to know the effect of mustard green, mustard bitter, squash, and mixture of mustard greens, mustard bitter, squash on growing rate in cricket *Gryllus mitratus.* The study was conducted in room of house, Kandang Limun Village, Bengkulu District from August until October, 2005. A number of cricket *Gryllus mitratus* used in the study were 200 cricket, 10 – 50 old days with 5 replications of which each replication consisted of 10 crickets. Parameter measured were the feed consumption (g), body weight growth (g), mortality (%) temperature and humidity of the chamber. The results showed that the mustard greens, mustard bitter, squash, and mixture of mustard greens, mustard bitter, squash were significant different ($P< 0.01$) on feed consumption in cricket *Gryllus mitratus*. The average feed consumption of mustard greens (1.664g) was not significantly different ($P>0.05$) to that mustard bitter (1.527g), and mixture of mustard greens, mustard bitter, l squash (1.605g), but it was significantly different ($P<0.01$) to that squash (1.099g). The average body weight growth was not significantly different ($P>0.05$) to mustard greens, mustard bitter, squash and mixture of mustard green, mustard bitter, squash. In conclusion, mustard green, mustard bitter, and mixture of mustard green, mustard bitter, squash was better body weight growth than squash.

Key words: cricket, mustard greens, mustard bitter, and squash

INTRODUCTION

In Indonesian, there are 123 species of cricket from 900 species classified in the world in the classification. From these 123 species of cricket, there are two species cultivated, namely *Gryllus mitratus* and *Gryllus testaceus* (Paimim, 1999*)*. Patton (1978) stated that cricket belong to omnivore insect that live in group, suitable for raise, and laboratory animal. In general, cricket is found in Asia Tropic, and not requently more live in humid dry, looses soil, agriculture soil and farm. It is like to live at range of temperature 20^0C – 32^0 C and moisture 60%-80% (Sukarno, 1999). Sukarno (1999) stated cricket have a good opportunity for development. Kumula (1999) stated that cricket can be used as feed sources of domestic animal, human food, materials of cosmetic.

The acceleration and development of insect were very influenced by many factors such as; sex, temperature, humidity and feed as well as feed quality (Chapman, 1975; Woording *et al*., 1979) in (Widiyaningrum, 2001). Widiyaningrum (2001), stated that species and kind of feed influenced on feed consumption. Ashari (1995), in Rahman (2002), The feeding of eggplant, bean and mustard greens is more like cricket than carrot, squash, and cabbage. And then, the cricket is feeding of mustard greens and bean more fast of growth and eggs production than tubers. Widiyaningrum (2001), mustard greens and papaya leaf was give $7g^{-1}$ $cricket^{-2}day^{-3}$ produced palatability more efficient than leafy vegetables, cassava leaf and amaranth.

Base on the this information, It is important to research the effect of many vegetables on growing rate in cricket *Gryllus mitratus*. The purpose of this research was to observe to influence mustard greens, mustard bitter, squash and mixture of mustard greens, mustard bitter, squash on growing rate in cricket *Gryllus mitratus*10 – 50 Day Old.

MATERIALS AND METHODS

The study was conducted in room of house, Kandang Limun Village, Bengkulu District from August until October, 2005. The materials used in the study were box with the measures of 50 cm x 40 cm x 30cm,

20 box, hand sprayer, thermometer, hygrometer, soil tester, analytic scales, banana leaf for hidden place. 200 crickets (*Gryllus mitratus)* 10 days old, mustard greens, mustard bitter, squash that given in fresh.

Stage of Study

1. **The box for raising**
   The box that used to in the study to do in plywood in 20 box; measure length 50cm x high 40 cm x width 30cm, in close and foot, and have ventilation.
2. **Species of ricket**
   The species of cricket was local species, *Gryllus mitratus* that obtain from livestock in Kebun Tebeng, Bengkulu District.
3. **Vegetables**
   Vegetables used were mustard green, mustard bitter, and squash. All of these vegetables were sold from selling from traditional market. The vegetable were used in fresh and given every morning. Before using, the vegetable were dressed.
4. **Variable measures**
   a. Feed Consumption; it was obtain from difference the vegetables that given with residue.
   b. Body Weight Growth; it was obtain from difference with the last body weight with first body weight.
   c. Mortality; it was calculated from percentage the total of diet.
   d. Temperature and humidity; it was obtain fromtemperature (^{0}C) and humidity (%) in the morning and afternoon
5. **Experiment design**
   The Experiment design was be used Completely Random Design (CRD) with four treatments and five replications. Each the replication consists of 10 crickets with 10 day olds. The treatment were T1; mixture of mustard greens, mustard bitter, T2; mustard greens, T3 mustard bitter, and T4 squash with given to each treatments 7 g^{-1} cricket $^{-2}$ day^{-3} The data were be obtain to test with statistic. It was differences that was result of analysis of variance, and then it was the test with Duncan's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Feed Consumption of Cricket *Gryllus mitratus* ($g^{-1}cricket^{-2}$ $week^{-3}$)

The average of feed consumption per week in each treatment; mixture of mustard green, mustard bitter, squash (T1), mustard green (T2), mustard bitter (T3), and squash (T4) were describe in Table 1. The result of analysis of variance indicate that the mixture of mustard greens, mustard bitter, squash (T1), mustard green (T2), mustard bitter (T3), and squash (T4) were significant different (P<0.01) on feed consumption in cricket *Gryllus mitratus*. It was significant different because of different of vegetables.

Table.1. The Avarage of Feed Consumption of Cricket (*Gryllus mitratus*) per Week in Fresh Matter

Weeks	Treatments			
	T1	T2	T3	T4
I	1.555	1.520	1.201	0.764
II	1.634	1.725	1.617	1.163
III	1.703	1.786	1.631	1.154
IV	1.483	1.643	1.484	1.137
V	1.649	1.643	1.703	1.280
Avarage	1.605[a]	1.664[a]	1.527[a]	1.099[b]
SD	0.116	0.087	0.097	0.093

Note: Supersrkip is different in the same row of indicate the very significant different (P<0.01). TI=mixture (mustard greens, mustard bitter,squash), T2= mustard green. T3= mustard bitter. T4= squash.

The result of the test with Duncan's Multiple Range Test (DMRT) indicated that feet consumption among vegetable mixture (T1) 1.605 g, mustard greens (T2) 1.664g, and mustard bitter (T3) 1.527 g were not significantly different (P>0.05) but it was significant different (P<0.01) to squash (T4) 1.099g. In The Table 1. above, it's high consumption; vegetable mixture, mustard greens, and mustard bitter; and lower consumption was squash. The high of consumption; vegetable mixture, mustard greens, and mustard bitter were correlated with the physical and texture of feeding that were soft. Although water contains were lower for mustard greens 91.6%, mustard bitter 91.8% and mixture vegetable 92.3% compare with squash 93%. It was influence of palatability (Rubatzky and Yamaguchi, 1999).

Besides of lower of water contain, It were high protein; mustard greens 2.69%, mustard bitter 2.65% and mixture vegetable 4.30%. It was lower of feeding consumption of squash because of high fiber, has gland

secretion, rough texture and lower of protein 0.69% and then it was difficult for cricket in bit and feeding. As well as, cricket more sensitive to smell and taste in feed (Gillot, 1995). It was agree with Soenanto (1999) state that cricket more like the mustard compare with squash, and then it's the more consumption.

Chapman in Widyaningrum (2001), state that base on reference for feeding of insect, not only protein contain, but also morphology include texture, water contain, shape, and color. Paimin (1999), cricket was more like the leaf , more contain water, because this insect was not consumption of water. It was suitable of it's characteristic, the cricket more like to consumption of feeding was more contain water like mustard. The mustard was palatable and stimulating, and then mustard greens has high consumption compare of another vegetable.

It was agree with Tillman *et al*. (1986), state that the shortage of water in animal body will decrease feed appetite and feet intake, however the water is suitable of feed appetite. In this research, although the water set aside in box, but the cricket will be like to consumption in vegetable contain.

The Average of Body Weight Growth of Cricket *Gryllus mitratus* ($g^{-1}cricket^{-2}day^{-3}$)

The average of body weight growth of cricket *Gryllus mitratus* per week in each treatment; mixture of mustard greens, mustard bitter, squash (T1), mustard greens (T2), mustard bitter (T3), and squash (T4) were describe in Table 2. The result of analysis of variance indicate that the mixture of mustard greens, mustard bitter, squash (T1), mustard green (T2), mustard bitter (T3), and squash (T4) were significant different ($P<0.01$) on body weight of cricket *Gryllus mitratus*. It was significant different because of different of vegetables.

The result of the test with Duncan's Multiple Range Test (DMRT) indicate that Body weight growth with vegetable mixture (T1), mustard greens (T2), and mustard bitter (T3) were not significant different ($P>0.05$) but it's significant different ($P<0.01$) in squash (T4). In the Table 2 below, the high of body weight growth was vegetable mixture 0.092g, mustard greens 0.089g, and mustard bitter 0.087; and lower body weight growth was squash 0.070g. The high of body weight growth in vegetable mixture was possibility of more high protein 4.30%, however mustard greens 2.69% and mustard bitter 2.65%.

Table 2. The average of Body Weight Growth of Cricket (*Gryllus mitratus*)

Weeks	Treatments			
	T1	T2	T3	T4
I	0.012	0.013	0.011	0.015
II	0.036	0.039	0.038	0.045
III	0.106	0.129	0.108	0.093
IV	0.149	0.152	0.174	0.125
V	0.156	0.111	0.106	0.074
Avarage	0.092^{a}	0.089^{a}	0.087^{a}	0.070^{b}
SD	0.023	0.019	0.015	0.021

Note: Supersrkip is different in the same row of indicate the very significant different ($P<0.01$). TI=mixture (mustard greens, mustard bitter,squash), T2= mustard green. T3= mustard bitter. T4= squash.

And then, it can assumption that the vegetable mixture contain organic matter and mineral very complete. It was visible to the body weight growth of cricket more high than squash. The different of body weight growth can be different of vegetable and free choose. Jumar (1997) and Natawigena (1990). The feeding is source of nutrition that needing by insect for growing and development.

The lower of body weight growth of cricket was feed in squash compare with another vegetables, because it was possibility of lower of nutrition, hard texture and gland secretion. And then the cricket is difficult to feed and digestion.It was agree with Soenanto (1999), mustard greens and mustard bitter were more like compare with squash, and cabbage. By the way, the cricket will be more consumption mustard greens and mustard bitter, it will influence to body weight growth.

Mortality

It was not mortality of cricket for this research, although cricket was given many treatments; mixture of mustard greens, mustard bitter, squash (T1), mustard green (T2), mustard bitter (T3), and squash (T4). It was not mortality, because of feeding of consumption enough and the temperature as well as humidity of compiled with condition. In this study was indicated that the temperature the chamber of average 26.40°C – 29.83°C, and humidity 65.50% - 76.20%.

The Temperature and Humidity of Chamber in Research

In this research, the average of the temperature and humidity of chamber were 26.40°C – 29.83°C, and 65.50% - 76.20%, respectelly. In normally, this condition was suitable with the live of cricket (Sukarno, 1999). In Indonesia, the cricket is usually development in condition temperature 20 – 32°C and humidity 65% - 80%, while in North America such as; *Acheta domesticus* has development in temperature 32^0C and humidity 70% - 75% (Patton, 1978). The environment temperature and humidity is needed to concert, because of one of the factor to influence to productivity.

CONCLUSION

Mustard greens, mustard bitter, and mixture of mustard greens, mustard bitter, squast was better body weight than squash.

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Ration with Different Dietary Cation Anion Difference to Mineral Status of Blood and Urine Garut Ewes

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ABSTRACT

The objectives of the present experiment were to evaluate the effect of dietary cation-anion difference (DCAD) on mineral status in blood and urine. Rations with DCAD value of -28, -18, 0, +14, and +32 mEq were offered to 15 ewes in a randomized complete block design. On day 21, blood samples were taken anaerobically using heparinized syringes from the coccygeal jugular venipuncture. Each syringe was capped and placed on ice immediately following collection to determine on plasma Na, K, Cl, Ca, and P concentrations. The DCAD values had no effect on plasma Na, K, Cl, Ca, and P concentrations indicating that there was homeostasis to maintain the physiological status of the body. The DCAD value of -18, 0, +14 and +32 mEq resulted in the normal blood with Na:K ratio closed to 20:1. Plasma Cl concentration was associated with plasma Na, but the concentration of Cl was lower than that of Na. The DCAD values significantly influenced P urine, but had no effect on urinary Na, K, Cl, S, and Ca. The DCAD value of -28 and -18 mEq resulted in the low acidity of urine at level of 5.73 ± 0.20 and 5.84 ± 0.27, respectively. The DCAD value of 0, +14, dan +32 mEq resulted in normal urinary pH. Rations with DCAD values of -18, 0, +14, and +32 mEq in garut ewes had normal ratio of plasma's Na^+:K^+ and were able to perform regulation of minerals control inside their blood to be homeostatis, and some excessive minerals would be secreted through urine. Rations with DCAD values of -28 and -18 mEq in garut ewes had the highest ratio of plasma's Ca^{2+}:P^{2-} which was 2.2:1.0, so it could be used as an action to prevent milk fever.

Key words: dietary cation-anion difference, blood, urin, ewe

INTRODUCTION

Consumed ration will affect physiological condition of the livestock. According to Stewart (1983), addition of anions (Cl and S) into the ration would lower the pH of body fluid. Blood condition is the result of acid base balance in body fluid and regulation of nutrition metabolism inside it. Blood is consisted of cells and plasma. Plasma contains water as much as 90% and anorganic minerals in form of soluted ion as electrolytes, proteins, metabolic waste products, respiration gases and hormones. Concentration of this combined ions is important for maintenance of blood osmotic balance. Acid base balance is highly affected by the function of lungs and kidneys.

Kidneys have vital role as controller of volume and composition of blood chemicals by secreting solution and water selectively. Vital functions of kidneys are done by the filtration of plasma through *glomerulus* followed with reabsorption some amount of solution and water with correct volume along the kidneys tubulus. The excess of solution and water will be secreted out as urine through collector system. Epitelic cells help maintaining constant pH of body fluid by controlling secretion of hydrogen ion. Secretion of acid in urine as the result of potential acid and H^+ formation rate from blood buffer. Acidic urine is also secreting Ca^{2+}.

In this research, the rations experimented with DCAD values of -28, -18, 0, +14, and +32 mEq. The objectives of this research was, to identify the effect of different DCAD to mineral in blood and urine of Garut ewes (*Ovis aries*).

MATERIALS AND METHODS

This experiment was conducted at the Pen Field Laboratorium A of Animal Husbandry Faculty and Integrated Laboratorium of Veterinary Faculty Bogor Agricultural University on January 11th - July 14th 2007.

Experimental Design and Animal Care

Fifteen Garut ewes were 2.50 ± 0.25 years old were assigned randomly to randomized complete block design. The ewes were blocked into groups of 3 according to (I)

ewes previously had twin female offsprings; (II) ewes previously had twin male offsprings; and (III) ewes previously had twin male and female offsprings. Ewes were housed and fed in individual cage. The composition of basal ration contained 89.30% dry matter, 8.12% ash, 15.00% crude protein, 5.12% ether extract, 14.73% cude fiber, and 57.03% nitrogen free extract (Tabel 1).

Determination of crude protein ration contents of 15.00 % based on Wodzicka-Tomaszewska *et al*. (1991), Na mineral of 0.09 - 0.18 %, K of 0.50 - 0.80 % (maximum 3.00 %), Cl had no clause (based on NRC, 1985).

The value of basal rations DCAD was +14 mEq/100 g of DM and treatment rations in this research with five dietary cation anion difference values (DCAD).

1. -28 mEq = basal ration added with 14.375 mEq S and 27.884 mEq Cl
2. -18 mEq = basal ration added with 14.375 mEq S and 17.884 mEq Cl
3. 0 mEq = basal ration added with 14.259 mEq S
4. +14 mEq = basal ration
5. +32 mEq = basal ration added with 10.21 mEq Na and 7.531 mEq K

Method of operating decreasing of DCAD value to 0 mEq/100 g of DM with basal ration was added $CaSO_4$ (Brataco Chemika, Cikarang, Jakarta). Decreasing DCAD to -28 and -18 mEq/100 g of DM with basal ration were added $CaCL_2$, dan $CaSO_4$ (Brataco Chemika, Cikarang, Jakarta). The value of DCAD increased to +32 by addition of Na_2CO_3 and K_2CO_3 in basal ration (Brataco Chemika, Cikarang, Jakarta). Treatment rations had been offered for three weeks before the samples were collected.

Sample Collection and Analysis

On 21st day after treatment rations were offered, blood samples were taken anaerobically using heparinized syringes (Franklin Lakes NJ USA) from the coccygeal jugular venipuncture. Then, the blood were centrifuged for 15 minutes at 3000 rpm. Afterward, the plasma were taken to be analyzed for its Na, K, and Ca contents by using Automatic Absorbance Spectrofotometer (AAS), while Cl, P, and S by titration.

Sample of urine were collected by using plastic apron in the morning around 07.00 - 08.00 o'clock. The urine were tested for its pH by using pH-meter pocket HANNA, then the urine were analyzed for its Na, K, Ca, Cl, P, and S mineral contents by using the same method with the blood sample.

Statistical Analysis

Data were analyzed with GLM procedure in SAS System for Windows 6.12. Treatments effects were compared using the multiple comparation approach of Duncan Multiple Range Test. Regression analyses were conducted with the Proc REG procedure, whereas correlation corficients were

Tabel 1. Composition and nutrient content of basal ration base on dry matter

		Proximate analysis (%)					Minerals (%)			
Feed type	Percent	Ash	CP	EE	CF	NFE	Na	K	Cl	S
Corn forage	35.0	2.81	3.24	0.43	10.18	18.34	0.020	0.305	0.011	0.070
Rice bran	6.0	0.63	0.59	0.84	0.65	3.30	0.000	0.079	0.014	0.008
Onggok	9.5	1.83	0.26	0.13	0.83	6.45	0.007	0.017	0.002	0.003
Corn meal	18.5	0.26	1.48	0.55	0.42	15.79	0.001	0.068	0.002	0.013
Coconut meal	7.0	0.43	1.17	1.01	1.08	3.31	0.007	0.144	0.053	0.017
Soybean meal	22.0	1.98	8.24	0.37	1.58	9.84	0.001	0.458	0.111	0.041
Fish oil	2.0	0.18	0.03	1.79			0.003	0.006	0.000	0.000
Total	100	8.12	15.00	5.12	14.73	57.03	0.039	1.077	0.192	0.152

Note: CP = crude protein Na = natrium
EE = ether extract K = kalium
CF = crude fiber Cl = chlor
NF = nitrogen-free extract S = sulfur

obtained from the Proc CORR procedure of SAS (Mattjik and Sumertajaya, 2006).

RESULTS AND DISCUSSION

Blood Acidity and Blood Plasma Mineral Status

Average data of Na^+, K^+, Cl^-, S^{2-}, Ca^{2+}, and P^{2-} of garut ewes' blood plasma offered with rations with various DCAD values were presented on Table 2. The DCAD values had no effect (P>0.05) on plasma's Na^+. The differences of DCAD values were consumed by garut ewes had no effect on plasma's Na^+ (P>0.05). It means that, the ewes succeeded in performing homeostatis. Average values of Na^+ of experimented ewes's plasma were ranging 17308 ± 3281 to 18397 ± 1915 ppm (Table 2). The amount of plasma's Na^+ was not related to DCAD value (r = 0.01). Hu and Murphy (2004) stated that there was no effect of DCAD on Na^+ of blood plasma. However, Roche *et al*. (2005) reported that the increase of DCAD value, would increase Na^+ of blood plasma. Ewes offered with rations with DCAD value of -28 mEq produced acidic blood (Fathul *et al.*, 2008) so the Na^+ of blood plasma in ewes offered with rations with DCAD value of -28 mEq were relatively lower than those offered with rations with DCAD values of 0, +14, dan +32 mEq.

The DCAD values had no effect (P>0.05) on plasma's K^+. The differences of DCAD values were consumed by garut ewes had no effect on plasma's K^+ (P>0.05). It means that, the ewes also succeeded in performing homeostatis. Average values of K^+ of experimented ewes' plasma were ranging 811 ± 268 to 983 ± 183 ppm (Table 2). The amount of plasma's K^+ was not related to DCAD value (r = -0.16).

At normal condition, extracellulic fluid performed balance between Na^+ and K^+ at constanta of 20:1 (Georgievskii, 1982). The ewes on this research performed homeostatis, the contents of Na^+ and K^+ of blood plasma were not affected by DCAD. But, the body regulated K^+ value inside the plasma to always lower than Na^+ value. The relatively highest average value of plasma's K^+ (983 ± 183 ppm) was found in ewes offered with rations with DCAD value of -28 mEq. This was because in those ewes there were occured metabolic acidosis indicated by the decrease of blood's HCO_3 concentration (-2.53 ± 2.42 mmol/L) (Fathul *et al.*, 2008). Therefore, the decrease of blood's HCO_3 concentration would be followed by alteration of plasma's K^+ so plasma's K^+ became relatively highest. On Table 2, ewes offered with rations with DCAD values of -28, -18, 0, +14, and +32 mEq had each ratio plasma's Na^+:K^+ of 18:1, 20:1, 20:1, 20:1, and 19:1, respectively. The values ratio plasma's Na^+:K^+ in ewes offered with rations with DCAD value of -18, 0, +14, and +32 mEq (approximately 19 : 1 − 20:1) were close to normal because the normal ratio plasma's Na^+:K^+ was 20:1. Ewes offered with rations with DCAD values of -28 had ratio plasma's Na^+:K^+ of 18:1, it meant that its plasma's Na^+ was lower and its plasma's K^+ was the highest compared with ewes offered with other DCAD values. Odongo *et al.* (2006) stated that metabolic acidosis was acid-base upset caused by the decrease of blood's [HCO_3^-] and generally, followed by alteration of [K^+] to become hyperkalemia (Weiderseiner *et al*., 2004). Ewes offered with rations with DCAD value of -28 mEq had the lowest blood's HCO_3^- and included in metabolic acidosis (Fathul *et al*., 2008), so it had relatively the

Table 2. Average of Na^+ , K^+, Cl^-, S^{2-}, Ca^{2+}, and P^{2-} of garut ewes' blood plasma offered with different DCAD

Variables	Dietary cation-anion difference (mEq)				
	-28	-18	0	+14	+32
Na^+ (ppm)	17308 ± 3281	18547± 1661	18397 ± 4940	16520 ± 516	18397 ± 1915
K^+ (ppm)	983 ± 183	926 ± 174	918 ± 104	811 ± 268	945 ± 55
Cl^- (ppm)	4580 ± 646	4449 ± 82	4509 ± 268	4307 ± 102	4698 ± 182
S^{2-} (ppm)	63 ± 18[a]	35 ± 2[b]	33 ± 2[b]	32 ± 5[b]	29 ± 3[b]
Ca^{2+} (ppm)	473 ± 27	471 ± 22	449 ± 20	421 ± 44	426 ± 43
P^{2-} (ppm)	211 ± 114	217 ± 51	320 ± 50	331 ± 51	339 ± 25
Na^+:K^+	18 : 1	20 : 1	20 : 1	20 : 1	19 : 1
Ca^{2+}:P^{2-}	2.2 : 1.0	2.2 : 1.0	1.4 : 1.0	1.3 : 1.0	1.3 : 1.0

Note: value with different letter on same row mean different (P<0.05).

most K^+ content. High content of K^+ in blood was called hyperkalemia. This, maybe because the ewes offered with rations with DCAD value of -28 mEq had very acidic blood pH and there was occured metabolic acidosis, eventually ratio Na^+:K^+ was not at normal condition. Ratio Na^+:K^+ had to be performed by the livestocks in order homeostatis. Determination of Na^+:K^+ homeostasis mechanism inside the body was done by kidneys. Regulation of Na^+:K^+ homeostasis was involving corticoid-aldosterone and deoxycorticosterone mineral which acted on K^+ secretion in consequence of reabsorption of Na^+ ion inside the kidneys' ducts. Corticoid mineral was also likely affecting the regulation of membrane permeability and Na^+ : K^+ pair mechanism (Pratas, 1992).

Block (1994) explained that the unbalance of one ion with another, will caused poisoning that produced alkalosis or acidosis. This was likely because the unbalance of HCO_3^- and H^+ variables. If the presence of Na^+ was not enough to initiate absorption of NaCl (neutral), the excess of HCO_3^- in blood vessels could drive to acidosis condition. Further explained by Horst *et al.* (1997) that Cl^- was absorped more than SO_4^{2-} so Cl^- was a stronger aciditive to acidified the blood. Acid-base balance was related to exchange of H^+ ion internal media component which was able to donate or recieve ion. Substances that was able to donate ion was acid, while the one that was able to bind hydrogen was base.

The DCAD values had no effect ($P>0.05$) on plasma's Cl^-. The differences of DCAD values consumed by garut ewes had no effect on plasma's Cl^- ($P>0.05$). It means that, the ewes succeeded in performing homeostatis. Average values of Cl^- of experimented ewes' plasma were ranging 4307 ± 102 to 4698 ± 182 ppm (Table 2). The amount of plasma's Cl^- was not related to DCAD value (r = 0.07). The content of plasma's Cl^- was following presence trend of plasma's Na, but the amount of Cl^- was lower than Na and the presence of Na^+ to form NaCl (neutral). Ewes offered with rations with DCAD value of -28 mEq had the lowest plasma's Na so to perform neutralization with Cl^- was relatively fewer than ewes offered with rations with other DCAD values.

The DCAD values had very high effect ($P<0.01$) on plasma's S^{2-}. Ewes offered with rations with DCAD value of -28 mEq had the highest plasma's S^{2-} ($P<0.05$) as much as 63.38 ± 17.94 ppm. Ewes offered with rations with DCAD value of -18, 0, +14, dan +32 mEq had no differences on plasma's S^{2-} ($P<0.05$). The amount of plasma's S^{2-} was highly related to DCAD value (r = -0.67).

The DCAD values had no effect ($P>0.05$) on plasma's Ca^{2+}. The differences of DCAD values consumed by garut ewes had no effect on plasma's Ca^{2+} ($P>0.05$). It meant that, the ewes also succeeded in performing homeostatis. Average values of Ca^{2+} of experimented ewes' plasma were ranging 421 ± 44 to 473 ± 27 ppm. The relatively highest average value of plasma's Ca^{2+} (473 ± 27 ppm) was found in ewes offered with rations with DCAD value of -28 mEq. This was because the ewes offered with rations with DCAD values of -28 and -18 mEq had very acidic blood. That acidic condition would increase cells of intenstines tissue's sensitivity to paratyroid hormone (PTH) so the absorption of Ca^{2+} on intestines increased. In addition, acidic condition increased synthesis of 1.25 dihydroxyvitamin D3 from 25 hydroxyvitamin D3 by 1α-hydroxylase enzyme in the kidneys so increasing reabsorption of Ca^{2+} from glomerular filtrate. Therefore, ewes offered with rations with DCAD value of -28 and -18 mEq had more plasma's Ca^{2+} than those offered with other DCAD values. Block (1994) stated that the increase of ration's anion, would increase reabsorption of osteoclastic bones and increase synthesis of 1.25 dihydroxyvitamin D3 regulated by PTH. Paratyroid hormone also regulated reabsorption of Ca^{2+} and HPO_4^{2-}.

Ewes offered with rations with different DCAD values performed ratio Ca^{2+}:P^{2-} inside the blood therefore obtained different number of ratio, depended on cells of intenstines tissue's sensitivity to PTH, 1.25 dihydroxyvitamin D3, and PTH utilization depended on its blood acidity. Experimented ewes offered with rations with various DCAD values had ratio of plasma's Ca^{2+}:P^{2-} ranging from 1.3:1.0 to 2.2:1.0 (Table 2). Ewes offered with rations with DCAD values of -28 and -18 mEq had the highest ratio of plasma's Ca^{2+}:P^{2-} (2.2:1.0) than ewes offered with rations with other DCAD values. Ratio of normal plasma's Ca^{2+}:P^{2-} balance was 2:1 (Georgievskii et al. 1982). If the ratio of plasma's Ca^{2+} : P^{2-} < 2:1, it was likely that the

livestock would have milk fever especially diary cattles with high diary production. Therefore, rations supplies at late months pregnancy (dry condition) could act as prevention to milk fever. The amount of plasma's Ca^{2+} was highly related to DCAD value (r = -0.59) and less related to plasma's K (r = 0.47).

Block (1994) explained that low DCAD value could reduce hypokalsemia peripartum by the increase of Ca^{2+} ion in blood and urine. Addition of Ca^{2+} in blood was caused by the decreasing DCAD value (low) which causing Ca^{2+} had homeostatis by increasing absorption on intestines so that also increasing the secretion (Schonewille *et al*., 1994; Roche *et al.*, 2003b). Findings of this research were appropriate with ideas of Moore et al. (2000), Roche *et al*. (2003), and Charbonneau *et al*. (2006) who stated that the decrease of DCAD value would cause increase of blood Ca^{2+}. Relation between plasma's Ca^{2+} and plasma's K was explained by Yingst *et al*. (2001) that K^+ ion increased the pump of Na^+ in order to increase the concentration of Ca^{2+} so that free Ca^{2+} in blood was increased by the pump of Na^+ in some cells. In the other part, polarization affected the decrease of K^+ concentration in order to perform K^+ balance (Quinn et al., 1987).

The DCAD values had no effect ($P>0.05$) on plasma's P^{2-}. The differences of DCAD values consumed by garut ewes had no effect on plasma's P^{2-} ($P>0.05$). It meant that, the ewes were trying to perform homeostatis. Average values of P^{2-} of experimented ewes' plasma were ranging 211 ± 44 to 473 ± 27 ppm. The amount of plasma's P^{2-} was quite highly related to DCAD value (r = 0.67) but not related to blood pH (r = 0.17).

In this research, the order of plasma's mineral from the most to the less were Na^+ which then followed by Cl^-, K^+, Ca^{2+}, P^{2-}, and S^{2-}. Isnaeni (2006) stated that in extracellulic fluid or blood plasma the order of mineral from the most to the less were Na^+, Cl^-, K^+, Ca^{2+}, P^{2-}, and Mg^{2+}.

Urinary Mineral Status

The DCAD values had high effect ($P<0.01$) on urinary pH. Ewes offered with rations with DCAD values of -28 and -18 mEq had the lowest urinary pH ($P<0.05$) each of 5.73 ± 0.20 and 5.84 ± 0.27, because in rations with DCAD values of -28 and -18 mEq there were addition of $CaCl_2$ and $CaSO_4$ anionic minerals. It known that body of livestock perform homeostatis so the excess of Cl^- and S^- were secreted outside the body. Secretion of S^- dan Cl^- excess were through urine so the urine would become more acidic because Cl^- dan S^- were acidic. Value of urinary pH was the picture of cation-anion of consumed rations. If the livestock consumed excessive anion, its urine will be acid. Otherwise, if the Na^+ was consumed excessively, urinary pH will became base. On Table 6, in rations with DCAD values of -28 and -18 mEq there were many addition of acidic anionic salts, while in rations with DCAD value of +32 mEq there were many addition of base cationic salts. So ewes offered with rations with DCAD values of -28 and -18 mEq had the lowest urinary pH than those offered with other DCAD values, but ewes offered with rations with DCAD values of +32 mEq had the highest urinary pH. This had been explained by Chan *et al.* (2006) that the decrease of urinary pH was reflection of the effect from anion contained in the rations. Urinary pHvalues caused by consuming ratios with DCAD value of -28 and -18mEq were 5.7373 ± 0.20 dan 5.84 ± 0.27, respectively; those urinary pH were acid because

Table 3. Average values of pH , Na^+, K^+, Cl^-, S^{2-}, Ca^{2+}, and P^{2-} in urine of garut ewes offered with different DCAD

Variables	Dietary cation-anion difference (mEq)				
	-28	-18	0	+14	+32
pH	5.73 ± 0.20^b	5.84 ± 0.27^b	7.60 ± 0.51^a	7.51 ± 0.78^a	8.28 ± 0.33^a
Na^+ (ppm)	68 ± 10	94 ± 43	121 ± 82	460 ± 661	907 ± 734
K^+ (ppm)	27397± 8162	33039±6704	21258 ± 8874	36697 ± 16258	39895±12109
Cl^- (ppm)	2374 ± 2528	2870 ± 3014	1400 ± 1329	2192 ± 778	3072± 990
S^{2-} (ppm)	977 ± 456	1274 ± 676	630 ± 574	7 ± 117	66 ± 23
Ca^{2+} (ppm)	2295 ± 1733	2119 ± 1951	224 ± 323	272 ± 118	17 ± 27
P^{2-} (ppm)	120 ± 67^b	95 ± 30^b	96 ± 36^b	203 ± 93^b	407 ± 191^a

Note: values with different letters on same row mean different ($P<0.05$).

the urinary pH were <6.0. Ewes offered with rations with DCAD values of 0, +14, and +32 mEq had urinary pH of 7.60 ± 0.51, 7.51 ± 0.78, and 8.28 ± 0.33, respectively; those urinary pH were normal because the urinary pH were between 7.50 - 8.50. Pratas (1992) stated that acidic urinary pH was <7.50; normal urinary pH was between 7.50 - 8.50; base urinary pH was >8.50. Moore *et al*. (2000) reported that if urinary pH was lower than 6.0, than the rations offered contained excessive anionic salts. Based on those facts above, the addition of anionic salts into the rations wih DCAD values of -28 dan -18 were excessive. Low urinary pH showed blood pH was also low (Vagnoni and Oetzel, 1998). This matched with this research, ewes offered with rations with DCAD value of -28 mEq had very acidic blood as well as the urinary pH. The urinary pH was highly related to DCAD value (r = 0.89). Roche *et al*. (2002) reported that the increase of K^+ consentration in rations would increase urinary pH. The increase of K^+ consentration in rations meant that there was an increase of DCAD value. This was similar to the findings by West *et al*. (1992), Moore *et al*. (2000), Riond (2001), Dersjant-Li *et al*. (2002), Roche et al. 2003, Borucki Castro *et al*. (2004), Hu and Murphy (2004), Roche *et al*. (2005), Apper-Bossard *et al*. (2006), Charbonneau (2006), and Kienzle *et al*. (2006); they stated that the increase of DCAD value would also increase urinary pH.

Body would always balancing its body fluids, in this case, the one that took role was kidneys. The excess of anion or cation carried by rations would be regulated by kidneys to be secreted through urine. Renal tubular cells responded directly to the changes in blood pH and intracellular pH. Kalium ion was moving from cells into the blood by releasing H^+. The body cells pumped the excess of H^+ ion into the urine. Ion of H^+ caused the decrease of pH. In this research, changes in urinary pH were matched with its rations' cation-anion balance. Escobasa *et al*. (1984) reported that the increase of Cl^- consumption on cattle would decrease its urinary pH.

The DCAD values had no effect (P>0.05) on urinary Na^+. The average values of experimented ewes' urinary Na^+ were ranging from 68 ± 10 to 907 ± 734 ppm (Table 3). Although the urinary Na^+ was not affected by DCAD value, but it close related (r = 0.66) with DCAD value. In Rations with DCAD value of +32 mEq there was addition of Na_2CO_3 salt, but there were no differences in plasma's Na^+ and urinary Na^+ (P>0.05) and normal urinary pH, so it meant that the addition of that Na_2CO_3 was not excessive.

The DCAD values had no effect (P>0.05) on urinary K^+. The average values of experimented ewes' urinary K^+ were ranging from 21258 ± 8874 to 39895 ± 12109 ppm (Table 3). Urinary K^+ content was not too related to DCAD (r = 0.35). In Rations with DCAD valeu of +32 mEq there was addition of K_2CO_3 salt, but there were no differences in plasma's K^+ and urinary K^+ (P>0.05) and normal urinary pH, so it meant that the addition of that K_2CO_3 was not excessive.

The DCAD values had no effect (P>0.05) on urinary Cl^-. The average values of experimented ewes' urinary Cl^- were ranging from 1400 ± 1329 to 3072 ± 990 ppm (Table 3). Urinary Cl^- content was not related to DCAD (r = 0.06), Cl^- consumption (r = 0.04), and blood Cl^- (r = 0.11).

The DCAD values had no effect (P>0.05) on urinary S^{2-}. The average values of experimented ewes' urinary S^{2-} were ranging from 66 ± 23 to 977 ± 456 ppm (Table 3). Urinary Cl^- content was highly related to DCAD (r = -0.72).

The DCAD values had no effect (P>0.05) on urinary Ca^{2+}. The average values of experimented ewes' urinary Ca^{2+} were ranging from 17 ± 27 to 2295 ± 1733 ppm (Table 3). Urinary Ca^{2+} content was highly related to DCAD (r= -0.65). Roche (1999) stated that there was an increase in absorption of Ca^{2+} and secretion of Ca^{2+} in diary cattle, if anionic salts were added in its DCAD rations.

The DCAD values had no effect (P>0.03) on urinary P^{2-}. The lowest urinary P^{2-} contents (P<0.05) were found in ewes offered with rations with DCAD values of -28, -18, 0, and +14 mEq each of 120 ± 67, 95 ± 30, 96 ± 36, and 203 ± 93 ppm, respectively, while the highest value (P<0.05) was found in ewes offered with rations with DCAD values of +32 mEq, which was 407 ± 191 ppm. This was because urinary P^{2-} was highly related (r = 0.82) to DCAD value. Secretion through urine was the main homeostatis in the regulation of Na^+, K^+, and Cl^- (Maltz and Silanikove, 1996) in order to maintain constant Na^+:K^+ ratio in extracellu-

lar fluids. On Table 2 appeared that ratio plasma's Na^+ : K^+ was close to normal balance (as much as 20:1) in rations with DCAD values of -18, 0, +14, and +32 mEq. It meant that the ewes consuming rations with DCAD values of -18, 0, +14, and +32 mEq were able to perform regulation of minerals control inside thier blood to be homeostatis, and some excessive minerals would be secreted through urine. Bannink *et al*. (1999) and Nennich *et al*. (2006) stated that that urine secretion was directly related with consumption of Na^+, K^+, and N. Maltz and Silanikove (1996) explained that urine secretion was the main method of homeostatis regulation. Furthermore, according to Price and Wilson (1995), minerals filtrated by kidneys were Na^+, K^+, Ca^{2+}, Mg^{2+}, and Cl^-.

CONCLUSION

Rations with DCAD values of -18, 0, +14, and +32 mEq in garut ewes resulting normal ratio of plasma's Na^+:K^+ and were able to perform regulation of minerals control inside their blood to be homeostatis, and some excessive minerals would be secreted through urine. Rations with DCAD values of -28 and -18 mEq in garut ewes resulting highest ratio of plasma's Ca^{2+} : P^{2-} which was 2.2:1.0, so it could be used as an action to prevent milk fever.

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Characteristics of Size and Shape of Body Dimension of Madura and Rote (Indonesia) Fat-Tailed Sheep Using Principal Component Analysis

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ABSTRACT

Fat-tailed sheep is one of the important livestock resources for meat production especially in dry area of Indonesia such as Madura and Rote Islands. One of criteria for good performance as meat type is body measurement which can be useful to show their characteristics and general body dimension. This experiment was done to identify the size and shape of body dimension of fat-tailed sheep in Madura and Rote Islands. Data of 9 body measurements have been analysed from 136 fat-tailed sheep, 50 rams and 86 ewes. The data obtained were analyzed with Principle Component Analysis (PCA). The results showed that chest deep is a representaion of body size for fat-tailed Madura and Rote rams with correlation coefficient between body size and chest deep were 0.924 and 0.842, respectively. There is a different representation of body size for both ewes. The tail width is the representation of body size for fat-tailed Madura ewes with correlation coefficient is 0.799; in contrast, the wither height is the representation of body size for fat-tailed Rote ewes with the correlation coefficient is 0876. The representation of body shape for both fat-tailed sheep are the cranium width (for fat-tailed Madura rams and ewes and also for fat-tailed Rote ewes) and chest circumference (foe fat-tailed Rote rams). A positive correlation coefficient between cranium width and body shape representation is found (0.785; 0.785; 0.630), but the fat-tailed Rote rams have negative correlation coefficient which is -0.648. Sheep possessing greater tail length and width have smaller body shape, or vice versa. It is concluded that the body size and shape of the rams and ewes of fat-tailed Madura sheep do not differed from those of fat-tailed Rote sheep.

Key word: fat-tailed sheep, principal component analysis (PCA), body size, body shape

INTRODUCTION

Indonesian fat-tailed sheep is one of the largest population of sheep which produce meat in dry area. There are two regions having the largest population which are the Madura Island in the west part of Indonesia, and Rote Island in the east part of Indonesia. The production of meat animals is associated with the growth and development. The development pattern is useful in the assessment of confirmation. Usually the size is measured as body weight while the shape is described by several body measurement or visual appraisal. The problem of size and shape of animal is that the weight does not adequately distinguish the different in body composition. Furthermore, visual appraisal is affected by individual biases and perceptual differences among observers (Carpenter, 1979). Statistical technique with multivariate technique of principal component analysis has been used to combine weight and body measurements into indexes for defining the size and shape (Brown *et al*., 1973; Carpenter, 1979). The concept of principal component analysis (PCA) has received limited attention, but it can be used to evaluate variation in body shape (Brown *et al*., 1973). MacFie (1979) used multivariate statistical techiques to quantify the differences in shape between breed. The use of PCA for analysing the size and shape in Indonesian sheep were rarely. Brown *et al*. (1973) has used the PCA of nine linier measurement and weight to elicit an objective description of different pre-yearling body shape. A similar PCA to that used by Brown *et al*. (1973) was also applied to measure the body size of Garut sheep (Erfan, 2004). The aim of the present paper is to quantify the difference in size and shape of fat-tailed sheep in Indonesia.

MATERIALS AND METHODS

Data

The data used for this study was taken from Madura island as a representative of dry area in the west part of Indonesia and Rote island as a representative of dry area in the

east part of Indonesia. The total data were collected from 146 head sheep which consisted of 86 head of Madura sheep (28 rams and 58 ewes) and 50 head of Rote sheep (22 rams and 28 ewes). The fat-tailed sheep of 3 - 4 years age were considered to be mature and used in this experiment.

Body Measurement

Nine body measurements (cm) were taken and this included:body length, wither height, chest width, chest depth, chest circumference, cranium widht, cranium length, tail lenght and tail width. All measurements were taken from the left side of the sheep while standing on a flat ground in right position with paralel legs. Circumference measurements were taken by a tape, while the other measurements were taken by a specially designed caliper. The prosedure and anatomical reference point for the respective body measurements are fully describe elsewhere in Diwyatno (1984), and Salako and Ngere (2002).

Statistical Analysis

The multivariate principal component analysis was used to combine weight and body measurements of sheep into size and shape indexes. The use of the technique in the analysis of biological measurement data has been reported by Jolicoeur and Mossiman (1960). Brown *et al*. (1973) and Carpenter *et al*. (1978). The purpose of the analysis was to determine simultanously the effect of all body measurements on performance rather than to examine each one singly. The principal component transformation involves computation of the Eigen value representing the generalized variance that is explained by each component. The first principal (PC 1) component which usually accounts for the largest proportion of variance may be interpreted as a size vector, provided that all coefficient are positive. The second principal components (PC 2) and also other components with positive and negative cofficients can be considered as shape vectors. In this study the PCA was run on an ovarall correlation matrix.

Principal component analysis is a method for transforming the variables in a multivariate data set, X1. X2. X3….Xp into new variables, Y1. Y2. Y3…Yp which uncorrelated with each other and account for decreasing proportions of the total variance of the original variables defined as:

$$Yj=a_11X_1+a_12X_2+a_{13}X_3+\ldots+a1pX$$
$$Yj=a_21X_1+a_22X_2+a_{23}X_3+\ldots+a2pX$$
$$Yj=a_21X_1+a_22X_2+a_{23}X_3+\ldots+a2pX$$

with the coefficients being chosen; so that Y1. Y2. Y3…Yp are accounted for decreasing proportions of the total variance of the original variables X1. X2. X3….Xp (Everitt *et al*. 2001) and Gaspersz (2007).

RESULTS AND DISCUSSION

Principal Component of Fat-tailed Madura and Rote Sheep

Principal component analysis show similar results for both fat-tailed sheep rams. The largest coefficient in the first principal component is found for the chest deep. The chest deep is a representation of body size for fat-tailed Madura and Rote rams with correlation coefficient between body size and chest deep were, respectively, 0.924 and 0.842. The cranium length had small similar magnitude coefficients. The PCA results for both ewes differed from those for the rams. The largest coefficient in the first principal component is the tail width for the Madura ewes and the wither height for the Rote ewes. The coefficient for the tail length is small and has similar magnitude (Tables 1 and 2). The tail width is a representation of body size for fat-tailed Madura ewes and the wither height is a reperesentation of body size for fat-tailed Rote ewes with correlation coefficient between body size and tail width for fat-tailed Madura ewes was 0.799 and correlation coefficient between body size and wither height for fat-tailed Rote ewes was 0.876.

The second principal component showed similar result for both fat-tailed Madura and Rote ewes. The cranium width has the largest coefficient. However, the chest circumference in fat-tailed Rote sheep received larger weight than cranium width.

In morfometric applications of the PCA, the first component is acceptable as size vector and the second one as shape vector (Hayasi *et al*., 1982). The first component accounted for about 55 % for the rams of fat-tailed Madura and Rote sheep with 41 % of generalized variance and the first eight principal components are required to reach 99% of the variance. In the ewes, the first compo-

Table 1. The first and second principal components for the rams of fat-tailed Madura and Rote sheep

Variables	Fat-tailed Madura sheep		Fat-tailed Rote sheep	
	PC 1	PC 2	PC 1	PC 2
Wither height (X_1)	0.384	-0.288	0.394	-0.134
Body length (X_2)	0.241	-0.321	0.358	-0.108
Chest width (X_3)	0.343	0.199	0.084	0.419
Chest deep (X_4)	0.394	-0.045	0.412	-0.130
Chest circumference (X_5)	0.337	-0.053	0.245	0.514
Cranium length (X_6)	0.202	0.549	0.182	-0.379
Cranium width (X_7)	0.213	0.644	0.312	-0.217
Tail length (X_8)	0.254	-0.150	0.236	0.507
Tail width (X_9)	0.361	0.010	0.303	0.213

Table 2. The first and second principal component for the ewes of fat-tailed Madura and Rote sheep

Variables	Fat-tailed Madura sheep		Fat-tailed Rote sheep	
	PC 1	PC 2	PC 1	PC 2
Wither height (X_1)	0.349	-0.128	0.493	-0.207
Body length (X_2)	0.350	-0.217	0.118	0.438
Chest width (X_3)	0.404	-0.091	0.202	-0.454
Chest deep (X_4)	0.269	-0.018	0.457	0.312
Chest circumference (X_5)	0.390	0.093	0.245	-0.172
Cranium length (X_6)	0.290	0.562	0.390	0.140
Cranium width (X_7)	0.252	0.593	0.265	0.494
Tail length (X_8)	0.205	-0.458	0.083	-0.113
Tail width (X_9)	0.430	0.204	-0.255	0.394

Table 3. Eigen values and cumulative proportion of variance for the rams of fat-tailed Madura and Rote sheep

Variables	Fat-tailed Madura		Fat-tailed Rote	
	Eigen value	Cumulative	Eigen value	Cumulative
PC 1	5.504	0.552	4.180	0.418
PC 2	1.514	0.702	1.595	0.578
PC 3	1.108	0.813	1.108	0.688
PC 4	0.630	0.876	1.056	0.794
PC 5	0.445	0.920	0.720	0.866
PC 6	0.288	0.949	0.515	0.918
PC 7	0.224	0.972	0.407	0.958
PC 8	0.169	0.989	0.214	0.980
PC 9	0.060	0.995	0.149	0.995
PC 10	0.053	1.000	0.050	1.00

Table 4. Eigenvalues and cumulative proportion of variance for the ewes of fat-tailed Madura and Rote sheep

Variables	Fat-tailed Madura		Fat-tailed Rote	
	Eigen value	Cumulative	Eigen value	Cumulative
PC 1	3.461	0.385	3.162	0.351
PC 2	1.755	0.580	1.629	0.533
PC 3	1.040	0.695	1.191	0.665
PC 4	0.899	0.794	0.819	0.756
PC 5	0.724	0.875	0.708	0.835
PC 6	0.516	0.932	0.521	0.893
PC 7	0.252	0.960	0.421	0.939
PC 8	0.211	0.984	0.384	0.982
PC 9	0.148	1.000	0.160	1.000
PC 10				

nent accounted for about 38 % for the ewes of fat-tailed Madura and Rote sheep and 35 % of generalized variance and the first eight principal components were required to reach

99% of the variance. Most of Eigen vectors have positive values indicating that these components may be acceptable as size vector (Table 3 and 4).

The PCA has made it possible of ranking the strains on the basis of their size. The first principal component for the two difference region and sexes clearly indicated that the strains could be divided into three body measurements. The chest deep, tail width, and wither height can be used to quantify differences in the size of fat-tailed sheep. The largest in the second principal component is different for the fat-tailed Rote sheep. The largest coefficient in fat-tailed Rote rams and ewes is tail length. The largest coefficient in the second principal component is found for the cranium width (x7) in fat-tailed Madura rams dan Madura ewes, and also in fat-tailed Rote ewes with with correlation coefficient between body shape and cranium width for them were 0.785; 0.785 and -0.648, respectively. The largest coefficient in the second principal component is found for the chest circumference in fat-tailed Rote rams with correlation coefficient between body shape and chest circumference was -0.648.

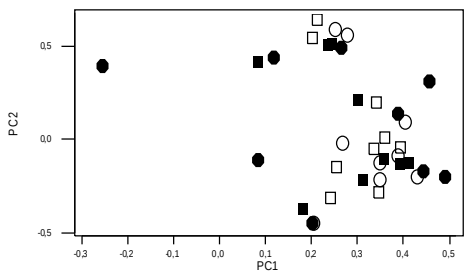
The coefficients for PC1 are all positive indicating that the first component contrasted the animals based on the overall size (Mc Curley *et al*., 1981). PC1 is interpreted as a measure of general size (Carpenter *et al*., 1971). PC 2 is composed of some positive and negative coefficients for body measurements. These components are interpreted as contrasting the animals based on their body shape arraying them from those that are short in stature and fat to those that are tall and thin. Weight received little emphasis. In bulls, a large positive PC2 value would typify the more comprest bull and a large negative PC2 value would represent a more rany type of bull (Brown *et al*., 1973).

These results show the important biological underlying the phenotypic relationships for many traits that are important for breeding program. The PCA can be used for all functional traits and can be useful in both dimension reduction and avoiding co-linearity problems that is common in the analysis of closely related functional trait such as body measurements or fertility (Karacoeren *et al*., 2006).

Principal component of chart of fat-tailed Madura and Rote sheep

The principal component chart (Figure 1.) shows overlapping of the groups of ram from fat-tailed Madura and Rote sheep. It is also clear that there is no difference observed in PC1 (size) and PC 2 (shape) between these two groups. No significant difference is also found in PC1 and PC2 between the ewes of fat-tailed Madura and Rote sheep. This result indicates that the fat-tailed Madura sheep is not distinguishable from that of fat-tailed Rote sheep by the external morphological character such as wool colour, shape, and body size etc., the two subspecies are similar to the common species peculiarity in its body conformation.

Body size and shape of fat-tailed Madura rams and ewes did not differ from those of Rote rams and ewes which are shown by the overlapping distribution. These can be due to genetic and environment factors that are almost the same in both regions. The Madura and Rote islands have the same environment as the majority of dry areas. Body size (size) is more influenced by the environment (Everitt and Dunn, 1999). On the other hand, the genetic factor shows a



□= fat-tailed Madura ram
■= fat-tailed Rote ram

○= fat-tailed Madura ewe

●= fat-tailed Rote ewe

Figure 1. The principal component chart of the first two transformed variable derived from the covariance matrix of body measurements fat-tailed sheep Madura and Rote

similarity between fat-tail Madura rams to that of Rote rams. The body shape is better todescribe the potential of animal genetic compared to the body size that is more influenced by the environment (Everitt and Dunn, 1999). A previous study reported that the fat-tailed Rote ram is predicted from the same breed as the rams that were imported from East Java (Madura); these imported rams were used to maintain the local ewe in dry area in Rote island (Gunawan *et al.*, 2007). The animal performance is an expression of genetic and environmental factors (Martojo, 1992).

CONCLUSION

The chest deep is a representation of body size for fat-tailed Madura and Rote rams with correlation coefficient between body size and chest deep were, respectively, 0.924 and 0.842. Both fat-tailed ewes differed in the representation of body size. The tail width is the representation of body size for fat-tailed Madura ewes with correlation coefficient is 0.799; in contrast, the wither height is the representation of body size for fat-tailed Rote ewes with the correlation coefficient is 0876. The representation of body shape for both fat-tailed sheep are the cranium width (for fat-tailed Madura rams and ewes and also for fat-tailed Rote ewes) and chest circumference (foe fat-tailed Rote rams). A positive correlation coefficient between cranium width and body shape representation is found (0.785; 0.785; 0.630), but the fat-tailed Rote rams have negative correlation coefficient which is -0.648. Sheep possessing greater tail length and width have smaller body shape, or vice versa. It is concluded that the body size and shape of the rams and ewes of fat-tailed Madura sheep do not differed from those of fat-tailed Rote sheep.

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The Profile Of Blood Transaminase Enzyme On Duck (Anas Sp.) Polluted By Lead (Pb) Textile Waste

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ABSTRACT

Duck which is raised traditionally in around of textile industry have a risk by lead (Pb) pollution from textile industry liquid waste, will caused hepatocite liver. Decrease liver function, has the impact to vitellogenesys that is synthesis of vitellogenin and β-lipoprotein as yolk precursor. Transaminase is enzyme which is indicated decrease in liver function. Objectives of this research was to know the profile of blood transaminase enzyme (SGPT= serum glutamate pyruvate transaminase, SGOT = serum glutamate oxaloacetate transaminase) on duck that polluted by lead (Pb) from textile waste. This experiment has been used survey method with purposive sampling, amount of sample used was 60 Tegal duck, consisted of 30 duck which was not polluted by Pb and 30 duck which was polluted by Pb. The data was analys by statistical analysis of T-students. Based on research showed that blood SGPT level which was polluted Pb higher significant different 76,74 ± 1,89 μmol/L against which was not polluted 47,93 ± 1,59 μmol/L, and so it is with SGOT level was higher significant different 78,73 ± 2,73 μmol/L polluted by Pb, against which was not polluted was 46, 52 ± 1,53 μmol/L.

Key words: Transaminase, Lead (Pb) and Duck

INTRODUCTION

Duck which is raised traditionally in around of textile industry have a risk by lead (Pb) pollution from textile industry liquid waste, that cause hepatocite to liver. Decrease in liver function, has the impact to vitellogenesys that is synthesis of vitellogenin and β-lipoprotein as yolk precursor. Transaminase is enzyme which is indicated decrease in liver function.

Accumulation of excess Pb can potentially cause liver damage that is clinically characterized by increased SGPT (serum glutamic-pyruvic transaminase) and AST (serum glutamic-oxaloacetic transaminase). Degree increase in these enzymes correlated positively with the level of liver damage. Biochemical changes due to liver damage, manifested by the increase of ALT levels from 20-200 times the normal levels (1-36 μM / L) and AST levels by 10-150 times the normal values (8-40 μM / L) (Bergmeyer and Bernt, 1971).

Pb concentrations in water, soil and air around industrial areas may reach 0.2 ppm and wastewater regulations limit the conditions 0.05 ppm (Amina, 2006), whereas Pb concentrations of wastewater based on preliminary research results is 0.207 ppm,

Pb content of blood contaminated duck waste textiles based on preliminary research results by using Atomic Absorption Spectrophotometer (AAS type) reached 0.07 ppm, whereas the Pb content of blood is not contaminated duck waste textiles reach 0.0005 ppm. Based on preliminary research results, there is a heavy metal content of Pb in waste water higher than the content of heavy metals other than Pb content of blood was contaminated ducks to reach 0.07 ppm indicate the occurrence of heavy metal pollution Pb in ducks raised in the neighborhood textile industry.

MATERIALS AND METHODS

Animals and Survey

Animals used in this research were 60 Tegal Ducks, 10-12 months age, average body weight 1.6 kg. Sampling method used was sampling purphosif sampling, consist of 30 ducks polluted by lead and 30 ducks not polluted by lead.

Survey have been made during 30 days and blood sample collected every week (forth time/ 4 weeks).

Parameters

1. Glutamate Pyruvat serum transaminase (SGPT/ALT).
   This enzyme produced by liver cells and

functions to catalyzed alpha-amino group of alanine to alpha-ketoglutaric acid.

2. Oxaloacetat Glutamate 2 Serum transaminase (SGOT/AST)
   This enzyme catalysis the transfer function for alpha-amino group of aspartic acid to alpha-ketoglutaric acid.

ALT and AST levels are determined by using Clinical Auto Analyzer Cobas Type C-111. The sample was measured using a photometric system with wavelength 340-659 nm.

Data Analysis

This study used analysis of T-student population :

- Population 1 = duck contaminated textile
- Pollution 2 = duck population is not contaminated textile pollution.

RESULTS AND DISCUSSION

Averages Level of SGPT and SGOT of duck blood polluted and not polluted of Pb

Serum OF Glutamate Pyruvat Transamnase (SGPT) and Serum Glutamate Oxaloasetat Transaminase (SGOT) were transaminase enzym that referable to evaluation of liver function. Average level of SGPT and SGOT of duckblood polluted and not pullted of Pb, showed in Table 1.

Table 1. Averages Level and Analysis Results of SGPT and SGOT of duck blood polluted and not polluted of Pb

Level	Environmental condition		
	Polluted	Not polluted	Results of Analysis
	……µM/L……		
SGPT	76,74 ± 1,89	47,93 ± 1,59	P < 0,01
SGOT	78,73 ± 2,73	46, 52 ± 1,53	P < 0,01

Table 1 showed of SGPT level average of duck blood polluted Pb were 76,74 ± 1,89 µM/L and not polluted by Pb were 47,93 ± 1,59 µM/L.

Analysis result showed that average of transaminase enzym (SGPT and SGOT) level was difference significant (P<0.01), between duck polluted and not polluted by Pb. It was showed that take effect higher Pb accumulation, so much so that caused reduced of liver function. Increasing SGPT and SGOT level would happened if there were releasing enzym in accordance with intracellular to into blood that caused hepatocyte, eg nekrosys hepatoseluler or infark miokardial (Bijanti, 2006).

Serum glutamic-pyruvic transaminase (SGPT/ALT) is an enzyme sitosolik contained in these organs function as catalyst of removal of the alpha amino acid alanine to alpha ketoglutrarat. ALT most abundant in the liver. ALT values are considered normal is 1 to 36 µM / L. These levels will rise rapidly and exceed the normal case of liver cell necrosis, or do not have this enzyme eliminated out (Darmono, 2001). Based on our research ALT levels average for ducks contaminated with Pb metal reached 76.74 ± 1.89 µM / L, this shows an increase of ALT levels reached three times the normal levels (1-36 µM / L), ALT levels whereas the average for the ducks that are not polluted by Pb metal was 47.93 ± 1.59 µM / L.

Serum glutamic-oxaloacetic transaminase (SGOT/AST) many founded in the heart, liver, muscle, panckreas, lung, eritrocite, brain cells. Althought this enzime used for lever testing, its high level founded in the muscle. Contain of SGOT in the blood are 8-40 µM/L. Function of SGPT was transfer catalys of alfa-amino group from aspartate acid to be alfa ketoglutarat acid (Darmono, 2001).

Based on our research AST levels average for ducks contaminated with Pb metal reached 76.3 µM / L, this shows an increase of AST levels reached twice normal levels (8-40 µM / L), while the average levels of AST average for the ducks that are not polluted by Pb metal was 46, 52 ± 1.53 µM / L.

Increased levels of transaminase enzymes infected duck blood Pb can be explained due to liver tissue damage occurs through a reduction in its function as a result of ion exchange of important minerals such as K, Na, P and others into Pb ions and the formation of the complex formation as Suhendrayatna (2008) suggests that the network bodies, contamination of Pb^{2+} ions bind to the cell membranes of two different ways, the first exchange of monovalent ions and divalent ions such as Na, Mg, and Ca on the cell membrane is replaced by ions of heavy metals (Pb), and second is the complex formation between Pb ions with functional groups like carbonyl, amino, thiol, hydroxyl, phosphate, and hydroxyl-carboxyl is located on the cell membrane, this phenomenon has led to de-

creased cell function until the death of cells (hepatosit).

Toxicity of lead (Pb) in various organs is mediated through several mechanisms including inactivation of enzymes and other macromolecules through bonds with sulfhydryl, phosphate, and carboxyl and interaction with cations, especially calcium, zinc and iron. Pathological processes can occur in the cell membrane and mitochondria, function and neurotransmitter synthesis, heme synthesis, cellular redox status and nucleotide metabolism. Adverse effects can occur in nerve, kidney, gastrointestinal tract, hematopoesis, reproductive and cardiovascular system. Pb metal including metal-metal bond is more reactive with the ligand in the cell, if the metal binding cells of (non-essential), it will cause damage to the catalyst capability (detoksikasi) of the cell itself (Darmono, 1995).

Related to this, Alifia and Djawad (2003) and Vodela, et al., (2007) suggests that the degeneration of parenchymal damage characterized by changes hepatosit or liver cell death that causes the specific enzymes involved in metabolism of protein migration into blood vessels. Associated with specific enzyme migration into blood vessels, Kimball (1983) and Linder (2006) suggested that the transaminase enzymes can be indicators of liver damage.

CONCLUSION

Based on research inferential that duck polluted by lead significantly undergo increased level of SGPT and SGOT

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Production Performance Of Etawah Cross Bred Goats In Turi – Sleman, Yogyakarta

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ABSTRACT

A study is conducted to investigate production performance of Etawah Cross Bred goats, which are raised by farmers in Turi, Sleman, Yogyakarta. This region has the highest population of Etawah Cross Bred goats in Yogyakarta Province. The study was done through a survey and investigation, lasting from September 2006 to February 2007. 115 farmers were interviewed to collect the data pertaining to socio economic aspects of goat management and 234 goats were used, 34 of these were female goats, as random samples from the goat population in that region. The result showed that most of the goats are raised in the communal goat houses with the average numbers of 5.3 ± 3.7 goats per farmer. The goats do not satisfy the required standard of Etawah Cross Bred body weight and size. The average milk yield per goat is 774 ml per day. The age of female goat at first mating is 14.9 months with kidding interval of 10 months and the average number of kids born is 1.9/doe. The kids are weaned after 4 months and reach weaning weight of 26.2 kg. On the average, the goat produces 1.0 kg of manure per goat per day. It is concluded that the production performance of Etawah Cross Bred goats based on their body size and weight cannot reach the high standard of Etawah Crossed goat production performance. It is recommended that the goats are raised to produce milk. Since the average of milk yield is low (less than 1 liter per day), there is a need to improve the quality of breeding stock and management.

Key words: Etawah cros bred goats, production performance

INTRODUCTION

The majority of goat production in Indonesia is managed by small scale farmers under traditional methods. Sleman Regency is one of the regions where Etawah Cross Bred goats are concentrated. By the end of January 2007, the population of goats in Sleman reaches 31.431 heads. The number of goats increased as a result of '*Kambingisasi*' program applied in 2005. Some of the goats are Etawah Cross Bred and most of which are concentrated in Turi numbering as many as 2,935 goats (Anonymous, 2007). In recent years, Etawah Cross Bred goats have become popular to be spread in new regions. The concentration areas for raising Etawah Cross Bred goats are upland regions, such as Kulon Progo and Sleman.

Etawah Cross Bred goats are the result of mating Etawah and Kacang goats. The characteristics of Etawah Cross Bred goat are combinations of those of Etawah and Kacang goats (Harjosubroto, 1994). For many years, the objective of raising Etawah Cross Bred goat tends to be multi purpose. In this situation, the farmers raise goats for producing milk, fertilizer, replacement stocks or for savings. As a result of the different purposes of raising goat, Etawah Cross Bred goats in different regions vary in their characteristics, i.e. weight, body size and productivity. The recent situation also leads farmers to keep Etawah Cross Bred goats as pet animals. Therefore, the price of goats increased unreasonably and the motives of raising goats have changes. It is expected that the situation will change and farmers will raise the goats for the appropriate goals.

This study is conducted to identify the characteristics and productivity of Etawah Cross Bred goats raised by groups of farmers in Turi, Sleman. The objectives are to determine the purpose of Etawah Cross Bred raising, to analyze the potentials of Etawah Cross Bred goat production and to recommend some techniques of raising management in order to obtain the optimum results.

MATERIALS AND METHODS

In this study, questionnaires were used to interview farmers. Materials for investigating production performance consisted of 234 goats, of which 34 goats were lactating ones,

feed, a scale, a measuring tape, a beaker glass, plastic bags and goat houses.

115 farmers were interviewed in Nganggring, Kemirikebo, Sukorejo, Nangsri and Babadan groups to collect data pertaining to socio economic aspects of goat management and the body size and body weight were measured from the 234 samples. Milk and manure production were recorded from 34 lactating goats for 3 consecutive days. The data were statistically analysed by calculating mean values and deviation standard.

RESULTS AND DISCUSSION

Goat Management

The total numbers of Etawah Cross Bred goats in Turi are 554. Those are raised by 115 farmers. The majority of farmers in this region are organized in groups and manage their goats in the communal goat houses. There are 3 communal goat houses in Turi, located in Nganggring, Kemirikebo and Sukorejo. In other groups (Nangsri and Babadan), there are no available communal goat houses; therefore, farmers here raise their goats in pens which are situated at the backyard.

On the average, each farmer in Turi has 5.3 ± 3.7 goats. Generally, the farmers raise goats as multipurpose animals (Table 1). 75.2% of farmers explained that their main purpose of raising goat is for saving, while 14.5% and 10.2% are to produce replacement stock and manure, respectively. Milk production is a secondary purpose which is only found in Nganggring and Kemirikebo groups. In these groups, several farmers have milked their goats and sold either in fresh or processed milk.

Table 1. Distribution of Farmers in Turi According to Main Purpose of Raising Goats

Main purpose of raising goat	Percentage of farmers to total population
For savings	75.2
For producing replacement stock	14.5
For producing manure	10.2

The purpose of raising goats for savings has caused negative and positive effects. The negative effect is shown by a decrease in goat quality. This occurs when farmers need a large amount of money, and the high qualities of goats are offered at high prices. In this situation farmers tend to sell the goats without considering the quality. As a result of continual selling of high standard goats, only low quality goats remain in the area and finally this situation decreases the quality of breeding stock.

The positive effect can be found when the prices decrease. While waiting for a good price for selling, farmers have the opportunity to obtain additional products, such as kids, manure and milk. In this situation, high quality bucks are possibly mating many females and produce kids during uncertain periods. Those kids can be selected to obtain replacement stock. This raising system needs to be supported by providing a guideline of selection, in order to get high standard replacement stocks. This guideline must contain the description of high grade Etawah Cross Bred goat characteristics and is useful for standard selection. Through this method farmers are expected to be able to select the goats properly.

In the group where the main purpose of raising goats is to produce manure (in Sukorejo group), farmers had been processing the manure and selling the compost. However, the farmers only compost a small part of the manure, as it is needed to fertilize their own land. In this area, the utilization of goat manure is mainly to support fruit production, especially for *Salak Pondoh*. Goat manure is important for Salak Pondoh production because these fruits are the main agriculture products which require goat manure as the fertiliser (Table 2).

Table 2. The Distribution of Farmers According to Their Main Job and Agricultural Products

Variables	Percentage of farmers to total population
Farmers main job:	
Producing Crops	94.4
Raising livestock	1
Others	4.6
Type of agricultural products:	
Salak Pondoh	70.2
Salak Pondoh and vegetables	11.6
Salak Pondoh and feed	12.1
Salak Pondoh and rice	6

In this study, adult goats produced 1 kg manure per day (Table 4). Based on the number of goat ownership, farmers in Turi can

produce 150 kg manure per month. This data showed that raising Etawah Cross Bred goats had contributions to support the farmer main job. The amount of available manure for selling depended on how much manure was required for fertilizing their land.

Characteristics of Etawah Cross Bred Goats in Turi

The result of investigation on 34 lactating goats (2 to 6 years old) indicates that the goats in Turi cannot reach the required physical standard of Etawah Cross Bred goats, especially for body length, height of wither and body weight (Table 3). According to Sumadi *et al*. (2003), the required standards of Etawah Cross Bred goats are 72 - 80 cm for body length, 76 - 85 cm for height of wither, 25 - 32 cm and 8 -10 cm, respectively, for the ear length and width; the standard body weight of adult female is 55 - 65 kg. In comparison to the size of strain of *Kaligesing* Etawah Cross Bred goats, these goats have 65 - 85 cm in length and 70 - 90 cm height of wither with the ear size is 25 - 41 cm long and 8 to 14 cm wide (Anonymus, 2005). These comparisons demonstrate that characteristics, i.e. body size, of goat in this study are smaller than the standard body size of Etawah Cross Bred goats, but similar to those of strain of *Kaligesing* Etawah Cross Bred goats. Kaligesing is a place of the origin of Etawah Cross Bred goats. The goat body weight of 43 kg is less than the average body weight standard, ranging from 50 to 65 kg. The small size of these goats indicates that the quality had decreased. This is probably affected by continual selling of high standard goats.

Table 3. Body Size of Lactating Etawah Cross Bred Goats in Turi

Variables	Average ± S.D	Range
Age of goat (year)	4.2 ± 1.7	2 – 6
Body length (cm)	66.5 ± 7.4	49 – 90
Height of wither (cm)	73.0 ± 4.9	62 – 86
Chest girth (cm)	80.5 ± 4.9	68 – 90
Length of ear (cm)	27.2 ± 2.7	22 – 31
Width of ear (cm)	9.2 ± 0.9	7 – 11
Length of teat (cm)	12.9 ± 3.7	7 – 20
Body weight (kg)	43.0 ± 6.4	28 – 59

The characteristics of Etawah Cross Bred goats are hereditarily received from Etawah goats. Therefore, to improve the quality, the selected goats should be compared to their ancestors, which are characterized by the height and size as well as other traits. The Etawah goats have Roman nose, long ears and neck. The ear length of adults is about 29 cm. The udder is capacious; the teats are long up to 12 - 14 cm in length. The weight of female Etawah at 6 months is 30 pounds and at 12 months reaches 65 pounds (Rout *et al.*, 2000).

Reproductive Performance

According to Sarwono (1999), the age of goat at puberty is around 6 to 8 months. Farmers in Turi tend to delay the age of first mating to around 15 months because of the priority to attain the proper body weight prior to mating. The age of goat at first mating in this study is similar to that reported by Devendra and Burn (1994) who suggested that mating should be delayed until 12 months for small breed and 15 months for large breed. In comparison to Etawah goats, the age of first conception is around 18 months, first kidding at 23 months, and kidding interval about 11 months (Rout *et al.*, 2002). The objective of delaying the first mating is to reach the maximum body weight at mature age. Howe (1980) indicates that at puberty the goat only reaches 2/3 of mature body weight. Amoah *et al*. (1996) state that an increase in body weight of mother at mating has significantly improved the litter size and there is correlation between body weight of doe at mating and the number of litter size.

Table 4. Reproductive Performance and Milk Production of Goats

Variables	Average
Age of goat at first mating (months)	14.9± 4.1
Kidding interval (months)	10.0± 3.3
Litter size	1.9± 0.6
Age of kids at weaning (months)	4.1± 2.4
Weight of kids at weaning (kg)	14.1± 5.8
Kid mortality (%)	10.7± 9.0
Adult mortality (%)	11.0± 2.0
Milk production (ml per day)	774 ±291
Manure production (kg per goat per day)	1.0± 0.6

Data shown in Table 4, female goats which start mating at 15 months of age with 10 months kidding interval, will reach the peak reproductive performance at 6.5 years old or at the 4th to 5th of parity. With an aver-

age litter size of 1.9, the goats can be expected to produce 9 to 10 kids during this period. The average litter size of goats in this study is higher than that of the Etawah breed, averaging 1.6 (Rout *et al*., 2000). However, Rout *et al*. (2002) also reports that kidding twins had 52% probability; triplets or quadruplets are also common for Etawah goats. According to Amewu *et al*. (1999), litter size increases with parity, with the greatest occurs at the 5th parity. Therefore, farmers should select the kids from the 2nd to 4th kidding in order to find the best quality of replacement stock.

Milk Production

In this study, milking is started one month after kidding. Milk production was measured from goats in 3 consecutive days. The average milk production is 774 ml per day. This result is comparable to the report of Sutama and Budiarsana (1997), showing that Etawah Cross Bred goat produce 158 kg of milk in 127 to 287 days of lactation. Baba *et al*. (2000) reports that the average milk production of Etawah Cross goats in Malaysia is 317 to 490 ml per day. In comparison to the ancestor, milk production of Etawah Cross Bred goats in this study is lower; the Etawah goat produces 2.5 pounds of milk per day. Milk yields increase up to the end of two months and then started to decline. The average lactation period is 260 days. Does with multiple kids usually produce more milk than those with single kids (Rout *et al*., 2002).

Etawah Cross Bred goats are dual purpose goats; therefore, the capability of producing milk is lower than those of pure dairy goats. Recently consuming goat milk has become more popular. In Turi, farmers in Nganggring and Kemirikebo have initiated to produce and process goat milk. This has motivated other farmers from the other groups to follow their activities. However, since the average milk yield is low (less than 1 liter per day), there is a need to improve the quality of breeding stock and management for milk production.

CONCLUSION

It is concluded that Etawah Cross Bred goat body size and weight do not meet the high standard. However, the goats show high reproductive performance indicated by the short kidding intervals and a tendency to produce twins. The goats have contributions to support agriculture production by producing manure for fertilizer. The average milk yield is low, i.e. less than 1 liter per day; therefore, there is a need to improve the quality of breeding stock and management.

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Isolation and Determination of Enzymatic Activity of Selected Fungi on Sugarcane Bagasse as Feed for Ruminant

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ABSTRACT

It was found that ester and covalent bond between lignin, polysaccharides, and protein could reduce the digestibility of cellulose and hemicelluloses of sugarcane bagasse. Objectives of this experiment were to identify and to isolate the fungi that capable of degrading the lignocellulosic materials in sugarcane bagasse and to determine the decomposition ability of enzymatic selected fungi. The method used to culture the fungi was enrichment and platting method, while the method used for selection of fungi was the enzymatic selection method. Isolated fungi in this experiment were: *Aspergillus* sp, *Penicillium citrinum*, *Penicillium* sp(2), *Penicillium* sp(3), *Penicillium* sp(4), *Penicillium* sp(5), *Penicillium* sp(6), *Memnoniella* sp(1), *Memnoniella* sp(2), dan *Helminthosporium* sp. Further test showed that these isolated fungi have cellulolytic activity.

Key words: fungi, cellulase, and sugarcane bagasse

INTRODUCTION

As a center of agro-based industry in Indonesia, Lampung Province has a very high fibrous agricultural residues and agro-industrial byproducts, including sugarcane bagasse. This resource could be used as a main feed for ruminants (goats, sheep, and cows) in the future. These animals have the ability to digest the cellulosic materials using microorganisms in the rumen to help in breaking down the feed and nutrients, so that the host animals can get the nutrients from it. However, lignocellulosic materials, such as sugarcane bagasse, have long been demonstrated to have high degree of resistance to ruminal degradation. Therefore, this abundant renewable biomass in fact still has a minimum benefit as a feed for ruminants. Kirby (2006) explained that lignin has a highly complex and relatively random structure that provides this organic material with a high degree of resistance to degradation. Their wide varieties of chemical bonds make specific cleavage by the active site of an enzyme difficult, and would require many enzymes, each with a specific active site, for degradation. Moreover, Taherzadeh and Karimi (2008) stated that lignin is a complex molecule constructed of phenyl propane units linked in a three-dimensional structure, which is particularly difficult to biodegradation.

Numerous attempts have been made to improve the utilization of cellulosic materials as a feed for ruminant, include pretreatment of cellulosic materials and optimizing the bioprocess in the rumen. The whole digestion process in the digestive tract of the ruminants, especially in the rumen, could be accelerated by application of feed treatment (pretreatment), including chemical and biological treatments. Mosier *et al*., (2005) stated that pretreatment is an important tool for improving cellulose conversion or degradation processes. Pretreatment is required to alter the structure of lignocellulosic biomass to make cellulose more accessible to the enzymes that convert the carbohydrate polymers into fermentable sugars. The ultimate goal of pretreatment is to break the lignin seal and disrupt the crystalline structure of cellulose.

A number of preliminary studies have investigated the benefit effects of fungal cultures on improving the lignocelluloses decomposition. Culture of *Trichoderma viride* in sugarcane bagasse could improve the availability of structural carbohydrate (Prayuwidayati, 2006) and improve the crude protein content of fermentation product (Prayuwidayati and Muhtarudin, 2006). However, the exact mechanism or process of the effects is still not yet explored. Moreover, early enzymatic exploration of several fungal that could be cultured in sugarcane bagasse (Prayuwidayati *et al*., 2008) revealed that *Trichoderma viride*, *Aspergillus niger*, *Aspergillus oryzae*, *Rhizopus oryzae* has cellulase activity of 0.034, 0.007, 0.007, 0.004 units/ml respectively. The main objective of

this study was to identify and to isolate the fungi that capable of degrading the lignocellulosic materials in sugarcane bagasse and to determine the decomposition ability of enzymatic selected fungi.

MATERIALS AND METHODS

This research was conducted at the Department of Animal Science, Faculty of Agriculture and Department of Biology, Faculty of Natural Science University of Lampung in April - October 2009.

Substrate. Substrate material used in this experiment is sugarcane bagasse obtained from the local sugarcane industry PT. Gunung Madu Plantation, in Center of Lampung District. In this experiment, this material was used as its original condition from the factory without any physical and chemical treatment.

Fungi Exploration. Exploration of the fungi that capable of degrading sugarcane bagasse was conducted through culturing the fungi using moist chamber method and then followed by direct plating method. Moist sugarcane bagasse as a substrate in petri dish was placed in incubator for several days until all potential fungi were grown. All grown fungi were then isolated and cultured with PDA medium in separated petri dish and then placed in incubator until the colony of fungi produced enough spore for further evaluation.

Cellulase Activity Test. After isolation and identification, enzymatic activity of all grown fungi were then qualitatively analyzed using Congo red indicator method. In this method, cultured fungi on CMC medium (on top side) and PDA (on below side) was dropped by 1 - 3 drop of Congo red. After at least 24 hour placed in refrigerator, cultured fungi were then washed with NaCl physiological solution. Cellulase activity produced by fungi was observed as halo or clear zone on the medium. Cellulase activity could also be observed as change in the color of medium from red to dark-blue.

RESULTS AND DISCUSSION

Isolation and Identification of Fungi

Isolated and indentified fungi that capable of degrading the sugarcane bagasse in this experiment were: *Aspergillus* sp, *Penicillium citrinum*, *Penicillium* sp(2), *Penicillium* sp(3), *Penicillium* sp(4), *Penicillium* sp(5), *Penicillium* sp(6), *Memnoniella* sp(1), *Memnoniella* sp(2), dan *Helminthosporium* sp. Picture of microscopic form of these isolated and identified fungi were presented in following figures:

a. Fungi 1 (F1): *Aspergillus* sp

Figure 1. *Aspergillus* sp

b. Fungi 2 (F2): *Memnoniella* sp(1).

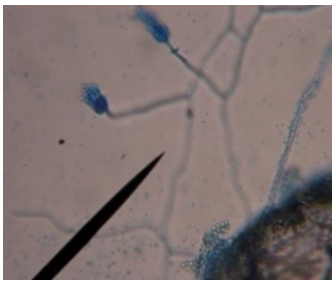


Figure 2. *Memnoniella* sp(1)

c. Fungi 3 (F3): *Penicillium citrinum*

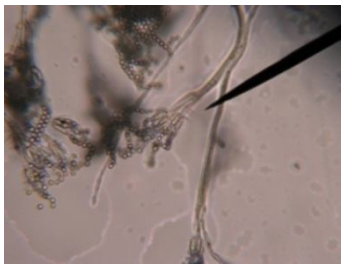


Figure 3. *Penicillium citrinum*

d. Fungi 4 (F4) : *Penicillium* sp(2)

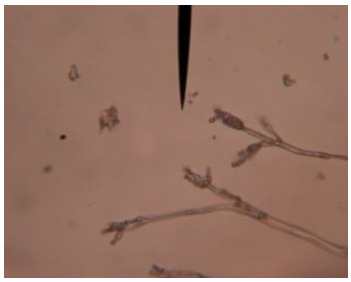


Figure 4. *Penicillium* sp(2)

e. *Helminthosporium* sp

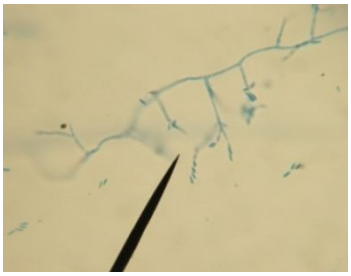


Figure 5. *Helminthosporium* sp.

g. *Penicillium sp*(3).

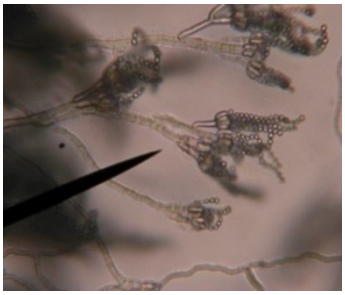


Figure 6. *Penicillium* sp(3).

g. *Penicillium* sp(4)

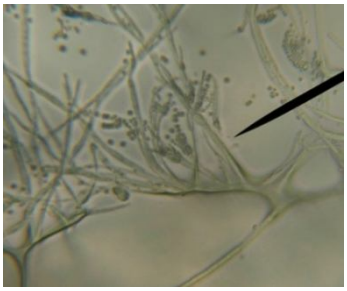


Figure 7. *Penicillium* sp.

h. *Penicillium sp* (5)

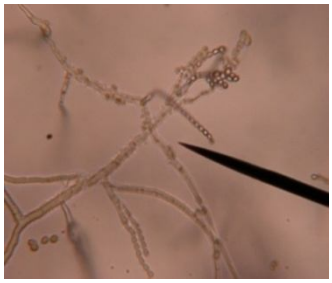
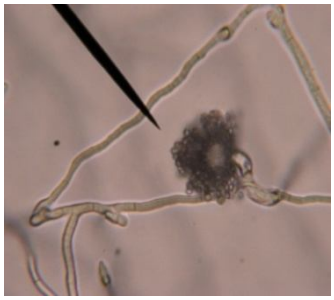


Figure 8. *Penicilluim sp*. (4)

i. *Memnoniella* sp(2).



Picture 9. *Memmoniella* sp (2)

j. *Penicilluim* sp (6)

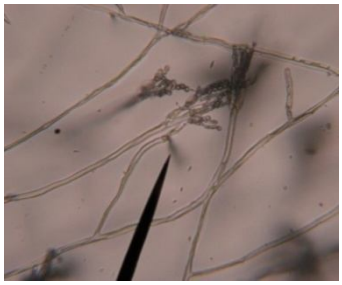


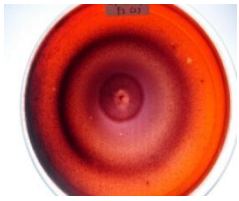
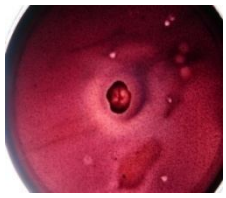
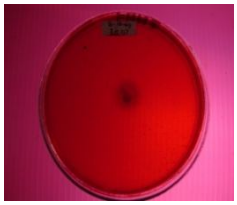
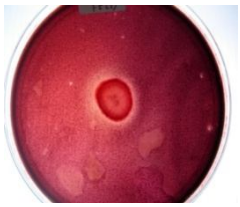
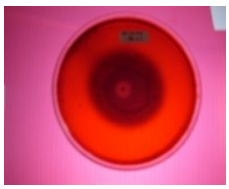
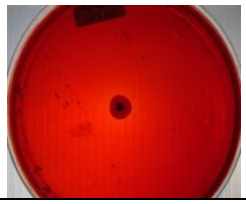
Figure 10. *Penicillium* sp(6).

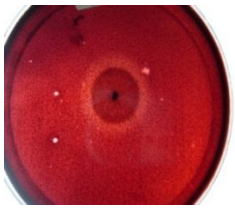
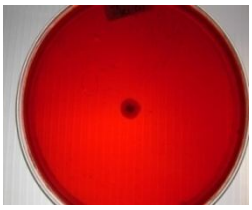
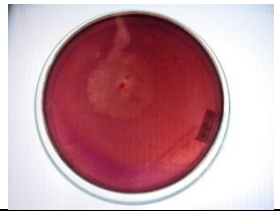
Table 1. Result of identification of isolate from sugarcane bagasse.

Codes	Colony Colors	Hyphae	Conidophore	Metula	Phialide	Spore/ Conidia	Name of Isolate
F1	black	septate	upright, with vesicle	-	-	Ovale, globose → ovoid to globose	*Aspergillus sp*
F2	green	septate	smooth-wall, nearly green, apex swelled	-	flask-shaped, each metula contains 6 to 10 phialide	spheric, chained, densed	*Penicillium citri-num*
F3	green	septate	dark-colored, simple, not branched	each conidiophore contains 2 to 6 metula)	short phialides, conidiophore contains 3 to 6 phialide	catenulate, spheric	*Memnoniella sp*
F4	green, densed	septate	smooth-wall, not swelled	each conidiophore contains 3 to 5 metula	each metula contains 2 to 4 phialide	globose to spheric	*Penicillium sp* (2)
F5	purple	septate	smooth, short, simple	-	-	thin, each two side with sharp point	*Helminthosporium*
F6	white at center , green at side	septate	not branched	-	-	spheric, dark-colored	*Penicillium sp* (3)
F7	green, in layers	septate	branched		each metula contains 3 to 6 phialide	spheric, catenu-late	*Penicillium sp* (4)

F8	nearly brown - green	septate	smooth-wall, apex swelled		each metula contains 2 to 4 phialide		*Penicillium sp* (5)
F9	dark green	septate	not branched, dark-colored		short phialides, conidiophore contains 2 to 4 phialide, chained	ovoid to spheric	*Memnoniella sp*
F10	green, densed	septate	smooth-wall apex not-swelled	each conidiophore contains 3 to 5 metula	each metula contains 2 to 4 phialide	globose to spheric	*Penicillium sp* (6)

Table 2. Activity of cellulolytic enzymes based on Congo red test

Fungi Code	Congo Red Test	Observation	+/-
F1		• Halo zone • Blue color	+
F2		• Halo zone Blue color	+
F3		• Halo zone • Blue color	+
F4		• Halo zone • Blue color	+
F5		• Halo zone • Blue color	+
F6		• Halo zone • Blue color	+
F7		• Halo zone • Blue color	+

F8		• Halo zone • Blue color	+
F9		• Halo zone • Blue color	+
F10		• Halo zone not clear • Blue color	+

+ = fungi produce cellulolytic enzymes;
- = fungi not produce cellulolytic enzymes.

Activity of Cellulolytic Enzymes

All isolated fungi were tested qualitatively for measurement of cellulase activity. Based on the result of the test, all isolated fungi have the cellulase activity. Qualitative analysis was conducted at the age of culture of 3 - 4 days. It could be seen clearly on the medium that the color of the colony were not so much different among the tested isolated fungi. The color of colony of all tested isolated fungi are close to white, because at this stage all the isolated fungi just form the miselium, or no spora produced yet. Results of Congo red test to measured the cellulase activity of all isolated fungi were presented in Table 2.

Colony of isolated fungi had different response to Congo red indicator. Congo red indicator could associate with the glycoside bound in cellulosic matter in CMC medium. Cellulase produced by fungi could break the glycoside bound and this process would be an indicator as formation of the halo zone or clear area around its colony. The color of Congo red indicator will be red in base environment and blue in acid environment. Therefore, decomposition of CMC by cellulase that produce organic acids will lead to the formation of blue color in medium. In other words, the blue color seen in the medium indicate that the isolated fungi could produce cellulase that enabling them to have decomposition ability.

CONCLUSION

It can be concluded from this experiment that *Aspergillus* sp, *Penicillium citrinum*, *Penicillium* sp (2), *Penicillium* sp (3), *Penicillium* sp (4), *Penicillium* sp (5), *Penicillium* sp (6),*Memnoniella* sp (1), *Memnoniella* sp (2), dan *Helminthosporium* sp produce cellulase that enabling them to decompose the cellulosic materials.

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Effects Copra Meal Fermented by *Aspergillus niger* and *Trichoderma Spp* on Performance of Broiler

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ABSTRACT

Inclusion of copra meal in the diet could impair the performance of birds due to its physical and nutritional problems. Improving feed quality by fermentation has long been believed. An experiment was conducted to examine the effects copra meal (CM) fermented by *Aspergillus niger* and *Trichoderma spp* on bird performance. One hundred twenty six day old unsexed Cobb chicks were used in this study. The birds were fed seven different diets (0% CM, 10% CM, 10% CM supplemented with 1% *Aspergillus niger* fermented CM, 10% CM supplemented with 1% *Trichoderma* fermented CM, 30% CM diet, 30% CM supplemented with 1% *Aspergillus niger* fermented CM, 30% CM supplemented with 1% *Trichoderma* fermented CM. Feed and water were available at all times. A completely randomised design was applied in this experiment with seven treatments and three replicate cages. Data indicated that body weight gain of birds fed the supplemented CM diet with 1% fermented CM tended to be higher than those of birds fed the un supplemented CM diet. This trend became evident when the birds fed 30% copra meal diet. The birds fed 30% copra meal diet supplemented with 1% Trichoderma fermented CM had higher body weight gain than those of birds fed the un supplemented 30% CM diet (1711 gram vs 1421 gram). Feed conversion ratio of bird fed 30% CM was also affected by fermentation with *Trichoderma spp*. Feed consumption was not affected by addition of copra meal diet with 1% fermented CM. Fermentation of copra meal with Trichoderma spp could improve the performance of bird in a diet containing 30% copra meal.

Key words: fermentation, copra meal and broiler

INTRODUCTION

Nutritionally, copra meal contains 21 - 25% protein and 7% lipid. The nutrient contents of copra meal appear quantitatively favourable. However, nutrient qualities are poor, possibly because of heat damage during the drying or oil extraction processes (Butterworth and Fox, 1963), the presence of indigestible polysaccharides, especially mannan and galactomannan (Balasubramanian, 1976; Saittagaroon *et al.*, 1983), and low levels of several limiting amino acids (NRC, 1994). These components impair the nutritive value of the diet when copra meal is added into the diet (Sundu *et al*., 2006).

Fermentation has been practiced for quite long time as a means to improve the quality of food. Fermentation process using filamentous fungi, such as Aspergillus niger, has been applied to improve nutritive value of soybeans (Chah *et al*., 1975; Mathivanan *et al*., 2006), guar meal (Nagra *et al*., 1998) and tofu waste (Rasud, 2009) for poultry. The fermentation process can create conditions for the growth of microorganisms that break down fibre and anti-nutrients.

Filamentous fungi, such as Aspergillus niger, has capacity to produce various enzymes such as hemicellulase, pectinase, lipase and tannase (Pinto *et al*., 2001; Mathivanant *et al*., 2006). Fermented feedstuffs (fermented copra meal using filamentous fungi) can simply be used as enzymes sources. Accordingly, the use of fermented copra meal in small quantity can improve nutritive value of copra meal based diet. This study was undertaken to examine supplementation of fermented copra meal in copra meal based diets on bird performance.

MATERIALS AND METHODS

Fermentation Process

Copra meal was used as solid substrate for fermentation. A total of 500 gram of substrate was placed in a plastic tray and moistenned with 250 ml distelled water. The medium was sterilized by steaming it for 1 hour. The substrate was then incubated with 1 gram fungi (*either Aspergillus niger or Trichoderma spp*). Those fungi were purchased from Laboratory of plant disease at Agriculture Faculty, University of Tadulako. The sub-

strate was placed in a cabin for 5 days at room temperature for fermentation.

Animal and Feed

The study was conducted in the animal house at The University of Tadulako, Palu, Indonesia. A total of 126 day-old unsexed Cobb chicks were available for use as experimental animals. They were placed in a brooder pen from days 1 to 21 and given a starter diet. After the 21 day, birds were transferred into floor pen and were offered a grower diet. Diets were formulated to meet the nutrient requirements of starter broilers (see Table 1.), using the UFFF computer program version 1.11 (Pesti *et al.*, 1986). The seven diets imposed are described in Table 2. Feeds were offered *ad libitum* twice a day at 08.00 and 16.30 hours, and water was available at all times. Feed intake and body weight were measured on day 1 and day 42.

Statistical Analysis

A completely randomized design was adopted with seven treatment, each with three replicate cages of six birds. Data were analysed by analysis of variance using the SAS 6.2 statistical program (SAS Institute, 1990). Differences among treatments were tested for significance by using Duncan's Multiple Range Test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Data of live weight gain, feed intake, feed conversion ratio (FCR) and growth uniformity are shown in Tables 3. Feed intake of broilers was not affected by addition of CM and fermented CM. Growth of birds fed the 10% CM diet was not significantly different ($P<0.05$) from the growth of birds fed the control diet. The inclusion of 30% CM significantly decreased the body weight gain of birds fed from 1 to 42 days. However, when a 30% CM diet was supplemented with either *Apergillus niger* fermented CM or *Trichoderma spp* fermented CM, weight gain of birds was improved to the level of body weight gain of birds fed control diet. The 30% CM added with 1% *Trichoderma spp*

Table 1. Ingredient and nutrient composition of the experimental diets (%)

Dietary components	Control diet		10% CM diet		30% CM diet	
	Starter	Grower	Starter	Grower	Starter	Grower
Copra Meal	0.0	0.0	10.0	10.0	30.0	30.0
Maize	51.7	54.0	44.2	48.0	31.2	32.9
Soybean	22.0	25.0	24.0	23.0	25.5	25.0
Fish Meal	13.5	9.5	12.5	9.5	8.0	6.0
Rice bran	11.0	10.0	7.5	8.0	0.2	0.1
Vegetable oil	0.0	0.0	0.0	0.0	2.5	3.5
Dicalcium Phosphate	1.0	0.7	1.1	1.0	1.8	1.8
Premix	0.3	0.3	0.3	0.3	0.3	0.3
DL-Methionine	0.3	0.3	0.3	0.1	0.3	0.2
L-Lysine	0.2	0.2	0.1	0.1	0.2	0.2
Calculated composition; ME (kcal/kg)	3078	3134	3086	3113	3032	3105
Protein	22.0	20.9	22.0	20.2	22.2	20.9
Methionine	0.7	0.6	0.7	0.5	0.7	0.6
Lysine	1.4	1.3	1.3	1.2	1.3	1.2
Calcium	1.1	0.8	1.1	0.9	1.0	0.9
Available phosphorus	0.7	0.6	0.8	0.6	0.8	0.6

Note: CM: Copra meal; ME: Metabolizable energy; kcal: Kilo kalori.

Table 2. Details of experimental treatments

Diet	Fermentation	Treatments
0% CM (control)	- Without fermented CM	- D1
10% CM diet	- Without fermented CM	- D2
10% CM diet	- With 1% Aspergillus niger fermented CM	- D3
10% CM diet	- With 1% Trichoderma spp fermented CM	- D4
30% CM diet	- without fermented CM	- D5
30% CM diet	- With 1% Aspergilus niger fermented CM	- D6
30% CM diet	- With 1% Trichoderma spp fermented CM	- D7

Table 3. The effect of fermented copra meal in the diets on broilers performance from day 1 to 42

Diet	Feed intake (g)	Weight gain (g)	FCR	Uniformity (% CV)
D1	3070	1729[a]	1.78[ab]	0.5
D2	2950	1568[ab]	1.88[ab]	2.1
D3	3283	1741[a]	1.89[ab]	4.6
D4	3083	1824[a]	1.69[b]	4.4
D5	3084	1421[b]	2.20[a]	14.7
D6	3143	1586[ab]	1.99[ab]	5.3
D7	2837	1711[a]	1.66[b]	3.3

Note: values with the same superscript within a column are not significantly different (P<0.05)
CV: Cofficient variation.

fermented CM had better FCR than the 30% CM diet without fermentation. The cofficient variation of weight gain was improved when the level of copra meal in the diet was reduced.

The earlier experiment by Sundu *et al*. (2004; 2005; 2006) indicated that birds fed 10% copra meal diet were consistently better than those birds fed 30% copra meal diet. Our current data on the use of 10% and 30% copra meal in this study also adds this consistency. It is not difficult to rasionalize these findings since the nutrients quality of copra meal is poor. Addition of this feedstuff in a greater quantity deteroriates nutritive value of the poultry diet (Sundu *et al*., 2009).

The results also demonstrate that birds fed the 10% copra meal supplemented with either 1% *Aspergillus niger* fermented copra meal or 1% *Trichoderma spp* fermented copra meal exhibit good performance in terms of body weight gain and feed conversion ratio. This indicates that the use of 10% copra meal did not impair the growth of birds, particularly when the diet was supplemented with fermented copra meal. The performance of 42 day old birds fed the 10% copra meal diet plus 1% *Trichoderma spp* fermented copra meal was increased by nearly 250 gram and 100 gram compared with the birds fed the unsupplemented 10% copra meal diet and control diet respectively. However, the differences were not significant.

Effectiveness of supplementing 1% *Trichoderma spp* fermented copra meal in copra meal based diet became evident when the concentration of copra meal in the diet was increased to the level of 30%. Possible reasons of the improvement in body weight gain are an increase in nutrients availability (Nagra *et al*., 1998) and the presence of enzymes in the diets due to addition of fermented feedstuff (Filler, 2001). These speculations need to be clarified.

The cofficient variation of weight gain of birds fed the control diet was 0.5%. It appears that the quality of control diet is homogenous from one cage to another and this led to uniform growth of birds. When the diet contained 10% of copra meal, the variation of growth was still below 5%. Further addition of copra meal in the diet to the level of 30%, variation of body weight gain of birds was ununiform, even reached 14.7% in birds consuming unsupplemented 30% copra meal.

The 1% *Trichoderma spp* fermented copra meal in the diet produced better feed efficency than the 30% copra meal diet without any supplementation. It appears that *Trichoderma spp* worked well in solid state fermentation when copra meal was used as a solid substrate. However, modus of operandi how fermented copra meal with this fungi could improve nutritional value is difficult to answer. The presence of enzymes in the fermented product, availability of nutrients and the possibility of this fungi as a probiotic in gastro intestinal of birds are some possible causes.

CONCLUSION

Supplementation of copra meal based diet with 1% Trichoderma spp fermented copra meal increased the nutritive value of the diet.

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The Changing of Broilers'blood Component at Various Environmental Temperatures and Times of Sampling

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ABSTRACT

This study was conducted to evaluate the effects of environmental temperatures and ages (time of sampling) on erythrocyte number (Er), hemoglobin concentration (Hb) and hematocrite value (Hm). Ninety 14-d old broilers were used in 3 x 4 completely randomized design in split plot 3 x 4 reared in three environmental chambers (25.55±1.45; 29.29±1.27 and 31.59±1.05°C as T1, T2 and T3 respectively),and four times of sampling (0, 4, 8 and 16 days after factor of treatment environmental temperature as S0, S4, S8 and S18 respectively). The results showed in general that T2 and T3 significantly increased in Er and Hm. The numbers of erythrocyte and presentation of hematocrite of T3S4 were higher and T1S8 were lower than all. The level of hemoglobin of S0, were higher than the others. It was concluded that a high environmental temperature and time sampling could affects the blood component of broilers.

Key words: temperature, time of sampling, blood component, broilers

INTRODUCTION

The global environmental temperature issue that will increase the environmental temperature is one of major concern for poultry producers. The increasing of environmental temperature will affect on industry of animal husbandry. It causes, besides will affect on hormonal system, digestibility of protein, availibility of antioxidant and the increasing of the free radical, the heat stress will affect the biochemistry and component of blood.

Lu *et al*. (2007) reported that feed consumption and body weight gain of broilers reared at temperature of 21°C (from 5 to 8 weeks of age) were 169.9 g/d, and 61.45 g/d respectively, significantly higher than for those reared at 34°C with feed consumption and body weigh gain of 93.6 g/d and 22.29 g/d respecticely. However, feed to gain ratio increased from 2.76 at low temperature to 3.92 at high of temperature.

Sugito *et al*. (2007) and Kusnadi *et al*. (2009) approved from their experiments that heat stress could reduce growth rate as well as level of the hormone triiodothyronin (T_3) in blood plasma of broiler chicken. As calorigenic factor T_3 has function to increase oxygen consumption for metabolisme through what the increment of growth rate could be gained.

Harlova *et al*. (2002) reported that erithrocyte, leucocyte, hemoglobin and hemotocrite of heat-stressed were significantly lower than control. Similar result was showed by Kusnadi (2008), that blood component of broilers 4 and 6 weeks of age reared at 33.5°C, significantly lower than 28.55°C. Zhang *et al*. (2007) reported that erithrocyte, hematocrit and hemoglobin of broiler reared at low altitude (100 m) were 1770000/mL, 29.73% and 9.49 g/mL, significantly lower than at high altitude (2900 m) of 2860000/mL, 36.49% and 10.45 g/mL respectively. The objective of the present study was to evaluate the effect of environmental temperatures and times of sampling on blood component of broilers.

MATERIALS AND METHODS

Ninety 14-d old broilers with 500 - 600 g of body weight were used as materials. The treatments had two factors, the first factors were three environmental temperatures (25.55±1.45; 29.29±1.27 and 31.59±1.05°C as T1, T2 and T3 respectively) and the second factors were four times of sampling (0, 4, 8 and 16 days after factor of environmental temperature as S0, S4, S8 and S18 respectively). The ration used was commercial feeding from Comfeed Industry. The ration and dringking water were always available or *ad libitum*.

The measured variables consisted of erythrocyte number, level of hemoglobin and presentation of hematocrite. All those variables were measured with autohematology analyzer used spectrophotometer. The expe-

rimental design used was a completely randomized design in split plot 3 x 4 (three environmental temperatures, four times of sampling) with six replications, respectively. Data collected were analysed with analysis of variance (ANOVA) and Duncan multiple range test (DMRT) was further used to test the significant differences (Steel and Torrie, 1993).

RESULTS AND DISCUSSION

Analisis of variance resulted that interactions of environmental temperature x time of sampling affected significantly ($P<0.05$) on erythrocyte and hematocrite, only factor of time of sampling affected significantly ($P< 0.05$) on hemoglobin. The average of erythrocyte, hematocrite and hemoglobin were showed in Table 1.

Table 1 showed that the average of erythrocyte of T3S4 was 2763000 pieces/mm^3. It was significantly higher than T2S4 and T1S4 (2193000 pieces/mm^3 and 1987000 pieces/mm^3 respectively). On 8 days of sampling, all of erythrocyte number decreased but the erythrocyte of T1S8 was still the lowest than the others. However on S16 , all of that erytrhocyte increased especially in T1S16. (2330000 pieces/mm^3) higher significantly than T2S16 (2093000 pieces/mm^3) and T3S16 (2103000 pieces/mm^3).

The increasing of erythrocyte of T3S4 (Table 1), may be related to reduced blood oxygen saturation. This result agree with research of Olanrewaju *et al*.(2007). The oxygen was needed to increase the metabolic activity to meet the energy demands for both maintenance and growth under relatively extreme stressfull condition (Luger *et al*., 2003). In others sampling, the erythrocyte of T2 and T3 especially at 16 days of sampling (T2S16 and T3S16) lower than of T1S16. This was caused, the birds had adapted with environmental temperatures and in turn decreased in erythrocyte number and their productivity (Harlova *et al*., 2002; Kusnadi, 2008).

Futhermore, Table 1 showed that the presentation of hematocrite of T3S4 and T2S4, significantly higher than T1S4 and presentation of hematocrite of T3S8 and T3S8, significantly higher than T1S8. This result equally with result of erythrocyte. The increasing of hematocrite in T2 and T3, may be related to the increased muscle activity and the concomitant movement of water from plasma to muscle, leading to an increase in erythropoiesis as a compensatory reaction to the lack of sufficient oxygen in the tissue, possibly because of impaired oxygen-carrying capacity in the blood. The increasing of hematocrite in T2 and T3, may be due to many factors, such as enchanced erythropoiesis because of high levels of corticosterone (CS) or diminished plasma volume (Maxwell *at al*., 1990; Yahav *et al*., 1997; Luger *et al* .,2003; Olkowski *et al*., 2005).

Table 1. Average of erythrocyte, hematocrite and hemoglobin of broilers at various environmental temperature (T1=25.55±1.45; T2=29.29±1.27 and T3=31.59±1.05°C) and times of sampling (0 day=S0, 4 days=S4, 8 days=S8 and 16 days=S16)

Environmental Temperature	Time of sampling (days)				
	S0	S4	S8	S16	average
	erythrocyte (piece/mm^3)				
T1	1980000^{b}	1986667^{b}	1756667^{a}	2330000^{c}	2013333
T2	2000000^{b}	2193333^{b}	2063333^{b}	2093333^{b}	2087500
T3	2019000^{b}	2763333^{d}	2050000^{b}	2103333^{b}	2233917
Average	1999667	2314444	1956667	2175556	
	hematocrite (% of blood)				
T1	28.33333^{c}	26.33333^{b}	23.33333^{a}	30^{cd}	27
T2	28^{c}	29^{cd}	27.33333^{b}	27.33333^{b}	27.91667
T3	27.58^{b}	30.33333^{cd}	26.33333^{b}	26.66667^{b}	27.72833
Average	27.97111	28.55556	25.66667	28	
	hemoglobin (mg/dL)				
T1	11.86667	10.56667	9.033333	11.5	10.74167
T2	11.567	11,5	10.6	10.86667	11.13342
T3	11.99	11.86667	10.5	10.53333	11.2225
Average	11.80789^{c}	11.31111^{bc}	10.04444^{a}	10.96667^{b}	

Note: mean with different suppercripts within a row/column differ ($P< 0.05$).

In this study, concentration of hemoglobin S8 were lowest than others. This result equally with decreasing in erythrocyte and hematocrite. The increasing of hematocrite in S16, may be caused the bird of S16 had adapted with environmental temperatures (Harlova *et al*., 2002; Kusnadi, 2008).

CONCLUSSION

The numbers of erythrocyte and presentation of hematocrite of T3 at 4 days sampling (T3S4) were higher than all, however it was the lowest at 8 days sampling of T1 (T1S8). The level of hemoglobin of S0, were higher than the others.

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Distribution of Population and Output Estimation of Some Cattle Breeds at Bawang Subdistrict Banjarnegara Regency Central Java Province

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ABSTRACT

This research was conducted from January 1st up to March 31st, 2009 to study the distribution of population and to estimate the output of some cattle breeds. Census method were used in this research located at Bawang subdistrict, Banjarnegara regency, Central Java Province. The object of this research were 1,369 respondents. The respondents were cattle farmers. The variables observed were cattle breed, amount of cattle every farmers, the age of cattle, sex of cattle, management of rearing, mortality, and birth. The breeding system of cattle were used to estimate requirement of replacement cattle and cattle composition. Natural Increase (NI) were calculated based on the difference of birth percentage and mortality percentage for a year. The output of cattle were calculated based on the difference of NI and requirement of replacement cattle. This research indicated that there was three cattle breeds: 630 Ongole Grade cattle (PO cattle) consisted of 68.15% adult cattle, 17.78% young cattle, 16.03% calves, 1,442 Simpo cattle consisted of 66.99% adult cattle, 8.04% young cattle, 24.97% calves, 20 Limpo cattle consisted of 66.54% adult cattle, 10.99% young cattle, 22.47% calves. In the PO, Simpo, and Limpo groups, every respondent had 1.44 cattle (1.21 UT), 1.49 cattle (1.18 UT), and 1.05 cattle (0.67 UT), respectively. NI of PO, Simpo, and Limpo cattle groups were 53.38%, 60.14%, and 45% , respectively with the average 59.48%. Estimation of cattle output at Bawang subdistrict were calculated based on the remainder of replacement stock and the old cattle. The remainder of replacement stock consisted of 28.54% male and 20.56% female PO cattle, 26.01% male and 17.68% female Simpo cattle, 22.50% male and 17.53 female Limpo cattle. The old cattle consisted of 7.98% female PO cattle, 8.33% female Simpo cattle, and 4.97% female Limpo cattle. Data of cattle population at Bawang subdistrict, Banjarnegara regency, Central Java Province based on information of government (3,188 cattle) were higher 52.85% than that were based on data of census (2,092 cattle). It could be concluded that Bawang subdistrict, Banjarnegara regency, Central Java Province was suitable as cattle breeds resource .

Key words: Distribution of population, Output , PO, Simpo, and Limpo

INTRODUCTION

Big cattle population was found in Central Java Province, the second after East Java Province. Batang Subdistrict in Banjarnegara Regency of Central Java were well known as cattle resource region. Most of population at Bawang subdistrict (94.41%) worked as farmer and cattle were raised as secondary commodity for sale when cash money were needed (Wibowo, 2009) . The population of beef cattle at Banjarnegara regency in 2007 were 38,501 cattle (Agriculture, Fishery, and Animal Husbandry Department (AFAD) of Banjarnegara Regency, 2007).

Ongole Grade cattle (Peranakan Ongole/ PO cattle) were raised by most farmers at the region resulted in additional numbers of crossbred animals. Introduction of new cattle breeds through artificial insemination resulted in additional on crossbred cattle. Simpo and Limpo were the examples of crossbred cattle found in the region so there were changes in numbers of PO cattle. Most of farmers chose Simpo and Limpo cattle because it had better performance than that of PO cattle.

Hardjosubroto (1994) stated that the growth, production and reproduction of Bos Taurus cattle were higher than that of Bos Indicus cattle. But Bos Taurus cattle had good resistance to heat weather and to parasites and had good mothering ability. The PO cattle had high adaptation, primarily to feed residue from agriculture waste product.

However, very limited information found on cattle population, production and reproduction performance, and the changes of proportion of population each breed in the region. The information is important for decision making in formulation development program.

This research was conducted to study population distribution and estimate on of the changes and proportion of each breed of cattle in the population in Central Java.

MATERIALS AND METHODS

This research was conducted at Bawang Subdistrict, Banjarnegara Regency, Central Java Province from January 1st up to March 31st, 2009. Survey method were used in this study and interview was conducted with 1,369 respondents to get data of farmer characteristic, composition of cattle population and distribution, the number of cattle owned by each farmer. Data of cattle population during 2004 up to 2008 was obtained from AFAD of Banjarnegara Regency. The number of cattle owned by each farmer were calculated in animal unit (AU) as followed: 1 head of adult cattle =1.00 AU, 1 head of young cattle = 0.67 AU, 1 head of calve =0.25 AU (Reksohadiprodjo, 1984).

Variables observed were distribution of cattle population, natural increase, and output of cattle. Population distribution were calculated based on population data of cattle obtained by survey method and then it were grouped based on breed, the age of cattle, and sex of cattle. Natural increase estimation (NI) were calculated by the formula as be recommended by Hardjosubroto (1994) as followed:

NI (%) = (% calving rate on cattle population) – (% mortality of cattle on cattle population)

The estimation of output were calculated by formula as followed:

Output (%) = NI (%) –Requirement of replacement cattle (%)

Data of cattle population obtained from AFAD of Banjarnegara Regency were analysed by Time Series with least square method (Supranto, 1993). The formula of that were $\hat{Y} = a + bx$ ($\hat{Y}$ = Time Series Data, x = Time (year), a and b = the constanta).

RESULTS AND DISCUSSION

Composition of Cattle Breeds and Number of Cattles Every Farmer

Data at Table 1 indicated that composition of cattle breeds at Bawang Subdistrict of Banjarnegara Regency consisted of 630 heads (30.11%) PO cattle 1,442 heads (68.93%) Simpo cattle, and 20 heads (0.96%) Limpo cattle. Percentage of Simpo cattle were highest than that of PO and Limpo cattle because the price of Simpo cattle

Table 1. Structure and composition of cattles breeds at Bawang Subdistrict of Banjarnegara Regency, Central Java

Groups of cattle	PO cattle		Simpo cattle		Limpo cattle		Beef cattle	
	Number	%	Number	%	Number	%	Number	%
Calves	**101**	**16.03**	**360**	**24.97**	**9**	**45.00**	**470**	**22.47**
-Male	27	58.70	118	90.08	0	0	145	81.92
-Female	74	12.67	242	18.46	9	45.00	325	16.97
Young	**112**	**17.78**	**116**	**8.04**	**2**	**10.00**	**230**	**10.99**
-Male	0	0	1	0.76	0	0	1.00	0.56
-Female	112	19.18	115	8.77	2	10.00	229	11.96
Adult	**417**	**66.19**	**966**	**66.99**	**9**	**45.00**	**1,392**	**66.54**
-Male	19	41.30	12	9.16	0	0	31	1.49
-Female	398	68.15	954	72.88	9	40.9	1,361	65.06
Total	**630**	**100**	**1,442**	**100**	**20**	**100**	**2,092**	**100**
-Male	46	7.30	131	9.08	0	0	177	8.46
-Female	584	92.70	1,311	90.92	20	100	1,915	91.54
Respondents (persons)	436		970		19		1,425	
Cattles (AU)	526.25		1,143.05		12.75		1,682	
Number of cattle owned by each farmer	1.21		1.18		0.67		1.18	

were highest than that of PO and Limpo cattle at the region. Despite that, at the region only frozen cement of Simpo sire were always available and the frozen cement of Limpo sometime were not available.

The condition at the region with percentage of Simpo cattle that were highest were similar with the condition at Sewon and Banguntapan Subdistricts, Bantul Regency, Daerah Istimewa Yogyakarta (DIY) Province. Hasbullah (2003) reported that most of farmers at Sewon and Banguntapan Subdistrict, Bantul Regency, DIY Province choose Simpo cattle to be raised than Limpo and PO cattle because Simpo cattle grew faster than that of Limpo and PO cattle. Simpo cattle need 166.7 days to reach the body weight about 100 kg with the average daily gain (ADG) 0.60 kg and PO cattle need 277.8 days to reach the same body weight with ADG 0.36 kg although to get the body weight 100 kg, it was needed low feed cost per gain at PO cattle (Rp11,780.00 every head of cattle) than that of Simpo cattle (Rp12,483.00 every head of cattle).

Decreasing of PO cattle percentage were similar with the condition at Daerah Istimewa Yogyakarta (DIY) and Jawa Tengah. Percentage of PO, Simpo, and Limpo cattle at DIY province were 25.75, 52.38, and 21.87%, respectively. Percentage of them at Jawa Tengah (Central Java) Province were 51.93, 36.50, and 11.57%, respectively. The condition indicated that there were decreasing PO cattle at Java Island (Sumadi, 2009).

The structure of cattle breeds at Bawang Subdistrict were 8.54% male cattle and 91.54% female cattle with the percentage of male adult cattle 1.49% of population. The percentage of male adult cattle were low because natural insemination had not been available in the region and fattening program to male cattle were not conducted at the region. The male cattle were sold when it was still calves.

Table 1 indicated that percentage of female adult of PO, Simpo, and Limpo cattle were 68.15%, 72.88%, 40.90%, respectively, and the average of female adult cattle were higher (65.06%) than that of male adult cattle (1.49%), so that the region could be stated as beef cattle resources. Percentage of female adult cattle that were higher than that of male were suitable with recommendation of Sumadi *et al*. (2004). They stated that composition of female adult cattle of beef cattle at Eastern Java (51.54%), Western Nusa Tenggara (48.88%), Eastern Nusa Tenggara (53.32%), North Eastern Sulawesi (29.64%), and Southern Sulawesi (42.83%) were higher than that of male. The result indicated that artificial insemination were available in that region so percentage of male adult cattle were lower than that of female.

Table 2 indicated that the number of cattle owned by each farmer were low, i.e. 1.47 heads (1.17 AU) that mean every farmer just had one head of adult cattle. The number of cattle owned by each farmer were low because the farmer in that location raised cattle just as secondary job and agriculture as their primary job. Sumadi *et al*. (2004) said that the number of cattle owned by each farmer at Eastern Java were lower (1.87 AU) than that of Western Nusa Tengggara, Eastern Nusa Tenggara, South Eastern Sulawesi, and Southern Sulawesi, i. e. 7.03 AU, 15.08 AU, 5.04 AU, 3.67 AU, respectively. The condition in Eastern Java were caused by limited of land, employment, and capital owned by each farmer so the farmers in the region just raised cattle in the small scale.

Natural Increase and Output

Estimation of Natural Increase (NI) value of PO, Simpo, and Limpo cattle were 58.38%, 60.14%, 45.00%, respectively (Table 2). Estimation of cattle output at Bawang Subdistrict were calculated based on the remainder of replacement stock and the old cattle (Table 2).

That value of NI and cattle output in the region were high as the recommendation of Sumadi *et al.* (2004). They stated that NI value were high and ideal if it obtained 20%. That value were affected by percentage of calving rate and mortality of cattle. The value of NI at Bawang Subdistrict were high because calving rate percentage of PO, Simpo, and Limpo cattle were high, i. e. 78.14%, 82.91%, 100%, respectively. Despite that, mortality percentage of PO, Simpo, and Limpo cattle were low, i.e. 0.19%, 0.54%, 0.00%, respectively. Another factors that affected the value of NI were type and management of rearing. Cattles raised to get off springs caused the percentage of calving rate high.

Table 2. Estimation of natural increase and beef cattle output at Bawang subdistrict

No	Variables	PO	SIMPO	LIMPO	Average
1	Number of adult female cattles (%)	63.17	66.16	45	65.06
2	Calving rate on number of dams (%)	78.14	82.91	100	81.63
3	Calving rate on number of cattles population (%)	58.57	60.68	45.0	59.91
4	Mortality percentage on population (%)	0.19	0.54	0	0.43
5	Natural increase (NI) (%)	58.38	60.14	45.0	59.48
6	Replacement stock (%/year)				
	Male	0	0	0	0
	Female	7.40	7.61	5.45	7.65
7	Replacement availability (%)				
	a. Male	30.91	31,60	9.9	31.56
	b. Female	27.36	27.22	35.10	27.66
8	The remainder of replacement stock (%)				
	a. Male	30.91	31,60	9.9	31.56
	b. Female	19.96	19.61	30	20.01
9	Old cattles (%)				
	a. Male	0	0	0	0
	b. Female	7.40	7.61	5.45	7.65
	c. Total	7.40	7.61	5.45	7.65

It was compared with the value of NI on PO, Simpo, Limpo cattle at Borobudur subdistrict, Magelang regency, Central Java province, i.e. 30.74%, 22.95%, and 32.39%, respectively, the value of NI at Bawang Subdistrict Banjarnegara Regency were higher than that of cattle at Magelang regency. The condition were caused by calving rate percentage on dam samples and that of on cattle population at Magelang regency were lower than that of Banjarnegara regency. Calving rate percentage on dam at PO, Simpo, Limpo cattle at Magelang regency were 37.02%, 31.09%, 39.18%, respectively. Calving rate percentage on cattle population at PO, Simpo, Limpo cattle at that location were 30.79%, 23.51%, 32.19%, respectively (Sumadi *et al.*, 2009).

Data on Table 2 indicated that Bawang subdistrict had the remainder of replacement stock that consisted of male and female cattle. That cattle can be sent to the other region need male and female cattle to breed. The old cattle only consisted of female cattle because the farmers didn't raise male cattle until it was old. The farmer didn't need male cattle to mate female cattle because the female cattle always mate by artificial insemination.

The output at Bawang subdistrict were high as statement of Sumadi *et al.* (2003). They said that the estimation of output of cattle were high if the value higher than 20 % on cattle population. The output of cattle at the research location were caused by high calving rate and low mortality percentage. Calving rate of PO, Simpo, and Limpo on dam population for a year were 78.14%, 82.19%, and 100.00%, respectively. Mortality rate of PO, Simpo, and Limpo on cattle population for a year were 0.19%, 0.54%, and 0.00%, respectively (Table 2).

The cattle output at Bawang Subdistrict as high as Borobudur Subdistrict, Magelang Regency, Central Java Province as was reported by Sumadi *et al*. (2009). They stated that the output of PO, Simpo, and Limpo cattle at Borobudur Regency were 28.20%, 22.95%, and 32.39%, respectively. The cattle output at Borobudur subdistrict were high because calving rate percentage of PO, Simpo, and Limpo cattle on cattle population for a year were high, i.e. 30.74%, 23.51%, and 32.59%, respectively, and mortality rate on cattle population of PO, Simpo, and Limpo for a year were low, i.e. 0.0%, 0.57%, and 0.20%, respectively.

Population Distribution of Beef Cattles

Data of cattle population at Bawang Subdistrict (2,092 heads) obtained by survey

method were lower than data from AFAD Banjarnegara Regency (3,188 heads), so there was a different 34.36%. The different were caused by different method to calculate population. This research used survey method and AFAD Banjarnegara Regency used the other method.

Beef cattle population obtained from AFAD Banjarnegara Regency in 2004 and 2008 were 2,866 heads and 3,188 heads, respectively, so there were improvement 2.74% every year (Table 3).

Table 3. Dynamic of beef cattle population at Bawang Ssubdistrict, Banjarnegara Regency from 2004 up to 2008

Year	Population (heads)	Improvement	
		Number (heads)	%
2004	2,866	-	-
2005	2,780	-86	-3.09
2006	2,897	117	4.04
2007	2,978	81	2.72
2008	3,188	210	6.59
Average	2,941.8	80.5	2.74

In 2004, beef cattle population were decreasing -3.09% (Table 3) because there were disposal many calves to the other regency. However, from 2005 up to 2008 there were increasing population (the average 2.74%) because Government gave many cattle to help farmers.

The formula to estimate dynamic of beef cattle population obtained by Time Series method were Y=2,941.8 + 84.2 X. Based on data of beef cattle population in 2008 (Table 3), dynamic of beef cattle population at Bawang Subdistrict from 2009 up to 2013 could be estimated (Table 4).

Based on Table 4, it could be estimated the number of beef cattle each breed at Bawang subdistrict, Batang regency from 2009 up to 2013 (Table 5). The beef cattle population at Bawang Subdistrict from 2009 up to 2013 will increase about 84.2 heads or 2.47% every years, so the beef cattle population in 2013 were predicted 3,531.20 heads.

Table 4. Dynamic of beef cattle population at Bawang subdistrict from 2009 up to 2013

Year	Population (head)	Increasing	
		Number (head)	%
2009	3,194.4	-	-
2010	3,278.6	84.2	2.57
2011	3,362.8	84.2	2.50
2012	3,447.0	84.2	2.44
2013	3,531.2	84.2	2.38
Average	3,362.8	84.2	2.47

Table 6 indicated distribution of cattle population at Bawang subdistrict. There were 630 heads (30.11%) PO cattle, 1,442 heads (68.93 %) Simpo cattle, and 20 heads (0,96%) Limpo cattle. The village with high population (299 heads) were Kutayasa village. The village with low population (7 heads) were Bandingan village. The low cattle population at Bandingan village were caused by limitation of the village. Most of the village area were used for building so there was not enough area to raise many cattle and to get grass for animal feeding.

CONCLUSION

Based on result and discussion, it could be concluded that Bawang Subdistrict, Banjarnegara Regency, Central Java Province were stated as beef cattles resource and there were decreasing of PO population.

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Table 5. Estimation of distribution of beef cattle population each breed (heads)

Year	Beef cattle population	PO cattle	Simpo cattle	Limpo cattle
2009	3,194.40	961.83	2,201.90	30.67
2010	3,278.60	987.19	2,259.94	31.47
2011	3,362.80	1,012.54	2,317.98	32.28
2012	3,447.00	1,037.89	2,376.02	33.09
2013	3,531.20	1,063.24	2,436.06	33.90
Average	**3,362.80**	**1,012.54**	**2,317.98**	**32.28**

Table 6. Distribution of cattle population at Bawang subdistrict

No	Village	PO		SIMPO		LIMPO			
		Number	%	Number	%	Number	%	Number	%
1	Kutayasa	60	9.52	227	15.74	12	60	299	14.29
2	Masaran	24	3.81	128	8.88	2	10	154	7.36
3	Gemuruh	1	0.16	82	5.69	0	0	83	3.97
4	Serang	62	9.84	57	3.95	1	5	120	5.74
5	Pucang	12	1.90	32	2.22	0	0	44	2.10
6	Bandingan	0	0.00	7	0.49	0	0	7	0.33
7	Bawang	95	15.08	60	4.16	0	0	155	7.41
8	Binorong	14	2.22	50	3.47	2	10	66	3.15
9	Joho	13	2.06	40	2.77	0	0	53	2.53
10	Mantrianom	32	5.08	48	3.33	0	0	80	3.82
11	Wanadri	133	21.11	40	2.77	0	0	173	8.27
12	Wiramastra	19	3.02	57	3.95	0	0	76	3.63
13	Depok	2	0.32	75	5.20	0	0	77	3.68
14	Kebondalem	35	5.56	262	18.17	0	0	297	14.20
15	Majalengka	13	2.06	127	8.81	0	0	140	6.69
16	Watuurip	59	9.37	29	2.01	0	0	88	4.21
17	Winong	12	1.90	27	1.87	0	0	39	1.86
18	Blambangan	44	6.98	94	6.52	3	15	141	6.74
	Total	**630**	**100**	**1,442**	**100**	**20**	**100**	**2.092**	**100**

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Effect of Different Drying Method and Maturity of Mulberry (*Morus alba*) Hay On *In Situ* Degradability of Sheep

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ABSTRACT

The experiment was conducted to elucidated the characteristics of the mulberry hay with respect to *in situ* degradation of dry matter (DM) and crude protein (CP). Four types of diets were used in the study differentiated by the drying methods and maturity of mulberry. These are, Mulberry harvested at 5 weeks of age and oven-dried (MHO5); Mulberry harvested at 7 weeks of age and oven-dried (MHO7); Mulberry harvested at 5 weeks of age and sun-dried (MHS5); Mulberry harvested at 7 weeks of age and sun-dried (MHS7). Samples (MHO5 and MHO7) were dried in the oven at 60°C for 48 h. Meanwhile, samples (MHS5 and MHS7) were directly dried in the sun until they reached a constant weight. Three rumen-fistulated mature sheep of 2.5 to 3 years old and average weighed 37±2.0 kg were used in this experiment. The sheep were kept in individual pens and fed twice daily in equal meals at 09:00 and 17:00 h and free access to water. The diet of the animals consisted of 30% mulberry hay (DM basis) and 70% of oil palm frond (OPF) (DM basis). The DM degradation of MHO5 and MHS5 was significantly ($P<0.05$) higher than MHO7 and MHS7 at 12, 24, 36 and 48 h of rumen incubation**.** Meanwhile, the CP degradation of MHO5, MHS5, MHO7 and MHS7 was not significantly ($P>0.05$) different at 0, 6, 12, 24, 36 and 48 h of incubation. The degradability of water insoluble (b), potential degradability (PD) and effective degradability (ED) of DM of MHO5 and MHS5 were higher than MHO7 and MHS7. Meanwhile, the PD and ED of CP were significantly ($P<0.05$) decreased with advancing plant maturity. These suggest that mulberry hay of five weeks maturity more fermentable and large potential for feeding sheep.

Key word: mulberry hay, degradability, maturity, sheep

INTRODUCTION

Mulberry hay of five weeks maturity contained higher CP and lower cell wall and lignin content than of seven weeks (Ali *et al*., 2007). The nutritive value of forage could be predicted from their degradation characteristics as they are strongly correlated to voluntary intake as compared to *in vivo* or chemical composition (Tolera and Sundstol, 2001).

Accurate estimation of the nutritive value of feed is important in animal production. The *in sacco* or nylon bag technique is commonly employed to estimate the degradation characteristics (Nordkvist *et al*., 1987) in particularly of protein and roughages and also for rumen environment studies (Ørskov and Shand, 1997). The bag technique is also a very robust and powerful tool to study several other aspects of nutrition in ruminants. The degradation characteristics of feeds, determined by the *in sacco* method, could be used in the predictions of feed intake, digestibility and animal performance in terms of growth rate (Ørskov and Ryle, 1990).

Studies on the degradation characteristic of fresh mulberry have been of great interest. Schmidek *et al*. (2002b) reported that mulberry leave showed high values of the soluble and potentially degradable fraction as well as the potential and effective degradation. It shows that mulberry foliage has a considerable potential for feeding ruminant.

The objective of this experiment was to elucidate the characteristics of mulberry hay with different drying and maturity respect to the *in situ* degradation of DM and CP.

MATERIALS AND METHODS

Experimental Diets

Four types of diets were used in the study differentiated by the drying methods and harvesting age of mulberry. These are:

MHO5 : Mulberry harvested at 5 weeks of age and oven-dried.

MHO7 : Mulberry harvested at 7 weeks of age and oven dried.
MHS5 : Mulberry harvested at 5 weeks of age and sun-dried.
MHS7 : Mulberry harvested at 7 weeks of age and sun-dried.

Samples (MHO5 and MHO7) were dried in the oven at 60°C for 48 h. Meanwhile samples (MHS5 and MHS7) were distributed and laid on a wooden frame tray (size: 3 m^2) covered underline with a fine plastic netting and directly dried under the sun until they reached a constant weight. The nutrient composition of the samples is shown in Table 1.

Animals and Diets

Three rumen-fistulated mature sheep of 2.5 to 3 years old and average weighed 37±2.0 kg were used in this study. The sheep were kept in individual pens and fed twice daily in equal meals at 09:00 and 17:00 h and free access to water. The diet of the animals consisted of 30% mulberry hay (DM basis) and 70% of oil palm frond (OPF as DM basis). The nutrient composition of the basal diet is shown in Table 2.

Experimental Design

The experiment was conducted in randomized complete block design with four treatments (MHO5, MHO7, MHS5 and MHS7) and three blocks (sheep). The sheep were adapted to the diet for 15 days. The degradation study was conducted for 7 days. In the rumen of each sheep, a total of four bags were put (four treatments). The bags were put in the rumen immediately before feeding in the morning (08:00 am).

Measurements of Degradation

Approximately 5 g of each sample (MHO5, MHO7, MHS5 and MHS7) were weigh and placed into the nylon bags (size 15 x 9 cm, pore size 45 µm) and then incubated in the rumen of fistulated sheep for 0, 6, 12, 24, 36 and 48 h. At the end of each incubation time the bags were removed from the rumen and washed in washing machine until the rinsing water was clear. All bags were then dried to constant weight at 60°C, and then analyze for DM and CP. The DM and CP were determined according to the methods of AOAC (1990). The bags for 0 h was not incubated in the rumen but washed directly in the washing machine until the rinsing water was clear.

The degradation characteristics of the samples were determined by using the NEWAY program based on the equation of $P = a + b (1- e^{-ct})$ (Ørskov and McDonald, 1979).

Where,
P: the degradation after t hours
a: the rapidly-soluble fraction at zero time,
b: the amount which in the time will degrade,
c: the degradation rate constant and
t: the incubation time

Statistical Analyses

Data on DM and CP degradation and degradation characteristics of the four treatments

Table 1. Nutrient compositions (% DM) of MHO5, MHO7, MHS5 and MHS7

Nutrient	MHO5	MHO7	MHS5	MHS7
Dry matter (%)	90.6	91.1	89.6	87.6
Ash (%)	9.5	9.9	8.8	9.3
Crude protein (%)	24.3	21.9	24.1	21.1
Neutral detergent fiber (%)	40.8	44.4	43.1	45.6
Acid detergent fiber (%)	27.7	32.1	28.0	33.4
Acid detergent lignin (%)	4.6	6.4	4.9	6.3

Table 2. Nutrient composition (%DM) of the basal diet fed to the canulated sheep

Nutrient	OPF	Mulberry hay
Dry matter (%)	98.4	96.4
Ash (%)	6.1	8.6
Crude protein (%)	4.5	20.4
Neutral detergent fiber (%)	82.3	47.2
Acid detergent fiber (%)	63.9	29.9
Acid detergent lignin (%)	14.2	6.9

MHO5, MHO7, MHS5 and MHS7) were analyzed by a completely randomized design using General Linear Models (GLM) procedure (SAS, 1997) and compared using Duncan's multiple range test (DMRT).

RESULTS AND DISCUSSION

Dry Matter Degradation

Table 3 shows the DM degradation of MHO5, MHO7, MHS5 and MHS7. The DM degradation was rapid with increasing time of incubation, particularly up to 12 h. The DM degradation was relatively constant after 12 h of incubation. At 0 and 6 h time of incubation DM loss was not significantly (P>0.05) different for all treatments.

The DM degradation of MHO5 and MHS5 was significantly (P<0.05) higher than MHO7 and MHS7 at 12, 24, 36 and 48 h of incubation. Meanwhile, DM degradation of Mulberry hay was not influenced by the method of drying.

Table 4 shows the degradation characteristics of DM of MHO5, MHO7, MHS5 and MHS7. The soluble fraction (a) was no significantly (P<0.05) different for all treatments. The highest degradation of water insoluble (b) value was obtained in MHS5 followed by MHO5, MHS7 and MHO7. The degradation rate (c) was not significantly (P>0.05) different for all treatments.

The highest potential degradability (PD) was recorded in MHO5 followed by MHS5, MHS7 and MHO7. The highest effective degradability (ED) was obtained in mulberry of 5 weeks stage of maturity i.e. MHO5 (65.9%) and MHS5 (65.8%). There were significant decreased (P<0.05) for "b", "PD" and "ED" values with advancing maturity but not significantly (P>0.05) different with the drying methods.

Crude Protein Degradation

Table 5. shows the CP degradation of MHO5, MHO7, MHS5 and MHS7. The CP degradation increased with increasing time of incubation particularly up to 12 h, after that it tended to plateau. The CP degradation for all samples was not significantly (P>0.05) different at 0, 6, 12, 24, 36 and 48 h of incubation.

The slow degradation was recorded after 24 h of incubation. The CP degradation was not significantly (P>0.05) different with advancing maturity and method of drying at all time of incubation.

Table 6 shows the degradation characteristics of CP of MHO5, MHO7, MHS5 and MHS7. The highest soluble fraction (a) was obtained for MHO5 followed by MHS5, MHS7 and MHO7. There were significantly decreased (P<0.05) with advancing stage of maturity at oven drying but not significant (P>0.05) different at sun drying. The highest degradation of water insoluble (b) obtained in MHO7 followed by MHS7, MHS5 and MHO5.

Table 3. Degradation of DM (%) of MHO5, MHO7, MHS5 and MHS7 incubated in sheep

Samples	Time of incubation (h)					
	0	6	12	24	36	48
MHO5	25.6 ± 1.4	45.7 ± 6.4	74.1 ± 6.2^{a}	83.1 ± 1.4^{a}	83.5 ± 1.0^{a}	83.5 ± 1.7^{a}
MHO7	23.4 ± 0.9	47.3 ± 5.6	68.0 ± 5.5^{b}	77.0 ± 2.4^{b}	78.0 ± 0.9^{b}	78.2 ± 1.8^{b}
MHS5	24.4 ± 2.4	46.1 ± 3.1	74.3 ± 6.7^{a}	82.8 ± 0.7^{a}	83.3 ± 1.3^{a}	83.3 ± 0.8^{a}
MHS7	25.2 ± 2.5	45.3 ± 7.7	67.5 ± 7.7^{b}	77.8 ± 1.8^{b}	78.5 ± 0.9^{b}	79.8 ± 6.2^{b}
SEM	1.1	1.3	0.8	0.5	0.5	1.6

Note: a,b means with the different superscript within rows differ significantly (P<0,05). SEM: Standard Error of Mean.

Table 4. Degradation characteristic of DM of MHO5, MHO7, MHS5 and MHS7

Degradation Characteristics	MHO5	MHO7	MHS5	MHS7	SEM
a (%)	23.6 ± 2.3	22.4 ± 0.8	22.5 ± 1.9	23.6 ± 1.1	1.1
b (%)	62.4 ± 2.9^{p}	57.3 ± 1.7^{q}	63.2 ± 1.7^{p}	57.6 ± 2.1^{q}	1.3
c (fraction/h)	0.11 ± 0.02	0.12 ± 0.03	0.11 ± 0.02	0.10 ± 0.03	0.003
a+b (%)	86.0 ± 1.0^{p}	79.7 ± 1.0^{q}	85.7 ± 0.7^{p}	81.2 ± 1.0^{q}	0.4
Effective degradability (%)	65.9 ± 3.0^{p}	62.1 ± 3.1^{q}	65.8 ± 2.3^{p}	62.2 ± 3.8^{q}	0.4

Note: p,q means with different superscripts within rows differ significantly (P<0.05);
SEM: Standard error of mean.

Table 5. Degradation of CP (%) of MHO5, MHO7, MHS5 and MHS7 incubated in sheep

Diets	Time of incubation (h)					
	0	6	12	24	36	48
MHO5	38.4 ± 1.2	56.9 ± 7.9	88.3 ± 7.0	95.7 ± 0.8	96.2 ± 0.3	96.4 ± 0.4
MHO7	24.3 ± 6.6	55.7 ± 6.0	86.1 ± 6.5	93.2 ± 1.3	94.7 ± 0.6	94.5 ± 0.4
MHS5	31.9 ± 3.3	56.6 ± 4.7	89.3 ± 6.0	96.0 ± 0.7	96.0 ± 0.5	96.8 ± 0.4
MHS7	27.3 ± 6.4	53.1 ± 5.0	84.5 ± 10.5	93.8 ± 1.4	94.4 ± 0.4	95.8 ± 1.2
SEM	3.3	2.8	1.31	0.2	0.2	0.3

Note: SEM = Standard error of mean.

Table 6. Degradation characteristic of CP of MHO5, MHO7, MHS5 and MHS7

Degradation Characteristics	MHO5	MHO7	MHS5	MHS7	SEM
a (%)	35.9 ± 1.9^{p}	22.8 ± 6.1^{q}	29.9 ± 2.7^{pq}	25.4 ± 6.9^{pq}	3.2
b (%)	63.2 ± 2.9	73.8 ± 5.5	68.8 ± 2.3	72.4 ± 6.4	4.3
c (fraction/h)	0.11 ± 0.03	0.13 ± 0.04	0.12 ± 0.02	0.11 ± 0.03	0.009
a+b (%)	99.1 ± 1.0^{p}	96.5 ± 0.6^{q}	98.8 ± 0.6^{p}	97.8 ± 0.7^{pq}	0.4
Effective degradability (%)	78.6 ± 3.6^{p}	75.3 ± 2.9^{q}	78.1 ± 2.5^{p}	74.9 ± 3.6^{q}	0.7

Note: p,q means with different superscripts within rows differ significantly (P<0.05);
SEM: Standard error of mean.

There was no significant (P>0.05) difference for "b" and "c" value with advancing maturity and drying method. The highest "PD" was obtained for foliage of five weeks stage of maturity compared with seven weeks stage of maturity. The "PD" value was significantly (P<0.05) decreased with advancing stage of maturity by oven drying, but not significantly (P>0.05) different by sun drying methods. The "ED" value was not significant (P<0.05) difference by drying method, but significant (P<0.05) different with advancing maturity.

This study showed that the DM and CP degradability of MHO5, MHO7, MHS5 and MHS7 were relatively constant after 12 h of rumen incubation. This is in agreement with Schmidek *et al*. (2002a) who obtained a high degradation rate within the first hours (6-12 h), followed by a stabilizing phase. The mean DM degradability of mulberry hay at five and seven weeks of maturity at 48 h of incubation was 83.4 and 79.0%, respectively. The degradability values were generally similar to the reported by Saddul (2005) with values of 83.9 and 81.9%, respectively, for fresh mulberry. The mean CP degradability of mulberry hay at five and seven weeks maturity were 96.7 and 95.2% that were also similar to the values of 96.3 and 96.2% as reported by Saddul (2005). Schmidek *et al.* (2002a) also found that the CP degradability of mulberry foliage at 48 h incubation was 96.8%. The result shows that the drying process did not influence the degradability of DM and CP of mulberry foliage.

The study showed that the plant maturity influenced the DM degradability. The DM degradability of MHO5 and MHS5 was higher than MHO7 and MHS7. This result is in agreement with findings of several researchers (Kawas *et al* (1990); Bal *et al* (2000); Akbar *et al*. (2002) and Kamalak *et al*. (2005) who found that DM degradability was significantly reduced with increasing maturity. Saddul *et al*. (2005) reported that the effect of plant maturity on degradation of mulberry might be associated with the corresponding increase in the structural fiber composition with advancing plant maturity. This may constitute a barrier for microbial attachment to the feed resulting in a decrease in degradability with advancing maturity. Increasing maturity of forage usually leads to higher cell wall content Akbar *et al*. (2002) and reduce CP and DM contents (Yu *et al*., 2003). When forage mature the leaf:stem ratio declines (Jung and Engels, 2002). Stem contain more cell walls material than leaves and degradability of cell walls by ruminant are lower than cell soluble components (Buxton and Brasche, 1991). Meanwhile, the CP degradability was not significantly different with advancing maturity. This result is in agreement with the finding of Balde *et al*. (1993) and Chaves *et al*. (2006).

The "b", "PD" and "ED" values of dry matter of MHO5 and MHS5 were higher than of MHS7 and MHO7, this may be due to the increase in fiber constituents with maturity (Akbar *et al*., 2002). Kamalak *et al*. (2005) also reported that the DM degradation and

degradation characteristic was significantly reduced with increasing maturity. The soluble (a) value of crude protein of MHO5 and MHS5 were higher than MHO7 and MHS7, and this indicate that the rapid degradation fraction of CP was not influenced by plant maturity. This result was consistent with the reports of Balde *et al* (1993); Kamalak *et al*. (2005) and Saddul (2005) who found a significant decline in the soluble a fraction of CP with advancing stage of maturity. The PD and ED of CP of mulberry hay were significantly ($P<0.05$) decreased with advancing stage of maturity. This result is similar with reported by Balde *et al*. (1993) who found that PD and ED of CP of alfalfa decreased with increasing maturity.

CONCLUSION

The DM degradability of mulberry hay was significantly decreased with advancing plant maturity from five weeks to seven weeks and the CP degradability was not influence by advancing plant maturity. Mulberry hay at five weeks old offers higher effective and potential degradability than seven weeks old. These suggest that mulberry hay at five weeks maturity more fermentable and large potential for feeding sheep.

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Production and Quality Of 15 Days Ages Of Corn Herbage as an Alternative Concentrate Ingredient for Young Calves Diet

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ABSTRACT

Feed takes 60% of production cost. Corn herbage can give high quality feed especially young forage because it contains high protein and it low lignification content. The objectives of this research are to get a methods of high quality forage production to increase cost feed efficiency, and to get an alternative concentrate for calves. This research is divided into three steps of experiment. The first experiment, analyzing production and nutrient corn herbage in 15 days ages; in the second, production of corn herbage and observe the effect of corn herbage on the calves; and third, analyzing forage production cost and compared it to concentrate cost. The Factorial Randomized Complete Design was selected for these experiments. The parameter including dry matter, nutrient contains, organic matter coefficient, palatability, and economic analyzis. The results showed that corn herbage production can did in small box. A tretment by soil medium and hydrophonic nutrient give dry matter production is 136 g DM/m^2, protein contained i.e: 18,30% and organic matter coefficient is 68%, fresh forage is very palatable and feed cost is Rp 858,-. It makes the corn herbage available to use as alternative concentrate.

Key words: forage, corn, calves, concetrate, alternative

INTRODUCTION

Forage is important ruminant feed, since it contains fibre that is very usefull for rumen health. Ruminant animals consume forage about 10% of body weight a day. Tropical forages are characterized by low quality. Fluctuation of biomass production is dominated by season. The peak of legume and grasses production are recorded in early dry season, and the lowest is in early rainy season (Hidayati, 2001). Low forage quality affects concentrate requirement by ruminant, particularly on dairy intensive farm. This is to maintain productivity and life spend of cows. Use of concentrate in dairy cattle diet leads to increase of ration cost. It is therefore use of high availability, such as 15 days corn herbage is one alternative to reduce diet cost.

Corn herbage can give high quality feed especially young forage because it contains high protein and it harvested before lignification. Corn production is fluctuative, it impacts on price. Corn get lower price when it gain higher production in rainy season since it high water contain. It be a problem for farmer to save the product. Young corn herbage is an alternative product to reduce farmer losses by cultivate corn, harvest on young age and save it for feed in dry season. As concentrate, corn will get a higher price. It can be concentrate alternative since it crude protein contains more than 16% and low fibre.

This research analyzed productivity 15 days age corn herbage and its nutrient contains to get the best feed formulation for young calves to gain cost efficiency, especially from feed. Aim of the research are to obtain appropriate method of corn herbage production in short time, has high quality, applicable for farmer to increasing efficiency of feed cost and to evaluate nutrition value of corn herbage as concentrate alternative

MATERIALS AND METHODS

This research was conducted at Animal Science Faculty, Bogor Agricultural Universityfrom May untill November 2008.

1. Corn Herbage Production

Local corn seeds were grown on soil and water media. In this stage observation on plant growth, biomass production, and optimum harvest time were conducted. This step was conducted at Laboratory of Agrostology. The herbage production then used to estimate the requirement of calves.

2. Nutrient Content of Trial Feeds

Nutrient content of corn herbage including: crude protein, crude fiber,

crude fat, calcium and phosphor content were analysed. Nutrient analysis of concentrate was also analysed before used.

3. **Corn herbage Production**
4. **In Vivo Test**

   In vivo test was done by involving 6 calves with 100 live weight kg. The calves were reared on metabolic stable. Two weeks before feces collecting, pre condition was conducted at the third week samples were collected. This research done at Ruminant Nutrition Laboratory

5. **Cost Analysis**

   Cost including feed production cost, processing cost, and transportation were calculated. The result can be a recomendation for farmer in order to increase efficiency of calves feed.

Data Analysis

This research use Factorial Completely Randomized Design consisting of two treatments:

1. Fertilizer, contained 3 level : without fertilizer (F0), phonska fertilizer (F1) and complete fertilizer (macro and micro) (F2)
2. Growing media, 2 level : soil (M1) and husk charcoal (M2)

The interaction of two factors result four combinations of treatments with three replicates.

RESULTS AND DISCUSSION

Corn Herbage Production And Nutrient Contents

Phonska fertilizer is a common fertilizer in used Indonesia, it can be purchased from agricultural shop. Complete fertilizer can be composed by farmer or bought. Fertilizer usage be diluted to efficiency.

Treatment of media showed a significant effect on the average value of the leaves lengths and crown dry matter. Corn herbage planted in the soil media with a complete nutrient (macro and micro) produced a higher average value of leave length than the husk charcoal medium. This is possible because the beginning of plant grown stunted in plant roots to absorb nutrients because of the influence of the media structure husk charcoal and soil itself has contain nutrient. Schwarz (1995) suggested that the plant will have a deficiency if the essential nutrients are not available in sufficient numbers or not in a form that can be absorbed by plants, and consequently the plants will not grow well and have the abnormal structure and colour.

Nutrient analysis of corn herbage result the best value on complete fertilizer and soil media. It contained crude protein i.e.: 18.24%. Crude fiber contained in these researches was not different significantly, but on complete fertilizer treatment showed NDF gained 65%. It means that crude fiber was digestible energy by animal (Jung, 1989). Therefore, forages containing high NDF can potentially be included in finishing diets at lower concentrations,

Table 1. Corn herbage Nutrient Analysis (15 days age)

Treatments	Parameters		
	Fat	Crude Protein %	Crude Fiber
Soil Media			
Without Fertilizer	3.70	11.90^{b}	21.59
Phonska Fertilizer	4.07	16.34^{a}	21.59
Complete Fertilizer	3.10	18.30^{A}	22.35
Husk Charcoal Media			
Without Fertilizer	2.98	8.41^{c}	22.78
Phonska Fertilizer	3.60	12.80^{b}	23.18
Complete Fertilizer	3.53	13.48^{b}	21.34

Table 2. Corn herbage Production Analysis (15 days age)

Treatments	Parameters		
	Leaves length (cm)	Crown dry matter (g)	Root dry matter (g)
Soil Media			
Without Fertilizer	43.8^{A}	10.0^{B}	11.1
Phonska Fertilizer	41.8^{B}	12.1^{B}	9.3
Complete Fertilizer	50.5^{A}	15.6^{A}	10.5
Husk Charcoal Media			
Without Fertilizer	40.0^{b}	9.8^{B}	12.1
Phonska Fertilizer	41.4^{B}	9.5^{B}	10.3
Complete Fertilizer	42.3^{B}	11.2^{B}	11.1

ultimately providing finishing diets with greater NE values while preventing digestive disorders (Peters, Montgomery, Bierman, 2009).

Vegetative phase of plant growth are closely related to three processes there are cell division, elongation and the first stage of differentiation (Harjadi, 1989). In this phase of nutrient absorption occurs more rapidly, so availability is absolutely necessary for optimal growth occurs. Nutrient availability of N, P and K is the element most absorbed in the vegetative phase, but with the addition of micro elements in a complete nutrient (macro and micro) can increase the protein content is very significant compared with other treatments.

In the noon observation, the corn herbage on husk charcoal medium looked stress especially in the first week after planting. The plants looked wilted, while the media remain fresh. Inhibition of nutrient absorption in the husk charcoal medium in the early phase of growth led to the slow-growing and small plant.

Effect on Animal

Fresh corn herbage has high palatability. In the palatability test for two days, the calves ate all the green corn, including the roots. This is because the roots of the corn herbage were still young. Feeding in the dry form caused decreased the palatability shown by the remained feed to the average of 40 g for 2 days as a part of the livestock adaptation (Morrison, 1986).

Forage digestibility is generally considered to be a very useful integrated measurement of forage quality. Dry matter and organic matter digestibility analysis by in vivo test showed that corn herbage fed give significant effect compared with elephant grasses (Table 3). Corn herbage feed decreased dry matter digestibility coefficient significantly compared elephant grasses due to dry matter of corn herbage very low (8% fresh matter).

Corn herbage increases organic matter digestibility coefficient (OMDC) significantly, gained 95% on 20% calves feed. It was because the crude fiber contained in corn herbage still not lignifined (Jung, 1989). On this research, corn herbage was fed as a part of introduction to forage because calves digestibility was very low (no more than 5 kg/day).

Table 3. Dry Matter and Organic Matter Digestibility Coefficient Analysis By In Vivo Test

Treatments	Parameter	
	DMDC (%)	OMDC (%)
Concentrate: Elephant Grasses= 80: 20	30.12[a]	83.39[c]
Concentrate: Elephant Grasses:Corn herbage= 80:10:10	22.04[b]	88.92[b]
Concentrate: Corn herbage = 80: 20	20.68[b]	95.47[a]

Economic Analysis

The cost for the first corn herbage production shown on table 4. Cost of fresh corn herbage production will reduce after second plantation. Cost production will decreased to be Rp 858,-/kg after third plantation. Price of concentrate in this research is Rp 2,223.9/kg.

Table 4. Cost Analysis of Corn herbage Production

Treatments	Cost (Rp/kg fresh matter)
Soil Media	
Without Fertilizer	1,380.1
Phonska Fertilizer	2,818.4
Complete Fertilizer	2,758.0
Husk Charcoal Media	
Without Fertilizer	1,312.8
Phonska Fertilizer	3,244.3
Complete Fertilizer	3,208.6

CONCLUSION

Corn herbage can be produced in 15 days by local seed, use simple batch or a square from mulch plastics as constraint of water and fertilizer for production efficiently. Corn herbage production (15 days age) by soil media and complete fertilizer contains crude protein 18.23% . Corn herbage has potency to improve as concentrate alternative and the ways of corn storage and as product variation for feed.

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A Study of Morphometric-Phenotipic Characteristic of Indonesian Chicken: Kampong, Sentul and Wareng-Tangerang, Based on Discriminant Analysis, Wald-Anderson Criteria and Mahalanobis Minimum Distance*

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ABSTRACT

An observation of linear body sizes was conducted in this study; this includes femur length (X_1), tibia length (X_2), shank length (X_3), shank circle or circumference (X_4), the third finger length (X_5), wing length (X_6), maxilla length (X_7), comb height (X_8) and sternum length (X_9). Grouping or classifying on the basis of morphometric characteristic between Indonesian native chicken: Kampong, Sentul and Wareng-Tangerang, is carried out using discriminant analysis, Wald-Anderson criteria and Mahalanobis minimum distance. Discriminant function equations for Kampong vs Wareng-Tangerang chicken, for males is $Y = 0.07X_1 + 0.29X_2 + 0.004X_3 + 0.38X_4 – 0.51X_5 + 0.04X_6 + 0.27X_7 + 0.27X_9$, and for females is $Y = 0.16X_1 + 0.28X_2 + 0.23X_3 + 0.67X_4 + 0.10X_5 + 0.03X_6 + 0.07X_7 – 0.06X_9$. Several numbers of female from Kampong and Wareng-Tangerang chicken is found not in the right group. In female group, data for Kampong chicken that is group as Wareng-Tangerang is 2.1%; on the other hand, data for Wareng-Tangerang chicken that is classified as Kampong chicken is 2.2%. The Mahalanobis minimum distances for male and female between Kampong and Wareng-Tangerang, respectively are 2.9925 and 2.9864. The greater distance for males indicates a non similarity of morphometric of males is greater than that of females. This means that actual separation for males is easier than that for females for both groups of chicken. The equation of discriminant function for Kampong vs Sentul chicken for males is $Y = 0.12X_1-0.05X_3+0.25X_5-0.12X_8$, and for females is $= 0.0005X_1 + 0.33X_2 + 0.64X_3 + 0.19X_5 – 0.09X_6 – 0.86X_7 – 0.10X_8 - 0.11X_9$. Calculation result at actual group shows that numbers of male and female from Sentul and Kampong chicken are found not to be in the right group. For male group, the data for Sentul chicken that is grouped as Kampong chicken is 4.3%, and for Kampong chicken that is grouped as Sentul chicken is 2.2%. For female group, the data for Sentul chicken that is grouped as Kampong chicken is 11.1%, and for Kampong chicken that is group as Sentul chicken is 5.2%. It is much more difficult to determine the males because of the numbers of determinant variables are smaller. The Mahalanobis minimum distances for males and females of Kampong and Sentul chicken are, respectively, 1.2801 and 1.6900. A non-similarity of morphometric for Kampong vs Sentul indicates that the size of females are greater than that of males. Therefore, actual separation is easier to be done at female group. For Wareng-Tangerang vs Sentul chicken, the discriminant function equation for males is $Y = -0.18X_1 -0.03X_2 + 1.20X_3 + 1.09X_4 + 0.20X_5 -0.36X_6 + 0.06X_7 – 0.54X_8 + 0.11X_9$, and for females is $Y = 0.02X_1 + 0.32X_2 + 0.93X_3 + 1.30X_4 + 0.69X_5 – 0.10X_6 – 0.22X_7 – 0.81X_8 – 0.29X_9$. There is only the male that is found not in the right group. Separation among males is more difficult than separation among females although all of variable observed are determinant variables for both males and females. For males, data for Wareng-Tangerang chicken which is classified as Sentul chicken is 4.3% and for Sentul chicken which is grouped as Wareng-Tangerang is not found. The Mahalanobis minimum distance for males and females between Wareng-Tangerang and Sentul chicken is, respectively, 2,9925 and 2,9864. A greater distance for males demonstrates a non-similarity of morphometric for males that is greater than for females. Actual separation for males should be easier than for females; however, a reverse situation is observed in this experiment. This is because of a similarity in feather colour of Wareng-Tangerang chicken to that of Sentul chicken. In conclusion, the highest distance of a non-similarity of morphometric is caused by the largest numbers of determinant variables; this has increased the accuracy of grouping separation. Wareng-Tangerang chicken is different from

Kampong and Sentul chicken. A similarity of morfometry between Kampong and Sentul chicken is closer than that with Wareng-Tangerang. Kampong and Sentul chicken are Indonesian native chicken; however, the Wareng-Tangerang chicken is originated from abroad that have adapted with Indonesian condition.

Key words: discriminant analysis, Wald-Anderson criteria, mahalanobis minimum distance, Kampong chicken, Sentul chicken, Wareng-Tangerang chicken

INTRODUCTION

Kampong and Sentul chicken are local chicken producing eggs and meat (dual-purpose) (Nataamijaya, 2000). Wareng-Tangerang chicken is claimed from Tangerang district having performance as fairly productive laying chicken; it is originally from abroad (Iskandar *et al*., 2004[b]). Kampong, Sentul, Wareng-Tangerang chicken have long been adapted and domesticated in Indonesia. They are included in the 31 clumps of local chicken in Indonesia (Nataamijaya, 2000). This pattern has lead to the group formation of specific and typical species of local chickens.

Variables of body skeleton sizes are sufficiently accurate to be used as distinguishing variables or markers that can give overviews of specifications of local chickens that are heterogeneous. The purpose of this study was to compare the distinguishing variables among Kampong, Sentul and Wareng-Tangerang chicken based on discriminant function. Phenotypic characteristics of body skeleton sizes in each group of chickens can be used to determine the groupings of individuals that are not matched with its actual groups based on Wald-Anderson grouping criteria, and to estimate genetic distance of morphometric non-similarity based on D^2 Mahalanobis minimum distance criteria. The results, then, can be used as one tool or selection criteria in policy making in the observed breeding chicken.

MATERIALS AND METHODS

Locations and Sampling Determination

The experiment was conducted at the Livestock Research Center (Balai Penelitian Ternak) Ciawi-Bogor for the measurement of Kampong and Wareng-Tangerang chicken, and at people farms in Cigembor and Ciulu villages, Ciamis district for the measurement of Ciamis-Sentul chicken. The study was conducted from August to September 2006. The determination of locations was conducted by purposive sampling in which the location for sampling is done on purpose based on the existence of Kampong, Wareng-Tangerang and Sentul chicken.

Materials

The observed chicken were 125 Kampong chicken (28 males and 97 females), 110 Wareng-Tangerang chicken (20 males and 90 females) and 50 Sentul chicken (23 males and 27 females). All of the observed chickens are foundation stocks that are developed through selection by the Livestock Research Center in Ciawi Bogor. All of the observed chickens have reached matured conditions.

Variables

The variables measured are femur length (X_1), length of the tibia (X_2), tarsometatarsus length (X_3), circumference tarsometatarsus (X_4), third finger length (X_5), length of wing (X_6), length of maxilla (X_7), comb hight (X_8) and the length of the sternum (X_9).

Procedures

All variables were measured in the body of Kampong, Wareng-Tangerang and Sentul.

Data Analysis

T^2-Hotteling Test

The average vector value of the two groups of chicken in each sex was tested to determine whether there were statistical differences between the average values of the tested traits (Gaspersz, 1992). Testing was done by formulating the following hypotheses:

$H_0 : U_1 = U_2$ meaning that the average vector value from the first population is equal to the second population if

$$T^2 \leq \frac{(n_1 + n_2 - 2)p}{n_1 + n_2 - p - 1} F_{\alpha;v_1,v_2}$$

$H_1 : U_1 \neq U_2$ meaning that the average vector value from the two populations are different if

$$T^2 \succ \frac{(n_1+n_2-2)p}{n_1+n_2-p-1} F_{\alpha;v_1,v_2}$$

Then the values

$$F = \frac{n_1+n_2-p-1}{(n_1+n_2-2)p} T^2$$

distribute following F values with the degree of freedom is $V_1 = p$ dan $V_2 = n_1 + n_2 - p - 1$ in which :

T^2 = T^2-Hotteling statistic value
F = calculated value for T^2-Hotteling
n_1 = amount of observed data in the first group
n_2 = amount of observed data in the second group
$\underline{\bar{x}}_1$ = the average vector value of the random variables of the first group
$\underline{\bar{x}}_2$ = the average vector value of the random variables of the second group
S_G^{-1} = pooled covariance matrix inverse (inverse of the matrix SG)
p = number of variables measured

If the results of testing the hypotheses are rejected, this shows both average values of the observed traits are different; so that the discriminant function is used to assess differences in the traits that are found among the chicken groups.

Grouping Based on Fisher Linear Discriminant Function

Fisher linear discriminant function according to Gaspersz (1992) is defined as follows:

$$Y = \underline{a}' \underline{X} = \left(\underline{\bar{x}}_1 - \underline{\bar{x}}_2 \right)' S_G^{-1} \underline{X}$$

Description :

$\underline{a}$ = loading vector coefficient for discriminant function
$\underline{X}$ = random variables vector identified in the discriminant function model
$\underline{\bar{x}}_1$ = the average vector value of the random variables of the first group
$\underline{\bar{x}}_2$ = the average vector value of the random variables of the second group
S_G^{-1} = pooled covariance matrix inverse (inverse of the matrix SG)
S_G = pooled covariance matrix

Classification Based on Wald-Anderson Statistical Test

Wald-Anderson statistical test can be used to classify individuals from the observed chicken group which is defined by Gaspersz (1992) as follows:

$$W = x' S_G^{-1} \left(\underline{\bar{x}}_1 - \underline{\bar{x}}_2 \right) - 1/2 \left(\underline{\bar{x}}_1 + \underline{\bar{x}}_2 \right)' S_G^{-1} \left(\underline{\bar{x}}_1 - \underline{\bar{x}}_2 \right)$$

Description:
W = Wald-Anderson statistical test value
$\underline{x}'$ = vector of individual random variables
$\underline{\bar{x}}_1$ = the average vector value of the random variables of the first group
$\underline{\bar{x}}_2$ = the average vector value of the random variables of the second group
S_G^{-1} = pooled covariance matrix inverse (inverse of the matrix SG)

Classification criteria based on the W statistics is:
1) allocation of x to the first group (population), if: $W > 0$
2) allocation of x to the second group (population), if: $W \leq 0$

Classification Based on the D^2-Mahalanobis Minimum Distance

D2- Mahalanobis minimum distance between any two chicken groups in each sex is calculated based on the quantitative characteristics of the body skeleton which is formed by discriminant function.

The minimum genetic square distance according to Gaspersz (1992) is defined as follows:

$$D^2_{(1/2)} = \left(\underline{\bar{x}}_1 - \underline{\bar{x}}_2 \right)' S_G^{-1} \left(\underline{\bar{x}}_1 - \underline{\bar{x}}_2 \right)$$

$D^2_{(1/2)}$ = D2- Mahalanobis minimum distance as a measurement of squared distance between the two chicken groups (between the first and the second group)
S_G^{-1} = pooled covariance matrix inverse (inverse of the matrix SG)
$\underline{\bar{x}}_1$ = the average vector value of the random variables of the first group

$\overline{x_2}$ = the average vector value of the random variable of the second group

RESULTS AND DISCUSSION

Classification of Male and Female in Kampong vs Sentul Chicken, Kampong vs Wareng-Tangerang Chicken, and Wareng-Tangerang vs Sentul Chicken

The results of T^2 Hotteling test show there are differences in vector average values of linear body size variables between the two groups of chicken that are observed ($P<0.05$). These are between Kampong and Wareng-Tangerang chicken; Kampong and Sentul chicken, and Wareng-Tangerang and Sentul chicken. The differences between Kampong, Wareng-Tangerang and Sentul chicken in its characteristic of linear body size variables at the adult aged are the results of factors such as genetic, gene mutation and adaptation (Iskandar *et al*., 2004[a]; Iskandar *et al*., 2004[b]). Herren (2000) explained that bone and muscle tissue grew on a regular basis during growth period, rapid growth occurred from birth up to adult matured body have been reached. Then, bone and muscle growth stopped and continued with the development of fat. Thus, the linear body size variables can be related with body weight in this study.

Among the chicken types, the largest linear measures of body size variables are found in Sentul chicken, which is followed by Kampong and Wareng-Tangerang chicken. This is consistent with the previous results obtained by Iskandar *et al*. (2004[b]) and Iskandar *et al*. (2006). Male and female adult body weights, respectively, were 2,500 g and 1,850 g for Sentul chicken, 1,815±353 g and 1,382±290 g for Kampong chicken, and 1,000 g and 841 g for Wareng-Tangerang chicken (Iskandar *et al.*, 2004[b]; Iskandar *et al*., 2006).

Significant differences in the vector average of linear body size variables are obtained in this study based on statistical tests T^2 Hotteling ($P<0.05$) between the two types of chickens, Kampong, Wareng-Tangerang and Sentul chiken. Thus, the discriminant function can be used to assess differences in morphometric characteristics between these two types of chicken.

Kampong vs Wareng-Tangerang Chicken

For the males between Kampong vs Wareng-Tangerang chicken, classification based on discriminant function showes that comb height (X_8) between the two types of male chickens is not different.

Table 1. Average, Standard Deviation and Coefficient Variation of Body Size Observed in Kampong and Wareng-Tangerang Chicken

Body size variables	Kampong Chicken (n=125)			Wareng-Tangerang Chicken (n=110)		
	♂(n=28)	♀(n=97)	♂+♀	♂(n=20)	♀(n=90)	♂+♀
			(mm)			
Femur Length	102.29 ± 6.45 (6.31)	83.48±3.79 (4.54)	87.69 ± 9.06 (10.33)	84.05±4.46 (5.31)	71.19±5.06 (7.12)	73.52±7.01 (9.53)
Tibia Length	152.95±10.24 (6.69)	123.14±5.92 (4.81)	129.82±14.34 (11.05)	120.90±5.17 (4.28)	103.33 ± 6.82 (6.60)	106.53±9.43 (8.85)
Shank Length	110.04±9.11 (8.28)	85.81±4.52 (5.27)	91.24±11.69 (12.81)	86.96±2.81 (3.23)	71.31 ± 3.96 (5.55)	74.15±7.14 (9.63)
Shank Circumference	53.29±7.44 (13.96)	39.64±3.02 (7.62)	42.70±7.20 (16.86)	35.47±2.29 (6.46)	31.67±2.38 (7.51)	32.36±2.78 (8.59)
Third finger Length	64.27±5.93 (9.23)	52.64±5.16 (9.80)	55.25±7.21 (13.05)	52.77±2.40 (4.55)	41.08±3.33 (8.12)	43.21±5.53 (12.79)
Wing Length	234.79±15.10 (6.43)	192.14±11.61 (6.04)	201.70±21.74 (10.78)	188.95±9.78 (5.18)	159.27±11.99 (7.53)	164.66±16.32 (9.91)
Maxilla Length	35.99±3.65 (10.14)	31.70±1.86 (5.87)	32.66±2.97 (9.09)	30.77±1.48 (4.81)	28.19±1.64 (5.82)	28.66±1.89 (6.59)
Comb Height	49.45±19.40 (39.23)	16.84±10.09 (59.92)	24.14±18.63 (77.17)	35.02±6.64 (18.96)	20.07±4.78 (23.82)	22.79±7.74 (33.96)
Sternum Length	130.76±10.31 (7.88)	105.24±8.08 (7.68)	110.96±13.71 (12.36)	103.18±6.53 (6.33)	88.49±7.08 (8.00)	91.16±8.99 (9.86)

Note: ♂= male, ♀= female, n = number of samples; numbers in parentheses express the coefficient of variability in percent (%)

This is because the value of the correlation coefficient of discriminant function is not significant at 95% of confidence interval. Thus the discriminant function formed for males between these two types of chicken352is $Y = 0.07 X_1 + 0.29 X_2 + 0.004 X_3 + 0.38 X_4 - 0.51 X_5 + 0.04 X_6 + 0.27 X_7 + 0.27 X_9$. The Variables that distinguish males between Kampong vs Wareng-Tangerang chicken are femur length, tibia length, shank length, third finger length, and wing length, length of maxilla and length of the sternum.

Classification of males for both type of chicken based on Wald-Anderson criteria shows none the male data deviates from the discriminant function (Table 2). This classification also indicates that high similarity in comb height of the males between Kampong and Wareng-Tangerang chicken did not cause errors in the separation between these two types of chicken.

Discriminant function which is formed in the females of Kampong vs Wareng-Tangerang chicken shows the same trend as the males. All the observed variables, except comb height (X_8), are distinguishing factors. Discriminant function equation that is formed between these chicken females is : $Y = 0.16 X_1 + 0.28 X_2 + 0.23 X_3 + 0.67 X_4 + 0.10 X_5 + 0.03 X_6 + 0.07 X_7 - 0.06 X_9$.

The results of Wald-Anderson criteria show that corrected data of females both in Kampong vs Wareng-Tangerang chicken are 97.9% based on distinguishing variables in discriminant function (Table 3). Corrected data for females of Wareng-Tangerang chicken classified as a group of female of Kampong chicken are 97.9%, and for females of Kampong chicken classified as a group of female of Wareng-Tangerang are 97.8%. These are because two females of Kampong chicken are included in female group of Wareng-Tangerang chicken, and two females Wareng-Tangerang chicken are classified into the female group of Kampong chicken. Corrected data of females of Kampong chicken which are classified as females of Wareng-Tangerang chicken is 2.1% (100% - 97.9%). The corrected data of females of Wareng-Tangerang chicken is 2.2% (100% - 97.8%) and classified as females of Kampong chicken. Based on calculation of actual group, some females of Kampong chicken and Wareng-Tangerang are found in groups that are not appropriate although eight variable sizes between these two groups are different. This indicates that the females are more difficult to be distinguished in comparison to the males in Kampong and Wareng-Tangerang chicken.

Mahalanobis minimum distance in males and females between Kampong and Wareng-Tangerang chicken, respectively, are 2.9925 and 2.9864. A greater distance in males shows that morphometrics dissimilarity in males among Kampong chicken vs

Table 2. Classification of Male Data of Kampong vs Wareng-Tangerang Chicken Based on Wald-Anderson Criterion

Actual Group	Classification		% Correction
	Kampong Chicken	Wareng-Tangerang Chicken	
Kampong chicken (n=28)	28	0	28/28 x 100% = 100.0%
Wareng-Tangerang chicken (n=20)	0	20	20/20 x 100% = 100.0%
Total (n=48)	28	20	48-(0+0)/48 x 100% = 100.0%

Note: n = number of samples.

Table 3. Classification of Females in Kampong vs Wareng-Tangerang Chicken Based on Wald-Anderson Criterion

Actual Group	Classification		% Correction
	Kampong Chicken	Wareng-Tangerang Chicken	
Kampong Chicken (n=97)	95	2	95/97 x 100% = 97.9%
Wareng-Tangerang Chicken (n=90)	2	88	88/90 x 100% = 97.8%
Total (n=187)	97	90	187-(2+2)/187 x 100% = 97.9%

Note: n = number of samples.

Wareng-Tangerang is larger than the females. This means that the actual separation is more easily done on the males than that on the females in these two types of chicken. Figure 1 and 2 present dendograms of the Kampong, Sentul and Wareng-Tangerang chicken in males and females.

Eight variables are used as body size variables that distinguish male and female groups in Kampong vs Wareng-Tangerang chicken. This indicates morphometric dissimilarity between these two chicken groups. Figure 2 shows that dissimilarity in males is larger than in females, so males between the two types of chickens are easily distinguished. Nishida *et al*. (1982) state that the length of wing and long bones (femur, tibia, shank or tars metatarsus) were effective to distinguish the body conformation of chicken. Campbell and Lack (1985) state that the origin, domestication process, selection and crossing, may influence phenotypic variation in body morphology of birds.

Morphometric dissimilarity distance found between the two types of chicken shows that the two types of chicken are from different breeds. Nataamijaya (2000) states that the kampong chicken was a local chicken natively to Indonesia, while Iskandar (2004[b]) states that the Wareng-Tangerang chicken came from abroad, namely from Russia. Genetically, these two chicken types are different but they can adapt well to the Indonesian environment.

Differences in selection goals result in morphometric phenotypic differences shown by the eight variables of differences. Sartika (2000) states that the diversity of phenotypic characteristics (productivity performance, egg quality, size and height comb) in Kampong chicken is still high in the basic population for selection purposes. Indirect selection at Kampong chicken for the purpose of dual-purpose also occurred among farmers or communities. Susanti *et al*. (2006) state that the Wareng-Tangerang chicken was the result of selection and crosses. This chicken has small body posture (posture Wareng) as laying chickens. Selection for egg production in Wareng-Tangerang chicken is followed by indirect selection on the linear body size. The result of this study shows that comb height is not distinguishing variables. This indicates that selection towards the establishment of Kampong and Wareng-Tangerang chicken does not consider the comb height although the Wareng-Tangerang chicken combs are uniform (single comb).

Kampong vs Sentul Chicken

On the basis of discriminant function classification, morphometric similarity is high for males of Kampong vs Sentul chicken. This is because of there are four distinguish variables from nine variables of linear body size variables. These are femur length, shank length, third finger length (X_5) and high cockscomb (X_8). These four variables are highly correlated to the discriminant function equation at 95% confidence interval. Discriminant function equation formed between the two types of chicken males is:

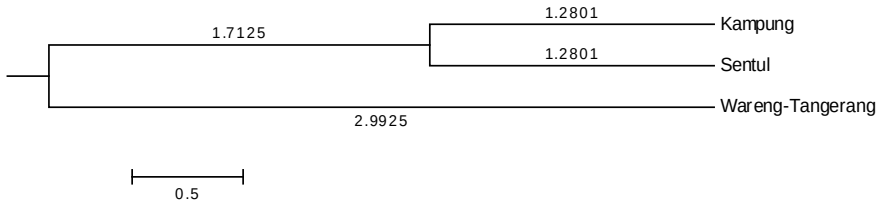

Figure 1. Dendogram of Linear Body Measurement Dissimilarity in Male of Kampong, Sentul and Wareng-Tangerang Chicken

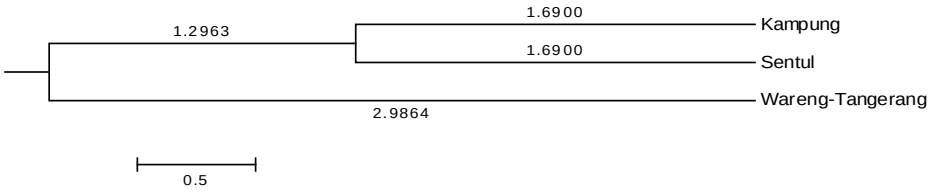

Figure 2. Dendogram of Linear Body Measures Dissimilarity in Female of Kampong, Sentul and Wareng-Tangerang Chicken

$Y = 0.12\ X_1\text{-}0.05X_3 + 0.25\ X_5\text{-}0.12X_8$. Table 4 presents the data classification of individuals Kampong vs Sentul chicken on the basis of Wald-Anderson criteria. Kampong chickens are 85.7%. This is because one male Sentul chicken is found in the male. The corrected male individuals data in the two groups are 90.2% based on the distinguishing variable in the discriminant function. Male individuals which are classified as group of Sentul chickens that have been corrected are found to be 95.7% and as a group of Kampong chickens are 85.7%. This is because one male Sentul chicken is found in the male Kampong chicken group based on Wald-Anderson classification, and four males of Kampong chicken are grouped in Sentul male chicken group. The corrected data of male Sentul chicken which are classified as male Kampong chicken are 4.3% (100% -95.7%). The 14.3% (100% -85.7%) data is the corrected data of male of Kampong chicken which is classified as male chicken Sentul.

Discriminant function that is formed in the female Sentul vs Kampong chicken shows that all variables of linear body size can be used as distinguishing variables, except for the shank circumference. These variables are the lengths of the femur, tibia, shank, third finger, wing, maxilla, and sternum, and comb height. These variables are significant ($P<0.05$) at 95% of confidence interval and has a strong correlation to the discriminant function scores.

Equation of discriminant function formed between the two types of chicken females is Y = 0.0005 X1 + 0.33 X2 + 0.64 X3 + 0.19 X5 - 0.09 X6 - 0.86 X7 - 0.10 X8 - 0.11 X9. Table 5 shows that the corrected data of individuals in both groups of females (Sentul vs. Kampong chicken), based on Wald-Anderson criteria is 93.5%. Those that are classified as a group of females of Sentul chicken that has been corrected is 88.9% and as a group of females of Kampong chicken is 94.8%. This is because of three females of Sentul chicken are included in the group of female of Kampong chicken, and five females of Kampong chicken are included in the Sentul female chicken based on Wald-Anderson classification. Female individual data of Sentul chicken corrected and classified as females Kampong chicken are 11.1% (100% -88.9%). The 5.2% (100% -94.8%) is the data for female individuals of Kampong chicken that are corrected and classified as female individual data of Sentul chicken.

Based on the calculation of actual group, several females and males from both in Sentul and Kampong chicken are found in groups that are not appropriate. Males are more difficult to be distinguished because they have less numbers of distinguishing variables.

Mahalanobis minimum distance in males and females between Kampong and Sentul chickens is 1.2801 and 1.6900 (Figures 1 and 2). Greater distances in females show that morphometric dissimilarity among females is larger than males in Kampong vs Sentul chicken. The actual separation is more easily done on the female group than on the

Table 4. Classification of Individuals Data in Males at Kampong vs Sentul Chicken Based on Wald-Anderson Criterion

Actual Group	Classification		% Correction
	Sentul chicken	Kampong chicken	
Sentul Chicken (n=23)	22	1	22/23 x 100% = 95.7%
Kampong Chicken (n=28)	4	24	24/28 x 100% = 85.7%
Total (n=51)	26	25	51-(4+1)/51 x 100% = 90.2%

Note: n = number of samples.

Table 5. Classification Group of Individual Data in Female of Sentul vs Kampong Chicken Based on Wald-Anderson Criterion

Actual Group	Classification		% Correction
	Sentul chicken	Kampong chicken	
Sentul Chicken (n=27)	24	3	24/27 x 100% = 88.9%
Kampong Chicken (n=97)	5	92	92/97 x 100% = 94.8%
Total (n=24)	29	95	124-(5+3)/124 x 100% = 93.5%

Note: n = number of samples.

male group and females is a result of differences in response to genetic variation, environmental, and genetic-environment interactions that relate to differences in selection goals and crossing (linebreed) in each type of chicken.

Mahalanobis minimum distance obtained in the present observations in males and females between Kampong vs Sentul chicken is much closer than between Kampong vs Wareng-Tangerang chicken. Sartika *et al*. (1997) and Sartika, *et al*. (2004) stated that Kampong and Sentul chicken had a close genetic distance because they come from the same family or ancestor based on observations of microsatellite DNA marker at locus Abr 359, Abr 297, Abr 339, Abr and 28 based on REML (Restricted Maximum Likehood Estimation) method. Similarity of the origin of the two groups were actualized in the discriminant function that is formed, especially for the males group that has only four distinguishing variables, of the nine observed variables. Differences in distinguishing variables in males and females of Sentul or Kampong chicken are due to differences in tightness of selection. Selection of males group is allegedly not as tight as the female group; it has been shown that distinguishing variables in the male group are less than those in female group.

Shank circumference is not a distinguishing variable in both in males and females of Kampong vs Sentul chicken. This is because of the purpose of selection in both types of chickens is directed toward the dual-purpose chicken with medium body size. Shank circumference has a role in supporting the body of a chicken. Indonesian territory demographic factors may be the possible causes of phenotypic differences and similarities of linear body measurement variables of these two types of chicken. Based on the origin of the chicken used in the observation, Kampong chicken comes from different locations in West Java i.e. the area of maintenance of Balitnak, Bogor, Depok, Garut and Jatiwangi (Sartika, 2000). Kampong chicken has a high phenotypic variation compared to that of Sentul chicken, and are from Ciamis, West Java (Table 1).

Wareng-Tangerang vs Sentul Chicken

The classification based on discriminant function indicates that high differences in males of Wareng-Tangerang vs Sentul chicken are because of all the observed variables are distinguishing variables. These are the length of the femur, tibia, shank, third finger, wing, maxilla, and sternum, shank circumfrerence, and comb height. All the variables are highly correlated to the discriminant function equation which is established through testing simultaneous confidence interval at 95%. Discriminant function equation which is formed between the two groups of male chickens is : $Y = -0.18\ X_1 - 0.03\ X_2 + 1.20\ X_3 + 1.09\ X_4 + 0.20\ X_5 - 0.36\ X_6 + 0.06\ X_7 - 0.54\ X_8 + 0.11X_9$. The results (Table 6) show that the corrected data of females in Wareng-Tangerang vs Sentul is 97.7%% based on the distinguishing variables in the discriminant function. Individuals who are classified as male group of Sentul chicken that have been corrected is 95.7% and as a male group of Wareng-Tangerang is appropriately corrected for 100%. One male Sentul chicken based on Wald-Anderson classification is included in the males of Wareng -Tangerang chicken.

The corrected data for males of Wareng-Tangerang chicken and classified as males of Sentul chicken is 4.3% (100% - 95.7%). The corrected data for male of Wareng-Tangerang chicken is 100%. A male of Wareng-Tangerang chicken is found in the male

Table 6. Classification of Individual Data on Male Group of Wareng-Tangerang vs Sentul Chicken Based on Wald-Anderson Criterion

Actual Group	Classification		% Correction
	Sentul chicken	Wareng-Tangerang Chicken	
Sentul Chicken (n=23)	22	1	22/23 x 100% = 95.7%
Wareng-Tangerang Chicken (n=20)	0	20	20/20 x 100% = 100.0%
Total (n=43)	22	21	43-(1+0)/43 x 100% = 97.7%

Description: n = number of samples.

of Sentul chicken. None of male of Sentul chicken is found in the males of Wareng-Tangerang chicken.

Discriminant function which is formed in the females group in Wareng-Tangerang vs Sentul chicken shows that all variables of linear body size are found to be distinguishing variables, as it occurred in the male group. Distinguishing variables of the females in Sentul vs Wareng-Tangerang chicken are femur length, tibia length, shank length, shank circumference, third finger length, wing length, maxilla length, comb height and length of the sternum. These variables are significant ($P<0.05$) in the simultaneous confidence interval at 95% and has a strong correlation to the discriminant function scores. Discriminant function equation that is established between these two types of female chicken is : $Y = 0.02\ X_1 + 0.32\ X_2 + 0.93\ X_3 + 1.30\ X_4 + 0.69\ X_5 - 0.10\ X_6 - 0.22\ X_7 - 0.81\ X_8 - 0.29\ X_9$. The results show that the data of individuals in both groups of females that have been corrected is 100% based on the discriminant function (Table 7). Females who are classified as a group of Sentul chicken that has been corrected is found to be 100% and as a group of Wareng-Tangerang is 100%. None of female of Sentul chicken is grouped into females of Wareng-Tangerang chicken, and vice versa.

The separation between the groups of Wareng-Tangerang and Sentul chicken in actual groups which is not appropriate, is only found in the males. Males are more difficult to be separated than females although all the observed variables are distinguishing variables in both sexes.

Mahalanobis minimum distance in males and females between Wareng-Tangerang and Sentul chicken is 2.9925 and 2.9864 (Figures 1 and 2). A greater distance in males showed that morphometric disimilarity among males in Sentul vs Wareng-Tangerang is larger than the females. The actual separation should be more easily done on the male group, but an opposite situation occur in this study. The discriminant function which is formed in males and females shows the same number of distinguishing variables.

Morphometric differences in linear body size of Sentul and Wareng-Tangerang chicken are indicated by the size of the body of Wareng-Tangerang chicken which is much smaller than Sentul chicken (Table 1). Discriminant function that is established shows all linear body size variables are distinguishing variables between these two groups of chicken, both in males and females. Differences of origin, selection destination and the difference in genetic variation, environmental and interaction between them, are the cause of these differences. According to Iskandar (2004[b]), Sentul chicken were the Indonesian native chicken, and Wareng-Tangerang chicken came from Russia having high adaptation through domestication and selection since the 1980s. Differences of the origin distinguish the phenotypic response based on the potential for additive genes controlling the linear body size of each type of chicken to the nature of growth, development and osteogenesis body frame. Selection to the superior layer type (mild type) in the Wareng-Tangerang chicken shown by linear size variables of the observation body is the smallest compared to the other two types of chicken and is the most uniform relative to the male group (Table 1). However, this is not the case in the female group. In Wareg chicken, selection toward the superior layer, also affect indirectly the linear measures of body variables. Susanti *et al*. (2006) states that the Wareng-Tangerang chicken is categorized as mild type of chicken having potential as productive layer type.

Table 7. Classification of Data Group of Females at Sentul vs Wareng-Tangerang Chicken Based on Wald-Anderson Criterion

Actual Group	Classification		% Correction
	Sentul chicken	Wareng-Tangerang Chicken	
Sentul Chicken (n=27)	27	0	27/27 x 100% = 100.0%
Wareng-Tangerang Chicken (n=90)	0	90	90/90 x 100% = 100.0%
Total (n=117)	27	90	117-(0+0)/117 x 100% = 100.0%

Description: n = number of samples.

Sentul chicken is selected as dual-purpose type. Sentul is laying chicken that have a large body size. North and Bell (1990) stated that one of the functions of bone-sustaining linear variable measures are body muscle attachment. This condition is found in Sentul as broiler chicken type. Sartika (2000) states that the correlation between the length of tarsometatarsus, tibia length and femur length with body weight is positive, with the highest closeness in the relationship between shank length and tibia length.

Differences of selection goals and crossing of the breeding program policies on Sentul and Wareng-Tangerang chicken are suspected to be factors causing differences in distinguishing variables from the discriminant function that is formed besides the differences of the origin. Susanti *et al.* (2006) stated that the Wareng-Tangerang was categorized as a lightly type of chicken with potential as productive layer type chickens, whereas Sentul chicken were selected for production purposes that was more directed as a dual-purpose type chickens (Iskandar, 2004[b]). This chicken has a greater skeleton size than Wareng-Tangerang chicken. Sentul chicken was the Indonesian native chickens, while Wareng-Tangerang chicken came from Russia which had undergone domestication and selection since the 80s (Iskandar (2004[b]).

Table 8 presents the minimum distance matrix of D^2-Mahalanobis between the two types of chickens that are observed in males. The highest minimum distance is found between Sentul and Wareng-Tangerang chicken in the males; and the lowest is found between the Kampong and Sentul chicken. The same phenomenon is also found in the female group in Table 9.

The dendogram constructed based on the minimum distance matrix D^2-Mahalanobis in males and females of Kampong, Wareng-Tangerang and Sentul chicken can be seen in Figures 1 and 2. Grouping between Kampong and Sentul chicken indicate that these two types of chicken have high similarity based on measures of body size variables that are observed. Separate grouping of Wareng-Tangerang chicken indicates that this type of chicken has different body size variables that are higher than those in other two types of chicken.

CONCLUSION

Eight distinguishing variables are found, both in males and females based on discriminant function in the Kampong vs Wareng-Tangerang chicken. Four distinguishing variables are found in males and eight distinguishing variables are found in females in in the Kampong vs Sentul chicken. Nine distinguishing variables are found both in males and females in the Wareng-Tangerang vs Sentul chicken.

The classification based on the actual group shows that the chicken are found in the wrong group. In females, some of the data of Kampong chicken are found in Kampong vs Wareng-Tangerang chicken and vice versa. Some of the data of Sentul chicken are found in Kampong chicken and vice versa both in males and females. Some of the data of Wareng-Tangerang chicken that are found in Sentul chicken are only found in males. Grouping between Kampong and Sentul chicken indicate that these two types of chicken have high similarity based on measures of body size variables that are observed. Separate grouping of Wareng-Tangerang chicken indicates that this type of chicken has

Table 8. Minimum Distance Matrix of D^2-Mahalanobis in Males between Kampong, Wareng-Tangerang and Sentul Chicken Based on Body Size Variable

Types of Chicken	Kampong	Wareng-Tangerang	Sentul
Kampong	0.0000		
Wareng-Tangerang	22.5180	0.0000	
Sentul	6.5544	52.1980	0.0000

Table 9. Minimum Distance Matrix D^2 of Mahalanobis in Females between Kampong, Wareng-Tangerang and Sentul Chicken Based on Body Size Variable

Types of Chicken	Kampong	Wareng-Tangerang	Sentul
Kampong	0.0000		
Wareng-Tangerang	17.7196	0.0000	
Sentul	11.4249	59.8460	0.0000

different body size variables that are higher than those in other two types of chicken.

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Carcass and Beef Characteristic from Brahman Cross Steers Fattened in Feedlot Prepared for Traditional Market

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ABSTRACT

Beef cattle feedlot is a fast growing industry in Indonesia. The industry supplies beef for particular market (hotel, restaurant and institution) as well as traditional market. This study was aimed to examine carcass and beef characteristics from Brahman Cross (BX) steers slaughtered at different slaughter weight, and also fat thickness categories prepared for traditional market. The study involved 40 heads of feeder cattle of Brahman Cross steer fattened on concentrate based ration and slaughtered at four slaughter weight categories (301-350 kg, 351-400 kg, 401-450 kg and 451-500 kg), and three fat thickness categories (2.5-4.5 mm, 5.0-7.0 mm and 7.5-9.5 mm). The carcass characteristics observed included hot carcass weight, dressing percentage, twelfth rib fat thickness, loin eye area, estimated lean weight and percentage and estimated fat weight and percentage. The beef characteristics observed included meat tenderness, cooking loss, water holding capacity, marbling score, meat and fat colors. The experiment was set up in a completely randomized design with slaughter weight category, and also fat thickness category as the treatment. Results of the study indicated that slaughter weight category significantly ($P<0.05$) affected hot carcass weight, estimated lean and fat weights, while dressing percentage, twelfth rib fat thickness, estimated lean and fat percentages were not significantly influenced by slaughter weight category. Fat thickness category did not have significant effects on hot carcass weight, dressing percentage, rib eye area and lean weight but this fat category significantly ($P<0.05$) affected estimated lean percentage, estimated fat weight and percentage. Neither slaughtered weight nor fat thickness categories had obvious effects on beef characteristics. It was apparent that slaughter weight and fat thickness categories were not a limiting factor for beef quality traits but the carcass productivity traits.

Key words: brahman cross steer, fattening, carcass and beef characteristics, traditional market

INTRODUCTION

Local cattle have been primarily supplying beef for traditional market. The high demand for beef has stimulated the fast growing feedlot industry using imported feeder cattle from Australia, which amounted to 400,000 heads annually (Direktorat Jenderal Peternakan, 2008). Cattle feedlot industry in Indonesia has grown rapidly in order to fulfill quality beef for supplying particular market such as hotels, restaurant and institution. Recently, the feedlot industry also supplies traditional market since there was a shortage of local cattle. Halomoan *et al.* (2001) reported finished cattle at lighter slaughter weight, approximately 372 kg, for traditional market and heavier slaughter weight, approximately 511 kg for particular market. Carcass weight and fat thickness have been identified as indictors of a carcass' productive traits (Johnson *et al*., 1997; Priyanto *et al*., 1997; Priyanto *et al*., 1999; Hafid and Priyanto, 2006). The two factors have long been used as a basis of beef carcass evaluation (Kempster *et al*., 1982). The following study examined the effects of slaughter weight and fat thickness categories on carcass and beef characteristics from Brahman Cross (BX) steers fattened in feedlot.

MATERIALS AND METHODS

Cattle and Procedures

The study involved 40 heads of 2 year-old Brahman Cross steers with initial liveweight averaging 220 - 335 kg. They were fattened on concentrate based ration containing 14 % crude protein and 75 % TDN for approximately two months. The steers were prepared for traditional market and sequentially slaughtered at four slaughter points; those were 301 - 350 kg, 351 - 400kg, 401 - 450 kg and 451 - 500 kg. All steers were fasted but access to water 24 hours prior to slaughter. They were then slaughtered according to common practice applied in the state slaughter house. Following dressing, the carcasses were weighed and divided into two

sides. Measurements were taken on the right side of the carcass. Based on twelfth rib fat thickness measurement, the carcasses were grouped into three fat thickness categories, namely 2.5 – 4.5 mm, 5.0 – 7.0 mm and 7.5 – 9.5 mm. The weights and percentages of lean and fat were estimated according to the regression equations described by Priyanto (1993). Meat sample was taken on the Longissimus dorsi muscle between 12th and 13th ribs in order to obtain measurements of beef properties.

Data Analysis

The experiment was set up in completely randomized design with slaughter weight and fat thickness categories as the factor. The data were statistically analyzed using analysis of variance. Significant differences between treatments were further tested by Duncan Multiple Range Test (Steel and Torrie, 1993). The carcass characteristics observed were hot carcass weight, dressing percentage, twelfth rib fat thickness, loin eye area, estimated lean weight and percentage and estimated fat weight and percentage. The beef characteristics observed included meat tenderness (shear force value), water holding capacity, cooking loss, marbling score, meat and fat colors.

RESULTS AND DISCUSSION

Carcass Characteristics

It is a common practice that slaughter weight was used to determine economic value of beef cattle in domestic market. Table 1 summarizes the carcass characteristics of lot fed Brahman Cross steers which were prepared for traditional market and grouped according to slaughter weight category.

As shown in Table 1, increased slaughter weight resulted in significantly ($p<0.05$) increased carcass weight and therefore estimated lean weight. The estimated fat weight increased significantly ($P<0.05$) with increasing slaughter weight despite the twelfth rib fat thickness was not obviously affected by slaughter weight of beef cattle. The beef carcass prepared for traditional market in this study had low fat thickness, averaging 5.24 mm (3 – 9 mm). Halomoan *et al.* (2001) reported that beef carcass destined for traditional market required lower fat thickness as high carcass fat thickness would resulted in excessive fat trimming and consequently lowered beef yield. Whilst there were significant differences ($P<0.05$) in loin eye area observed between slaughter weight classes, the values did not follow any particular pattern (Table 1). It was suggested that fat thickness and loin eye area were inadequately associated with carcass weight. In light weight carcasses, as in this study, carcass weight was strongly associated with carcass lean and fat weights (Johnson *et al.*, 1997). Nevertheless, overall carcass composition was not influenced by slaughter weight category. This was indicated by similar values of lean percentages and fat percentages among slaughter weight points.

Unlike the effects of slaughter weight category, the effects of fat thickness category was significant ($P<0.05$) for estimated carcass fat weight and percentage, and estimated carcass lean percentage. The other carcass characteristics as carcass weight, dressing percentage, loin eye area and carcass lean weight were not markedly influenced by fat thickness category (Table 2).

Increases in fat thickness of the beef carcass in this study would be followed by significant increases of the weight and percentage of carcass fats and conversely significant decrease of carcass lean percentage. In light weight carcasses, Johnson *et al.* (1997) reported that

Table 1. Carcass characteristics of brahman cross steers according to slaughter weight category

	Slaughter Weight Category (kg)			
Parameter	3001-358	351 – 400	4001 – 450	451 – 500
Carcass weight (kg)	169.64^{a}	192.42^{b}	207.43^{c}	224.00^{d}
Carcass dressing (%)	49.90	50.32	49.70	48.62
12th rib fat thickness (mm)	4.73	5.58	5.32	5.33
Loin eye area(inch^2)	16.30^{a}	13.38^{b}	14.74^{a}	12.29^{a}
Estimated lean (kg)	98.48^{a}	111.42^{b}	120.95^{c}	130.92^{d}
Estimated lean (%)	64.14	63.68	63.82	63.81
Estimated fat (kg)	27.75^{a}	33.18^{b}	35.36^{bc}	38.33^{c}
Estimated fat (%)	17.62	18.50	18.23	18.24

Note: means in the same rows followed by a different superscripts indicate significant differences ($P<0,05$).

Table 2. Carcass Characteristics of Brahman Cross Steers According to Fat Thickness Category

Parameter	Fat Thickness Category (mm)		
	2.5 – 4.5	5.0 – 7.0	7.5 – 9.5
Carcass weight (kg)	193.17	193.00	198.60
Carcass Dressing (%)	50.12	49.19	51.21
Loin eye area($inch^2$)	14.43	15.45	12.53
Estimated lean (kg)	114.05	111.38	112.03
Estimated lean (%)	64.63^a	63.52^b	62.27^c
Estimated fat (kg)	30.07^a	33.95^b	39.02^c
Estimated fat (%)	16.68^a	18.81^b	21.18^c

Note: means in the same rows followed by a different letter differ significantly (P<0.05).

subcutaneous fat thickness alone could predict carcass composition with high degree of accuracy.

Carcass weight and fat thickness are two main factors determining the value of a carcass. They are used for carcass classification in beef marketing (Kempster *et al*., 1982). It has been well established that subcutaneous fat thickness was shown to be the most important variable in percentage-based prediction while carcass weight was the major contributor in weight-based prediction (Butterfield, 1965; Johnson *et al*., 1997). Beside other indicators, carcass weight and subcutaneous fat thickness have been used as the main indicators of carcass productivity in carcass evaluation scheme (AMLC, 1991; USDA, 1997).

Beef Characteristics

Beef evaluation was accentuated particularly on physical characteristics including tenderness (shear force value), water holding capacity (WHC, measured from percentage water loss), cooking loss, marbling score, meat and fat colors. The effects of slaughter weight and fat thickness categories on the physical characteristics of beef were summarized in Table 3 and Table 4 respectively.

Neither slaughter weight nor fat thickness categories had significant influence on all physical characteristics of beef. The overall means of meat tenderness, WHC, ooking loss, marbling score, meat and fat colors were 6.27kg/cm^2, 29.86%, 42.25%, 1.78, 4.05 and 1.83 respectively. Wahyuni (1998) reported tenderness, WHC and cooking loss of beef from Brahman Cross 8.03 kg/cm^2, 46.59% and 46.63% while Amri (2000) reported on steer of the same breed that those values were 7.80 kg/cm2, 22.68 % and 41.18%. The slightly different values of the respective traits might be due to the variation in live weight and age of cattle. These results indicated that such categories failed to differentiate beef quality properties in light weight carcass.

Table 3. Beef Characteristics of Brahman Cross Steers According to Slaughter Weight Category

Parameter	Slaughter Weight Category (kg)			
	3001-358	351 – 400	4001 – 450	451 – 500
Meta tenderness (kg/cm^2)	6.01	7.02	5.87	6.09
WHC (%)	30.98	29.29	20.77	28.40
Cooking loss (%)	42.59	42.78	39.34	39.16
Marbling score	1.64	1.92	1.79	1.67
Meat color	3.82	4.92	3.79	4.33
Fat color	2.09	2.00	1.64	1.00

Table 4. Beef Characteristics of Brahman Cross Steers According to Fat Thickness Category

Parameter	Fat Thickness Category (mm)		
	2.5 – 4.5	5.0 – 7.0	7.5 – 9.5
Meat tenderness (kg/cm^2)	5.97	6.43	6.81
WHC (%)	29.90	30.13	28.77
Cooking loss (%)	42.61	38.33	46.31
Marbling score	1.94	1.71	1.40
Meat color	3.89	4.23	5.00
Fat color	2.17	1.35	2.20

CONCLUSION

In feed-lot Brahman Cross steer prepared for traditional market, it is concluded that slaughter weight and fat thickness categories were not a limiting factor for beef quality traits but the carcass productivity traits. Lean and fat weights increased with increasing carcass weight while lean percentage decreased and conversely fat percentages increased with increasing carcass fatness.

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Performance and Marketing of Garut Sheep, West Java

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ABSTRACT

Sheep is concentrated in West Java with the population of approximately 48% of the total sheep population in Indonesia (9.514.184 heads). Garut Regency contributed around 5% and 9% respectively to West Java and Indonesia population (DGLS. 2007). A steady increase with very little fluctuation of sheep population within 3 years (2004-2007) in West Java (10%/year) and Garut (17%/year) indicates its economical contribution and cultural values to the region. However, Garut sheep gained little attention and support for it's development.

A study was carried out in two representative villages in Garut, aiming at obtaining information on sheep production characteristics including, ownership, types of management, production capacity and marketing. The results showed that the numbers of animals owned was ranging from 1-5 heads/family. Ratio between males and females of 1 : 1.7, providing enough chances for mating activities. Simple housing, feeding and health management were applied by farmers.

Fifty four percent of ewes were kept for breeding purpose, while lamb were raised for sale. The average body weight gain of 100gr/day. Reproductive characteristics was significantly high: 1) sexual maturity for males and females was achieved at 6 m and 10 m respectively; 2) age at first mating around 1 year of age; 3) lambing interval was 9 months and litter size was 2.8 lambs/year, with high percentage of single birth (41%) and twining birth (46%). High mortality rate (75%), mostly occurred at young age.

Sheep were sold as live animals to other farmers, consumers or middle-man. The marketing channels by middle men went through several steps before reaching the final consumers.

Key words: *Garut sheep, performance, marketing chain*

INTRODUCTION

Sheep is mainly concentrated in West Java with the population of approximately 48% (4,605,417 heads), of the total sheep population in Indonesia (9.514.184 heads), of which Garut Regency contributed around 5% and 9% respectively to West Java and Indonesia population (Statistik Peternakan, 2007). Garut sheep plays significant roles in farmer daily activities, has good productivity, economical contribution and high cultural values to the region. Sheep has been part of the farmers activity since the Dutch colonialism era. From biological and economical point of view, sheep has high reproductive performance with litter size up to 150% per year, meat production, could achieve 50% of bodyweight, very adaptive to local environment. Based on approach and information from Indonesia Trade Promotion Center (ITPC), importer from Arab Saudi Association is ready for marketing collaboration. The animal has potency for domestic market and also export to Saudi Arabic country, especially during Idhul Adha.

Despite its popularity, in fact, the Garut sheep, gained little attention and support for it's development toward a sustainable sheep farming. Therefore information on production characteristics is essential. This study was initiated, aiming at obtaining information on sheep production systems and characteristics including, ownership, types of management, production and reproduction capacity, including the marketing system applied by farmers in the region.

The objective of this study was to evaluate sheep production systems in Garut, West Java, to assess production and reproduction characteristics of Garut sheep, and to identify the marketing system applied by farmers.

MATERIALS AND METHODS

A study was carried out in representative villages in two districts (Wanaraja and Tarogong) of Garut Regency involving sheep owners, groups of farmers in both locations, livestock officers and village representatives (kepala desa and local key persons). Pre-survey was conducted prior to data collection, to evaluate regional potency which was

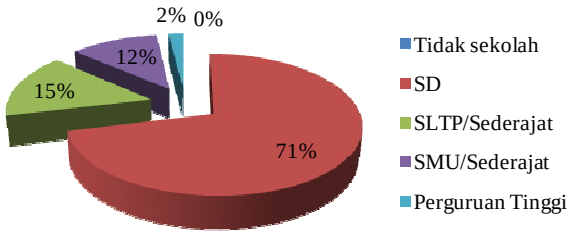
necessary for site selection and sampling purposes. Secondary data was obtained from regional statistics, previous reports on sheep and district data. Questionnaires were used for interview with 2 groups of sheep farmers for data collection on sheep production and. performance, marketing system applied including organization and farmers characteristics. Four hundreds and fifty eight (458 heads) of sheep were used as samples for assessment and direct observation was made on the sheep housing and management, productivity and biological performance. Based on data and information collected, depth discussion was done with related institutions including key personnel, and analyses was made on the economics and the marketing system applied during the last year of the study period.

RESULTS AND DISCUSSION

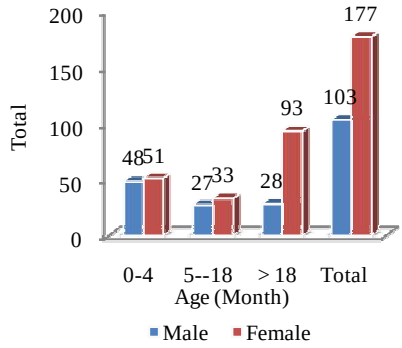
Production and Management

The results showed that most farmers in the region reared sheep on individual basis, the numbers of animals owned ranging from 3 - 13 heads with an average of 8 heads /family. Beside individual rearing, sheep were raised by groups of farmers in each district; 280 heads of sheep were kept by group 1 and 178 heads by group 2. Commonly, sheep were raised for specific reasons, i.e: in Wanaraja, for meat production, while in Tarogong most farmers kept so called *"domba tangkas"*, for fighting purposes. Every year, the community held a big traditional event, in which sheep keepers brought their well trained animal for fighting. Therefore, the sheep keepers were mostly men, with low education level (71% finished elementary school) (Picture 1). According to the opinion of respondents, education had little benefit on their daily activities as small farmers, which was resulted in such low motivation for sending their kids to a higher level of education. Realizing the great potency of sheep, informally-applied training would be needed to improve their knowledge and skill in sheep management, for improving their daily income.

Sheep population in Garut Regency during the study period (2002), was 69,274 Animal Units (AU), of which Wanaraja and Tarogong accounted for approximately 1.6% (1.112 AU) and 0.6% (446 AU) respectively. Based on regional potency, the caring capacity of Garut Regency for ruminant animals was 606,779 AU, indicating a good opportunity for increasing the ruminant population including sheep. Picture 2 and 3 describe the proportion of sheep reared by each group of farmers, based on age and sex, by which, females were preferred to be kept in the flock for breeding purpose.

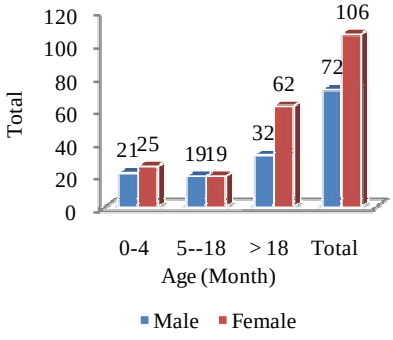


Picture 1. Level of Education of Respondent Farmers in Wanaraja



Source: Laporan Tahunan (2001)

Picture 2. The Number of Sheep Raised by Group 1 of Farmers Based on Age and Sex



Source: Laporan Tahunan (2001).

Picture 3. The Number of Sheep Raised by Group 2 of Farmers Based on Age and Sex

Ratio between males and females in each flock was 1:1.8 and 1:1.5 respectively, providing enough chances for mating activities. Mostly, sheep were housed or reared near the household, with simple feed and feeding management and health and disease control. Approximately 54% of ewes were kept for breeding purpose, while lamb were raised for sale. The average body weight gain of 100gr/day, which was considered low. Reproductive characteristics was significantly high: 1) sexual maturity for males and females was achieved at 6 m and 10 m respectively. 2) age at first mating ranged between 11m-12m of age; 3) lambing interval was 9 months and litter size was 2.8 lambs/year. Data in Table 1 showed that high percentage was found in single birth (41%), twining birth (46%), with small cases of tripled and quartered. However, high mortality rate up to 75% during the study, was very high. The cases was mainly occurred at young age (lamb), due to various factors including high litter size, low birth weight and also the unavailability of sufficient feed and nutrients during the extremely-long dry seasons, particularly, for ewes during pregnancy. The extreme information of mortality resulted from the study was very different to the normal condition and information obtained from various resources such as Garut Agricultural office (2002), local personal and farmers which was less than 5 %. This indicated an unusual climate change during the alternate years. In addition, samples used for this study was limited, influencing the figure.

Based on the production and reproduction characteristics, with an assumption that the environmental condition was stable, the mortality rate was 2%, and without big changes in the population, the expected number of lambs produced per year would be 430 heads. Calculation on population dynamics indicates that within a period of 5 years (Table 2), the population would increase by 234%, 5,358 heads of sheep. Ideally, if the small holding farmers were provided with enough knowledge and skills in sheep rearing, under a proper management, sufficient feed provision and disease control, mortality rate was less than 5%, then sheep development is promising. According to Hartz and Knipscheer (1987), under rural condition, proper management supported by skilled farmers and appropriate technology would contribute to the improvement of socio-economic status of region.

Sheep

Sheep were sold as live animals to other farmers, consumers or middle-man, either in the village or nearby local market for cash

Table 1. The average number of lambs produced classified by group of farmers and types of birth

Type of birth	Group 1		Group 2	
	No of birth (times)	No of lamb (heads)	No of birth (times)	No of lamb (heads)
Singgle	33	33	15	15
Twin	37	74	26	52
Triplet	8	24	9	27
Quartered	2	8	2	8
No of lambs per ewe per birth)	1.76		1.96	

Table 2. Population dynamics of sheep in the 2 localities based on age

Time/Period	No of lamb (head)		No of young lamb (head)		No of mature animals (head)	
	male	female	male	female	male	female
No of animal at starting year	69	76	46	52	60	155
Year 1	215	215	67	74	104	203
Year 2	281	281	210	210	167	271
Year 3	376	376	275	275	369	471
Year 4	653	653	368	368	630	730
Year 5	1013	1013	640	640	977	1075
The condition when males were sold alternately within the years	1013	1013	45	640	68	1075

money. For sheep trading between farmers and middle man or sheep collector, prices were determined by sheep owners based on age and the condition of animals, which was always lower than the timely market price and differed between age, The marketing channels by middle men went through several channels before reaching the final consumers. Table 3. and Figure 4. showed the number of animal bought and sold by respondent farmers during the period of 2002.

CONCLUSION

Garut sheep were reared by farmers as secondary activities in agricultural production with the numbers of animal owner ranging between 3-13 heads/family. Simple housing, feeding and health management were applied which was resulted in low productivity. Realizing the existing sheep production and performance and regional potency, improvement of farmers skill and sheep management, Garut sheep could be developed, toward a sustainable sheep farming. The existing marketing system indicates that sheep were sold as live animals through several steps and channels, for cash money.

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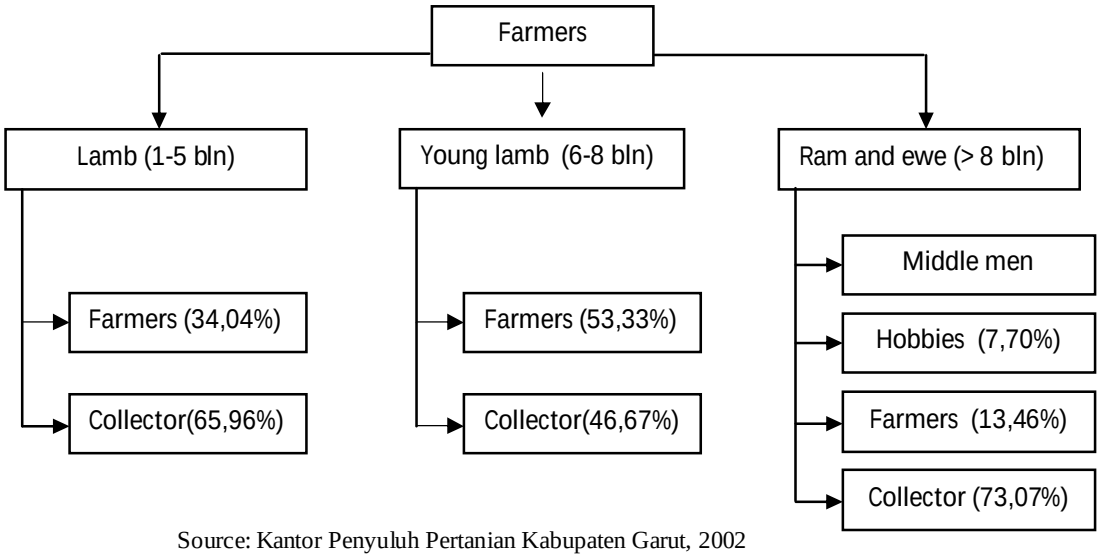
Table 3. The number of animal bought and sold during the study period (2002)

Age	Bought		Sold	
	Male	Female	Male	Female
Lamb	23	1	29	25
Young lamb	5	10	29	32
Ram and Ewe	18	8	43	27
Total	46	19	101	84

Source: Kantor Penyuluhan Pertanian Kabupaten Bogor (2002).



Source: Kantor Penyuluh Pertanian Kabupaten Garut, 2002
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Figure 4. Marketing channels based on sheep age, in Wanaraja

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Physical Properties and Palatability of Cassava Peel Wafer Complete Ration for Sheep

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ABSTRACT

Cassava peel are waste product from cassava plant which have high carbohydrates that can be used such as source of energy for cattle. Percentage of cassava peel has 0.5-2% from total weight of fresh cassava and inside cassava peel has 8-15%. The usage of forage and agriculture by product increased with feed processing technology as wafer ration complete. The aim of this experiment was evaluate physical characteristic of wafer ration complete for sheep. The parameters observed were water content, water absorption, swelling and density. Analysis data that used were Completely Randomize Design, with four treatments and three replications. The treatments were R1 (70% concentrate + 0% cassava peel + 30% field grass), R2 (70% concentrate + 10% cassava peel + 20% field grass), R3 (70% concentrate + 20% cassava peel + 10% field grass) and R4 (70% concentrate + 30% cassava peel + 0% field grass). The results were subjected to ANOVA and Contrast Orthogonal Test (Steel and Torrie, 1993). The result of this experiment indicated that treatment has significantly influenced to water content, water absorption, swelling and density. The average of water content was 10.060-13.137%, average of water absorption was 82,490-169,780%, average of swelling was 35.697-102.295%, average of average density was 0.855-0.870 g/cm^3, and palatability wafer ration complete was 769-866 g/day/head. It concluded that cassava peel is able to be utilized field grass until 30% in wafer ration complete for sheep.

Key words: wafer complete ration, cassava peel, sheep, physical properties and palatability

INTRODUCTION

Quality and quantity of feed is sometimes constraint which need effort to requirement of maintenance, growth, and animal production, thus it need to look for some alternative raw material sources which do not compete with human requirement, having nutrient, cheap price, easy to get and safe consumed for animal. Agricultural waste, plantation and agro industrial can be processed to become feed, example: sugar cane sprout, cassava peel, coffee peel, bagasse, rice bran, copra meal and tofu waste (Mariyono, 2007).

Cassava production in Indonesian reaches 16,723,257 tons (Badan Pusat Statistik, 2002). Percentage of total peel waste is 0.5 - 2% of total weight fresh cassavas and inner skin wastes 8-15% (Grace, 1977) and if it converted by inner cassava skin amount that can be utilized as much as 2,508,489 tons of cassava production at Indonesian.

Cassava as feedstuff has many weaknesses for example low palatability and low cyanide acid contents (HCN) then constitutes curb factor in good usage for animal and human. Normal HCN content on cassava as weight as 15-400 ppm HCN/kg heavy fresh and human consumption cannot be more than 1 mg HCN/kg body weight per day (Balagopalan *et al*., 1988). One of the methods to remove or decrease of HCN on cassava is by soaking into deep water, boiling and drying on the sun shines or hot weather.

Efficiency increasing of foodstuff utilization has to be done through various technologies. Technology can be used for applying and increasing utilization of foodstuff. The utilization of field grass or agricultural waste can be increased by processing technology which is mixing between field grass or agricultural waste and concentrate to be wafer complete ration. Wafer complete ration has physic of compact type then it provided easy for handling and transportation, that inside of have food nutrition completes.

Garut sheep is a local sheep from Indonesia that spread widely in West Java, particularly in Garut regency which the sheep population reaches 337.036 head. Garut sheep has high profilic, having good potency to be developed as source of meat and has been

made region tourism affinity (Mansjoer *et al*., 2005). According to Syukur (2006), molasses can be used as binding and raw materials of feed or to be processed to become single cell protein and amino acid. Molasses has BETN'S content of high dry matter (Bata, 2008).

The aim of this experiment was to know and study cassava peel as supplementary field grass in wafer complete ration of sheep palatability and to evaluate physical characteristic (water content, water absorption, swelling and density).

MATERIALS AND METHODS

Equipment that was used with weights capacity 1, 2, 5 kg, analytic weights, Chopper, Hammer mill swing's type, meter skidders, pressing machine wafer (temperature 120^0C, pressure 12 kg/cm ^{2} , up to 10 minutes).

Grass source that was used was field grass and agricultural waste, to replace as inner cassava peel. Raw material for concentrate were corn, rice bran, soybean meal, onggok, coconut meal, copra meal, molasses, urea, Na_2SO_4 and $CaCO_3$.

Palatability of wafer complete ration that was utilized by Garut local sheep as much 12 head, with body weight around 30-40 kg. Housing of sheep that was utilized has footage 120 cm, wide 80 cm and high 186 cm. That housing made from wood which completed with feed bucket with wide 40 cm and high 45 cm.

Formulation of Wafer Complete Ration

Ration that was utilized in this research consisting of grass source and concentrate with compare 30:70%. Ration formulation was arranged by using trial and error methods that crude protein content more than 20%. Nutrient composition ration adjusted by sheep requirement with body weight around 30-40 kg. Formulation and Nutrient Composition of The Wafer Complete Ration on Table 1. and 2.

Data Analysis

Data Analysis that used was Completely Randomize Design, with four treatments and three replications. The results were subjected to ANOVA and Contrast Orthogonal Test (Steel and Torrie, 1993). The treatments were R1 (70% concentrate + 0% cassava peel + 30% field grass), R2 (70% concentrate + 10% cassava peel + 20% field grass), R3 (70% concentrate + 20% cassava peel + 10% field grass) and R4 (70% concentrate + 30% cassava peel + 0% field grass).

The parameters observed of wafer complete ration were:

1. Water content (AOAC, 1984)
2. Density (Widarmana, 1997)
3. Water absorption and swelling (SNI, 1991)
4. Palatability

Palatability test of wafer complete ration utilized by Garut local sheep as much 12 head , with body weight around 30-40 kg. Feed application during 2 days and final stage of this test were weight and measuring rest one hour of wafer complete ration that was used (Edney, 1982) :

Consumption (kg) = total application – feed rest.

Table 1. Formulation of The Wafer Complete Ration(%)

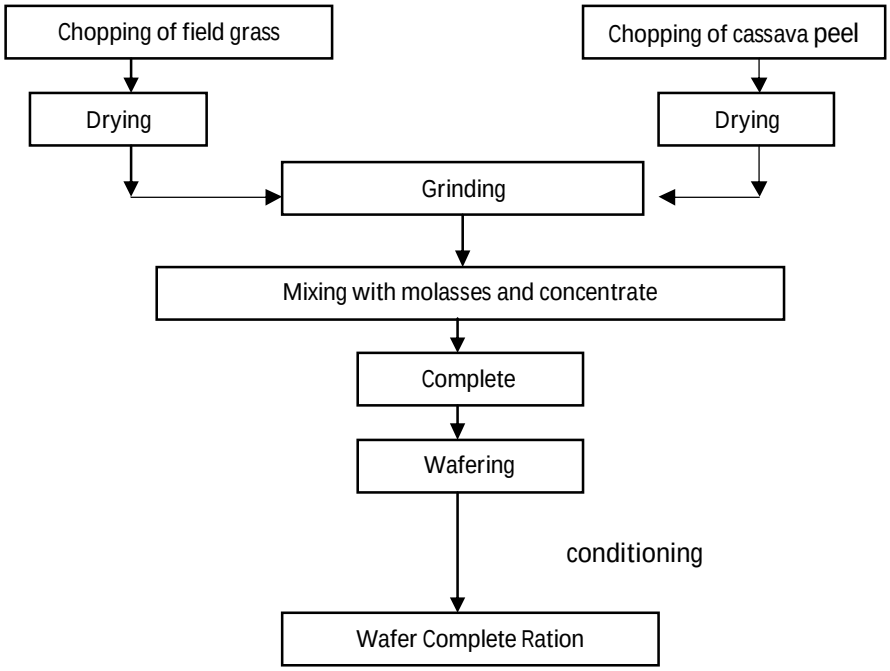
Raw materials	R1	R2	R3	R4
Cassava peel	0	10	20	30
Field grass	30	20	10	0
Corn	6	6	6	6
Rice bran	8	8	8	8
Onggok	5	5	5	5
Soyabean meal	26	26	26	26
Coconut meal	14	14	14	14
Copra meal	4	4	4	4
Molasses	5	5	5	5
$CaCO_3$	1	1	1	1
Na_2SO_4	0.5	0.5	0.5	0.5
Urea	0.5	0.5	0.5	0.5
Total	100	100	100	100

Table 2. Nutrient Composition of the Wafer Complete Ration

Nutrition Composition	Treatment			
	R1	R2	R3	R4
Crude Protein (%)	22,27	22,18	22,08	21,99
Crude Fibre (%)	15,69	13,33	10,97	8,60
Fat (%)	4,07	4,46	4,85	5,24
Ash (%)	7,90	7,39	6,86	6,33
BETA-N (%)	50,33	52,38	54,43	56,48
TDN	69,25	72	74,75	77,50
Phosphorus (%)	0,59	0,60	0,62	0,64
Phosphor (%)	0,52	0,53	0,53	0,54

Note: R1 = 70% Concentrate* + 0% cassava peel + 30% field grass; R2 = 70% Concentrate* + 10% cassava peel + 20% field grass; R3 = 70% Concentrate* + 20% cassava peel + 10% field grass; R4 = 70% Concentrate* + 30% cassava peel + 0% field grass

Diagram Process of Wafer Complete Ration



RESULTS AND DISCUSSION

Water Content

Water content is percentage of water that can declare for to base wet or dry weight (Syarief and Halid, 1993). Water content of wafer is total water that remains stay in intra cellular cell cavity and particle squire since pressing process. Statistics Analysis (Table 3.) shows that conduct gives influence that significant (P<0.01) water content. Verma *et al*. (1996) report that water content as 8-12% is optimum. Ortogonal's contrast test showed that water content on conduct R1 was not significant with R4, but R1 was really significant with R2 and R3. Wafer content determined by particle water rate before wafering process, total contained water in binder and secretor water amount of binder system when get heat energy on pressing processes that as pressure and temperature of wafering process.

Table 3. The Average of Water Content (%)

Treatment	Water Content (%)
R1	11.150 ± 0.544[a]
R2	13.137 ± 0.908[b]
R3	12.877 ± 0.710[b]
R4	10.060 ± 0.448[a]

*Really significant (P<0.01).

Density

Wafer density determine dimension stability and physical performance of wafer complete ration (Jayusmar *et al*., 2002). Density value of wafer complete ration is in Table 4. Statistics Analysis shows that treatment was significant (P>0,05). Average of density value is around 0,855 until 0,870 g/cm^3 which the highest density value is R1 and the lowest is R4. Density value tends to decrease with using cassava peel. It was caused by field grass that had crude fiber content higher than cassava peel, it decreased the absorbing ability of wafer.

Table 4. The Average of Density (g/cm^3)

Treatment	Density (g/cm^3)
R1	0.870 ± 0.004[b]
R2	0.866 ± 0.005[b]
R3	0.861 ± 0.005[b]
R4	0.855 ± 0.012[b]

*Really significant (P<0.01).

Wafer complete ration that has high density will give texture that thick and hard, so edge out in stored good handle and also shocking at the moment transportation and is estimated more long-lasting deep stored (Trisyulianti *et al*., 2003). Wafer with high density will be difficult to consume by sheep then it should be added by water prior to consume by sheep, because usually animal prefer to choose soft feed or wafer with low density. According to Djalal (1984) wafer density can cause of particles formation.

Water Absorption and Swelling

Water absorption is parameter that shows to ability to absorb water in surround to get tied up with material particle (Jayusmar *et al*., 2002). Swelling is parameter that shows wafer ration complete can swell if absorb water and gets to be utilized to predict in as much as which wafer complete ration if mix with saliva in animal (Rakhma, *et al*. , 2003). In this research, water absorption character was added water content on wafer complete ration while that wafer is soaked deep water up to 5 minutes. Value of water absorption and swelling can be seen on Table 5.

Table 5. The Average of Water Absorption and Swelling (%)

Treatment	Water Absorption (%)	Swelling (%)
R1	169.780 ± 10.698[d]	102.295 ± 4.386[d]
R2	134.420 ± 10.090[c]	75.369 ± 7.276[c]
R3	113.126 ± 6.360[b]	55.310 ± 4.890[d]
R4	82.490 ± 7.775[a]	35.697 ± 1.575[d]

*Really significant (P<0.01).

Statistics Analysis showed that treatment was really significant (P<0.01) to water absorption and swelling. Average value of water absorption is around 82,490% until 169.780% with the highest on R1 and the lowest on R4. Average value of swelling is around 35.697% until 102.295% with the highest on R1 and the lowest on R4.

Orthogonal's contrast test at each treatment give in contrast influence with water absorption that tending decrease in step at the increase cassava peel, also that swelling. Water absorption time and swelling on R4 different from R1, R2 and R3. Quantitatively, R4 cannot measured water absorption and swelling because wafer example test while soaked by water will destroy, but qualitatively, R4 more crumbly strikes by water compare with R1, R2 and R3.

According to Djalal (1984) there are several factors that regard absorption water which are (1) blank space volume that can keep all water between particle, (2) mark sense capillary channel that link room one by the other blank space, (3) particle extents that can't be covered by pastes and (4) in its paste penetration to particle.

Palatability

Wafer or feed application has substance nutrient composition that needed with exact total, that feed shall also measure up as safe as to be consumed, palatable, economic and gets nutrient rate that adequately to meet the need it. Palatability is sensed of raw material or feed that alone so regarding in height feed consumption (Scot *et al*., 1982). Palatability on wafer complete ration of sugar cane sprout and field grass more preferred by FH cows than bagasse and also sprout and bagasse combine (Retnani *et al* , 2009). Palatability test of wafer complete ration on each conduct can be seen on Table 6.

Table 6. Range of Palatability Test Result of Wafer Complete Ration

Treatment	Dry Matters Consumption (g/head/day)	% Sheep Weight (30-40 kg)
	769-819	1.7-1.9
R2	794-821	1.8-2.4
R3	829-851	2.3-2.5
R4	849-866	2.4-2.6

Consume dry matters (g/head/day) on each treatments R1, R2, R3 and R4 were 769-819; 794-821; 829-851 and 849-866. R4 treatment had highest consumes dry matters of wafer complete ration and the lowest on R1 treatment. It shows that cassavas peel purpose until level 30% preferred by sheep because the increase of cassava peel purposes will impact to increase the wafer consumption.

Dry matter consumption in this research among 1,7% until 2.6% of body weight. This value was lowest compared to standard requirement sheep as 3% of body weight (NRC, 1985). It was caused by the sheep in this research was unhabitual consumption wafer, which previously it consumes conventional feed, i.e. field grass or concentrate.

CONCLUSION

The treatment has significantly influence water content, water absorption, swelling and density. The average of water content was 10.060-13.137%, average of water absorption was 82.490-169.780%, average of swelling was 35.697-102.295%, average of average density was 0.855-0.870 g/cm^3, and result of palatability was 769-866 g/day. It concluded that cassava peel is able to be utilized field grass until 30% in wafer complete ration for sheep.

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Identification Of Alabio Ducks (*Anas Platyrhynchos* Borneo) Beak And Shanks Colour In Two Farming Center In South Kalimantan

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ABSTRACT

Alabio ducks (*Anas platyrhynchos* Borneo) as germ plasma of Indonesia were highly populated in South Kalimantan. These ducks developed rapidly and well known as an excellent duck breed with high genetic potential for egg production. However, presumably of neglected crossing in the fields, the originality (purity) and the egg productivity of Alabio ducks decreased. The study was conducted in two Alabio centre regions in South Kalimantan: Hulu Sungai Utara (district of Sungai Pandan) and Hulu Sungai Tengah (district of Labuan Amas Utara) to identify and to provide information about the colour of feather, beak, and shanks of Alabio ducks. The observed ducks were at the same age (around 20 weeks of age). The results indicated the colour variation of feather, beak, and shanks of Alabio ducks. The feather colour varied as *mengelaras*, *membatik* (stripe), or non *membatik* (non stripe) with cream as the basic colour. Meanwhile, the colour of the beak and shanks spread between light yellow, dark yellow, and orange.

Key words: Alabio ducks (Anas platyrhynchos Borneo), identification, and colour variation

INTRODUCTION

Alabio duck (*Anas platyrhynchos* Borneo) as germ plasma in South Kalimantan is well known as laying duck type. Nowadays, Alabio ducks are also oriented as meat producer (meat type). Registered duck population in South Kalimantan is around 3.487.002 head which are spread in 13 regencies of South Kalimantan (Dinas Peternakan Kalimantan Selatan, 2006). Concerning the benefits of Alabio duck, several research have been done. Through several Livestock Research Center, the researches in Alabio ducks are growing widely which produce crossbred of Alabio and other local ducks (Prasetyo and Susanti, 2007).

Several crossing have been done to produce high quality of duck as egg and meet types. Suharno and Amri (2003) *in* Rahmatullah (2008) reported that Alabio ducks can produce around 200 – 250 eggs/year with an average of 65 – 70 gram/egg and body weight standard of 1.8 – 2.0 and 1.6 – 1.8 kg for male and female, respectively.

The Alabio crossing with other ducks outside Kalimantan was rapidly developed from year to year. This crossings improved the genetic quality of Alabio but it can change the origin characteristics of Alabio duck such as the colour of feather, beak, and shank. Meanwhile, this characteristics belong to be specific for Alabio duck.

There were several factors affecting the colour pattern. Especially in poultry, the feather colour was determined by pigment, physical structure, and combination between them. Meanwhile, beak and shank colour was derived from skin pigment such as lipochrom (Winter and Funk, 1960).

The objective of this study was to find information about the colour variation of beak and shank of Alabio ducks in two different areas in South Kalimantan.

MATERIAL AND METODES

Time and Place

This study was carried out for four months (1 January to 30 April 2008) in two Alabio duck farming centres in South Kalimantan (Sungai Pandan District/ Alabio (Hulu Sungai Utara Regency); and Labuan Amas Selatan District / Pantai Hambawang (Hulu Sungai Tengah Regency).

Materials and Equipment

This study involved the farmers and the male and female Alabio ducks (≤ 6 months old) from the farmers in Sungai Pandan District / Alabio (HSU Regency) and Labuan Amas Selatan District / Pantai Hambawang (HST Regency). Several equipment were used such as digital cameras, writing utensils and a Roche yolk colour fan which was used

to measure the yellow colour level of the beak and shank of Alabio duck.

Methods

Purposive sampling as survey method was applied to determine the location sample and to interview the local farmers through questionnaire. An observation of Alabio ducks (male and female) in these locations was conducted without experimental design.

Data Analysis

This research using description data for explan result, because in experimental research only observation Alabio duck in 2 (two) farming duck in South Kalimantan.

RESULTS AND DISCUSSION

Characteristics of Alabio Duck Famers

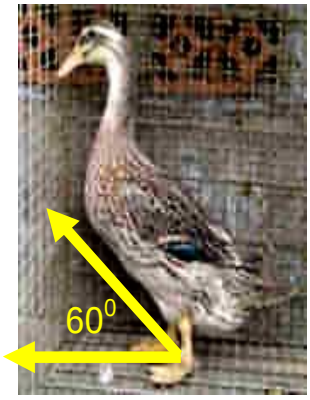
Several characteristics of Alabio duck farmers are presented in Table 1. The farmers as respondents aged 47,2 years old in average (farmer from HSU, age is 52, and farmer from HST age is 42) and belong to the productive age. At this age he farmers still have ability and good skill to keep ducks intensively or extensively. The respondents from HSU have been involved and experienced for about 18 years in duck farming following the tradition of inherited knowledge and the respondent from HST have been experienced for about 10,2 years.

All of DODs (day old ducks) were originated from Mamar village in Amuntai region as this region produced good quality of DODs. Meanwhile the farmers from coastal area of Hambawang obtained the DODs either from the market or from hatchery in Mamar village.

Concerning the aim of farming to produce eggs, the majority of respondent chose the female ducks. However, there were several male ducks were included as the sex was relatively difficult to determined at 2 weeks of age. Actually, the DOD was sexed by differentiation of voice or protrusion in cloacae.

General Characteristic of Alabia Duck

General characteristics of Alabio ducks were obtained from age, sex, body weight, body position (elevation angle), and body form as performed in Picture 1 and Table 2. The body weight was different according to sex. According to farmer's information 127 samples female Alabio at age ≤ 6 months (20 – 24 weeks) weight between ≤ 1,5 – 1,6 kg (in HSU and HST). This finding was in line with Abdul (1992) who reported that Alabio duck entering eggs production phase weighed 1,5 – 1,6 kg. Meanwhile, at the age of < 5 months, the duck weighed 1,3 – 1,4 kg which is in line with Gunawan (1987) who found that the Alabio duck weighed 1,405 kg at the age of 8 - 16 weeks. Based on farmer's information, at the age of > 6 months, the duck weight was 1,6 kg. This finding was in line with Suharno and Amri (2003) who reported of 1,6 kg at the age > 24 weeks until 40 weeks.



Picture 1. General characteristics of Alabio ducks with elevation angle

Concerning the elevation of body posture, the ducks demonstrated the same level of 60^{o} to the ground. Suharno and Amri (2003) and Wasito and Rohaeni (1994) found the same case.

The Alabio ducks body showed the bottle shape form when they observed from head to foot in upright position. From beside and at stand still position, meanwhile, the ducks formed the triangle. This finding was also observed by Marhiyanto (1996) and Suharno and Amri (2003): like bottle formed and triangle shaped.

Feather Colour Characteristics of Alabio Duck

The result of feather colour characteristic that was observed from the back, tail, breast, neck and wings was presented in Table 3. The characteristic of back colour was differentiated from dotted and branched black fleck: dotted/ branched 1; dotted/ branched 2, and dotted/ branched 3. This finding proved the occurrence of black fleck variation in Alabio duck as shown in Picture 2.

Table 1. Description of Respondents

No	Description	Data	
		HSU	HST
1	Farmer age (year/s)	52	42
2	Farming experience (year/s)	18	10,2
3	Education	SD / SR = 50 % (3 farmer) SMP = 50 % (3 farmer) SMA = - (0 farmer) PT = - (0 farmer)	SD / SR = 25 % (1 farmer) SMP = 25 % (1 farmer) SMA = 25 % (1 farmer) PT = 25 % (1 farmer)
4	Age of ducks and percentage	Age ≤ 6 months (100%)	Age ≤ 6 months (100%)
5	Number of ducks (head) and percentage	10- 50 heads 33,3 % (2 farmer) >50 – 100 heads 33,3 % (2 farmer) >100 – 200 heads 0 % (0 farmer) >200-400 heads 16,7 % (1 farmer) > 400 heads 16,7 %(1 farmer)	10- 50 heads 40 % (2 farmer) >50 – 100 heads 40 %(2 farmer) >100 – 200 heads 10 % (1 farmer) >200-400 heads - (0 farmer) > 400 heads - (0 farmer)
6	Modus of obtaining DOD a. From the farmer breeder b. From the market c. From self hatchery	a. 83,3 % (5 farmer) b. 0 % (0 farmer) c. 16,7 % (a farmer)	a. - (0 farmer) b. 100 % (5 farmer) c. - (0 farmer)
7	The DOD origin (farmer or market)	Mamar village	Mamar village
8	Frequency of DOD buying	Depends on financial status and need (for example once in 3 or 6 months)	Depends on financial status and need (for example once in 3 or 6 months)
9	Dominant species chosen	Alabio duck	Alabio duck
10	Dominant duck sex chosen	Female	Female
11	Method of sexing a. Self sexing b. Other help	Self sexing	Self sexing
12	Aim of Alabio duck farming: a. Production of consumption eggs b. Fattening for meat production c. Breeding	Egg production (2 farmer) Egg production and fattening (3 farmer) Fattening (1 farmer) Egg production and breeding (1 farmer)	Egg production (4 farmer) Egg production and fattening (0 farmer) Fattening (0 farmer) Egg production and breeding (0 farmer)
13	Method of sexing a. Differentiation of voice b. Protrusion in cloacae c. Colour of feather, beak, and shank d. Direct information from the seller	From: c.Colour of feather, beak, and shank	From: c.Colour of feather, beak, and shank

In HSU, back feather was varied in colour: greyish brown (95,71%) and greyish black (4,29%), and in HST, back feather was varied in colour almost finding greyish brown. Suharno and Amri (1996) reported that the colour of Alabio duck was greyish yellow and greyish black for male and female, respectively. Meanwhile, Puslitbangnak (2007) found there were two colours (greyish brown and greyish black).

This finding showed that the pigment played a big role especially melanin (the black pigment). Stevens (1991) mentioned that besides pigment, the sight elevation and light effected the colour perception. Therefore, the green feather colour was observed.

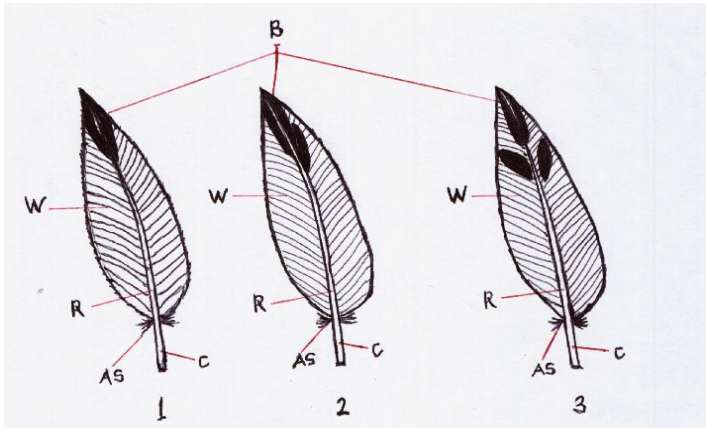
The greyish brown or greyish black was found at the edge of the tail feather. This colour was well known among the farmers as *membatik* (batik coloured) or *mengelaras* (the colour of dry banana leaf). All ducks owned "*mengelaras*" in tail feather. In HSU, Black spotted differentiated between male and female, a total of 4,29 % was greyish black (male) and 95,71% was greyish yellow (female), but in HST, 57 samples (100 %), finding was greyish yellow.

The breast feather was divided into two type of colours: *membati*k (batik coloured) and non *membatik*. Furthermore, this feather colour was divided into the occurence and absence of black shield. The breast colour became a criteria in breed selection. It was observed that in HSU, 71,42 % were *membatik* and 28,57 % non *membatik*, but not much different observed in HST result, 70,17 % were *membatik* and 29,82 % non *membatik*.

The neck coloured was uniform of greyish yellow (*mengelaras*). The wing feather was distinguished in 2 types, light *cerminan* and dark *cerminan*. *Cerminan* feather was located at the end of the wing and lighted to bluish green if it is exposed to the light. From this *cerminan*, the farmers predicted the beginning of laying period. This cerminan was well developed if the ducks had access to water. According to the farmers, the more shine the cerminan feather, the worst the egg production.

Table 2. General descriptionof Alabio duck

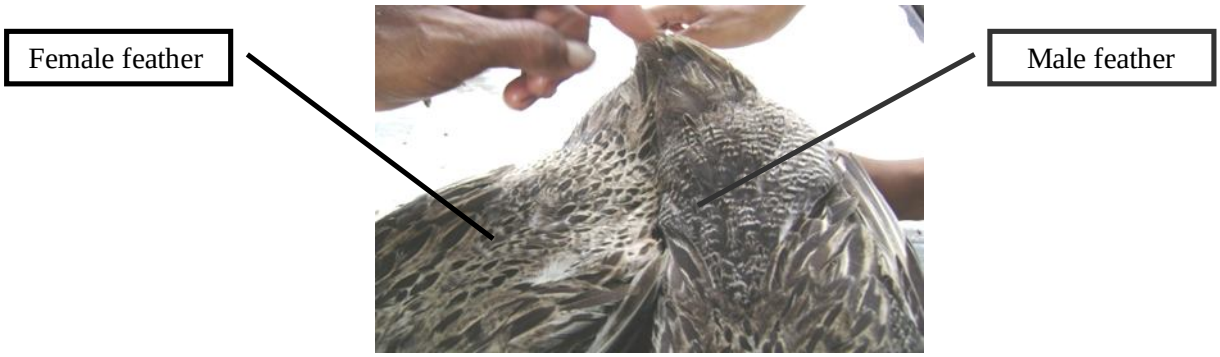
No.	Characteristics	Percentage	
		HSU	HST
1	Age		
	a. ≤ 6 months	100 % (70 samples)	100 % (57 samples)
2	Sex		
	a. Female	95,71 % (67 samples)	100 % (57 samples)
	b. Male	4,29 % (3 samples)	-
3	Body weight		
	a. ≤ 1,5–1,6 Kg	95,71 % (67 samples)	100 % (57 samples)
	b. > 1,6 Kg	4,29 % (3 samples)	-
4	Body position (elevation)		
	a. 60^{o}	100 %	100 %
5	Body form		
	a. Bottle form	100 %	100 %



Picture 2. The characteristic of back colour was differentiated from dotted and branched blackfleck. Figure 2. dotted/branched 1, Figure 3. dotted/branched 2

Table 3. Observation of Feather Colour of Alabio Duck

Parameter	Parameter Detail	Percentage	
		HSU	HST
Feather colour of the back	1.Black fleck with branch		
	a. 1 (one)	34,29% (24 samples)	- (0 samples)
	b. 2 (two)	1,43 % (1 samples)	- (0 samples)
	c. 3 (three)	64,29 % (45 samples)	100% (57 samples)
	2. Colour:		
	a. Greyish brown	95,71 % (67 samples)	66,67 %(38 samples)
	b. Greyish black	4,29 % (3 samples)	33,33 %(19 samples)
Feather colour of the tail	1.Greyish brown (at feather peak)	95,71 % (67 samples)	100 % (57 samples)
	2. Greyish black (at feather peak)	4,29 % (3 samples)	- (0 samples)
Feather colour of the chest	1. Batik formed:		
	a. occurred / *mengelaras*	71,42 % (50 samples)	70,17 % (40 samples)
	b.absence/*tidak mengelaras*	28,57 % (20 samples)	29,82 % (17 samples)
	2. Black shield on feather:	25,71 % (18 samples)	- (0 samples)
	a. occurred	74,29 % (52 samples)	100 % (57 samples)
	b. absence		
Feather colour of the neck	Batik coloured	All sample were uniform in colour	All sample were uniform in colour
Feather colour of the wing	1. Glanced	52, 86 % (37 samples)	100 % (57 samples)
	2. Doped	47,14 % (33 samples)	- (0 samples)



Picture 3. The characteristic of Feather Colour of Alabio duck male and female.

Characteristic of Beak Colour of Alabio Duck

The colour of beak was measured by Roche yolk colour fan. The observed colour variation ranged between yellow or orange which is the specific beak colour of Alabio duck. His finding was in line with Suharno and Amri (2003) *in* Rahmatullah (2008). The observation results are shown in Table 1 and Picture 2.

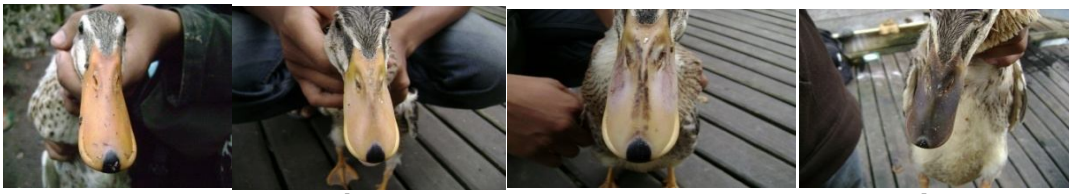
In Alabio region, the beak colour of the light yellow scored 1 – 5, dark yellow scored 5 –10, orange scored 10 - 15, and black were found accordingly 44,29%; 2,86%; 50%; and 2,86% from the sample population.

The orange colour was mainly found in semi intensive farming (Rohaeni, 2005). The farmers choosed this beak colour based on their experience that these ducks had good laying intensity independed from feed influence. The farmers used mix ration from sago, kalambuai, salted fish, and rice hull with maximum 25% commercial feed.

The population of Alabio ducks with black beak colour was relatively smaller compared to other beak colour. This was corresponding with the recessive gene. This

Table 4. Beak Colour of Alabio Duck.

Observation		Percentage (%) in Region	
Colour	Score	Alabio (HSU)	Pantai Hambawang (HST)
Light yellow	1 – 5	44,29 (31 samples)	49,12 (28 samples)
Dark yellow	5 – 10	2, 86 (2 samples)	12,28 (7 samples)
orange	10 – 15	50 % (35 samples)	35,09 (20 samples)
Black	≥ 15	2, 86 (2 samples)	3,51 (2 samples)



4a 4b 4c 4d

Picture 4. The characteristic of Beak Colour of Alabio duck, Picture 4a. Orange colour, Picture 4b. dark yellow colour, Picture 4c. light yellow colour, Picture 4d. black colour.

recessive gene occurred as the result of interaction between homozygote and heterozygote genes during breeding. This black colour might be occurred because of inbreeding which was happened in SPAKU that has large population with uncontrolled breeding program.

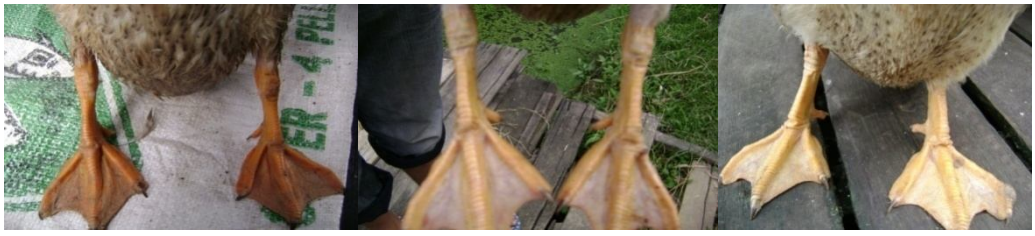
In Pantai Hambawang region, the beak colour of light yellow and dark yellow was 49,12% and 12,28%, respectively. The orange and black beak coloured was only found in small number (35,09% and 3,51%). The farmers had no experience and knowledge that the orange beak colour of Alabio duck was corresponding with better laying intensity. The farmers expected the good laying intensity only by using commercial feed. Other influencing factor showed that the farmers in Pantai Hambawang kept also Mojosari-Alabio ducks as egg producer.

The beak colour variation was affected by the laying rate. More productive ducks showed more pale beak colour as the xanthophylls pigment was absorb from the beak (originated from the ration) to give yellow pigmentation in egg yolk (Tanudimadja, 1974).

Characteristic of Shanks Colour of Alabio Duck

The shanks colour of Alabio duck is yellow (Suryana, 2007). Comparing the Tegal and Mojosari ducks, the shanks colour as well as beak colour belongs to the specific characteristic of Alabio ducks. The shanks colour was determined by Roche yolk colour fan.

There was variation in shanks colour of Alabio duck in two different regions (Alabio and Pantai Hambawang). The shanks colour varied between light yellow until orange (Picture 5).



5a 5b 5c

Picture 5. The characteristic of Shanks Colour of Alabio duck, Figure 5a. Orange colour, Figure 5b. dark yellow colour, Figure 5c. light yellow colour.

Table 5. Shanks Colour of Alabio Ducks

Observation		Percentage (%) in Region	
Shanks Colour	Score	Alabio (HSU)	Pantai Hambawang (HST)
Light yellow score	1 - 5	14,29 (10 samples)	52,63(30 samples)
Dark yellow	5 - 10	34,29 (24 samples)	26,32(15 samples)
Orange	10 - 15	51,43 (36 samples)	21,05(12 samples)

From the sample population Alabio (HSU), 14,29%, 34,29%, and 51,43% of the shanks colours were light yellow (scored 1 – 5), dark yellow (scored 5 – 10), and orange (scored 10 -15), respectively.

The light and old yellow coloured shanks were not choosed as laying type ducks as the farmer knew that this shanks colour was correlating with the beak colour. Therefore, it was rarely found the ducks with these shanks colours in semi intensive farming.

The light and dark yellow coloured shanks were primarily found in SPAKU and Pantai Hambawang regions, meanwhile, the orange coloured shanks was mainly observed in Alabio region.

Most of the Alabio ducks in Alabio region had orange shanks as the assumption that this colour pattern was correlating with high laying intensity and yellow until orange coloured yolk. The observation of shanks colour was presented in Table 2

The scientists and farmers mentioned that yellow is the specific colour of the Alabio shanks. Only few mentioned that Alabio shanks colour is orange. Based on this study, the most colour of Alabio shanks was yellow. However, reported that the specific shanks colour of Alabio ducks was orange.

In Pantai Hambawang region, 52,63 %, 26,32 % and 21,05 % of shanks colour found were light yellow, dark yellow and orange, respectively. This data was corresponding with beak colour as the yellow beak colour was correlated with yellow shanks colour (Suharno and Amri, 2003) *in* Rahmatullah (2008). In fact, the majority of shanks colour was yellow either light or dark. The occurrence of light shanks colour was higher in Pantai Hambawang region (52,63%). This number was relatively the same with the beak colour.

The farmers in this region were rarely used this shanks colour as laying intensity indicator. They believed that shanks colour had no correlation with higher laying intensity. However, they selected the laying ducks from the body shape. The farming knowledge was not traditionally inherited from the family, but from the try and error learning method. Therefore, they dependt the rate of laying intensity through the commercial feed but not derived from the genetic basic.

The shanks yellow and orange colours were influenced by lipochrom pigment (Winter and Funk, 1960) which was reduced gradually during egg production period. The shank colour reduction started from dorsal to the plantar (Tanudimadja, 1974).

CONCLUSION

This study concluded that the feather, beak and shank colours of Alabio ducks varied in two different regions. The feather colour showed black fleck at the feather edge with greyish brown and greyish black colours variation. The breast feather colour was differentiated by membatik or non membatik pattern; and the occurrence or absence of black shield. The beak colour varied between light yellow, dark yellow, orange and black. The shanks colour varied between light yellow, dark yellow, and orange.

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Antimicrobial Clove Bud Oil for Inhibiting *Salmonella sp*. Isolated from Broiler Carcass Samples

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ABSTRACT

The aim of this study was to determine the inhibition effect of clove bud oil against the growth of *Salmonella sp*. isolated by in vitro method. Three isolates of *Salmonella sp*. I, II and III were obtained from the broiler carcasses in Central Java used in this study. Every isolates was tested against several concentrations (25, 20, 15, 10 and 6% of c*love bud oil*. The results indicated that the higher of oil concentration the higher the growth inhibition of *Salmonella enteritidis* obtained (P<05). The growth inhibition produced by 25, 20, 15, 10 and 5% oil concentrations respectively were 18.7, 15.7, 14.3, 9.3 and 6.7 mm. The most sensitive *Salmonella sp*. isolate to clove bud oil was *Salmonella sp.* isolate II (P<05). Growth inhibitions of *Salmonella sp*. I and III and I respectively were 7.2, 11.6 and 13.4 mm respectively. It was concluded that clove bud oil could inhibit the growth of *Salmonella sp.*isolated from broiler carcass samples.

Key words : a*ntimicrobial, clove, Salmonella sp.*

INTRODUCTION

Clove bud oils have biological activities, such as antibacterial, antifungal, insecticidal and antioxidant properties, and are used traditionally as flavoring agent and antimicrobial material in food (Huang *et al.*, 2002, Lee and Shibamoto, 2001 and Velluti *et al*., 2003). For example, clove oil was effective against *L. monocytogenes* and *S. enteritidis* in tryptone soya broth (TSB) and cheese (Smith-Palmer *et al.*, 1998; Smith-Palmer *et al*., 2001). The high levels of eugenol contained in clove essential oil give it strong biological activity and antimicrobial activity. This phenolic compound can denature proteins and reacts with cell membrane phospholipids changing their permeability (Briozzo, 1989; Deans and Ritchie, 1987).

The development of antimicrobial resistance among pathogenic bacteria has emerged as a major public health concern, which has led to an intensification of discussion about the prudent use of antimicrobial agents, especially in veterinary medicine, nutrition and agriculture (Caprioli *et al*., 2000).

The utilization of antimicrobial drugs has played an important role in animal husbandry, since they are used in prophylaxis, treatment and growth promotion. Overall, there are largest quantities of antimicrobials that are used as regular supplements for prophylaxis or growth promotion in the feed of animal herds and poultry flocks. This result in the exposure of a large number of animals, irrespective of their health, has shown frequently sub therapeutic concentrations of antimicrobials (Dupont and Steele, 1987 and Franco *et al.*, 1990). Furthermore, antibiotics given to animals and closely related compounds used in human therapy have been exerting selective pressure on their target bacteria for decades (Witte, 1998), and can generate a reservoir of antimicrobial resistant bacteria (Endtz *et al*., 1991 and Smith *et al*., 1999).

Antimicrobial-resistant bacteria in food animals threaten the efficacy of human drugs if antimicrobial-resistant bacteria or antimicrobial-resistance genes become incorporated into human bacterial populations (Smith *et al*., 2002). Agricultural antibiotic use increases the frequency of antibiotic resistant zoonotic pathogens such as Salmonella (Smith *et al*., 2002). Most antimicrobial-resistant Salmonella infections are acquired from eating contaminated foods of animal origin (Angulo *et al*., 2000). The husbandry practices used in poultry industry and the widespread use of medicated feeds in broiler and layer operations made poultry a major reservoir of antimicrobial-resistant Salmonella (D'Aoust *et al*., *1992*). Resistance in Salmonella limits the therapeutic options available to veterinarians and physicians in the treatment of certain human cases of salmonellosis (Witte, 1998). Furthermore, if there is a confection with HIV, it may result in

rapid disease progression in the infected individual and has a potential multiplier effect on the dissemination of the resistant pathogen to the rest of the population (WHO, 2001).

Therefore, the aim of this study was to determine the inhibition effect of clove bud oil against the growth of *Salmonella sp.* isolated by *in vitro* method. Three isolates of *Salmonella sp*. I, II and III were obtained from the broiler carcasses in Central Java used in this study.

MATERIALS AND METHODS

This study was assigned to 3 x 5 factorial arrangements of treatments to examine the effect of three isolates of *Salmonella sp*. and five concentrations of clove bud oil. Each treatment was replicated three times. Duncan's Multiple Range Test was used to estimate the differences between treatment means.

The *Salmonella sp.* isolates (I. II and III) used in this experiment were *Salmonella sp*. isolates that are collections of Balitvet-Bogor. Clove bud oil (*Oleum caryophylli*) was collected from PT. Phytochemindo extract, Central Java. Concentrations of the clove bud oil (*Oleum caryophylli*) for this investigation were 25, 20, 15, 10 and 5%. Mueller Hinton agar and broth media were used as the growth media for the three bacterial isolates for this study.

Anti bacterial study was done at Balitvet- Bogor. Fifteen microlitres of clove bud oil were dropped into sterile disk. The disk then were laid on Mueller-Hinton-Agar Medium that had been inoculated with *Salmonella sp*. isolates and incubated at 37^0C overnight. The next day, the growth inhibition on every plate was observed.

RESULT AND DISCUSSION

Clove bud oil concentration

Increasing clove bud oil concentrations resulted in significantly increased growth inhibition of *Salmonella sp*. isolates (P <.05) (Table 1).

The result indicated that the higher concentrations of clove bud oil, the higher its abilities to inhibit growth of *Salmonella sp*. isolates (P<0.5). This might be due to the increase in concentration of usnat acid as bacteriostatic component in the clove bud oil. This result agrees with Windholz *et al*. (1983) who showed that the clove bud oil contains usnat acid as antibiotic and bacteriostatic.

Table 1. The effect of *Usnea spp*. extract concentration on growth inhibition of *Salmonella sp*. isolates

Extract concentration (%)	Growth inhibition (mm)
25.0	18.65^a
20.0	15.65^b
15.0	13.31^c
10.0	9.31^d
5.0	6.65^e

Note: means within column with different superscripts are significantly different (P<0.5).

Salmonella sp. Isolates

Three *Salmonella sp.* isolates (I, II and III) used in this study had significantly different sensitivity (P<0.5) to clove bud oil (Table 2). Growth of *Salmonella sp*. isolate II was the most inhibited by clove bud oil (P<0.5), while growth of *Salmonella sp*. isolate I was the least inhibited. These results have indicated that *Salmonella sp.* isolate II was the most sensitive to the clove bud oil. The sensitivity differences among three *Salmonella sp*. isolates used in this study might be due to the effect of antibiotic that was applied previously to the affected animal. Poeloengan *et al.* (1992) showed that continuity of antibiotic application resulted in resistency.

Table 2. Growth inhibition of three *Moraxella* isolates

Salmonella sp. isolates	Growth inhibition (mm)
I	$9{,}18^c$
II	$15{,}38^a$
III	$13{,}58^b$

Note: means within column with different superscripts are significantly different (P<0,5).

Table 3. The effect of the clove bud oil on growth inhibition of three *Salmonella sp*. isolates

Extract concentration (%)	Type of isolate	Growth inhibition (mm)
25	I	16
20	III	18
15	II	11
10	I	8
5	III	11
25	II	16
20	I	4
15	III	24
10	II	16
5	I	12
25	III	16
20	II	8
15	I	6
10	III	8
5	II	17

Note: means within column with different superscripts are significantly different (P<0,5).

Table 3 shows that *Salmonella sp.* isolate II was the most sensitive isolate to clove bud oil especially at high concentrations (25 and 20%), while the isolate I of *Salmonella sp* was the least sensitive to the clove bud oil at all concentrations (P<0.5). This result suggested that to inhibit the growth of *Salmonella sp* isolate II would be more effective at high concentration of the clove bud oil.

CONCLUSION

Increasing clove bud oil concentrations resulted in increasing the growth inhibition of *Salmonella sp* isolates significantly (P<0.5). Isolate II of *Salmonella sp* was the most sensitive to clove bud oil at 25% and 20% concentration.

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Screening for Antibacterial Properties of Some Plants and Chemical Antibiotic Against Two Isolates of *Escherichia coli* from Diarhea Calves in Indonesia

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ABSTRACT

Plants used in factory medicine of Indonesian native people were collected. Ethanol extract and powder were prepared and evaluated in a test against two isolates of *Escherichia coli* from herbal plants mahkota dewa (*Phaleria macrocarpa*), daun sembukan (*Paederia foetida*), daun sirih/betel vine (*Piper betle*), kencur /greater galingale (*Kaempferia galangal*), garlic (*Allium sativum*) and jinten hitam (*Nigella sativa*). The results showed anti *E.coli* activity at 20% concentration with the most active plants with diameter of inhibition zones of 15 mm (*Allium sativum*) powder, 11 mm (*Piper betle*) extract and 14 mm (*Nigella sativa*) extract. Extract *Paederia foetida* and extract *Phaleria macrocarpa* had no inhibition effects. The two *E. coli* isolates were sensitive to chloramphenicol at 30 µg.

Key words: a*ntimicrobial, Escherichia coli, Allium sativum, piper betle, Nigella sativa, Paederia foetida, Phaleria macrocarpa, Kaempferia galangal*

INTRODUCTION

The spread of multi-drug resistant pathogens is one of the most serious threats to successful treatment of microbial diseases. Down the ages, spices have evoked interest as sources of natural products for their potential uses as alternative remedies to heal many infectious diseases (Parekh *et al*., 2005).

According to the reports of many researchers, antibacterial resistance is a worldwide growing-problem. Isolation of microbial agents less susceptible to regular antibiotics and recovery of resistant isolates during antibacterial therapy is increasing throughout the world. One of the measures to combat the increasing rate of resistance in long run, is to have continuous investigation for new, safe and effective antimicrobials as an alternative agents to substitute with no-effective ones. Natural resources, especially plants and microorganisms were the potent candidates for this rum. Usage of plants in curing illnesses has deep roots in man's history since plants are sources of many life-sustaining metabolites.

Colibacillosis incidences in cattle, pig and other farm animals were well documented in Indonesia. These bacterial incidences in young calves and piglets were reported in Bali (Hartaningsih and Hasan, 1985), Lampung (Suastama, 1983) and Central Java (Setiawan, *et al*., 1982). Piglet neonatal diarrhea associated with enterotoxigenic (ETEC) *Escherichia coli* was commonly observed in intensive piggeries in Bogor and Kapok areas. Here diarrhea occurred at the rates of 13 to 40 percents within the first two weeks of life. The associated mortality rates were from 12 to 30 percents (Supar *et al*., 1989). In turn, this young animal mortality contributed considerable losses to the national farm income.

As an effort to control diarrhea and other gastro-intestinal disorders. farmers regularly added antibiotics to farm animal feeds, especially in poultry and swine rations. In the long run, this practice may damage the animal health. Supar *et al*. (1990) proved that several *E. coli* isolates were resistant to commonly use antibiotics. including Ampicillin, Streptomycin, Trimethoprim. and Sulphamethoxazole. Further observation show that 100 *E. coli* strains were resistant to at least one antibiotic. The highest percentages being attained for resistance was to Penicillin. Tetracycline and Cephalothin (Carvalho *et al*., 1992).

Plants used in factory medicine of Indonesian native people were collected in this experiment. Ethanol extract and powder were prepared and evaluated in a test against two isolates of *Escherichia coli* from herbal plants; mahkota dewa (*Phaleria macrocar-*

pa), daun sembukan (*Paederia foetida*), daun sirih/betel vine (*Piper betle*), kencur/greater galingale (*Kaempferia galangal*), garlic (*Allium sativum*) and jinten hitam (*Nigella sativa*).

MATERIALS AND METHODS

Materials

Methanol was used to make four concentrations of the plants drug (*Phaleria macrocarpa* extract, *Paederia foetida* extract, *Piper betle* extract, *Kaempferia galangal* extract, *Allium sativum* powder, and *Nigella sativa* extract) for this investigation. Mueller Hinton blood agar and broth media were used as growth media for the four bacterial isolates for this study. Additionally the blood agar media were also used as a purification control.

Isolates of *Escherichia coli* were collected from diarrhea calves in Indonesia by small farmers in Bogor, West Java. These specimens were later used for bacterial verification.

The obtained bacterial specimens were brought to BALITVET laboratory at Bogor. Here they were cultivated in the blood agar medium plates. The inoculated blood agar plates were incubated for 24 hours at 17^{o}C. The bacterial isolates grown in the blood agar plates were identified by employing Cowan and Steel methods (1973).

Extracting the Plants Drug

Dried the plants drug (*Phaleria macrocarpa* extract, *Paederia foetida* extract, *Piper betle* extract, *Kaempferia galangal* extract, *Allium sativum* powder, and *Nigella sativa* extract) were ground into powder. Methanol was then added to *Allium sativum* powder. To homogenize the mixture. the liquefied the plants drug (*Phaleria macrocarpa* extract, *Paederia foetida* extract, *Piper betle* extract, *Kaempferia galangal* extract, *Allium sativum* powder, and *Nigella sativa* extract) was shaken for one hour. This agitation was necessary to accelerate the solution of the plants drug (*Phaleria macrocarpa* extract, *Paederia foetida* extract, *Piper betle* extract, *Kaempferia galangal* extract, *Allium sativum* powder, and *Nigella sativa* extract) active compounds in the methanol solvent. The mixture was kept for 24 hours. The liquefied of the plants drug was filtered by paper filter. The obtained methanol solution containing the plants drug active compounds, was poured into a Florentine tube. The tube was placed in a rotary evaporator to evaporate the methanol solvent at 40^{o}Celsius at 140-160 rpm and at 15-20 lbs of pressure.

Sterile aquadest was added to the obtained extracts to make four concentrations of the plants drug i.e. 20, 15, 10 and 5 percents. Then 15 micro liters of each concentration was dropped into a sterile paper disks. Each disk was laid on the MEU blood agar media that had been previously inoculated with each of the three bacterial isolates and were incubated for 24 hours at 37 ^{o}C.

The bacterial growth inhibition zones were observed and measured. The size of the growth inhibition zones indicate the effectiveness of the plants drug (*Phaleria macrocarpa* extract, *Paederia foetida* extract, *Piper betle* extract, *Kaempferia galangal* extract, *Allium sativum* powder, and *Nigella sativa* extract) in controlling the bacterial infection.

Experimental Design and Data Analysis

The first factor observed in this *in vitro* test was the type of the plant drug (*Phaleria macrocarpa* extract, *Paederia foetida* extract, *Piper betle* extract, *Kaempferia galangal* extract, *Allium sativum* powder, and *Nigella sativa* extract) on the inhibition growth of *Echerichia coli* isolates. There were four levels of this factor, i.e. *E. coli* isolates taken from diarhea calves. The second factor was the concentrations of the plant drug. This factor had five levels which was 20, 15, 10 and 5%. The observed dependent variable of this investigation was the determine differences among the means of the diameters of growth inhibition zones.

The Analysis of Variance (Anova) was used to analyze the data, the diameters of the bacterial growth inhibition zones. The Duncan Multiple Range Test (DMRT) procedure was used to determine differences among the means of the diameters of growth inhibition zones.

RESULTS AND DISCUSSION

The analysis of variances shows that the effects of plant drugs (*Phaleria macrocarpa* extract, *Paederia foetida* extract, *Piper betle* extract, *Kaempferia galangal* extract, *Allium sativum* powder, and *Nigella sativa* extract) growth inhibition zones were significant (Table 1). The results also showed that the

Table 1. The effect of plant drug 20% concentration on two *E.coli* growth inhibition

Plants drug at % concentration	Dried	Diameter of growth inhibition zone (mm)	
		E.coli (1)	*E.coli* (2)
Phaleria macrocarpa	extract	0	0
Paederia foetida	extract	0	0
Piper betle	extract	11	11
Kaempferia galangal	extract	0	0
Allium sativum	powder	15	15
Nigella sativa	extract	13.30	13.30

greatest effects on bacterial growth inhibition were obtained when plant drugs of *Piper betle*, *Allium sativum* and *Nigella sativa* were used.

The effects of plant drugs of *Piper betle*, *Allium sativa* and *Nigella sativa* on bacterial growth inhibition were confirmed by the results in Table 2. The highest effect was obtained from *Allium sativa* powder, then was followed by *Nigella sativa* and *Piper betle* extracts (P<0.05).

Table 2. The effects of type plant drugs of *Nigella sativa* extract, *Allium sativum* powder, and *Piper betle* extract on the bacterial growth inhibition zones

Plants drug	Diameter of growth inhibition zone (mm)	Level of significant*
Nigella sativa extract	8.83	b
Allium sativum powder	11.15	a
Piper betle extract	7.25	c

*Different alphabet code indicated a significant difference at (P<0.5).

Table 3 demonstrates results of effects of different concentrations of plant drugs applied on growth inhibition zones. The results indicate that the increase in concentrations from 5 up to 20% produced greater effects in growth inhibition zones. The greatest growth inhibition zone was obtained at the greatest concentration, i.e. 20%.

Table 3. The main effect of the plant drug concentration increase on the bacterial growth inhibition

Plants drug concentration	Diameter of bacterial growth inhibition zone (mm)	Level of significant*
20.00	13.11	a
15.00	10.44	b
10.00	7.67	c
5.00	5.11	d

*Different alphabet codes indicate a significant difference at (P<0.5).

Table 4 indicates the combine effects of concentration and the three plant drugs (*Piper betle* extract, *Allium sativum* powder, and *Nigella sativa* extract) on *E. coli* growth inhibition zones. The results demonstrated that *Allium sativum* powder produced the largest growth inhibition zones which differed significantly from those obtained by *Nigella sativa* and *Piper betle* extracts at all concentrations. The largest growth inhibition zones was obtained by using *Allium sativum* powder at 20% concentration.

Table 4. Effects of combination between extract concentration and types of several plant drugs on *E.coli* growth inhibition zones

Extract concentration (%)	Type of plant drugs	Diameter of growth inhibition zones (mm)	Significant level
20 %	*Nigella sativa* extract	14.3	b
	Allium sativum powder	15.0	a
	Piper betle extract	11.0	c d
15 %	*Nigella sativa* extract	10.0	d
	Allium sativum powder	12.3	b c
	Piper betle extract	9.3	e
10 %	*Nigella sativa* extract	7.0	f g
	Allium sativum powder	10.0	d
	Piper betle extract	6.0	g
5 %	*Nigella sativa* extract	5.0	h
	Allium sativum powder	7.3	f
	Piper betle extract	3.3	i

*Different alphabet code indicated a significant difference at P<0,5 DMRT.

CONCLUSION

The plant drugs *Phaleria macrocarpa* extract, *Paederia foetida* extract, *Piper betle* extract, *Kaempferia galangal* extract, *Allium sativum* powder, and *Nigella sativa* extract had bactericidal effect on *E. coli.*

The higher the concentration of the plant drugs (*Phaleria macrocarpa* extract, *Paederia foetida* extract, *Piper betle* extract, *Kaempferia galangal* extract, *Allium sativum* powder, and *Nigella sativa* extract), the larger the diameter of the bacterial growth inhibition zones obtained.

Of the three plant drugs tested, *E. coli* growth was the most affected by *Allium sativum* powder at all concentrations.

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Domestic Grasses as Cattle Main Feed on Coastal Area at Desa Ujung Genteng, Kecamatan Ciracap, Kabupaten Sukabumi

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ABSTRACT

In general, forage for cattle in Indonesia comes from the domestic availability of grass, only efforts to improve the culture is still very limited. And also research related to domestic grass. Therefore, basic research is needed to feed the domestic grasses to determine its potential as a plant cultivation. The purpose of this study is to identify potential types of grass as a source of forages for cattle. Research conducted by survey method, direct observation, sampling plants, shooting, ex-situ preservation, creation and identification of herbarium. In general, there are cattle in the desa Ujung Genteng is Peranakan Ongole (PO). Cattle released for 24 hours in open areas along the coast. In addition to cattle, there are also sheep and goats. Performance of livestock in general either do not seem thinness indicated. Depends entirely forage available, particularly domestic grasses. There are 16 types of grass found along the coast, divided into 3 belt: Belt-1 (directly adjacent to the sea) consists of: *Dactyloctenium aegyptium* (L) wild, *Cynodon dactylon* (L) Pers., *Digitaria sanguinalis* (L.) Srop, *Ischaemum muticum* (L.) dan *Imperata cylindrica* (L.) P. Beauv.Gaertn,, Belt-2 (sand-dominated soil) consists of: *Chrysopogon aciculatus* (Retz.) Trin., *Brachiaria subquadripara* (Trin.) A. Hitchc., *Brachiaria distachya* (L.) Stapf., *Chloris barbata* Swartz., *Themeda triandra* Forssk., *Paspalum cartilagineum* Presl., *Digitaria nuda* Schumacher, *Paspalidium flavidum* A. Camus, *Eragrostis amabilis* (L.) Wight & Arnott ex Nees and *Eleusine indica* (L.) and Belt-3 (predominantly clay soil) consists of: *Eulalia leschenaultiana* (Decne.) Ohwi. Based of observation on field and ex-situ there are 5 types of grass that is cultivated potential for *Digitaria sanguinalis* (L.) Srop, *Ischaemum muticum* (L.), *Brachiaria subquadripara* (Trin.) A. Hitchc., *Brachiaria distachya* (L.) Stapf.dan *Paspalum cartilagineum* Presl.

Key words: domestic grass, sandy beaches, beef cattle

INTRODUCTION

In general, beef cattle feed in Indonesia comes from the domestic availability of grasses, only an effort to improve the culture is still very limited. Likewise, research related to domestic grass. Utilization of the beach as cattle grazing fields practically no attention. South Coast of West Java has a huge potential as a source of beef cattle. Coastal villagers in Kecamatan Ciracap utilize marginal land along the coast as livestock grazing areas, especially cattle. Beside that, they graze sheep and goat too. Fodder which is the most dominant are domestic grasses, just in general have relatively low productivity. This is related to the existing pastoral systems, climatic, edaphic and genetic potential of the grass itself. Basic data to determine the potential for much needed domestic grass, among others, the potential to be cultivated grass, adaptation to the saline environment and the response to fertilization. Therefore, basic research needed to find out its potential as grass cultivation. The purpose of this study is to identify potential types of grass as a source of green feed beef cattle.

MATERIALS AND METHODS

Research conducted in Desa Ujung Genteng, Kecamatan Ciracap, Kabupaten Sukabumi in October 2009 with the survey method. Implementation research is divided into 3 Belt: Belt-1: The area directly adjacent to the sea. Plants directly affected by the tidal sea water. Media grow sand. Observation area 5-10 m from the beach grass and bushes, Belt-2: Areas not directly adjacent to the sea. Growing media is dominated by sand. Coconut-dominated woody vegetation. There is also a rainfed rice farming area. Distance of 10-300 m from the beach, and Belt-3: Areas with growing media dominated clay. Distance from the beach 300-1000 m.

All types of grass that is observed along the coast, carried out shooting and

making planting materials. Next planting material is divided in two parts, the first made herbarium and second planted in Bogor. Ex-situ maintenance carried out to see the speed of growth, ease of maintenance, and response to fertilization. Herbarium grass and ex-situ cultivation then used in the process of identification through the study of literature and cross-check in Botany LIPI Research Center.

RESULTS AND DISCUSSION

Sandy beach is the extreme land for the plant because of the limited nutrients, high salinity and high porosity. Only plants that have a high adaptation that can grow or plant is very specific.

Belt-1 is the most extreme conditions and only 5 species of grass found in the area of, ie: *Dactyloctenium aegyptium* (L) Willd, *Cynodon dactylon* (L) Pers., *Digitaria sanguinalis* (L.) Srop, *Ischaemum muticum* (L.) dan *Imperata cylindrica* (L.) P. Beauv. Gaertn.

In general, grass can be maintained ex-situ that were located ± 250 km and a location with an altitude of 225-250 above sea level. There are only 2 types of death that is *Dactyloctenium aegyptium* (L) Willd. and *T. triandra* Forssk. Possibilities of death are 2 types of grass not because of low adaptability, but due to the planting material from pols are less good. Based on the collections of the two types of grass can grow well, just about the response to fertilization. Naturally both types are found in Bogor, especially *T. triandra* Forssk In the Belt-2 is more diverse grass species, 10 species were recorded there that have nothing to Belt-1, and vice versa on the grass that grows only 1 Belt-1 is not present in the Belt-2 and 3 of *I. muticum* (L.). Thus there is only one specific types of grass on the sandy beach of the Ujung Genteng village of tile *I. muticum* (L.).

Based on field observations and ex-situ, there are 5 types of potential for grass grown for livestock feed is *Digitaria sanguinalis* (L.) Srop, *Ischaemum muticum* (L.) *Brachiaria subquadripara* (Trin.) A. Hitchc., *Brachiaria distachya* (L.) Stapf. and *Paspalum cartilagineum* Presl. Especially for sandy beaches (belt-1) developed grass *I. muticum* (L.).

CONCLUSION

There are 5 types of grass as a potential domestic source of forage on the sandy beach of *Digitaria sanguinalis* (L.) Srop, *Ischaemum muticum* (L.) *Brachiaria subquadripara* (Trin.) A. Hitchc., *Brachiaria distachya* (L.) Stapf. and *Paspalum cartilagineum* Presl.

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.Tabel 1. Grass Type on Sand Beach at Desa Ujung Genteng, Kecamatan Ciracap, kabupaten Sukabumi.

No.	Grass Type	Belt	Maintenance of ex-situ		
			Live/dead	Grow Speed	Response to Fertilizer
1.	*D. aegyptium*	1	dead	-	-
2.	*C. dactylon*	1	Live	poor	poor
3.	*D. sanguinalis*	1	Live	well	well
4.	*I. muticum*	1	Live	very good	very good
5.	*I. cylindrica*	1	Dead	-	-
6.	*C. aciculatus*	2	Live	poor	poor
7.	*B. subquadripara*	2	Live	average	well
8.	*B. distachya*	2	Live	average	well
9.	*C. barbata*	2	Live	poor	poor
10.	*T. triandra*	2	Dead	-	-
11.	*Paspalum sp*	2	Live	poor	poor
12.	*D. nuda*	2	Live	well	well
13.	*E. unioloides*	2	Live	average	Well
14.	*E. unioloides*	2	Live	poor	well
15.	*E. indica*	2	Live	well	well
16.	*E. leschenaultiana*	3	Live	poor	well

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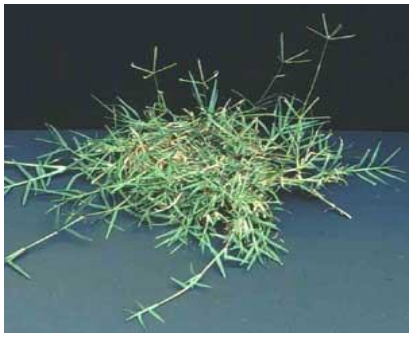
APPENDIX

Dactyloctenium aegyptium (L) wild



Cynodon dactylon (L) Pers

Digitaria sanguinalis (L.) Srop

Ischaemum muticum (L.)

Imperata cylindrica (L.) P. Beauv.Gaertn

Chrysopogon aciculatus (Retz.) Trin.

Chloris barbata Swartz.

Themeda triandra Forssk

Paspalum sp.

Digitaria nuda Schumacher

Panicum geminatum Forssk.

Eragrostis unioloides (Retz.) Nees ex Steudel

Eleusine indica (L.)

Eulalia leschenaultiana (Decne.)

Brachiaria subquadripara (Trin.) A.

Brachiaria distachya (L.) Stapf.

The Condition of Desa Ujung Genteng's Beach

The Kind of Cow in Desa Ujung Genteng

Contributions of *Bos Indicus* Breed to Genetic Diversity of Sumatra Native Cattle Based on Y-Chromosome Microsatellite Marker

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ABSTRACT

The genetic composition of cattle in the world generally tend to two dominated breeds, *Bos taurus* (European cattle) and *Bos indicus* (Asian cattle). From morphological phenotype could be differed by hump for Asian cattle and humpless for European cattle. Molecular study indicated that any alleles or haplotypes which tend to those dichotomy. Similar condition in Indonesian cattle, including Aceh and Pesisir cattle, previous study showed that any indication introduction of Taurine and Indicine breeds in those cattle, while in the other hand Indonesian native cattle is descendent from the one of common ancestor cattle in the world, Banteng (*Bibos banteng*). For advancing study in genetic introduction of other breeds in Aceh and Pesisir cattle, we assayed those Sumatra's breeds by using molecular marker of Y-chromosome microsatellite. The using of Y-chromosome marker by assumption could be a model for detection of male introduction in breed. From this research showed that all of locus have low allele number, both in Aceh and Pesisir cattle. Also from *Polymorphic Information Content (PIC)* value, this marker has lower value (less 0.5) than FAO recomended. But, in these result indicated that *B. indicus* is the one of the genetic composition of Aceh and Pesisir cattle. Because locus INRA 124 was could amplification in those breeds and these locus also the one of *B. indicus* specific allele.

Key words : Bos taurus, Bos indicus, Bos javanicus, Y-chromosome microsatellite, Aceh cattle, Pesisir cattle

INTRODUCTION

Asia continent has more or less three hundred millions cows and two hundred millions of them reside in India sub-continent. Cattle breeds in Asia and Africa are generally categorized into hump and humpless. About 170 breeds are already known, including Bali cattle (domesticated from Banteng or *Bibos banteng*) in Southeast Asia, including Indonesia and Philippine. So, Asian farmers have been famous in their role in cattle domestication process and agriculture ecology in this continent (Schearf, 2003).

Bali cattle and other breeds have genetic relationship with Banteng (just like Madura cattle) and also have genetic admixture with *B. taurus* and *B. indicus*. Because of economic and political factors in the past (during colonialism era or after the independence), they are brought to Indonesia and finally could adapt to the environment and become part of local cattle. Aceh cattle are believed to be the local breed, but the previous studies by Muhamad *et al*. (2007) and Ugla (2008) shows that the genetic compositions of those cattle came from *B. indicus*. So, the genetic study of native cattle in Indonesia is interesting because the genetic variations are great. This is important because it is related to the efforts characteristics improvement and keep genetic characters conserved. Finally, the quality of those native cattle does not decrease or even extinct.

The recent development on genome and genetic analysis on human population shows a tendency to haplotype of Y-chromosome which is an important tool in studying population naturally (Hurles & Jobling, 2001). Without neglecting pseudo-autosom, Y-chromosome action in general is a non-recombinant unit, which is male specific and effective haploid. This is to make sure that the combinations mutation along male offspring is concentrated as a single unbiased haplotype. Y-chromosome characters are needed in its context as male lineage just like mitochondrial DNA in female lineage. The level of polymorphism characters in non-recombinant area of Y-chromosome area is started from the lowest, that is biallele event in point mutation of single nucleotide polymorphisms (SNPs) to the most common

event in locus characters minisatellite or microsatellite (Short Tandem Repeat [STR]). Eventhough, the polymorphism of SNPs in Y-chromosome is often founded in specific population (Hammer *et al*. 1997). However, data of Y-chromosome in non-primate (like cattle, goat and lamb) genetic population is rare because of its rare marker in Y-chromosome in sequence information (Petit *et al.* 2002) and its low variation level (Hellborg & Ellegren 2004; Meadows *et al*. 2004; Queney *et al*. 2001).

Domestic cattle show indicated that individual variation into population based on microsatellite molecular marker is specific character to their sex chromosome (Y-chromosome), the existence of breed hybridization and their migration (McHugh *et al*. 1997; Bradley *et al*. 1998). Therefore, the existence of polymorphism in microsatellite specific to Y-chromosome can be used as a starting point in providing information about paternal genetic variation study on cattle or related species. The indication that growth hormone pseudogen (GH) is found in male domestic cattle shows that sex chromosome can influence individual differentiation process. It is important to know the role of sex chromosome in male cattle, for examples, in their reproduction ability.

Hanotte *et al*. (2000) study using microsatellite characters in Y-chromosome of INRA23 locus showed that there is any introduction of Zebu male in local cattle, Mozambique and Zimbabwe. It is suspected that their alleles came from Mozambique bay. In the past, Mozambique bay is an international trading area including cattle trading, so that was able to make genetic introduction of cattle from other breeds.

Edward *et al.* (2000) study using four characters microsatellite DNA marker (INRA124, INRA126, INRA 189 and BM 861) showed an evidence that any male genetic flow from *B. Taurus* or Zebu on hybrid population of bovidae species, including cattle. A study on African cattle using microsatellite DNA on Y-chromosome showed that there is a high different in cattle in central Africa and Southwest Africa.

The specific aim of this study is to know the pattern of Y-chromosome microsatellite DNA allele polymorphism in Aceh and Pesisir cattle, and to get Y-chromosome microsatellite DNA specific alleles of Aceh and Pesisir cattle.

METHODOLOGY

Collection of DNA Samples

DNA was extracted from total blood-cells which were collected from cattle as many as 10 ml per sample and preserved using EDTA 10%. The two areas where the samples came from were Aceh cattle from Breeding Center of Promising Aceh Cattle (*Balai Pembibitan Ternak Unggul (BPTU) Sapi Aceh),* Indrapuri Sub District, Aceh Besar Regency, Nangroe Aceh Darussalam Province and Pesisir cattle from Painan Sub District, Pesisir Selatan Regency, West Sumatera Province.

DNA Amplification and Allele Microsatellite Detection

DNA extraction using phenol-chloroform standard method (Sambrook *et al*. 1989) was then preserved in TE buffer. Locus-microsatellite amplification used seven primers which is flanks Y-chromosome microsatellite locus (INRA008, INRA057, INRA062, INRA 124, INRA 126, DYS 199, and INRA 189). Microsatellite allele PCR product were separated using PAGE 10% and observation was done manually (Leung *et al*. 1993) after silver staining (Tegelstrom 1986).

Y-Chromosome Microsatellite Allele Analysis

Microsatellite alleles were obtained from observation result and were analyzed statistically to get frequency and distribution value of allele, heterozygousity *(h)* allele and *Polymorphic Information Content* (PIC) in sample population.

Total number and frequency of microsatellite alleles were calculated to get the value of heterozygousity and alleles frequency per locus using formula:

$$f(A) = \frac{A}{2n}$$

where :

f (A) = Frequency of microsatellite allele
A = Total number alleles of i^{th} locus
N = Total number of individual observed

Locus is considered polymorphic if the value of f(A) is the same or less than 0,9. Genetics variation is determined from the average value of heterozygousity (*h*) for all locus. Heterozygousity value per locus is

calculate according to formula bellow (Nei 1987):

$$h = 2n\frac{\left(1-\sum Xi^2\right)}{\left(2n-1\right)}$$

where:
h = locus heterozygousity
Xi = alleles frequency of i^{th} locus
N = total number of individu observed

Allele frequency is also used to get Polymorphic Information Content (PIC) index, i.e. value to calculate marker informative level, calculated according to Botstein *et al.* (1980) formula:

$$PIC = 1 - \sum_{i=1}^{k} P_i^{\,2} - \sum_{i=1}^{k-1}\sum_{j=i+1}^{k} 2P_i^{\,2}P_j^{\,2}$$

Where k is total alleles number, P_i and P_j are allele frequency of i^{th} and j^{th} allele respectively at microsatellite locus observed.

RESULTS AND DISCUSSION

The developments of some genetic markers that are used in cattle breeding because of the molecular markers are more discriminative and accurate than their phenotypic characters. But, the utilizing of this marker has not been maximally yet. By using this marker would have helped in handling cattle breeding management system (Ge *et al.* 2002).

Breed specific allele information for Indonesian domesticated cattle until recently is limited. From previous studies (Muladno *et al.* 2000; Noor *et al.* 2000; Winaya 2000; Winaya *et al.* 2000; Mohamad *et al.* 2007; Ugla 2008) that use DNA microsatellite marker has been showed that these markers can also give description of breed specific allele and genetic relationship between Indonesian native cattle (i.e. Aceh, Pesisir, Madura, Bali, PO and PFH), but this result can not shows yet the description about the genetic variation of Indonesian native cattle based on Y-chromosome DNA microsatellite in more detail. So, this study may be consideration as a preliminary data base to determine the genetic composition of Indonesian native cattle in general.

PCR product using seven primers which is flanks microsatellite sequence were loaded using polyacrilamide gel electrophoresis / PAGE 10% then continued with silver staining. Microsatellite allele was detected manually either its number and its size and next analysis. Separation result using polyacrylamide gel generally can determine number and allele frequency as shown in Table 1 below.

Table 1 showed that we found only two alleles for all of locus by means 1.7, both in Aceh or Pesisir cattle. So, this allele still could not be determined as polymorphic allele yet, because according to FAO minimum standard locus has minimally four different alleles to be used as judgment in determining genetic differentiation between groups of

Table 1. Number of cattle assayed, allele number and frequency of seven Y-chromosome microsatellite locus in population of Aceh and Pesisir cattle

LOCUS	ACEH			PESISIR		
	1	2	3	1	2	3
INRA 008	18	2	A = 3 (0.17) B = 15 (0.83)	15	2	A = 3 (0.20) B = 12 (0.80)
INRA 057	18	1	A = 18 (1)	15	2	A = 2 (0.13) B = 13 (0.87)
INRA 062	18	2	A = 6 (0.33) B = 12 (0.67)	15	2	A = 7 (0.47) B = 8 (0.53)
INRA 124	18	2	A = 5 (0.28) B = 13 (0.72)	15	2	A = 3 (0.20) B = 12 (0.80)
INRA 126	18	1	A = 18 (1)	15	2	A = 1 (0.07) B = 14 (0.93)
DYS 199	18	2	A = 3 (0.17) B = 15 (0.83)	15	1	A = 15 (1)
INRA 189	18	2	A = 4 (0.22) B = 14 (0.78)	15	1	A = 15 (1)

Note: 1) number of cattle assayed; 2) allele number; 3) allele frequency.

Table 2. Heterozygosity *(h)* and Polymorphic Information Content *(PIC)* value of Y-chromosome microsatellite allele between Aceh and Pesisir cattle population

LOCUS	ACEH		PESISIR	
	h	*PIC*	*h*	*PIC*
INRA 008	0.30	0.24	0.34	0.27
INRA 057	0	0	0.24	0.20
INRA 062	0.47	0.34	0.53	0.37
INRA 124	0.43	0.32	0.34	0.27
INRA 126	0	0	0.14	0.12
DYS 199	0.30	0.24	0	0
INRA 189	0.36	0.28	0	0
Mean $\pm$ SD	0.27 $\pm$ 0.19	0.20 $\pm$ 0.14	0.23 $\pm$ 0.20	0.18 $\pm$ 0.14

cattle (Pandey *et al*. 2006). From this study it is also revealed that genetic composition of Aceh and Pesisir cattle are also contain of *B.indicus* genetic. Because INRA124 also the one of *B. indicus* locus (Hanotte *et al*. 2000; Edwards *et al*. 2007). These result, therefore, are in line with the previous research results that *B indicus* is one of genetic source of the genetic composition of Aceh and Pesisir cattle (Mohamad *et al*. 2007; Ugla 2008).

From previous study (Winaya *et al*. 2008), the highest heterozygousity *(h)* value has been found in microsatellite locus of DYS 199 (68%) in Madura cattle population. It shows that the higher the number of allele, higher the heterozygousity tendency will be. Again, according to FAO guideline, here must be four different allele locus minimum to justify the differentiation or genetic variation between breed (Pandey *et al*. 2006).

From polymorphic information content *(PIC)* value, the Y-chromosome microsatellite marker has reveals *PIC* value from 0.18 to 0.23. This result could be explained that in generally all of the locus could not be determined as an informative allele for population genetic analysis, because the PIC value less than 0.50 (Botstein *et al.* 1980). As Meadows *et al*. (2006) study has been found the low value in nucleotide variation sequence of specific Y-chromosome area in some animal species, including cattle. So, from this study we still need more locus of Y-chromosome microsatellite marker for genetic analysis of Indonesian native cattle in generally. As we know that until recently for genetic evaluation is trend to use the Y-chromosome microsatellite marker. So, eventhough this study was not found the polymorphic locus, but for future study we still need more number of Y-chromosome DNA microsatellite marker, because this marker could be as a tool to determine the genetic specific of Indonesian native cattle in much more detail.

CONCLUSION

The genetic flow of both *B taurus* and *B indicus* influencing genetic composition of Indonesian native cattle, including Aceh and Pesisir cattle, eventhough, we knew that Indonesian native cattle breed generally still have a lineage from Banteng (*Bos banteng*), the one of ancestor cattle in the world until nowadays. This trend has also been described in this study, by using Y-chromosome microsatellite markers, those cattle populations have indicated any genetic composition of *B indicus*, with locus INRA124 as reference.

The use of molecular marker of Y-chromosome microsatellite has generally described potentials and genetic variations of Aceh and Pesisir cattle, eventhough there was not found the polymorphic and informative allele. But, for the next study we can use more locus of Y-chromosome DNA microsatellite marker, because we still need more data base for genetic potential of Indonesian native cattle, especially the genetic composition of Aceh and Pesisir cattle. So, the genetic existence of those cattle as specific cattle can be improved.

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Measuring the Responses of Different Genotypes of Slow Growing Broilers Toward Short-Term Heat Challenge Test

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ABSTRACT

This study was performed to evaluate the responds of different genotype of slow growing broilers with regard to heat stress. A number of 102 females from the slow growing broiler hybrids (Hubbard ISA I657, S757N and I957) were raised from hatch until week 5 in 3 pens under the same room temperature of 30°C beginning from week 3 until 5. Twenty four experimental birds of each genotype were individually exposed for 15 minutes to a short-term heat test at 30°C (control) and 35°C between weeks 3, 4, and 5. The rectal temperatures before and after heat exposure were measured and the latency until panting was recorded. Strain differences were significant for body weight, daily weight gain and relative growth rate ($P<0.01$). For I657, S757N and I957, respectively, body weight in week 5 averaged 815.8±81.2, 924.0±87.9 and 1269.3±136.3 g. Daily gain averaged 22.0±9.8, 25.5±13.1 and 34.9±17.6 g/d, whereas relative growth rate ranged between 11.5±5.5, 13.9±6.9 and 13.0±6.1 %. Rectal temperatures after short-term heat stress were 42.4±0.7°C, 42.4±0.7°C and 42.7±0.7°C, with strains differing significantly ($P<0.01$). The level of heat stress temperature significantly influenced latency until panting ($P<0.01$). When exposed to 35°C, birds started panting within 10.95±2.43 (I657), 12.26±2.61 (S757N) and 10.16±2.36 (I957) minutes. The chi-square analyses revealed significant influences of the heat level and the strain on the frequency of birds panting ($P<0.01$). After 35°C test, 96% (I657), 100% (I957) and 67% (S757N) of birds demonstrated panting ($P<0.01$), while strain differences were not significant for frequency of birds panting exposed to 30°C.

Key words: slow growing broilers, short-term heat stress, rectal temperatures, panting, growth

INTRODUCTION

Heat stress is one of the important stress factors especially in tropical and subtropical environments which effects the productive performance of broilers. High mortality decreased feed consumption and poor body weight gain as disadvantages have been reported by many authors. Beside high ambient temperature, the large contribution to heat production occurs in the bird itself since metabolic production increases as the body weight of bird progresses (Lott *et al*., 1998).

Under hot environment, heat production decrease whereas heat dissipation increase. When air temperature climbs, the breathing frequency of birds increases and the evaporative heat loss enhances significantly (Wiernuz and Teeter, 1996) and dissipated through respiratory evaporation as the main avenue (Hillman *et al.*, 1985).

Increased heat tolerance is reflected in lower body temperature and the limit of temperature tolerance is affected by body weight. Sykes and Fataftah (1986) reported the index of heat tolerance is the increasing rate of rectal temperature from the start and after one hour of exposure. Value of 2^oC/h or more reflects rapidly rising body temperature meanwhile, value of $\leq 0.5^oC$/h indicates effective heat tolerance.

The intensive genetic selection for rapid growth rate has been associated with increased susceptibility of broilers to heat stress. Birds selected for rapid growth demonstrate higher body temperature (low heat tolerance) compared to slow growing birds which have a greater tolerance to high temperatures (Cahaner and Leenstra, 1992, Berong and Washburn, 1998).

The present experiment was conducted to develop a suitable method to measure reactions of slow growing broilers towards heat stress and to evaluate the differences between three genotypes of commercial slow growing broiler hybrids with regard to heat stress reactions.

MATERIALS AND METHODS

A total of 102 day old chicks from 3 commercial broiler hybrids (Hubbard ISA, France) differing in growth patterns hatched were imported from France. Genotypes used

were I657 (slow growth, red), S757N (slow growth, Nn) and I957 (medium slow, white). All chicks hatched in the same hatchery on the same day and sexed and only females were used from hatch until 5 weeks of age. Birds were wing banded and housed in the same room in 3 pens (measuring 6.40 m^2/pen) having 34 chicks in each (stocking density of 5.3 birds/m^2) with strains separated. The ambient temperature was maintained at 30°C in week 3 until the end of the experiment. Birds were fed *ad libitum* with starter crumble diet (22.76% CP and 11.92 MJ/kg ME) during the first 3 weeks and a 2 mm grower pellets diet (19.67% CP and 12.13 MJ/kg ME) afterwards. Water was freely available. Productive traits measured were hatch weight, individual body weight, weight gain, relative growth rate, feed intake and feed conversion for each pen.

Birds were individually placed into a heat challenge compartment and exposed to short term heat stress (35°C or 30°C as control) between 3, 4, and 5 weeks of age to evaluate the reaction of birds to high temperatures. From each strain, 24 experimental birds were randomly chosen per week. Temperature during heat challenge varied between 30±0.2°C and 35±0.2°C.

Before subjecting the birds to heat challenge test, the rectal temperature was measured using a digital thermometer to the nearest 0.1°C. The bird was then individually placed into the test apparatus for 15 minutes at 30°C or 35°C. The sequence of temperature within the tests was at random for the individual bird. During the heat challenge test, the latency until panting (minute) was directly measured using a stopwatch. After 15 minutes, the bird was taken out of the cage and its rectal temperature was immediately measured. For birds that did not start panting within 15 minutes, latency until panting was set to 15 minutes. In addition, the proportion of birds panting was calculated (%).

Data were analyzed using the General Linear Model (GLM) procedure of SAS (SAS Institute Inc., 1990). Least Square Means (LSM) were calculated and differences among mean were differentiated by Duncan's multiple range test. Strain differences in frequencies of birds panting were analyzed by Chi square tests (Siegel, 1985).

RESULTS AND DISCUSSION

Growth Performance

The three strains differed significantly in body weight, daily weight gain and relative growth rate until 5 weeks of age. At week 5 the white strain weighed the heaviest followed by the Nn and the red strain. The weight gain and relative growth rate associates with the growing capacity and the level of feed intake and conversion. The white birds (medium slow) consumed most feed and tended to convert it much efficient than the red and the Nn birds. Although the red and the Nn birds are slow growing broilers, starting from week 3 to the end of the experiment, the Nn birds tended to consumed more feed than the red birds with the same level of conversion resulted in more weight gain and higher relative growth rate than the red birds. At high ambient temperature, feather coverage was negatively correlated with body weight gain because a higher body temperature results in a larger growth depression because of heat (Cahaner *et al*., 1993; Eberhart and Washburn, 1993). This result is in line with Bordas *et al*. (1978) and Yahav *et al*. (1998) that reported that the naked neck birds tend to gain more weigh at high ambient temperature and consume more feed. At the end of the rearing period, the red and the Nn birds weighed respectively about 64% and 72% from the body weight of the white strain (*Table 1*). The body weight development of each strain of birds from hatch until 5 weeks of age is illustrated in *Figure 1*.

Deep Body (Rectal) Temperatures

The strain, age and level of heat challenge influenced significantly on the rectal temperatures before (T0) and after (T1) heat challenge. The white birds have higher body weight than other counterparts and generate more heat and enhance deep body temperature which is reflected in higher rectal temperatures (T0 and T1) since metabolic production increases as the bird progresses in body weight (Lott *et al*., 1997).

Although the Nn are heavier than the red birds, the reduction about 20% of feather coverage around the neck allows the Nn birds to dissipate higher rate of irradiation of the internally metabolic body heat to the environment through the unfeathered skin better than the feathered surface, thus improving

Table 1. Productive performance of different strains of birds (LS-Means ± SD) by week

Traits	Age (d)	I657 (Red)	I957 (White)	S757 (Nn)	F	P
Body weight (g)	29-35	815.8 ± 81.2 [c]	1269.3 ± 136.3 [a]	924.0 ± 87.9 [b]	183.27	0.000
Weight gain (g/d)	29-35	33.9 ± 5.1 [c]	55.0 ± 7.3 [a]	42.3 ± 4.9 [b]	110.45	0.000
	Ø 1-35	22.0 ± 9.8	34.9 ± 17.6	25.5 ± 13.1		
Relative growth rate (%)	29-35	5.8 ± 0.6 [c]	6.2 ± 0.6 [b]	6.7 ± 0.5 [a]	21.79	0.000
	Ø 1-35	11.5 ± 5.5	13.9 ± 6.9	13.0 ± 6.1		
Average feed intake(g/d)	4-5	67.3	106.9	83.7		
	Ø 1-35	39.9 ± 21.5	62.0 ± 36.4	47.0 ± 27.3		
Feed conversion	4-5	1.98	1.94	1.96		
	Ø 1-35	1.74 ± 0.22	1.70 ± 0.21	1.77 ± 0.18		
% BW from white	29-35	64.3		72.8		

Note: [a, b, c] Means within the same row with different superscript differ significantly (P<0.05).

Table 2. Rectal temperatures (°C) before, after and the difference of birds subjected to short-term heat stress in 3, 4 and 5 weeks (LS-Means ± SD)

Item		Rectal temperature (°C)		
		T0	T1	TC
Strain	I567 (Red)	41.83 ± 0.64 [b]	42.43 ± 0.66 [b]	0.61 ± 0.31 [a]
	I957 (White)	41.95 ± 0.63 [a]	42.66 ± 0.66 [a]	0.71 ± 0.37 [a]
	S757N (Nn)	41.79 ± 0.66 [b]	42.40 ± 0.75 [b]	0.60 ± 0.31 [a]
		P=0.020	P=0.000	P=0.208
Age	3 weeks	42.67 ± 0.25 [a]	43.31 ± 0.34 [a]	0.64 ± 0.33 [a]
	4 weeks	41.51 ± 0.27 [b]	42.12 ± 0.45 [b]	0.61 ± 0.37 [a]
	5 weeks	41.39 ± 0.31 [c]	42.06 ± 0.37 [b]	0.67 ± 0.30 [a]
		P=0.000	P=0.000	P=0.639
Challenge temperature	30°C	41.79 ± 0.65 [b]	42.30 ± 0.69 [b]	0.51 ± 0.30 [b]
	35°C	41.92 ± 0.63 [a]	42.70 ± 0.64 [a]	0.77 ± 0.33 [a]
		P=0.003	P=0.000	P=0.000

Note: [a, b, c] Means within the same column with different superscript differ significantly (P<0.05);
T0= rectal temperature before heat challenge; T1= rectal temperature after 15 minutes of heat challenge; TC= temperature difference between T0 and T1.

thermoregulation under high ambient temperature as reflected by lower body temperature (Yahav *et al*., 1998) and reduces body temperature change when ambient temperature was elevated from 24 to 32°C (Deeb and Cahaner, 1999).

Increased age decreased T0 and T1. Younger birds had higher T0 and T1 and decreased within the next age. All birds exhibit higher T0, T1 and TC when they were exposed to higher level of temperature (*Table 2*).

Latency Until Panting and Frequency of Panting

When ambient temperature raises and approaches body temperature the evaporative heat loss is significantly enhanced (Wiernuz and Teeter, 1996). The respiratory evaporation becomes the main avenue of heat dissipation (Hillman *et al*., 1985) and noticeable as panting in birds. Latency until panting of birds revealed significant strains and levels of heat challenge differences. The red and white birds started panting at the same time but earlier than the Nn birds. All strains began panting much earlier if they were challenged to higher level of heat stress (Table 3). The higher rate of the internally metabolic body heat and heat received from the environment can be more efficiently dissipated by means of sensible heat throughout the unfeathered skin around the neck in Nn birds. Decreasing of feather cover allows the Nn birds to dissipate more heat by means of sensible heat loss better than the other full feathered white and red strain. Therefore, the Nn birds started panting much later than the other counterparts, although over 3 weeks of age the Nn birds have more weight than the red birds.

The chi square analyses revealed a significant influence of the level of heat challenge on frequency of birds panting (P≤0.001). In red and white birds, about 12.5% and 8.3%; and 96% and 100% started panting if they were exposed to 30°C and35°C, respectively. None of the Nn birds started panting if they were exposed to 30°C, and about 67% to 35°C. The frequency of birds panting differed significantly if the birds were exposed to heat challenge at 35°C

Table 3. Latency until panting (min) of birds subjected to short term heat stress (LS-Means ± SD)

Item		Latency (min) ± SD	P
Strain	Red (I657)	12.86 ± 2.63 [b]	0.011
	White (I957)	12.44 ± 2.92 [b]	
	Nn (S757N)	13.63 ± 2.29 [a]	
Challenge temperature	30 °C	14.83 ± 0.76 [a]	0.000
	35 °C	11.12 ± 2.58 [b]	
30°C	Red (I657)	14.77 ± 0.83	0.067
	White (I957)	14.72 ± 1.03	
	Nn (S757N)	15.00 ± 0.00	
35°C	Red (I657)	10.95 ± 2.43	
	White (I957)	10.16 ± 2.36	
	Nn (S757N)	12.26 ± 2.61	

Note: [a, b, c] Means within the same column with different superscript differ significantly (P<0.05).

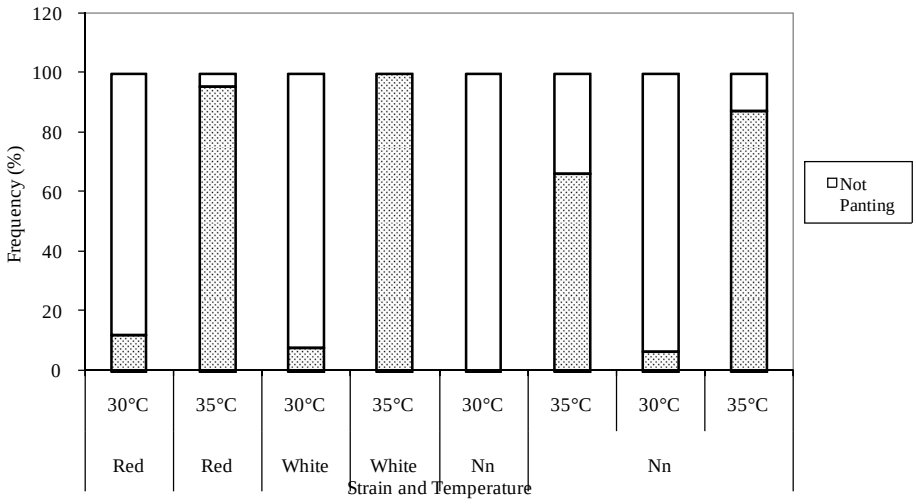

Figure 1. Distribution of frequency birds panting by strain and challenge temperature

(P≤0.001). About 7% and 88% of birds exhibited panting if they were challenged to 30°C or 35°C, respectively (*Figure 1*).

CONCLUSION

Naked neck birds exposed higher adaptability and heat tolerance to high ambient temperature. Short-term heat challenge test was suitable to detect clear differences between genotypes.

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Productivity of Local Pigeon Fed with Cafetaria Method in Intensive Rearing

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ABSTRACT

This experiment has been done to evaluate productivity of local pigeon fed with corn or commercial feed using cafeteria method in pre-laying, hatching and production (squab suckling) phases in intensive rearing. There are 68 couples of local pigeon used in this experiment, and each couple is placed in individual cages. The results indicate that average of egg production is 1.8 eggs/couple/period, average of egg weight is 17.7 g, fertility is 96.6%, hatching rate is 77%, embryo mortality rate is 23%, interval period from laying up to hatching and suckling is 51 days, 31.4 days for period of hatching, and 17.6 days for period without (non) hatching and suckling. Each couple of local pigeon need 73.04 g feed/day in pre-laying phase, 60.38 g feed/day in hatching phase, and 91.75 g feed/day in suckling two squabs; these are based on the total feed consumptions of corn and commercial feed. During hatching phase, corn consumption is the same as commercial feed consumption in week I, II and III. During non-hatching phase, corn consumption differs from that of commercial feed consumption. During this phase, corn consumptions are the same in all weeks (I=II=III=IV); commercial feed consumption at week II is the lowest, but there is no different in commercial feed consumption at the other weeks (II<I=II=III). During squab suckling phase, there are differences in pattern of corn consumption from that of commercial feed consumption; corn consumption increases from week I up to week III (I<II<III=IV), commercial feed consumption at week I-III are lower than that at week IV (I=II=III<IV). In all phases, corn is more preferred than commercial feed, but the commercial feed can still be given and the best ratio between corn and the commercial feed is 60:40%. Squab weight increases up to the 4^{th} week, then decrease in the 5^{th} week. Growth rate is the highest at the 1^{st} week, but then decreases from the 2^{nd} up to the 5^{th} weeks with the negative growth rate occurs at the 5^{th} week. Squab growth rate follows a quadratic pattern with this formula : $Y = 11.2 + 121t - 13.3t^2$. Feed conversion ratio up to the age of 4^{th} week is 5.7. It is concluded that squab selection on the basis of slaughter weight can be done at the 4^{th} week old.

Key words: pigeon, productivity, consumption, squab growth rate, and cafeteria feeding

INTRODUCTION

Commonly, the owner of pigeon feed their pigeons just with corn or other grains such as rice grain. Corn or other grains do not meet pigeon nutrient requirement for reproduction. However, there is limited information of feed requirement of local pigeon.

Feeds that are suitable with the pigeon needs in intensive rearing are necessary to obtain their productivity as expected. It is expected that the pigeon lay 1-3 eggs per period with average is 2 eggs per period (Levi, 1945). Cock and hen hatch the eggs with hatching time allocation for hen are longer than the cock. The first egg hatch 17-18 hour after the eggs are laid, and the second egg hatch 48 h after the first eggs are laid (Blakely and Bade, 1989). Hen will lay again after the squab reaching the age of 2 weeks. Both cock and hen suck their squab.

The pigeon is able to consume simple feeds consisting of grains and a little good grit; the pigeon also needed clean water (Anggorodi, 1995). Drevjany (2001) also reports that pigeon could be fed with feed that was made up of crumble ration or mixed of grains, minerals, grit and water. Among the feeds, pigeon liked grains such as corn, soya bean, peanut and wheat grain (Alwazzan, 2000). A good feed for pigeon contains nutrient composition as follows: 13.5% crude protein, 65% carbohydrate, 3.5% fibre and 3.0% fat; minerals, vitamins and grit also need to be added.

There are no pigeon feeds available in poultry shop that is produced commercially. A mixed of corn and broiler diet can be given to the pigeon during production phase. Those feeds are expected to be able to meet the nutrient requirement for the pigeons in intensive rearing. The commercial feeds given to the pigeon is also needed to increase the produc-

tivity of pigeon and to replace the use of grains such as corn. The pattern of pigeon consumption for those feeds needs to be observed as well as the parental productivity and squab growth per week. This information is important for rearing the pigeons especially those in intensive rearing.

Therefore, the purpose of this experiment is to study the effects of giving feeds consisting of corn and commercial broiler diet in cafeteria feeding on pigeon productivity, consumption patterns of pigeons in pre-laying, hatching and suckling phases, and squab growth in intensive rearing. The advantage of this experiment is to provide information to pigeon owner and hobbies about pigeon feeds that are given in cafeteria feeding in intensive feeding.

MATERIALS AND METHODS

This experiment used 68 couples of adult local pigeons (*Columba livia*). Each couple of pigeon was placed in a cage (60x50x50 cm^3) that was made up of wire (1.2x1.2 cm^2). Feeds and water troughs were placed in each cage. As the pigeon was fed with cafeteria feeding, there were two feed troughs available, one for corn and the other for commercial broiler diet. Feeds and water were given *ad libitum.*

Variables that were observed included parental productivity, feed consumption for each kind of feed in different phase and squab growth that were recorded every week. The data were analysed descriptively; however, data for feed consumption was examined by T-test (Steel and Torrie, 1995).

RESULTS AND DISCUSSION

Pigeon productivity

Productivity of local pigeon is shown in Table 1, the productivity data included productivity characteristics and parental reproductions.

There are 12 out of 68 couples of pigeon that do not hatch. The percentages of pigeon that hatch are 82.4%. This hatching variation is due to genetic factor and there is no selection for good hatching characteristic from the maternal ability. Hatching variation is also affected by other factors, such as the pigeons do not like the provided nest although they lay the eggs.

Commonly, a pigeon lays two eggs per period of laying. In this experiment, the average of egg production per couple is 1-3 eggs. It is observed in this experiment that there are three hens laying one egg, and one hen laying three eggs.

This result is in agreement with that was obtained by Levi (1945). Egg production is influenced by factors such as poultry instinct to adapt to the environment; on the other hand, poultry laying capacity is determined by genetic capability and environment (Rasyaf, 1985). Egg weight is about 12-20 g with the average is 17.7 $\pm$ 1.6 g and coefficient variation is 90% in this experiment. Ensminger (1992) indicates that poultry egg weight is influenced by genetic, body weight and age. Egg numbers that have been produced in a year is affected by clutch, feed protein content, feed and water, temperature, cage type and diseases. The heritability off egg weight is 0.6 (Noor, 2000), and 0.4-0.85 (Etches, 1996). The egg weight has high heritability. This means that individual selection for selecting parents having big eggs is effective for increasing pigeon egg weight.

Tabel 1. Productivity of local pigeon

Variables	Mean $\pm$ sd	Range	Coefficient of Variation (%)
Maternal ability (%)	82.4	-	-
Egg weight (g)	17.7 $\pm$ 1.6	12-20	9.0
Egg production (egg)	1.8 $\pm$ 0.6	1-3	33.0
Shape index (%)	75.7 $\pm$ 5.6	-	7.5
Fertility (%)	92.5	-	-
Hatchability (%)	77.0	-	-
Day old pigeon (g)	14.0 $\pm$ 1.2	10.9-16.2	8.0
Embryo mortality rate (%)	23.0	-	-
Laying interval period (days)			
1. hatching and suckling	51.0	-	-
2. hatching only	34.1	-	-
3. non hatching	17.6	-	-

Pigeon egg shape, egg round with shape index, is 75.5 ± 1.6% with coefficient of variation is 7.5%. In this experiment, the egg shape is almost identical. The colour of pigeon egg shell is also identical which is white.

Fertility and hatching capacity, respectively, are 92.4% and 77% in this experiment. This means that there is small percentage of unfertilized eggs or not fertilized eggs (7.6%). However, not all the fertile eggs are hatched to be squab. This indicates that there is 23% of fertile eggs died during hatching phase.

Pause is interval laying time in one period with the next laying period. The average of pause in non-hatching period in this experiment is 17.6 days, pause in hatching period is 34.1 days, and totally the pause from laying, hatching and up to suckling the squab is 51 days.

Pigeon Feed Consumption

Pigeon feed consumption during hatching, suckling and pre-laying in this experiment is divided into two types of feeds that are given, i.e. corn and commercial broiler diet. Feed pattern for each of feed types is useful for pigeon breeding. Pigeon consumption of corn and commercial broiler feed is shown in Table 2.

During the phase of not hatching (non-hatching phase), the amount of corn consumed is 283 g/couple/week in week I. Corn consumption decreases in week II reaching 267 g; this corn consumption increases to 321 g in week III, but there is no significant increase in week IV (323 g). These patterns are also observed when commercial broiler feed is consumed by the pigeon and when the total consumption is calculated. However, the corn consumption is higher than that of commercial feed. Comparison between corn consumption and commercial feed consumption indicates that the difference is not significant in week I. There are significant differences observed in week II and III ($P<0.01$) and week IV ($P<0.05$). This means that corn consumption produces more effect than that of commercial feed and this could be due to the form of corn, i.e. grains, vs crumble in commercial feed.

During the phase of hatching, corn consumption reduces linearly from 236 g in week I to 226 g in week II ant to 219 g in week III. This pattern differs from that of commercial feed consumption which tends to follow quadratic pattern; the consumption is 168 g in week I, decrease to 148 g in week II, and then increases up to 171 g in week III. When the corn consumption is added with that of commercial feed, the total consumption also has quadratic pattern, but in the reverse pattern of commercial feed consumption. This means that the corn consumption produces a greater effect than that of commercial feed. The ratio of corn consumption to commercial feed consumption is 60 : 40% in hatching phase.

In suckling phase, a hen with two squabs consumed corn at 331 g at week I which increases linearly to 365, 383 and 397 g, respectively at weeks II, III and IV. A similar pattern to corn consumption also occurs when commercial feed is given. These linear increases in corn and commercial feed also cause linear increases in total consumption of corn and commercial feed for week I up to week IV. Differences between com and commercial feed are significant for week I up

Table 2. Feed consumption per couple

Phase	Feed	Week				Week				
		1	2	3	4	1	2	3	4	Average
		g/week				g/day				
Not hatching	Corn	283	267	321	323	40.43	38.14	45.86	46.14	42.64
	Commercial feed	233	151	228	239	33.29	21.57	32.57	34.14	30.39
	T-test	ns	**	**	*	ns	**	**	*	
	Corn + commercial feed	516	418	549	562	73.71	59.71	78.43	80.29	73.04
Hatching	Corn	236	226	219		33.71	32.29	31.29		32.43
	Commercial feed	168	148	171		24.00	21.14	24.43		23.28
	T-test	**	**	**		**	**	**		
	Corn + commercial feed	404	474	390		57.71	67.71	55.71		60.38
Suckling (2 squabs)	Corn	331	365	383	397	47.29	52.14	54.71	56.71	52.71
	Commercial feed	248	261	288	296	35.43	37.29	41.14	42.29	39.04
	T-test	**	**	**	**	**	**	**	**	
	Corn + commercial feed	579	626	671	693	82.71	89.43	95.86	99.0	91.75

to week IV. The highest consumption of commercial feed in week I is due to requirement of squab to smooth feed that contains high nutrient concentration. This can be provided by milk crop produced by the parents, and by feeds eaten by the parents; feeds in bigger size than commercial feed, such as corn, can only be eaten by the squabs after 6 days old in this experiment. Mire and Plate (2009) indicate that pigeon fan can be fed with 15% protein (pellet) or a mixed of grains and layer hen feed consisting of 16-17% protein.

This experiment indicates that the average of feed consumption in non hatching phase is 73.04 g/couple/day, in hatching phase is 60.38 g/couple/day and in suckling phase is 91.75 g/couple/day.

Squab body weight gain and feed conversion ratio

Table 3. indicates body weight of squabs. Squabs grow quickly during the first up to third weeks, growth rates then decrease until the squab is weaned at 35 days old. High growth rate occurs during week I – II, growth rates decrease linearly with the lowest growth rates with negative result occurs at week V.

This experiment also indicates that squab growth curve from hatching up to weaning in 35 days old followed quadratic pattern, i.e. $Y = 11.2 + 121\ t - 13.3\ t^2$; Y = weight and t = time (age). Figure 1. shows that the highest growth rate occurs until the squab reaching 14 days old, then decrease with negative result after 28 days. The results of squab carcass indicate that selection should be done when the squabs reaching 21-28 days old and before the squab is weaned at 35 days. This is also because of growth rate is low, but the squab weight is the highest in the fourth week, it then decreases in fifth week.

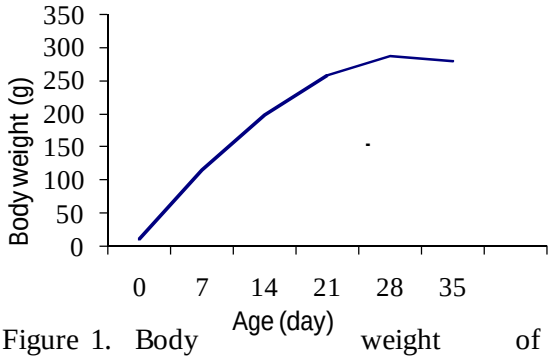

Figure 1. Body weight of squab from 0 – 35 days

Feed conversion rate for squab is high. This is because of high growth rate occurs during the first three weeks. It then decreases at the fourth and fifth weeks due to reductions in growth rate of squab (Table 4). This indicates that feed efficiency decreases with the increases in age.

CONCLUSION

There are variations in egg production which is about 1-3 egg/couple/period. Fertility is 96.9% with hatching capacity 77% selection for good iparental is good to increase hatching capacity and fertility. Each couple of local pigeon need 73.04 g feed/day in pre-laying phase, 60.38 g feed/day in hatching phase, and 91.75 g feed/day in suckling two squabs; these are based on the total feed consumptions of corn and commercial feed. The ratio between corn and commercial feed is 60:40%.

Weaning weight of squab in the 4th week is the highest, i.e. 290.4 g; growth rate reduces at the 5t^{h} week; the growth rate followed quadratic pattern. Slaughter weight selection can be done at four weeks old.

Table 3.Body weight and growth of squab

Week	Body weight (g/bird)		Coefficient of variation (%)	Period between week	Growth rate (%)*
	Means ± sd	Range			
0 (hatched)	14.02±1.20	10.9- 16.2	8.56		
I	112.22±27.98	60.0- 80.0	24.93	0-I	155.58
II	202.77±47.51	93.0-306.0	23.43	I-II	62.57
III	256.16± 8.24	192.0-355.0	22.74	II-III	23.27
IV	290.40±27.98	170.0-340.0	9.63	III-IV	16.92
V	282.17±44.43	135.5-340.0	15.74	IV-V	-2.87

*) Growth rate is calculated based on this formula : $[(W_2-W_1)/0.5(W_1+W_2)]x100\%$, W=body weight of k-th. measurement (Bokhari, 2002).

Table 4. Feed conversion ratio

Week	Feed conversion ratio
I	2.95
II	3.42
III	6.30
IV	10.16

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