



3rd AINI INTERNATIONAL SEMINAR

In conjunction to 50th Anniversary Faculty of Animal Science
Andalas University



THE ROLE OF NUTRITION AND FEED IN SUPPORTING SELF SUFFICIENT
IN ANIMAL PRODUCTS, FOOD SAFETY AND HUMAN WELFARE
Padang, 24 – 25 September 2013

PROCEEDING

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"The Role of Nutrition and Feed in Supporting Self Sufficient in Animal Products, Food Safety and Human Welfare"

PROCEEDING
3rd International Seminar and 9th Biennial Meeting of AINI
"The Role of Nutrition and Feed in Supporting Self Sufficiency Animal
Products, Food Safety and Human Welfare"
in conjunction with
the 50th Anniversary of the Faculty of Animal Science
University of Andalas, Padang West Sumatera
Grand Inna Muara Hotel, Padang 24-25 September 2013

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"The Role of Nutrition and Feed in Supporting Self Sufficient in Animal Products, Food Safety and Human Welfare"

FOREWARD President AINI

Assalamu 'alaikum Wr. Wb.,
The Honourable Rector of The University of Andalas,
The Dean Faculty of Animal Science, University of Andalas
Distinguish guests, participants, ladies and gentlemen,

First of all, on behalf of the Indonesian Animal Nutritionist and Feed Scientist Association (AINI), I would like to extend our warmest welcome, and indeed it is a great pleasure to see you all in this room, participating in the 3rd *International Seminar and 9th Biennial Meeting of AINI* in conjunction with the 50th anniversary of the Faculty of Animal Science University of Andalas, Padang West Sumatera. AINI that was firstly established in 1996 with the objective to gather all of the animal nutrition and feed scientists in Indonesia permitting to the exchange of knowledge and experiences under spirit of brotherhood, to stimulate the advancement of science and technology in nutrition and feed science, thus benefiting to the competitiveness of livestock agribusiness.

Since its establishment 1996, AINI has been conducting regularly the biennial scientific meeting. From 2007, the scientific meeting was upgraded to the International level and the first International seminar was conducted at Jenderal Sudirman University, Purwokerto Central Java and then the second International seminar was held in Padjajaran University, Bandung West Java, while this third event is conducting here with the theme **"THE ROLE OF NUTRITION AND FEED IN SUPPORTING SELF SUFFICIENCY IN ANIMAL PRODUCTS, FOOD SAFETY AND HUMAN WELFARE"**

Distinguish guests, participants, ladies and gentleman,

The role of feed and nutrition is primordial in the livestock agribusiness, not only due to the fact that more than 70% of production cost is coming from the feed cost, but also the feed safety that affect the food safety is becoming the great issues in recent years and become a great concern by many countries in the world. Animal products such as egg, meat and milk are subjected to the government policy to reach the self sufficiency. Indonesian government has payed attention and put high priority especially in meat self sufficiency for 2014. Unfortunately, effort made by the government ie. Ministry of Agriculture since many years has facing now the difficulty to succeed, due to some reasons such as the meat price volatility, and also the low exchange rate of rupiah to the US dollar at this time being. Indeed, the demands on the animal products will be increasingly in the future as the population and income per capita are growing. We should take a part and do our best to support the government policy in fullfiling the food of animal products, quantitative and qualitatively. In this regards, role of nutrition and also Nutritionist and Feed Scientist are very important.

I would like also to take this opportunity to share the idea with all you, that AINI as the organization of scientist, to have a international scientific journal is a must. The Journal deals with all aspects of nutrition and feed issues in tropical conditions. The Management board of AINI has taken the decision for revitalizing the AINI Journal to become the Journal of Nutrition and Feed Science, internationally recognized, by involving the International committee of lecture as the reviewers. To this end, we need



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fully your support and encourage the scientists especially the young scientists to publish their work in English. The accomplishment of this task will bring the association be more respected in national and international level.

Distinguish guests, participants, ladies and gentleman,

For this opportunity, I should express my sincere thanks to the Dean of the Faculty of Animal Science, University of Andalas, the organizing committee, sponsors, and all party that cannot be listed since we are deeply in debt to all of your effort and sacrifice to the success of this seminar. Our sincere thanks must go to the Directorate General for Higher Education Department of National Education for the grant awarded. For our invited speakers, Prof. Tamo Fukamizo (Kinki University, Japan), Dr. Robert L. Payne (Evonik-US), Dr. Yuwares Ruangpanit (Thailand), Prof. Abdul Razak Alimon (UPM Malaysia), Prof. Yose Rizal (University of Andalas, Indonesia), Prof. Ali Agus (University of Gajah Mada, Indonesia), Prof. Suhubdy (Mataram University, NTB) we are indebted to your effort and your participation in this event. Your views will enlighten and inspire how to empower our local feed resources in sustaining the feed security for the future. Also, on behalf of the AINI, I must express my deepest gratitude to the Director General of Livestock Services Department of Agriculture for his willingness to give the key note speech to this seminar.

Distinguish guests, participants, ladies and gentleman,

I hope you will have the fruitful meeting and gaining many new ideas and perspectives to be developed in the future. I do hope also, we will see you again in the 4th International seminar and Xth Biannual meeting 2015 that will be hosted by AINI member and colleagues from Sam Ratulangi University, Manado, North Sulawesi as the organizing committee.

Finally and surely, please enjoy your stay with west Sumatera culture and nature, tradition and hospitality, in addition to your scientific activities. Thank you,

Wassalamu 'alaikum Wr. Wb.

Padang, September 24th, 2013

President AINI

Prof. Ali Agus

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FOREWORD ORGANIZING COMMITTEE

Assalamu'alaikum Wr. Wb.,

The Honourable Rector of The University of Andalas,
The Dean Faculty of Animal Science, University of Andalas
Distinguish Guests, Seminar Participants, Ladies And Gentlemen,

First of all, we are very grateful for Allah the Almighty, who has allowed us to get together in the prestigious 3rd AINI International Seminar which is held by the Faculty of Animal Science, University of Andalas in conjunction to celebrate the 50th Anniversary of the Faculty of Animal Science, collaborated with Indonesian Association of Nutritionist and Feed Scientist (AINI). We would like to welcome all participants who have come from different provinces in Indonesia, and especially to our distinguished guests and participant from overseas (USA, Japan, Thailand, Malaysia, Philippine and Australia).

The animal protein consumption of the people in Indonesia and other developing countries around the world as well is still low. The Indonesian Government has performed many efforts to increase this animal protein consumption. One of them is through the launching of a program called the self sufficient in beef (program swasembada daging sapi = PSDS), that has been targeted to be achieved in 2014. Besides, other attempts are also to develop poultry and other animal industries that have contributed to the fulfillment of animal protein requirement. However, based on the animal industry condition nowadays it would be rather complicated to achieve it, due to the low in farm animal productivity in Indonesia. Among the problems of low in animal productivity are the nutrition and feed they obtain during their life cycles. Besides, the price of feed for animal industries could reach 60 to 70% of the total cost of animal production. Indonesia has very limited range land for cattle grazing and limited feed sources for poultry feeding. The cattle feeding are very dependent on the utilization of agriculture waste/by-product as the source of feed. Most of these available feedstuffs are low in quality, so that they require further processing before feeding them to cattle. Meanwhile, the poultry and other farm animal feeding are depending on imported feeds. The other problem is the concern in utilization of in-organic feedstuffs or feed additives for growing farm animals.

The theme of this seminar is very relevance to the nowadays national as well as international issues of feeding safety feed for livestock and poultry, and conserving nutritious, safety and hygienic food for human health. This nutritious, safety, hygienic and healthy food of animal origin will be obtained from the high quality of feed that is fed to animals. The feed and food processing technology will support the high quality of feed and food. This 3rd AINI International Seminar on nutrition and feed is held to collect the information and to share the ideas from nutritionists, scientist and practitioners on the nutrition, feed processing technology and its utilization for producing high quality of feed and food which are available in other part of the world to contribute to the human welfare.

Prof. Dr. Novirman Jamarun

Chairman of the Organizing Committee

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WELCOME SPEECH

Rector of Andalas University

Bismillahirrahmaanirrahim,
Assalamu'alaikum wrwb, Peace be with you!
Your Excellency, Governor of West Sumatra Province.
Your Excellency, Chairman of House of Representative of West Sumatera Province
The Honorable, Dr. Mursyid Ma'sum, M.Agr, Director of Animal Feed, Direktorat General
of Livestock and Animal Health, Ministry of Agriculture.
The honorable, The Chairman of Indonesian Association of Nutritionist and Feed
Scientist (AINI), Prof. Dr. Ali Agus DAA, DEA, from Gajah Mada University.
Honorable guest, the Dean of The Faculty of Animal Science.
The Honorable guests all keynote speaker.
Seminar Committees, Participants, Ladies and Gentlemen,

Good evening.

First of all, let us say a merciful for Allah the Almighty who has given us a chance to meet each other at this 3rd AINI International Seminar which is held by the Faculty of Animal Science University of Andalas in conjunction to celebrate of 50th Anniversary Faculty of Animal Science, Andalas University.

On this occasion, I welcome all of the seminar participants who come from different places in the world, as well as participants from different uiversities and agencies in Indonesia.

On this opportunity, I would like to introduce to all of you about the University of Andalas. It was the oldest university in Indonesia, outside the Java Island that was founded in 1956. This university has 15 Faculties with 38 study programs for Sarjana degree, and 34 Graduate Study Programs for Master's and Doctor's degrees. The Faculty of Animal Science is one of the faculties at the University of Andalas which was established in 1963. I am very proud of this International seminar, which is conducted by the Faculty of Animal Science.

It indicates that the Faculty of Animal Science, University of Andalas, has the capability to create a link or a network with national as well as international level institutions, in which it is a kind of initiation toward the world class university.

Ladies and Gentlemen,

From this 3rd AINI International Seminar, I hope that it will result in the fruitful thoughts and brilliant ideas which could be implemented by the government and animal industrial community for the development of the Animal Feed industries in Indonesia as well as in West Sumatra. The Faculty of Animal Science University of Andalas plays a role in the development of feed industries in cooperation with the government, and livestock as well as animal nutritionist organizations.

Feed Industries contribute to the fulfillment of animal development in Indonesia because Feed is one most important factors to develop animal production and animal population and with cheap in price of feed will give high benefit could be reached by the



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farmer. The development of feed and animal industries needs science and technology, and through this seminar, it is hoped that the scientists from all over the world could contribute the information and technology in disciplines in feed sciences.

Ladies and Gentlemen,

This seminar is in concomitant with the 57 year University of Andalas, and the 50 year Faculty of Animal Science Anniversaries. Considering the age of this institution, it is the appropriate time for this institution to play its role at the international level. The progress toward the world class university is a dream of every institution, including the University of Andalas. That is why I hope that this international seminar could be performed routinely, so that the development of science and technology in the field animal science could always be monitored and implemented by the animal community in Indonesia.

Finally, I would like to address my special thanks to the committees who have work very hard to prepare this seminar, congratulation and good luck for all of you.

Wassalammualaikum wr.wb.

Dr. Werry Darta Taifur, SE, MA
Rector of Andalas University

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WELCOME SPEECH **Governor of West Sumatera**

Assalamualaikum. ww

Your Excellency, Chairman of House of Representative of West Sumatera Province. Ir. Yulteknil, MM

The Honorable, Dr. Mursyid Ma'sum, M.Agr, Director of Animal Feed, Direktorat General of Livestock and Animal Health, Ministry of Agriculture.

The honorable, Prof. Dr. Ali Agus DAA, DEA, The Chairman of Indonesian Association of Nutritionist and Feed Scientist (AINI) from Gajah Mada University.

Honorable guest, the Rector of the University of Andalas.

The Honorable guests all keynote speaker.

Honorable guest, the Dean of The Faculty of Animal Science.

Seminar Committees, Participants, Ladies and Gentlemen,

Seminar Committees, Participants, Ladies and Gentlemen,

First of all we are very grateful for Allah the Almighty, who has allowed us to get together in the prestigious 3rd AINI International Seminar which is held by the Faculty of Animal Science University of Andalas in conjunction to celebrate of 50th Anniversary, Faculty of Animal Science, Andalas University.

I would like to say 'welcome' to all of participants who have come from different areas in Indonesia, and especially to the participants from several countries (USA, Malaysia, Thailand, USA and Japan).

West Sumatra is one of 33 provinces in Indonesia which is also called "Ranah Minang" or Minang Area, because this area mostly inhabited by Minangkabau ethnic. This province is well known with its beautiful scenery and culture, because it possess Sianok canyon, marvelous beach in Mentawai Island with its high wave that is suitable for surfing, gorgeous Harau Valley, four beautiful lakes (Singkarak, Maninjau, Upper and Lower Lakes), and several other places for tourism. We have two international regular events, the first is Tour de Singkarak, and the second is Padang International Dragon Boat competition. Tour de Singkarak, a bike racing event every year followed by many bicyclers from all over the world, got its name from this lake's name 'Singkarak'.

The population of West Sumatra province is approximately 4 million people who mostly are moslems. Besides, Ranah Minang also well known with its specific hot and spicy foods. One of the menus is RENDANG, which is the most delicious food in the world. Rendang is made of varieties of meat (beef, chicken, or egg) which is mixed up with coconut milk, chili, and other ingredients. That is why, after this seminar I suggest you to spare your time visiting some of those beautiful and marvelous places while enjoying the specific menu I mentioned.

Furthermore, I would like to address that this 3rd AINI International Seminar is an important event for us, because it is a place where the experts from all over the world get together, informing their research findings to the others and sharing the ideas in order these findings and ideas to be useful for the development of science and technology in animal nutrition. The information from this seminar will be very useful for the development of animal industry in West Sumatra, Indonesia as well as in other countries around the world.



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Ladies and Gentlemen,

The target of the Indonesian Government nowadays is to achieve the self-sufficient in meat in 2014 in order to fulfill the demand for animal protein for the Indonesian people. For supporting the achievement of this target, the West Sumatra province is implementing a program called “Satu Petani Satu Sapi” or one farmer one cow which is funded by government and private. The purposes of this program are to motivate farmers to raise cattle, to accelerate the increase in the population of cattle, to accelerate the achievement of target in fulfilling the demand for animal protein, to vary the source of income for farmers, and to increase the farmers’ income.

Ladies and Gentlemen,

Finally, I hope this seminar will produce the fruitful thoughts which could be implemented in the development of animal industry around the world as well as in Indonesia. Please enjoy this seminar, congratulation to the 50th Anniversary Faculty of Animal Science, University of Andalas, and I wish it will be continued with the other international seminars in different field.. Good luck for you all!!! And by saying:

Bismillahirrahmaanirrahim, I officially open this seminar.

Wabillahitaufik walhidayah, Wassalamualaikum warahmatullahi wabarakaatuh.

Governor of West Sumatera Province

Prof. Dr. Irwan Prayitno, PSi, MSc

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SEMINAR PROGRAM

TIME	ACTIVITIES	PIC	LOCATION
	Participants arrive in Padang	Transportation and Accommodation	Airport and Hotels
19:00–22:00	Opening Ceremony and Welcome Dinner Welcome address by Chairman of the 3 rd AINI International Seminar (Prof. Dr. Novirman Jamarun) Welcome address by President of AINI (Prof. Dr. Ali Agus) Welcome address by Rector of Andalas University Welcome address by Governor of West Sumatera Province Inaugural of Regional Representative of AINI	MC Chairman President of AINI Rector Unand Governor AINI	Governor House, Jl. Sudirman, Padang

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DAY-1 (WEDNESDAY, SEPTEMBER 25, 2013)

TIME	ACTIVITIES	PIC	LOCATION
08:00 – 08:30	Re-registration	Secretariate	Assembly Hall
08:30 – 12:30 KEYNOTE SPEAKERS			
08:30 -9:00	Dr. Ir. Mursyid Ma'sum, M.Agr (Director of Animal Feed, Directorate General of Livestock and Animal Health Services, Ministry of Agriculture - Indonesia) Title: "Policy And National Program For Feed Development"	Moderator : Dr. Maria Endo Mahata	OMBILIN HALL
9:00 – 9:30	Prof Dr. Tamo Fukamizo (Kinki University Japan). Title: "The Mode of Action of Chitinolytic Enzymes: Production of Bioactive Oligosaccharides as Animal Nutrients"	Dr. Maria Endo Mahata	
9:30 – 10:00	Dr. Yuwares Ruangpanit (Kasetsart University,Thailand) Title: "Improving Egg Nutritional Value By Dietary Marine Sources – A Current Update"	Dr. Maria Endo Mahata	
10:00 – 10:30	Prof. Dr. Yose Rizal (Unand, Indonesian) Title: "The Utilization of Juice Waste Mixtures in Poultry Diets: A Review"	Dr. Maria Endo Mahata	
10:30 – 11:00	Prof Dr Abdul Razak Alimon, Universiti Putra Malaysia, Malaysia) Title: "Utilization Of Herbs As Growth Promoters In Animal Feed"	Dr. Rusmana Ningrat	
11:00 – 11:30	Dr. Robert L. Payne, Ph D, PAS (Evonik – US) Title: ' The Role Of Amino Acids In Sustainable Poultry Production "	Dr. Rusmana Ningrat	
11:30 – 12:00	Prof. Dr. Suhubdy Yasin(University of Mataram, Indonesia) Title: " Rangelands and Pastures of Indonesia for Ruminant Production: a Poor Attention and Neglected Resources "	Dr. Rusmana Ningrat	
12:00 – 12:30	Prof. Dr. Ali Agus, DAA, DEA (University of Gajah Mada, Indonesia). Title: "Food And Feed Safety Issues In Indonesia : Reducing Mycotoxins Toxicity And Its Carry Over From Feed Into Animal Products"	Dr. Rusmana Ningrat	
12:30 –13:30	LUNCH BREAK		

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SESSION 1. RUMINANT NUTRITION (ROOM OMBILIN 2-3)

CHAIR: DR. IRSAN RYANTO H, UNAND, INDONESIA

1	13:40– 13:50	Estimation of Rumen Microbial Nitrogen Supply Based on Purine Derivatives Excreted in the Urine of Kejobong and Bligon Goat Feed by King Grass and Peanut Straw, <i>Yusiati, L. M., and C. Hanim, UGM, Indonesia</i>
2	13:50 – 14:00	The Effect Of NDF Ratio in the Diet on the Performance of Philippine Native Goats (<i>Caora hicus</i> Linn), <i>Nugroho, D., C.C. Sevilla, AA. Angeles, F. Setyawatie, Animal Science and Dairy Cluster, UPLB, Philippine</i>
3	14:00 – 14:10	Meat Physical Properties of Local Lamb Fed Urea-Impregnated Zeolite Ration, <i>Kardaya, D., E. Dihansih, D. Wahyuni, Djuanda University, Bogor, Indonesia</i>
4	14:10 – 14:20	In vitro study of <i>sardinella lemuru</i> oil based calcium-soap supplementation effects on the sheep’s rumen digestibility <i>Asep Sudarman</i>
5	14:20 – 14:30	Milk Composition Of Etawah Crossbred Goat Fed Forage And Leaves Pellet <i>Suryanindyah, Y. Y., N. Umami, Nurliyani, Y. S. Muthoharotin, Y. P. Oktaviani, UGM, Indonesia</i>
6	14:30 – 14:40	Rumen fermentability and digestibility of lingzhi (<i>ganoderma lucidum</i>) and organic chromium supplementation in high and low forage rations <i>Dwierra Evvyernie¹, Toto Toharmat¹, Sumiati¹ and Dian Astriana¹</i>
7	14:40 – 14:50	Ruminal Degradation Characteristics Of Maize (<i>Zea Mays</i>) Leaves, <i>Rusdi, Mustaring, M. Salman, Animal Husbandry Dept, Tadulako University, Palu Indonesia</i>
8	14:50 – 15:00	The Effect Of Concentrate Offered In Ratio Based On Rice Straw To The Performance Of Bali Cattle, <i>Trisnadewi, S., T. G. O. Susila, I W. Wtjana, Faculty of Animal Husbandry, Udayana University, Bali</i>
9	15:00 – 15:10	Enhancing Performance of Sheep by Feeding Corn Leaf Biscuit. <i>Yuli Retnani, Sobri, D. K. Putra, and T. Toharmat, IPB Bogor, Indonesia</i>
10	15:10 – 16:20	Effect of Black Tea (<i>Camelia sinensis</i>) Waste on Rumen Degradation of Feed Protein, <i>Kurniawati, A., B. Nugroho and C. Hanim, UGM Indonesia</i>
11	15:20 – 15:30	Supplementation of Solid Ex-Decanter Multi-Nutrient Block on Simbrah Breed Weaned Calves Performances as Integrated Farming System with Palm Fruit Agroindustry, <i>Fariani, A., A. Abrar, G. Muslim, E. D. Y. Astuti and L. Warly, Sriwijaya University, Palembang, Indonesia</i>
	16:50 – 17:00	CLOSING CEREMONY AT OMBILIN 2-3
		FAREWELL DINNER AT MAJOR HOUSE OF PADANG CITY

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“The Role of Nutrition and Feed in Supporting Self Sufficient in Animal Products, Food Safety and Human Welfare”

SESSION 2. NON-RUMINANT NUTRITION (ROOM ANAI 1-2)

CHAIR: PROF. DR. KHALIL AND PROF. DR. YUSRIZAL, MSC, UNAND AND UNJA, INDONESIA

1	13:30 – 13:40	Productivity Of Local Chicken In Growth Periods And Carcass Characteristics By Inclusion Of <i>Moringa Oleifera</i> Leaves Meals In The Diets <i>Hafsah, S. Sarjuni, T. Riske, I. Kumbok</i>
2	13:40 – 13:50	The Effect Of Palm Kernel Meal Contain Probiotic To Reduce The Fecal Ammonia Emmission In The Laying House, <i>Yusrizal, F. Manin, Yatno and Noverdiman, University of Jambi, Indonesia</i>
3	13:50 – 14:00	Contribution Of Lysin And Calcium Of Azolla Microphylla On Egg Shel Calcium Deposition In Arab Hen, <i>Wulandari, E., C., R.H. Prawitasari, N. Suthama, W. Murningsih, V.D Yuniato, I. Estiningdriati and H.I. Wahyuni, Agriculture Diponegoro University, Semarang, Indonesia</i>
4	14:00 – 14:10	Japanese Quail Eggs Quality Fed Fermented <i>Jatropha Curcas</i> Meal, <i>Sumiarti, R. Mutia, and R. Khalim, IPB Bogor, Indonesia</i>
5	14:10 – 14:20	Evaluation in the presence of black tea waste extract on different level of energy-protein rations in the performance and carcass parameters of broiler <i>Dilla marestia fassah¹*, supadmo² and rusman³</i>
6	14:20 – 14:30	Efforts for the meat quality of bali ducks through offering purple sweet potato (<i>ipomoea batatas</i> l) fermented <i>aspergillus niger</i> in diets <i>Tjokorda gede belawa yadnya, ida bagus sudana, i gede mahardika and i m. Mastika</i>
7	14:30 – 14:40	Effect of avocado seed meal and fermented of banana peel meal to laying quail <i>Hera dwi triani, ade djulardi, ahadiyah yuniza</i>
8	14:40 – 14:50	Layer Ducks Performances Fed Katuk Leaf Meal, <i>Hermana, W., M. Septiana and Sumiarti, IPB Bogor, Indonesia</i>
9	14:50 – 15:00	The Profile Of Corn-Cob Nutrient As Prospective Poultry Feed In Upper And Lower Land Area In West Sumatra <i>Maria Endo Mahata, Ahadiyah Yuniza, Nuraini I and Yose Rizal</i>
10	15:00 – 15:10	Performance of Broiler Chicks Fed Waste Canned Oil Fish in Ration. <i>Jola J.M.R. Londok, John E.G. Rompis dan Youdhie H.S. Kowel. Fakultas Peternakan Unsrat Manado</i>
11	15:10 – 16:20	The dynamics of indigenous lactic acid bacteria probiotics on carcass yield, abdominal fat And intestinal morphology of broilers <i>Sri Harimurti, Miftahul Huda, and Anisa Dwi Kistiani</i>
12	15:20 – 15:30	The effects of xylanase supplementation on meat quality, carcass recovery and blood cholesterol in broilers fed on wheat-based diets <i>Chusnul hanim, lies mira yusiati, ali wibowo, and muhamad nur cahyanto</i>
13	15:30 – 15:40	Digestive organ's growth of local duck fed high dietary fiber during post-hatch: effect on allometric measurement <i>Hanny Indrat Wahyuni, Istna Mangisah, Nyoman Sutham and Maulana Hamonangan Nasution</i>
	17:30 – 18:00	CLOSING CEREMONY AT OMBILIN 2-3
	19:00 – 22:00	FAREWELL DINNER AT MAJOR HOUSE OF PADANG CITY

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SESSION 3. FEED SCIENCE AND TECHNOLOGY, PASTURE AND RANGE LAND, NUTRITION AND REPRODUCTION, SOCIO-ECONOMIS OF FEED AND FOOD (ROOM OMBILIN 1)

CHAIR: PROF. DR. MARDIATI ZAIN, MS, UNAND, INDONESIA

1	13:30 – 13:40	Effect of Somatotropin Hormone on Macroscopic and Microscopic Semen Quality of Simbrah Breed Bulls <i>Gatot Muslim, A. Fariani, A. Abrar, E. D. Y. Astuti and L. Warly</i>
2	13:40– 13:50	Effect of vegetable and animal-derived ingredients on the physical pellet properties of extruded feed <i>Suluh nusantoro, erfan kustiawan, and nurkholis</i>
3	13:50 – 14:00	Isolation and identification of lactic acid bacteria from feces of young calves as a potential Candidate of probiotic <i>Ismail Jasin, Z. Bacrudin H. Hartadi</i>
4	14:00 – 14:10	Effect Of Shade On Growth And Productivity Of Torbangun, <i>Karti, IPB, Bogor, Indonesia</i>
5	14:10 – 14:20	Evaluation Of Availabiliy And Quality Of Forage At Limau Manis Campus Of Andalas University, Padang, West Sumatra, <i>Khalil, Unand, Indonesia</i>
6	14:20 – 14:30	Feed Intake And Efficiency In Mice (<i>Mus Musculus</i>) Given Treated – <i>Jatropha Curcas</i> L. Seed Meal, <i>Tjakradidjaja, Anita S., P. H. Siagian And Hadriyanah IPB, Bogor, Indonesia</i>
7	14:30 – 14:40	Effect Of The Extracted Cinnamin Stick And Ground Cinnamon Stick On The Rancidity Of Palm Oil Decanter Meal, <i>M. Afdal, A. Kasim, Ar. Alisman and N. Abdullah, University of Jambi, Indonesia</i>
8	14:40 – 14:50	Feed Intake, Nutrient Digestibility and Blood Glucose of Sheep Supplemented with Organic Chromium From Fungi <i>Ganoderma lucidum</i> . <i>Agustina, F., D.Evyvernie, D.Taniwiryono, S.Tarigan , T.Toharmat</i>
9	14:50 – 15:00	Analisis Performances Of Egg Poultry Industries In 50 Kota Regency As A Based Sector Of West Sumatra <i>Rahmi, E. W. Sartika. Unand, West-Sumatra</i>
10	15:00 – 15:10	Comparison Of Corn Stover Nutrient Content In Lower And Upper Land Areas In West Sumatra <i>Rusmana W. S. Ningrat1, Irsan Ryanto1, Montesqrit1and Giovani M. Turchini2</i>
11	15:10 – 16:20	Nutrients intake and their relation to milk production and qualities under traditional and Small scale indonesian dairy farms enterprises <i>Despal*, A. Lestari, Y. Destianingsih, Z. Malyadi, H. Hartono, L. Abdullah</i>
12	15:20 – 15:30	Utilization Onggok Enriched With Egg Powder To Making Nutritious Instant Food <i>Sukma, A. K. Sayuti, Novelina. UNAND West- Sumatra</i>
13	15:30 – 15:40	Study On In Vitro Digestibility Of Soaked Oil Palm Fiber By Filtrated Oil Palm Fruit Bunch Ash <i>Ari L. Darmawan, Asep Irawan, Tidi Dhalika, Ana R. Tarmidi, Mansyur, Atun Budiman, Kurnia A. Kamil and Iman Hernaman</i>
14	15:40 – 15:50	Productivity Of Bali Cattle Based On Scrotum Size, Body Weight And Feed Quality <i>Abyadul Fitriyah1, Nurul Hilmia2, Lalu Muhammad Kasip3, Sukmawat1, Totok.B.Julianto2</i>
15	15:50 – 16:00	Quality of rennet from rabbit stomach during cold and frozen storage <i>Nurliyani¹, Indratiningsih², Mufti Tri Matra</i>
16	16:00 – 16:10	<i>IN VITRO</i> CULTURE FOR THE SUPPLY OF MATERIAL GENETIC TRANSFORMATION ON DWARF NAPIERGRASS (<i>Pennisetum purpureum</i> cv. Schumach) <i>Nafiatul Umami</i>
17	16:10 – 16:20	Eggs rendang characteristic by addition of gambier catechin antioixidan <i>Deni novia, Afriani Sandra Indri Juliyarsi Anwar Kasim and Azhari Nuridinar</i>
17:30 – 18:00		CLOSING CEREMONY AT OMBILIN 2-3
19:00 – 22:00		FAREWELL DINNER AT MAJOR HOUSE OF PADANG CITY

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POSTER SESSION (OMBILIN ASSEMBLY HALL)

CHAIR: PROF. DR. IR. NURAINI, MS AND PROF. DR. IR. YETTY MARLIDA, MS, UNAND, INDONESIA

12:00 – 14:00

P-01	The effect of concentrate ratio based on palm kernel cake on pH on, VFA and NH3 In-Vitro Rumen. <i>Arief, N. Jamarun, Elihasridas, F. Achmad</i>
P-02	Integrated Farming System With Empowerment Of Cattle Farmers Group In Village Kinomaligan. <i>F.H. Elly, A.Makalew, F.N.S. Oroh dan D. Polakitan</i>
P-03	The Effect Bioprocess of Banana Peels With The Different of incubation lenght and the source of Local microorganisms (MOL) on Crude Protein, Crude fiber and lignin content. <i>Tri Astuti and S. Amir</i>
P-04	The content of phytochemical and antibacterial activity of cinnamon leaf (<i>Cinnamomum burmanii</i>) and Noni Fruit and Leaf (<i>Morinda citrifolia L</i>) mixture extract to Replace Antibiotic. <i>Yuniza, A and Yuherman</i>
P-05	The Effect of Supplementation Lamtoro (<i>Leucaena Leucocephala</i>) Based on Rice Straw Amoniation Ratio on In-Vitro Rumen Characteristics. <i>Herawati, R., N. Jamarun, M. Zein, Arnim</i>
P-06	Dry Matter And Organic Matter Digestibility Of Java Wood (<i>Lannea Coramandelic</i>) Leaves By Use In Sacco. <i>Fatmawati</i>
P-07	Improving Quality Soybean Waste by Fermentation as Poultry Ration. <i>Mirnawati, Ade Djulardi and Helmi Muis</i>
P-08	Effect of total time marketing on microorganisms in cattle Meat Marketed In Padang Great Market, West Sumatera. <i>Yuherman, Eva Umar, and John Farlis</i>
P-9	The Application Of Science And Technology For Cattle Farmers Group For Improving Integrated Farming System In Village Ongkaw. <i>A.H.S. Salendu., F.H. Elly., M.A.V. Manese dan D. Polakitan</i>
P-10	Performances And Hematological Profile Of Broiler Under Heat Stress Fed Diet Containing Carica Papaya L. Leaf Meal And Curcuma Domestica Val. <i>Dwi Margi Suci, Dewi Apri Astuti, F.Kumala. Dewi and D. Kuncoro Sakti</i>
P-11	Mix Of Lingzhi (<i>Ganoderma Lucidum</i>), Organic Chromium And Roasted Soybean Evaluated As Feed Supplement For Laying Hen. <i>Tania Perdana Putri, Dwierra Evvyernie, Dwi Margi Suci And Muhammad Lukmannulhakim</i>
P-12	Fermentability And Degradation Of Concentrate Contents Dry Carboxylate Salt Or Methyl Ester In Rumen Liquid. <i>A.M. Tasse, E.B. Laconi, D. Agustina</i>
P-13	Comparative Analysis Of Nutrient Composition Of Different Sorghum Varieties After Ensilage Processes. <i>Awistaros Angger Sakti, A. Sofyan and H. Julendra</i>
P-14	Viability Of Lactic Acid Bacteria Isolated From Rumen Liquor On Molasses Mixture Medium. <i>Ema Damayanti, N. A. Hermawati, A. Pangastuti and A. Sofyan</i>
P-15	Effect Of Formic Acids In Silage Processing From Shrimp Head Waste As Animal Feed. <i>Mirzah, Montesqrit and Suslina A Latif</i>
P-16	Effects Of Saga Leaves And Yellow Leaves On Rumen Microbes And In Vitro Digestibility. <i>Dwierra Evvyernie, H. Ahmad Sukria, E. Harlina, E. Rachmi, A. Winarni And U. Nurjannah</i>
P-17	Flushing With Different Sources Of Energy Quality Ration On Reproductive Performance Local Sheep, <i>Khotijah, L., K.G. Wiryawan, K.B.Satoto, D. Diapari, N.B.Sitpu and N.E.K.Santi, IPB, Bogor, Indonesia</i>
P-18	The use of <i>trichoderma harzianum</i> in the fermentation of tofu waste product <i>Burhanudin Malik; Anggraeni; Sawarni Hasibuan; Rudiana¹</i>
P-19	Indigofera Zollingeriana Adaptation To Drought Stress And Mycorrhiza Inoculation <i>S. Sowmena), L. Abdullah), P.D.M.H. Kartib), D. Soepandic</i>
P-20	Effect Of Cocoa Pod And Cocoa Leaf On In Vitro Fermentation And Nutrient Digestibility <i>J. Rahman, M. Zain, and Erpomen</i>

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P-21	Effect Of Vitamin E Supplementation In The Extender On Frozen – Thawed Semen Preservation Of Pesisir Cattle <i>Zaituni Udin; Ferdinal Rhim; Jaswandi and Ayu Fadillah</i>
P-22	Green livestock development: the role of lactic bacteria for improvement and utilisastion Of total mixture forage <i>Zaenal Bachruddin¹, Supadmo¹, Lies Mira Yusiati¹, Chusnul Hanim¹, Asih Kurniawati¹, Dimas Hand Vidya Paradhipta² and Mayansari²</i>
P-23	The effects of roasted coriander seeds in the diet on carcass trait and cholesterol content of broiler <i>R.Mutia, R.G. Paminda, and N.Ramli</i>
17:30 - 18:00	CLOSING CEREMONY AT OMBILIN 2-3
19:00 - 22:00	FAREWELL DINNER AT MAJOR HOUSE OF PADANG CITY

INVITED SPEAKERS
**Invited Speakers at 3rd AINI International Seminar,
Padang, West Sumatera, Indonesia**



Dr. Mursyid Ma'sum, M.Agr

Director of Animal Feed, Directorate General of Livestock and Animal, Health Services.

Prof Dr. Tamo Fukamizo (Kinki University Japan)

graduated his Bachelor and Master course of Agricultural Chemistry of Kyushu University, Japan, in 1977 and 1980, respectively. He completed his Ph.D. in Kyushu University in 1983. Currently, he is a full professor of Enzyme Chemistry at Department of Advanced Bioscience, Kinki University, Japan. His research of interest is,

1. Crystal structure analysis of the chitinase-oligosaccharide complex
2. NMR analysis of the interaction of chitin-binding proteins
3. Calorimetric analysis of the interaction of chitin-binding proteins
4. Conversion of chitinase into a glycosynthase by protein engineering technique

5. Biomass conversion from fungal cell wall by enzymatic digestion
Recently, in collaboration with Dr. Maria Mahata, University of Andalas, he successfully produced partially N-acetylated chitooligosaccharides, which might be used as animal food ingredients, directly from fungal cell wall. In today's his lecture, mechanism of oligosaccharide production from chitin and chitosan biomass will be presented, and the utilization of the products will be discussed.



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Dr. Robert L. Payne, Ph D, PAS (Evonik - US)

Regional Director of Nutrition and Technical Services for Evonik Health & Nutrition. Rob joined Evonik-Degussa in 2004, and since that time, he has served Evonik's Health and Nutrition group as Animal Nutrition Services Manager, Technical Services Manager for US and Canada, and Director of Nutrition and Technical Services for North America. In 2011, Rob moved to Singapore to become Director of Nutrition and Technical Services for the Asia South region. As technical director, Rob is responsible for guiding Evonik's value-added technical services team, which provide tools and consulting for nutritional, analytical, and feed production issues. Rob has authored numerous peer-reviewed, popular press articles, and invited talks, and currently serves on the editorial boards for the Journal of Animal Science and Poultry Science.



Prof. Dr. Ali Agus, DAA, DEA (University of Gajah Mada, Indonesia)
 Graduated from the Faculty of Animal Science, University of Gajah Mada in 1989, and completed his DAA, DEA (1993) and Doctorate (1996) from Ecole Nationale Supérieure Agronomique de Rennes (ENSA), Rennes, France in Nutrition and Physiology of Dairy Cattle He is also a member of National Feed Commission, Department of Agriculture, Republic of Indonesia. He published several books and articles in peer reviewed international journals, presented papers in international meeting and published in Proceedings. His research interest are in animal nutrition, feed toxicology, mycotoxins and community developments.



Dr. Yuwares Ruangpanit (Thailand)

graduated her Bachelor and Master in Animal Science from Kasetsart University, Thailand in 1992 and 1995, respectively. She completed her Ph.D. in Nutrition from North Carolina State University in 2004. Currently, she is a lecture of Mono-gastric animal nutrition at Department of Animal Science, Kasetsart University, Thailand. Her research of interest is nutritional evaluation and application of alternative energy and protein source for poultry, especially, a high fiber by-product from Agro-industry. Her responsible research also involves in the application of feed additive in mono-gastric animal under tropical conditions



Prof. Dr. Abdul Razak Alimon (Malaysia)

obtained his Bachelor of Science and Masters of Science in Agriculture from the Faculty of Rural Science, University of New England, NSW, Australia in 1971 and 1980, respectively, and completed his Ph.D degree in 1989 at University of Reading, United Kingdom. He is currently a Professor of Animal Nutrition at the Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia. His current interest is in the utilization of herbs as growth promotants in poultry and also agricultural byproducts as animal feed.

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Prof. Dr. Yose Rizal (Indonesia)

graduated from the Faculty of Animal Science, University of Andalas, Padang, West Sumatra, Indonesia with a Sarjana degree in 1981, and completed his Master and Ph.D degrees in Animal Nutrition, at the Department of Animal Science, University of Illinois, USA in 1987 and 1989. He is currently a Professor at the Faculty of Animal Science, University of Andalas, Padang, West Sumatra, Indonesia. Now, he is also responsible for the Quality Assurance at the University of Andalas. His area of interest is in the utilization of agriculture wastes/by- products for poultry feeds.



Prof. Dr. Suhubdy Yasin (Indonesia)

Is highly distinguish profesor in ruminant nutrition science, awarded as Ph.D. From the University of Queensland, Australia 2002. He was a fullbright visiting profesor at Utah State University, USA 2008/2009. He is Director a Research Center Of Tropical Rangelands and Grazing Animal Production Systems, Indonesia.

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“The Role of Nutrition and Feed in Supporting Self Sufficient in Animal Products, Food Safety and Human Welfare”

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KEYNOTE SPEAKERS

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1. POLICY AND NATIONAL PROGRAM FOR FEED DEVELOPMENT

Mursyid Ma'sum

Director of Animal Feed, Directorate General of Livestock and Animal Health Services, Ministry of Agriculture – Indonesia

Abstract

The policy of the Directorate of Animal Feeding has been formulated to respond two main issues ie; 1). Feed Security, the availability of feeds includes feed ingredients or raw material of feeds, 2). Feed Quality and Feed Safety; to meet the need of nutrition and free from feed hazards/contaminants (physical, chemical and biological hazards). In fact, Indonesia as an agricultural country has a big potency to meet the demand and to respond the global issue related to animal feeds ie. Highly competitive among feed, food and fuel, climate change and the increasing need for feed safety that will affecting food safety from animal origin.

Issues on national animal feed have been identified as follows: 1. **Ruminant Feed.** The classic issue on forage is still happen, over production in wet season and scarcity during dry season, limited land for animal farm and less adoption of feed handling, processing and preservation technology. Meanwhile, the production of concentrate for ruminant animals is left behind compared to poultry feed production. The products could not meet the national quality standard. 2. **Poultry Feed.** Although poultry feed production has been implement the Good Manufacturing Practice and developed significantly, the producers are challenged to improve feed efficiency to produce better quality feed for affordable price. The Strategic Program to support Self-Sufficient Program for Beef and Buffalo 2014(PSDSK) in livestock center areas is

conducted with different activities such providing good seed, integrated farming with forestry, accelerate development of pasture and “cut and carry system”, the use of applied technology based on local feed. The Directorate of Animal Feeding has formulated the strategy for development of poultry feeds based on conventional and non-conventional feedstuff, facilitate the establishment of mini feedmill for local livestock based on local available feedstuffs. The strategy for ruminant feeding is approached by providing good quality forages and concentrate by providing feed processing units, establishing grazing field, water supply and technology and feed management supervision. The policy for development of feed quality and quality control has been established.

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2. THE MODE OF ACTION OF CHITINOLYTIC ENZYMES: PRODUCTION OF BIOACTIVE OLIGOSACCHARIDES AS ANIMAL NUTRIENTS

Tamo Fukamizo

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Abstract

Chitinolytic enzymes (chitinase and chitosanase)—Chitin, a β -1,4-linked polysaccharide of *N*-acetylglucosamine (GlcNAc), is hydrolyzed by chitinases (EC 3.2.1.14), producing chitin oligosaccharides (GlcNAc)_n, which are biologically active and can be utilized for animal nutrients. Chitinases are divided into two families (family GH18 and family GH19) according to the CAZy database. We have been analyzing the crystal structures of GH-18 and GH19 chitinases from various plant species in a complex with chitin oligosaccharides, (GlcNAc)_n. GH18 chitinases hydrolyze (GlcNAc)₆ into (GlcNAc)₂+(GlcNAc)₄ with net retention of the anomeric form, while GH19 enzymes hydrolyze the hexamer into (GlcNAc)₃+(GlcNAc)₃ with anomer inversion. On the other hand, chitosans, deacetylated products obtained from chitin, are usually found in fungal cell wall together with chitin, and hydrolyzed by chitosanases (EC 3.2.1.132). Our group first reported the structure-function relationship of a chitosanase from *Streptomyces* sp. N174. The enzyme hydrolyzes chitosan hexamer (GlcN)₆ into (GlcN)₃+(GlcN)₃ with inversion of the anomeric form.

Enzymatic digestion of fungal cell wall—The intact cells of *Rhizopus oligosporus* NRRL2710, whose cell walls are abundant source of *N*-acetylglucosamine (GlcNAc) and glucosamine (GlcN), were digested with three chitinolytic enzymes, a GH-46 chitosanase from *Streptomyces* sp. N174 (CsnN174), a chitinase from *Pyrococcus furiosus*, and a chitinase from *Trichoderma viride*, respectively. Solubilization of the intact cells by CsnN174 was found to be the most efficient from solid state CP/MAS ¹³C-NMR spectroscopy. Chitosanase products from *Rhizopus* cells were purified by cation exchange chromatography on CM-Sephadex C-25 and gel-filtration on Cellulofine Gcl-25m. NMR and MALDI-TOF-MS analyses of the purified products revealed that GlcN-GlcNAc, (GlcN)₂-GlcNAc, and (GlcN)₂ were produced by the enzymatic digestion of the intact cells. The chitosanase digestion of *Rhizopus* cells was found to be an excellent system for the conversion of fungal biomass without any environmental impact.

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3. IMPROVING EGG NUTRITIONAL VALUE BY DIETARY MARINE SOURCES – A CURRENT UPDATE

Yuwares Ruangpanit

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Abstract

Value added egg is considered one strategy to increase egg consumption. Our current researches indicate that egg nutritional value could be improved by the utilization of dietary marine sources. It is known that an increase in ω -3 poly-unsaturated fatty acids (PUFA ω -3) consumption could lead to a benefit for consumer health. PUFA ω -3, especially DHA (docosahexaenoic acid) is found mainly in marine plants (algae, microalgae) and marine animals. Beside essential fatty acid, dietary marine sources contain also a great quantity of several carotenoids and antioxidants. Two experiments were conducted by supplementing microalgae and krill meal in laying hen diets to produce ω -3 and vitamin A enriched eggs. **Exp. 1** Microalgae, *Schizochytrium sp.*, containing DHA 40% of total fat was used in this study. A total of 192, 33 week old, Lohmann Brown laying hens were divided into 4 dietary treatments with 6 replications per treatment. Experimental diets were supplemented with the microalgae *Schizochytrium sp.* at 0, 0.5, 0.75 and 1 %. All diets were isocaloric and isonitrogenous (2,750 kcal/kg and 17.5% CP). During the first 28 days, there was no significant effect of *Schizochytrium sp.* supplementation on production performance. Alpha-linolenic acid (ALA) and eicosapentaenoic acid (EPA) was not different among dietary treatments. However, DHA content in egg yolks increased significantly with an increase in the level of *Schizochytrium sp.* in the diet ($P < 0.01$). **Exp. 2** Krill is a small marine crustacean found in Antarctic waters (Antarctic krill or Whale krill). Krill contains high quality protein, rich in marine phospholipids and PUFA ω -3 as well antioxidants. It contains 10-140 ppm of astaxanthin, a carotenoid (pink-red pigment) that is commonly used in aquaculture. A total of 240 Lohmann Brown laying hens were divided into 5 treatments including; corn-soy basal diet (CS); CS with 7.5% cassava meal (Low Pigment; LP) and LP with 1, 3, and 5% krill meal, respectively. Each treatment consisted of 6 replications with 8 birds each. Birds were raised in an evaporative cooling system house with feed and water provided *ad libitum* for a 3-28 day period. There was no significant difference in production performance and egg quality among dietary treatments. Feeding the LP diet lowered yolk color score ($P < 0.05$). Using krill meal in the LP diet increased redness ($P < 0.01$) and decreased yellowness ($P < 0.05$) of egg yolk resulted in a higher yolk color score ($P < 0.05$). Feeding higher levels of krill meal tended to increase astaxanthin content ($P = 0.1089$) and significantly increase vitamin A content of egg yolk. Under the conditions of the present studies, microalgae and krill meal could be used to produce value-added eggs, giving consumers more of choice and better health.

Key words: Microalgae, Krill meal, PUFA ω -3, Astaxanthin, Vitamin A, Egg

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4. THE UTILIZATION OF JUICE WASTE MIXTURES IN POULTRY DIETS: A REVIEW

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Abstract

The utilization of fruit or vegetable wastes mixture is one of solutions to overcome the shortage of feeds in poultry industry. A series of experiments had been conducted to utilize the juice wastes mixture (JWM) in the chicken diets. This JWM consists of carrot (*Daucus carotta*), apple (*Mallus sylvestris*), mango (*Mangifera indica*), avocado (*Persea americana*), orange (*Citrus* sp.), melon (*Cucumis melo L*), and tree tomato (*Cyphomandra betacea* Sendtn.) in the same proportion. Results of experiments showed that the JWM contained crude protein similar to corn, some of its amino acids were superior to corn, but its metabolizable energy was lower than corn. This JWM could be included up to 20% in diet to effectively replace 40% corn in broiler diets. The main problem was the height in its crude fiber content. Three methods (physical, chemical and biological) were performed to improve its nutritive value. All the three methods could improve this JWM nutritive value when it was compared with the untreated JWM. However, the chemical method by using rice-hull ash filtrate was superior to the physical method (high pressure steaming in autoclave) and biological method (fermentation with *Trichoderma viride*) in improving the JWM nutritional value. This improved juice wastes mixture could be included up to 40% in the diet to effectively replace 80% corn in broiler diets, and up to 30% to substitute for 60% corn in layer diets. Further investigations are still needed to increase its utilization in poultry diets.

Keyword: *juice wastes mixture, rice-hull ash filtrate, steaming, fermentation, trichoderma viride, broiler, layer*

INTRODUCTION

The conventional feeds in the diet of poultry such as corn, soybean meal and fish meal are often limited in their availability, especially in the developing country like Indonesia. Diversification of feeds is one of the alternative solutions to overcome the shortage of these feeds. This diversification can be obtained from the utilization of varieties of agricultural wastes or agricultural-industry by-products.

Some experiments about the utilization of wastes from fruits and vegetables had been performed. Apple by-products could be included up to 10% in the broiler diet (Zafar *et al.*, 2005). Al-Betawi (2005) found that tomato pomace could be used in broiler rations at a level of 10% from the total diet. While, Ghazi and Drakhshan (2006) reported that the inclusion of up to 15% of untreated tomato pomace could be recommended for practical poultry diet formulation, and more investigations are still needed on this subject. The other study on the utilization of fruit wastes by Al Khawajah (2003) has shown that the citrus pulp could be supplemented up to 7.5% in the diet without affecting body weight gain and feed conversion of broilers. The sun dried sweet orange (*Citrus sinensis*) rind can be used to replace dietary maize in the diet of broilers at 15% level (Oluremi *et al.*, 2006). Further experiment by Mourao *et al.*

(2008) found that 10% citrus pulp in the diet decreased the daily weight gain of broilers by about 26%. Agu *et al.* (2010) reported that sweet orange peel meal could be included up to 20% for maize replacement in broiler diets. Diarra *et al.* (2011) recommended that the boiled mango kernel meal could replace up to 50% of the maize in the starter and grower diets, and up to 75% in the finisher diet of broilers without adverse effects on growth, carcass measurements and blood parameters.

However, none of these experiments used the available fruit or vegetable wastes in a form of mixture or in combination. Besides, less information is available on the utilization of other fruit or vegetable wastes for poultry diets. This paper will review the utilization of juice wastes mixture (JWM) in the chicken diets from the results of a series of experiments.

THE JUICE WASTES MIXTURE (JWM) IN CHICKEN DIETS AND ITS OBSTACLES

The JWM is the combination of carrot (*Daucus carotta*), apple (*Mallus sylvestris*), mango (*Mangifera indica*), avocado (*Persea americana*), orange (*Citrus sp.*), melon (*Cucumis melo L.*), and tree tomato (*Cyphomandra betacea* Sendtn.) in the same proportion. The nutrient and energy contents of this JWM are as follows: crude protein 8.44%, crude fiber 17.1%, ether extract 6.24%, Ca 0.09%, P 0.01% and metabolizable energy 1747 kcal/kg, while its amino acids content as compared with corn is figured out in Table 1 (Rizal *et al.*, 2010a). The crude protein content of this JWM is similar to corn (NRC, 1994). The tryptophan content of this JWM is 4.0 times, lysine 1.6 times, glycine 1.6 times and threonine 1.3 times higher than that of the corn (Rizal *et al.*, 2010a). Results of fiber analysis by using Van Soest *et al.* method (1980) indicated that the concentrations of NDF 34.3%, ADF 24.4%, cellulose 12.2%, hemicellulose 9.9%, and lignin 11.8% in this JWM (Rizal and Mahata, 2009).

Table 1. The amino acid contents in JWM as compared with corn.*

Amino Acids	Amino Acids Concentration (% DM)	
	Corn	JWM
Aspartate	-	0.71
Glutamate	-	0.98
Serine	0.37	0.46
Histidine	0.23	0.14
Glycine	0.33	0.54
Threonine	0.29	0.39
Arginine	0.38	0.37
Alanine	-	0.54
Tyrosine	0.30	0.43
Methionine	0.18	0.13
Valine	0.40	0.44
Phenylalanine	0.38	0.37
Iso Leucine	0.29	0.34
Leucine	1.00	0.54
Lysine	0.26	0.42
Proline	-	0.68
Cysteine	0.18	0.05
Tryptophan	0.06	0.23

*Rizal *et al.* (2010a).

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The utilization of this JWM in the diet of broilers had been performed. The results of this experiment (Table 2) showed that up to 20% of JWM could be included in broiler diets to effectively replace 40% corn in the diet (Rizal *et al.*, 2010a). High crude fiber content in this JWM could be one of chemical compounds that limit its utilization by chickens. Further investigations are necessary to optimize the inclusion of this JWM in the broiler diet.

Table 2. Mean of growth performances and physiologic organ sizes as affected by treatments.*

Parameters	Treatments (JWM Levels)					SE ¹
	A=0%	B=10%	C=20%	D=30%	E=40%	
Feed Consumption (g/head/day)	63.08 ^a	63.53 ^a	69.19 ^b	69.89 ^b	69.29 ^b	1.77
Average Daily Gain (g/head/day)	32.37 ^a	33.21 ^a	39.06^b	40.26^b	39.93^b	1.41
Feed Conversion	1.95 ^a	1.92 ^{ab}	1.78^{bc}	1.74^c	1.74^c	0.05
Abdominal Fat Pad (%)	1.41 ^b	1.76 ^a	1.52 ^{ab}	1.32 ^b	1.28 ^b	0.11
Carcass (%)	68.61	70.73	69.03	68.56	67.53	1.20
Liver Percentage	1.70	1.87	1.73	1.77	2.02	0.11
Pancreas Percentage	0.23	0.26	0.33	0.24	0.24	0.05
Gizzard Percentage	2.49	2.56	2.45	2.56	2.27	0.15
Heart Percentage	0.60	0.58	0.55	0.50	0.55	0.04

¹ Standard Error of the Mean.

^{a,b,c} The means with different superscripts at the same row differ significantly (P<0,05).

*Rizal *et al.* (2010a).

IMPROVING THE NUTRITIVE VALUE OF JUICE WASTE MIXTURE

It has already been mentioned in the previous chapter that the utilization of this JWM is still limited due to the height in its crude fiber content. Several efforts could be conducted to reduce this obstacle, for examples: physical, chemical, or biological treatment. According to Nour *et al.* (1987), Guo *et al.* (2002), and Abebe *et al.* (2004) the physical, chemical and biological treatments could improve the nutritive value of feedstuffs.

Physical treatment such as using autoclave (high pressure steaming) is more effective than the other physical treatments in improving the crop residue nutritive value (Guo *et al.*, 2002). It could reduce the crude fiber content in the feedstuff. He *et al.* (1989) reported that high pressure steaming could decline the crude fiber content, but increase the crude protein content of wheat straw. Heat treatment reduced the anti-nutrition content of the feed (Nyirenda *et al.*, 2003).

Chemical treatment such as the utilization of alkali or hydrogen peroxide also decreased the crude fiber content of feed stuffs (Owen and Jayasuriya, 1989). Malekkhahi *et al.* (2012) found that NaOH and urea treatment declined the NDF and ADF contents of sesame residues. Mao and Feng (1991) reported that Ca(OH)₂ + urea treatment increased the crude protein of rice straw and wheat straw. The 10% use of the rice-hull ash filtrate for soaking the shrimp waste for 48 hr, decreased its chitin content (Mirzah, 2006). According to Suwandiyastuti and Bata (2010), the use of 10% rice-hull ash filtrate improved volatile fatty acid (VFA) production, ammonia nitrogen and microbial protein synthesis in ruminants.

In biological treatment, fermentation is one of several solutions to decrease the fiber content of the feed. Fungi of the species of *Trichoderma viride* is often used for fermenting feeds. This fungi produces several enzymes such as protease, lipase,

pectinase and cellulase (Pelczar and Reid, 1974; Wiseman, 1981; dan Rogers, 2002). Utilization of *Trichoderma viride* decreased the crude fiber, NDF, ADF, cellulose and hemicellulose content of cassava leaves (Rizal *et al.*, 2006). There is no information available on the utilization of *Trichoderma viride* for reducing the fiber content of the JWM.

EXPERIMENT 1

1. Physical Treatment (High Pressure Steaming in Autoclave)

The effects of the length of steaming in autoclave (0, 15, 30, and 45 minutes) on the crude fiber, crude protein and ether extract contents of JWM are depicted in Table 3.

Table 3. The effect of the length of steaming in autoclave on crude fiber, crude protein, and ether extract contents of JWM.*

Length of Steaming (minutes)	Crude Fiber (%)	Crude Protein (%)	Ether Extract (%)
0	13.36b	13.07b	5.42a
15	13.31b	13.60b	5.46a
30	12.02c	13.35b	4.62b
45	14.15a	15.17a	5.34a
SE ¹	0.17	0.35	0.11

¹ Standard Error of the Mean.

^{a,c} Means with different superscripts at the same column are differed (P<0.05).

* Mahata *et al.* (2012).

The result of experiment indicated that the length of steaming up to 30 minutes decreased the crude fiber content, but when the length of steaming was increased to 45 minutes, there was an increase in the crude fiber content of this juice waste mixture again. The crude protein content of JWM increased when the length of steaming reached 45 minutes. The ether extract content of JWM declined when the length of steaming was increased up to 30 minutes. However, when the length of steaming reached 45 minutes, the ether extract content increased again. The results of this experiment were in accordance the results of experiments by Wong *et al.* (1974), Pate (1982), Kling *et al.* (1987), Mirzah (1990), and Mahata and Rizal (2000), who found that high pressure steaming improved the nutrient contents of feed stuffs.

2. Chemical Treatment (Rice-hull Ash Filtrate)

a. The effect treatments on the crude fiber content of JWM

The effect of rice-hull ash filtrate doses (10, 20 and 30%) and soaking length (0, 24, 48 and 72 hours) on the crude fiber content of JWM is figured out in Table 4.

There was an interaction between doses and length of soaking on the crude fiber content. When the dose was increased, there was a reduction in crude fiber content at the same soaking length, but when the soaking length was also increased, there was a dramatic reduction in the crude fiber content of JWM. The reduction of crude fiber content occurred at the dose of A2 (20%) and soaking length of B3 (72 hr).

Table 4. The effect of rice-hull ash filtrate dose and soaking length on the crude fiber content of JWM (%).*

Treatment	B0(0)	B1(24)	B2(48)	B3(72)	Average
A1(10%)	16.31 ^{Aa}	16.19 ^{Aa}	15.09 ^{Ba}	14.49 ^{Ca}	15.52
A2(20%)	14.99 ^{Ab}	15.40 ^{Ab}	13.38 ^{Bb}	12.70^{Cc}	14.12
A3(30%)	14.62 ^{Ab}	14.61 ^{Ac}	12.80 ^{Bc}	13.33 ^{Bb}	13.84
Average	15.31	15.40	13.76	13.51	

^{a,b,c} Means with different superscripts at the same column are significantly differed (P<0.05).

^{A,B,C} Means with different superscripts at the same row are significantly differed (P<0.05).

* Rizal *et al.* (2010b).

b. The effect of treatments on the crude protein content of JWM

The effect of rice-hull ash filtrate doses (10, 20 and 30%) and soaking length (0, 24, 48 and 72 hours) on crude protein content of JWM is depicted in Table 5.

Table 5. The effect of rice-hull ash filtrate dose and soaking length on the crude protein content of JWM (%).*

Treatment	B0(0)	B1(24)	B2(48)	B3(72)	Average
A1(10%)	9.58 ^{Ca}	11.92 ^{Ba}	12.22^{ABa}	12.53^{Aa}	11.56
A2(20%)	9.88 ^{Ba}	11.00 ^{ABa}	10.69 ^{Bb}	12.22^{Aa}	10.95
A3(30%)	8.68 ^{Ba}	11.61 ^{Aa}	11.91 ^{Aab}	9.78 ^{Bb}	10.50
Average	9.38	11.51	11.61	11.51	

^{a,b} Means with different superscripts at the same column are significantly differed (P<0.05).

^{AB} Means with different superscripts at the same row are significantly differed (P<0.05).

* Rizal *et al.* (2010b).

The interaction between dose and soaking length were detected. When the dose was increased at the same soaking length, there was no difference in crude protein content of JWM among treatments. When the soaking length was increased at the same level of dose, there was an increase in crude protein content of JWM. When the dose and soaking length were augmented to certain levels, the crude protein content went up. However, at the peak level of dose and soaking length, the crude protein content went down again. In this study, the dose of 20% and the soaking length of 72 hr were enough to increase the crude protein content of JWM.

c. The effect of treatments on ether the extract content of JWM

The effect of rice-hull ash filtrate doses (10, 20 and 30%) and soaking length (0, 24, 48 and 72 hours) on the ether extract content of JWM is illustrated in Table 6.

Table 6. The effect of rice-hull ash filtrate dose and soaking length on the ether extract content of JWM (%).*

Treatment	B0(0)	B1(24)	B2(48)	B3(72)	Average
A1(10%)	6.18 ^{Aa}	5.90 ^{Ba}	5.63 ^{Cb}	5.45 ^{Da}	5.79
A2(20%)	6.14 ^{Aa}	5.72 ^{Bb}	5.81 ^{Ba}	5.50 ^{Ca}	5.79
A3(30%)	5.91 ^{Ab}	5.70 ^{Bb}	5.38^{Cc}	5.22^{Cb}	5.55
Average	6.08	5.77	5.61	5.39	

^{a,b,c} Means with different superscripts at the same column are significantly differed (P<0.05).

^{A,B,C,D} Means with different superscripts at the same row are significantly differed (P<0.05).

* Rizal *et al.* (2010b).

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There was an interaction between doses and soaking length on the ether extract content of JWM. When the dose was increased, there was a decrease in the ether extract content at the same soaking length. However, when the soaking length was augmented, a very significant decline in ether extract content occurred. In this experiment the rice-hull ash filtrate dose of 30% (A3) and soaking length of 72 hours (B3) was appropriate to reduce the ether extract content of the JWM.

3. Biological Treatment (Fermentation by Using *Trichoderma viride*)

a. The effect of treatments on the crude fiber content of JWM

The effect of inoculum dose (5, 7 and 9%) and fermentation length (0, 5, 7, 9 and 11 days) on the crude fiber content of JWM is depicted in Table 7.

Table 7. The effect of inoculum dose and fermentation length on crude fiber content of JWM (%).*

Treatment	B0(0)	B1(5)	B2(7)	B3(9)	B4(11)	Average
A1(5%)	15.10 ^{ABa}	15.21 ^{Aa}	13.97 ^{Cb}	14.34 ^{Bab}	15.34 ^{Aab}	14.79
A2(7%)	13.72 ^{Bb}	12.23 ^{Cb}	14.18 ^{Bb}	13.73 ^{Bb}	15.04 ^{Ab}	13.78
A3(9%)	13.26 ^{Cb}	14.58 ^{Ba}	15.42 ^{Aa}	14.55 ^{Ba}	15.90 ^{Aa}	14.74
Average	14.03	14.03	14.52	14.21	15.43	

^{a,b,c} Means with different superscripts at the same column are significantly differed (P<0.05).

^{A,B,C} Means with different superscripts at the same row are significantly differed (P<0.05).

* Rizal *et al.* (2012).

There was an interaction between inoculum dose and fermentation length. When the inoculum dose was increased at the same fermentation length, the crude fiber content declined. When the fermentation length was also increased, the crude fiber content increased. The lowest crude fiber content of JWM was obtained at the inoculum dose of 7% and fermentation length of 5 days (A2B1).

b. The effect of treatments on the crude protein content of JWM

The effect of inoculum dose (5, 7 and 9%) and fermentation length (0, 5, 7, 9 and 11 days) on the crude protein content of JWM is illustrated in Table 8.

Table 8. The effect of inoculum dose and fermentation length on crude protein content of JWM (%).*

Treatment	B0(0)	B1(5)	B2(7)	B3(9)	B4(11)	Average
A1(5%)	11.00 ^{Ba}	10.02 ^{Cb}	11.84 ^{Aa}	10.14 ^{Cc}	10.44 ^{Bc}	10.69
A2(7%)	11.00 ^{Ba}	11.29 ^{ABa}	10.05 ^{Cb}	11.60 ^{ABb}	11.94 ^{Ab}	11.18
A3(9%)	10.69 ^{Ca}	10.88 ^{Ca}	11.21 ^{Ca}	13.30 ^{Ba}	15.69 ^{Aa}	12.35
Average	10.90	10.73	11.03	12.30	12.69	

^{a,b,c} Means with different superscripts at the same column are significantly differed (P<0.05).

^{A,B,C} Means with different superscripts at the same row are significantly differed (P<0.05).

* Rizal *et al.* (2012).

The interaction between inoculum dose and fermentation length was detected. The increase in the inoculum dose did not influence the crude protein content of JWM

at the same fermentation length. However, when the fermentation length was augmented, there was a very significant increase in this crude protein content of JWM. The highest crude protein content was obtained from the inoculum dose of 9% and the fermentation length of 11 days (A3B4).

c. The effect of treatments on ether the extract content of JWM

The effect inoculum dose (5, 7 and 9%) and length of fermentation (0, 5, 7, 9 and 11 days) on the ether extract content of JWM are depicted in Table 9.

Table 9. The effect of inoculum dose and fermentation length on ether extract content of JWM (%).*

Treatment	B0(0)	B1(5)	B2(7)	B3(9)	B4(11)	Average
A1(5%)	4.25 ^{Ba}	4.41 ^{Ba}	4.31 ^{Bb}	5.37 ^{Aa}	4.39 ^{Bb}	4.55
A2(7%)	4.77 ^{ABa}	3.72 ^{Cb}	5.16 ^{Aa}	5.31 ^{Aa}	4.30 ^{BCb}	4.65
A3(9%)	4.52 ^{Ba}	4.24 ^{Ba}	4.89 ^{ABab}	4.85 ^{ABb}	5.43 ^{Aa}	4.79
Average	4.51	4.12	4.79	5.18	4.71	

^{a,b,c} Means with different superscripts at the same column are significantly differed (P<0.05).

^{A,C} Means with different superscripts at the same row are significantly differed (P<0.05).

*Rizal *et al.* (2012).

The inoculum dose and fermentation length were interacted in the ether extract content of JWM. The increase in the level of inoculum dose did not influence the ether extract content of JWM at the same fermentation length. However, when the fermentation length was increased to a level of 5 days, there was a decline in the ether extract content of this JWM. When the increase in this fermentation length more than 5 days, the ether extract content augmented again. The lowest ether extract contain was reached at the inoculum dose of 7% and the fermentation length of 5 days (A2B1).

EXPERIMENT 2

1. The nutrient content, anti-nutrition content, nitrogen retention, and metabolizable energy of untreated vs. treated JWM.

The effect of treatment on nutrient content, anti-nutrition content, nitrogen retention, and metabolizable energy are depicted in Table 10.

In physical treatment, the utilization of autoclave (high pressure steaming) treatment numerically reduced the crude fiber, NDF, ADF and ether extract contents of JWM. However, this treatment numerically increased crude protein content and improved nitrogen retention and metabolizable energy of JWM. He *et al.* (1989) also found that high pressure steaming decreased crude fiber content of wheat straw.

In chemical treatment, the utilization of rice-hull ash filtrate reduced crude fiber, NDF, ADF, hemicelluloses and tannin contents of JWM. The reduction of NDF and ADF was in accordance with the experiment by Madrid *et al.* (1998) who found that the NDF content of wheat straw declined when treated with NaOH + urea. According to Chaudhry (1998), the reduction of this NDF was due to the declining in hemicelluloses.

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2. The amino acids content of untreated vs. treated JWM.

The amino acids content of untreated vs. treated JWM (physical, chemical and biological) is figured out in Table 11.

Table 10. Nutrient content, anti-nutrition content, nitrogen retention and metabolizable energy of untreated vs.treated (physical, chemical andbiological) JWM.*

Compound and Energy	Untreated (%)	Treated (%)		
		Physical	Chemical	Biological
Crude Fiber	17.10	12.02	12.70	12.23
NDF	34.30	32.62	32.57	31.55
ADF	24.40	22.05	21.90	22.43
Cellulose	12.20	10.50	12.50	11.15
Hemicellulose	9.90	10.57	6.67	9.12
Lignin	11.80	11.55	11.40	11.28
Crude Protein	8.40	13.35	12.22	11.29
Ether Extract	6.24	4.62	5.50	3.72
Starch	0.09	1.04	1.28	1.15
Cellulose	0.01	0.01	0.13	0.21
Phytate	0.84	1.15	1.28	1.27
Tannin	1.6	0.08	0.10	0.18
Nitrogen Retention	59.99	65.51	67.57	63.64
Metabolizable Energy (kkal/kg)	1747	2550	2717	2599

* Rizal *et al.* (2010b).

Table 11. Amino acids’ profile of untreated vs. treated JWM (% DM).*

Amino Acids	Untreated	Treated		
		Physical	Chemical	Biological
Aspartate	0.71	0.61	0.79	0.60
Glutamate	0.90	0.77	0.92	0.78
Serine	0.32	0.32	0.40	0.34
Histidine	0.13	0.12	0.17	0.08
Glycine	0.41	0.39	0.50	0.41
Threonine	0.36	0.35	0.48	0.37
Arginine	0.31	0.25	0.34	0.26
Alanine	0.43	0.36	0.47	0.37
Tyrosine	0.23	0.21	0.27	0.25
Tryptophan	0.13	0.12	0.17	0.14
Methionine	0.06	0.07	0.09	0.10
Valine	0.41	0.39	0.51	0.41
Phenylalanine	0.31	0.31	0.39	0.32
Iso-leucine	0.34	0.32	0.43	0.33
Leucine	0.48	0.45	0.58	0.48
Lysine	0.34	0.20	0.42	0.27
Cystine	0.01	0.02	0.02	0.03
Cysteine	0.02	0.02	0.03	0.02
Proline	0.50	0.50	0.54	0.51

* Rizal *et al.* (2010b).

Amino acids content of JWM was numerically different among untreated and treated physically, chemically and biologically. When this JWM was treated physically by using autoclave, most of its amino acids content declined when compared to

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untreated. Mean while, when this juice wastes mixture was treated chemically by using rice-hull ash filtrate at the level of 20% for 72 hours, there was an increase in most of the amino acids content of the JWM. Biological treatment by using *Trichoderma viride* at the level 7% for 5 days of fermentation length also reduced some amino acids content of the juice wastes mixture compared to the untreated one. So, the chemically treated by using rice-hull ash filtrate at the level of 20% for 72 hours of this JWM was the best among others treated and untreated in its amino acids content. All of its amino acids content were higher than the untreated juice wastes mixture.

IMPROVED JUICE WASTES MIXTURE (IJWM) IN THE CHICKEN DIETS

Two experiments were performed to evaluate the utilization of IJWM in chicken diets. The first experiment was to evaluate the IJWM in broiler diets, and the second was to evaluate the IJWM in laying hen diets.

The IJWM used in the diet of broilers and laying hens was the juice wastes mixture which was treated with rice-hull ash filtrate. This treatment was selected due to the nutritive values of this IJWM such as nitrogen retention, metabolizable energy and amino acid profile were better than the other treatments.

A. IJWM in Broiler Diets

The means of feed consumption, average daily gain, feed conversion, abdominal fat pad percentage and carcass percentage of broilers were depicted in Table 12.

Table 12. The effect of treatments on feed consumption, average daily gain, feed conversion, abdominal fat pad and carcass percentage of broilers.*

Treatments	Feed Consumption (g/head/day)	Average Daily Gain (g/head/day)	Feed Conversion	Abdominal Fat Pad (%)	Carcass (%)
A (0% IJWM)	63,04 ^a	32,54 ^d	1,94 ^a	1,20 ^a	67,78
B (20% IJWM)	60,91 ^b	38,45^a	1,60^b	0,94^b	72,00
C (25% IJWM)	58,72 ^c	36,67^b	1,60^b	0,88^{bc}	70,60
D (30% IJWM)	52,23 ^f	32,80^{cd}	1,60^b	0,80^c	69,28
E (35% IJWM)	54,61 ^e	34,23^c	1,58^b	0,84^c	70,23
F (40% IJWM)	57,42 ^d	36,22^b	1,58^b	0,88^{bc}	70,68
SE ¹	0,23	0,49	0,02	0,03	1,61

^{a,b,c,d,e,f} Means with different superscripts at different column indicate significantly different (P<0,05).

¹ Standard Error of the Mean.

^{*} Mahata *et al.* (2013)

The feed consumption of the broilers was very significantly affected (P<0.01) by treatments. The utilization of IJWM decreased the feed consumption of broilers. 30% IJWM in the diet was the lowest feed consumption of broilers. However, when the level of IJWM was increased to 40% in the diet, the feed consumption of broilers also increased.

Average daily gain of broilers was influenced (P<0.01) by treatments. Utilization of IJWM in the diets augmented the average daily gain of broilers. The 20% utilization of IJWM in the diet has the highest average daily gain of broilers. The 30% level of IJWM in the diet had the same average daily gain of broilers as the 0% level of

IJWM. However, all other levels of IJWM addition in the diets have higher average daily gain of broilers when they are compared with no IJWM addition.

The feed conversion of broiler was highly affected ($P < 0.01$) by the treatments. The utilization of IJWM in the diets decreased the feed conversion or improved the efficiency of feed utilization of the broilers. The increase in the level of IJWM in the diets improved the efficiency of feed utilization. However, there was no difference in the feed conversion among the levels of addition of IJWM in the diets. It means that the IJWM could be included up to 40% in the diets of broilers to effectively replace 80% corn in the diet of broilers.

B. IJWM in Laying Hen Diets

The means of feed consumption, egg production and feed conversion of laying hens as affected by treatments were illustrated in Table 13.

Table 13. Effects of treatments on feed consumption, egg production, feed conversion, egg weight, and hen-day egg production of laying hens.*

Treatments	Feed Consumption (g/head/day)	Egg Mass Production (g/head/day)	Feed Conversion	Egg Weight (g/egg)	Hen-day Egg Production (%)
A(0% IJWM)	90.23 ^b	25.56 ^c	3.53 ^b	51,72	48.42 ^b
B(10% IJWM)	95.48 ^a	33.26 ^a	2.87 ^c	53,41	62.23 ^a
C(20% IJWM)	96.73 ^a	29.51 ^b	3.29 ^{bc}	53,31	54.69 ^b
D(30% IJWM)	97.80 ^a	25.53 ^c	3.85 ^b	52,26	47.29 ^b
E(40% IJWM)	100.01 ^a	24.43 ^c	4.28 ^a	52,90	44.90 ^b
SE	1.57	0.90	0.12	0,67	2.09

^{a,b,c} Means with different superscripts at the same column are differed ($P < 0,05$).

SE = Standard Error of the mean.

*Rizal *et al.* (2011).

The feed consumption of the laying hens was very significantly influenced ($P < 0.01$) by the treatments. The feed consumption of laying hens receiving the control diet was lower than that of the feed consumption of laying hens receiving the treated diets. The addition of IJWM in the diets up to 40% increased the feed consumption of the laying hens. However, there is no difference in the feed consumption of laying hens receiving 10, 20, 30 and 40% IJWM in the diets.

The egg mass production of laying hens was very significantly affected ($P < 0.01$) by the treatments. The egg mass production of laying hens receiving the control diet was not differed from those receiving 30 and 40% IJWM in the diets. However, the egg mass production of laying hens receiving diets containing 10 and 20% IJWM was higher than those of the control, 30% and 40% IJWM in the diets. The egg mass production of laying hens receiving 10% IJWM in the diet was the highest among the treatments.

The feed conversion of of the laying hens was significantly influenced ($P < 0.05$) by the treatments. The feed conversion of laying hens receiving control diet was not differed from those receiving 20 and 30% IJWM in the diets. However, it was higher than those receiving 10% IJWM in the diet, and lower than those receiving 40% IJWM in the diet. The best feed conversion was obtained from the laying hens receiving 10% IJWM in the diet. The high in feed conversion of broilers receiving the 40% IJWM in the diet means that the efficiency of feed utilization of broilers receiving this diet was

low. So, utilization of IJWM in the diet of laying hens was only up to 30% to effectively replace 60% corn.

The egg weight of laying hens was not influenced ($P>0.05$) by treatments. Eventhough the feed consumption increased due to the increase in the level of IJWM in the diets, the egg weight did not differ.

The hen-day egg production of laying hens was highly affected by treatments. The hen-day egg production of laying hens receiving 20, 30 and 40% IJWM in the diets was not different from those of control diet, on the other hand the hen-day egg production of laying hens receiving 10% IJWM was significantly higher than those of the control diet. So, increasing the level of IJWM in the diets did not influence the hen-day egg production of laying hens. The highest hen-day egg production was obtained from laying hens receiving 10% IJWM in the diet.

From the results of the experiment with laying hens, it could be concluded that the IJWM could be included up to 30% to effectively replace 60% corn in the diet of laying hens.

CONCLUSION

The JWM can be included up to 20 in the diet to effectively replace 40% corn in broilerdiets.

The obstacle in the utilization of JWM in broiler diets is due to the height in crude fiber content.

The improvement of the nutritive value of JWM can be performed through physical(high pressure steaming), chemical (rice-hull ash filtrate), or biological (fermentation with *Trichoderma viride*) treatment.

The improvement of this nutritive value of the JWM by using rice-hull ash filtrate is superior to high pressure steaming or fermentation treatment.

The improved juice wastes mixture can be included up to 40% in the diet to effectively replace 80% corn in broiler diets, and up to 30% to effectively replace 60% corn in layerdiets.

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5. UTILIZATION OF HERBS AS GROWTH PROMOTERS IN ANIMAL FEED

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Abstract

Herbs are whole plants or part of plants or plant extracts that are used for specific purposes such as spices in food flavouring, medicinal purposes, and recently as growth enhancers, in human and animal nutrition. Herbs that are used as feed additives, are known to contain organic compounds that affect animals metabolically and physiologically improving feed efficiency and utilization, and subsequently promote growth. With the ban on the use of antibiotics as growth promoters imposed in many parts of the world, scientists are looking for alternatives such drugs, organo-chemicals or herbs that serve the same functions. There are several herbs known to possess anti-microbial properties and these herbs have been used for human medications for the traditional treatment of several diseases. Traditional medicine has been long practiced in India and China not only for humans but also for animals. Poor farmers in India apply Ayuverda medicine for the treatments of common diseases as it is cheap and easily available. Recent studies have shown that several herbs, especially *Andrographis paniculata*, *Orthosiphon stamineus*, *Curcuma longa* and *Euphorbia hirta* are useful as growth promoters in the feeding of poultry. This paper discusses some of the work published on the role of herbs in livestock and aquaculture feeding.

Keywords: Herbs, herbals, essential oils, livestock, growth promoters

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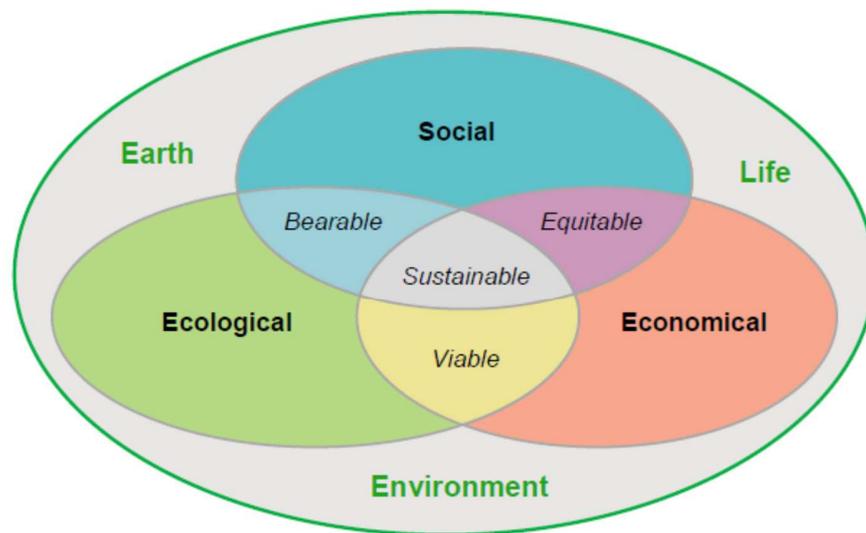
6. THE ROLE OF AMINO ACIDS IN SUSTAINABLE POULTRY PRODUCTION

Robert L. Payne and Michael Binder

Evonik Industries - US

Introduction

The world and its inhabitants face many challenges ahead. Population growth is rapidly expanding, and it is expected that we will reach a global population of 9 billion people by 2050. This population expansion is accordingly putting enormous pressure on our natural resources. Today, we can no longer debate if the inhabitants of earth are impacting its resources, but rather we must focus on how to sustain higher living standards for an increasing world population while dealing with less natural resources and more climate change. To do this, one must quickly realize that these social, environmental, and economical issues are not independent, but rather co-dependent as shown in Figure 1.



Source: Wikipedia

Figure 1. Sustainability Model

To add life to this model, consider the following scenario: While there is growing sentiment for climate protection, the world population is steadily growing and thus requiring a sustainable supply of high quality and safe food. To further complicate this, the growing population is also becoming more affluent, especially in developing countries. The direct result is increased demand for meat and milk products, which further intensifies the discussion on climate change. Ehrlich and Holdren (1974) introduced a simple equation to describe the relationship between environmental impact, population size, consumption level and technology. Their equation:

$$I = P \times A \times T$$

where: I = environmental impact; P = population; A = affluence (consumption); T = technology

Basically stated asks: “what is the environmental impact, given the relationship between population size, consumption level (affluence) and technology?”

Undoubtedly, animal agriculture is a major player in these global environmental issues. The huge demand for feed crop production shapes entire landscapes and can reduce natural habitats, causing degradation in some areas. The result is that agricultural production of meat and milk products for human nutrition is increasingly blamed as one of the main sources of detrimental greenhouse gases. The objective of this paper is to review the role of poultry production in this sustainability challenge and then to provide insight into how technology, such as the use of supplemental amino acids, can benefit the world's population, our environment, and finally poultry production long-term.

The Role of Animal Production

It is simply not enough to think of poultry production as just feeding and growing birds anymore. Today, there is so much more to poultry production including but not limited to: crop production and its inputs, production of feed additives and their inputs, feed and livestock production themselves, manure handling, meat processing, retailing, and finally the consumer. Additionally, important structural changes are taking place in poultry production, including intensification, the vertical integration and up-scaling of production, and shifts towards increased consumption of poultry meat. There are also geographic shifts, with production moving away from local natural resources. All of these inputs ultimately have an impact of the relatively simple task of growing chickens or eggs. To that end, four high- impacting megatrends on animal agriculture have been identified: demographic transition, economic growth, the nutrition transition and technological change.

The first factor, demographic transition, is shaped not only by the population explosion, but also by urbanization. Today, more people are migrating to urban areas from rural communities and there is a strong trend towards people living in mega cities. The result is increased urban sprawl and potentially decreased arable land available for agricultural production. Furthermore, this increased urban population demands convenient, affordable, safe, and healthy food options, which not only taxes the production of that food, but also becomes a significant challenge to the food supply chain. The second factor, economic growth, has a strong influence on meat consumption. As per capita income increases, meat consumption increases. This is illustrated in Figure 2. This relationship is especially seen in people with a low income; they tend to increase their meat consumption disproportionately as their income increases.

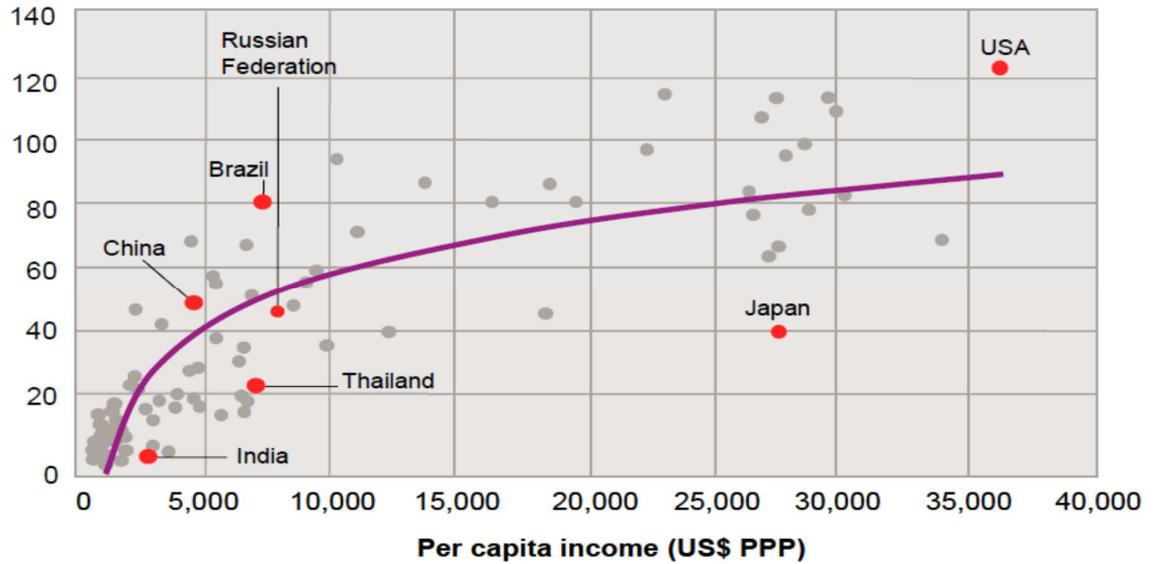
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Percapitameatconsumption(kg)

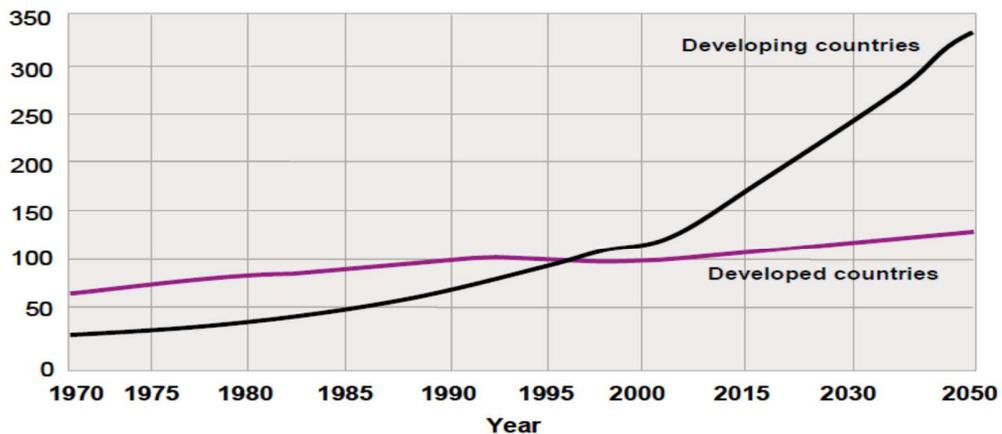


Note: National per capita based on purchasing power parity (PPP) - Source: World Bank (2006) and FAO (2006)

Figure 2. Relationship between per capita income and meat productionPer capita meat consumption (kg)

Further support to this increase in meat consumption is shown in Figure 3, which reports the projected meat production for the developed and developing countries from 1970 to 2050. There is a relatively small growth in the developed countries but a tremendous growth in the developing countries. This again highlights that there is a disproportionate increase in meat consumption driven by increasing purchasing power in those countries. As a reminder, almost two-thirds of the world’s population resides in these “developing” countries.

Milliontonnesofmeat



Source: FAO, 2006

Figure 3. Projected meat production: developed and developing countries.Million tonnes of meat

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The trend of shifting towards consuming more meat is the third factor, nutrition transition. In this factor, the shift from undernourishment to richer and more varied diets is accelerated. Table 2 illustrates the changes in food consumption. Globally substantial progress has been made in providing high quality protein however not all regions have benefited to the same degree.

Table 2. Changes in food supply in selected regions* Source: FAO, 2010

		1961	1970	1980	1990	2000	2007
China	Milk	2.51	2.25	3.04	6.01	9.71	28.70
Africa	Meat	13.27	13.41	14.13	14.16	14.55	15.51
Africa	Milk	28.39	31.06	35.41	33.88	33.85	37.92
World	Meat	22.96	26.87	30.40	33.36	37.90	40.09
World	Milk	74.93	75.36	76.37	76.57	77.61	84.93

*kg/capita/year.

Technology is a key driver of global animal production

The fourth megatrend, technological improvement, is a key driver of global livestock production. Growing productivity has been achieved through advanced breeding and feeding technology, and through irrigation and fertilizer technology in crop production, leading to higher yields per hectare. The use of concentrate feed, more productive breeds through better genetics, animal health improvements and developments in the post-harvest sector can also be counted as technological improvements. Many of these have been helped by the application of modern information technology.

On a global scale, efficiency improvements of up to 35 % have been seen between 1980 and 2005 (Table 3). The development in chicken meat is truly remarkable as it not only reflects the increased demand for this meat, but also highlights the outstanding performance in that sector due to technological and biological progress.

Table 3. Key productivity parameters for livestock from 1961-2008 Source: FAO, 2010

	Chicken meat*		Egg yield**		Pig meat*		
	1961	2008	1961	2008	1961	2008	
Africa	1.15	2.40	77	109	32.63	32.01	
Eastern Asia	1.03	2.58	105	189	21.20	106.29	
European Union	2.16	6.65	201	248	98.20	142.46	
Northern America	3.41	7.97	209	264	94.25	155.59	
Oceania	2.08	7.62	185	203	55.93	95.90	
South America	1.26	7.37	112	178	25.72	80.14	
World	1.95	4.31	142	183	60.93	109.63	

* kg output/year/number of livestock

** eggs/layer/year

A theoretical example highlighting the importance of technology is provided in Table 4. This example shows that a high consumption level per capita is not necessarily a

negative as it also depends on technology and on the number of people. A small population with a high consumption level but under-developed technology can cause much more environmental damage than a very large population with medium size consumption level but progressive technology. It depends on the combination of these variables and technology is the critical factor. Although consumption is doubled, the impact on the environment can be kept constant or reduced by improving the technology.

Table 4. Influence of technology on relative environmental impact is demonstrated in this theoretical calculation

Population	100	100	100	100	100	100	100
Affluence	5	5	5	10	10	10	10
Technology	1	0.5	0.1	1	0.5	0.1	0.05
Impact	500	250	50	1000	500	100	50

Reducing the impact of poultry production

Globally, around 9 % carbon dioxide, 35–40 % of methane, 65 % of nitrous oxide and around 64 % of ammonia are derived from animal production. Animal production also has an enormous impact on water use as illustrated in Table 5. Advanced nutrition can alter the impact of poultry production significantly in these respects via balanced feeding strategies. As a general rule, a one percentage point reduction in dietary protein will lead to reductions of 10% nitrogen in manure, 10 % ammonia emissions into the air, 3 % water consumption and 5 % manure volume.

Table 5. Liters of water required to produce 1 kg of product or 1 kg of protein in various species or crops.

Species	Beef	Milk	Pork	Eggs	Poultry	Corn	Rice	Wheat
1 kg product	10,200	1,200	5,200	2,900	2,900	1,200	1,700	1,800
1 kg protein*	56,900	37,700	26,100	22,800	12,000	14,600	19,700	14,800

*This does not reflect quality of the protein

Source: Evonik calculations from Mekonnen and Hoekstra (2010)

Due to the increasing demand for meat, milk and eggs, it is unlikely that there will be an alternative to intensive livestock production. Therefore, we must look at how to mitigate or minimize the environmental impact in high density livestock areas. Two potential goals would be: 1) to reduce nutrient loading to the environment and 2) to improve the animal’s feed conversion, i.e. the efficiency of feed and water into animal products, e.g. protein, milk, eggs etc. Reducing the nutrient loading to the environment would have a direct impact on biodiversity of both land and aquatic species of both animal and plant origin, while improving feed conversion would reduce the total amount of feed needed per animal to reach a certain body weight. This would ultimately mean that our need for grain and oilseed crops could be reduced if producing same number of animals or the ability to produce more animals using existing crops.

Animal diets are typically a mixture of various feed ingredients, such as corn, wheat, soybean meal, rapeseed, fishmeal, plus concentrated sources of vitamins and minerals. The intention of such of diet is to provide the animal with the nutrients needed to grow and produce. Unfortunately, protein-containing feed ingredients are often

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unbalanced protein for animal nutrition, i.e. the amino acid profile does not fit with the actual physiological demand of the animal. As such, a compound feed based on various components such as corn, wheat and soybean meal (SBM) will also likely share this deficiency of essential amino acids.

In typical corn-soy based diets for broilers, methionine, lysine, and threonine are the first three limiting amino acids. A specific supplementation with these limiting amino acids can easily compensate deficits in compound feed and lead to improved growth performance. Lemme et al. (2002) demonstrated this by showing that feed conversion improved as dietary methionine content increased (Figure 4). In addition to improving the efficiency of the feed to provide growth, fortification with these essential amino acids also distinctly reduces the nitrogen emissions as well as the emissions of relevant greenhouse gases.

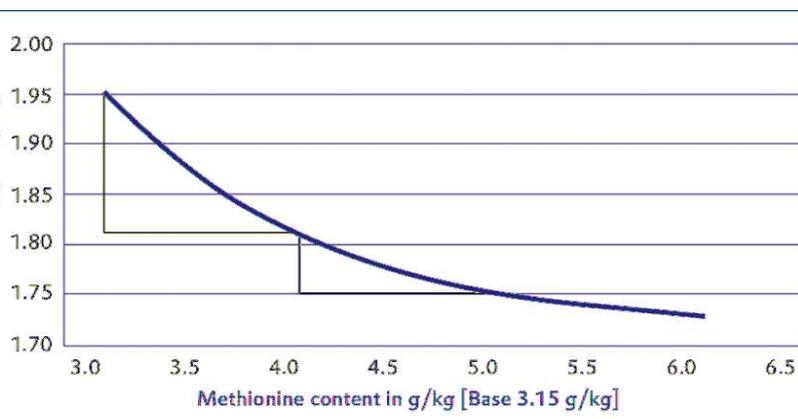


Figure 4. Effect of supplementing DL-methionine to a corn-soybean meal based diet on feed conversion (Lemme et al., 2002).

To further illustrate how improving feed efficiency and reducing the nutrient excretion can help mitigate the overall impact of poultry production, a life cycle assessment (LCA) for a typical broiler production scenario has been completed. Life cycle assessments describe the complete fate of a product by compiling and evaluating all ecological input and the consequences for the environment during each phase in the life cycle of the product. This covers the production of raw materials for the manufacturing process, the use by the consumer, and including the disposal of the used product. The methods in life cycle assessment follows clearly defined internationally harmonised norms (DIN EN ISO14040/44:2006).

A life cycle assessment is broken down into the definition of a framework, the target definition, the life cycle inventory and the environmental impact assessment. When setting up the life cycle inventory the factors of influence are then classified into categories according to their impact. The environmental impact assessment then puts a weight on the individual factors of the life cycle inventory within each of the impact categories. Finally the LCA is checked for conformity with the norms by an independent critical review panel.

As mentioned above, compound feed usually shows an unbalanced amino acid profile corresponding to the quality and composition of the feed ingredients used. The deficit in the essential amino acids can either be compensated by increased feed consumption, higher feed protein levels or by specific supplementation with the

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respective amino acid. In the LCA, feed mixes based on local cereals such as wheat and barely and with oilseeds such as soybean meal (SBM) or rapeseed meal are typically supplemented with the necessary quantities of DL- methionine, L-lysine, L-threonine, and L-tryptophan in order to meet the nutritional requirements. The LCA considers the entire production process of the feed ingredients of agricultural origins, the production of the amino acids, the production of the compound feed as well as the conventional animal production in Germany or Europe “from the cradle to the grave”

In order to compare the different production systems feed mixes need to be identified and defined which provide identical nutritional value to the animals. The gap for the essential amino acids can either be compensated by specific supplementation of the respective amino acids (Table 6; Option 1) or by increasing a certain ingredient in the ratio such as soybean meal or rapeseed meal (Options 2 and 3). For the latter part of this exercise, the wheat was partially replaced by either soybean meal or rapeseed meal.

Table 6. Alternatives for broiler feeding to meet specific animal requirements

	Description
Option 1	Supplementation with 3 amino acids: DL-methionine, L-lysine, and L-threonine in a wheat compound feed
Option 2	Compound feed based on SBM without amino acid supplementation
Option 3	Compound feed based on rapeseed meal without amino acid supplementation

The LCA evaluates the impact of the fortification of an amino acid deficient poultry feed by one kg of digestible amino acids in a pre-mixture per ton of feed. This is the functional unit used as a reference. The functional units in the other options provide the same amount of amino acids from natural protein so that all options provide an identical nutrient supply.

Life cycle inventory considers all contributions for the different options for each category of impact. The categories of impact primarily cover those areas of ecological relevance according to the current state of the science or as far as they can be described in a scientifically reliable manner (Table 7). The life cycle inventory covers the entire course of each option along all steps. As shown in Figure 5, all options have a common LCA after the processing in the feed mill. In the subsequent part of the LCA, relating to animal production and the disposal of the manure, only the nitrogen containing compounds such as ammonia and nitrate were considered in detail as these parameters should show the clearest difference between the three options.

The comparison of the systems is performed on the basis of the impact categories as shown in Table 7. The results for the impact categories are provided in the order of their importance in the bar graphs shown in Figure 6 to 10. The height of the bar represents the impact of the respective option on the category of environmental impact. The higher the bar the higher is the respective emission in the environmental category.

The results of this LCA indicate that feeding a diet containing supplemental amino acids (option 1) significantly reduces the global warming potential (Figure 6), acidification potential (Figure 7), eutrophication potential (Figure 8), primary energy demand (Figure 9), and resource consumption (Figure 10) relative to a diet containing either soybean meal or rapeseed meal.

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Interestingly, in the case of global warming, acidification, and eutrophication potentials, a diet using rapeseed meal as the primary source of amino acids was better than one using soybean meal. The reason for this is simply higher nitrogen emission from diet containing soybean meal. While soybean meal is often considered to have a good protein profile in terms of quality relative to the animal’s needs, it is higher in protein content than rapeseed meal, which in turn means that it contains higher levels of all dietary essential and non-essential amino acids. As there is no means to store excess amino acids in the body, then these must be deaminated and the nitrogen excreted via uric acid or urine. The result is increased nitrogen containing emissions such as ammonia, nitrates and N₂O. These N containing emissions amplify the acidification and eutrophication potential significantly.

Table 7. Selected impact categories as per the international scientific state of the art, also applied by the German environmental administration (UBA 2000)

Impact Category	Life Cycle Inventory Parameter
Resources consumption	Cumulated energy demand CED _{fossil} CED _{nuclear} CED _{regenerative}
Global warming potential Green house gas effect:	CO ₂ , N ₂ O, CH ₄
Acidification	NO _x , NH ₃ , SO ₂ , HCl, HF
Eutrophication	NH ₃ , NO ₃ ⁻ , CSB, N-compounds, P-compounds

Conversely, in the case of primary energy demand, a diet with rapeseed has the higher impact than one with soybean meal. This is likely related to the costs of fat extraction and processing of these oilseeds into meals.

Next steps

The data presented in this paper suggest that a diet using supplemental amino acids in place of either rapeseed or soybean meal has a significantly reduced environmental impact, and is thus more sustainable. Supplementation of amino acids to compound feed allows a more effective utilization by the target animal. Replacing imported oilseeds by regionally produced cereals combined with amino acid supplementation also reduces generation of environmentally harmful gases substantially. This contributes to reducing the greenhouse gas effect and avoiding unnecessary land use change. Also, less energy will be consumed for processing the feed ingredients.

In conclusion, the use of balanced amino acids and protein profiles is critical to achieving long-term sustainable animal production. The time for action is now. We must examine our feeding strategies, including but not limited to phase feeding, formulating on a digestible basis using ideal protein profiles, etc. and change those as necessary to achieve a goal to provide healthy, safe, affordable, and convenient food to 9 billion people by 2050.

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7. Rangeland and Pasture of Indonesia for Ruminant Production: a Poor Attention and Neglected Resources

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Native rangelands and/or grasslands and pasture of Indonesia are potential and essential for ruminants or non-ruminant herbivores production if properly managed. Currently, there is uncertain data of their location, area, and use. Domestic and/or wild herbivores rely very much on rangelands or grasslands for fulfilling their nutritional requirements and social interaction. Dynamic and sustainability of rangelands are influenced by climate change, pastoralists/ranchers activities, and herbivores population dynamic. Indonesian rangelands must be managed and recorded to maximize their production and strategic role in ruminant production. This paper reviews the existence and importance of Indonesian natural rangelands and pastures for the sustainability of ruminants production and the balance of the globe.

Keywords: rangeland, grassland, pasture, ruminant, herbivore, sustainability, wildlife

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8. FOOD AND FEED SAFETY ISSUES IN INDONESIA : REDUCING MYCOTOXINS TOXICITY AND ITS CARRY OVER FROM FEED INTO ANIMAL PRODUCTS

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Abstract

Nowadays, ensuring food safety has been a major focus of many countries in the world. Among chemical hazards, the contamination of food and feed by mycotoxins has been a major food safety issue due to its serious effects on human and animal health. Worldwide surveys indicated 81% of food or feed sample was contaminated at least by one mycotoxins. This data explained that mycotoxins issue not only resulted on public health hazard but also implicate on great economic losses due to losses in agricultural trading and animal productivity. High temperature and humidity in tropical climate are very conducive for several fungus to produce mycotoxins. Surveys on agricultural products in Indonesia showed high occurrence and level aflatoxin B1 (AFB1) contamination, the most toxic and carcinogenic toxin among group of mycotoxins. Since AFB1 in feed can be transferred into animal products as its hydroxylated metabolite, aflatoxin M1 (AFM1), the carry-over of AFB1 from contaminated feed into animal products has been a special interest. Further studies showed that AFB1 transfer from contaminated feed into AFM1 in milk by lactating cow can be a principal way of aflatoxin transfer from feed into food chain. Limited surveys in Central Java and Yogyakarta provinces indicated 100% of dairy ration was contaminated by AFB1 and then resulted in 100% of raw milk sample was containing AFM1. Due to its cancerogenity, aflatoxin intake by foods should be as low as possible, therefore many research have been conducted to find an effective and applicable method to reduce aflatoxin transfer from contaminated feed. The addition of adsorbents in the diet is the most recent approaches and widely applied way to prevent mycotoxicoses in the livestock. Several adsorbents have high affinity to AFB1, such as activated carbons, zeolites, bentonites, and certain clays may bind AFB1 in the gastrointestinal tract and reduce its bioavailability. However further studies are needed to examine the effects of these aflatoxin adsorbents in reducing aflatoxin transfer and on dairy cattle performance.

Key words: mycotoxins, aflatoxin B1, aflatoxin M1, aflatoxin transfer

INTRODUCTION

Mycotoxins are secondary metabolites produced by filamentous fungi, primarily *Aspergillus*, *Fusarium* and *Penicillium* genera. Mycotoxins can contaminate food and feed supply chain through fungal growth prior to and during harvest, or post harvest from improper storage conditions (Bhatnagar *et al.*, 2004). Mycotoxicosis is a disease caused by mycotoxins. Several health risks associated with mycotoxins are gastrointestinal problems, reduced immunity and reproduction, and in some cases, death might occurs (Bhat *et al.*, 2010).

Mycotoxins have adverse effects not only on human and animal health but also results on tremendous loss in economic and international trade. Globally, it is estimated that more than 25% of the world's crops are contaminated by mycotoxins (Gowda *et al.*,

2013). A worldwide surveys in 2009-2011 by Rodriques and Nahrer (2012) showed 81% feedstuffs and feed sample was contaminated by at least one mycotoxin. The most prevalence groups of mycotoxins are aflatoxin (Af), zearalenone (ZEN), deoxynivalenol (DON), fumonisins (FUM) and ochratoxin A (OTA) (Bryden, 2012).

Fungi infection in standing crop or stored feed is depending on agronomic practices, the composition of commodity, and the conditions of harvesting, handling and storage. Moisture and temperature have a major influence on fungi growth and mycotoxin production. In order to produce mycotoxin, they have to be stressed by some factors such as nutritional imbalance, drought or water excess.

Among group of mycotoxins, aflatoxin has been a special interest due to its occurrences and detrimental effects. The International Agency for Research on Cancer (IARC) has classified aflatoxin B1 (AFB1) as carcinogenic to human (Group 1) (IARC, 2002). Therefore, this paper will be focused on the occurrences and levels of AFB1 contamination in feed and feedstuffs in Indonesian, as well as the possibilities to reducing its toxicity on animal and its carry over into animal products.

AFLATOXIN CONTAMINATION IN AGRICULTURAL PRODUCTS AND FEEDSTUFFS IN INDONESIA

Aflatoxins are secondary metabolites produced mainly by species of fungus *Aspergillus flavus* and *A. parasiticus*. The toxigenic strains of those species are commonly found in important animal feeds such as groundnut cake, cottonseed cake, copra cake, and maize (Piet, 1992; Gowda *et al.*, 2013). The most toxic and frequent mycotoxin contaminant produced in those feedstuffs is aflatoxin B1 (AFB1) (Coulombe, 1993).

Occasionally, *A. flavus* and *A. parasiticus* contaminate grains, such as barley, oat, wheat, rice, peanut, soybean and cotton seed. Toxigenic strains of *A. flavus* and *A. parasiticus* require specific conditions of moisture and feed substrate to produce appreciable levels of its toxin. In order to avoid aflatoxins production by *A. flavus* or *A. parasiticus*, the moisture content of grains at the storage condition should be less than 14% at relative humidity less than 70% (Kabak *et al.*, 2006). Grains stored under high moisture (humidity >14%) at warm temperatures (> 20°C) and or inadequately dried can potentially become contaminated by aflatoxins (Richard, 2007).

Aflatoxins are toxic, mutagenic, teratogenic, and carcinogenic compounds. Consumption of food/feed contaminated with high level of AFB1 may lead to acute aflatoxicosis with fatal outcome. Regular intake at low dose of AFB1 is reported responsible for development of liver cancer, kwashiorkor, and impairment of child growth (Bhat *et al.*, 2010). Aflatoxin is a cofactor for human hepato-cellular carcinoma (liver cancer), it is estimated that aflatoxin exposure may contribute on 28.2% of all liver cancer case worldwide (Peng and Chen, 2009; Liu and Wu, 2010). The major symptom of acute aflatoxicosis in mammal includes lethargy, ataxia, rough hair coat, and enlarged fatty liver. Ingestion of aflatoxins-contaminated feeds in farm animal is reported reduced feed intake and feed efficiency that lead to decreased in productivity (Kabak *et al.*, 2006; Bhat *et al.*, 2010; Bryden, 2012)

Several surveys have been carried out in Indonesia to study the occurrence and levels of regional aflatoxin contamination in agriculture products. Sardjono *et al.* (1992) found that 28% of the peanut and 64% of the corn samples collected at farms, from middlemen, and retailers from several areas in Indonesia, were contaminated with A.

flavus/A. parasiticus. Ali *et al.* (1998) found 11 of 16 corn samples (69%), collected in Indonesia, were contaminated by total aflatoxin with a mean level of 119 µg/kg and a maximum level of 487 µg/kg. Goto *et al.* (1999) investigated 26 agricultural samples from markets in Central and East Java and Bali, Indonesia. They found that AFB1 was presented in five of eight peanut (62.5%) and four of five corn (80%) samples. They also reported that the highest level of AFB1 found approached 6000 µg/kg in a peanut sample and 300 µg/kg in a corn sample. In other study, Purwoko *et al.* (1991) found 91% out of the 34 corn samples tested were contaminated with aflatoxins, with concentrations ranging from 22 to 6171 µg/kg.

Apparently, level of AFB1 contamination in agriculture products will be higher by longer market chain. Survey at Blitar, Klaten and Tasikmalaya by Yuniarta and Agus (2008) found the average of AFB1 content in corn sample obtained from farmer was 18.6 µg/kg that is relatively low compare to corn sample obtained from retailer that was 139.81 µg/kg.

Survey on feed and feedstuffs indicated high occurrence and level of AFB1 contamination in Indonesia. Study on feedstuffs and feed for poultry by Bahri *et al.* (2005) in Lampung and East Java provinces of Indonesia concluded that AFB1 was mostly found in feedstuffs and commercial feed with occurrence between 70%-100% and levels between 2.2-198.4 µg/kg. Survey on dairy feed by Agus *et al.* (2013a) in Central Java and Yogyakarta provinces found 100% of dairy ration was containing AFB1 that was ranging from 4.15 to 84.82 µg/kg and the average of 46.60 µg/kg. In this study 24 of total sample (82.76%) contained AFB1 above USDA maximum tolerance limit for AFB1 in dairy ration (20 µg/kg). These results suggested that aflatoxin contamination in Indonesia is a crucial problem.

CARRY OVER OF AFLATOXIN RESIDUE FROM FEED INTO ANIMAL PRODUCTS

A metabolite of AFB1 is excreted in the milk of lactating cow that consumes feed contaminated by AFB1. This compound (AFM1) has high genotoxic activity and is also known to be hepatotoxic and carcinogenic (van Egmond, 1989). Recently, AFM1 was also detected in meat and egg of animal consuming feed contaminated by AFB1. However, the carry over of AFB1 into AFM1 in milk is a special interest because its prevalence, carry over rate, and milk is consuming by juvenile consumers (Voelkel *et al.*, 2011).

In the dairy cows, the amount of AFM1 excreted into the milk can be up to 3% of the AFB1 intake (Diaz *et al.*, 2004). Van Egmond (1989) stated that cows ingesting a quantity of AFB1 less than 40 µg/cow/day produce milk with an AFM1 content less than 0.05 µg/kg. Study by Sumantri *et al.* (2012b) showed Indonesian Friesian Holstein (IFH) consuming 350 µg AFB1/day and has milk production between 6.8-7.3 kg/day will transfer 0.10-0.12% of AFB1 intake into the milk. Surveys on dairy farming in central Java and Yogyakarta provinces was also showing COR value of IFH those were 0.32% in farmer group and 0.85% in individual farmer (Agus *et al.*, 2013a). This COR for IFH was much lower compared to estimated COR value for dairy cow in sub tropical region. Britzi *et al.* (2013) reported high COR values of high yielding lactating cows in Israel those were 5.8% and 2.5% for mid-lactation and late-lactation cow, respectively. Survey Some variables affect the COR value, namely: levels of contamination (van Egmond, 1989), stage of lactation (Veldman *et al.*, 1992; Britzi *et*

et al., 2013), milk yield (Masoero *et al.*, 2007; Britzi *et al.*, 2013), species differences (Battacone *et al.*, 2003) and individual variability (van Egmond, 1989).

In contrast to AFB1, only few data of AFM1 contamination on dairy milk or milk products in Indonesia has been published. Nuryono *et al.* (2009) reported that 100% of samples that were taken in Yogyakarta Province contained AFM1. However, levels of contamination were relatively low compared to results from other Asian countries. Result showed that 42.5% of samples contained AFM1 less than 5 ng/L milk, 27.4% of samples contained between 5 and 10 ng/L, and 30.1% contained more than 10 ng/L of milk. Compared to study on AFB1 in feedstuffs, surprisingly none of samples exceeded the EU regulation limits for AFM1 in food for infant and adult, 25 and 50 ng/L respectively.

High occurrence of AFM1 contamination in milk is a result of high occurrence of AFB1 contamination dairy feed. Survey by Agus *et al.* (2013a) on dairy farming and milking unit in Central Java and Yogyakarta provinces showed all of raw milk samples were containing AFM1 that were ranging from 14.6-220.7 ng/l. this study indicated high level of AFM1 contamination in raw milk. According to EU regulation, 95.2% of sample was not allowed for infant consumption (25 ng/L) and 68.25% of sample was not allowed for adult consumption (50 ng/L). High AFM1 concentration in this study was closely related to high AFB1 concentration which was found in corresponding feed sample.

STUDIES ON REDUCTION OF AFLATOXIN TOXICITY AND ITS CARRY OVER INTO ANIMAL PRODUCT

In the food chain, the best way to avoid risk of mycotoxicosis in human or animal is to reduce mycotoxins production levels by controlling harvesting conditions, grain maturity, or the use of biological and chemical agent in storage (Huwig *et al.*, 2001; Kabak *et al.*, 2006). However, tropical climate conditions and poor post-harvest management result in a high occurrence of mycotoxin contamination in agricultural products in developing countries such as Indonesia (Bryden, 2012). Therefore, several methods are developed to reduce the aflatoxicosis in animal and aflatoxin transfer to milk.

Utami (2010) studied garlic extract to reduce negative effect of AFB1 in broiler. Pure AFB1 inclusion in broiler diet until 1500 µg/kg for 42 days significantly reduced body weight and feed conversion and garlic extract supplementation minimized these negative effects. Apparently, Allicin, an active compound in garlic extract, could reduce AFB1 toxicity in broiler.

Since some detoxification methods (e.g. heating processes) have not shown to be applicable to reduce aflatoxicosis and aflatoxin transmission into the milk, inhibition of absorption of AFB1 in the animals gastro-intestinal tract is widely adopted for those purposes (Diaz *et al.*, 2004; Kabak *et al.*, 2006; Moschini *et al.*, 2008). Addition of non-nutritionally adsorbents in feed may reduce bioavailability of AFB1 in the gastro-intestinal tract. Due to its high affinity to mycotoxins, adsorbents (such as activated carbons, hydrated sodium calcium aluminosilicate, zeolites, bentonites, and certain clays) are widely used and are the most studied adsorbents to protect animals against mycotoxins (Kabak *et al.*, 2006).

In vitro study by Sumantri *et al.* (2012a) showed natural bentonite has the highest binding capacity and stability in rumen medium compared to natural zeolite and

activated carbon. Recent *in vivo* study indicated 0.1% natural bentonite inclusion on highly contaminated dairy feed (46 µg/kg) could maintain milk production level of IFH cow better than cow in group without bentonite (Agus *et al.*, 2013b).

CONCLUSION

Surveys on mycotoxin contaminations in feed and food in Indonesia, especially AFB1, indicated high level of aflatoxins exposures both on human and animal. The evidence on the carry over of aflatoxin from feed to food, increased consumers risk in Indonesia for adverse effects of aflatoxin. Therefore, it is important to emphasize studies on methods for reducing aflatoxin toxicity and transfer into food chain.

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RUMINANT NUTRITION

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01. ESTIMATION OF RUMEN MICROBIAL NITROGEN SUPPLY BASED ON PURINE DERIVATIVES EXCRETED IN THE URINE OF KEJOBONG AND BLIGON GOAT FED BY KING GRASS AND PEANUT STRAW

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Abstract

The aims of this experiment was to compare the rumen microbial nitrogen supply in three group of goat, namely male Kejobong goat, female Kejobong goat and male Bligon goat fed by the same diet, King grass and peanut straw. Five male Kejobong goats, six female Kejobong goats and six male Bligon goats were put in the metabolism cages, fed *ad libitum* twice a day at 08.00 am and 15.00 pm. The feeding trial was run for one-week collection period, after couple month of adaptation period. During collection period, samples of feed, uneaten feed as well as feses were taken out for dry matter and organic matter analysis. Daily urine collection was done for purine derivatives (PD) including allantoin and uric acid analysis. The data of total urinary PD excretions were used to estimated microbial nitrogen supply based on the equation postulated by Chen and Gomes (1992) with modification in endogenous purine derivatives excretion for Kejobong and Bligon goat (Purwati *et al.*, 2013). The result showed, there were no significant differences in dry matter and organic matter intake as well as digested dry matter and digested organic matter intake between groups animal. Urinary PD excretion in male Kejobong goat was higher compared with male Bligon goat (10^7 vs. $52 \mu\text{mol}/\text{W}^{0.75}/\text{d}$). Estimated microbial nitrogen supply in Kejobong goat showed the tendency higher compared with Bligon goat (0.985 vs 0.384 g/d) No differences were found for PD excretion and estimated microbial nitrogen supply between male and female Kejobong goat. The highest microbial N supply when expressed per gram rumen digested organic matter in Kejobong goat compare with Bligon goat indicated the higher feed efficiency in male Kejobong goat. In conclusion, response of goat fed by the same diet for rumen microbial nitrogen supply could be different among breeds.

Key words: Rumen microbial nitrogen supply, Purine derivatives, Kejobong goat, Bligon goat.

INTRODUCTION

In Indonesia, generally ruminant received only poor quality diet such as rice straw and other agriculture products. Due to the low protein content in this feedstuff, microbial protein would be the primary protein source to meet the animal need. Therefore, it attracts nutritionist to give a special notice for the rumen microbial synthesis as well as the contribution of the rumen microbes to the protein supply for the host animal.

The estimation of microbial protein supply based on the purine derivatives (PD: allantoin, uric acid, xanthine and hypoxanthine) excretion in the urine of ruminants is considered to be the simple method and non invasive as it does not need the digesta flow measurement as well as rumen-duodenal cannulated animal. Therefore it is being used by researchers all over the world for animal nutrition studies. The method has been adopted for Bali cattle, Ongole crossed bred cattle, Fresian Holstain crossed bred cattle as well as buffalo.

Chen *et al.* (1990) postulated a model to estimate rumen microbial protein synthesis for European cattle. The model based on his finding that there was correlation between purine absorbed (X) and purine derivatives excreted in the urine (Y), which was showed by equation $Y = 0.85 X + 0.385 W^{0.75}$. Yusiati (2005) found the different model to estimate rumen microbial protein synthesis among the different Indonesian cattle. It might be due to the differences in the enzyme activities of xanthine oxidase and uricase involved in the purine metabolism, such as their activities in the liver, plasma as well as in the intestinal mucous. The different model of rumen microbial protein synthesis also reported between Indonesian goat, namely Bligon and Kejobong goat (Purwati *et al.*, 2013). It is considered that we have to start using the model to evaluate the microbial protein supply of Indonesian ruminants. Therefore, in this present study the microbial protein supply of Bligon and Kejobong goat fed by the same diet (King grass and peanut straw) would be compared.

MATERIALS AND METHODS

Animals and biological trial

Two years old male of six Kejobong and six Bligon goats were used in this study to estimated microbial protein synthesis when the animals fed by King grass and peanut straw. All animals were kept in metabolism cages to get the good separation feces from urine samples and fed twice a day at 08.00 am and 15.00 pm. Diet and drinking water were served *ad libitum* for 2 months. The feeding trial was run for one-week collection period. During the time, 500 g of feed samples were taken daily as well as 10% of individual uneaten feed. The samples were dried, bulked and grinded. Sub samples were taken for nutrient analysis, including dry matter (DM), organic matter (OM) analysis according to the AOAC (2005) method. Daily individual feces samples as much as 5% of total excretion were taken and put in fridge through the collection period. The samples were made into composite and sub samples as much as 10% of the total samples were taken for DM and OM analysis.

Urine was collected daily into plastic bucket placed under the cages and containing 10% sulfuric acid solution to reached the urine pH below 3 to avoid microbial growth. Urine volumes were measured and filtered through the 2 layers of gauze. Daily sub samples were taken, put into 20 ml plastic vials and stored at -20°C . At the end of collection period, the samples were analysis for PD (allantoin and uric acid) concentration. Allantoin was determined by spectrophotometric method according to the procedure of Yang dan Conway (Chen and Gomes, 1992), while uric acid was determined by spectrophotometric method using Kit reagent.

Microbial protein synthesis was estimated according to the equation $Y = 0.84X + 0.150 W^{0.75} e^{-0.25X}$ proposed by Chen and Gomes (1992) with modification in endogenous PD excretion for Kejobong and Bligon goat (Purwati *et al.*, 2012). The equations was used for goat to describe the quantitative relationship between absorption of microbial purines (X mmol/d), and excretion of PD in urine (Y mmol/d).

Estimated Microbial Nitrogen Supply (EMNS, g/ d) = $(X \text{ mmol/d} \times 70) / (0.83 \times 0.116 \times 1000) = 0.727X$.

The efficiency of the microbial protein synthesis in the rumen was expressed as grams of microbial N per kilogram of digestible organic matter apparently digested in the rumen (DOMR).

Statistical analysis

Data were analyzed using the analysis of variance with one way (SPSS) and Duncan’s New Multiple Range Test was used to compare treatment mean values (Rosner, 1990).

RESULT AND DISCUSSION

The dry matter intake (DMI), organic matter intake (OMI), digested dry matter intake (DDMI) and digested organic matter intake (DOMI) are shown in Table 1. Average DMI, OMI, DDMI as well as DOMI were not significantly different among the animals.

Table 1. Nutrient intake and digested nutrient of Kejobong and Bligon goat fed *ad libitum* by King grass and peanut straw ((g/d, mean ± SE).

	Male Kejobong	Female Kejobong	Male Bligon
Dry matter intake ^{ns}	571 ± 6.39	558 ± 13.19	565± 13.72
Organic matter intake ^{ns}	502 ± 5.62	492 ± 10.81	497± 11.20
Digested dry matter intake ^{ns}	398 ± 19.44	378 ± 21.44	365± 10.84
Digested Organic matter intake ^{ns}	368 ± 15.16	353 ± 16.87	341± 8.35
DOMR ^{ns}	239 ± 9.85	239 ± 10.97	221 ± 5.43

^{ns} in a row, mean values are not significantly differ (P ≤ 0.05).

*DOMR, digestible OM fermented in the rumen, calculated as 0.65 · DOM intake (Chen and Gomes, 1992).

Allantoin, uric acid and total purine derivatives concentration in the urine of female Kejobong goat were not significantly different compared with the male. The differences also did not found between Kejobong and Bligon goat (Table 2.). The relative proportion of allantoin to uric acid in the urine were 76/24; 78/22 and 62/38 respectively for male Kejobong, female Kejobong and male Bligon goat. The urinary allantoin proportion to PD were slightly lower compared the finding (86%)by Jetana (2005)

Uric acid concentration in the urine of the Bligon goat 20% lower compared with Kejobong goat. The differences might be due to the enzymes activities involving in the nucleic acid catabolism, uricase which catalyze the uric acid to allantoin and xanthine oxidase which catalyze xanthine-hypoxanthine to uric acid. The amount of reaction products depend on the enzyme velocity.

Table 2. Purine derivative concentration in the urine of Kejobong and Bligon goat fed *ad libitum* by King grass and peanut straw (mean ± SE).

	Male Kejobong	Female Kejobong	Male Bligon
Allantoin ^{ns}	655.85 ± 191.14	591.74 ^c ± 256.61	219.52 ± 100.01
Uric acid ^{ns}	205.52 ± 32.53	167.53 ± 25.30	135.62 ± 7.01
Purine derivatives ^{ns}	861.37 ± 216.28	759.27 ± 280.45	355.15 ± 97.14

^{ns} in a row, mean values are not significantly differ (P ≤ 0.05).

The proportion of urinary xanthine and hypoxanthine was 6.57% of total purine derivatives (Belenguer, 2002). It also reported by Purwati (2013) proportion of urinary xanthine dan hypoxanthine to total purine derivatives was very low, 0.32% and 0.36% in the urine of Kejobong dan Bligon goat respectively. Therefore the excretion of xanthine and hypoxanthine in the recent experiment did not be measured.

Table 3. Purine derivative excretion in the urine, estimated microbial nitrogen supply of Kejobong and Bligon goat fed *ad libitum* by King grass and peanut straw (mean \pm SE).

	Male Kejobong	Female Kejobong	Male Bligon
Allantoin ($\mu\text{mol}/\text{W}^{0.75}/\text{d}$)	79.98 ^a \pm 13.99	54.47 ^{ab} \pm 20.03	27.91 ^b \pm 11.32
Uric acid ($\mu\text{mol}/\text{W}^{0.75}/\text{d}$ ^{ns})	26.86 \pm 2.41	18.35 \pm 3.07	23.82 \pm 4.96
PD ($\mu\text{mol}/\text{W}^{0.75}/\text{d}$)	106.83 ^a \pm 12.81	72.82 ^{ab} \pm 20.83	51.73 ^b \pm 9.37
EMNS ^{ns} (mg/d)	984.80 ^a \pm 175.82	561.00 ^{ab} \pm 218.24	384.00 ^b \pm 110.00
EMNS/DOMR ^{ns} (mg/g)	4.11 ^a \pm 0.67	2.49 ^{ab} \pm 0.99	1.80 ^b \pm 0.58

^{a,b,c} in a row, mean values with the different superscript were significantly differ ($P \leq 0.05$).

Allantoin excretion of male Bligon significantly lower compare with male Kejobong (Table 3.), but did not differ compare with the female Kejobong. There was no significant difference in allantoin excretion between female and male Kejobong goat. Total purine derivatives excretion shown the same patern with allantoin excretion, although there was no differences effect in uric acid excretion. The differences of breed seemed having dominant effect on purine derivatives excretion. These result were similar to those obtained in same goat by other authors (Purwati, 2012), which also found that Kejobong excreted higher PD compare with Bligon (117.96 vs 72.40 $\mu\text{mol}/\text{W}^{0.75}/\text{d}$) although the endogenous PD excretion Kejobong was lower than Bligon (8.85 vs 19.33 $\mu\text{mol}/\text{W}^{0.75}/\text{d}$). Allantoin excretion in urine of Kejobong goat was closed to the value reported by Fujihara *et al.* (1999) in sheep urine (92.79 $\mu\text{mol}/\text{W}^{0.75}/\text{d}$). The recent finding on urinary allantoin excretion of Kejobong as well as in Bligon goat were within the range value found by Chen *et al.* (1990) in the goat urine (32-208 $\mu\text{mol}/\text{W}^{0.75}/\text{d}$). Uric acid excretion in the urine of the both goat were higher compare with the excretion in the urine of goat (12.5-20.4 $\mu\text{mol}/\text{W}^{0.75}/\text{d}$) reported by Yanez-Ruiz *et al.* (2004). The efficiency of the microbial protein synthesis in the rumen, expressed as mg of microbial N per g of digestible organic matter apparently digested in the rumen of male Kejobong significantly higher compared with the male Bligon.

Estimated microbial nitrogen supply in Kejobong goat showed the tendency higher compared with Bligon goat (0.985 vs 0.384 g/d). No differences were found for PD excretion and estimated microbial nitrogen supply between male and female Kejobong goat. The highest microbial N supply when expressed per g rumen digested organic matter in Kejobong goat compare with Bligon goat indicated the higher feed efficiency in male Kejobong goat.

CONCLUSION

It could be concluded that Kejobong goat were more efficient compare with Bligon goat in supplying rumen microbial protein to the host.

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02. THE EFFECCT OF NEUTRAL DETERGENT FIBER RATIO IN THE DIET ON THE PERFORMANCE OF PHILIPPINE NATIVE GOATS (*Caprahircus*Linn.)

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Abstract

The objective of this research is to determine the optimum of feed utilization and growth performances based on the ratio of NDF in the diets. This research study was used 18 female Native goats, around 7.96±2.21 kg of body weight (BW) and 3 male of cannulated goats forin *situ* digestibility. A RCBD (Study 1 and 2) and latin square (Study3) were applied. The treatments were applied as follows: T1 is NDF Forage-Concentrate with ratio: 75.0%-25.0%); T2 (ratio: 67.5%-32.5%); and T3 (ratio: 60.0%-40.0%). Different ratio of NDF forage in the diet was not affecting the rate of degradability of dry matter (DM) at 0 hours, levels of b or Potentially degradable fraction of dry matter degradability and the rate of degradation of b(p>0.05). The total intake of DM and crude protein (CP) were not significantly affected by treatment (p>0.05). The influence of the treatment didn't affect the level of digestibility (DM, CP and TDN) of native goats in each treatment (p>0.05). Differences in the treatment didn't significantly affect the performance of the native goats (ADG and FC) (p>0.05). All of the treatment give same feed utilization and growth performances.

Key words : NDF forage, ration, goat

INTRODUCTION

The opportunities to increase production of ruminant are still very open to be developed. These opportunities as seen in the dairy industry sector, where the goverment have a plan to raise the national milk production in order to increase of self-sufficiency from 30% to 50% (Morey, 2011). But these opportunities are also accompanied by a lack of efficency production process applied by farmers, it causes decreasing population and un-optimal management ruminant feed (problem in quality and quantity of diets) (Syamsu et al., 2003).

One of the steps to be taken is the improvement of feeding management. The farmers use high ration of concentrates compairing with the forage in the diets. Although it has a positive impact on production from an economic standpoint, the use of excessive concentrations will lead to an increase in production cost. Therefore cost is not worth with the revenue that is obtained. It is estimated that the gained revenue is lower than the cost of production, which will bring to negative income to the farmers. Study of dairy production relating the used of higher portion of concentrate in the diets depresses the percentage of milk fat. Approximately 60% of the cost of milk production can be attributed to the concentrates fed to the animals (Chantaprasarn and Wanapat, 2008).

Nutrients are needed for the maintenance and production. Aside from crude protein (CP), energy and minerals, the content of neutral detergent fiber (NDF) in the feed ration should also be considered. The sources for NDF are concentrates and forage. The proportion of forage NDF in the ration also plays a role in ruminant production. It is associated with chewing activity, saliva production, fermentation rate and yield and digestibility of feed. Chewing time is highly influenced by NDF content, compared with

the particle size (PS) of forage (Beachemin, 1991 cited by Moon et al., 2004). NDF content from roughages or forage well know as a tool to maximize production of ruminant and to maintain health by sustaining a stable environment in the rumen (Tafaj et al., 2005).

Level and ration of NDF in the diet can be used as standard to formulate porportion of forage and concentrate in the diets, so with optimized the value of NDF, it can improve the performance of ruminant. In general, optimizing forage as source of NDF will indirectly decrease the production costs while increase revenue without reduce the quality and quantity of production. Theoretically, value of NDF from forage is more useful around 50%, than concentrates (NRC, 2001). The ration of forage and concentrate of the diet should contain NDF around 75%, but the minimum level of the NDF in temperate zone is around 25% to 28%. That recommendation of NDF level is relatively difficult to maintain in the tropics, because of the poor quality of forage.

A minimum of dietary NDF level and proportion of NDF forage (25% DM and 75% to 60% in the diet) still provides sufficient utilization of fiber for production and maintains fat corrected milk (Kanjana pruthipong et al., 2001). In dairy experiment, a high ratio of forage to concentrate will decrease milk production and increase the percentage of milk fat. It is because the precursors of fatty acid derived from acetic acid, which can be produced more by highly fibrous forage than the concentrate. NDF from forage triggers elevated levels of fat in milk (NRC, 2001; Yang et al., 2001). The effect of ratio of NDF forage in the diets as a ration forage and concentrate in the diets was reported to decrease the digestibility of crude fat and NDF of animal fed with low fiber hay. When the ratio of concentrate in the diets was increased from 20% to 50%, it resulted negative effect to the animal (Tafaj et al., 2005). Higher production of ruminant especially in milk production (40 liters/ head/ day) reduces their production if the NDF content of the diets around 32%, however NDF content in the diets around 44% are not affecting the ruminant with low production around 20 liters/ head/ day (NRC, 2001). It is important to observe and evaluate the impact of the ration of NDF forage in the diets as recommended by NRC, with some adjustment based on the tropical condition and practical habits in the farm, especially in dairy farm.

The objective of this research is to determine the optimum feed utilization and growth performances in dairy ruminants' diets based on the ratio of NDF forage and concentrate using Philippine native goats (*Capra hircus* Linn.) as media with the following response variables: feed utilization, growth performances and feed cost efficiency.

MATERIALS AND METHODS

The experiment was conducted at the Animal and Dairy Sciences Cluster, College of Agriculture, University of the Philippines Los Baños, College, Laguna from December 15th, 2012 to February 1st, 2013 covering all of the adaptation and phases of data collection in Study 1, 2 and 3.

Materials

Eighteen (18) native female goats were grouped into 6 weight classes for the feeding trial and in the *in vivo* experiment. The average body weight (BW) of the animals was around 7.96 ± 2.21 kg (CV = 27.76%) and were approximately 1 year of age. For their *in situ* digestibility, three (3) male of mature goats that were surgically fitted with cannulated rumen were used. The goats were kept in individual cages. The

equipment that were used in this study includes, hygiene kits, buckets, weighing scales, metabolic cages and a set of tools for collection of feces.

Methods

Experimental Design

A randomized complete block design (RCBD) was applied for study 1 and 2; a 3x3 latin square design was used for study 3 that performed in 3 trials; with 3 treatment levels of NDF in forage-concentrates ration within 6 block (study 1 and 2). The dietary content of crude protein (13.71±0.14%) and total digestible nutrients (TDN) (66.61±4.81%) for study 1, 2 and for study 3 were of the same amount with other nutrients expect in NDF level. The levels of protein and TDN that were used in each treatment were in similiar based on the range as reported by Nugroho (2009), Sommart et al (2000) and Chanjula et al (2007). Nutrient content of feedstuffs that were used can be seen in Table 1.

The composition and nutrient content of treatments are shown in Table 2 and Table 3. The proportion or level of forage NDF and concentrates as a basis in this study are shown in Table 4.

Table 1. Nutrient content of feed ingredients.

Ingredients*	CP (%)	EE (%)	CF (%)	ASH (%)	NFE(%)	NDF(%)
<i>P. purpureum</i> ¹	12.88	1.44	29.32	18.40	35.64	63.87
Concentrate ¹	17.62	7.33	12.28	8.10	54.67	58.06
Urea ²	281.00	0.00	0.00	0.00	0.00	0.00
Molasses ³	3.94	0.30	0.40	11.00	84.36	0.00

Note : * Based on DM basis

¹from Animal Nutrition Laboratory; Animal and Dairy Science Cluster, UPLB. ² NRC (1988). ³ NRC (2001). CP is crude protein; EE is extract ether; CF is crude fiber; NFE is nitrogen free extract; NDF is neutral detergent fiber.

Table 2. Composition of feed ingredients in the dietary treatments (%).

Compositions	T1	T2	T3
<i>P. purpureum</i>	67.41	60.71	54.00
Concentrate	27.09	35.24	43.40
Urea	0.50	0.30	0.10
Molasses	5.00	3.75	2.50
Total	100.00	100.00	100.00

Research Procedure in Study 1 and 2 (Feeding Trial and *In Vivo*)

Research procedures were carried out in four phases: preparation, adaptation, introduction and application of the treatments. Preparation process includes preparation of feed, cages, equipment and goats. Stage of adaptation of goat to the environment and feed was then followed by the preliminary stage. The preliminary stage consists of seven days, the goats were randomly assigned to 3 treatments among the group. The objectives of this stage is to clear the digestive tract of the animal of its previous diet. At this stage, the ability of goat for consuming feed was observed. At the end of the

preliminary stage, goats were weighed to obtain initial body weight. Stage of application of treatment was the final step wherein feeds were given as much as 3% of goat’s body weight (according to the capability of goat). The goats were fed three times a day: morning (8:00 and 11:00 am) and afternoon (3:00 pm). Concentrates were fed first, followed by feeding of forages one hour after. Drinking water was be provided *ad libitum*. Before giving feeds and water, residue of feeds and water were calculated. Goats were weighed every week to adjust the amount of rations given to the animal.

Nutrient content of treatment diets were analyzed using proximate analysis (AOAC, 1984) and NDF analysis, following procedure of Van Soest et al (1991). Evaluation of feed quality during the study was conducted by collecting samples of offered feed every day during the experiment and then mixed. Three hundred (300) grams of collected feed sample was obtained and it analyzed using proximate analysis method (AOAC, 1984).

Table 3. Nutrient contents of treatment’s diets (%).

Nutrient contents	NP	C1	C2	C3	D1	D2	D3
DM	100.00	100.00	100.00	100.00	100.00	100.00	100.00
CP	12.88	19.00	19.00	16.52	13.54	13.81	13.77
TDN	56.64 ¹	81.16 ¹	80.11 ¹	77.48 ¹	65.93	65.55	68.37
NDF	63.87	52.31	55.29	55.21	58.94	60.14	60.65
EE	1.44	4.88	5.51	1.51	2.19	2.73	3.28
CF	29.32	8.63	9.58	9.75	19.98	20.39	19.17
ASH	18.40	7.66	7.56	7.28	14.15	13.62	13.14
NFE	35.64	56.40	54.11	61.71	48.19	47.78	48.93

Notes :NP : Napier grass or *Pennisetum purpureum*
 C1 : Concentrate was mixed with urea and molasses based on treatment 1
 C2 : Concentrate was mixed with urea and molasses based on treatment 2
 C3 : Concentrate was mixed with urea and molasses based on treatment 3
 D1 : Napier was mixed with mixed concentrate based on treatment 1
 D2 : Napier was mixed with mixed concentrate based on treatment 2
 D3 : Napier was mixed with mixed concentrate based on treatment 3
¹ : TDN value based on the equation from Sutardi (2001)

Table 4. The proportion or level of forage NDF and concentrates each treatment

	T1	T2	T3
NDF Total (g)	133.92	131.77	133.90
NDF Forage (g)	96.64	84.27	57.21
NDF Cons. Mix (g)	37.28	47.50	56.94
NDF Forage (%)	72.07	63.87	57.21
NDF Cons. Mix (%)	27.93	36.13	42.79
Forage portion (%)	67.89	60.48	53.41
Concentrate portion (%)	32.11	39.52	46.59

The treatments were applied as follows:

T1 = NDF Forage-Concentrate (Ratio: 72.07%-27.93%)

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rumen fluid was determined immediately after before and 3 hours after feeding using a pH meter.

Response variables

Responses variables were: (1) average daily gain (ADG); (2) consumption of dry matter (DM) and crude protein (CP); (3) apparent digestibility of DM and CP also total digestible nutrients (TDN); (3) DM, NDF and CP degradability of the diets and (5) conversion of feed.

Data Analysis

The data were analyzed using analysis of variance (ANOVA). Then the data was tested with a variety of analysis according to the instructions Gaspersz (1991) and, if there were significant facts between treatments, it were followed by Turkey's test with error level (α) 5%. The MINITAB 14 was used for running analysis of variance and Turkey's test for other analyses if necessary.

RESULTS AND DISCUSSIONS

Nutrient contents and the ration of the diets in the experiment

The data indicate that *P. Purpureum* had lower NDF content (63.87%). This can be due to the younger age of *P. Purpureum* given during experiment. According to Celberg (1956), the age of the plant or forages affects their nutritional value because it is related with the phase of their maturity. It was also said that leaves have high nutritive value than the stem. NDF content that stated by Hartadi et al. (2005) is around 70% and 68% mentioned by PHILSAN (2010). Supported by Januartiet al. (2009), nutrient content of *P. Purpureum* ranges around 11.66% (CP) and 73.76% (NDF).

Table 5. Nutrient contents and the ratio of the diets on the experiment

Parameters*	Forage ¹	T1	T2	T3
%.....			
NDF	63.87	58.94	60.14	60.65
Raito of NDF forage in the diet	-	72.07	63.87	57.21

Note : * based on DM basis and ¹ is *P. Purpureum*

Changes in the dietary NDF was due to the lower NDF content in *P. Purpureum*. The NDF content of *P. Purpureum* was lesser than the ideal. So it changes content of NDF in the diets. NDF in Treatment 1 decreased from 62.91% to 58.94%, while in Treatment 2 decreased from 62.96% to 60.14% and in Treatment 3 to 60.65% from 50.44%. Decline value of dietary NDF was measured from the difference value on its expected and actual value. It also encourages a change in the ratio of forage NDF in the diets from the expected value. Aside from changes in the NDF diets, the decrease can be attributed to the limitations of goats to consume all the given feed. Ratio of NDF forage in the diets decreased from the expected value. Treatment 1 changed from 75.00% to 72.07%, Treatment 2 decreased from 67.50% to 63.87% and also Treatment 3 changed from 60.00% to 57.21%. Based on Lu et al. (2005), NDF content used in the diets was higher than the ideal 41% NDF. That value is nutritionally adequate for high producing lactating dairy goats.

In Situ Study

Dry matter degradability on 0 hour, or degradability caused by the washed nylon bag is shown in Table 6. Treatment 1, 2 and 3 were not significantly affecting the rate of degradability of dry matter at 0 hours ($p > 0.05$). The average dry matter degradability at 0 hours, at T1, T2 and T3 were 30.98, 29.01 and 29.38%, respectively.

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Treatment 3 (103.14 g/d). Total dry matter intake in all treatment were relatively the same, 222.58, 217.84 and 223.63 g/d for Treatment 1, 2 and 3, respectively. Increase in dry matter intake, especially of concentrates further enhance fermentation resulting in increased protein synthesis (Rotger et al., 2006; Bourquin et al., 1994). DMI was relatively uniform as described by Cantalapiedra-Hijar et al. (2009) that the diets were prepared to have the same palatability. Contrary to the results of this study, Haddad (2005) reported dry matter intake increased with increasing the concentrate portion and averaged 585, 630, and 676 g day⁻¹ for the high forage, forage and medium medium high low forage diets, 60:40, 45:55 and 30:70 forage: concentrate ratios, respectively.

Table 7. Ration of intake and nutrients intake of the treatment’s diets

Parameters	T1	T2	T3
Dry Matter Intake, g/d			
Forage	151.31±46.40 ^a	131.94±36.50 ^{ab}	120.49±39.00 ^b
Concentrate	71.27±20.21 ^a	85.90±22.95 ^b	103.14±30.10 ^c
Total	222.58±66.50	217.84±59,10	223.63±69.10
% of forage intake on DMI (%)	67.89±0.56 ^a	60.48±1.37 ^b	53.62±1.20 ^c
Crude Protein Intake, g/d			
Forage	19.49±5.97 ^a	16.99±4.70 ^{ab}	15.52±5.02 ^b
Concentrate	13.54±3.84 ^a	16.32±4.36 ^{ab}	17.04±4.97 ^b
Total	33.03±9.81	33.31±9.01	32.56±9.99

Notes : ns = not significant with (p>0.05); and * = significant with (p<0.05) with different superscripts within rows denote significant differences

Crude protein intake from forage was higher in Treatment 1 (19.49 g/d), followed by Treatment 2 (16.99 g/d) and Treatment 3 (15.52 g/d). Meanwhile, the intake of crude protein from concentrate was found to decrease from the largest on Treatment 3 (17.04 g/d), then 16.32 g/d in Treatment 2, and Treatment 1 (13.54 g/d). Intake of total crude protein T1, T2 and T3 were relatively the same. This was due to the intake of dry matter intake which was relatively equal, the total content of CP in the diets relatively similar and the accumulation of CP intake supplied from forage and concentrate uniform in the range (32.56 to 33.31 g/d).

Based on data from forage and concentrate intake, the data was converted to a ratio of forage in diets. Thus, the ratio of forage intake in the total intake or diets was higher in Treatment 1 (67.89%), while Treatment 2 only had a ratio of 60.48% and the lowest ratio was found in Treatment 3 (53.62%). Differences in forage intake ratio in the total intake with the expected ratio was as discussed previously.

From the data above, it can be concluded that the quality of the diets on each treatment were relative the same because of the total of intake (dry matter and their nutrition) relatively similiar. It can also be assumed the diets used in this experiment have almost the same palatability. Feeds with good quality are usually consumed by animal in larger quantities compared to low-quality feed (Tillman et al., 1984). The diets were arranged for fullfil their requirement, and composed as isoprotein and isoenergy. The content of NDF has been reported to affect the level of consumption

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through physical effects (filling effect), so it can be used as a variable in predicting consumption (Waldo, 1986; Merten, 1994).

Digestibility study

Data on fecal dry matter, content of CP in it, is shown in Table 8. Differences in the ratio of forage NDF in the diets did not provide a significant influence on the dry matter in the feces, as well the nutritional content on it. The influence of the ratio of forage NDF in the diets did not affect the level of digestibility of native goats in each treatment ($p > 0.05$). This was because of the intake and the amount of nutrients that enter the digestive tract were relatively uniform in all treatments, which was also the cause of the reason for the similar rate of degradability of the diet in this study. This supports that the utilization rate of feed, which caused the rate of degradability of feed and microbial activity in the rumen, it did not directly affect the degradability of feed resulting residue levels in the feces and feed digestibility (digestibility of crude protein and TDN) relatively similar. Forage : concentrate ratio in this experiment had similar digestibility, it had different report from Haddad (2005), feeding growing Baladi kids diets with lower forage:concentrate ratios resulted in improving DM, OM (organic matter) and CP digestibilities.

The mean dry matter Digestibility in Treatment 1 was 66.46%, and also ranged from 64.69% in Treatment 2 and Treatment 3 was around 64.95%. Cantalapiedra-Hijar et al. (2009) stated that in some cases, concentrate supplementation may decrease digestibility of forage-containing diets, that depend on the nature and proportion of the concentrate as well as the quality of the forage. Its mean, adding more concentrate in this experiment, still resulted to equal quality of the diets. Mulligan et al. (2002) stated that Holstein-Frisien steers fed with different feeding level of grass silage and soya hulls (50:50 and 15:85) has lower digestibility when the steers fed with high proportion of soya hulls (15:85), around 67.25% of DM digestibility, compare with 50:50 of grass silage and soya hulls (73.80%). Adding grain from 0 to 50% as supplementation could improve the DM digestibility of the diets, from 76.6 to 84.0% (Cerrillo et al., 1999)

Table 8. Nutrient contents in the feces and digestible nutrients

Parameters	T1	T2	T3
Feces			
Dry matter of feces (g/d)	72.03±9.92	74.96±17.07	75.44±15.30
Crude protein of feces (g/d)	8.67±1.17	8.97±1.93	9.10±1.53
Digestible Nutrient			
Dry matter digestibility (%)	66.46±5.29	64.69±6.82	64.95±5.88
Crude protein digestibility (%)	72.85±3.85	72.40±4.55	70.83±5.11
Total digestible nutrient or TDN (%)	65.93±4.21	65.55±5.53	68.37±4.95

The digestibility of crude protein in Treatment 1 was 72.85%, Treatment 2 was 72.40%, while Treatment 3 had a value of 70.83%. Degradation of proteins vary depending on the source of protein. The protein bound in the carbohydrate structure has a low degradation. Protein digestibility was also influenced by the amount of rumen microbes (Soeharsono and Winarni, 2005). Digestibility of crude protein in every treatment were almost the same as explanation by Rotger et al. (2006), protection of the cellulose structure in the diets are equal, then the degradability of DM and NDF in every diets show equal passage rate and availability of diets. The benefit of increasing

concentrate level in the diet, especially in terms of achieving greater urinary PD excretion and greater N retention efficiency (Cantalapiedra-Hijar et al., 2009). CP digestibility in this study better than was done by Rapetti and Bava (2004) feeding goats with different level of starch in the treatment diets, only achieved around 64.03 to 69.58%. Also when Holstein-Frisien steers were fed with different feeding level of grass silage and soya hulls (50:50 and 15:85), just only have CP digestibility around 58.30 to 53.85, respectively (Mulligan et al., 2002). The similiar result with Cerrillo et al. (1999), adding grain 50% in the diets could decrease CP digestibility from 59.80 to 55.80%.

TDN values at each treatment in this experiment was relatively similar, with a range of 65.93% (T1), 65.55% (T2) and 68.37% (T3). Total digestible nutrients are the percentage of nutrients that digested in the gastrointestinal tract. Total digestible nutrients include crude protein digestibility, crude fat that multiple by 2.25, NFE, crude fiber, in this case total digestible nutrients reflects digestibility of energy (Parakkasi, 1999). Mulyaningsih (2006) reported on feeding with different rasio of forage and concentrate (0 to 75% proportion of forage in the diets) has TDN around 55.05 to 57.92%. TDN in this experiment was higher comparing with Purbowati et al. (2007) using 4 treatments with different level of dietary CP and energy, only has TDN around 50.46 to 58.60%.

Performance of the native goats fed by the experimental diets

Performance of native goats given 3 kinds of diets with different forage NDF ratio in the diet, it can be seen from the aspect of average daily gain (AVG) and feed conversion can be seen in Table 10.

Table 10. Performance of the native goats fed by the experimental diets

Parameters	T1	T2	T3
Performance			
Average daily gain or ADG (g/d)	12.24±9.04	11.43±3.71	17.69±10.77
Feed conversion	25.26±15.43	18.91±4.17	16.50±11.40

Differences in the ratio of forage NDF in the diets did’t significantly affect the performance of the native goats (p> 0.05). The mean ADG in Treatment 1, 2 and 3 were 12.24, 11.43 and 17.69 g/d, respectively. The mean of feed conversion in Treatment 1, 2 and 3 were relatively similar, ranging from 25.26, 18.91 and 16.50 g/d. Weight gain by goats is sensitive to protein and energy content of forages (Ash and Norton, 1987). In contrast to the results of Haddad (2005), a linear increase was observed for ADG with increasing levels of dietary concentrates. Dønnem et al. (2011) reported that Norwegian dairy goats supplemented with a low (LC; 0.6 kg per goat daily) or normal (NC; 1.2 kg per goat daily) level of concentrate, have a body gain around 25 vs 94 g during the their experiment. By ADG ranged 13.92 g/d obtained in this study, a shortage of DMI around 73.71 g/d of which is determined by Kears (1982) and a shortage of TDN around 7.59. While going surplus around 6.38 g/d for digestible of protein.

CONCLUSIONS

Based on the interpretation of the data obtained in this study, the effect of differences in the ratio of NDF in the diets had no effect on dry matter and crude protein intake of the diets. This was also supported by the dry matter, crude protein digestibility and TDN, which was relatively uniform between treatments, due to differences in the

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ratio of NDF in the diets has no effect. Uniformity utilization of diets was shown by the uniformity of degradability rate between diets, which was supported by the relatively uniform blood glucose between treatment. Uniform feed utilization resulting in treatment did not significantly affect the performance of native goats with an average ADG 13.92 g/d and FC of native goats worth 20.30.

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03. MEAT PHYSICAL PROPERTIES OF LOCAL LAMB FED UREA-IMPREGNATED ZEOLITE RATION

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Abstract

The purpose of this study was to determine the meat physical properties of lamb fed rations containing urea, zeolite, and urea-impregnated zeolite. Twelve local male lambs aged about 1 year old were used. A randomized block design with 4 treatments and 3 replications groups based on body weigh was used. Animals were fed 60% forage and 40% concentrate. Treatment rations were as follows: forage + concentrate without zeolite without urea (NU) forage + concentrate of urea (U), forage + concentrate of zeolite (Z), and forage + concentrate urea-impregnated zeolite (UZ). The variables measured were pH, tenderness, cooking loss, water holding capacity, and color indexes (L*, a*, b*, C*, h*). Results showed that feeding urea, zeolite, and urea-impregnated zeolite rations did not significantly affect pH, tenderness, cooking loss, water binding capacity, and color indices (L*, a*, C*, h*) except yellowness (b*). The average values for all variables measured were 5.53, 2.33 kg/cm², 45.18%, 40.04%, 41.16, 16.36, 6.93, 17.80, 23.09 for pH, tenderness, cooking loss, water holding capacity, L*, a*, b*, C*, h*, respectively. It was concluded that rations contained urea, zeolite, or urea-impregnated zeolite fed to local male lambs could maintain meat quality as shown by the pH value, cooking loss, water holding capacity, and meat color that showed high levels of lightness (L*), redness (a*), the chroma (C*), and hue (h*). Moreover, the value of yellowness (b*) increased in the meat of the lamb fed zeolite ration.

Keywords: local male lambs, urea, zeolite, urea-impregnated zeolite, meat physical properties.

INTRODUCTION

Meat physical properties determine consumer acceptability of the meat because some physical properties are easily detected by the consumer. Therefore, any indicator related to meat physical qualities, i.e. color, tenderness, pH, water holding capacity (WHC), and cooking loss, need to be improved in order to increase the consumer acceptability. For this purpose, zeolites are expected to show a potential ability to improve the meat physical properties and in turn, increase the consumer acceptability.

Zeolites with its cation exchange capacity may act as a cation exchanger and a molecular sieve (Mumpton, 2006). Moreover, its physical properties, i.e. porosity and surface area (in form of smaller granular) may absorb gas and fluid materials. Based on its porosity, zeolites were impregnated with liquid urea to provide non-protein nitrogen (NPN) source for rumen microbe of lamb in this study. The study aimed to reveal the efficacy of the urea-impregnated zeolites in improving meat physical properties of lamb.

MATERIALS AND METHODS

Twelve heads of one-year local male lamb were allocated into four treatments and three groups of live weight-based replications within a randomized block design (RBD). Rations consisted of 60% forage and 40% concentrate formulated in iso-nitrogenous (Table 1, Kardaya *et al.* 2012). Treatment rations were as follows: forage + concentrate

contained neither zeolite nor urea (NU), forage + concentrate contained urea (U), forage + concentrate contained zeolite (Z), and forage + concentrate contained urea-impregnated zeolite (UZ). The three groups of live weight-based replications were lambs those its live weight within a range of 17 – 19 kg (Group 1), 20 – 21 kg (Group II), and 22 – 25 kg (Group 3). The variables measured were pH, tenderness, cooking loss, water holding capacity, and color indexes (L*, a*, b*, C*, h*).

Protocol

All lambs were weighed to determine its initial live weight and then placed in individual pen according to RBD. The lambs were fed the treatment rations at 0800 and 1600 daily within 60 days following a seven-day adaptation period. Drink water was provided *ad libitum*. At the end of experimental period, all pre-fasting lambs were slaughtered according to Islamic procedure. The pre-slaughtered lambs were carcassed and then divided into left and right carcasses. All carcasses were then chilled at 4⁰ C for 18 hours in a chilling room. Pre-chilled left carcasses were cut into commercial cuts and then each of the commercial cut was deboned. Meat samples for analysis purpose were obtained from loin and top side cuts.

Laboratory Analysis

Meat samples were analyzed for pH-value with a pre-calibrated meat pH-meter. The pH-meter was inserted into top side of the meat sample with three replications and the pH reading was recorded. Meat color was measured by a Chromameter type CR-200. Results of color indexes (L*, a*, b*, C*, h*) reading of each meat sample was then printed for further statistical analysis. Water holding capacity (WHC) of meat samples were measured by a press method of Hamm (1972) as explained by Soeparno (2005) and the values were obtained according to formula as follows:

WHC = % water content of meat sample - % water freed from meat sample

Meat tenderness was measured by Warner-Bratzler shear. A 200 g meat sample was inserted by bimetal thermometer and sink into boiling water in order to reach meat internal temperature of 80 – 81⁰C and then allowed to cool in about 60 minutes before analyzing with Warner-Bratzler. Cooking loss value was obtained by subtracting cooked meat weigh from sample meat weigh and presented as percentage. A 50 g meat sample was inserted by bimetal thermometer and sink into boiling water in order to reach meat internal temperature of 80 – 81⁰ C and then the cook meat was allowed to cool in about 24 hours at room temperature before it was weighed. The cooking loss value was determined by the following formula:

Cooking Loss = {(g meat sample – g cooked meat sample)/g meat sample} x 100%

Statistical Analysis

Collected data were analyzed by analysis of variance (ANOVA) to reveal the effect of the treatment to the variables measured. Duncan multiple range-test was applied only if the treatment showed significant effect (P<0.05) to reveal the differences among the treatments.

RESULTS AND DISCUSSION

Ultimate pH

Meat ultimate pH values of lambs fed experimental rations were within a range of 5.45 – 5.68 (Table 2) and fall within normal range of meat ultimate pH value (5.4 –

5.9) when recommendation of Ekiz *et al.* (2009) was applied. Experimental rations did not affect meat ultimate pH value of the lambs significantly. Thus, urea, zeolite or urea-impregnated zeolite ration showed similar effect to urea ration on ultimate meat pH value. It was previously expected that cation exchange capacity of zeolites would create an alkalogenic effect that would influence ultimate meat pH value. Presumably, even if the alkalogenic effect of zeolites occurred, decreasing pattern of the pH value occurred in normal rate and resulted in normal meat ultimate pH value as proposed by Aberle *et al.* (2001).

Tenderness

Meat tenderness of lambs fed experimental rations showed similar shear force value and range between 2.00 – 2.67 kg/cm² with average shear force value was 2.33±0.65 kg/cm² (Table 2). This average value was considered as very tender because it less than 3.3 kg/cm² if recommendation of Suryati *et al.* (2008) was applied. However, lambs fed ration supplemented Ca-fish oil resulted in lower shear force value (1.55 kg/cm²) as reported by Sudarman *et al.* (2008).

Water Holding Capacity

Water holding capacity (WHC) of meat from lambs fed experimental ration was in range of 37.93 – 41.49% and the average value was 40.04 ± 4.49% (Table 2). However, the WHC was not affected significantly by the experimental rations. Siagian *et al.* (2004) obtained higher WHC of meat (93.88%) from pig fed zeolite rations although the result was not significantly different among the treatments. Apparently, lower WHC relates to lower pH value and lower pH may denature meat protein and impact on lower WHC. This presumption was in agree with Lawrie (2003) who suggested that higher pH value was relatively able to hold more water than lower pH value.

Cooking Loss

Meat cooking loss of lambs fed experimental rations extended from 41.67 to 48.23% with the average cooking loss value was 45.18% and fallen within a normal range of cooking loss (15% - 54.5%) as proposed by Soeparno (2005). In present study, cooking loss did not affected significantly by experimental rations. According to Shanks *et al.* (2002), cooking loss related to WHC where lower WHC would result in higher cooking loss. Thus, as previously stated that lower pH value resulted in lower WHC and in turn, increased cooking loss.

Color

The only meat physical characteristics affected by experimental ration was yellowness index (b*) (P<0.05) whereas another color parameters, i.e. lightness (L*), redness (a*), chroma (C*), or hue (h*) did not change significantly (P>0.05, Table 2). Yellowness index of meat color related to carotenoids deposited in fat component of lamb meat as stated by Joy *et al.* (2008) that higher b* related to the carotenoids deposited in fat as a result of pasture intake. Thus, it is very possibly that the higher b* values of meat from lambs fed zeolite ration is a result of zeolite role in improving carotenoid utilization contained in field grass, and in turn, increase carotenoids concentration deposited in fat component of lamb meat. Redness index (a*) and lightness index (L*) of lamb meat in present study fallen in recommendation range of Khilji *et al.* (2010), i.e. redness (a*) and lightness (L*) values should equal to or exceed 9.5 and 34, respectively, in order for the consumer to consider meat as fresh.

CONCLUSION

Rations contained urea, zeolite, or urea-impregnated zeolite resulted similar effect with control ration (contained no urea or zeolite) on ultimate pH, WHC, cooking loss, and some color indexes (lightness, redness, chroma, and hue) of lamb meat. However, zeolite ration increased yellowness index of lamb meat.

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Table 1. Feed composition and nutrient contents of local lamb rations

Components	Ration (DM basis)			
	NU	U	Z	UZ
 %			
Field grass	60	60	60	60
Pollard	18	12.2	10	3.6
Yellow corn	2	11	6	16
Soybean meal	10	6	11.5	8.5
Coconut meal	10	9	11.5	8.79
Molasses	-	1	-	1
Zeolite	-	-	1	-
Urea	-	0.8	-	-
Urea-impregnated zeolite	-	-	-	2.11
Nutrient contents:				
DM	65.13	64.52	65.12	64.92
 % DM			
NDF	52.55	49.82	51.61	48.36
ADF	28.04	27.35	27.74	29.29
Hemicellulose	24.51	22.47	23.87	19.07
Cellulose	21.17	20.56	20.33	21.03
Lignin	5.38	5.39	5.03	6.18
Ash	7.70	7.31	8.33	8.24
OM	92.30	92.69	91.67	91.76
CP	16.41	16.53	16.49	16.12
CP/DM	16.80	16.21	16.82	15.80
EE	3.07	3.12	3.48	2.66
NFE	56.90	56.82	55.18	58.63

*NU: no urea, U: urea Z: zeolite, UZ: urea-impregnated zeolite, urea N represented 16% of total natural feed protein (N x 6.25). (Kardaya *et al.* (2012)

Table 2. Physical characteristics of meat from lambs fed experimental rations

Variables	Experimental Rations				Mean
	NU	U	Z	UZ	
pH	5.49±0.36	5.68±0.20	5.49±0.27	5.45±0.16	5.53±0.24
Tenderness (Kg/cm ²)	2.00±1.00	2.67±0.58	2.33±0.58	2.33±0.58	2.33±0.65
Cooking loss (%)	41.67±2.40	45.77±1.86	48.23±4.58	45.07±3.96	45.18±3.79
Free water:					
mg	104.03±15.86	110.20±13.95	105.60±8.34	108.80±16.37	107.16±12.21
%	34.70±5.29	36.73±4.62	35.17±2.78	36.27±5.46	35.72±4.07
WHC (%)	41.02±4.39	37.93±5.52	41.49±4.35	39.73±5.54	40.04±4.49
Color Indexes:					
Lightness (L*)	41.07±7.81	40.08±0.81	41.98±3.58	41.53±9.54	41.16±5.52
Redness (a*)	15.25±0.46	17.81±2.10	15.71±3.55	16.67±1.63	16.36±2.16
Yellowness (b*)	6.23 ^a ±0.27	7.05 ^{ab} ±0.19	8.09 ^b ±0.78	6.34 ^a ±1.10	6.93±0.97
Chroma (C*)	16.47±0.35	19.17±1.89	17.69±3.47	17.87±1.23	17.80±2.03
Hue (h*)	22.15±1.41	21.69±2.68	27.59±3.54	20.93±5.03	23.09±4.02

Means in the same row with different superscript differ significantly (P<0.05).NU: no urea, U: urea Z: zeolite, UZ: urea-impregnated zeolite, WHC = water holding capacity.

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04. IN VITRO STUDY OF *SARDINELLA LEMURU* OIL BASED CALCIUM-SOAP SUPPLEMENTATION EFFECTS ON THE SHEEP’S RUMEN DIGESTIBILITY

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Abstract

The objective of this experiment was to evaluate the lemuru oil based calcium-soap supplementation effects on the sheep’s rumen digestibility *in vitro*. It is well known that fat is a good source of energy for ruminant livestock especially those reared in tropical region due to its low heat increment. However, addition more than 5% fat in ruminant diet would disturb digestion process in rumen. Therefore the use of fat more than 5% must be protected which one of them is by making calcium-soap. Twelve sheep weighing 16.91 kg \pm 1.66 were allocated into four treatments and three replicates of block randomized design. They fed a basal diet consisted of field grass and concentrate diet in ratio of 25 : 75 given at 0700 and 1600. The nutrient content of concentrate diet was dry matter 61.47%, TDN 59.55% (equal to 2.15 Mkal ME), crude protein 16.96% and crude fat 4.52%. Sample of feses were collected at the last seven days of feeding trial. Rumen liquor was collected 4 hr. after a.m.feeding using vacuum pump and was strained through cheesecloth before use. The treatment diets were R0: control diet, R1: R0 + 1.5% ca-soap, R2: R0 + 3% ca-soap, R3: R0 + 4.5% ca-soap. The variables observed were concentration of NH₃, and total VFA production, dry matter digestibility (DMD), organic matter digestibility (OMD), crude protein digestibility (CPD) and digestibility of energy (DE). Data were analyzed using analysis of variance (ANOVA) and any significant difference were further tested using contrast orthogonal. The results showed that the treatments significantly (P<0.01) affected the concentration of NH₃ and VFA (P<0.05) production, but DMD, OMD, CPD and DE were not different among the treatments. It is concluded that calcium-soap supplementation increase VFA concentration, reduce NH₃ concentration, but has no effects on ration digestibility. Calcium-soap supplementation at level of 1.5% gives better digestibility efficiency.

Key words: Calcium-soap, digestibility, Lemuru fish oil, NH₃, Sheep, VFA

INTRODUCTION

In hot environments livestock appetite is usually low so that feed consumption decreased and ultimately resulted in low productivity of livestock. High feed intake produces a high heat production that is not needed by the livestock in hot environment. A high proportion of forage in the diet of ruminants also result in a higher heat production (Sudarman and Ito, 2000). This condition is further exacerbated by the low quality forage, which is commonly grown in the tropics. This is due to low feed quality is difficult to digest and produce body heat higher than the good quality feed though in the same amount of energy consumption (NRC, 1981). In a hot environment, such type of feed resulted in decreased feed consumption were more severe.

To overcome the negative effects of high ambient temperature on appetite can be achieved by formulating rations that contain more dense nutrients and produces low heat increment. The addition of fat to the ration is recommended, because it produces low heat increment and containing high dense energy. One of available fat sources in

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Indonesian is lemuru fish oil, a byproduct of lemuru canning process that is widely available in Banyuwangi. Fish oil which is rich in unsaturated fatty acids are good for human health. When fish oil was given to the sheep, it might improve lipid profiles of mutton flesh become richer content of unsaturated fatty acids.

However, fat supplementation is often lower digestibility of forages by disrupting normal conditions of rumen microbes when it is given above 5% in the diet (Preston and Leng, 1987; Bunting et al., 1996). Moreover if the fat given is in the form of unsaturated fat, because unsaturated fat is more toxic to rumen microbes than saturated fat (Galbraith and Miller, 1973; Henderson, 1973) and inhibit microbial growth (Palmquist and Jenkins, 1980). Wachira et al. (2002) reported that administration of fish oil without being protected in sheep lower dry matter intake and body weight gain.

So as not to disturb the rumen microbe, fish oil needs to be protected, one of which is the technique of saponification to produce calcium-soap. Further, it is expected that the Ca-soap can be digested and absorbed in the post-ruminal digestive tract. Fat protection can also mean that the fat can be added to ruminant rations with more than 5% without causing negative impacts on forage digestibility.

The objective of this experiment was to evaluate the effect of polyunsaturated fat protection by the formation of calcium-soap on digestibility of dry matter, organic matter, crude protein and energy as well as the production of VFA and NH₃ in the rumen of sheep.

MATERIALS AND METHODES

Twelve local male lambs with initial weight 16.91 ± 1.66 kg were reared for 3 months. They were divided into 4 groups and each group consists of 3 heads. Grouping was based on average body weight intended to minimize the variability between groups. Sheep were placed in individual cages equipped with buckets for feed and drink. Feed was given twice daily at 7 am and 4 pm as much as 3% of body weight based on the dry matter. Concentrate was first given and then the grass that had been chopped into pieces of ± 5 cm long.

Experimental diets used consisted of field grass and concentrate with 25:75 ratio based on dry matter. Feed ingredients used in the concentrate consists of cassava tube waste, cassava flour, palm oil cake, fermented soybean waste, soy sauce waste, CaCO₃, premix, urea, salt and molasses with a dry matter content of 61.47%, 59.55% TDN (2.15 Mkal ME), 16.96% crude protein and 4.52% crude fat. Calcium soap evenly mixed into the concentrate. Calcium soap was made using modified double decomposition method (Jenkins and Palmquist, 1984). The nutrient content of concentrates diets was showed in Table 1. The treatments applied were R0 = Field grass + concentrate (0% Ca-soap), R1 = R0 + 1.5%Ca-soap, R2 = R0 + 3.0%Ca-soap, and R3 = R0 + 4.5%Ca-soap

Faecal sampling was conducted during the last seven days of experiment. The feces were weighed in the beginning and taken about 10% for composite samples then dried aired for 2-3 days and weighed again as the air dry weight. Air-dried feces were then used for analysis of dry matter content, organic matter, crude protein and energy.

Tabel 1. Nutrient content of experimental diets¹⁾

Nutrient	Treatments			
	R0	R1	R2	R3
	----- %DM -----			
Dry metter (DM)	85.54	85.40	85.26	85.13
Crude protein (CP)	14.21	14.01	13.82	13.63
Ether extract (EE)	3.58	3.60	3.62	3.64
Crude fibre (CF)	15.62	15.40	15.18	14.97
Ash	9.85	9.94	10.03	10.13
FDN	32.81	33.53	34.23	34.91

Calculation result based on Hartadi *et al* (1997)

The variables measured in this study were the digestibility of the ration (dry matter, organic matter, protein and energy), the concentration of total VFA and NH₃. The method of steam destilation (General Laboratory Procedures, 1966) was used to analyze VFA and Mikrodifusi Conway method (General Laboratory Procedures, 1966) was used to analyze NH₃.

Experimental design used was randomized blocked design (RBD) with four treatments and three groups based on body weight. Data obtained were analyzed using analysis of variance (ANOVA) (Steel and Torrie, 1982). Any means significant differences were further tested using Duncan Multiple Range Test.

RESULTS AND DISCUSSION

Organic and Dry Matter Digestibility Coefficient

Effect of the addition of calcium soap on the dry matter digestibility coefficient (DMDC) and organic matter digestibility coefficients (OMDC) are presented in Table 2.

The average value of dry matter digestibility coefficients of the four treatments ranged from 67.39 to 73.89%. While the average value of the coefficient of digestibility of organic matter in the range of 67.33 to 73.67%. The highest value on both digestibility coefficients was in R1 treatment and the lowest value was in R3 treatment. The higher the calcium soap added into the diet, the lower the digestibility of the diet. This indicate that the addition of calcium soap into the diet in level of 1.5% slightly improved the digestibility coefficient value of feed compared to control group, but elevated levels (3% and 4.5%) cannot reduce the negative effect of fat on the rumen microbe especially on fiber digestibility.

Table2. Average digestibility coefficient of dry matter and organic matter

Treatments	Digestibility coefficient	
	Dry matter	Organic matter
	----- (%) -----	
R0	71,91 ± 0,55	72,94 ± 0,40
R1	73,89 ± 2,60	73,67 ± 2,64
R2	70,65 ± 8,15	70,88 ± 8,18
R3	67,39 ± 4,95	67,33 ± 4,91

The addition of calcium soaps did not give significant effect on digestibility coefficients of dry matter. This is consistent with the results of previous studies that the addition of protected fat does not affect the ration dry matter digestibility coefficient (Garg, 1997), and the coefficient of organic matter digestibility (Reddy et al., 2003). This may also precaution that calcium soap originated from fish oil should not be added more than 3%.

Crude Protein and Energy Digestibility Coefficient

Digestibility coefficient of crude protein ration for each treatment study are presented in Table 3.

Tabel 3. Average digestibility coefficients of energy and crude protein

Treatments	Digestibility coefficient of Energi	Digestibility coefficient of crude protein
	----- % -----	
R0	75,32 ±0,10	73,54 ±4,72
R1	77,96 ±2,45	73,75 ±7,34
R2	75,22 ± 8,35	70,07 ± 12,30
R3	72,83 ± 4,36	71,23 ± 3,25

Results of analysis of variance showed that the treatment effect was not significant on crude protein digestibility (CPD). This is due to the protein content in the diet is relatively the same. Crude protein digestibility coefficients depending on the percentage of crude protein in the ration (McDonald, 2002), so that when the protein content of the rations relatively similar, microbial activity in the rumen to digest nutrients, especially protein consumed is also similar.

Energy digestibility coefficient values of treatments were shown in Table 3. The results showed that the treatment effect was not significant on energy digestibility. It is thought that the energy content of the ration used in the treatment (Table 1) were relatively similar. Provision of 1.5% calcium soap (R1) tends to increase the digestibility coefficients of energy, in contrast the provision of 4.5% (R3) tends to lower energy digestibility coefficients. This may be due to the energy content of the R3 ration was higher. If the higher energy content of feed can be regarded to be similar to the higher feed intake, therefore the result of present experiment may be explained by the result of Tilman (1998) that the highest digestibility was obtained in feed consumption that was slightly lower than that of maintenance level.

Ammonia (NH₃) and VFA Production

Effect of the addition of calcium soaps into the diet on NH₃ and VFA concentrations was shown in Table 4.

Table 4. Average VFA and ammonia (NH₃) concentration obtained during experiment

Treatments	VFA concentration	NH ₃ concentration
	----- mM -----	
R0	73,47 ^a ± 17,59	9,08 ^c ± 0,88
R1	38,41 ^b ± 6,30	11,65 ^d ± 1,36
R2	63,46 ^a ± 7,65	7,20 ^b ± 1,68
R3	72,65 ^a ± 6,63	4,62 ^b ± 1,16

Mean with different superscript within the same column differed significantly (p<0.05).

Average concentration values of the four treatment NH₃ are in the range of 4.62 to 11.65 mM (Table 4). The treatments significantly ($P < 0.05$) affected the concentration of NH₃ in the rumen. Ammonia in the rumen is the result of protein and non protein nitrogen degradation (Arora, 1989). Ammonia acts as a source of nitrogen for the formation of microbial cells in the rumen (Hungate, 1966). According to Sutardi (1997) NH₃ concentrations for optimum rumen microbial growth ranged of 4 - 14 mM. This means that all treatments produced enough concentrations of NH₃ to support rumen microbial growth.

Calcium soap addition significantly reduced the concentration of NH₃ from R1 to R3. This decrease in concentration may be due to higher calcium soap disturbed the microbe in the rumen, especially proteolytic microbes. Other possibility was that higher calcium soap protected protein feed stronger, so it is no longer available to be utilized by the bacteria for the synthesis of their body proteins.

The addition of 1.5% calcium soap resulted in a higher concentration of NH₃ (11.65 mM) compared to control or higher addition levels of calcium soap. Therefore the addition of 1.5% calcium soap seemed to be the optimum level in providing ammonia for rumen bacterial growth.

VFA is one of end products of carbohydrate fermentation and is the main energy source for ruminants. The treatment gave significant influence ($P < 0.05$) on total VFA concentration in the rumen. The average of total VFA concentration of the four treatments ranged from 38.41 to 73.47 mM.

Increasing addition of calcium soap from 1.5% (R1) to 4.5% (R3), increase VFA concentration. The higher VFA concentration of R3 group might due to the fact that calcium-soap made still contained 3.78% unhydrolysed lipid (Sudarman, et al., 2008). Lipid was hydrolyzed in the rumen into glycerol and further fermented to volatile fatty acids (Preston and Leng, 1987).

The average concentration of total VFA in the R1 treatment significantly ($P < 0.01$) lower than other treatments. This may indicate that VFA produced in the rumen were utilized as an energy source by microbes to synthesize their body proteins. Hungate (1966) revealed that ammonia, as for carbon framework, and energy source of VFA are simultaneously used for body protein synthesis of microbial rumen. This is in accordance with that reported by Arora (1989) that soluble sugars available in the rumen were fermented into VFA and were used by the microbes to utilize ammonia. On the other hand, if Kim's et al. (1993) report is the case that the addition of calcium soaps can interfere with the performance of rumen microbes and the availability of glucose. Hence, the R3 group should show the lowest VFA production, but in fact the present result is in contrary with that of Kim's et al. (1993) result. The data of dry matter and organic matter digestibility in the present experiment support this argument.

CONCLUSION

The addition of calcium soap in sheep rations had no effect on feed digestibility. Increasing level of fat addition from 1.5% to 4.5% increase total VFA production and decrease the concentration of NH₃. Giving as much as 1.5% calcium soaps most effectively used in the ration because it produces the highest NH₃ as well as the highest digestibility coefficients of dry matter, organic matter, crude protein and energy.

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05. MILK COMPOSITION OF ETAWAH CROSSEDBRED GOAT FED FORAGE AND LEAVES PELLETT

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Abstract

The study aimed to investigate the effect of substitution forage with leaves pellet on goat milk composition. Calliandra and Cassava leaves pellet were used in this study. Pellet substituted 12,25% of forage dry matter in the ration. The study used 9 lactating Etawah Crossed Bred goats, divided into 3 groups (Control, Treatment I and Treatment II). Feed in Control group consisted of grass, Calliandra and concentrate (21:49:30) of dry matter. In Treatment I and II the goat was given the similar ration, but 25% of Calliandra was substituted with Calliandra pellets and Cassava leaf pellets. Data were collected for 60 days, consisted of feed and nutrient consumption, milk composition (total solid, fat and protein content). The result showed there was a significant effect of substitution of forage with leave pellets on dry matter, crude protein, crude fiber and energy consumption ($P < 0.05$). Milk production, fat and protein was not affected by substitution. The study concluded there was no effect of feeding leaves pellets as forages substitution on the composition of goat milk.

Key words: leaves pellet, milk composition, goat

INTRODUCTION

In smallholder condition Etawah Crossedbred goat was commonly depends on forages. Calliandra, Gliricidia and Cassava leaves composed the main feed. Those type of leaves are known to be nutritious for goats. The protein content is 22,29% in Calliandra (Matara, 2001) and 23,42% in Cassava leaves (Purwanti, 2005). Problem commonly occurs in the dry season, that was the condirion when forages production decreased, so that the availability of good quality forage was low. In this situation there was need an effort to preservate forage in order to overcome the problem of feed shortage. The effort should be expectable to maintain the availability of high quality forages such as Calliandra, Gliricidia and Cassava leaves, therefore significance to the sustainability of goat farming systems, depending on natural vegetation.

Pelleting is one of the methods of feed preservation. The process was a combine of mixing of the raw material, forming and a thermal treatment to hard the pellet. To produce forage pellet there was need to add concentrate and filler such as gelatin and sort of flour (Anonim, 2000). According to Parakkasi (1999) there are advantages of pellets. It can be stored in small areaand there is no waste. Anonimous (2013) stated that animal fed pellets is free to eat at different times or several times a day and therefore can increase milk production. Sanz Sampelayo *et al.* (1998) reported the advantageous of feeding alfalfa pellet in compared with alfalfa hay, such as increased casein milk protein, improved the utilization of nitrogen and metabolizable energy for milk production. It was also indicated that amount of fat and protein in the milk depended on energy intake.

Milk composition of is one of the major factors determining its nutritive value (Morand-Fehr *et al.* 2007). Some factors including feed have been known to determine milk production and composition. In this recent situation where goat milk being

promoted to consume, there is need to investigate the composition. An evaluation on Calliandra and Cassava leaves pellets for forage substitution was necessary to be done, since those type of forage were majority produced by farmers.

MATERIAL AND METHODS

Materials:

Goat and feed. The study carried out with 9 lactating Etawah Crossedbred goats in the village of Sukorejo, Turi, Yogyakarta. The goats were in the second lactation with body weight around 40 to 46 kg. The goats were separated from their kids 7 days after kidding. Feed consisted of grass (*Pennisetum purpureum*), *Calliandra calothyrsus*, concentrates and leaves pellets (Calliandra and Cassava leaf pellets). Concentrates composed of rice bran (30%), wheat pollard (40%) and soy bean hull (30%) of dry matter. The component of leaves pellets are Calliandra or Cassava leaves mill (80%), rice bran (20%) of dry matter and filler component (tapioka flour and mollasses).

Equipment. Individual slatted goat houses completed with feed trough and bucket for water feeding. Goat scale, equipments for processing leaves pellets and chemical analyses of feed and goat milk.

Methods:

The goats were divided into 3 groups (Control, Treatment I and Treatment II). Feed of goats in the Control group consisted of grass (21%), Calliandra (49%) and concentrates (30%) of dry matter. Similar feed was given to goats in Treatment I and Treatment II, but 25% of Calliandra was substituted with Calliandra pellets or Cassava leaf pellets, respectively. The quantity of substituted pellet corresponded to 12.25 % of Calliandra (DM). Total dry matter of feed given to all groups was 4% of body weight. Feed was offered twice a day in the morning and the afternoon. Pellets was given after feeding concentrates.

Feed consumption was measured by weighting feed offered subtracted by feed refused after 24 hours. Samples of feed, feed refused and milk were taken every 3 days during 60 days then dried and composited for determination of nutrient content. Milk samples were taken for analyses of spesific grafiti (lactodensimeter), total solid, milk fat (Babcock tets) and milk protein (Kjehldalh). Body weight of goats was measured by weighing at the beggining and the end of study period. Data of feed and nutrient consumption, body weight change, milk production and composition were collected during the study and statistically analysed by SPSS Statistical Package Programme 17 followed Completed Randomised Design.

RESULT AND DISCUSSION

Feed and nutrient consumption

Composition of feed and nutrien content were presented in Table 1 and 2. Table 1 showed the average of nutrient consumption by goats. Dry matter intake (DMI) in Treatment I and Treatment was higher than that in the Control (85.78 and 96.40 g/kg BW^{0.75} compared with 70.41 g/kg BW^{0.75}). Those value corresponded to 3.35 and 3.74% compared with 2.77% of body weight. The data indicated an effect of substitution of Calliandra (25% DM) with Calliandra or Cassava leaves pellets

($P < 0.05$). The highest DMI found in Treatment I (substitution with Calliandra pellets). Leaves pellets in this study considered to be palatable. It was proved by no refused pellets. This condition contributed to maintain DMI. The level of DMI of goats in this study was higher than average DMI of goats fed forage in free range as 43.6 g/kg BW^{0.75} corresponded to 2% of body weight (Njoroge, 1996) and 2.8 to 4.9% of bodyweight for tropical goats (Atabany, 2002). The level of DMI in Treatment I and Treatment II was slightly under the capacity of DMI of goats as about 4 to 7% of body weight (Harris and Springer, 2013). Based on the palatability, pelleting Calliandra and Cassava leaves is expectable preservative method for forage. In the dry season it might be helpfull for farmer to solve the problem of feed shortage.

There as an effect of substitution fresh Calliandra with leaves pellets on nutrient intake ($P < 0.05$). The highest intake of crude protein (CP), crude fiber (CF) and total digestible nutrient (TDN) was found in the Treatment I where the goats received Calliandra pellets. According to Muinga *et al.* (1995) cited by Kato *et al.* (2006) and Mpairwe *et al.* (2003) supplementation with adequate CP to ruminants has promoted dry matter intake (DMI), rumen degradation and nutrient flow to the small intestine and culminated in higher animal performance. In this study, 20% of pellets components also concentrates (rice bran, flour and mollasses) in small amount. Therefore apart from substituting fresh Calliandra, leaves pellets in this study also supplemented the ration in a small amount. Those materials took part in increasing DM and nutrient intake for goats in the Treatment groups.

In this study, the purpose of pelleting was to reduce tannin content, apart from preservate. *Calliandra calothyrsus* has been identified and recommended as the most suitable species feed. However, it contains antinutritional factors, which could be reduced by ensiling (Sabiiti, 2001; Bareeba and Aluma, 2000 cited by Kato *et al.*, 2006). According to NRC (1981) high water content in forage could restrict energy availability for goat. In this study, the form of leaves pellets was solid and high density. Dry matter content (58.60% and 58.40%) was higher than fresh leaves. According to Carvalho *et al.* (2006) cited by Rufino *et al.* (2012) forages have low energy density and slow rate of degradation and passage, which limit the forage intake. In this study, the density of pellet probably increased degradation and passage in the rumen. There was not bulky in the rumen and therefore contribute to increase dry matter and nutrient intake. The increasing of CP and CF consumption as a result of pellets substitution possibly affected milk production and composition, especially on milk fat and milk protein.

Milk composition

Milk composition of lactating goats (presented in Table 4) was not significantly affected by treatment. Milk gravity varied between 1.026 to 1.029. Milk fat and protein were 4.40%, and 4.55% in Control goats; 4.19% and 5.16% in Treatment I; 3.69% and 4.41% in Treatment II. The concentration of fat and protein of goat milk in this study was similar to the average milk composition. According to Strzalkowska *et al.* (2009) the concentration of milk fat varied between 3.67 to 3.85% and milk protein 2.98 to 3.66% while other report was 3.3 to 7.7% and 3.1 to 4.5% (Pambu *et al.*, 2011). The characteristic of breed was also reported, such as superiority in milk quantity of fairy goat and quality in indigenous breed. The data showed similarity of milk

composition in Control and Treatment goats. The increased nutrients including CP, CF and energy in the Treatment groups did not show significant effect on the main milk composition (fat and protein). Sanz Sampelayo *et al.* (1998) showed the advantageous of feeding alfalfa pellet, such as increased casein milk protein, improved the utilization of nitrogen and metabolizable energy for milk production in compared with alfalfa hay. It was also indicated that amount of fat and protein in the milk depended on energy intake. According to Morand-Fehr and Sauvant (1980) supplementary concentrates during midlactation increased intakes of dry matter and energy and milk production. Milk protein percentage was increased, but milk fat contents were lower. Increased intakes of energy raised percentages of palmitic acid and decreased carbon-18 acids. In this study, feeding Calliandra pellets resulted the highest energy intake (in the form TDN), however there was no improvement in milk fat and protein content. There were many reasons of those condition. Variation of breed character, which affected milk production and milk composition. There was no record of goat samples origin. Forage quality was also uncertainly and samples were not taken daily. In this study Calliandra, Cassava leaves and grass were provided by farmers, so that very depending on the place and age of plantation. This because energy potential and protein content in forages are inversely related to maturity. The cellulose in young forages is generally more digestible because rumen microbes are able to more quickly break it down and ferment it, which resulted in more volatile fatty acids. Those reason was probably determine the digestibility of feed in rumen and nutrient absorbtion in the intestine.

The average milk yield was 540 ml/day (Control); 400 ml/day (Treatment I) and 460 ml/day (Treatment II). The result in Table 4 showed the highest milk fat and protein yield were achieved by goats in the Control group because this group produced the most quantity of milk. The yield of fat and protein were 24.49 and 25.39 g/kg BW^{0.75} (Control); 17.27 and 21.21 g/kg BW^{0.75} (Treatment I); 17.43 and 20.79 g/kg BW^{0.75} (Treatment II). The benefit of leaves pellets utilization in this study could be emphasis on its capability to maintain nutrient intake and milk composition. This meant no negative effect of feeding leaves pellets on consumption and the quality of milk. Since the leaves pellets were palatable, pelleting could be considered as preservative method. This point was probably helpfull for farmers to overcome shortage feed problem during the dry season.

CONCLUSION

The study conclusion were (1) Calliandra and Cassava leaves was palatable and good substitution for fresh forages, (2) the effect of substitution 25% of forages dry matter with Calliandra and Cassava leaves pellets increased nutrient consumption significantly, (3) the susbtitution was not affected goat milk composition.

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06. RUMEN FERMENTABILITY AND DIGESTIBILITY OF LINGZHI (*Ganoderma lucidum*) AND ORGANIC CHROMIUM SUPPLEMENTATION IN HIGH AND LOW FORAGE RATIOS

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Abstract

The objective of this research was to study the supplementation effect of lingzhi (*Ganoderma lucidum*) and organic chromium in high and low forage ratios through evaluation of in vitro fermentability and digestibility. Factorial Randomized Block Design 2 x 5 with 4 replications was used in this experiment. The first factor consisted of two levels of forage in the ration *i.e.* high level (60% forage + 40% concentrate) and low level (35% forage + 65% concentrate); and the second factor consisted of five supplements *i.e.* R0 (control); R1 (R0 + 3 ppm organic Chromium); R2 (R0 + 3 ppm organic Chromium); R3 (R0 + 0.01% BW of Lingzhi); and R4 (R0 + 0.01% BW of Lingzhi + 3 ppm organic Chromium). Four times taken of cow rumen fluid were used as block/replication. Parameter were fermentability (NH₃ and Total VFA concentrations) and digestibility (dry matter/DMD and organic matter/OMD percentages). Data were analyzed using ANOVA (Analysis of Variance) and significant differences were further tested using contrast orthogonal. The result findings showed that the increasing of total VFA concentration was achieved by supplementation of Chromium (R1 and R2) in all of forage levels (P<0.01). However, Lingzhi supplementation (R3) seem increased total VFA concentration when ration containing high level of forage. There were no differences in NH₃ concentration for all of treatments. The highest percentage of DMD and OMD were achieved by low level of forage or high level of concentrate for all of treatments (P<0.05). There was indication that supplementation decreasing in vitro digestibility especially OMD. There was no interaction between level of forage and kind of feed supplement in ration. There were two conclusion of this in vitro research, firstly, the supplementation of Chromium increased energy of ruminant when high level of forage contain in the ration; and secondly, the increasing of digestibility was supported by high level of concentrate in the ration.

Keywords: chromium, digestibility, forage, *Ganoderma lucidum*, VFA

INTRODUCTION

Climate change in the tropics is often the cause of the growing proliferation of pathogenic bacteria in the rainy season and declining quality of feed in the dry season. These conditions resulted in a decline of ruminant productivity due to of declining their health. One effort to overcome this problem is addition of feed supplement in their rations. Some of supplements suitable to one organism, but others refused by similar organism due to its content or level required. Natural plant or herb is one of famous supplement for human and animal cause of no risk to consume in high doses. However, the use of herb in animal ration is still restricted. Variety of supplement that consume in short or long time impact to improving the health and immunity of ruminants and also their productivity.

Lingzhi (*Ganoderma lucidum*) is a group of white rot fungi which contain active compounds such as β -D-glucans, triterpenoids and ganoderic acid which functions as an immunomodulator (Syabana, 2002), a 'carrier' in the manufacture of organic chromium (Yang *et al.*, 2005), its mycelium when palm waste fibre as substrates can

replace 50% of napier grass in sheep ration (Evyvernie *et al.*, 2002), and its fruit body reduced the occurrence of subclinical mastitis that measuring through the somatic cell count in dairy lactation (Evyvernie *et al.*, 2011). However, due to lingzhi has function as antibacteria, giving excess amount or improper form of supplement in the ruminant ration causing rumen microbes ceased where in turn decreasing the animal host productivity.

Chromium is one of essential trace element that function supporting animal production. Its availability important in carbohydrate, fat and protein metabolism. Chromium (Cr) is an active component in Glucose Tolerance Factors (GTF) that function as stabilizer of blood glucose. Organic chromium supplemented to dairy cattle concentrate stabilized physiological activities and T3 and T4 concentrations in warm condition (Bestari *et al.*, 2011). Effect of chromium and its metabolism in the rumen are still need investigation.

The aim of this research was to study the fermentability and digestibility in vitro of lingzhi and chromium supplementation in ruminant rations containing different of forage-concentrate ratios (high and low forage levels in the ration).

MATERIALS AND METHODS

Materials Specification. Two kind of rations consisted of different level of forage contain performed two ratio between forage and concentrate, *i.e.*, 60 : 40 and 35 : 65 were applied in this research. The ingredients, composition and nutrition of both rations were presented in Table 1. The average of energy ration (TDN) was 67%, and average of crude protein was 12,8%. The component substances of the supplement consisted of 100 ppm of Lingzhi fruit body (*Ganoderma lucidum*), 3 ppm of inorganic Chromium (inorgCr) and 3 ppm of organic Chromium (orgCr). Organic Cr was made by incorporated inorganic-Cr to mycellium of *Ganoderma lucidum* with rice straw as substrate within 4 weeks fermentation.

Experimental Design and Data Analysis. This experiment was conducted using in vitro analysis with factorial randomized block design 2 x 5 with four blocks (that means four times taken of cow's rumen fluid). The first factor was two types of rations that different in level of forage *i.e.* rasio between forage and concentrate (high forage= 60: 40 and low forage= 35 : 65). The second factor was five kinds supplement as treatment *i.e.*: R0= basic ration (control), R1= R0 + OrgCr, R2= R0 + inorgCr, R3= R0 + Lingzhi, and R4= R0 + Lingzhi + OrgCr. Four parameters were measured in this in vitro experiment *i.e.*, dry matter digestibility (DMD), organic matter digestibility (OMD), concentrations of total volatile fatty acids (VFA) and ammonia nitrogen (NH₃-N). In vitro digestibilities (DMD and OMD) were determined according to Tilley and Terry method (1963). Concentration of total VFA was analyzed by steam distillation method. Concentration of total NH₃-N was analyzed by Conway microdiffusion method. The findings data were analyzed using *analysis of variance* (ANOVA) and contrast orthogonal test (Mattjik and Sumertajaya, 2002).

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RESULTS AND DISCUSSION

Effect of Treatments on Volatile Fatty Acids and Ammonia Nitrogen Concentrations

Table 2 showed the results of the in vitro fermentability of rations supplemented with chromium and lingzhi. Total VFA of R0 (control ration) in high level of forage (60 : 40) was lower than R0 in low level of forage (35 : 60), due to high readily available carbohydrate (RAC) content in concentrate, although in high forage level might high amorphous substances content of napier grass and also lack of lignin portion in its cell wall compared than corn leaf in low forage level which one of corn plant by-product after harvest. Supplementation of chromium for both ration types (R1 and R2) produced total VFA were higher than control and other treatments ($P < 0,01$). Addition of Cr caused increasing of total VFA in high forage ration higher than those in low forage ration (23% vs 10%). Chromium is an essential mineral for carbohydrate metabolism and also it function as important active compound of GTF (glucose tolerance factor) to stabilize blood glucose in correlated to insulin. The role of Cr in the rumen is still unclear. However, in this research we used fine grinding ration that usually support amilolytic bacteria to produce like propionate or lactate. Addition of Cr supposed activate more of those bacteria to degrade the amorphous substance content of forage or ration in the short time (4 hours in vitro fermentation for VFA and $\text{NH}_3\text{-N}$ analysis). In usual carbohydrate metabolism, EMP (Embden-Meyerhof-Parnas) is one of pathway to produce ATP yield, but in case of *Propionibacterium shermanii*, phosphofructokinase was replaced by a pyrophosphate-6-phosphofructokinase, where the pyrophosphate bond has a free energy of approximately 4.6 kcal (Hobson and Stewart, 1997). We need more investigation to know more of Cr role's to increase energy (VFA) in the rumen.

Supplementation of lingzhi (R3 and R4) seem suppressed the production of total VFA due to might a function of lingzhi's active compound such as polysaccharide as antimicrobes which causing some microbes died. Addition of organic chromium (R4) and lingzhi together caused more decreasing total VFA in high forage ration because of the role of antimicrobes and high metabolism of ration due to role of chromium causing pH reduction in the fermentor. Arora (1995) said that decreasing of total VFA was influenced by the number of microorganisms, microbial fermentation and feed consumption. However, the average of total VFA production in this study were 108.8 to 159.5 mM, which were still fulfill the optimum range of energy requirement in the rumen around 80-160 mM (Suryapratama, 1999).

Concentrations of ammonia nitrogen were stable and not affected by the all supplementations and they fulfill the needs of the optimum (3-12 mM) microbial activity in the rumen (Sutardi, 1980), or 6 to 17.65 mM (McDonald *et al.*, 2002). Orskov (1992) stated that the concentration of $\text{NH}_3\text{-N}$ were derived from degradation of feed protein. Feed protein in the rumen undergo proteolysis by rumen microbial enzymes into oligopeptides and amino acids, and both will undergo deamination to produce α -keto acid, CO_2 , VFA and $\text{NH}_3\text{-N}$ (McDonald *et al.*, 2002).

Effect of Treatments on Dry Matter and Organic Matter Digestibility

Table 3 showed in vitro digestibility of treatment rations with different level of forage and supplemented with chromium and lingzhi. Ratio of forage and concentrate

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significantly affected the dry matter digestibility (DMD) ($P < 0.05$) and very significantly affected the organic matter digestibility (OMD) ($P < 0.01$). The highest of DMD and OMD were achieved by control (R0) in low forage level (35 : 65). Both digestibility of all treatment and all ratio of forage and concentrate rations were lower than control. These results seem supported results of total VFA. By short time fermentation of VFA analysis, addition of chromium supposed encourage activity of non-cellulolytic bacteria, that impact to reduced the activity of cellulolytic bacteria to degrade forage completely. In vitro fermentor tubes were a closed system fermentation without absorption, after a part of VFA and $\text{NH}_3\text{-N}$ were used to synthesis of microbes protein, the rest might be accumulated as well as end product of fermentation that to be toxic. In this situation, decreasing pH and increasing toxin in environment causing the bacteria ceased and then affected decreasing of digestibility. This occurrence was almost similar than digestibility results of lingzhi supplementation (R3 and R4). The population of bacteria to degrade forage and concentrate were reduced by addition of lingzhi in the ration. Lingzhi contains active compound like polysaccharide that function as antibacteria. The active compound of *G. lucidum* was potent against Gram positive bacteria than against Gram negative (Yoon *et al.*, 1994). Some of plant fibre-degrading-bacteria like *Ruminococcus spp*, *Streptococcus bovis* are Gram positive as well as some of methanobacterium, and some of the polysaccharide-degrading-bacteria in the rumen are Gram negative which can produce propionate, butyrate or succinate, such as *Prevotella ruminicola*, *Butyrivibrio fibrisolvens*, and *Fibrobacter succinogenes* (Hobson and Stewart, 1997). Based on those researches, digestibility of rations containing lingzhi could not achieved the maximum due to antibacteria compound of lingzhi suppressed bacteria viability and accumulation of acids as fermentation products caused pH reduction.

In vitro research has disadvantage because no absorption of VFA and $\text{NH}_3\text{-N}$ productions as fermentation products. Accumulation of those end products cause decreasing of rumen pH, further causing death of microbes, and in turn influence rations digestibility and life of the host. From those findings suggesting that supplementation of chromium in the ration containing high forage (60 : 40) like ration for dairy lactation cow, should consider level of chromium in the ration to avoid decreasing the digestibility. Although increasing of milk production may occurred due to increasing of VFA absorption through the rumen wall, but it should avoid decreasing of milk quality because of low nutrients absorbed in the digestion after rumen. Supplementation of chromium in low forage ration (35 : 65) like ration for beef cattle, can be applied to maximize animal host growth, but this investigation should be continued to find the optimum level of chromium in the ration. Supplementation of lingzhi that contain antimicrobes should more investigated to find the accurate way to add lingzhi in the ration, in order to achieve maximum result due to functions of other compounds of lingzhi content are able to encourage the health and immunity of the animal host.

CONCLUSION

Supplementation of Chromium increased the total VFA higher in ration contain high level of forage than ration contain low level of forage. Ration contains low level of forage or in the other word ration contains high concentrate showed the highest digestibility. Supplementation of Lingzhi decreased total VFA and digestibility of both rations.

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Table 1. Ingredient, composition and nutrition of treatment rations

Ingredients	Treatments (%)	
	60 F : 40 C	35 F : 65 C
Napier grass	60	-
KPS Concentrate	40	-
Corn leaf	-	35
Rice bran	-	21,50
Corn	-	19,65
Soybean meal	-	13,60
Coconut meal	-	8,00
Corn oil	-	2,00
Treac	-	0,25
Crude Protein	12,1	13,5
Crude Fiber	25,68	17,0
TDN	67	67

Note: Proximate analysis from Biological Resources Research Center and Biotechnology, IPB (2008); F= forage; C= concentrate; TDN= total digestible nutrient

Table 2. In vitro fermentability of rations containing Lingzhi and Chromium (mM)

Ration	Average of VFA		Average of NH ₃ -N	
	60 F : 40 C	35 F : 65 C	60 F : 40 C	35 F : 65 C
R0	129,0 ± 31,0 ^A	134,8 ± 10,9 ^A	12,6 ± 2,7	13,6 ± 3,0
R1	159,5 ± 21,3 ^B	137,9 ± 21,1 ^B	13,9 ± 3,0	10,3 ± 2,0
R2	159,2 ± 11,1 ^B	147,9 ± 9,2 ^B	12,7 ± 2,4	11,3 ± 4,1
R3	137,2 ± 17,6 ^A	112,6 ± 10,4 ^A	11,0 ± 3,7	13,0 ± 3,0
R4	108,8 ± 17,8 ^A	122,9 ± 16,4 ^A	11,6 ± 1,5	11,9 ± 3,2
Average	138,6 ± 21,4	131,2 ± 13,7	12,4 ± 2,0	12,0 ± 3,3

Notes: Mean in the same column with different superscript differ very significantly (P<0,01). VFA = volatile fatty acids, NH₃-N = ammonia-nitrogen, F = forage, C = concentrate. R0 = control, R1 = R0 + organic Cr, R2 = R0 + inorganic Cr, R3 = R0 + Lingzhi, R4 = R0 + organic Cr + Lingzhi

Table 3. In vitro digestibility of rations containing Lingzhi and Chromium (%)

Ration	Average of DMD		Average of OMD	
	60 F : 40 C	35 F : 65 C	60 F : 40 C	35 F : 65 C
R0	58,4 ± 8,4	61,6 ± 5,5	58,0 ± 8,6	63,7 ± 6,1
R1	55,5 ± 4,7	57,4 ± 5,8	54,1 ± 5,0	59,2 ± 6,2
R2	57,9 ± 7,5	60,2 ± 4,6	57,3 ± 8,5	62,0 ± 3,7
R3	57,5 ± 8,3	60,2 ± 7,0	56,0 ± 9,5	61,3 ± 5,9
R4	56,6 ± 9,0	58,3 ± 4,6	55,7 ± 10,5	59,6 ± 4,4
Average	57,2 ± 7,3 ^a	59,5 ± 5,9 ^b	56,2 ± 8,1 ^A	61,2 ± 5,5 ^B

Note: Means in the different column with different small superscript differ significantly (P<0,05). Means in the different column with different capital superscript differ very significantly (P<0,01). DMD = dry matter digestibility, OMD = organic matter digestibility, F = forage, C = concentrate. R0 = control, R1 = R0 + organic Cr, R2 = R0 + inorganic Cr, R3 = R0 + Lingzhi, R4 = R0 + organic Cr + Lingzhi

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07. RUMINAL DEGRADATION CHARACTERISTICS OF MAIZE (*Zea mays*) LEAVES

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Abstract

A research has been conducted to evaluate degradation characteristics of maize leaf through *in sacco* method using nylon bag technique. Three varieties of maize were involved in this study: local variety, hybrids variety and sweet variety. Leaves from three varieties were chopped and dried before it was being grinding to get a homogen sample with 1 mm of particle size. Two gram samples of each variety in nylon bags were incubated in permanently fistulated goats within three replicates. The incubation times were 0, 8, 16, 32, 64 and 72 h. Degradability of dry matter and protein data generated from incubation process were analysed using exponential equation of Orskov and McDonald (1979) and using “Neway” program. The mean values of a,b,c and Effective Degradability (ED) were analysed using an analysis variance according to Completely Randomized Design. The results indicated that dry matter and crude protein degradability of sweet variety are higher than hybrids variety ($P<0.05$) and local variety ($P>0.05$). The characteristics degradation values of sweet variety were 9.26%, 15.66% and 3.86%/h for fraction a, b and c respectively. Local variety degradation characteristics were 10.69%, 15.04% and 3.04%/h, while hybrids variety were 5.18%, 8.72% and 4.04%/h for fraction a, b and c respectively. Effective degradability of sweet variety was higher than hybrids variety ($P<0.01$) and local variety ($P>0.05$). The values of ED were 19.70%, 14.57% and 20.40% for local, hybrides and sweet variety, respectively. In conclusion, maize leaves from sweet variety seemed to be more potential as forages source than two others varieties.

Keywords: degradation, dry matter and crude protein

INTRODUCTION

Adequate nutrition of animals includes knowledge of the characteristics of used feeds, their nutritive value, nutritional needs, possibility for consumption and utilization of food. Primary task of nutritionists is to adequately investigate and evaluate the quality of feed to be used in the diets for livestock production. Basic knowledge of degradation characteristics of feed allow us to estimate the feeding value of particular feed without involving experimental feeding. For instance, Orskov and Ryle (1990) produced feeding values as an index for feed consumption based on its degradation characteristics. This index could be developed to estimate *in vivo* digestibility and live weight gain depending on generated data from feed index evaluation. While, Mgheni *et al.* (2001) used degradation characteristics of feed to predict dry matter intake of cows.

Rumen degradation characteristics of feed entirely depends on feed property, solubility, outflow rate, intake level, fermentation substrate, microbial population, particle size, physical form of feed and rumen pH (Orskov, 1982). Protein solubility has a positive correlation with rumen degradation (Madsen and Hvelplund, 1990), and a good indicator to predict nitrogen availability (ammonia) for microbial growth in the rumen. High soluble proteins produced high rumen-ammonia and stimulate an optimal microbial growth. A degradation characteristic has a main role to formulate diets for ruminant nutrition. However, some local potential feed do not have degradation characteristics, particularly maize-leaf from three varieties. Therefore, the present study

evaluated degradation characteristics of leaves from three maize varieties through incubation of experimental materials using nylon bag technique in the goat rumen.

MATERIALS AND METHODS

Feed samples degradation. Characteristics degradation evaluation was conducted *in sacco* method using nylon bag technique. A mature male goat permanently fistulated was used to determine rumen degradability of maize leaf samples. The goat fed with elephant grass (*Pennisetum purpureum*) at maintenance. Present study involved three varieties of maize: local, hybrids and sweet variety. Maize-leaf samples were harvested from local farmer after 40 days of plantation. The leaves samples were chopped and dried to reduce moisture and then ground to 1 mm of particle size. About 2g samples were placed in nylon bags (4x6 cm with pore size of 60 μ m). Filled bags were properly sealed and tied up in a sequence of incubation times to a 30 cm small chain. All filled bags were incubated in the rumen of permanently fistulated goat within three replicates. The bags were removed after 8, 16, 32, 64 and 72 hours of incubation. The zero hour bags were soaked in water instead of rumen to estimate the soluble fraction of DM. After removal, bags were immersed in cold water to cease microbial fermentation and washed manually in tap water until effluent water was clear. The washed bags were dried to constant weight at 60°C and weighed and stored for chemical analysis. Procedures, handling samples before and after incubation were based on Kristensen *et al.* (1982).

Chemical and statistical analysis. Leaves samples before and after incubation were analysed for dry matter, crude fibre and nitrogen according to AOAC (1990). Degradability of dry matter and protein data generated from incubation process were analysed using exponential equation of Orskov and McDonald (1979) and using “Neway” program of Chen (1994). Further calculation to produce values of soluble fraction (a), potential fermented insoluble fraction (b), rate of degradation (c) using equation $P = a + b(1 - e^{-ct})$ and P disappearance rate at time (%), noted as degradability rate at the present study. The effective degradability (ED) of crude protein (CP) was calculated by using the following equation of Ørskov and McDonald (1979). $EDCP = a + \{(bc)/(c+k)\}$ where, k = the rate of particulate outflow from the rumen, assuming is 0.02/ h. The mean values of a,b,c and ED were analysed using an analysis variance according to Completely Randomized Design of Steel and Torrie (1980) with statistical packages of Minitab-11. Mean values different were analysed using Least Significant Difference (LSD).

RESULTS AND DISCUSSION

Chemical composition of maize leaves was different for all variables analysed (see Table 1). On average, the lowest value of protein was 6.1% on hybrids variety and the highest value of protein was 7.11% on sweet variety. The values of protein, except for hybrids variety, were higher than values of protein to about 6.25% for tropical forages that may reduce microbial activities and intake (Djajanegara and Doyle, 1989). Philpau and Michalet-Doreau, (1997), Cone *et al.*, (2008) and Hetta *et al.*, (2012) reported that type of maize produce a different in chemical composition at the same maturity. Interestingly, this study demonstrated that the highest value of protein was followed by the lowest value of crude fibre and *vice versa*. This phenomenon had a marked effect on ruminal degradation and ruminal digestibility (Orskov, 1982;

Philppeau and Michalet-Doreau, 1997), and therefore it influenced feeding values of the leaves.

In sacco degradability is a good indicator for fermentation and degradation rate in rumen. Degradability values of the feed will vary depending on chemical composition and feed property as mentioned above. Mean values of *in sacco* degradability is presented in Table 2. The results indicated that dry matter degradability and protein degradability increases as incubation time increases. This trend could be explained that rumen microbial have more time to penetrate particle feed as incubation time increases. Maize variety provided a significant different in dry matter and protein degradability ($P < 0.01$). For all incubation time, dry matter and protein degradability of hybrids variety was lower than two others varieties of local and sweet maize ($P < 0.01$). Furthermore, dry matter and protein degradability of sweet variety tended to be higher than local variety ($P > 0.05$). The higher value of degradability on sweet variety is related to its chemical composition as noted on Table 1. Degradability variation between type of maize has been reported in many studies (Philppeau and Michalet-Doreau, 1997; Cone *et al.*, 2008; Hetta *et al.*, 2012). Chemical compositions from each type of maize has a significant contribution for the differences in their degradability.

Degradation characteristics generated from three varieties are similar to the degradability trend in which sweet variety had the highest value of potential degradation in rumen (b) followed by local and hybrids variety ($P > 0.05$). The b values were 15.66, 15.4 and 13.72 % for sweet, local and hybrids respectively. However, the water soluble fraction (a) of sweet variety was lower than local variety ($P > 0.05$) and it was higher than hybrids variety ($P < 0.05$). The water soluble fraction (a) values were 10.66, 5.18 and 9.26% for local, hybrids and sweet variety respectively. The highest value of rate of degradation (c) was achieved by hybrids variety at the level of 4.04%/h ($P > 0.05$). Degradation characteristics values in present study are still comparable to the values reported by Nsahlai *et al.* (1995) for the teff (*Eragrostis tef*) straw. The values are 11.1%, 49.7% and 2.2%/h for fraction a, b and c respectively. The values in present study are values for straw category. Degradation characteristics values of leaves from three varieties are summarized in Table 3. The differences in degradation characteristics are due to the difference in chemical composition of leaves samples as presented in Table 1. The results current study were supported by the evidence in leaves of sorghum that the partial differences in degradation characteristics was due to the chemical composition of leaves in different varieties Fadel-Elseed *et al.*, 2007). A similar evidence has been reported in maize variety (Philppeau and Michalet-Doreau, 1997; Cone *et al.*, 2008).

The values in Table 3 are not categorized as extreme values that may have a significant consequence on effective degradability of protein in particular feed (Orskov, 1982). The more interesting findings in the current study are degradation characteristics of hybrids variety where fraction a value achieved the lowest value and rate of degradation reached the highest value, respectively. In fact, outflow rate change on hybrids variety base diet will produce a significant change in effective degradability of protein. However, at the outflow rate of 0.02/h, effective degradability values of sweet variety were higher than two others varieties. The values were 20.0, 19.7 and 14.5% for sweet, local and hybrids variety respectively. These values of effective degradability of protein indicated that protein fraction of the experimental leaves in present study are

categorized as by-pass protein. Importantly, there was a negative relationship between effective degradability and chemical properties of feed samples in present study. This negative relationship stands solely for ruminal degradation and should not be compared with total tract digestibility studies (Bal *et al.*, 1997) or macro *in situ* trials (Johnson *et al.*, 2003)

The values are presented in Table 2 and Table 3, are clear evidence for maize leaves potential of sweet variety as better feed source compared with two others varieties. This phenomenon is related to chemical composition of sweet variety, where protein content was higher than two other varieties with the lowest value of fibre (Table 1). McDonald *et al.* (1994); Philippeau and Michalet-Doreau, (1997), and Cone *et al.*, (2008) reported that chemical composition influenced the extent and the rate of rumen degradation and digestibility of particular feed. The property of sweet variety is vapourable for ruminal microbe development. However, sweet variety is not entirely good protein sources for microbe because its effective degradability of protein is only about 20%. It means that more protein fraction is categorized as “by-pass” protein and therefore maize leaves base diet should be provided an additional protein or nitrogen sources to enhance microbial growth in rumen. This demonstrates the importance of degradation characteristics knowledge in particular feed for better understanding of varietal difference in feeding value of the feed and better diet formulation in ruminant nutrition.

Conclusion, degradation characteristics varied between three varieties of tested maize leaves. Sweet variety was more degradable than two others varieties. The highest value of degradation potential and effective degradability of protein was sweet variety followed by local and hybrids variety.

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Table 1. Chemical composition of the leaves from three varieties maize.

Maize variety	Dry matter	Crude protein	Crude fibre
	%		
Local	51.50	6.88	63.57
Hybrids	49.66	6.17	68.09
Sweet	49.01	7.11	61.15

Table 2. Mean values of *in sacco* dry matter and crude protein degradability of leaf maize from three varieties with different in incubation times.

Incubation times (h)	Dry matter degradability (%)			Crude protein degradability (%)		
	Local	Hybrids	Sweet	Local	Hybrids	Sweet
8	13.58 ^a	9.53 ^b	13.37 ^a	13.46 ^a	9.46 ^b	13.53 ^a
16	16.48 ^a	11.58 ^b	16.45 ^a	16.54 ^a	11.60 ^b	16.37 ^a
32	19.14 ^a	15.96 ^b	20.59 ^a	19.21 ^a	15.90 ^b	20.66 ^a
64	21.79 ^a	17.79 ^b	22.45 ^a	21.81 ^a	17.61 ^b	22.28 ^a
72	23.97 ^a	18.52 ^b	24.88 ^a	23.98 ^a	18.39 ^b	24.87 ^a

Values within the rows for dry matter degradability or protein degradability followed by different superscript letters are significantly different (P<0.01).

Table 3. Mean values for degradation characteristics of a, b, c and ED (effective degradability) protein of maize leaves from local, hybrids and sweet variety.

Varieties	a (%)	b (%)	c(%/h)	ED# (%)
Local	10,69 ^A	15,04 ^a	3,04 ^a	19,70 ^A
Hybrids	5,18 ^B	13,72 ^a	4,04 ^a	14,57 ^B
Sweet	9,26 ^A	15,66 ^a	3,86 ^a	20,40 ^A

assumption outflow rate is 0.02/h; values within the columns followed by different superscript letters are significantly different (capital letters for P<0.01 and small letters for P<0.05).

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08. THE EFFECT OF CONCENTRATE OFFERED IN RATION BASED ON RICE STRAW ON THE PERFORMANCE OF BALI CATTLE

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Abstract

The experiment was aimed to study the optimal level of concentrate offered on male bali cattle which given rice straw as a basis ration. The experiment used block randomized design (BRD) that consist of three treatments and three blocks, so it was nine units experiment. The range body weight of male bali cattle 168-182 kg. The three treatments were level of concentrate offered on 1.0; 1.5; and 2.0% from body weight for treatment P1, P2, and P3 respectively. Rice straw and drinking water were given ad libitum. Variables observed were dry matter, organic matter, crude protein, and crude fiber consumption, body weight gain, feed conversion ration (FCR), and feed cost to raise 1 kg of body weight. Results of the experiment showed that the increase of concentrate offered from 1.0 to 1.5% of body weight could increase crude fiber consumption, weight gain ($P < 0.05$), increase feed cost to raise 1 kg of body weight, suppressed crude fiber consumption and FCR value, but not affected to dry and organic matter consumption ($P > 0.05$). Increasing of concentrate offered from 1.0 to 2.0% of body weight could increase dry and organic matter, crude protein consumption, and weight gain ($P < 0.05$), increase feed cost to raise 1 kg of body weight, however suppress crude fiber consumption and FCR value ($P < 0.05$). It can be concluded that the best performance was 2.0% concentrate level, but the cheapest of feed cost to raise 1 kg of body weight was 1.0% concentrate level in ration with rice straw as a basis ration.

Keywords: concentrate, rice straw, performance, bali cattle

INTRODUCTION

The animal protein requirement in Indonesia is increase with the increase number of people and realize of the important of animal protein. But cattle industry not yet ability to fulfill the increase requirement of meat. The import of cattle and meat from foreign couldn't be avoided as a consequence. It is a big challenge and chance for cattle industry in Indonesia to raise availability of protein from animal.

Bali cattle is one of Indonesia native germplasm, not selective characteristic with available feed, adapted with environment in Indonesia, high percentage of pregnancy and birth, and high percentage carcass with good meat quality (Gunawan and Ronjali, 2010). In the future bali cattle could be hope as a meat supply in Indonesia, but developing of bali cattle in Bali is faced on the difficulties of forage availability. On the other side, the availability of agriculture waste such as rice straw is high and potential to use as feed. Rice straw production in Bali was 5.53 ton dry matter/ha/production period. The main difficulty of rice straw usage as feed are high lignin and silica content, low protein and digestibility (Sutrisno, 1988). Offering rice straw as single feed is not enough to fulfill the maintenance of bali cattle, eventhough giving ad libitum. So, bali cattle needs supplement if offer rice straw as a basal ration.

Feed supplement that possible to cover the lack nutrient in rice straw is concentrate. Concentrate could be able to cover the lack nutrient in rice straw and make nutrient contain in ration is appropriate with the cattle requirement and cattle could grow appropriate with its genetic potential.

The exceed offer of concentrate will stimulate volatile acid and lactic acid in rumen and decrease of rumen acidity (pH). The decrease of rumen pH under normal pH will decrease productivity and activity of rumen microbes. The decrease of rumen pH caused the decrease of cellulose digestive activity and bigger decrease if pH under 6.0. The decrease of cellulose digestive in rumen will make a bad impact to the cattle productivity. Its proved from the result of Damayanti's research (2004) found that crude fiber digestibility on goat which offer 60% concentrate in ration caused 19.18% significant lower than goat which offer 20% concentrate in ration. Based on it, it needed a research about level of concentrate supplementation in ration based on rice straw to yield the maximal productivity.

MATERIAL AND METHODS

Material:

The experiment used bali cattle with 168-182 kg body weight and were placed in individual stall and completed with feeding and drinking water equipment. The stall is colony cage with 175 cm × 125 cm wide, asbes roof, concrete floor with 5% slope angle to make easier to sweep the floor.

Ration consist of rice straw and concentrate. Rice straw was obtained from field rice around the location of the experiment and contain of 93% dry matter, 2.93% crude protein, 19.83% crude fiber, and 75.43% organic matter (Nutrition Laboratory, Animal Husbandry Udayana University). Concentrate was commercial concentrate (LSP) and mixed with 0.15% pignox (product of PT. Medion) as multivitamin mineral source before giving to cattle. The content of concentrate were 97.83% dry matter, 16.89% crude protein, 87.56% organic matter and 9.16% crude fiber. Source of drinking water from well around the research place.

Method

The experiment was conducted in Batubulan Kangin Village, Gianyar Regency, and consist of field and laboratory experiment. Field experiment conducted for eight weeks, and sample analysis in laboratory for four weeks.

The experiment used block randomized design (BRD) with three treatments and three blocks as replication based on body weight difference, so it was 9 units trial. Each unit used one cattle and nine cattle total. The treatments were 1.0: 1.5: and 2.0% of concentrate from body weight for P1, P2, and P3 treatment, respectively. Rice straw and drinking water were given ad libitum. The cattle were adapted to ration about two weeks with rice straw and concentrate in same amount and given worm medicine one week before the experiment begin.

Ration mixture carried out each week, LSP concentrate were mixed well with pignox and placed in plastic bag and ready to give to cattle. Concentrate was given twice a day in the morning and afternoon. Rice straw and drinking water were given ad libitum

Sample withdrawal of rice straw and concentrate was conducted every day, and at the end of the experiment sample were composited appropriate with the treatments. Subsample collected 200 g for analyzing at laboratory. The ration residue only analysis on dry matter and ration analysis on dry matter, organic matter, crude fiber, and crude protein used AOAC method (1980).

Variables observed were dry matter and nutrient consumption, body weight gain, and feed conversion ratio (FCR):

- Dry matter and nutrient consumption. Dry matter consumption is calculated by counting the different of total ration offer with ration residue which not consume, and conducted every day. Nutrient content were measured by sample analysis, and nutrient consumption counting with formula:
$$\text{Nutrient consumption} = \text{total rice straw consumption} \times \% \text{ dry matter of rice straw} \times \text{rice straw nutrient} + \text{total concentrate consumption} \times \% \text{ dry matter of concentrate} \times \text{concentrate nutrient}$$
- Weight gain is calculated by counting the different of final body weight and initial body weight. Body weight was measured every week in the morning before giving feed and drinking water. The weighing used electronic scale with 300 kg capacity.
- Feed Conversion Ratio (FCR) is calculated by dividing total feed consumption (kg) and body weight gain (kg) along the experiment.
- Feed cost to raise 1 kg body weight was measured by calculating rice straw consumption (kg) to raise 1 kg body weight \times price of 1 kg rice straw + concentrate consumption (kg) to raise 1 kg of body weight \times price of 1 kg concentrate.

The obtain data were analysis with analysis of variances. In case the results showed significant differences ($P < 0.05$) in treatments, so further analysis will be examined with Duncan Multiple Range Test (Steel and Torrie, 1986).

RESULT AND DISCUSSION

The increase of concentrate supplementation on bali cattle which given rice straw as based ration could increase dry matter consumption. Dry matter consumption on P3 treatment was 12.81% higher compared with P1 treatment. Dry matter consumption of ruminant is determined with two factors: cattle effort to fulfill energy requirement and fully of rumen as a consequence of high crude fiber in ration (Parakkasi, 1999). The increase of dry matter consumption on treatment P3 probably caused by supplementation of 1% concentrate from body weight not yet be able to fulfill cattle requirement for maximizing growth, so the cattle effort to eat more to support its growth. The increase of dry matter consumption on treatment P3 caused by the increase of concentrate consumption (Table 1). The increase of concentrate consumption increased nutrient consumption for rumen microbe, so could increase the activity of feed digesting. It caused increase the rate of passage to digestive tract post rumen and dry matter consumption.

The increase of dry matter consumption could increase organic matter consumption because organic matter is the biggest composition of dry matter. It appropriate with Tillman et al. (1998) stated that organic matter is the biggest part of dry matter, so the increase of dry matter consumption could increase organic matter consumption.

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Table 1. The effect of concentrate offer in ration based on rice straw on the performance of bali cattle

No.	Variables	Treatments ¹⁾			SEM ²⁾
		P1	P2	P3	
1.	Initial body weight (kg)	175.33a	176.03a	175.63a	2.15
2.	Final body weight (kg)	199.69b	205.09ab	209.53a	2.02
3.	Body weight gain (kg)	24.35c	29.05b	33.99a	2.27
4.	Dry matter consumption (kg)				
	- Concentrate	98.77	154.21	200.69	
	- Rice straw	173.80	134.15	106.82	
	- Total	272.58b	288.36ab	307,51a	2.69
5.	Organic matter consumption (kg)				
	- Concentrate	86.36	134.84	175.48	
	- Rice straw	131.22	101.31	80.64	
	- Total	217.58b	236.15ab	256.12a	0.79
6.	Crude protein consumption (kg)				
	- Concentrate	16.58	25.9	33.71	
	- Rice straw	5.40	4.17	3.32	
	- Total	21.98c	30.07b	37.03a	0.22
7.	Crude fiber consumption (kg)				
	- Concentrate	9.03	14.10	18.35	
	- Rice straw	33.37	25.56	20.50	
	- Total	42.4a	39.66ab	38.85b	0.23
8.	FCR	11.58a	9.93b	9.23b	1.25
9.	Feed cost to raise 1 kg of body weight (Rp.)	11236,23	12547.57	15339.53	

Note:

- 1) P1 = rice straw ad libitum + concentrate 1% from body weight; P2 = rice straw ad libitum + concentrate 1.5% from body weight; P3 = rice straw ad libitum + concentrate 2% from body weight
- 2) SEM = Standard Error of Treatment Means

Crude protein consumption on treatment P2 and P3 were higher compared with treatment P1. It because of higher consumed of concentrate on treatment P2 and P3 (Table 1). Concentrate contain of rich degradable energy and protein (Tillman et al., 1998), so the increase of concentrate consumption increased protein consumption. On the other hand, the increase of concentrate could suppress crude fiber consumption, because the increase of concentrate could suppress rice straw consumption (Table 1). Rice straw is rich of crude fiber feedstuff (Sutrisno, 1988).

The increase of concentrate supplementation on bali cattle which offer rice straw based could increase weight gain. Treatment P2 and P3 resulting 19.54% and 39.22% higher weight gain compared with treatment P1. The increase of concentrate supplementation could increase concentrate consumption (Table 1), so could increase nutrient consumption to synthesis of body tissue, and finally will increase weight gain of cattle. The increase of nutrient consumption could increase productivity of cattle (Nitis, 1982; Suyasa et al., 2004; and Susila et al., 2007).

FCR of treatment P2 and P3 were 19.24 and 20.29% lower than FCR on treatment P1. It caused by crude fiber consumption on treatment P2 and P3 were lower compared with treatment P1 or on the contrary, concentrate consumption on P2 and P3 were higher compared with P1 (Table 1), so quality of ration that consumed by cattle on treatment P2 and P3 were less compared with P1. It means that use of feed efficiency on cattle P2 and P3 were higher than P1. Feed efficiency on bali cattle which offer crude fiber could be increased through supplementation of concentrate supplementation on certain level (Nitis, 1982).

Supplementation of concentrate could increase feed cost to raise 1 kg body weight. Feed cost to raise 1 kg of body weight on treatment P2 and P3 were 11.67% and 36.51% higher than treatment P1. It because of higher consumed of concentrate on treatment P2 and P3 and the price of 1 kg concentrate is more expensive than 1 kg of rice straw (Rp. 2500 vs Rp. 100).

CONCLUSION

Based on the above discussion, it could be concluded that:

1. The increase of concentrate supplementation from 1,0 to 1.5% from body weight could increase 36.80% of crude protein, 19.54% of body weight gain, and 11.67% of feed cost to raise 1 kg of body weight, suppress 6.46% of crude fiber consumption, and 14.24% FCR, but not effect to dry matter and organic matter consumption
2. The increase of concentrate supplementation from 1,0 to 2.0% from body weight could increase 12.81% of dry matter consumption, 17.71% organic matter consumption 68.47% of crude protein, 39.22% of body weight gain, and 36.51% of feed cost to raise 1 kg of body weight, but suppress 8.37% of crude fiber consumption, and 20.29% FCR
3. The best performance was obtained on bali cattle which offer rice straw as based ration supplemented with 2% concentrate from body weight, but the cheapest feed cost to raise 1 kg of body weight was obtained on bali cattle which offer rice straw based ration supplemented with 1.0% concentrate from body weight

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09. ENHANCING PERFORMANCE OF SHEEP BY FEEDING CORN LEAF BISCUIT

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Abstract

The objective of this study was to apply feeding of corn leaf biscuit to enhance growth performance and digestibility of sheep by measuring its feed intake, body weight gain, feed conversion, digestibility of dry matter, organic matter, crude fiber, neutral detergent fiber and acid detergent fiber of sheep. This research was conducted at laboratory of Feed Industry , Faculty of Animal Science, Bogor Agricultural University, Indonesia on June - October 2010. Nine heads of thin tail sheep with initial body weight was around 12-18 kg were randomly assigned to three dietary treatments (three heads of sheep / treatment) for three months with the adaptation period for two weeks. Experimental design used Completely Randomized Design. The treatments were biscuit composition i.e T1 = 100% field grass ; T2 = biscuit 50% field grass + 50% corn leaf; T3 = 100% corn leaf. The results showed that 100 % corn leaf biscuit (T3) had the best nutrient quality with crude protein 17.97%, crude fiber 28.20%, crude fat 1.09% and NFE 40.99%. The application of biscuit feeding on sheep showed that the dry matter intake was not significant ($P>0.05$) with the highest value was 100% corn leaf biscuit (263.18 g/head/day). Feeding of corn leaf biscuit was not significant ($P>0.05$) on body weight gain and feed conversion. The body weight gain of sheep fed with corn leaf biscuit was 61.90 g/head/day or 44.60% higher than sheep fed with field grass biscuit. The corn leaf biscuit was not significant effect ($P>0.05$) on digestibility of dry mater, organic mater, fiber, neutral detergent fiber and acid detergent fiber. It was concluded that corn leaf could be processed to produce biscuit as a fiber source in ruminant feed.

Keywords : *corn leaf, biscuit, growth performance, digestibility, sheep*

INTRODUCTION

Corn leaf waste is an alternative feed source and low cost raw material for producing various feed product. Corn leaf waste can be used for animal feed especially ruminant (Engel et al., 2008). The major constraints of corn plant waste usage as a ruminant feed are as follows: (i) due to its characteristics, corn plant waste is bulky and perishable which may cause difficulties in the handling, distribution, and feeding process, (ii) the stocks of corn leaf waste fluctuates during dry and rainy seasons, (iii) in the feeding process, corn leaf waste is low palatability and low digestibility for ruminants. Therefore, it is necessary to develop technology to produce ruminant feed which is more durable, easier to handle, to distribute and to feed.

Corn leaf Biscuit is a biscuit form of corn leaf waste which is processed by heating and pressing technique. This biscuit could replace forage during the dry season. Biscuit is a dry product that has relatively long lasting storage period and easy to handle on the way (Whiteley, 1971). The advantage of the corn leaf biscuit is its a compact form, so that it can be directly fed to the ruminant. Moreover, due to its long lasting storage, such biscuit overcomes the scarcity of sheep forage during dry season. Meanwhile, there is none of previous researches about processing forage into a form of biscuit.

The process in making of corn leaf biscuit consists of such stages as chopping, grinding, mixing, pressing and heating in temperature 95⁰C for 5 minutes and finally cooling in room temperature. Grinding treatment can change the particle size of feed, so that its digestibility will improve. A decrease in the particle size of feed can improve digestibility values as more surface areas are covered by enzymatic activity (Kitessa et al., 1999). The process in making of corn leaf biscuit may cause change in nutrient content due to heating and pressing technique, so it is necessary to evaluate digestibility of ruminants. Nutrient digestibility can be affected by the age of sheep, feed conversion, and the nutrient content of feed. Although ruminant is able to digest some form of structural carbohydrates cellulose and hemicellulose by using rumen microbes, the changes in nutrient content will affect the digestibility.

The objective of this study was to apply feeding of corn leaf biscuit to enhance growth performance and digestibility of sheep by measuring its feed intake, body weight gain, feed conversion, digestibility of dry mater, organic matter, fiber, neutral detergent fiber and acid detergent fiber of sheep.

MATERIALS AND METHODS

The research was conducted at Laboratory of Feed Industry, Faculty of Animal Science, Bogor Agricultural University, Indonesia on June-October 2010. The experiment used nine heads of thin tail sheep with initial body weight was around 12-18 kg. The experimental sheeps were maintenance individually. Dry matter and crude protein content of feed can be seen in Table 1.

Tabel 1. Nutrient Composition of Corn Leaf and Field Grass (% Dry Mater)

Nutrient	Corn Leaf	Field Grass
Ash (%)*	10.78	10.21
Crude Protein (%)*	16.17	8.20
Fat (%)*	3.24	0.67
Crude Fiber (%)*	20.34	28.57
NFE (%)*	35.76	36.26

*Laboratory Analysis of Feed Science and Technology (2010)

Diagram Process of Biscuit Production

Figure 1 showed that a diagram process of biscuit production from raw material i.e. corn leaf and field grass processed by chopping, mixing, pressing and heating with temperature 95⁰C for 5 minutes to form biscuit and than cooling in room temperature.

Experimental design

The experimental design used in this research was Completely Randomized Design with three treatments and three replications, the treatments were biscuit composition i.e: T1 = 100% field grass, T2 = 50% field grass + 50% corn leaf, T3 = 100% corn leaf. The data method used was Analysis of Variance. The differences among treatments were examined with orthogonal contrast test (Steel and Torrie, 1993). The variables that would be measured were:

- (i) Feed intake was calculated from the difference between the amounts of feed given with the rest of the feed that was not consumed
- (ii) Body weight gain was calculated by:

$$\text{Body weight gain (g/head/day)} = \frac{\text{Final body weight again (g)} - \text{initial body weight gain (g)}}{\text{gain (g)}}$$

(iii) Feed conversion was calculated from feed consumption (kg) divided by body weight again (kg)

(iv) Digestibility of dry matter, digestibility of organic matter, digestibility of crude fiber, digestibility of NDF, and digestibility of ADF were calculated using the formula:

$$\text{Digestibility}(\%) = (A-B) / A \times 100\%$$

Description:

A= the amount consumed (dry matter/organic matter/crude fiber/NDF/ADF/day

B= the amount of feces (dry matter/organic material/crude fiber/NDF/ADF/day.

RESULT

Growth Performance

The application of biscuit feeding for sheep showed that dry matter intake was not significant ($P>0.05$). The dry matter intake of sheep fed with field grass biscuit had dry matter intake was 691 g/head/day. The dry matter intake of sheep fed with 50% field grass+50% corn leaf biscuit had dry matter intake was 817 g/head/day and the dry matter intake of sheep fed with 100% corn leaf biscuit 872 (Table 4).

The body weight gain of sheep fed with field grass biscuit had dry matter intake was 53.33 g/head/day. The body weight gain of sheep fed with 50% field grass+50% corn leaf biscuit had dry matter intake was 64.29 g/head/day and the body weight gain of sheep fed with 100% corn leaf biscuit 70.71 g/head/day (Table 4) and statistically was not significant ($P>0.05$). The average of body weight gain of sheep fed with corn leaf biscuit was 24.58% higher than sheep fed with field grass biscuit.

The results of analysis of variance showed that on feed conversion was not significant differences ($P>0.05$). The feed conversion of sheep fed with 100% field grass biscuit had 12.96. The feed conversion of sheep fed with 50% field grass+50% corn leaf biscuit had dry matter intake was 12.70, and the feed conversion of sheep fed with 100% corn leaf biscuit had dry matter intake was 12.33 (Table 4).

Digestibility

The treatments was not significantly ($P>0.05$) on digestibility of dry matter. The sheep fed with 50% field grass+50% corn leaf had highest digestibility of dry matter among the other treatments was 46.461%, meanwhile sheep fed with 100% corn leaf biscuit had digestibility of dry matter was 45.78% and sheep fed with 100% field grass biscuit had digestibility of dry matter was 44.461% (Table 5).

The treatments were given in this study was not significantly affect ($P>0.05$) on digestibility of organic matter. The sheep fed with field grass biscuit had digestibility of organic matter was 56.670%, meanwhile sheep fed with 50% field grass+50% corn leaf biscuit had dry matter intake was 55.882% and sheep fed with 100% corn leaf biscuit 55.647% (Table 5).

The results of analysis of variance showed that on digestibility of crude fiber was not significant differences ($P>0.05$). The sheep fed with field grass biscuit had digestibility of organic matter was 37.574%, meanwhile sheep fed with 50% field

grass+50% corn leaf biscuit had dry matter intake was 55.882% and sheep fed with 100% corn leaf biscuit 55.647% (Table 5).

The results showed that digestibility of Neutral Detergent Fiber was not significant ($P>0.05$). Digestibility of Neutral Detergent Fiber values derived in this study range between 45.06% - 46.64% (Table 5).

The results showed that digestibility of Acid Detergent Fiber was not significant ($P>0.05$). Digestibility of Acid Detergent Fiber values derived in this study range between 25.15% - 37.45% (Table 5).

DISCUSSION

Growth Performance

The application of biscuit feeding for sheep showed that dry matter intake was not significant ($P>0.05$) since the particle size of biscuit among treatment was not significant ($P>0.05$) (Table 3). The process in making of corn leaf biscuit consists of such stages as chopping, grinding, mixing, pressing and heating. That process can change the particle size of feed and can decreased chewing activity. Fonseca et. al. (2000) stated that reduction of particle size will increase dry matter intake and microbial protein synthesis caused by an increase in the rate of emptying of the rumen.

According to NRC (1985) body weight gain was influenced by several factors, such as the total consumption of protein, sex, age, genetic, environmental, physiological condition of livestock and management. The average of body weight gain of sheep fed with corn leaf biscuit was 70.71 g/head/day or 24.58% higher than sheep fed with field grass biscuit. Body weight gain was high in the sheep fed with corn leaf biscuit caused by high dry matter consumption. Such high feed intake can promote to grow a new cell. Meanwhile, increase in body weight gain was correlated with nutrition content of biscuit feed. Therefore, the high nutrient quality of corn plant biscuit induces sheep grow fast. Nutrient content of field grass biscuit T1 were 10.57% of ash, 13.05% of crude protein, 30.68% of crude fiber, 1.76% of crude fat and 30.19% of NFE, while nutrient content of corn leaf biscuit (T3) were 11.79% of ash, 17.97 of crude protein, 28.20% of crude fiber, 1.76% of crude fat and 40.99% of NFE.

Feed conversion is affected by feed quality, digestibility value, and efficiency. Increase in feed quality will improve body weight gain, so feed conversion value will decrease, meaning that the application of feed is efficient (Pond *et al.*, 1995). Feed conversion depends on dry matter intake and body weight gain. Feeding of corn leaf biscuit was not significant ($P>0.05$) with feeding of field grass biscuit on feed conversion with average of feed conversion value was 12.33 until 12.09. Feed conversion was not significant in this research caused by insignificant increase in body weight gain.

Digestibility

The digestibility is defined as the feed that has been entered into the alimentary tract and feces are not disposed of with, this section is assumed to be absorbed in the body of animal (McDonald *et al.*, 2002). Digestibility of dry matter coefficient values in this study ranges between 44.46% - 46.10% (Table 5). This is because the ration of dry matter consumed by sheep also showed was not significant. Feed consumption is influenced by the digestibility of feed since an increase in the digestibility of feed also improve feed intake. Factors affecting the digestibility are as follows: (i) feed

composition, (ii) digestibility of crude protein and fat, (iii) feed processing, (iv) kind of animal, and (v) feed intake (Tillman *et al.*, 1997).

Digestibility of organic matter is described as the components of proteins, carbohydrates and fats that can be digested by ruminant. The treatments were given in this study also was not significant on digestibility of organic matter ($P > 0.05$). Digestibility of organic matter coefficient values in this study ranges between 55.65% - 56.67% (Table 5). The digestibility of dry matter and organic matter can be influenced by the forage species. The age of forage will affect the fiber content which may impact on the digestibility of forage. Church (1983) also mentions that high digestibility is correlated with an increase in feed intake, due to faster rate of the digestivity.

The results of analysis of variance showed that on digestibility of crude fiber was not significant ($P > 0.05$). Digestibility of crude fiber values obtained in this study ranges between 37.57% - 38.14% (Table 5). Van Soest (1994) stated that crude fiber content will influence the value of digestibility. Digestibility of crude fiber is closely related with the ability of ruminant to produce energy. Crude fiber can be digested about 20% - 70% by ruminant, while the rest is excreted through the feces (Cuthbertson, 1969). Comparing to Cuthbertson's study (1969), the result of this study performs well since the average value of crude fiber digestibility was 30%-80%.

Acid detergent fiber is a component of cell walls consists of cellulose, lignin and silica. Cellulose is the ADF component easily digested, while lignin is difficult to digest because it has a double bond. The coefficient of digestibility will be low, if the feed contains a high lignin. Ruminant have the privilege in digesting plant cell walls or Neutral Detergent Fiber. The plant cell wall is composed mostly of hemicellulose, cellulose, lignin and silica. Hemicellulose and cellulose can be digested by rumen microbes in the relative long time, while lignin and silica can not be digested (Parakkasi, 1999). The results showed that digestibility of Acid Detergent Fiber values derived range between 25.15% - 37.45%, while digestibility of Neutral Detergent Fiber values derived range between 45.06% - 46.64% (Table 5). The results showed that the digestibility of neutral detergent fiber was greater than the digestibility of Acid Detergent Fiber. This is due to hemicellulose content of the NDF is quite large compared to the ADF. Hemicellulose more digestible than NDF because it has a polysaccharide structure consisting of various monomers, whereas most of the hemicellulose of the ADF only consists of one monomer which forms a sort of glucose so it is difficult to digest.

CONCLUSION

Performance sheep by feeding field grass and corn leaf biscuit was not significant effect on dry matter intake, body weight, feed conversion, digestibility of dry matter, organic matter, fiber, neutral detergent fiber and acid detergent fiber. It was concluded that corn leaf could be processed to produce biscuit as a fiber source in ruminant feed.

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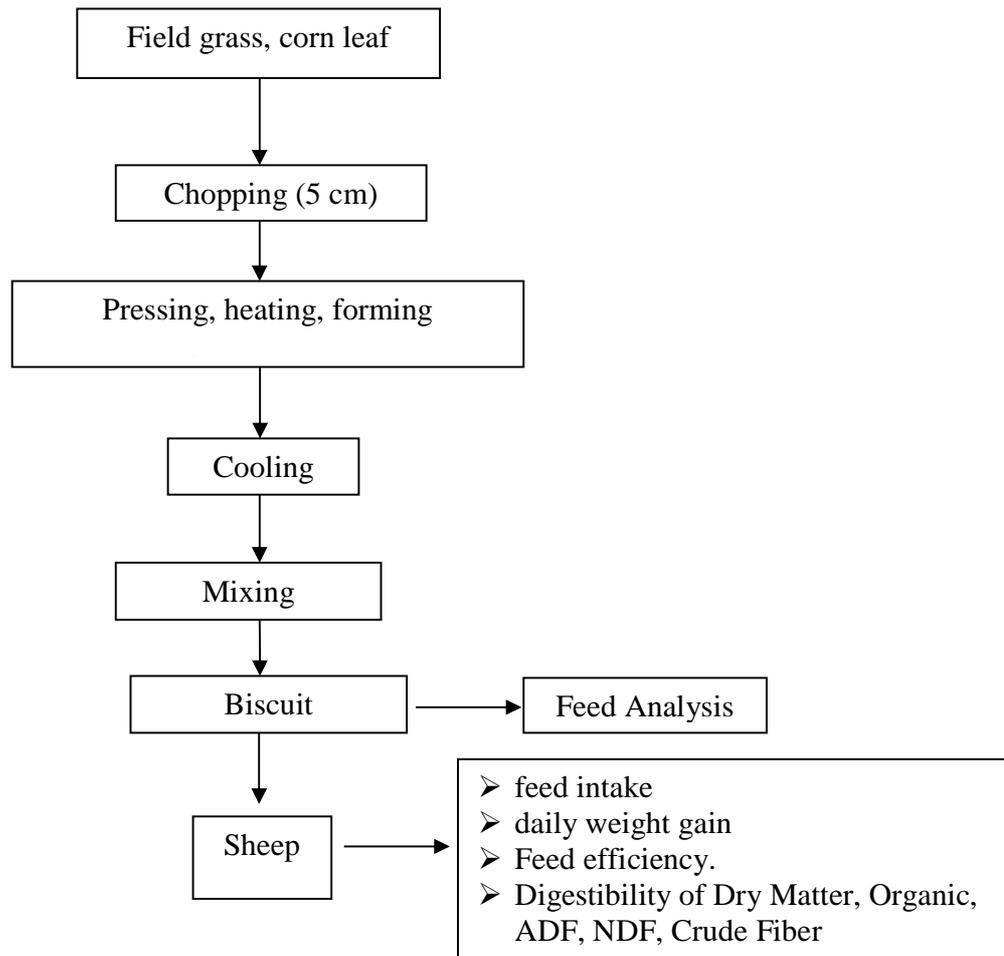


Figure 1. Diagram Process of Biscuit Production

Tabel 1. Nutrient Composition of Corn Leaf and Field Grass (% Dry Mater)

Nutrient	Corn Leaf	Field Grass
Ash (%)*	10.78	10.21
Crude Protein (%)*	16.17	8.20
Fat (%)*	3.24	0.67
Crude Fiber (%)*	20.34	28.57
NFE (%)*	35.76	36.26

*Laboratory Analysis of Feed Science and Technology (2010)

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Tabel 2. Nutrient Composition of Biscuit Corn Leaf and Field Grass (% Dry Mater)

Biscuit	Nutrient (%)				
	Ash (%)	Crude Protein	Crude Fiber	Crude Fat	Nitrogen Free Extract
T1	10.42	12.89	41.34	0.21	35.14
T2	9.79	14.51	31,90	0.20	43.60
T3	8.84	16.12	29.45	1.04	44.56

Description: Laboratory Analysis of Feed Science and Technology, Bogor Agriculture University (2010). Biscuit composition : T1 = 100% field grass; T2 = 50% field grass + 50% corn leaf; T3 = 100% corn leaf.

Table 3. Particle Size of Biscuit Field Grass and Corn Leaf

Variable	Treatment		
	T1	T2	T3
Modulus of Fineness	2.10±0.02	2.42±0.05	2.68±0.05
Modulus of Uniformity (C:M:F)	0 : 3 : 7	0 : 5 : 5	0 : 6 : 4

* Biscuit composition : T1 = 100% field grass; T2 = 50% field grass + 50% corn leaf; T3 = 100% corn leaf.

Table 4. Dry Matter Intake (g/head/day), Body Weight Gain (g/head/day), Feed Conversion

Variables	Treatments*		
	T1	T2	T3
Dry matter intake (g/head/day)**)	691 ± 82	817 ± 97	872 ± 34
Body weight gain (g/head/day)**)	53.33 ± 24.44	64.29 ± 13.57	70.71 ± 19.21
Feed conversion**)	12.96 ± 23.56	12.70 ± 3.32	12.33 ± 4.47

* Biscuit composition : T1 = 100% field grass; T2 = 50% field grass + 50% corn leaf; T3 = 100% corn leaf.

**) Non significant (P>0,05)

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Table 5. Nutrient Digestibility of sheep

Variables	Treatments*)		
	T1	T2	T3
Digestibility of Dry Matter**)	44.461 ± 0.18	46.103 ± 0.15	45.78 ± 1.38
Digestibility of organic matter**)	56.670 ± 0.28	55.822 ± 0.11	55.647 ± 0.78
Digestibility of Crude Fiber**)	37.574 ± 8.77	38.137 ± 2.52	36.687 ± 4.23
Digestibility of ADF**)	25.152 ± 1.28	37.449 ± 2.73	33.530 ± 2.74
Digestibility of NDF**)	46.173 ± 10.28	45.060 ± 3.21	46.644 ± 13.31

*Biscuit composition : T1 = 100% field grass; T2 = 50% field grass + 50% corn leaf ; T3 = 100% corn leaf.

**) Non significant (P>0,05)

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10. EFFECT OF BLACK TEA (*Camelia sinensis*) WASTE ON RUMEN DEGRADATION OF FEED PROTEIN

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Abstract

In order to evaluate the effect of black tea (*Camelia sinensis*) waste (BTW) on rumen protein degradability, soybean meal as protein source was incubated for rumen fermentation using in vitro Tilley and Terry method. Four level of BTW addition were made equal to tannin level of 0, 0.25, 0.5, 0.75 and 1% of soybean dry matter. Ammonia level in the medium of added BTW groups were lower significantly than control although pH was not affected. Protein degradability tends to decreased in line with the increasing of BTW level, and significant decreasing occurred at BTW level equal to tannin 1% ($p < 0.05$). Dry matter and organic matter degradability also declined by BTW addition ($P < 0.05$). This study demonstrated the possibility of black tea waste to be used for protecting feed protein from rumen microbial degradation.

Keywords: Black tea waste, Protein ruminal degradation, Protein protection, Tannin

INTRODUCTION

Ruminant can utilize both protein and non protein nitrogen as nitrogen sources. About 60 to 80% of feed protein are degraded in the rumen to amino acid then fermented to ammonia, whereas non protein nitrogen is rapidly and almost totally degraded (McDonald, 2002). Furthermore amino acid required by ruminant come both from dietary protein that escapes rumen fermentation and microbial protein produced during rumen fermentation.

Even though ammonia in the rumen liquor is the key intermediate in microbial protein synthesis, which is one of protein sources for the animal, degradation of protein is an inefficiency of feed protein utilization. In high production animal, ruminal synthesized protein is not sufficient to cover amino acid requirement (Kaldmäe *et al.* 2006) thus supplementation of rumen undegraded protein is needed.

Several method have been used to protect feed protein from microbial degradation in the rumen, such as non enzymatic browning reaction by gamma irradiation (Sang-He *et al.* (2005), Sang-He *et al.* (2006), Zegita *et al.* (2006) microwave irradiation (Sadeghi and Shawrang (2007) heat treatment (Kaldmäe *et al.* (2006) and chemical treatment by formaldehyde (Gulati *et al.* 2005).

Plant natural bioactives have being extensively explored recently to be used as feed protein protector from rumen microbial degradation, due to the less side effect on animal and human as consumer of animal product. Tannin is plant secondary metabolite, particularly condensed tannin, shows reduction effect on ruminal digestibility of feed protein. Tannin binds protein mainly by hydrogen and hydrophobic bond in a pH reversible manner (Min *et al.* 2003). Formation of bounding occur at near neutral pH and the bounding will dissociated releasedat pH less than 4 and more than 7 (McSweeney, 2001).

Beneficial effects of consuming condensed tannin (CT) by ruminant at low to moderate levels have been shown by several researches. Consuming CT at certain level begins to affect feed intake. The levels are varies depend on chemical nature of tannin and animal species (Rochfort *et al.* 2008). Waziry *et al.* (2005) reported the addition of

tannic acid on soybean meal at levels 1.2 and 3% of DM reduced ammonia concentration in the fermentation liquid as well as the gas production that represent of protein degradability. Goat consuming diet supplemented *Lespedeza cuneata* (content of condensed tannin 17.7%) showed the higher dry matter intake, dry matter digestibility, lower ruminal ammonia N, without affected on VFA production (Puchala *et al.* 2005). Low concentration of CT (20-45 g/kg DM) reduce rumen forage protein degradation and CT from several forage plants e.g. *L. Corniculatus* and sulla have been shown to advantages for ruminant i.e. increased milk production, wool growth, ovulation rate, reducing bloat risk and reducing internal parasites burdens. However high CT more than 55g/kg DM generally reduce feed intake, nutrient digestibility, and decrease of body and wool growth (Min *et al.* 2003)

Black tea waste (BTW) is the byproduct of black tea processing which consist of rejected steams and leaves. The proportion of BTW is 5 to 10% of total material of tea (PPTK Gabung, 2006). Due to BTW tannin content, 4.5%, this material have the potency to be apply as feed component to protect feed protein from extensive degradation in the rumen (Narasari, 2004). Moreover BTW protein content of BTW (27.94%) is higher than protein of *Leucaena leucocephala* (22.73%). BTW have not being used as animal feed. Hence this research objective was to evaluate the effect of BTW on rumen soybean protein degradability. Soybean meal is a commonly used protein supplement for ruminants

MATERIALS AND METHODS

Sample preparation

Black tea waste which used in this research was obtained from Research Center for Tea and Quinine, Gabung, Bandung, West Java, Indonesia. Soybean meal as protein source and BTW were dried at 55°C to get air dry sample and grounded to pass 2 mm screen for chemical analysis including crude protein (CP), dry matter (DM) and organic matter (OM) content and also evaluation of in vitro degradability.

In vitro incubation

Soybean meal samples added BTW which equivalent to tannin level of 0, 0.25, 0.50, 0.75 and 1% of dry matter were incubated on in vitro rumen fermentation according to procedure of 1st stage of Tilley and Terry (1963). Ruminal fluid was collected before feeding from two fistulated adult Ongole crossbreed cattle. Ruminal fluid was transported in the preheated vacuum flask to maintain the temperature and filtered through a double layer of cheese cloth and bubbled with CO₂ before commencement of incubation. Medium consist of ruminal fluid and synthetic saliva (McDougall 1948 cit. Tilley and Terry 1963) in ratio 1:4. Sample (0.5 g) were weighed and put into three 100 ml tubes for each parameter. Three tubes without substrate were included as blanks to correct solid fermentation residues originating from ruminal fluid. Sample and blank tubes then were added 50 ml medium. Inoculated tubes were close with rubber stopper, incubated at 39°C for 48 h. Tubes were manually homogenized every 8 h. At the end of incubation residual feed were separated from fermentation fluid by filtration. Feed residues were dried for dry matter, organic matter and crude protein analysis. Filtrates were collected for determination of pH and NH₃ concentration. Sample for further analysis of NH₃ were preserved with NaCl 10% at ratio of 1:1 and stored at -20°C.

Laboratory Analysis

DM content was measured by dried the sample at 105°C in dry oven (Memert UNB 100-500), OM and CP content was determined according to AOAC (2005). DM, OM and CP content of soybean meal, BTW and residual sample were used to calculate the digestibility of DM, OM and CP. pH of fermentation liquid measured by pH meter (Hanna Instrument HI 5520), whereas NH₃ concentration in the fermentation fluid was determined by phenol-hypochlorite method according to Weatherburn (1987).

Statistic Analysis.

CP, DM and OM digestibility and also pH and NH₃ data were analyzed by one way analysis of variance (ANOVA) continued by Duncan’s Multiple Range Test (DMRT).

RESULT AND DISCUSSION

Addition BTW in soybean meal equal to tannin level 1% reduced protein degradability (P<0.05) while other level 0.25, 0.5, 0.75 were not significantly differ from control (Table 1).

Table 1. Crude protein, dry matter and organic matter in vitro digestibility of soybean meal added different level of black tea waste as tannin source.

Parameters	Tannin level (% DM feed)				
	0	0.25	0.5	0.75	1
Crude protein digestibility (%)	73.03±4.42 ^b	70.41±8.33 ^b	65.60±5.56 ^b	68.11±3.76 ^b	51.32±7.47 ^a
Dry matter digestibility (%)	74.64±2.39 ^c	68.85±3.49 ^b	66.52±1.08 ^{ab}	62.63±0.91 ^a	64.09±3.31 ^{ab}
Organic matter digestibility (%)	76.71±2.53 ^c	71.97±2.95 ^{bc}	68.02±0.95 ^{ab}	64.59±1.18 ^a	65.94±4.93 ^a

^{a,b,c} different superscripts in the same row indicated significant different

The reducing value of protein degradation at BTW level 1% was 29.73% of control value. Animut *et al.* (2008) reported the protein digestibility by Boer goats fed *Kobe lespedeza* was drop from 19.2% to 0.51% meanwhile goat fed *Sericea lespedeza* fall from 21 to 0.28%. *Kobe lespedeza* and *Sericea lespedeza* contain of condensed tannin of 15.1 and 14% respectively. In vitro N disappearance declined linearly with increasing levels of CT extracted from *Leucaena leucocephala* at tannin level 0 (control), 10, 15, 20, 25 and 30 mg/500 mg feed (Tan *et al.* 2011). Feeding forages containing CT has also been reported decreased ruminal protein degradation (Barry and McNabb, 1999; Min *et al.* 2003, Carulla *et al.* 2005). Maximum CT concentration to inhibit proteolysis in vitro rumen without negative effect is about 4% or between 2 -4.5%, CT concentration more than 5.5% DM generally reduces digestibility, feed intake, growth of body and wool. The reducing feed protein degradability in CT present occurred due to reversible binding between tannins and protein that limiting contact of proteolysis microbes to the protein and to reducing population of proteolytic rumen bacteria (Min *et al.* 2003). Patra and Saxena (2009) reported that tannins inhibit protein degrading bacteria in the rumen because ability of tannins to forms complexes with the cell wall and extracellular enzyme. Decreasing protein degradability in this study was appeared at tannin level 1% which was lower than previous studies. The differences level of tannins which affect protein degradability was depending on the chemical structure,

nutrient composition of diets and microbial component in the rumen (Patra and Saxena, 2009).

Digestibility of DM and OM also reduced significantly by BTW addition in the soybean meal rumen fermentation (Table 1). Reduction of DM digestibility appeared at all BTW level, but the reduction of OM digestibility significant at level 0.50% and up. The decreasing value of DM digestibility at tannin level 0.25, 0.50, 0.75 and 1% DM were 7.76, 10.87, 16.72 and 14.13% respectively compared to control. Meanwhile the reductions of OM digestibility respectively were 6.19, 11.33, 15.81 and 14.05%. The decreasing of DM and OM degradability might be related to the decreasing of protein degradability due to the fact that protein is part of the dry matter and organic matter. More over the declining of digestibility of DM and OM were caused by reduction of other component DM and OM digestion including carbohydrate and lipid. Bae *et al.* (1993) resumed that cell associated and extracellular endoglucanase activities of *F. succinogenes* were inhibited by CT (100-400µg/ml in vitro fermentation fluid) from *Lotus corniculatus*. Inclusion of 30% *Calliandra* leaves containing tannins in the diet significantly reduced total cellulolytic bacteria including primary fiber degrading *F. succinogenes* and *Ruminococcus* spp. (McSweeney *et al.* 2001). Inhibition several rumen microbes were resulting reduction overall nutrient. Decreasing of in vitro DM digestibility evidence also has been reported by Tan *et al.* (2011) addition extracted CT from *Leucaena leucocephala* at level 20-30 mg/500 mg feed (equal to 4-7.5% DM) reduced DM digestibility 22–37%, while at a lower CT level of 15 mg (3%), the reduction was only 7%. At the tannin level 1% in this reseach, lower than Tan *et al.* (2011) level reported, the reduction of DM digestibility was greather.i

pH values of the fermentation liquid were not affected by addition of BTW (Table 2). It was range from 7.03 to 7.32. Ammonia concentration significantly declined by addition of BTW (Table 2). Significant decreasing of ammonia concentration occurred at all BTW level compared to control but there were no significant differences amongst treatments. Ammonia concentration ranged from 38.52 to 55 mg/100 ml with the highest value at control group and the lowest at 1% tannins group. This result in agreement with reseach that explored CT respectively from *Kobe lespedeza* and *Sericea lespedeza*(Animut *et al.*2008), CT from *Lespedeza cunneata*(Puchala *et al.* 2005) and extracts of *Plumbago zeylanica*, *Moringa oleifera*, *Picrorhiza kurroa*, and *Terminalia bellerica* (Alexander *et al.* 2008).Ammonia pool in the rumen depends on protein degradation and uptake ammonia by rumen microbe for microbial protein synthesis.

Table 2. Medium pH and ammonia concentration of in vitro rumen fermentation of soybean meal added different level of black tea waste as tannin source.

Parameters	Tannin level (% DM feed)				
	0	0.25	0.50	0.75	1.00
pH	7.21±0.25	7.12±0.20	7.32±0.05	7.30±0.05	7.03±0.19
NH ₃ (mg/100ml)	55.00±5.60 ^b	51.69±2.00 ^a	40.50±2.68 ^a	39.09±3.77 ^a	36.17±5.12 ^a

CONCLUSION

The present study showed that addition of black tea waste equal to 1% tannin of protein source dry matter reduced protein digestibility. Dry matter and organic matter digestibility, as well as ammonia concentration reduced at black tea waste level equal to

tannin concentration 0.25-1%. These result indicated that black tea wastes have possibility to be used as protein protector from microbial rumen degradability.

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11. SUPPLEMENTATION OF SOLID EX-DECANTER MULTI-NUTRIENT BLOCK ON SIMBRAHBREEDWEANED CALVES PERFORMANCES AS INTEGRATED FARMING SYSTEM WITH PALM FRUIT AGROINDUSTRY

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Abstract

The objectives of this study was to evaluate the potential of palm fruit agroindustry by product as feed supplement to increase weaned calves performance of Simbrah Breed. Solid ex-decanter were combined with multi mineral and utilized as feed supplement to form a lick block. Ten weaned calves 6 months old ($122,34 \pm 20,25$ kg) were randomly categorized into 2 groups. Control weaned calves receive no Solid Ex-Decanter Multi-Nutrient Block (SEDMB) supplementation and treated calf were given continuously. The experiment was conducted in July to September and calves fed total mix ratio formulated with palm fruit by product based. Measured parameter were daily weight gain (DWG), body condition score (BCS), feed consumption, feed conversion, *in vivo* dry matter and organic matter digestibility. The treatment were significantly different ($P < 0.05$) on daily weight gain, body condition score, and feed conversion but did not significantly different ($P > 0.05$) on feed consumption, dry matter and organic matter digestibility. SEDMB supplementation on weaned calves performance of Simbrah Breed showed the effectiveness of feed consumption.

Key word: Solid ex-decanter, multi-nutrient block, weaned calves, supplement, palm fruit agroindustry

INTRODUCTION

The world's palm oil production was 36.85 million metric tons, while Indonesian as lead of oil palm producers in the world with an estimated planted area 6.07 million hectares (USDA report, 2007). The oil palm industry offers two opportunities to promote animal production, firstly feed source from oil palm industry by-product, palm press fibre, palm kernel cake and secondly from oil palm plantation, the forages in the inter-rows and oil palm fronds after regular pruning. These are potential feeds source as the yields, palatability and nutritive values are adequate for cattle. The objectives of systematic integration of beef cattle and palm fruit agroindustry are cattle maximized palm fruit agroindustry by product through optimal use of resources and also utilize cattle feces as fertilizer to reduce the use of synthetic fertilizers.

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). Several study shown that integrated farm system between cattle and oil palm plantation under systematic management shows good potential as a livestock production system (Jalaludin, 1997; Wahab, 2003; Devendra and Leng, 2011).

Livestock production in developing countries is largely dependent on fibrous feeds – mainly crop residues and low quality pasture – that are deficient in nitrogen, minerals and vitamins (Makkar, 2007). The advantages of the use of multi-nutritional blocks, in diets based on crop by-products or pastures of typical low quality, are well known in terms of providing adequate non-protein nitrogen in the rumen, improving both function and efficiency, which is reflected in higher voluntary intake by the animal and better digestibility of fibre. Nutrition programs are requirement to minimize use of purchased mixed rations that the cost is generally higher and finally calves get to consume some feed provide protein and minerals. Later calves provide nutrition to promote some gain but maintain health status.

MATERIALS AND METHODS

Ten weaned calves 6 month old (122.1±19.27 kg) were randomly categorized into 2 groups. Control weaned calves receive no Solid Ex-Decanter Multi-Nutrient Block (SEDMB) supplementation and treated calf were given continuously or *ad libitum*. The design used in this study were T test assuming a two-way where if $P < (+/-) 0.05$ is significantly different results, while if $P > (+/-) 0.05$ then the result is not significantly different. The experiment was conducted in July to September 2012 and calf fed total mix ratio formulated with palm fruit by-product based.

Table 1. Ration composition based on palm palm agrindustry by-product

No	Feed ingredients	Composition (%)
1	Native pasture	75
2	Solid ex-decanter	10
3	Palm midrib mill	14.5
4	Palm press fibre	0.5
Total		100

Parameters observed were daily weight gain (DWG), body condition score (BCS), feed consumption, feed conversion, in vivo dry matter and organic matter digestibility. All calves were weighed before feeding on d-0 and at d-7 intervals thereafter. On weigh days, drinkers were turned off and emptied. Number of days on test required to attain the predetermined market weight was recorded. Total ADG was computed as the difference between initial BW and final BW divided by number of days on test. Each morning the feed bunks were observed and adjustments were made in the amount of feed offered daily depending on the amount of residual feed in the feed bunks. Body condition was scored using standard procedures based on a scale of 1 to 9, as describe by Eversole *et al.* (2009). Calves were scored for body condition every 2 weeks.

RESULTS AND DISCUSSION

Daily Weight Gain (DWG)

Beef producers face the challenge of remaining economically viable despite continuously changing paradigms in beef cattle production. Cow calf producers may want to consider early weaning as a management practice when traditional production systems cannot effectively address adverse conditions such as lack of forage, unfavorable market trends, noncompetitive freight rates, and poor weather conditions.

Weaning weight can be increased by genetic (crossbreeding, growth potential) and environmental (creep feeding) manipulations or by age of calf (calving earlier

and[or] weaning later). Production efficiency can be enhanced by using nonharvested forage (Lamb et al., 1996), but nutrient availability in forage can be limiting as forage matures. Nutritional status of cattle grazing mature native range forage can be increased with appropriate supplementation (Kartchner, 1980; Adams et al., 1986; Sanson et al., 1990).

Table 2. Body weight gain of Simbrah crossbred weaned calves on integrated farm system between cattle and palm fruit plantation

Calves	Control			Treatment		
	Initial weight (kg)	Final weight (kg)	Daily weight gain (kg)	Initial weight (kg)	Final weight (kg)	Daily weight gain (kg)
1	87	107	0.408	100	128	0.571
2	110	135	0.510	143	170	0.551
3	119	135	0.326	146	182	0.734
4	125	143	0.367	125	140	0.306
5	116	128	0.244	150	182	0.653
SD	111.4±11.9	129.9±11.2	0.37±0.08	132.8±16.8	160.4±20.3	0.563±0.13*

* = significant difference (P<0.05), ns = not significant difference (P>0.05)

SD = Standar deviation

Test results of daily weight gain of weaned calves after supplemented by solid ex-decanter multi-nutrient block (SEDMB) were statistically significant (P=0.026). Based on Table 2 (statistical test) that SEDMB gives higher average daily weight gain (0.563±0.13 kg) than control (0.37±0.08 kg). Leadley and Sodja (2003) reported that rates of weight gain up through weaning are lots of variations. The lowest rate of gain reported was 0.7 pounds (0.317 kg) per day for 42days. The highest rate was 1.3 pounds (0.589 kg) per day for 56 days. Fluhartyet. al. (2000) also reported about daily weight gain of weaned calves after 36-70 day after weaning ± 0.8 kg/day on normally weaned (205 day old).

Body Condition Score (BCS)

Body condition scores (BCS) are numbers used to suggest the relative fatness or body composition of the cow. For BCS to be most helpful, producers need to calibrate the system under their own conditions with their own cattle. A nine-grade system is commonly used by researchers in the United States. Body condition was scored using standard procedures basen on a scale of 1 to 9, with a score of 1 representing very thin body condition and 9 extreme fatness (Eversoleet al., 2009).

SEDMB supplementation on weaned calves were significantly different (P<0.05) than control (Table 3). BCS obtained were in line with increase of daily weight gain of Simbah breed weaned calves. Fluhartyet. al. (2000) reported body condition score of weaned calves with normally weaned (205 day old) were 4.4 after 99 day weaned. This score were lower than calves with early weaned (100 day old).

Body condition at weaning also is related to reproductive performance. Bowman and Sowel (1998) reporting nine-year summary of data from more than 77,000 cows clearly shows that cows that are thin at weaning are less likely to become pregnant during the following breeding season. Mathis et. al. (2002) suggested that every beef operation is different, and producers using BCS as a tool should set BCS targets based

on their willingness to assume risk. It probably is most effective to sort out thin cows at weaning and provide them with additional energy directly after weaning when their requirements are low.

Table 3. Average Body Condition Score, Feed Consumption and Feed Conversion of Simbrah Breed on integrated farm system between cattle and oil palm plantation

Measured Parameters	Control	Treatment	P-Value
Body Condition Score	3 ^a	3.75 ^a	0.00021*
Feed Consumption, kg	12.71	12.44	0.294 ^{ns}
Feed Conversion	35.22	23.39	0.042*

* = significant difference (P<0.05), ns = not significant difference (P>0.05)

^a = Body condition score: 1 = severely emaciated, 2 = emaciated, 3 = very thin, 4 = thin, 5 = moderate, 6 = good, 7 = very good, 8 = obese, 9 = very obese.

Feed Consumption and Feed Conversion

The key variables affecting the profitability of feedlots are: store cattle purchase price; finished cattle sale price; cost of feed consumed; and liveweight gain. This fact sheet deals with feed consumption and liveweight gain.

Based on Table 3, shows that the level of consumption on treated weaned calves were not significantly different (P=0.294). Control calves were higher average feed consumption (12.71 kg per day). Although the average feed consumption of treated calves are lower than control, the daily weight gain of treated weaned calves shows higher value (Table 2) and gives significantly effect (statistical test). SEDMB supplementation on weaned calves also affecting feed conversion. Higher feed conversion (Table 3) shows on control weaned calves (35.22) than treated weaned calves (23.39). T test results shows a significantly different (P=0.042). It suggest that solid ex-decanter multi-nutrient block gives feed efficiency. Calves will instinctively lick a solid ex-decanter multi-nutrient block if felt needed mineral intake.

Dry Matter and Organic Matter Digestibility

Apparent digestibility is a naturally feed digestibility occurring in the digestive tract in the body of the ruminant livestock. The process of digestion occurs in the rumen were assisted by microorganisms in it. Digestion by microorganisms also performed by enzymatic that the enzyme produced by the cells of microorganisms in the rumen (Tillman et.al. 1991).

Commodities used in feedlot rations vary considerably in dry matter content (DM). Hay and grain are approximately 90% DM, molasses 75% DM and silage 40% DM. A basic guide for estimating dry matter consumption of feedlot animals is to calculate 2.7% to 3.0% of their liveweight (in kilograms). Therefore, an animal consuming a grain based diet of 90% DM, would have an estimated intake of fresh feed between 3.0% (2.7% x100/90) and 3.33% (3.0% x100/90) of their live weight. The dry matter content of a ration refers to the amount of dry material available in a given ration. A number of factors influence the average daily dry matter consumption of lot-fed cattle. These include, liveweight (their required maintenance energy requirements),

body condition, energy concentration of the ration, health status, and ration palatability (Sarah, 2012).

Table 4. *In vivo* Dry Matter and Organic Matter Digestibility

Measured Parameters	Control	Treatment	P-Value
Average Dry Matter Digestibility, %	76.92	74.16	0.095 ^{ns}
Average Organic Matter Digestibility, %	63.39	64.48	0.296 ^{ns}

* = significant difference (P<0.05), ns = not significant difference (P>0.05)

Based on the data in Table 4, it shows that the treatment were not statistically different (P>0.05) on dry matter and organic matter digestibility. Dry matter digestibility of control weaned calves were higher than treated weaned calves. However, organic matter digestibility of weaned calves supplemented with SEDMB were higher even the statistical test were not significantly different (P>0.05). It suggests that SEDMB supplementation promotes rumen microbial activity to reach optimal digestibility with lower dry matter intake.

CONCLUSION

Based on the results of the study it can be concluded that the supplementation of solid ex-decanter multi-nutrient block as a libitum can improve weaned calves performance. The treatment were significantly different (P<0.05) on daily weight gain, body condition score, and feed conversion. However, solid ex-decanter multi-nutrient block did not affect the apparent digestibility of the ration.

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01. PRODUCTIVITY OF LOCAL CHICKEN IN GROWTH PERIODS AND CARCASS CHARACTERICS BY INCLUSION OF *MORINGA* 02. *OLEIFERA* LEAVES MEALSIN THE DIETS

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Abstract

Chicken growth is influenced by genetic and environmental factors. One of the major environmental factors that play a role in supporting the maximal growth is feed. Availability of feed stuffs in animal industry has always been a problem due to the limited and fluctuating prices of the feed, while there is a local feed available, but has not been used optimally due to lack of information about the use of such materials in poultry feed. *Moringa oleifera* leaves are one type of plant product that can be used as an ingredient in chicken feed. This study aims to assess the productivity of local chickens in growth phase with the use of *Moringa* leaves in the diets. The experiment was designed using completely randomized design with 4 treatments and 5 replications. Treatment consists of: R0=basal diet (without the use of *Moringa* leaves meals), R1=used of 5% *Moringa* leaves meals in basal diets, R2= used of 10% *Moringa* leaves meals in basal diets, R3= used of 15% *Moringa* leaves meals in basal diets. Variables were observed as performance (weight gain, protein and energy consumption, feed efficiency), and carcass yield (slaughter weight, carcass, abdominal fat, skin color index). Results of the experiment were shown no significant effects on weight gain, protein consumption, feed efficiency, slaughter weight, and carcass characteristics. However, it was found highly significant ($P < 0,01$) effects on energy consumption, and abdominal fat. The conclusion of this experiment was found the inclusion of *Moringa* leaves meals in the diets of local chickens can reduce energy consumption and abdominal fat pad and increase growth performance and carcass.

Keywords: local chickens, *moringa oleifera*, growth, carcass

INTRODUCTION

Diets is a primary requirement of poultry farms, it's took costs for about 70% of the total cost of poultry production (Amrullah, 2003). The price of feed ingredients will determine the cost of production. In addition to the price of feed and nutrient value also play an important role to optimize the productivity of an animal. A good nutrient value of feed corresponding with optimal genetic potential. Meanwhile, there are still some poultry feed stuffs are still imported at high prices. Ma'sum (2011) reported that the imported of poultry feed ingredients such as corn in 2011 (1.43 million tons) and soybean meal (1.07 million tons). To reduce the cost of production, raw materials of the diets needed are fairly inexpensive and easy to obtain with sufficient nutrient value. *Moringa* plant is one of the local plants in Central Sulawesi, it has leaves that used for local food and possibility for poultry feed. *Moringa oleifera*, is a member of *Moringaceae* family with 12 deciduous tree species native to semi-arid habitats (Mabberley, 1997). *Moringa oleifera* is a small, fast-growing evergreen or deciduous tree that usually grows up to 10 or 12 m in height. It has a spreading, open crown of

drooping, fragile branches, feathery foliage of tripinnate leaves, and thick, corky, whitish bark. The leaves, rich in vitamin A and C, are considered useful in scurvy and respiratory ailments; they are also used as an emetic. The juice extracted from the leaves has strong antibacterial and antimalarial properties (Gbeassor and Meddjagni, 1990). Luthfiya (2005) reported that the nutrient content of *Moringa* leaves has a crude protein (22%), fat (2.3%), crude fiber (19.2%), carbohydrate (53.5%), ash (7.6%), and calories 2005 kcal / kg. Results of research on the use of *Moringa* leaves powder in poultry rations has been reported by Labaso (1987), which states that the use of *Moringa* leaves powder 12% in broiler rations resulted in good performance and carcass quality. Additionally, Rosdiana (2011) reported that the use of 6% *Moringa* leaves powder in the diets of arab chicken give yolk color index yellowness. This study was designed to investigate the possible effects of *Moringa oleifera* leaves meals in the diets on the overall growth performace and carcass characteristics of local chicken.

MATERIALS AND METHODS

In this study were raised 60 local chickens (*Buras Super Strain*) with 7-day old. Its were allocated to four experimental diets in a Completely Randomized Design with 4 treatments and 5 replicates. The treatments were R_0 = basal diets; R_1 = 95% basal diets + 5% *Moringa oleifera* meals; R_2 = 90% basal diets + 10% *Moringa oleifera* meals; R_3 = 85% basal diets + 15% *Moringa oleifera* meals. The basal diets consist of yellow corn grain, fish meals, soybean grain, ricebrand, mineral mix, and *moringa oleifera* meals. The chemical compounds of *moringa oleifera* meals as crude protein (22%), crude fiber (19.2%), crude fat (2.3%), and ME (2005 kcal/kg). The composition of basal diets as shown in Table 1 and chemical composition of treatment diets in Table 2. All the diets were made up with essential ingredients such that supplied the required nutrients of the local chicken recommended by Yuwanta (2007) with the range 20-22% of protein and ME 2900-3000 kcal.

Feed and water were given *ad libitum*. The birds were reared in the plot with the sawdush litter and size 60x60x75cm, and put 3 birds for each plot during 14 weeks. Performance parameter were observe weekly. At the end of the experiment, two chicken for each plot were weight individually prior to slaughter. After slaughter, feather were removed by dipping the chicken in to the warm water (app. 60-70 °C). Carcass yield was weight of the dead chicken without feathers, head, neck, legs, and digestive organs. The chickens were cut in to the parts according to the standard procedure of dissection (Jensen, 1989). Variables determined were growth performance (weigh gain, protein intake, energy intake, and feed efficiency) and carcass yields (slaughter weight, carcass percentage, carcass component (breast meat, drumstick, thigh, back, wings), and abdominal fat pad. Abdominal fat can be defined as the fat surrounding the gizzard and lay between the abdominal muscles and the intestines. Data for all variables were subjected to an analysis of variance, based on designed experimental used (Steel and Torrie, 1991). The treatment means with significant differences were compared using Least Significant Different (Hanafiah, 2005).

Table 1. Composition of Basal Diets

Basal diets	Composition (%)
Yellow corn grain	49,5
Ricebrand	19
Soybean grain	16
Fishmeal	15
Mineral mix	0,5
Total	100
Nutrien composition:	
Protein, %	21,44
ME, kcal/kg	3011
Fat, %	6,51
Crude Fiber,%	4,83

Table 2. Chemical Composition of Treatment Diets

Components	Diets Treatments			
	R ₀	R ₁	R ₂	R ₃
Basal Diets, %	100	95	90	85
<i>Moringa olievera</i> meals, %	0	5	10	15
Total	100	100	100	100
Chemical Composition:				
Crude Protein,%	21.45	21.47	21.50	21.53
ME, kcal/kg	3011	2960	2910	2860
Crude Fiber, %	4.83	5.54	6.26	6.98
Crude fat, %	6.51	6.29	6.08	5.87

RESULTS AND DISCUSSION

Growth Performance

The effects of *Moringa oliefera* meals in the diets on growth performance (weigh gain, protein intake, energy intake, and feed efficiency) during the experiment as shown in Table 3.

Table 3. Growth Performance (weigh gain, protein and energy intake, feed efficiency) per bird during experiment

Variables	Diet Treatments				P value
	R ₀	R ₁	R ₂	R ₃	
Weigh gain, g	1039.40	1086.80	1072.00	1043.00	0.13 ^{ns}
Protein intake, g	916.42	912.24	911.40	901.59	0.63 ^{ns}
Energy intake, MJ	54.96 ^a	53.74 ^b	52.78 ^c	51.35 ^d	10.94 ^{**}
Feed efficiency	0.24	0.25	0.25	0.24	0.89 ^{ns}

^{a,b,c,d} Means in each column with different superscripts are significantly different (P>0.05)

Results of variance analysis showed that the treatment effect was not significant on weight gain, protein intake, and feed efficiency during the study. This was caused by the nutrient content of the ration was relatively similar for each treatment. *Moringa* leaves has nutrient content in high quality, caused the diets that inclusion of *Moringa* leaves provide

a good influence on the metabolism of nutrients that have an impact on weight gain, although was not significant effects. However, the treatment was affected in a highly significant ($P < 0.01$) on energy consumption. The higher the level of use of *Moringa* leaves in the diet tends to decrease energy consumption, but did not affect feed efficiency. Standard nutrient needs, especially metabolic energy, depending on the ambient temperature. Mechanisms of adaptation to environmental temperature can be observed from the increase and reduced of feed intake. It was caused by thermodynamic mechanisms which control the energy intake to maintain the stability of body temperature. Azma and Azahan (2011), states that the efficiency of energy utilization in poultry varied, it was very closely related to environmental conditions. In this study, it appears that the inclusion of *Moringa* leaves meals in the ration was affected the energy consumption in high significant effects but not to affect the consumption of protein. Hertiandryani (2011) stated that the inclusion of *Moringa oleifera* leaves meal 10% in broiler diets had no negative effect on growth performance, income over feed cost, and carcass weight. *Moringa* leaves was a supplement that has a high composition of vitamins A, B, mineral and protein (Vietmeyer, 1996), when it was added to broiler feed can be promote the growth rate (Labaso, 1987).

Carcass Characteristics

The carcass characteristics results of the experiment for each treatment are presented in Table 4.

Table 4. The average of carcass yield for each treatment

Variables	Diet Treatments				P value
	R ₀	R ₁	R ₂	R ₃	
Slaughter weight, g	1130.8	1120	1119.1	1129.7	0.03 ^{ns}
Carcass, %	64.40	64.22	64.64	63.93	0.22 ^{ns}
Carcass Components:					
Breast meat, %	25.52	25.73	25.91	25.85	1.49 ^{ns}
Drumstick, %	16.75	16.85	16.71	17.04	1.16 ^{ns}
Thigh, %	16.82	16.40	16.48	16.76	1.43 ^{ns}
Back, %	24.83	24.95	25.12	25.00	2.68 ^{ns}
Wings, %	16.09	16.07	15.79	15.65	1.89 ^{ns}
Abdominal Fat Pad, %	0.22 ^a	0.18 ^b	0.17 ^c	0.14 ^d	12.95 ^{**}

^{a,b,c,d} Means in each column with different superscripts are significantly different ($P > 0.05$)

Results of variance analysis was shown no significant effect on slaughter weight, carcass percentage, and carcass components. However was found highly significant ($P < 0.01$) effect on abdominal fat pad content. The percentage of breast meat yield was improved by increasing the level of *Moringa oleifera* meals in the diet, although not in a significant level. In this study, the ranges of carcass (63.93-64.64%), breast meat (25.52-25.91%), and abdominal fat pad (0.14-0.22%). This result closed to Lippens et al. (2000), found that 26.5% of breast meat in broiler chicken based on carcass weight, and Selle et al. (2003), reported the composition of breast meat 24% based on carcass weight of broiler. However, this experiment resulted more high carcass and breast meat compared to Sarjono (2008) were carcass percentage 62.07-63.20% and breast meat 24.11-28.44%. The high level of *Moringa oleifera* meals in the diet was reduced the abdominal fat pad (0.14-0.22%). Panda et al. (2005) was reported the abdominal fat pad 1.36% in broiler chicken.

CONCLUSION

The conclusion of this experiment show that the positive effect of inclusion *Moringa oleifera* leaves meals in the diets, it's may improved of the productivity of local chickens included of growth performance and carcass characteristics, although not in a significant effect. This suggest that more research is needed, particularly under practical condition. Future research in this area should focus on the attempt to obtain the maximum level, and the quality of carcass and meat of the chicken.

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02. THE EFFECT OF PALM KERNEL MEAL CONTAINING PROBIOTIC TO REDUCE THE FECAL AMMONIA EMISSION IN THE LAYING HOUSE

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Abstract

The aim of these experiments were conducted to study the effect of direct application of the palm kernel meal containing probiotic in the feces and in the laying diets to reduce the fecal ammonia emission. The experiments were conducted in two phases. Palm kernel meal as sources of prebiotic with six culture of probiotic bacteria such as *B. subtilis*, *B. cereus*, *B. thuringiensis*, *L. acidophillus*, *L. bugaricus* and *S. thermophilus* were used in the first experiment and three culture bacteria such as *B. cereus*, *L. acidophillus*, *L. bugaricus* were used in the second experiment in the laying diets. In the first experiment, for each treatment-replication, 50 g of fecal material were collected from the poultry farm and placed in 400 ml beakers. The fecal sample was then treated with 2%, 4%, 6%, 8% containing probiotic and prebiotic (*symbiotic*) and covered with plastic wraps. The volatile ammonia and excreta pH were measured after twenty four hours of standing and repeated at 48 hr. In the second experiment, 180 laying hens were used and the palm kernel meal containing probiotic were applied 0%, 10%, 20% and 30% in the laying diets. Similar procedure were applied in the second experiment for ammonia measurement. Ammonia emissions were measured using Kitagawa Toxic Gas Detector (Kitagawa, aspirating Gas Pump AP-20). Experimental design used was Complete Randomized Design (CRD) consisting of five treatments with five replicates in the first experiment and four treatments with five replications in the second experiment. The result of the first experiment indicated that the palm kernel meal containing of six bacterial cultures and three of best combination significantly ($P < 0,05$) reduced the fecal ammonia, excreta pH and moisture content. The result in the second experiment showed that the increasing palm kernel meal containing probiotic to 30% in the diets were significantly ($P < 0,05$) increased the *Lactobacillus spp.* counts and reduced the ammonia emission and excreta pH of fresh fecal. The conclusion of these experiments were that the direct application the palm kernel meal containing of probiotic in the fresh fecal and application in the laying diets were effective to reduce ammonia emission in the laying house.

Key Word: Probiotic, Prebiotic, Palm kernel meal, Ammonia, Laying hens

INTRODUCTION

Ammonia is the aerial pollutant from livestock operations, with domestic animals being the largest global contributor of atmospheric NH_3 emissions (Aneja et. al., 2006) and poultry (including laying hens) being the largest contributor among domestic animal in the United State (EPA, 2004). Ammonia is noxious gas that is produced by the microbial breakdown of uric acid in poultry manure. The deleterious effects of ammonia on chicken health, productivity and malodor problem in housing are well documented. Therefore, controlling ammonia in poultry house is essential to ensure a better environment, better health, performance of the laying hens and operators. The United Egg Producer 2006 Animal Husbandry Guidelines stated that atmospheric

concentrations of NH_3 should ideally be below 25 ppm and should not exceed 50 ppm (United Egg Producers, 2006).

Coufal et. al. 2006 mentioned that regarding ammonia (NH_3) concentrations and ammonia emission within poultry housing and poultry operation, and subsequent negative environmental effects of excessive ammonia emission have emphasized the need for research to find ways to reduce the volatilization of ammonia from poultry housing.

Several type probiotics have been used either in the fecal or poultry diets to reduce urease activity in poultry intestines, which in turn may reduced fecal ammonia emission. Yusrizal and Aziz, 2010, reported that use a combination of bacteria *B. cereus*, *B. Thuriengiensis*, *S. Thermophilus*, *L. Acidophilus* (acid bacteria) and *L. Bulgaricus*, *B. Subtilis* were significantly ($P < 0.05$) reduced fecal ammonia during 24 hours of incubation at room temperature. Unfortunately, ammonia emission after 24 hours of incubation tended to increase drastically. This probably because of the probiotic bacteria count was declined rapidly, so It was not effective anymore to produce acid and antibiotics to suppress gram-negative bacteria playing important role in producing ammonia. To extend the effectiveness of ammonia reduction, it is necessary the probiotic to be supported by prebiotic as a source of energy and nutrients, so it will increase the microbial life and total microbial count. This condition is known as symbiotic, a combination of probiotics and prebiotics (Collins and Gibson, 1989; Schrezenmeir and De Vrese, 2001). This combination may increase the livability of probiotic organism, because its specific substrate available for fermentation.

Palm kernel meal had a potential to be used as a prebiotic (colonic food). This is due to palm kernel meal contains mannan oligosaccharide that serves as a prebiotic (a source of energy and nutrients of probiotic). Daud and Jarvis (1992) reported that the mannose content (mannan-oligosaccharides) of palm kernel meal reached 56.4% of total cell wall. Palm kernel meal containing mannan oligosaccharide that was fermented by probiotic (symbiotic) and applied directly either in the fecal or used in the laying diet were expected to prolong the survival of probiotic bacteria in the fecal, so It will extend the effectiveness of the ammonia reduction of poultry feces in the laying house.

MATERIAL AND METHODS

A. Experimental

Two experiment were conducted in these studies. Six cultures of probiotic bacteria were used in the first experiment. *L. Acidophilus*, *S. thermophilus* (acid producing bacteria), *B. cereus*, *B. thuringiensis* (proteolytics' bacteria) and *L. Bulgaricus* *B. subtilis* (Antibiotic bacteria). Palm kernel meal as source of prebiotic were fermented with those bacteria (probiotic) to produce palm kernel meal containing probiotic (symbiotic fermentation). Those symbiotic fermentation either individually or combination were applied to the laying fecal. Fresh fecal was collected from laying house. For each treatment, five replications were made, 50 g of fecal material were collected from the laying hens and put into 400 ml beakers and covered with plastic wraps. The following treatments were assigned as follows: 1) Control feces, 2) Control feces + 2% symbiotic (w/w) 3) Control feces + 4% symbiotic (w/w), 4). Control feces + 6% symbiotic (w/w), 5). Control feces + 8% symbiotic (w/w). The volatile ammonia emission were measured on 24 hr and 48 hr on the same beakers. Fecal pH was also

measured on 24 hr and 48 hr. The beakers were incubated at room temperature prior to the first measurement of ammonia on 24 hr and for the second measurement on 48 hr.

In the second experiment, One-hundred and eighty 52-wk laying hens were allotted in 180 cages (1 bird/cages). Four level diets were assigned randomly in 9 cages each. Palm kernel meal containing probiotic were applied 0%, 10%, 20% and 30% in the laying diets. Diets were isocaloric (2,674 to 2,704 kcal/kg of ME) and iso protein (17.10 to 17.67% CP) and were fed for 6 weeks. Hens were randomly assigned to one of the following treatments:

P-0= Control feed, diets containing 0% symbiotic fermentation,

P-10= Diets containing 10% symbiotic fermentation,

P-20= Diets containing 20% symbiotic fermentation,

P-30= Diets containing 30% symbiotic fermentation.

Laying hens were housed in a open side of laying house. During the experiment laying hens had free access (*ad libitum*) to drinking water and dietary treatment (Table 1).

B. Analysis

1. Volatile ammonia.

Faecal material ammonia emissions were measured by using Kitagawa Toxic Gas Detector (Kitagawa, aspirating Gas Pump AP-20) and ammonia detecting tubes (0.2 to 2.0/260 ppm capacity, Komyo Rikagaku Kogyo KK). The detecting tubes were broken at both ends by using a tip cutter and inserted into the detector. One hundred milliliter air samples were used for each measurement. Fifty grams of each laying feces were collected from laying house and placed into a 400 ml beaker and covered with plastic wrap. The beakers were incubated at room temperature prior to the during ammonia measurement. The volatile ammonia contents were measured after 24 hr and then were repeated at 48 hr.

2. Fecal pH.

The pH of fecal content was measured by using a pH meter (Hanna). Fifty grams of each laying-faecal material were collected and placed into a 400 ml beaker and brought to the lab for pH measurement, just right after ammonia measurement. The pH was measured after 24 hr and repeated at 48 hr.

3. Fecal moisture content.

Faecal moisture contents were determined according to the method as described in AOAC (AOAC, 2005). Two grams of laying-faecal material were measured into aluminum dishes and dried in an oven at 103 C for at least 3 hr. Readings were taken after samples were cooled in a desiccators. The moisture content was measured after one hour of standing 24 hr and repeated at 48 hr.

Tabel 1. Ingredient and formulated nutrient composition of the basal diets (0 PKM containing probiotic), (10% PKM containing probiotic), (20% PKM containing probiotic), (30% PKM containing probiotic)

Ingredient (%)	Dietary Treatment*			
	P 0	P10	P 20	P 30
Yellow Corn	46.50	45.00	41.00	38.00
Rice bran	7.00	7.00	5.00	3.00
Comercial feed	24.50	24.00	21.80	18.50
Fish meal	8.00	9.50	8.00	7.00
Coconut kernel meal	9.00	0.00	0.00	0.00
Vitamin-mineral premix	3.00	3.00	3.00	3.00
Palm oil	2.00	1.50	1.20	0.50
Palm kernel meal (containing probiotic)	0.00	10.00	20.00	30.00
Total	100.00	100.00	100.00	100.00
Calculated nutrient ^a				
Dry matter, %	84.90	85.62	85.82	86.34
Total ash, %	16.73	16.77	15,55	14,09
Crude protein,%	17,39	17.67	17.39	17.10
Crude fiber,%	3.99	5.29	5.83	6.38
Extract ether, %	2.98	2.64	2.81	2.99
Calcium, %	4.79	4.94	4.59	4.17
Pospor, %	0.12	1.36	1.28	1.21
Gross energy (GE) Kcal/kg	3,688.00	3,729.00	3,711.00	3,681.00
Metabolism energy (ME) kcal/kg ^b	2674.00	2,704.00	2,690.00	2,669.00

Ket:* Calculated Analysis.

Note : a. Laboratory analysis, Faculty of Animal Science, Jambi University, 2012.

b. ME=0,725xGE (NRC, 1994)

RESULTS AND DISCUSSION

The effect of palm kernel meal containing probiotic *Lactobacillus acidophilus* and *Streptococcus thermophilus* on the volatile ammonia, pH and moisture content of fecal layer.

Generally, the direct application of palm kernel meal containing probiotic to fecal layer, reduced the ammonia emission either in individual or combination of probiotics. The ammonia emission was significantly affected ($P < 0.05$) by direct application of the palm kernel meal containing probiotic. The fecal pH and moisture content also were influenced significantly ($P < 0.05$) by direct application of the palm kernel meal containing probiotic.

It was showed that the application palm kernel meal containing probiotic of acid-producing bacteria (*Lactobacillus acidophilus*) on fecal layer was more effective in reducing ammonia emission than the bacteria *Streptococcus thermophilus*. The data showed that the effectiveness of ammonia reduction on average was 37.15% (*Lactobacillus acidophilus*) compared to 33.26% (*Streptococcus thermophilus*). (Table

2) This probably was due to the availability substrates of mannan oligosaccharide (prebiotic) coming from palm kernel meal combined with probiotic of *Lactobacillus acidophilus* resulted in higher acid producing and low pH of fecal broiler. So the amount of availability H⁺ ions was sufficient. The H⁺ ions in the fecal layer be able to convert ammonia into ammonium (NH₄⁺), so that the emission of ammonia could be prevented. Mobley and Hausinger, (1989) reported that pH feces is a major determinant of the rate and extent of NH₃ volatilization from animal waste because lower manure pH shift the NH₃ equilibrium toward NH₄⁺ which more water soluble and therefore less volatile than ammonia (NH₃).

Table 2. Volatile ammonia (ppm), pH and Moisture Content of fecal layer as effected by direct application of palm kernel meal containing probiotic *Lactobacillus acidophilus* and *Streptococcus thermophilus*

Spesies	Level	Ammonia	Ammonia	Ammonia	Ammonia	pH	pH	Moisture	Moisture
		(ppm)	(ppm)	Reduction (%)	Reduction (%)	24 hr	48 hr	Content (%)	Content (%)
<i>Lactobacillus acidophilus</i>	0%	452.00 ^a	1072.00 ^a			8.64 ^a	8.48 ^a	70.14 ^a	71.37 ^a
	2%	352.00 ^b	756.00 ^b	22,12	29.48	8.62 ^a	8.46 ^a	69.15 ^{ab}	70.73 ^{ab}
	4%	328.00 ^b	592.00 ^c	27,43	44.78	8.31 ^b	8.31 ^{bc}	67.69 ^{cb}	69.41 ^{bc}
	6%	336.05 ^b	648.00 ^c	25.66	39.55	8.40 ^b	8.42 ^{cb}	66.74 ^c	69.23 ^{bc}
	8%	216.00 ^c	472.00 ^d	52.21	55.97	8.30 ^b	8.26 ^c	66.44 ^c	68.24 ^c
					31.86	42.44			
		Avg. Ammonia Reduction (%)		37.15					
<i>Streptococcus thermophilus</i>	0%	456.00 ^a	1055.00 ^a			8.78 ^a	8.80 ^a	70.86 ^a	71.87 ^a
	2%	352.00 ^b	800.00 ^b	22.81	24.24	8.62 ^b	8.40 ^b	69.69 ^b	71.85 ^a
	4%	340.00 ^b	624.00 ^c	25.44	40.91	8.46 ^c	8.46 ^b	69.37 ^b	71.60 ^a
	6%	340.00 ^b	624.00 ^c	29.82	40.91	8.28 ^d	8.26 ^c	67.95 ^c	70.71 ^{ab}
	8%	272.00 ^c	616.00 ^c	40.35	41.67	8.28 ^d	8.24 ^c	67.29 ^c	69.75 ^b
					29.61	36.93			
		Avg. Ammonia Reduction (%)		33.26					

Note: Means in the same row with different superscript differ significantly (P<0.05).

The effect of direct application of palm kernel meal containing probiotic *Bacillus thuringiensis* and *Bacillus cereus* on the volatile ammonia, pH and moisture content of fecal layer.

In the Table 3, for a group of proteolytic bacteria showed that the palm kernel meal containing probiotic *Bacillus cereus* was more effective than *Bacillus thuringiensis* in reducing ammonia. It showed that a higher of the effectiveness of ammonia reduction was 27.72% (*Bacillus cereus*) compared to 16.79% (*Bacillus thuringiensis*). Beside the low of fecal pH, the higher of the reduction of ammonia probably was because of the *Bacillus cereus* consumed the uric acid as a nutritional substance. It resulted in a limited availability of uric acid to be converted into ammonia.

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Tabel 3. Volatile ammonia (ppm), pH and Moisture Content of fecal layer as effected by direct application of palm kernel meal containing probiotic of *Bacillus thuringiensis* and *Bacillus cereus*

Spesies	Level	Ammonia	Ammonia	Ammonia	Ammonia	pH	pH	Moisture	Moisture
		(ppm)	(ppm)	Reduction (%)	Reduction (%)	24 hr	48 hr	Content (%)	Content (%)
<i>Bacillus thuringiensis</i>	0%	512.00 ^a	1088.00 ^a			8.63 ^a	8.92 ^a	72.59 ^a	73.76 ^a
	2%	488.00 ^a	1056.00 ^a	4.68	2.04	8.56 ^a	8.68 ^b	71.14 ^b	73.30 ^{ab}
	4%	415.00 ^b	1056.00 ^a	18.75	2.04	8.54 ^a	8.64 ^b	69.41 ^c	72.35 ^{bc}
	6%	356.00 ^c	864.00 ^b	30.47	18.37	8.36 ^b	8.50 ^c	68.85 ^c	71.60 ^b
	8%	336.60 ^c	824.00 ^b	34.38	23.56	8.30 ^b	8.42 ^c	67.76 ^d	70.10 ^d
					22.07	11.50			
		Avg Ammonia Reduction (%)		16.79					
<i>Bacillus cereus</i>	0%	472.00 ^a	1616.00 ^a			8.46 ^a	8.98 ^a	72.89 ^a	73.25 ^a
	2%	400.00 ^b	1344.60 ^b	15.25	16.83	8.38 ^b	8.70 ^b	71.05 ^b	72.72 ^a
	4%	352.00 ^{bc}	1184.00 ^b	25.42	26.73	8.32 ^{bc}	8.62 ^b	68.67 ^c	71.26 ^b
	6%	320.00 ^{cd}	1280.00 ^b	32.20	20.79	8.23 ^{cd}	8.50 ^c	68.33 ^c	69.76 ^c
	8%	280.00 ^d	908.00 ^c	40.68	43.81	8.22 ^d	8.38 ^c	67.31 ^c	69.63 ^d
					28.39	27.04			
		Avg Ammonia Reduction (%)		27.72					

Note: Means in the same row with different superscript differ significantly (P<0.05).

The effect of direct application of palm kernel meal containing probiotic *Lactobacillus bulgaricus* and *Bacillus subtilis* on the volatile ammonia, pH and moisture content of fecal layer.

In the Table 4 showed that direct application of palm kernel meal containing probiotic (antibiotic-producing bacteria) *Lactobacillus bulgaricus* on fecal layer was more effective in reducing ammonia compared to *Bacillus Subtilis*. This was observed by the higher effectiveness of the ammonia reduction (37.88%) on *Bacillus bulgaricus* compared to *Bacillus Subtilis* (34.31%). This probably was not only due to the *Lactobacillus bulgaricus* in addition to produce acids that converted of ammonia to ammonium (NH₄⁺), but also produced more bacteriocin (antibiotic) to suppress the growth of gram (-) bacteria. Suppression of growth of these bacteria resulted in reducing the production of enzyme urease that could be used to convert uric acid into ammonia.

The effect of direct application of palm kernel meal containing probiotic *L. Acidophillu*, *B. Cereus* and *L. Bulgaricus* on the volatile ammonia, pH and moisture content of fecal.

It showed in Table 5, the effect of palm kernel meal containing a combination of three bacteria (*L. Acidophillus*, *B. Cereus* and *L. Bulgaricus*) on fecal layer in reduction of ammonia emission significantly (P<0.005) was observed for 24 hr and 48 hr of incubation. However, the reduction of ammonia emission by the bacteria combination was less effective during the treatment. It showed that the percentage of effectiveness in decreasing ammonia emission in which of the three bacteria such as *L. acidophilus*, *B. Cereus* and *L. bulgaricus* (28.64%) compared to the other of six bacteria such as *L. Acidophillus* (37.15%), *S. Thermophilus* (33.26%), *B. Cereus* (27.72%), *B. Thuringiensis* (16.79%), *B. Bulgaricus* (37.88%) and *B. Subtilis* (34.31%), and This because of the combination of acid-producing bacteria, proteolytic bacteria and

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antibiotic-producing bacteria had no synergism effects to reduce the fecal ammonia emission.

Table 4. Volatile ammonia (ppm), pH and Moisture Content of fecal layer as effected by direct application of palm kernel meal containing probiotic of *Lactobacillus bulgaricus* and *Bacillus subtilis*

Bacteria	Level	Ammonia (ppm)		Ammonia Reduction (%)		pH		Moisture Content (%)	
		24 hr	48 hr	24 hr	48 hr	24 hr	48 hr	24 hr	48 hr
<i>Lactobacillus bulgaricus</i>	0%	922.00 ^a	2048.00 ^a			8.26 ^a	8.38 ^a	72.89 ^a	74.69 ^a
	2%	768.00 ^b	1504.00 ^b	22.00	26.56	8.00 ^b	8.10 ^b	71.35 ^{ab}	73.90 ^a
	4%	624.00 ^c	1440.00 ^b	37.09	29.69	7.86 ^c	8.00 ^c	69.33 ^{bc}	72.04 ^b
	6%	592.00 ^{bc}	1024.00 ^c	40.32	50.00	7.78 ^d	7.98 ^c	67.99 ^c	70.86 ^c
	8%	528.00 ^d	1024.00 ^c	46.77	50.00	7.73 ^d	7.88 ^d	65.91 ^d	70.45 ^c
				36.69	39.06				
		Avg. Ammonia Reduction (%)		37.88					
<i>Bacillus subtilis</i>	0%	960.00 ^a	1888.00 ^a			8.06 ^a	8.22 ^a	74.92 ^a	75.40 ^a
	2%	784.00 ^b	1512.00 ^b	18.33	19.92	7.94 ^b	8.10 ^b	73.21 ^b	74.34 ^a
	4%	544.00 ^c	1212.00 ^{bc}	43.33	35.81	7.84 ^c	8.08 ^b	71.34 ^c	72.40 ^b
	6%	608.00 ^c	1248.00 ^{bc}	36.67	34.75	7.78 ^{cd}	7.98 ^c	70.06 ^d	71.13 ^{bc}
	8%	544.00 ^c	1088.00 ^c	43.33	42.37	7.74 ^d	7.86 ^d	69.36 ^d	70.82 ^c
				35.42	33.21				
		Avg. Ammonia Reduction (%)		34.31					

Note: Means in the same row with different superscript differ significantly (P<0.05).

Table 5. Volatile ammonia (ppm), pH and Moisture Content of fecal layer as effected by direct application of palm kernel meal containing probiotic

Bakteri Spesies	Level	Ammonia (ppm)		Ammonia Reduction (%)		pH		Moisture Content (%)	
		24hr	48hr	24 hr	48hr	24 hr	48hr	24hr	48hr
<i>L. Acidophilus</i> + <i>B. Cereus</i> + <i>L. Bulgaricus</i>	0%	680.00 ^a	1024.00 ^a			7.86 ^a	8.16 ^a	66.97 ^a	67.27 ^a
	2%	544.00 ^{bc}	752.00 ^b	20.00	26,56	7.72 ^b	7.76 ^{bc}	65.51 ^{ab}	66.35 ^a
	4%	608.00 ^{ab}	712.00 ^b	10.59	30,47	7.74 ^b	7.80 ^b	64.21 ^{bc}	65.87 ^{abc}
	6%	496.00 ^c	688.00 ^{bc}	27.06	32.81	7.56 ^c	7.68 ^{cd}	62.34 ^c	65.46 ^{bc}
	8%	380.00 ^d	640.00 ^c	44.12	37.50	7.42 ^d	7.58 ^d	63.75 ^{bc}	64.21 ^c
				25,44	31,84				
		Avg. Ammonia Reduction (%)		28.64					

Note: Means in the same row with different superscript differ significantly (P<0.05).

The data showed that the higher the level of palm kernel meal containing probiotic resulted in significantly reduce fecal-ammonia emission. The lowest level of reducing ammonia was achieved at 6% and 8% of treatment of fecal layer. Theses result indicated that palm kernel meal containing probiotic either individual or combined bacteria that is applied directly to the fecal layer prevented the ammonia emission. Furthermore, It was observed that the fecal moisture content of feces was

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reduced significantly as the treatment increased up to 8% in fecal layer. The reduction of moisture content was because of the palm kernel meal containing probiotic absorbed the moisture content of fecal layer.

The effect of utilization of palm kernel meal containing probiotic in the laying diet on the ammonia emission, pH and moisture content of fecal layer.

The effect of utilization of palm kernel meal containing probiotic in the laying diet on the ammonia emission, pH and moisture content of fecal layer are shown in Table 6. Results indicated that the ammonia emission was significantly reduced ($P < 0.05$) by the dietary treatment. The utilization of the palm kernel meal containing probiotic in layer diet also resulted in reducing ($P < 0.05$) fecal pH, moisture content of fecal layer.

The effect of palm kernel meal containing probiotic in the laying diet was significantly effect ($P < 0.05$) in reducing of fecal layer. The higher level 30% palm kernel meal containing probiotic in the layer diet (P3) resulted in the lowest of ammonia emission that incubated for 1 hour, 24 hours and 48 hours. It showed that the lowest

Table 6. The effect of palm kernel meal containing probiotic in the laying diet on the volatile ammonia, pH and moisture content of fecal layer.

Level	Fresh Fecal (1 hr after collection)			Fresh Fecal (24 hr after collection)		
	Ammonia (ppm)	pH	Moisture Content (%)	Ammonia (ppm)	pH	Moisture Content (%)
	1hr	1hr	1hr	24 hr	24 hr	24 hr
P-0	336.00 ^a	7.80 ^a	76.60 ^a	1024.00 ^a	7.94 ^a	76.09 ^a
P-10	165.20 ^b	7.66 ^b	71.82 ^b	928.00 ^a	7.88 ^a	71.30 ^b
P-20	102.40 ^c	7.44 ^c	71.44 ^{bc}	294.40 ^b	7.68 ^b	70.12 ^b
P-30	66.40 ^c	7.28 ^d	69.65 ^c	108.80 ^c	7.30 ^c	68.26 ^b
Level	Fresh Fecal (48 hr after collection)			<i>Lactobacillus sp</i> (cfu/gr feses)		
	Ammonia (ppm)	pH	Moisture Content (%)	48 hr	48 hr	48 hr
P-0	1792.00 ^a	7.94 ^a	78.48 ^a	8.73 ^b		
P-10	768.00 ^b	7.82 ^a	74.98 ^b	8.70 ^b		
P-20	285.60 ^c	7.56 ^b	73.64 ^{bc}	8.65 ^b		
P-30	109.20 ^c	7.24 ^c	72.24 ^c	9.00 ^a		

Note: Means in the same row with different superscript differ significantly ($P < 0.05$).

level of fecal ammonia emission 66.40 ppm at P-30 treatment compared to the P-0 or control treatment (336.00 ppm) for 1 hours of incubation. Similar result also showed at 24 hours fecal incubation in which the control treatment (P-0) produced 1024.00 ppm ammonia emission whereas the P-30 treatment was only 108.80 ppm. Decreasing ammonia emission was continuously at 48 hours, in which the control treatment produced at 1792.00 ppm whereas the P3 treatment was stayed in low level at 109.20 ppm. This result was similar to Yusrizal et al. (2013) that reported the use palm kernel meal at concentration of up to 30% in the diet for native laying hens reduced NH₃ emission.

Basically, the reducing of ammonia emission is closely related to a decrease in fecal pH because of palm kernel meal containing probiotic in the layer diet may reduce the pH of the fecal layer. These was due to the prebiotic mannan oligosaccharide derived from palm kernel meal as colonic food may increase the metabolism and growth of bacterial population in the intestine and produce short chain fatty acid (SCFA). SCFA caused a lower pH of fecal layer and result in the extend of decreasingly ammonia (NH₃) emission. Additionally, bacterial enzymes that involved in the breakdown of uric acid to ammonia (NH₃) have relatively high optimum pH and are

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therefore less active when manure pH is decreased (Mobley and Hausinger, 1989). These results were similar to as reported Yusrizal and Chen, (2003) that administration Raftifeed prebiotics in the broiler diet reduced ammonia emission and fecal pH in the fourth week of rearing of broiler chickens.

CONCLUSION

Direct application of palm kernel meal containing probiotic up to 8% was effective in reducing fecal ammonia emission in which *B. Cereus*, *L. Bulgaricus* and *L. Acidophilus* were more effective in reducing ammonia. Synergistic combination of those bacteria were not effective in reducing fecal ammonia emission. Effect of palm kernel meal containing probiotic up to 30% in the laying diet was effective in reducing fecal ammonia in fecal layer. The conclusion of this research was that the direct application of palm kernel meal containing probiotic in the fresh fecal and in the layer diet were effective to reduce ammonia emission in laying house.

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03. CONTRIBUTION OF LYSINE AND CALCIUM OF *Azolla microphylla* ON EGG SHELL CALCIUM DEPOSITION IN ARAB HEN

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Abstract

The aim of the present research was to evaluate the contribution effect of lysine and calcium derived from *Azolla microphylla* on egg shell calcium deposition in Arab hen. Eighty birds of Arab hen, age was \pm 34 weeks, with an average body weight of 1250 ± 124.52 g were used as experimental animals, and were divided randomly into a randomized block design with 4 treatments and 5 replications. Feedstuffs used for dietary treatments were rice bran, yellow corn, poultry meat meal, *Azolla microphylla*, CaCO_3 , oyster shell, and top mix. The rations were formulated approximately iso metabolizable energy and iso protein of 2700 kkal/kg and 16% respectively. Inclusion levels of *Azolla microphylla* in the ration were categorized as treatments, namely, none of control (T0), 3 (T1), 6 (T2), and 9% (T3). Feed, calcium, ADF and lysine consumptions, calcium and lysine retention, and egg shell calcium mass were variables recorded. Calcium retention and protein digestibility were measured by the combinations method of total collection and indicators. Lysine retention was calculated by comparing lysine percentage in the protein and protein retained. Protein retention was obtained from the protein digestibility multiplied by protein consumed. Calcium mass of eggshell were calculated by multiplication of Ca contents of eggshell and eggshell weight at dry matter basis. The data were subjected to analysis of variance and the differences between means were determined by Duncan's test. The results showed that feeding *Azolla microphylla* up to 9% significantly ($P \geq 0.05$) affected all parameters, except feed consumption. Inclusion of 9% *Azolla microphylla* in the diet compared to control diet increased consumptions of calcium (1,09 vs. 0,39 g/hen/day), ADF (4,41 vs. 0,39 g/hen/day) and lysine (1,05 vs. 0,94 g/hen/day); retention of calcium (0,75 vs. 0,16 g/hen/day) and lysine (0,99 vs. 0,91 g/hen/day), while egg shell mass (4,21 vs. 1,37 g/hen/day). The result suggest that the increasing level of *Azolla microphylla* brought about the increase in ADF consumption, but it did not affect metabolism of Ca. The improvement of Ca metabolism, indicated by Ca retention and deposition (Ca eggshell mass), were absolutely possible to be dependent on the presence levels of lysine (intake and/or retention). Conclusion of the research is that lysine and calcium retentions are not inhibited by the increase in ADF consumption due to the higher level of *Azolla microphylla* inclusion, moreover it can increase calcium deposited in egg shell.

Key word: Arab hen, *Azolla microphylla*, calcium retention, lysine retention, egg shell calcium mass

INTRODUCTION

Arab chicken is claimed as a local poultry in Indonesia which is able to produce relatively higher egg as compared to other native chickens. Egg production of Arab chickens can achieved about 63% as reported by Wulandari *et al.* (2012). It has been known that Arab chicken produce egg with white color of shell similar to the majority of modern laying chickens. Egg with white shell is given preference over the brown one due to its nutritional content is believed to be higher. Considering the taste of Indonesian consumers which is commonly prefer to consume egg with white egg shell,

it is greatly promising that Arab chickens can be possibly developed towards a commercial poultry industry in the future.

The successful of developing poultry farming, including Arab chicken, can not be separated from the important contribution of feed and/or nutrients supply. Feed with low price but contain adequate nutrients is the primary choice in order to achieve production efficiency. Therefore, local source of non conventional feedstuff with good category in quality is quite necessary to search for the component of poultry feed. *Azolla microphylla* as one locally non conventional feedstuff have a good quality, cheap and possible to be applied for poultry diet. *Azolla microphylla* is an aquatic plant species that can easily be developed. At the beginning, *Azolla microphylla* was developed by the Indonesian peoples as raw materials for fertilizer and a few for animal feed. The superiority possessed by *Azolla microphylla* is that protein, lysine, calcium, phosphor contents are categorized high about 23,91, 0,98, 2,10, and 0,77%, respectively (Wulandari and Prawitasari, 2012; Alalade and Iyayi, 2006). However, on the other side, *Azolla microphylla* has a weakness due to its high content of crude fiber, about 40,79% (Wulandari and Prawitasari, 2012). Fraction of the fiber is also high, namely acid detergent fiber (ADF) about 47,08% and neutral detergent fiber (NDF) is approximately 36,88%.

The metabolism of calcium is closely related to dietary lysine and fiber, especially acid detergent fiber (ADF). The mobilization of dietary Ca is negatively affected by ADF, and on the other hand, is positively linked with lysine. Seventy percent of animal body ashes consist of calcium which mainly deposited in the bone, meat, and metabolic function for eggshell formation. Calcium is effectively absorbed in the intestine, particularly in the duodenum and jejunum. The rate of calcium absorption is higher than do any other minerals (Vercese *et al.*, 2009). At the duodenum and jejunum, calcium absorption is closely related to protein, especially lysine. Lysine acts an important role as the activator of Ca^{2+} -ATPase-aminophospholipid for the regulation and absorption of Ca at the duodenum and jejunum. On the contrary, ADF may have a negative impact on calcium absorption, because of its affinity to form complexity polymer compound with calcium. Based on these phenomena, the present research was conducted to evaluate the contribution effect of lysine and calcium derived from *Azolla microphylla* on egg shell calcium deposition

MATERIALS AND METHODS

A total of eighty birds of Arab hen (\pm 34 weeks old) were used in the present study, and were randomly allocated into 4 dietary treatments, based on the inclusion levels of *Azolla microphylla* (0, 3, 6, and 9%), with 5 replicates (4 birds each). Diets were formulated on the basis of iso protein (16%) and metabolizable energy (2700 kcal/kg). Fiber components (neutral detergent fiber/NDF and acid detergent fiber/ADF) of the diets ingredients were determined according to the method of Gadberry (2009). Lysine components of the diets were determined based on the table of feedstuff composition of Hartadi *et al.* (1987) and research results of Iyayi and Alalade (2006). Composition of the experimental diet and nutritional contents are indicated in Table 1.

Tabel 1. Composition of experimental diet and nutritional content

Feedstuff	Treatment			
	T0	T1	T2	T3
	----- % -----			
Yellow corn	33,00	34,00	35,00	32,75
Rice bran	40,00	36,75	33,50	34,00
Poultry Meat Meal	5,00	5,50	5,00	5,50
Soybean meal	18,25	17,00	16,75	15,00
Oyster shell	2,50	2,50	2,50	2,50
CaCO ₃	1,25	1,25	1,25	1,25
<i>Azolla microphylla</i>	0,00	3,00	6,00	9,00
Total	100,00	100,00	100,00	100,00
Composition of Nutrient				
Energy Metabolism (kkal/kg)	2739,00	2736,00	2709,00	2706,00
Crude protein	15,98	16,13	16,19	16,18
Crude fiber	15,67	15,65	15,61	15,98
Lignin ^{a)}	2,87	3,00	2,88	3,09
Crude fat	5,34	5,16	4,90	4,93
Lysine ^{b) c)}	1,04	1,07	1,09	1,11
Arginine	1,18	1,21	1,23	1,24
Methionine	0,32	0,32	0,33	0,33
Calcium	0,5	0,54	1,02	1,22
Phosphorus	0,80	1,05	1,16	1,35
Ash	8,56	8,60	8,49	8,93
NDF (<i>Neutral Detergent Fiber</i>) ^{a)}	33,22	32,27	31,19	32,26
ADF (<i>Acid Detergent Fiber</i>) ^{a)}	0,43	1,87	3,24	4,68

Note : a). Calculated value based on the table composition feed of Gadberry (2009)
 b). Calculated value based on the research result of Iyayi and Alalade (2006)
 c). Calculated value based on the table composition feed of Hartadi *et al.* (1980)

Calcium retention and protein digestibility were measured by the combinations method of total collection and indicators. Lysine retention was calculated by comparing lysine percentage in the protein and protein retained. Protein retention was obtained from the protein digestibility multiplied by protein consumed. Calcium mass of eggshell were calculated by multiplication of calcium contents of eggshell and eggshell weight at dry matter basis. The data were subjected to analysis of variance and the differences between means were determined by Duncan’s test at the probability level of 5%.

RESULT AND DISCUSSION

The result showed that feed consumption was not affected by the feeding *Azolla microphylla*, but there were similar patterns of calcium and lysine retentions between T2 and T3, although consumptions of both indicated difference trend (Table 2). The increase in dietary inclusion of *Azolla microphylla* the higher levels of lysine and calcium contributed to the metabolic processes, because feeding dietary *Azolla microphylla* donates higher portion of lysine and calcium. According to previous studies, calcium content of *Azolla microphylla* was about 2.1% (Wulandari and Prawitasari, 2013) and lysine was approximately 0.98% (Alalade and Iyayi, 2006). Calcium retention between T0 and T1 was not different and also similar between T2 and T3, but that of both T0 and T1 compared to that of T2 and T3 were significantly different

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($P < 0.05$). The pattern of calcium distribution based on calcium consumption and retention was incomparable, but calcium retention appeared to be in proportion to lysine retained. However, it was greatly different when looking at the pattern of acid detergent fiber (ADF) consumption which was indicated significantly difference ($P < 0.05$) amongst treatments. The increase in ADF consumption is due to the significant contribution supply of ADF derived from *Azolla microphylla* by about 1.4, 2.82, 4.68% for T1, T2 and T3, respectively. Considering acid detergent fiber (ADF), comprises cellulose, silica, and lignin, which could not be digested by poultry, therefore, this phenomenon give an understanding that calcium retention was affected by lysine retained, but not by ADF consumption. The present results were disagree with the finding of Saura-Calixto *et al.* (1992) that cellulose and lignin could inhibit absorption of calcium by forming a complex polymer in the small intestine. Factor that accelerates calcium absorption in general, known as calcium-binding protein (Ca-BP) formed in the intestinal lumen. However, according to Harland and Oberleas (2001) there was more specific calcium-binding involving components of protein, namely lysine and arginine, that accelerate calcium absorption. Calcium can be absorbed through two mechanisms, namely trans-cellular and cellular. Trans-cellular mechanism is typically involving certain cell surface transporter or molecule acceptor which enables the absorption between cells occurred. Calcium transportation can be effectively achieved when the protein and/or amino acid used by the intestinal epithelium or basolateral membrane is sufficient for the circulatory system (Kotler, 2012).

Table 2. Feed, calcium, lysine and ADF consumptions, and calcium and lysine retentions, and calcium utilization for egg shell of Arab hen

Parameters	Levels of <i>Azolla microphylla</i>			
	0% (T0)	3% (T1)	6% (T2)	9% (T3)
Feed consumption (g/bird/day)*	90,32	93,30	94,42	94,22
Calcium consumption (g/bird/day)	0,39 ^c	0,44 ^c	0,88 ^b	1,09 ^a
Lysine consumption (g/bird/day)	0,94 ^b	1,00 ^{ab}	1,03 ^a	1,05 ^a
ADF consumption (g/bird/day)	0,39 ^d	1,75 ^c	3,06 ^b	4,41 ^a
Calcium retention (g/bird/day)	0,16 ^b	0,21 ^b	0,69 ^a	0,75 ^a
Lysine retention (g/bird/day)	0,91 ^b	0,97 ^{ab}	1,01 ^a	0,99 ^a
Calcium egg shell mass (g)	1,37 ^c	1,60 ^c	3,14 ^b	4,21 ^a

Values within the same row with different superscript indicates significantly difference ($P < 0.05$)

*)Data have been presented at the “Seminar National Peternakan Berkelanjutan 4”, Padjajaran University, Bandung (2012).

Calcium absorbed due to the inclusion of *Azolla microphylla* was able to be utilized for maintaining the stability of the production. This was demonstrated that calcium derived from *Azolla microphylla* really contributed to the improvement of calcium mass of egg shell. The facts can be proven with the feeding effects of *Azolla microphylla* between the levels of 6% (T2) and 9% (T3) indicated similar calcium retention but calcium egg shell mass was significantly different (Table 2). One factor assumed to be involved in such process is lysine retained in relation to the status of calcium presence in the body. Calcium is absorbed and passed into the blood and further transported to the target tissues is presence in three forms, namely in the form of free ions, protein-binding form, and ions which can not be dissolved because it bound

with other mineral (Pond *et al.*, 1995). The present finding was in accordance with the result reported by Vergara *et al.* (1982) and Joshi and Ghosh (2008) that calcium at the reproductive tract is secreted along with amino acids, especially lysine. Calcium-regulating activation through parathyroid hormone (PTH) in the sarcoplasmic reticulum needs the supply of aminophospholipid that can build Ca^{2+} -ATPase-aminophospholipid. Ca^{2+} -ATPase-aminophospholipid in turn might affect PTH and estrogen in the regulation of calcium ions secretion by cells of the uterine mucous. According to Nesheim *et al.* (1979), and Lewis-McCrea and Lall (2007) that the formation of egg shell is related to the availability of calcium and carbonate ions in the uterus fluid that can form calcium carbonate. Ion carbonate is formed under the presence of CO_2 in the blood, as a result of uterus cell metabolism, and the existence of ion H_2O . The reaction result of both is then broken down by the enzyme carbonic anhydrase into bicarbonate ions and finally change into carbonate ions after hydrogen ion is discharged.

CONCLUSION

Lysine and calcium retentions are not inhibited by the increase in acid detergent fiber (ADF) consumption due to the higher level of dietary *Azolla microphylla* inclusion, moreover, it can increase calcium deposited into the egg shell.

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04. JAPANESE QUAIL EGGS QUALITY FED FERMENTED JATROPHA CURCAS MEAL (*Jatropha curcas L.*)

Sumiati¹, R. Mutia² and I. R. Khalim³

Abstract

The purpose of this study was to examine the eggs quality of Japanese quail (8-14 weeks of age) fed *Jatropha curcas* meal fermented using *Rhizopus oligosporus* (JCMF). A completely randomized design with 5 treatments and 3 replications (10 birds per replication) was used in this experiment. The treatment diets were T0= diet contained 0% JCMF, T3= diet contained 3% JCMF, T6= diet contained 6% JCMF, T9= diet contained 9% JCMF and T12= diet contained 12% JCMF. The parameters observed were egg weight, percentage of egg components (proportions of yolk, albumen and shell to the weight of whole egg), shell thickness, haugh unit, and yolk colour score. The data were analyzed using analysis of variance (ANOVA), and if any significant differences among treatments, the data were further analysed using Duncan Multiple Range Test. The results indicated that feeding JCMF at the level of 9% and 12% significantly reduced ($P < .05$) the egg weight. Feeding JCMF at the level of 12% significantly reduced ($P < .05$) the percentage of albumen. However, feeding JCMF at the level of 6% up to 12% significantly increased ($P < .05$) the percentage of yolk. Feeding JCMF up to the level of 12% did not influence the percentage of egg shell, shell thickness, haugh unit as well as yolk colour score. The conclusion of this research was that *Jatropha curcas* meal fermented using *Rhizopus oligosporus* could be used up to 6% in the Japanese laying quail diets.

Keywords: eggs, *Jatropha curcas* meal, quails, *Rhizopus oligosporus*

INTRODUCTION

According to General Directorate of plantation of Indonesia /DITJEN Perkebunan (2011), production of *Jatropha curcas* in Indonesia was 7,081 tons dried seeds with planting area of 50 106 hectares, so the potential of *Jatropha curcas* meal in Indonesia was 4248.6 tons. *Jatropha curcas* meal (JCM) has potential as feed, Sumiati *et al.* (2010) reported that JCM contain high protein, about 24.7% (hull-seed) and 45-50% (unhull-seed). JCM contain up to true 90% protein, 3893 kcal / kg gross energy, 2115 kkal / kg metabolizable energy, 1.0% Ca and 0.99% P. However, according to Sumiati *et al.* (2010) JCM contain *phorbol esters* (24.33 mg / g), and *curcin* (0.09%) those hazardous to livestock (Sumati *et al.* 2011), so the using of JCM as feed ingredient could be done after going through the detoxification process.

The detoxification of toxins in JCM could be done through three methods, namely: chemical, physical and biological methods. According to El Rafei *et al.* (2010) ozone treatment was able to reduce levels of phorbol esters up to 78.53% and γ -irradiation treatment was able to reduce 71.35% phorbol esters levels, while the Na HCO₃ treatment was able to reduce 44.54% phorbol ester levels. According to Belewu and Sam (2010), fermentation used *Rhizopus oligosporus* could reduce a variety of anti-nutritional in JCM, namely: the trypsin inhibitor (20.51% to 8.15%), curcin (34.36% to 14.75%), saponins (2.47% to 0.33%), phytic acid (9.10% to 4.18 %) and phorbol ester (0.013% to 0.012%). As biologically side, the fermentation used *R. oligosporus* showed the usefulness of the protein efficiency, retention of Ca and P, as well as metabolic energy were better than physical treatment by heating and chemical treatment with NaOH (Sumiati *et al.* 2008).

The purpose of this study was to examine the eggs quality of Japanese quail (8-14 weeks of age) fed *Jatropha curcas* meal fermented using *Rhizopus oligosporus* (JCMF)

MATERIALS AND METHODS

The fermentation method of this study used the methods of Sumiati *et al.* (2009). JCMF was added with plain water until 60% of moisture. JCMF was steamed up to one hour in boiling water. After being cooling (about 38 ° C), JCMF was *tempeh fungi*. *Jatropha curcas* meal was fermented using *R.O ligosporus* (JCMF) and harvested after 48 hours. Then dried in the oven at a temperature of 60 ° C for 24 hours. JCMF was ground and mixed into the ration.

This study used 150 laying quails (8 weeks of age). This study used a completely randomized design (CRD) with five treatments and each treatment consisted of 3 replicates. The treatment diets were T0= diet contained 0% JCMF, T3= diet contained 3% JCMF, T6= diet contained 6% JCMF, T9= diet contained 9% JCMF and T12= diet contained 12% JCMF. The ration contained iso- calory and iso-protein (Leeson and Summers,2005). The composition and nutrient content of the ration are presented in Table 1.

The parameters observed were egg weight (g), proportion of egg components (yolk, albumen, and eggshell) and interior egg quality (shell thickness (μm), *yolk* color score and Haugh units of eggs). The data of egg quality was obtained from 12, 13, 14 weeks of age. The data were analyzed using analysis of variance (ANOVA), and if any significant differences among treatments, the data were further analysed using Duncan Multiple Range Test (Mattjik and Sumertajaya, 2000).

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Table 1. Composition and nutrient content of the diet

Feed Ingredients	T0	T 3	T6	T9	T12
	----- (%) -----				
Yellow corn	50	50	50	50	50
Rice bran	8	6	4	1.8	0
Soybean Meal	22	20.8	19.6	18.5	17.3
JCMF	0	3	6	9	12
Fish meal	6	6	6	6	6
CPO	6.2	6.4	6.6	6.8	7
CaCO ₃	6.1	6.1	6.1	6.1	6.1
D/CP	0.8	0.8	0.8	0.8	0.8
Salt	0.2	0.2	0.2	0.2	0.2
Premix	0.4	0.4	0.4	0.4	0.4
DL-Methionin	0.3	0.3	0.3	0.3	0.3
Total (%)	100	100	100	100	100
Nutrient content (<i>As fed</i>):					
Protein (%)	18.19	18.17	18.12	18.13	18.12
Metabolizable energy kcal / kg	2957.42	2956.08	2954.74	2951.85	2954.31
Fat (%)	8.27	8.43	8.59	8.75	8.92
Crude fiber (%)	2.33	3.07	3.82	4.54	5.31
P _{av} (%)	0.57	0.55	0.54	0.55	0.54
Ca (%)	3.01	3.04	3.06	3.11	3.09
Methionine (%)	0.57	0.56	0.56	0.55	0.54
Cystine (%)	0.29	0.29	0.28	0.27	0.26
Methionine + cystine (%)	0.86	0.85	0.83	0.82	0.80
Lysine (%)	1.06	1.03	1.01	0.99	0.97
Na (%)	0.14	0.14	0.14	0.14	0.13
Cl (%)	0.21	0.20	0.20	0.20	0.19

T0: diet without JCMF (control), T 3: diet contained 3% JCMF, T6: diet contained 6% JCMF, T9: diet contained 9% JCMF, T12: diet contained 12% JCMF.

RESULTS AND DISCUSSION

The whole eggs weight and percentages of quail egg weights component at 12-14 weeks of age are presented in Table 2.

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production compared with 15%, 17.5%, and 22.5% crude protein at 2,900 kcal / kg ME. The average of *yolk* weight in the study was $3,18 \pm 0.18$ g, and according to Song *et al.* (2000) the weight of quail egg *yolk* was 3.25 ± 0.40 g. The average of *yolk* percentage fed 3% and 9% JCMF was not significantly different ($P > 0.05$) compared to that of control (T0). The used of 6% and 12% JCMF in the ration significantly ($P < 0.05$) increased the *yolk* weight percentage. The increasing of *yolk* weight in this study was affected ($R^2 = 0.619$) by phorbol ester consumption. Phorbol ester can soluble in the fat that contained in JCMF. Besides that, the use of CPO also increase the fat content in the diet and also increasing fat intake. According to Stadelman and Cotterill (1995), the main components of *yolk* is fat (about 31.8% - 35.5% fat). Song *et al.* (2000) mentioned that the range of the *yolk* percentage was 29.42% - 33.38%.

The average of quail egg albumen weight fed fermented *Jatropha curcas* meal (JCMF) 3% and 6% were not significantly different ($P > 0.05$) compared to that of T0 (without using JCMF). The using 9% and 12% JCMF in the ration significantly ($p < 0.05$) decreased the albumen weight. The decreasing of albumen weight due to the strong relationship ($R^2 = 0.93$) between the albumen weight and whole egg weight which has a negative correlation with the consumption of phorbol ester and crude fiber. The albumen weights in this study was 5.71 ± 0.35 g, and according to Song *et al.* (2000) the quail egg albumin weight was 6.33 ± 0.59 g. The using 3%, 6% and 9% JCMF in the ration resulted the albumen proportions were not significantly different ($P > 0.05$) compared to T0. The using of 12% JCMF in the ration reduced the percentage of egg albumen. The decreasing of albumen percentage was caused by the increasing of *yolk* percentage. Dry matter and fat content in the ration T12 were higher than those of the other treatments, so the water and protein that the main component of albumen are lower than the other treatments. According to Cotteril Stadelman (1995), the main components of albumen are water (88 %) and protein (9.7% - 10.6%). Wiradimadja *et al.* (2009) reported that the use of *Sauropus androgynus* L Merr leaves starch in the diet also decrease the albumen weight, although the crude protein in the ration is higher. The average of albumen weight percentage obtained in this study was lower than that of resulted by Song *et al.* (2000) was ranged 58.88% - 63.52%.

The average of egg shell weight given 3% JCMF in the diet did not different ($p > 0.05$) compared to that of T0 (without JCMF). The using 6, 9, and 12% JCMF in the ration significantly ($p < 0.05$) decreased eggshell weights. The decreasing was due to the eggshell weight highly correlated ($R^2 = 0.609$) with whole egg weight. The average of eggshell weight in this study was 0.92 ± 0.07 g, and according to Song *et al.* (2000) quail eggshell weight was 0.76 ± 0.01 g. The percentage of eggshell weight fed 3%, 6%, 9%, 12% JCMF were not significantly different ($P > 0.05$) compared with control (without JCMF). The using of CaCO_3 in the ration was similiary ranged 3.01% - 3.11%, so the eggshell percentage was relatively same. The consumption of ration in this study was ± 19.58 g / bird / day, so the consumption of Ca approximately was 0.59 g / bird / day. Ca intake was sufficient to quail Ca requirement for producing an egg shell which is 0.38 g. Stadelman and Cotteril (2005) stated that the main component for producing the eggshell was calcium. The average of eggshell percentage in this study was bigger than that of resulted by Song *et al.* (2000) that quail eggshell percentage was 6.61% - 7.99%. The results of egg interior quality is presented in Table 3.

Table 3. The Interior quality of quail eggs percentage (12-14 weeks of age) fed JCMF

Variables	T0	T3	T6	T9	T12
Eggshell thickness (μm)	169.52 \pm 10.07	164.33 \pm 8.28	158.11 \pm 12.41	155.56 \pm 9.77	154.44 \pm 14.01
Yolkcolor score	6.04 \pm 0.56	6.44 \pm 1.01	6.35 \pm 0.68	6.09 \pm 0.77	6.76 \pm 0.76
Haugh unit	88.02 \pm 2.73	90.06 \pm 1.78	89.65 \pm 2.51	90.27 \pm 2.05	88.70 \pm 3.31

T0: diet without JCMF (control), T3: diet contained 3% JCMF, T6: diet contained 6% JCMF, T9: diet contained 9%JCMF, T12: diet contained 12% JCMF

The average of quail egg interior quality was not affected by the treatments. The quail able to maintain the eggs quality, as a compensation of decreased egg production and whole egg weight. The results showed that the average of eggshell thickness was $164.28 \pm 30.24 \mu\text{m}$. According to Song *et al.* (2000), the normal of eggshell thickness is 159.3 to 190.3 μm . It also suggests that Ca in JCMF was enough to produce eggshell. The main component of eggshell is calcium (98.2%), magnesium (0.9%), and phosphorus 0.9% (Stadelman and Cotterill, 1995). Based on that literature, the biggest composition of egg shell is calcium.

The yolk color scores were measured with *RocheYolk color fan*. The yolk color score obtained in this study was ranged 5-8. Ghazvinian *et al.* (2011) reported that the reduction in the use of maize in the ration despite increased protein content but able to reduced yolk color score, because the yolk color was obtained from carotenoid compounds in the diet that transferred to the egg yolk. According to Leeson and Summers (2005), the feedstuff that contain *xanthophyll* are CGM(275 mg / kg), yellow corn (20 mg / kg), wheat (4 mg / kg), shorgum (1 mg / kg), alfalfa flour (175 mg / kg), marigolds (7.000 mg / kg). Wiradimaja *et al.* (2009) measured the average of yolk color in quail ages 8, 12, and 16 weeks fed *Sauropus androgynus* L. Merr, with a score of 8. That results was higher because of the role of β -carotene in the *Sauropus androgynus* L Merr leaves. The pigments that affect to the yolk color is carotene pigment (Yuwanta, 2004). The color pigment in feed ingredients are *xanthophyll*, *zeaxanthin*, *canthaxanthin*, *astaxanthin*, β -apo-8-carotenoic, *cryptoxanthin* and β -carotene (Leeson and Summers, 2005).

According to Song *et al.* (2000), Haugh unit of quail eggs was ranged from 82.17 to 86.21. Another study conducted by Hazim *et al.* (2011), the average value of HU was 87.57. Haugh unit values in this study was 89.34 ± 2.56 . It shows that the grade quality of quail egg is AA (USDA, 2000). Haugh unit values were greatly influenced by storage time and temperature of the environment. The measurement of HU in this study was conducted at the same storage period and temperature (± 24 hours at a temperature of 27-28 $^{\circ}\text{C}$), so the storage condition was similar.

The used of JCMF up to 12% in the diet did not affect ($P > 0.05$) interior quality (shell thickness, yolk color score, and Haugh unit) quail eggs. The eggshell percentage also was not affected ($P > 0.05$) due to the use JCMF up to 12% in the ration. The percentage of albumen fed 3%, 6%, and 9% JCMF did not different ($P > 0.05$) to that of 0% JCMF, but the percentage of albumen was increased ($P < 0.05$) on the use of 12% JCMF in the ration. The percentage of quail egg yolk fed of 6% JCMF was higher ($P < 0.05$) than that of feeding 0%, and 3% JCMF. The whole egg weight fed 9% JCMF ($P < 0.05$) was lower than that of 0%, 3% and 6% JCMF, so that the proper use of JCMF in the quail laying period ration was 6%.

CONCLUSION

Jatropha curcas meal could be used as a source of protein feed after detoxification. *Jatropha curcas* meal fermented using *R. oligosporus* (JCMF) could be used up to 6% in the ration of quail laying period without resulted negative effects in the quality of quail eggs.

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05. EVALUATION IN THE PRESENCE OF BLACK TEA WASTE EXTRACT ON DIFFERENT LEVEL OF ENERGY-PROTEIN RATIIONS IN THE PERFORMANCE AND CARCASS PARAMETERS OF BROILER

Dilla Mareistia Fassah^{1*}, Supadmo² and Rusman³

Abstract

This experiment was designed to evaluate the effect of black tea waste extract (BTWE) and level of energy-protein rations as well as the interaction between them on the performance and carcass production of broiler feeding with different level of energy-protein rations. This experiment used an analysis of variance factorial pattern 3 x 3 (3-levels factor providing BTWE and 3-types of rations) and if there are significant differences, it is continued with Duncan Multiple Range Test (DMRT). The first factor was the level of BTWE (0, 500 and 1000 ppm) and the second factor was the level of energy-protein rations, namely: Type 1: low energy - protein, Type 2: medium energy - protein, Type 3: high energy - protein. The experiments were conducted for 42 days using 45 chickens, each treatment consisted of 5 birds as replication. Birds were housed in individual wire cages and offered water and feed *ad libitum*. Average daily gain (ADG), feed intake (FI) and feed conversion ratio (FCR) were determined weekly. At 42 days the birds were sacrificed, the carcass weight, the percentage of carcass, and abdominal fat were measure. The experiment results showed that supplying BTWE and energy-protein rations level significantly affected broiler gain ($p < 0.05$). Supplying BTWE significantly decreasing FCR at 500 ppm ($p < 0.05$) and didn't affect on gain and FI. Increasing of energy-protein level significantly increasing gain, FI, live weight, carcass weight and decreasing FCR ($p < 0.05$). It was concluded that BTWE as feed additives has positive in the performance and carcass production of broiler.

Key Words : Antioxidant, Extract, Broiler, Performance, Carcass

INTRODUCTION

The rapid growth of broiler chicken, needs to be balanced with sufficient nutrients in their feed, especially protein to support the growth process. Protein consumption is associated with energy content in the feed. High energy content of feed will decrease the consumption of protein. Increase in feed energy will generate excess abdominal fat accumulation, so that in the preparation of animal feed is necessary to consider the level of energy - protein feed. Feed with a high energy content increased lipid peroxidation and oxidative stress in the body tissues, which has negative effect in broilers growth.

Tea is the most widely consumed beverage in the world, and the polyphenolic compounds have many biological functions and health benefits. Black tea is obtained through the fermentation process by the polyphenol oxidase which present in tea leaf. The fermentation causes mayor of polyphenols (catechin) oxidized become theaflavins and thearubigins. Tea polyphenols have antioxidant capabilities by chellating metal and

acts as a scavenger of reactive oxygen species (Yang *et al.*, 2010). Polyphenols function has used as antioxidant source prevent the unsaturated fatty acid, the main components of cell membrane to the damage. Integrity of cell membranes will help the process of absorption and metabolism in animal body.

Tea polyphenol in high concentration also has negative effect in digestion process. Polyphenols in tea can interfere with the absorption of nutrients. Its ability to bind to proteins (protein binding capacity) would reduce protein digestibility and increased fecal excretion of nitrogen. Tannins, one of polyphenol compounds, can bind to the enzyme in the gastrointestinal tract. Bajerska *et al.* (2011) demonstrated that the chicken apparent protein digestion was decreased with fed green tea extract. It's explained that phenolic group of catechin mainly associated with lower efficiency of nutrients, inhibition digestive enzymes and increased excretion of endogenous protein.

Polyphenols have antioxidant functions, but besides that it also can act as pro-oxidants. It is related to various factors such as the ability to reduce metal, pH and solubility. The toxic levels of polyphenols depend on the specific kinds of polyphenols as well as the number of free radicals are formed (Babich *et al.*, 2011). The objective of the present study was to evaluate the effect of different level of BTWE in combination with the levels of energy-protein in the ration to broiler performance and several carcass parameters.

MATERIALS AND METHODS

Experimental animal and diets

A total of 45 1-d-old male broilers (Lohmann) were used in 42-d of experiment. Birds were randomly divide into 9 treatments in a factorial design. Each treatment consisted of 5 birds as replication. Birds were housed in individual wire cages with feeder and drinker. Yellow corn, rice polishing, soybean meal, fish meal, palm oil, premix, dicalcium phosphat, CaCO_3 and filler were used as feed ingredients of basal diets.

Black tea waste from The Research Institute for Tea and Cinchona of Gambung, Bandung, West Java, Indonesia. An extract of black tea waste (BTWE) was prepared by maceration. A total 2.5 kg of black tea waste powder was extracted overnight with and 5 L of ethanol 75% by maceration. Extract was filtered with Whatman paper. This step was repeated 3 times and filtrate was concentrated by vaccum evaporator and then the concentrate was made as powder form.

Three types of basal diets were formulated in *iso ratio* with different level of metabolizable energy (ME) and crude protein (CP) (Table. 1). Each dietary treatment was given three level of BTWE (control, 500 ppm, and 1000 ppm). BTWE was given from 7-d of experiment. All diets were fed in mash form with feed and water were being provided *ad libitum* throughout the experimental period.

Sampling and measurements

Birds body weight and feed intake were recorded weekly. This information was used to calculate ADG and FCR. On 42 day, birds were weighed individually and slaughtered. Carcass was weighed after removing feathers, head, shank, digestive tract and internal organs (except spleen and lungs) from the bird body. The abdominal fat was removed and weighed. Carcass percentage and abdominal fat was expressed as a relative percentage to whole body weight.

Statistical analysis of the data was carried out by ANOVA using general linear procedure. Computation was performed using SPSS 15.0 for windows.

RESULTS AND DISCUSSION

Results

Broiler Performances

Feed intake and ADG of broiler didn't significantly affected when BTWE at levels of 500 and 1000 ppm was given to the broiler. FCR was significantly affected by the level of given BTWE in broiler ($p < 0.05$). Giving 500 ppm of BTWE to broiler show the best result in FCR (Table 2). The level of energy-protein ratios significantly increased in feed intake, average daily gain and FCR of broiler ($p < 0.05$). An increase in feed intake and average daily gain of broiler in line with the increase of the energy-protein ratios level (Table 2). Giving 500 ppm of BTWE to broiler show the best result in FCR (Table 2). The combination of 500 ppm BTWE and high level of energy-protein ratios show the best interaction in average daily gain of broiler ($p < 0.05$). However, there was no interaction between the level of BTWE and the level of energy-protein ratios to feed intake and FCR of broiler.

Carcass parameters

BTWE didn't significantly affect on final body weight, carcass weight, carcass percentage and abdominal fat percentage. The level of energy-protein ratios significantly increase the final body weight, carcass weight and abdominal fat percentage of broiler ($p < 0.05$), but didn't significantly affect the carcass percentage. The results in Table 3 showed no interaction effect between BTWE level and the level of energy-protein ratios in final body weight, carcass weight, carcass percentage and abdominal fat percentage of broiler.

Discussion

Black tea waste is agricultural wastes that can potentially uses as a source of natural antioxidants. Farhoosh *et al.* (2007) reported that black tea waste is the potential agricultural waste that has an advantage to be used as natural antioxidant. The main

antioxidant compound in black tea is polyphenols, especially some of flavanoid compound such as catechin, theaflavin and thearubigin. The black tea waste extract used in this experiment was made by maceration.

The feed intake and average daily gain didn't significantly affect by the level of BTWE. In line with our studies, previous studies also find the same results. Green tea waste on broiler ration didn't significantly affect on feed intake of broiler at 6 weeks of ages (Yang *et al.*, 2003). It is presumably that the level of BTWE until 1000 ppm is still in low level. The polyphenols, tannin in BTWE had not effect on digestion and metabolism in animal body. polyphenol compounds, can bind to the enzyme in the gastrointestinal tract, thereby reducing the digestibility of protein, fat and starch, increases the excretion of fat and reduces cholesterol absorption into the body (Dufresne and Farnworth, 2001).

The results from this study can also presumably that polyphenol can be used as antioxidant by prevent the oxidation process of unsaturated fatty acid in membrane cell. Integrity of cell membranes will help the process of absorption and metabolism in animal body. Feed intake is the main factor that has effect in average daily gain and feed efficiency (Ferket and Germat, 2006). Feed intake didn't influence by level of BTWE, so it is causing the same results in average daily gain. The FCR is ratio of average daily gain and feed intake. Five hundred ppm of BTWE on ration is giving the lowest FCR of broiler. Average daily gain in the level of 500 ppm BTWE is increasing, although it didn't significantly effect by the level of BTWE.

Increase in level of energy-protein rations in line with the results of feed intake, average daily gain and FCR in broiler. Giving higher level of energy-protein rations was causing in increase in feed intake and average daily gain. It is followed by the decrease of FCR. In line with this study, Dairo *et al.* (2010) reported that feed intake will be decreased if broiler fed by the low level of energy protein rations. Feed with low energy levels are usually bulky so it makes the digestive tract become full faster and the animal stop consuming feed even though the nutrient requirements unfulfilled.

Increase in crude fiber in feed will effect on feed intake (Randa, 2007). The low feed intake results in low amounts of consume nutrient. It's causing a lower final body weight gain and carcass weight, due to changes in the body tissues synthesis. The lowest FCR obtained in the high level of energy-protein rations. This result is consistent with the opinion of Golian *et al.* (2010), which states that increased body weight gain and decreased FCR in line with an increase in levels of energy - protein rations. This is due to an increase in feed consumption in line with the increase in energy levels - protein feed.

Level of BTWE were not significant effect on the final body weight, carcass weight, carcass percentage and the percentage of abdominal fat. This is presumably because of the levels of polyphenols in BTWE up to 1000 ppm of rations don't affect

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the process of digestion and metabolism in the animal body, which is reflected in feed intake and body weight gain of broiler. Mahfouz *et al.* (2009) stated that the carcass weight is closely associated with slaughter weight and body weight gain.

The level of energy - protein feed significantly affect slaughter weight and carcass weight ($p < 0.05$). Increased level of energy - protein in the ration was accompanied by an increase in final body weight and carcass weight. This can be explained due to an increase in feed intake and body weight gain (Table 2). The result shows that carcass percentage between the levels of energy-protein treatment significantly different. This is possible because of the increased in final body weight and carcass weight don't come from the gain of muscle, but due to an increase in fat tissue. Soeparno (2005) stated that during the period of growth, muscle development can be hampered because of the limited size of muscle fibers at different ages. This limitation still can't be exceeded, even though animals kept eating the high quality feed.

The percentage of abdominal fat was significantly different between the treatment level of energy - protein rations. Higher levels of energy - protein in the ration is likely to increase the percentage of abdominal fat. This is presumably due to an increase in slaughter weight and body weight gain along with increased levels of energy - protein in the ration. The energy used by the body is generally derived from carbohydrates and fat reserves. Increased feed intake causes excess of energy consumed, and it will be stored as body fat reserves in animal's body (Sujana *et al.*, 2007). There is a positive correlation between weigh cut with abdominal fat percentage (Ojedapo *et al.*, 2008).

Broiler average daily gain is influenced by the interaction between the treatment level of BTWE and the levels of energy - protein . The best average daily gain is produced by the combination of 500 ppm BTWE and the high levels of energy - protein ration. Kamran *et al.* (2008), which states that the provision of low energy - protein will have a lower body weight gain. In different concentration of energy - proteins at a fixed ratio, the animals that received a low density feed will have the lower weight gain (Sahraei, 2012). Tannins has affect on the digestive tract enzymes, but 500 ppm BTWE, doesn't effect on enzymatic digestion. The combination of 500 ppm BTWE with high level of energy-protein in the ration showed the best performance and carcass paramaters.

CONCLUSION

Black tea waste extract has the potential to be used as natural antioxidant in broiler. Combination of black tea waste extract at 500 ppm and high level of energy-protein rations showed the best results by increasing the average daily gain and also didn't make any change on carcass parameters. The presence of polyphenols in 500 ppm BTWE not only can be used as good natural antioxidant , but also hasn't negative effect in digestion and metabolism process in animal body.

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Table 1. Composition of basal diets (as-fed basis)

Feed stuffs	Proportion (%)		
	Low energy-protein level	Medium energy-protein level	High energy-protein level
Yellow corn	47,00	47,00	48,50
Rice polishing	23,00	19,14	6,04
Soya bean meal	20,00	22,05	26,00
Fish meal	3,00	5,00	7,70
Palm oil	0,00	3,30	8,74
Dicalcium phosphat (DCP)	0,50	0,80	0,83
L-Lysine HCl	0,50	0,40	0,24
DL- Methionine	0,15	0,10	0,05
CaCO ₃	2,00	1,71	1,40
NaCl	0,25	0,25	0,25
Premix	0,25	0,25	0,25
Filler	3,35	0,00	0,00
Total	100,00	100,00	100,00
Calculated nutrient content			
Metabolizable energy (kcal/kg)	2769,41	3013,54	3351,62
Crude protein (%)	18,03	20,03	22,09
Crude fiber (%)	3,27	3,09	2,37
Crude fat (%)	6,03	9,10	13,38
Calcium (%)	1,05	1,05	1,00
Phosphor av. (%)	0,52	0,58	0,56
Methionine (%)	0,38	0,38	0,38
Lysine (%)	1,01	1,03	1,00

Laying hens were housed in a open side of laying house. During the experiment laying hens had free access (*adlibitum*) to drinking water and dietary treatment (Table 1).

Table 2. Effect of BTWE and level of energy-protein ration on performance of broilers

Parameter	BTWE (ppm)			Sig. ^m	Level of energy-protein rations			Sig. ^m	I ⁿ
	0	500	1000		Low	Medium	High		
Feed intake (g)	2084,28 ± 494,82	2049,03 ± 733,60	2176,13 ± 512,34	ns	1577,18 ^a ± 456,46	2127,40 ^b ± 308,83	2604,86 ^c ± 429,26	*	
ADG (g)	1011,63 ± 291,75	1125,80 ± 415,11	1055,40 ± 366,96	ns	753,37 ^a ± 96,44	976,40 ^b ± 277,13	1463,07 ^c ± 175,70	*	I
FCR	2,14 ^b ± 0,53	1,85 ^a ± 0,28	2,17 ^b ± 0,50	*	2,10 ^b ± 0,54	2,27 ^b ± 0,45	1,78 ^a ± 0,22	*	

^{ns} non significant (p>0,05) ; ^{a,b,c} Means in the same row with different superscript differ (p<0,05) ; ^{##} significance ; ⁿ interaction; ^I the interaction

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Table 3. Effect of BTWE and level of energy-protein ration on carcass parameters of broilers

Parameter	BTWE (ppm)			Sig. ^m	Level of energy-protein rations			Sig. ^m	I ⁿ
	0	500	1000		Low	Medium	High		
Body weight	1179,40 ± 294,97	1238,47 ± 412,08	1140,67 ± 315,60	ns	923,20 ^a ± 96,43	1139,27 ^b ± 281,28	1496,07 ^c ± 306,35	*	
Carcass weight (g)	756,19 ± 147,25	866,75 ± 344,30	767,53 ± 231,43	ns	580,50 ^a ± 109,45	786,59 ^b ± 216,19	1023,38 ^c ± 181,35	*	
Carcass percentage	64,24 ± 6,34	63,94 ± 3,30	63,97 ± 2,36	ns	64,13 ± 6,36	63,05 ± 3,01	64,97 ± 2,27	ns	
Abdominal fat (%)	1,01 ± 0,54	1,28 ± 0,76	1,26 ± 0,63	ns	0,60 ^a ± 0,30	1,13 ^b ± 0,44	1,82 ^c ± 0,42	*	

ns non significant (p>0,05) ; ^{a,b,c} Means in the same row with different superscript differ (p<0,05) significance; ⁿ interaction

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06. EFFORTS FOR THE MEAT QUALITY OF BALI DUCKS THROUGH OFFERING PURPLE SWEET POTATO (*IPOMOEA BATATAS L*) FERMENTED *ASPERGILLUS NIGER* IN DIETS

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Abstract

An experiment was carried out to determine efforts for improving meat quality of Bali ducks through offering purple sweet potato (*Ipomea batatas L*) fermented by *Aspergillus niger* in diets. The experiment was designed using a completely randomized design (CRDD) with seven treatments consisted of: (A) diet without purple sweet potato; (B) diet containing 10% purple sweet potatoes without fermentation; (C) diet containing 20% purple sweet potatoes without fermentation; (D) diet containing 30% purple sweet potatoes without fermentation; (E) diet containing 10% fermented purple sweet potatoes; (F) diet containing 20% fermented purple sweet potatoes; and (G) diet containing 30% fermented purple sweet potatoes. Each treatment consists of four ducks with homogenous age and body weight. The variables observed were physical meat quality (meat colour, water holding capacity, and cooking loss), chemical meat quality (water concentration, protein, and fat), and meat characteristics (colour, flavor, and texture). The study showed that diet containing 10% up to 30% with or without fermentation could increase physical meat quality ($P < 0.05$), but in B treatment no significant differences found in the water holding capacity of meat ($P > 0.05$). However, pH meat did not affect compared to the treatment in A diet. Ducks fed with or without fermented purple sweet potato diets (*Ipomea batatas L*) will produce a better texture of meat, characterized by wider *endomysium* and *perimysium* with offered of purple sweet potato (*Ipomea batatas L*) diets is better than A diet. It can be concluded that offered of purple sweet potato (*Ipomea batatas L*) diets could increase meat quality of Bali ducks..

Keywords: *fermented purple sweet potato (Ipomea batatas L), meat quality, chemical meat quality, organoleptic, and bali ducks*

INTRODUCTION

Duck is a source of animal protein which support society needs, but putrid smell, greasy and tough meat will be produced when ducks get older (Setyawardani *et al.*, 2001). These problems can be revealed with fermented purple sweet potato in diets. Yadnya and Trisnadewi (2011) conveyed that fermented purple sweet potato (*Ipomea batatas L*) could increase nutrients containing 3.97% to 3.97% of protein content, 0.69% to 0.31% of fat, 4.53% to 2.99% crude fiber, especially *tannin* and *cellulose* content could significantly decrease ($P < 0.05$). If large number of potatoes were used, so these should be formed in fermentation to fulfill nutrients requirement of protein.

Fermentation of purple sweet potato (*Ipomea batatas L*) could improve nutrients and contain antioxidant (Ishida *et al.*, 2000). Antioxidant could neutralize free radicals to inhibit oxidation of unsaturated fatty acid. Hustiany (2001) mentioned that putrid smell of duck meat derived from the process of lipid oxidation. Duck meat contains high fatty acids. Unsaturated fatty acids are susceptible oxidation materials which produce free radicals. These free radicals caused peroxides emerged. Peroxides will

decompose and produce compounds of aldehydes, alcohol, keton, carboxylic acids and hydrocarbons with specific odor. Lipid oxidation could be prevented by using antioxidant.

Febrina (2006) conveyed that a young male duck meat fed with 1% additional of dry *beluntas* leaves could produce less putrid smell compared to those without *beluntas* leaves. Randa (2007) tried giving synthetic antioxidant of vitamin C and E to young male ducks that caused less putrid smell than without antioxidant. Greasy meat is less popular for consumer since it could cause high susceptible of cholesterol and diseases, such as: hypertension, coronary attack, atherosclerosis (Hasim and Jusuf, 2008). It is necessary for food to contain antioxidant in order to reduce fat level of cholesterol, as those found in purple sweet potato (*Ipomea batatas L*) since it contains 110 mg/ 100 g up to 209 mg/ 100 g fresh weight (Suprpta *et al.*, 2003). Besides, it is also containing vitamin A, C, and E as antioxidants and completed with Ca, P, Fe, Mg and Se (Ratih, 2010). Antioxidants in purple sweet potato could inhibit oxidation of fat by free radicals (Kumalaningsih, 2007). In that case, fat will be bound by antioxidants and others are excreted into feces so fat absorbed and fat content in meat could be decreased.

An old duck often produce putrid smell, greasy and tough meat. In order to solve these problems, so its meat could be treated in pre and post harvest (Murtidjo, 1988). Before in post harvest or during the process of rearing, especially in ration formulation could be carried out i.e. adding antioxidant. Ducks will produce more collagen tissues which could cause tough meat when they are getting older. Thus, additional of antioxidants in diets is essential to inhibit biosynthesis of collagen tissue (Boniface *et al.*, 1982). In order to determine its histology of meat structure related with meat tenderness, so diameter of collagen fiber tissues, *endomysium*, *perimysium*, and *meatepimisium* are needed to be examined.

Based on the above descriptions, the research were carried out entitled: “Efforts for improving the meat quality of Bali ducksthrough offering purple sweet potato (*Ipomea batatas L*) fermented by *Aspergillus niger*in Diets”.

MATERIAL AND METHODS

Place and Period of Experiment

The experiment was conducted for 16 weeks at Guwang village, Gianyar regency, Bali. Meanwhile, determination of physical and organoleptic meat qualities conducted for 4 weeks in the Laboratory of Animal Result Technology, Faculty of Animal Science, Udayana University. Meat chemical quality conducted for 2 weeks in the Laboratory of Chemical and Microbiology, Faculty of Agriculture Technology, Udayana University. However, meat structure with Histology method was conducted for 2 weeks at Balai Besar Veteriner, Pegok, Denpasar, Bali.

Materials and Equipment

This research was using 16 weeks of age male Bali ducks owned by I Wayan Pegeg, at Guwang village in which duck breeders obtained from Bringkit, Badung regency.

Tubers of purple sweet potatoes (*Ipomea batatas L*) obtained at Banyuwangi, whereas *Aspergillus niger* from the Institute of Agriculture Technology (BPTP), Denpasar.

The diets composed were based on nutrient content which recommended in Scott *et al.* (1982) using yellow corn, coconut meal, soybean, purple sweet potato, premix, and NaCl. Meanwhile, analysis of nutrient contents in purple sweet potatoes based on Yadnya and Trisnadewi (2011). Materials and composition were presented in Table 1 and Table 2. Diets and water were fed in *ad libitum* with source of water taken from the local drinking water firm (PDAM).

This study was using birds and treatments in a completely randomized design. The treatments were (A) control treatment; (B) diet containing 10% purple sweet potatoes; (C) diet containing 20% purple sweet potatoes; (D) diet containing 30% purple sweet potatoes without fermentation; (E) diet containing 10% fermented purple sweet potatoes; (F) diet containing 20% fermented purple sweet potatoes; and (G) diet containing 30% fermented purple sweet potatoes. Each treatment consists of four replications with four ducks per replications.

Table 1. Feed Composition of ducks (16- 32 weeks of age)

Ingredients (%)	Treatment ¹⁾						
	A	B	C	D	E	F	G
Yellow corn	55,36	49,98	42,32	35,5	49,98	42,32	37,20
Soybean	9,37	12,45	13,88	15,40	12,45	13,88	15,40
Coconut meal	11,31	9,82	7,28	3,06	9,82	7,28	3,06
Fish meal	10,13	8,10	10,29	11,14	8,10	8,29	8,14
Rice bran	13,26	9,00	5,56	4,25	9,00	7,58	5,25
Coconut oil	1,00	-	0,50	1,00	-	1,00	1,00
Premix	0,50	0,50	0,50	0,50	0,50	0,50	0,50
NaCl	0,15	0,15	0,15	0,50	0,15	0,15	0,15

Variable Measurement

The variables were measured as follows:

1. Observation of physical meat quality was based on USDA (1977); meat cooking loss measured with heat method (Soeparno, 2005); water holding capacity (WHC) measured with Centripuge Clement 2000 (Soe[parno,2005) and meat texture method measured with histology method (Luna, 1968).
2. Chemical meat quality was observing water concentration with heat method (AOAC, 1979); acidity (pH) determined with standard of pH-meter method (Apyrantono *et al.*,1989); protein concentration based on Kjedral (AOAC, 1979); Ether extract measured with extraxy soxhlet method (AOAC, 1979).
3. Organoleptic observation was based on subjective method (Larmond, 1977).

Table.2 Chemical Composition of Ducks (16 – 32 weeks of age)

Nutrient	Treatment ¹⁾							Standard ²⁾
	A	B	C	D	E	F	G	
Metabolic Energy (Kcal/kg)	2907.07	2878,2	2904.93	2814,0	2886,1	2912.25	2905.2	2800 - 2900
Crude Protein (%)	17.03	16,68	17,18	16,97	16,67	17.01	16.99	15 - 17
Ether Extract (%)	5,75	5,92	5,61	5,38	5,85	5,84	5,17	4 – 7
Crude Fiber (%)	4,56	4,42	4,20	4,00	4,36	4,23	4,0	4 – 7
Calcium (%)	1,00	0,94	0,97	0,96	0,94	0,92	0,91	0,80
Phosphor available(%)	0,60	0,50	0,50	0,50	0,51	0,50	0,50	0,70
Methionine(%) + Cystine (%)	0,82	0,86	0,87	0,90	0,80	0,85	0,86	0,55
Lysine (%)	1,37	1,35	1,41	1,43	1,34	1,28	1,34	0,80
Methionine (%)	0,52	0,56	0,59	0,71	0,57	0,61	0,65	0,30

Note:

¹⁾ A : Control treatment (without purple sweet potato), B : diet containing 10,0 % purple sweet potatoes, C: diet containing 20,0 % purple sweet potatoes, D : diet containing 30,0 % purple sweet potatoes, E: diet containing 10,0 % fermented purple sweet potatoes, F : diet contain 20,0 % fermented purple sweet potatoes, and G : diet containing 30,0 % fermented purple sweet potatoes

²⁾ Scott *et al.*(1982)

Statistical Analysis Method

Data collected was analyzed statistically using variance analysis. The analysis will be continued by using Duncan’s multiple range tests to compare two treatment means in case statistical differences were found (Steel and Torrie, 1989).

RESULT AND DISCUSSION

Characteristic of Physical Meat Quality

The variables of physical meat quality characteristic observed were colour, cooking loss, and water holding capacity. The observation was conducted at the end of the experiment. Meat colour was determined by using USDA chard (1977). The study showed that meat colour in control treatment (A) was 3.33 (see in Table. 3). The score of meat colour in treatment B, C, D, E, F, and G were significantly increased compared to control treatment (P<0.05). Purple sweet potatoes with or without *Aspergillus niger* fermentation contain *antocyanin* and *carotenoid* pigment which could increase *myoglobin* accumulation.

The pigment tissues caused a red colour of meat. A meat colour consumed by consumer is bright red derived from oxymyoglobin pigment and range of meat colour is 4 – 6 (Lawrie, 1995).

It showed that in Table 3, water holding capacity (WHC) was 51.64%. Diet in treatment B did not affect WHC of duck meat (P>0.05). However, water holding capacity (WHC) in treatment C, D, E, F, and G could increase as of: 2.92; 3.96; 5.67; 6.71; and 8.05 compared to treatment A. WHC effected meat containing protein and pH (Soeparno, 2005). Increase of WHC could apply water to meat which causes an increase of protein, so it could also improve WHC. Meat has its capability to bring water molecule, depend

Table 3. Meat quality of Bali Ducks implemented Purple Sweet Potato Diets (*Ipomea batatas L*) with fermented *Aspergillus niger*

Variables	Treatments ¹							SEM ³
	A	B	C	D	E	F	G	
Colour	3,33 ^c	4,00 ^b	4,16 ^b	4,33 ^{ab}	4,33 ^{ab}	4,83 ^a	4,83 ^{a2}	0,19
Water Holding Capacity (%)	51,64 ^d	52,13 ^d	53,15 ^c	53,69 ^c	54,57 ^b	55,11 ^a	55,80 ^a	0,25
Cooking loss(%)	33,84 ^a	33,35 ^{ab}	32,99 ^b	32,8 ^{bc}	32,37 ^{cd}	31,98 ^d	31,62 ^c	0,20
Texture meat(μm)								
Endomesium	8.77 ^c	9.25 ^c	12,80 ^{bc}	13.54 ^a	11,21b ^c	13.01 ^a	14,76 ^a	0,85
Perimesium	^c 25,23 ^e	31,24 ^{de}	58,58 ^c	76,84 ^d	44,51 ^{cd}	88 ^{ab}	95,54 ^a	4,74

Note:

- 1) Treatment A : control treatment (without purple sweet potato); diet containing 10%, 20%, and 30% purple sweet potatoes without fermentation (B, C and D treatments); diet containing 10%, 20% dan 30% fermented purple sweet potatoes (E, F, and G treatments).
- 2).Different superscript on the same row is significant differences (P<0.05)
- 3). SEM : Standard Error of the Treatment Means.

the amount of protein activity (Purnomo and Palaga, 1989). Cooking loss (CL) of duck meat score with control treatment was 33.84% (see in Table 3.). Those offered with treatment B did not affect CL of duck meat, whereas with C, D, E, F, and G treatments could decrease CL of duck meat. Apparently, this is due to decrease of meat fat content. Cooking loss could be measured when there was decrease of WHC value. Lawrie (1995) conveyed that high cooking loss could produce more nutrients leak during the process of boiling.

Connective tissues composed around the muscles that contained perimiesium. The perimiesium is located between fasikuli, whereas endomesium surrounding muscle cells or muscle fibers. Fibers of each tissue, consists of: collagen fibers, very small endomiesium called reticulate.

In figure 1, histology structure of Bali duck meat musculus pectoralis profundus on longitudinal skeletal fibers microscope observation with 400x magnification. The endomesium of ducks meat implemented in A diet was 8.77 (see in Table 3). There were no effect found in the implementation of diets B and C (P>0.05), whereas within diets D, E, F, and G could significantly increase endomesium meat. The increase of endomesium meat might be caused by capacity content of antioxidant meat, so connective of meat fibers could easily loosen (see in attachment 1). Robert *et al.* (1979) reported that implementation of bilberry as antisianin source

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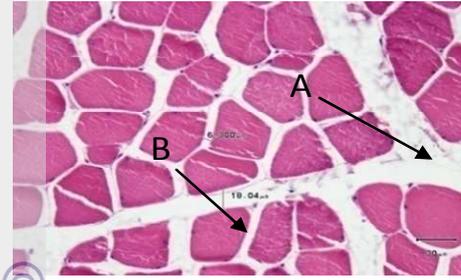


Figure 1.1 Histology A

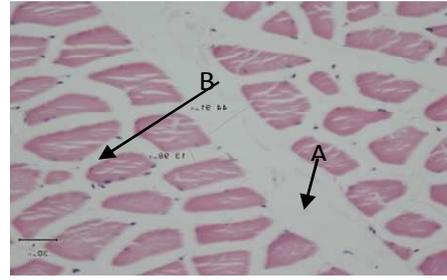


Figure 1.2 Histology B

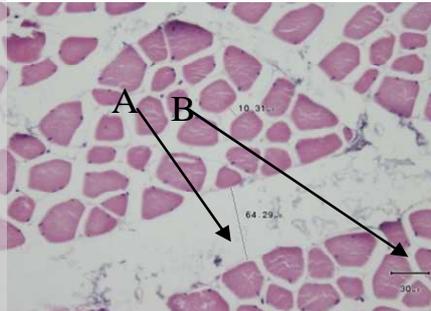


Figure 1.3 Histology C

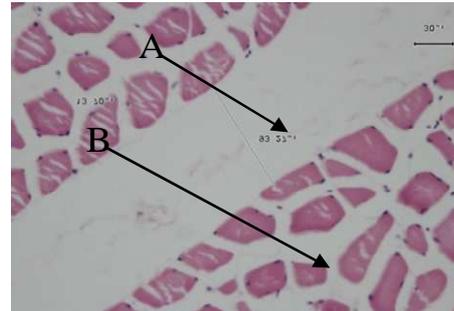


Figure 1.4 Histology D

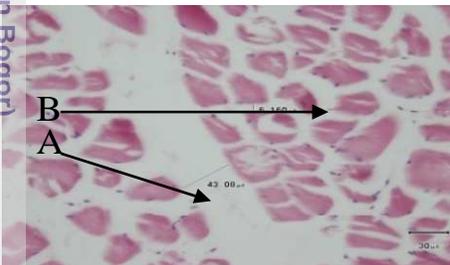


Figure 1.5 Histology E

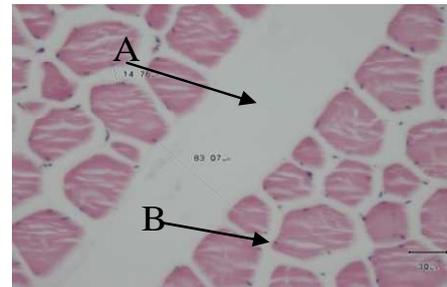


Figure 1.6 Histology F

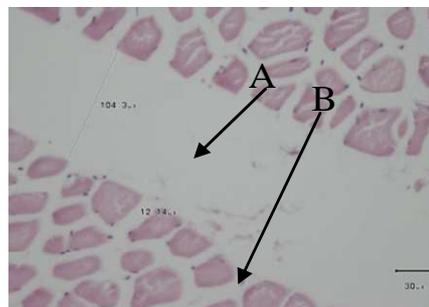


Figure 1.7 Histology G

Figure 1. Meat Texture with Histology Method
(A) Perimesium, (B) Endomesium

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could inhibit proteolytic enzymes such as elastase. It has a bound with collagen metabolism, particularly a cross bound on collagen fibers, and could reduce biosynthesis of collagen polymer (Boniface *et al.*, 1982).

Perimiesium of ducks meat in treatment A was 25.23 μ m (Table 3). There were no significant differences found in treatment B ($P>0.05$), whereas higher perimesium of meat were significantly ($P< 0.05$) found in C, D, E, F, and G treatments compared to treatment A. Easing that occurred on perimiesium and endomiesium due to the antioxidant capacity of the larger meat, followed by an increase in *superoxide dismutase* (SOD) meat . Endomiesium magnitude and perimiesium meat is determined by the species is, breed, and sex (Cacaci, 2007). Skeletal muscle fibers can have a diameter big size or longer because it is determined an increase in the amount of myofibril - myofibril constituent (Lawrie, 1995).

Organoleptic and Chemical Meat Qualities

The score of meat colour in treatment A was 5.40 (see in table 4). However, score of meat colour in B, C, D, E, and G treatments were significantly higher as of: 2.20; 4.25; 5.0; 5.37; 5.55; and 8.15% ($P<0.05$) compared to treatment A.

The score of meat flavor in treatment A was 5.40 (see in Table 4). Mean while, scores in treatment B, C, D, E, F, and G as of: 4.44; 5.00; 6.29; 7.41; and 8.33% respectively higher than treatment A. The decrease of putrid smell on ducks meat caused by the implementation of purple sweet potato diets since it contains compound of antioxidant that could prevent oxidation reaction by free radicals (Hustainy, 2001). Rukmiasih *et al.* (2011) conveyed that implementation of diets material which contain antioxidants could reduce the odor-off meat, including its aroma. This is totally different from putrid smell of chicken meat that caused by *Trimethyl amine* (Suranaya, 2010). *Trimethyl amine* is lipoprotein decomposition into choline, then choline reformed into *Trimethyl amine oxide*, and changed into *Trimethyl amine dehydrogenase enzyme*. *Trimethyl amine* could not be converted into N-oxide because there is a deficiency of *monooxygenase flavin* enzyme in the liver (Yuliana, 2001).

The score of duck meat taste in treatment A was 5.02 (see in table 4.), whereas in B, C, D, E, F, G could significantly increase duck meat flavor as of: 9.76; 11.55; 12.35; 12.74; 13.54; and 15.13 % ($P<0.05$). Organoleptic assessment of duck meat flavor showed that treatment G is the most preferable treatment. This is due to the lowest shrinkage of duck meat cooking, so non-protein substance which dissolves in water and fat, as a greater precursor of meat flavor among other treatments (Soeparno, 2005). Winarno (1986) mentioned that the taste of meat is determined by its smell, taste, and flavor. Antioxidants in diets fermented sweet purple potatoes could reduce oxidation by free radicals. In that case, less saturated fatty acids are oxidized could reduce off-odor intensity.

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Table 4. Bali ducks meat quality implemented with diet containing fermented purple sweet potato (*Ipomoea batatas* L)

Variable	Perlakuan							SEM
	A	B	C	D	E	F	G	
1. organoleptic quality								
Colour	5,40 ^e	5,52 ^d	5,63 ^c	5,67 ^{bc}	5,69 ^{bc}	5,74 ^b	5,84 ^a	0,02
Smell	5,40 ^d	5,64 ^c	5,67 ^{bc}	5,74 ^{abc}	5,76 ^{abc}	5,80 ^{ab}	5,85 ^a	0,04
Taste	5,02 ^c	5,51 ^b	5,60 ^{ab}	5,64 ^{ab}	5,66 ^{ab}	5,7ab	5,78 ^a	0,08
Total Texture	5,47 ^c	5,58 ^{bc}	5,66 ^{abc}	5,72 ^{abc}	5,75 ^{ab}	5,77 ^{ab}	5,86 ^a	0,08
	5,37 ^f	5,49 ^e	5,55 ^d	5,59 ^{cd}	5,62 ^{bc}	5,67 ^{ab}	5,69 ^a	0,02
2. Chemical Meat Quality								
Water (%)	56,26 ^a	56,31 ^a	56,24 ^a	56,27 ^a	56,36 ^a	56,51 ^a	56,38 ^a	0,09
Protein (%)	29,41 ^d	31,44 ^c	31,71 ^{bc}	31,73 ^{bc}	32,41 ^{ab}	32,56 ^{ab}	32,73 ^a	0,24
Fat (%)	12,17 ^a	11,11 ^{bc}	11,02 ^{bc}	11,46 ^b	10,74 ^c	10,69 ^c	10,62 ^c	0,22
pH	5,52 ^a	5,54 ^a	5,63 ^a	5,65 ^a	5,49 ^a	5,60 ^a	5,60 ^a	0,04

Note:

- 1) Diet without purple sweet potato (treatment A); diet containing 10% without fermented purple sweet potatoes (treatment B); diet containing 20% without fermented purple sweet potatoes (treatment C); and diet containing 30% without fermented purple sweet potatoes (treatment D); diet containing 10% fermented purple sweet potatoes (treatment E); diet containing 20% fermented purple sweet potatoes (treatment F); and diet containing 30% fermented purple sweet potatoes (treatment G).
- 2) Different superscript in the same rows indicate significant differences ($P < 0,05$)
- 3) SEM : *Standard Error of The Treatment Means*

The score of duck meat texture in treatment A was 5.47 (see in table 4). Mean while, ducks obtained B, C, and D diets achieved a better score but not significantly different ($P > 0,05$), whereas diets in treatment E, F, and G could improve the meat texture, i.e. 5.12; 5.26; and 6.03%, respectively ($P < 0,05$) compared to A treatment. Yadnya *et al.* (2012) found that implementation of *salam* leaves to ducks could produce a better meat texture in organoleptic compared to those without *salam* leaves. This due to *salam* leaves content flavonoid as an antioxidant that could assist in severing the bound of sulfahidril (-SH), so collagen will change into a digestible elastin (Gerindra, 1990). Tenderness of meat is determined by 3 components, such as: myofibrils and its status of contraction, connective tissue content of cross linking, water holding capacity of protein in meat juice (Soeparno, 2005).

The study showed that total acceptance of duck meat in treatment A was 5.37 (see in table 4). However, in B, C, D, E, F and G treatments could significantly achieve higher score ($P < 0,05$) compared to treatment A. Ducks in G treatment achieved the highest score compared to other treatment. Sutji and Sulandra (1994) conveyed that the total acceptance is a combination from some variables of organoleptic test on meat product, so the existence of a better meat variables component could improve the total acceptance scoring.

Chemical meat test observed in this study consists of: water content; pH; protein; and lipids. Moisture content of duck meat in treatment A was 56.26% (table 4),

where as in B, C, D, E, F and G did not affect of meat compared to treatment A ($P>0.05$).

pH duck meat in diet A was 5.62 (see in table 4), whereas in B, C, D, E, F and G treatments did not affect on pH duck meat compared to treatment A ($P>0.05$). pH meat alteration is caused by glycogen muscle reserve, it might be similar to pH score. This is due to water content of meat score nearly similar, so the number of H^+ ion of meat is equal because $pH = -\text{Log } H^+$ (Winarno, 1986), so the result of pH is equal. This result of study obtained pH meat in normal range: pH5.49 – 5.65 as the same condition found by Soeparno (2005).

Protein content of duck meat in treatment A was 29.41% (see in table 4). Meanwhile, in B, C, D, E, F and G treatments could increase protein content as of: 6.90; 7.82; 7.88; 10.20; 11.05; and 11.08% compared to A treatment ($P<0.05$). The highest protein content on ducks was in G treatment, since the implementation in this treatment has higher diet digestibility and protein diet digestibility. In that case, nutrient and protein substances could be absorbed which could also increase protein of meat. Soeparno (2005) conveyed that meat protein content determined by the diets protein content and livestock traits.

Fat content of ducks meat in diet A was 12.17% (see in table 4), whereas in B, C, D, E, F and G treatments significantly produced lower meat fat content compared to treatment A. The decrease of meat fat content caused by antioxidants contain in purple sweet potatoes which could inhibit oxidation by free radicals (Ischida *et al.*2000). Then, fat will be bound by antioxidants and some of them are excreted in feces, so fat absorbed will decrease and affect the decrease meat of fat content. Yadnya *et al.* (2012) reported the implementation of *Syzygium polyanthum* walp as antioxidant source could increase meat protein content and reduce fat. In addition, antioxidant could significantly increase protein digestible in the body and meat protein content.

CONCLUSION

Based on this study, it can be concluded as follows:

1. Diet containing 30% fermented purple sweet potatoes could improve physical quality of meat, especially the increase of colour, water holding capacity, endomisium, and perimisium. However, it could decrease the cooking loss.
2. Diet containing 30% fermented purple sweet potatoes could improve chemical and organoleptic qualities of Bali duck meat.

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07. EFFECT OF AVOCADO SEED MEAL AND FERMENTED OF BANANA PEEL MEAL TO LAYING QUAIL

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Abstract

Quail is a type of poultry that has the potential to be developed as a good egg producers, but the the problem of the high price of feed ingredients. Avocado seeds and a banana peel waste may be used as unconventional feed of energy sources to reduce corn in the diet of quail it can to lower costs. The avocado seed soaked by rice husk ash filtrate for 48 hours, then be dried and processed to be meal. The banana peel fermented by EM4 (dose 15 ml/100g) for 6 days, then be dried and processed to be meal . The research using quail aged 5 weeks maintained for 8 weeks by using completely randomized design (CRD) with 6 treatments and 4 replications. 6 treatments consisting of (A = control, B = 20% Avocado seed meal, C = 20% Banana peel meal, D = 10% avocado seed meal+10% banana peel meal, E = 15% avocado seed meal+5% banana peel meal and F = 5% avocado seed meal+15% banana peel meal), If there is a real difference followed by DMRT. Parameters observed in this research were feed consumption, Hen day production and age of sexual maturity. The results showed that the consumption of quail on diets C and F are not significantly different from the control, whereas feed consumption B, D and E was lower ($P < 0.01$) in the control. Hen day production on C is not significantly different from the control and hen day production at the B, D, E and F lower ($P < 0.01$) than control. This study also shows that the age of sexual maturity at C, D and F not significantly with the control, while B and E for longer than control ($P < 0.01$).

Keywords: consumption, hen day production, age of sexual maturity, ASM (Avocado Seed Meal), BPM (Banana Peel Meal)

INTRODUCTION

Quails one of various types of poultry have good prospects to be developed because the quail have good produce of egg. On maintenance equail, farmers have been facing the problem of high feed prices because most materials of feed are still imported and food availability are limited, It is encouraging farmers to look for alternative feed ingredients or feed materials that are unconventional waste or agricultural by-product. One of the waste that can be used is an avocado seed and banana peels can be used as one source of energy feed stuffs.

Seed avocado and banana peels have the potential to be used as energy source feed. According to Centarl Agency Statistic (2011), West Sumatra Province in 2010 produced as much as 29 457 tons of avocado fruit, banana fruit 160 516 tonnes while that avocado seed produced approximately 7,364 tonnes and 48 154 tonnes of banana peel, it because avocado seed weight is 25% of the avocado and banana peels is 30% of the banana.

Avocado seed in poultry can reduce the use of corn and rice bran. According Djulardi (1997) the metabolism energy of the avocado seed is 3570 kcal/kg, however the use of avocado seed as a feed ingredient in the diet of quail cannot directly because of the avocado seeds contain high tannins (1.47%). Poultry can tolerate only 0.5% tannin (Wahyu, 1997), so as to reduce levels of tannin avocado seed processing is done

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before and as soaking with water or alkali solution. Rice husk ash filtrate can be used as a substitute for NaOH alkaline solution is expensive and easy to obtain.

In addition to other waste avocado seeds can also be used as poultry feed is a banana peel. Banana peels have gross energy content of 4363 Kcal / kg and 8.36% crude protein and very high in vitamin A, especially pro-vitamin A, beta-carotene 45 mg per 100 g dry weight (Nurkholis, 2005). Utilization of banana peel in the diet of quail could not be maximum because the crude fiber content is high enough that 15% (Susilowati, 1997), then to lower crude fiber content and improve the nutritional value of banana peels can be done with fermentation. Fermented with EM 4 can lower crude fiber of feed ingredients. The use of waste avocado seeds are soaked with rice husk ash filtrate and banana peels fermented with EM4 to laying quail as feed ingredient energy sources can reduce consumption of corn to lower the cost of feed.

MATERIALS AND METHODS

Avocado seeds used in quail diets is avocado seeds soaked with rice husk ash filtrate 30% for 48 hours, then dried and processed to be meal, called the avocado seed meal (ASM). Banana peels used are banana peels fermented with EM4 for 6 days at a dose of 15ml/100grams, which is then dried and processed to be meal called banana peel meal (BPM).

Quails used in this research were 240 quails aged 5 weeks were placed in battery cages as many as 24 units with size of 30 cm x 40 cm x 30 cm. At cage unit consists of 10 quails. Quail diets will be prepared with 20% crude protein and metabolism energy (ME) 2800 kcal.

The diets treatments were:

- A : control (without ASM and BPM)
- B : 20% ASM + 0% BPM
- C : 0% ASM + 20% BPM
- D : 10% ASM + 10% BPM
- E : 15% ASM + 5% BPM
- F : 5% ASM + 15% BPM

Research methods using experimental methods completely randomized design with 6 treatment diets (A, B, C, D, E and F) and 4 replications. The mathematical model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + \epsilon_{ij}$$

Variables measured in the research were:

1. Diet Consumption (g / quail)
2. Hen Day Production (%)

$$\text{Hen day production} = (\text{Total of eggs} / \text{Total of quails}) \times 100\%$$

3. Age of Sexual Maturity.

The diet of quail in this research presented in the Table 1.

Table 1 : The diet of quail

feed ingredients	Treatments					
	A	B	C	D	E	F
Yellow corn (%)	42	21.5	23.5	21.5	21.5	23.5
Rice bran (%)	15	15	13	15	15	13
Soybean meal (%)	20	20	20	20	20	20
Fish meal (%)	15	15	15	15	15	15
ASM (%)	-	20	-	10	15	5
BPM (%)	-	-	20	10	5	15
Coconut oil (%)	3.5	4	4	4	4	4
Stone meal (%)	4	4	4	4	4	4
Topmix (%)	0.5	0.5	0.5	0.5	0.5	0.5
Jumlah (%)	100	100	100	100	100	100
Nutrient Content of diet						
Metabolism energy (Kkal /kg)	2844.4	2830.4	2834.0	2817.2	2823.6	2835.9
Crude Protein (%)	20.27	20.01	20.33	20.31	20.07	20.26
Crude Fiber (%)	4.22	4.75	6.33	5.65	5.19	5.90
Crude Fat (%)	6.02	7.83	7.32	7.50	7.64	7.42
Ca (%)	2.58	2.56	2.67	2.63	2.59	2.63
P (%)	0.81	0.83	0.86	0.85	0.84	0.85

RESULTS AND DISCUSSION

The average consumption, hen day production, and age of sexual maturity in this research presented in Table 2.

Table 2. Average Feed Consumption, Hen Day Production, and Age of Sexual Maturity

Treatments	Parameter		
	Feed Consumption (g/quail/days)	Hen Day Production (%)	Age of Sexual Maturity (days)
A (control)	21,44 ^a	51,16 ^a	49,25 ^b
B (20% ASM)	14,08 ^c	5,02 ^d	71,75 ^a
C (20% BPM)	21,20 ^{ab}	43,04 ^{ab}	53,25 ^b
D (10% ASM, 10% BPM)	19,90 ^b	22,38 ^c	59,50 ^b
E (15% ASM, 5% BPM)	15,45 ^c	9,69 ^d	70,10 ^a
F (5% ASM, 15% BPM)	21,65 ^a	37,59 ^b	55,25 ^b
SE	0,46	3,32	3,18

Description: superscript different in the same row indicates significant different

(P < 0.05), and very significant (P < 0.01)

SE = Standard Error

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The Influence Avocado Seed meal and Fermented of Banana Peel Meal to Feed Consumption of Quail

Feed consumption C and F not significantly with control (A), this is because the control diet (A) and a diet containing 20% fermented of banana peel meal (C) and 15% fermented of banana peel meal (F) have the same level of palatability of diets so that consumption is also not significantly different. Banana peel meal fermented that has a nice smell and flavor for quail, thus reducing the use of corn substituted with BPM 20% and 15% in the diet had no effect on feed intake the quail. According to Scott et al. (1982) that consumption is influenced by the addition of nutrients and energy of feed, animal health, are also influenced by the smell and shape of diets.

Feed consumption B, D and E very lower significant than A (control) caused by tannins in avocado seed meal that cause the bitter taste resulting in lower levels of palatability that can reduce feed intake to quail. According with the opinion of Fuller (1967) which states that the bitter taste caused by the presence of tannins can lead to lower feed consumption of chicken.

The results of this research shows that the higher the level of avocado seed meal in quail diets decreased feed consumption. The use 5% Avocado seed meal in quail diets can still be tolerated by the quail, it is seen by the not significant feed consumption with control diets (A), while the level 20% fermented of banana peel meal in the diet quail did not reduce feed consumption the quail.

The Influence Avocado Seed meal and Fermented of Banana Peel Meal to hen day production of Quail

Hen day production in treatment C was not significantly different from A (control), this is due to the consumption of C and A are also not significantly different, so the nutrients are consumed for the production of eggs is the same. Hen day production in quail who consumed diets B, D, E and Flower significantly ($P < 0.05$) than (control), this is due to the low consumption because there are tannin content in the diet derived from avocado seeds. Low consumption causes nutrients consumed to produce not sufficient that lower hen day production. Although the tannin in seed avocado lowered by soaked with rice husk ask filtrate but soaked with rice husk ask filtrate in avocado seed meal still there are tannin 0,84%.

Table 2 show that hen day production to A (control) was significantly higher than F, although consumption between A and F were not significantly different that because is not only tannin in the seed avocado but there are others alkaloid that is triterpenoids cause lower hen day production to quail. The results Zuhrotun (2007) also found that triterpenoids in avocado seeds, further C haroon Pokphan (2007) explains that the 0.9% triterpenoids in the diet cause lower the growth and digestibility of fat. Hen day production in treatment B lower significantly ($P < 0.01$) than the others because of very low consumption and high tannin levels in the diet.

The Influence Avocado Seed meal and Fermented of Banana Peel Meal to age of sexual maturity of Quail

The sexual maturity of quail if the quail have period of production or laying, that are the quail to produce age the first time. Research result showed that the age of sexual maturity in quail consuming diets B and E longer significantly ($P < 0.01$) than the control (A), while C, D and F the same as with control, This is because the enough feed

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consumption in treatments C, D and F that the nutrients to produce enough in laying, that are sexual maturity treatments C, D and F not significant with control (treatment A). The quails who consumed diets B and E the feed consumption is very low so that the nutrients needed for production in laying period are not met so slows sexual maturity. The low consumption so causes weight gain does not meet the quail to produce, in accordance with the opinion of Abbas (1999) that sexual maturity in addition is influenced by animal health and food governance was also influenced by body weight. Low weight will cause a longer sexual maturity.

CONCLUSION

The use of avocado seed meal on quail laying diets only at the level of 5%, while the use of fermented banana peel meal can be up to 20% which can substitute 44% corn consumption.

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08. LAYER DUCKS PERFORMANCES FED KATUK LEAF MEAL(*Sauropus androgynus* L. Merr)

Widya Hermana^a, Mahareni Septyana^b and Sumiati^c

Abstract

The objectives of this study was to evaluate the effect of using katuk leaf meal in the diet on performances of local ducks. Forty-eight layer local ducks (*Anas domesticus*) of 21 weeks of old were divided into 4 diet treatments with 4 replications and 3 ducks of each replication. The ducks were raised during 6 weeks. The diet treatments were: R1 (diet without katuk leaf meal), R2 (diet contained 5% katuk leaf meal), R3 (diet contained 10% katuk leaf meal) and R4 (diet contained 15% katuk leaf meal). Parameters observed were feed consumption, energy consumption, protein consumption, duck day egg production, egg mass production, egg weight, and feed conversion. A Completely Randomized Design was used in this research. The data were analyzed using ANOVA and any significant difference was further tested using Duncan's multiple range test. The results showed that feeding 15% katuk leaf meal significantly decreased ($P<0.05$) feed consumption, energy and protein consumption, feed conversion as well as egg mass production, and highly significantly decreased ($P<0.01$) the duck day production. Feeding 10% katuk leaf meal significantly decreased ($P<0.05$) the feed conversion and highly significantly decreased ($P<0.01$) the duck day production. The treatments did not effect the egg weight. It was concluded that katuk leaf meal could be used 5% in the layer ducks diet without interfere the performances.

Key words: katuk leaf meal (*Sauropus androgynus* L. Merr), local duck, performances

INTRODUCTION

Katuk leaf meal (*Sauropus androgynus* (L.) Merr.) is known to contain protein, beta carotene, vitamin C, and vitamin E that important to improve the reproduction function (Subekti *et al.*, 2008). Aziz and Muktiningsih (2006) reported that in 100 grams katuk leaf contained 59 cal energy, 6.4 g protein, 1.0 g fat, 1.5 g fiber, 1.7 g ash, 233 mg Ca, 98 mg P, 3.5 mg Fe, 10,020 mcg vit A and 164 mg vit C. Katuk also contains volatile oil, sterol, saponin, flavonoid, organic acid, amino acid, alkaloid, and tannin (Malik, 1997).

Feeding katuk leaf meal increased the feed consumption and egg production and egg quality of laying hens, also increased carotene and vitamin A in egg, but decreased cholesterol content in yolk, meat and liver (Saragih, 2005).

The objectives of this study was to evaluate the effect of using katuk leaf meal in the diet on performances of local ducks

MATERIALS AND METHODS

The experiment used 48 layer local duck, 20 weeks of age, 1,144-1,538 grams of body weight. The birds rared during 6 weeks in 16 cages. Diet and water gave *ad libitum*. Katuk leaf was sun dried and ground to be mash form. Nutrient composition of katuk leaf meal (KLM) showed at Table 1.

The experimental diet was formulated isoenergy (2,850 kcal/kg ME) and isoprotein (16% CP) as recommended by Leeson and Summers (2005). Composition of layers duck's diet showed at Table 2. Nutrient and tannin content of layers duck's diet showed at Table 3.

The diet treatments were: R1 (diet without katuk leaf meal), R2 (diet contained 5% katuk leaf meal), R3 (diet contained 10% katuk leaf meal) and R4 (diet contained 15% katuk leaf meal). A Completely Randomized Design, with 4 treatments, 4 replication and 3 ducks was used in this research. The data were analyzed using ANOVA and any significant difference was further tested using Duncan's multiple range test. Parameters observed were feed consumption(g/bird/day), tannin consumption (g/bird/day), energy consumption (kcal/bird/day), protein consumption(g/bird/day), duck day egg production (%), egg mass production (g/bird), egg weight (g/egg), and feed conversion.

RESULTS AND DISCUSSION

Feed consumption of duck fed 5% katuk leaf meal (KLM) the same as control diet. Feed consumption decreased as increasing KLM level (10% and 15%). It might be due to the tannin content from KLM. Tannin consumption significantly increased ($P<0.05$) as increasing of KLM content. The energy and protein consumption also significantly decreased ($P<0.05$) as well as increasing KLM content in the diet.

Duckday production of R2 (5% KLM) was the same as control and highly significantly different ($P<0.01$) of R3 and R4. Egg mass production of R2 (5% KLM) was the same as control and significantly different ($P<0.05$) of R4 (15% KLM). Egg weight was not affected by treatment.

Feed conversion of R2 (5% KLM) was the same as control and significantly different ($P<0.05$) of R3 (10% KLM) and R4 (15% KLM).

Feeding 15% katuk leaf meal significantly decreased ($P<0.05$) feed consumption, energy and protein consumption, feed conversion as well as egg mass production, and highly significantly decreased ($P<0.01$) the duck day production. Feeding 10% katuk leaf meal significantly decreased ($P<0.05$) the feed conversion and highly significantly decreased ($P<0.01$) the duck day production.

CONCLUSION

Katuk leaf meal could be used 5% in the layer ducks diet without interfere the performances.

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Table 1. Nutrient composition of katuk leaf meal

Nutrient	Content
Dry matter (%)	82.41
Ash (%)	7.76
Crude protein (%)	33.11
Crude fiber (%)	15.52
Ether extract (%)	3.51
NFE (%)	22.51
Ca (%)	1.38
Total P (%)	0.44
Gross energy (kcal/kg)	4,028

Table 2. Composition of layers duck’s diet

Ingredient	R1	R2	R3	R4
Corn	56.00	56.00	56.00	56.00
Rice bran	13.00	10.02	7.75	6.00
Soybean meal	11.03	8.66	5.00	2.26
Fish meal	7.00	7.00	7.00	7.00
MBM	1.85	1.85	1.85	1.85
Katuk leaf meal/ KLM	0	5	10	15
Palm oil	4.20	4.70	5.63	5.7
CaCO ₃	6.32	6.16	6.01	5.60
L-lysine	0	0	0.09	0.14
Dl-methionine	0.10	0.11	0.17	0.21
Premix	0.50	0.50	0.50	0.24
TOTAL	100.00	100.00	100.00	100.00

R1 = Control diet without katuk leaf meal; R2 = Diet contained 5% katuk leaf meal; R3 = Diet contained 10% katuk leaf meal; R4 = diet contained 15% katuk leaf meal

Table 3. Nutrient and tannin content of layers duck’s diet

Nutrient	R1	R2	R3	R4
ME (kcal/kg)	2,851.84	2,852.12	2,862.28	2,852.52
Dry matter (%)	87.03	86.74	86.53	86.17
Crude protein (%)	16.07	16.30	16.05	16.28
Crude fiber (%)	3.59	3.86	4.11	4.50
Ether extract (%)	8.59	9.22	10	10
Ca (%)	3.00	3.00	3.00	2.90
Available P (%)	0.40	0.39	0.38	0.38
Lysine (%)	0.88	0.80	0.78	0.75
Methionine (%)	0.43	0.42	0.45	0.46
Meth+Cyst (%)	0.69	0.66	0.66	0.65
Linoleic acid (%)	1.76	1.64	1.54	1.47
Tannin’s KLM (%)	0.00	0.17	0.35	0.52

R1 = Control diet without katuk leaf meal; R2 = Diet contained 5% katuk leaf meal; R3 = Diet contained 10% katuk leaf meal; R4 = diet contained 15% katuk leaf meal

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Table 4. Average of layer duck performance during 6 weeks (20-26 weeks of age)

Parameters	R0	R1	R2	R3
Feed consumption (g/bird/day)	140.10±6.02 ^a	138.09±7.01 ^a	126.83±13.75 ^{ab}	113.64±15.63 ^b
Tannin consumption (g/bird/day)		23.82±1.21 ^a	43.75±4.74 ^b	58.81±8.09 ^c
Metabolizable energy consumption (kcal/bird/day)	399.54±17.17 ^a	393.87±19.94 ^a	361.71±39.23 ^{ab}	324.96±44.60 ^b
Protein consumption (g/bird/day)	22.51 ± 0.10 ^a	22.19 ± 0.11 ^a	20.38 ± 0.22 ^{ab}	18.26 ± 0.25 ^b
Duckday Production (%)	29.88 ± 8.45 ^A	31.23 ± 12.43 ^A	8.73 ± 5.26 ^B	7.66 ± 3.06 ^B
Egg mass production(g/bird)	16.85 ± 5.79 ^a	17.52 ± 7.31 ^a	9.53 ± 5.80 ^a	4.47 ± 2.43 ^b
Egg weight (g/egg)	59.45 ± 2.85	59.63 ± 5.32	58.22 ± 11.26	64.05 ± 1.31
Feed conversion ratio	9.09 ± 3.06 ^a	8.88 ± 3.48 ^a	16.67 ± 7.61 ^b	36.06 ± 29.89 ^c

R1 = Control diet without katuk leaf meal; R2 = Diet contained 5% katuk leaf meal; R3 = Diet contained 10% katuk leaf meal; R4 = diet contained 15% katuk leaf meal

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09. THE PROFILE OF CORN-COB NUTRIENT AS PROSPECTIVE POULTRY FEED IN UPPER AND LOWER LAND AREA IN WEST SUMATRA

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Abstract

The corn-cob is a waste from corn harvesting. The availability of corn-cob is abundance in West Sumatra province, and it could be as prospective poultry feed because it is still has the nutrient content. Now the corn-cob is a pollutant in environment since it is not used for another need. This research was conducted to analyze the corn-cob nutrient profile in lower land area (<250 m above sea level: Pesisir Selatan, Padang Pariaman, Pasaman Barat, and Dharmasraya), and in upper land area (> 250 m: Agam, 50 Kota, Tanah Datar and Solok) in West Sumatra Province. The corn-cob sample were collected from nine different locations in each location in upper and lower land area. The nutrient content of corn-cob: protein, crude fiber, Ca, P, the fiber profile of corn-cob: Acid detergent fiber (ADF), Neutral detergent fiber (NDF), cellulose, hemicelluloses and lignin were compared between upper and lower land area by using t-test. Furthermore the corn-cob samples from each lower and upper land area were composited and then they were analyzed for amino acid content by using HPLC, the fatty acid content by chromatografi and β -carotene and phytic acid by HPLC. It concluded that the nutrient content, fiber profile except cellulose, amino acid, β -carotene, phytic acid, and some of fatty acid of corn cob are higher in lower land area in comparing with in upper land area.

Keywords: corn-cob, upper land, lower land, poultry, feed

INTRODUCTION

The harvesting of corn grain will produce the corn cob as a by-product. It is a potential source for animal feed because it is still has the nutrient content that useful by animal. Iraslina (2004) found the chemical composition of the corn cob as follows: crude protein 3.03%, crude fiber 36.32%, ether extract 2.14%, nitrogen-free extract 56.56%, and ash 1.95%. According to Preston (2006) the corn cob contained crude protein 3.0%, crude fiber 36.0%, ADF 39.0%, NDF 88.0%, ether extract 0.5%, ash 2.0%, Ca 0.12%, P 0.04%, K 0.8%, S 0.4% and Zn 5 ppm.

West Sumatra Statistics (2011) stated, the corn production in West Sumatra was scattered in several areas (districts) with the three districts of higher production were Pasaman Barat, Pesisir Selatan and Tanah Datar. There is no information about the availability of corn-cob statistically in West Sumatra, but it is predicted the availability of corn-cob is higher in corn production district in several areas in West Sumatra province. According to Sudjana *et al.*, 1991, the corn-cob is 20 % from the whole corn.

The districts in West Sumatra are located in the different elevation ranging from 0 to >1000 m above the sea level. The corn plantation in West Sumatra is grown in two areas of different elevation (from 0 to <200 m above the sea level and >200 m above the sea level). The difference in elevation will bring up to the difference in temperature of the areas which in turn will affect the growth and development of plants in those areas. Swenson (1970) reported that the area at the higher elevation has the low temperature when compare to the area at the lower elevation, in which increases the elevation of the area every 100 m above the sea level, decreases the temperature of that area

approximately 0.65 °C. The elevation will affect the nutrient content of plant. Morecroft and Woodward (1996) found that elevation affected the crude protein content of the leaf in which it was higher in the high elevation compared with the low elevation. Meanwhile, the crude protein content of grass was higher in the lower land areas than those in the upper land areas (Aoetpah, 2002). According to Alam *et al.* (2010) the sugar content of cacao was higher in the lower land areas than those in the upper land areas. There is not to much information about the effect of elevation in nutrient content of the corn-cob. The aim of this study to analyze the nutrient content of corn-cob in upper and lower land area in West Sumatra as the prospective poultry feed.

MATERIAL AND METHODS

The experiment for analyzing nutrients profile of corn-cob in this research was divided in two different area; lower and upper land area. The lower land is an area that lies less than 250 m above sea level (Pesisir Selatan, Padang Pariaman, Pasaman Barat, and Dharmasraya), and upper land is an area that lies more than 250 m above sea level (Siam, 50 Kota, Tanah Datar and Solok). The corn-cob samples were collected from nine different locations in upper and lower land area. The nutrient content of corn-cob are protein, crude fiber, Ca, P were analyzed according to AOAC (2002). The fiber profile of corn-cob: acid detergent fiber (ADF), neutral detergent fiber (NDF), cellulose, hemicelluloses and lignin were analyzed according to Goering and Van-Soest (1970) and all data from both nutrient content and fiber profile were analyzed by t-test (Steel and Torrie, 1980). Furthermore the corn-cob samples from each lower and upper land area were composited and analyzed for amino acid content by using HPLC according to AOAC (2002), the fatty acid content by chromatografi according to Garces and Mancha (1993). Phytic acid was analyzed based on the colorimetric method developed by Gao *et al.* (2007), and β -carotene was determined according to Mathiasson *et al.* (2002).

RESULT AND DISCUSSION

Nutrient Content of Corn-cob in Lower Land Versus Upper Land of West Sumatra.

The analysis of corn-cob's nutrient content for crude protein, crude fiber, ether extract, calcium and phosphor was showed in Table 1. The crude fiber, calcium and phosphor of corn-cob were significant higher ($P < 0.05$) in lower land area in comparing with in upper land area, while for crude protein was significant higher ($P < 0.05$) in upper land area than in the lower land area, and ether extract was not different between lower and upper land area.

The higher protein content of corn-cob in upper land area was in accordance with the study that founded by Morecroft and Woodward (1996) that the nitrogen content of *Alchemilla alpina* leaf was higher in the high altitude in comparing with in lower altitude. The higher crude fiber in lower area was due to the temperature. The temperature reduced the vegetative phase and increased the generative phase of plants resulted in augmented the crude fiber synthesis. The higher of Ca and P contents of corn cob in the lower land areas was due to the leaching process in the upper land areas in

which it increased the acidity of soil in the upper land areas. The acid soil usually contains high Al and Fe which can bind P, so that P is not available for plants. The high in crude protein, crude fiber, Ca and P content of corn cob in the lower land areas was in accordance with the study by Fernando (2001) who found that crude protein, crude fiber, Ca and P contents of palm kernel cake from lower land areas was higher than that in the upper land areas.

Table 1. Nutrient content of corn-cob in lower land versus upper land of West Sumatra.

Nutrient Contents %	Lower Land	Upper Land
Crude Protein	3.16 ^b	4.93 ^a
Crude Fiber	39.88 ^a	26.48 ^b
Ether Extract	0.19 ^a	0.23 ^a
Calcium	0.47 ^a	0.36 ^b
Phosphor	0.03 ^a	0.01 ^b

^{a,b} Means with different superscripts at the same row indicated that they were significantly differed (P<0.05).

Fiber Profile of Corn-cob in Lower Land Versus Upper Land of West Sumatra

The analysis of fiber profile is showed in Table 2. The NDF and cellulose in the lower and upper land area did not differ significantly (P>0.05). However, the ADF and lignin contents of corn cob in the lower land were significantly higher (P<0.05) than that in the upper land of West Sumatra. Meanwhile, the hemicelluloses content of corn cob in the lower land was significantly lower (P<0.05) than that of the upper land of West Sumatra. Lignification was affected by temperature, daylength, light and plant stress (Van Soest, 1982). High temperature in the lower land areas will increase the rate of plant maturity. Mature plants contain more lignin than the immature plant of the same age. Onim *et al.* (2012) reported that altitude influenced the lignin content of grasses in which every 20 m reduction in elevation increased the lignin content of grasses 1%.

Table 2. Fiber profile of corn-cob in lower land versus upper land of West Sumatra

Fiber Components (%)	Lower Land	Upper Land
NDF	75.9 ^a	74.1 ^a
ADF	42.4 ^a	37.9 ^b
Cellulose	30.3 ^a	30.5 ^a
Hemicellulose	33.5 ^b	36.2 ^a
Lignin	10.3 ^a	7.6 ^b

^{a,b} Means with different superscripts at the same row indicated that they were significantly differed (P<0.05).

Amino acid profile of corn-cob in lower land versus upper land of West Sumatra

The amino acids profile of corn-cob are higher in upper land area in comparing with in lower land area (Table3).

Table 3. Amino acid profile of corn-cob in lower land versus upper land of West Sumatra

Amino Acids %	Lower Land	Upper Land
Aspartic acid	0.20	0.34
Glutamic acid	0.22	0.39
Serine	0.10	0.17
Histidine	0.03	0.06
Glycine	0.10	0.18
Threonine	0.08	0.14
Arginine	0.09	0.16
Alanine	0.13	0.24
Tyrosine	0.06	0.12
Methionine	0.01	0.04
Valine	0.12	0.21
Phenylalanine	0.10	0.18
Isoleucine	0.10	0.17
Leucine	0.19	0.34
Lysine	0.14	0.23

All of the amino acids contents of corn cob in the upper land areas of West Sumatra were numerically higher than those in the lower land areas. This result was in accordance with the crude protein content of corn cob, in which it was also higher in the upper land areas than that in the lower land areas. The height in crude protein content of corn cob in upper land areas resulted in the increase in its amino acids content.

Fatty Acid Profile of Corn-cob in Lower Land Versus Upper Land of West Sumatra.

The fatty acid profile of corn-cob is showed in Table 4. Some of fatty acid of corn-cob in upper land area such as myristic acid, palmitic acid, heptadecanoic acid, stearic acid, elaidic acid, oleic acid, linoleic acid, arachidic acid, cis-11-eicosanoic acid, linolenic acid, behenic acid, tricosanoic acid, lignoceric acid, and fat conternt are higher in upper land are in comparing withlower land area, while the fatty acids erucic acid, is higher in lower land area in comparing with upper land are, and the lauric acid fatty acid, pentadeconic fatty acid, cis-10-Heptadecanoic acid is the same as in both upper and lower land area.

Thus, most of fatty acids contents of corn cob in the upper land were higher than those in the lower land areas. It was in accordance with the ether extract content of corn cob in the upper land that was also numerically higher than that in the lower land areas. While, Mountousis *et al.* (2006) found the variation of the effect of altitude on the ether extract content of herbages in Northern Greece.

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Table 4. Fatty acid profile of corn-cob in lower land versus upper and of West Sumatra.

Fatty Acids	Lower Land	Upper Land
Lauric acid, C12:0	0.10	0.10
Myristic acid, C14:0	0.28	0.36
Myristoleic acid, C14:1	Nd	Nd
Pentadecanoic acid, C15:0	0.18	0.18
Palmitic acid, C16:0	7.93	12.23
Palmitoleic acid, C16:1	0.10	0.19
Heptadecanoic acid, C17:0	0.33	0.42
Cis-10-Heptadecanoic acid, C17:1	0.04	0.04
Stearic acid, C18:0	3.33	4.78
Elaidic acid, C18:1n9t	0.03	0.05
Oleic acid, C18:1n9c	4.82	6.62
Linoleic acid, C18:2n6c	2.57	4.02
Arachidic acid, C20:0	0.75	0.94
Cis-11-Eicosanoic acid, C20:1	0.09	0.23
Linolenic acid, C18:3ω3	0.12	0.22
Heneicosanoic acid, C21:0	0.10	0.08
Cis-11, 14-Eicosadienoic acid, C20:2	0.05	0.03
Behenic acid, C22:0	1.19	1.20
Erucic acid, C22:1n9	0.13	0.11
Tricosanoic acid, C23:0	0.56	0.59
Cis-13, 16-Docosadienoic acid, C22:2	Nd	0.04
Ignoceric acid, C24:0	1.62	1.72
Cis-5,8,11,14,17-Eicosapentaenoic acid, C20:5ω3	0.07	0.03

β-Carotene and Phytic Acid of Corn-cob in Lower Land Versus Upper land of West Sumatra.

The β-carotene and phytic acid contents of the corn cob in the lower land versus upper land areas of West Sumatra is presented in Table 5. The β-carotene and phytic acid contents of the corn cob in the lower land areas were numerically higher than that in the upper land areas

Table 5. β-carotene and phytic acid of corn-cob in lower land versus upper land of West Sumatra.

Chemical Composition	Lower Land	Upper Land
β-carotene (ppm)	131,8	102,4
Phytic acid (ppm)	3700	3300

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CONCLUSION

The crude protein content, most amino acids and fatty acids content of corn cob in the upper land areas were higher than those in the lower land areas. Meanwhile, the crude fiber, fiber fractions, β -carotene and anti nutrient (phytic acid) in the lower land of the West Sumatra were higher than those in the upper land areas.

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10. PERFORMANCE OF BROILER CHICKS FED WASTE CANNED FISH OIL IN RATION

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Abstract

This research was conducted to study the effect of supplementing waste canned fishoil (WCFO) in diet on broiler performance. One hundred three-weeks broiler chicken were randomly assigned to twenty pen to receive four dietary treatments with five replicates (5 birds/replication). By using Completely Randomized Design (CRD), the treatments were conducted as follows: R₀ = Ration contains 4% WCFO; R₁ = Ration contains 6% WCFO; R₂ = Ration contains 8% WCFO; R₃ = Ration contains 10% WCFO. Data analyzed with Analysis of variance (ANOVA) and Honestly Significant Difference (HSD) (Kusriningrum, 2008). Water and feed were provided ad libitum. The feed stuff material consisting of corn, soybean oil cake, fish meal, coconut oil cake, ground bran, crude bran, mineral, DL methionine and lysin. Ration is arranged according to broiler's life period. The result showed that there were no significant difference (P > .05) in feed consumption. Supplementation of waste canned oil fish increased (P < .05) body weight gain and feed efficiency until 8%. Among the treatment, 8% waste canned oil fish revealed the best results with the highest body weight gain and feed efficiency.

Keywords: waste canned fishoil, feed efficiency of broiler.

INTRODUCTION

Indonesian societies consumption pattern for animal production, especially for meat consumption was faced a problem, on one hand meat consumption per capita is still low, but in the other hand some consumer disposed to limit consumption of poultry meat caused the opinion that fat would make negative effect on food quality (cholesterol-phobia). Foods for healthy live recommendations were risen in many countries, and even WHO as an International Institution was extend instruction to peoples to consumption more patty and fiber in contrary little fat and cholesterol. Certainly, this issue was become a challenge for animal nutritionist to produce animal product as a safety food for consumer.

Feed availability in an animal exertion (animal business) was the primary problem up to now. An effort that used to do was diversification of potential feed. North Sulawesi was known as fish source region. Fish oil was a nature marine substance that known contain omega-3. Omega -3 could prevent generative diseases like coroner heart disease and artery-sclerosis, wherein could decrease cholesterol level and repair HDL/LDL ratio. In 1988, recommendation to eat fish for Indonesian with 50 kg body weight was 18 kg/capita/year (Wiryanti, 1990). Fish consumption averages for North Sulawesi was 28 kg/capita/year, while in Java as lower, 5 kg/capita/year (Berhimpon, 1990). In Adisukresno, 1995). As a fish producer, canned fish fabric in North Sulawesi had a high capacity production. Canned fish processing was result some solid and liquid waste. Solid waste were head, red meat, bone, innards and fin, while liquid waste were pre-cooking by product and boil water (Buckle *et al.* 1985). Fish oil from canned fish fabric was liquid waste origin (Irianto, 1992). From about 5.8 millions km² in wide and 7.2 millions tonnage/year fish sources, only 40% was used. North Sulawesi was very potential in sea sources including fish as omega-3 fatty acid source. Some researchers found that in North Sulawesi oceans, unsaturated fatty acid was higher (approximately 54-62 %) than saturated fatty acid (Harikedua, 1992 and Berhimponet *al.*, 1994,

In Adisukresno, 1995). According to Wiryanti (1990), fish contain 5 – 20 % fatty acid and of it contain unsaturated fatty acid with double bond, including omega-3. There were many kind of fish catching in North Sulawesi ocean, for instant: “cakalang” (*Katsuwonus* sp.), “tuna” (*Yellow fin*), and “Deho” (*Rastrelliger* sp). Brodeur (2000) said that fresh meat tuna contains 1.504 g omega-3 fatty acid in each 100 g (0.363 EPA, 1.141 g DHA). Canned Tuna contain 0.202 g omega-3 fatty acid in each 100 g fat (0.027 g EPA, 0.101 g DHA). Meat chicken was a high nutrient of food product, so make an important role to meet a demand of animal protein. In addition of the nutrients, broiler has another superiority namely need a relatively short in time to rearing (in 5 weeks or 35 days could reach out ideal market weight) and reach out market price. Fast increasing of body weight gain in broiler, always followed by high fat deposit and cholesterol in meat. Cholesterol has many roles in embryo growth, structural component of cell membrane, as a precursor of adrenal, vitamin D and bile salt. Food efficiency showed the biology ability of an animal to result a product from feeds. Food efficiency influenced by some factors namely feed consumption, digestibility, nutrients using, energy and protein in ration.

According to all opinion before, a research had done to study the effect of omega-3 from some waste canned fish oil sources on broiler feed efficiency. Fish oil waste from canned fish was potentially as a source of omega-3, had known its utility and recently many studies about that was conducted. Its utility as a component of ration was possible. Omega-3 in fish oil could decrease cholesterol and would influence the animal body weight gain. The problem was not known yet how far its influence on feed efficiency of broiler which consumption some fish oil waste from canned fish as omega-3 source.

MATERIAL AND METHODS

This research was conducted to study the ration conversion of broiler fed Waste Canned Fish Oil (WCFO), in order to reach optimum growth. In this study, 100 three weeks broiler chickens was chose from 200 DOC in order to met homogenous weight. By using Completely Randomized Design (CRD), twenty (20) units cages were assigned into four (4) treatments group, each treatment in five (5) replicates. The treatments were as follow: R0 = Ration contains 4% WCFO; R1 = Ration contains 6% WCFO; R2 = Ration contains 8% WCFO; R3 = Ration contains 10 % WCFO. The effects of the treatments were evaluation by using the Analysis of Variance (ANOVA) of Completely Randomized Design (CRD). Experimental diet composed of yellow corn, fishmeal, rice bran, soybean meal, crude palm meal, minerals, DL-methionine and Lysin. Ration was arranged according to broiler age periods. Broiler chickens were reared during fourty (40) days, consist of 14 days starter period, fed 24% protein and 2400 kcal/kg level energy ration, and grower period start at age 2 weeks up to finisher fed ration which agree with protein treatments ration and level energy that balance to protein (Table 1). Feed consumption was observed everyday by measure the amount which given and decrease the residue during 24 hours. Body weight was measure at the beginning and end of the experiment period. Parameter performance that measured in this experiment were feed consumption, body weight gain and feed efficiency of broiler. The effect of the treatments were evaluated by using the Analysis of Variance (ANOVA) of Completely Randomized Design, and the differences among treatments were analyzed using Honestly Significant Difference (HSD) (Kusriningrum, 2008).

RESULTS AND DISCUSSION

Effect of Treatments on Feed Consumption

Averages of feed consumption at each treatment during these experiment was showed at Table 2. The averages of experiment feed consumption were $104.44 \pm 1,10$ until $106.37 \pm 2,26 \text{ g.head}^{-1} \text{ .day}^{-1}$. These were suitable to Wahyu (1992) recommendation, that male finisher chicken broiler consumption were 100 to $135 \text{ g.head}^{-1} \text{ .day}^{-1}$. Result of analysis of variance show that treatments make a non-significantly differences ($P > 0.05$) on feed consumption. It was mean that waste canned fish oil up to 10 % in chicken broiler ration was gave same effect on feed consumption, cause the experimental chicken broiler consumption protein – energy ration balance that relatively same, namely 142 – 143. Tillman *et al.* (1977) said that feed consumption was influence by body weight, feedstuff quality, management, environment climate, and animal healthy. Feedstuff quality was show by nutrients balancing in ration, primary protein-energy balance. This statement was supported by Suprijatna *et al.* (2005) that the amount of feed consumption were depend on feedstuff quality that utilized in ration, nutrients composition, suitable to the needed for optimum growth and productivity, and reared at the same environment condition. Another factor that influence feed consumption was ration physic shape. All experiment chicken broilers were gave same physically ration, namely mash, so it was not surprising that feed consumption was not influencing by using waste canned fish oil.

Effect of Treatment on Body Weight Gain

Averages of body weight gain of each treatment during the experiment were show at Table 2. The averages of chicken broiler body weight gain during the experiment were $41.57 \pm 0,69$ – $45.75 \pm 0,46 \text{ g.head}^{-1} \text{ .day}^{-1}$. This was suitable to Wahyu recommendation (1992), that chicken broiler body weight gain was 35.7 – $48.6 \text{ g.head}^{-1} \text{ .day}^{-1}$. Result of analysis of variance show that treatment gave a significantly differences ($P < 0.01$) on chicken broiler body weight gain. The highest body weight gain was reach at treatment R2 with 8 % WCFO. HSD test show that there were significantly differences ($P < 0.01$) between R0 compare to R1, R2 and R3; between R1 and R2 was significantly difference ($P < 0.05$), while between R1 and R3, also R2 and R3 were non significantly difference ($P > 0.05$). This result showed that WCFO could increase ration quality. According to Parakkasi (1985), ration quality would influence body weight gain. Fish oil tined waste in ration could increase ration fat level. Waste Canned Fish Oil was rich in *icosapentaenoic acid* (EPA), which could increase muscle mass and so influence body weight gain (Suprijatna, *et al.*, 2005). Chicken broiler could deposit energy as fat in its body up to 40 %. Consumption fat overbalance would cause fat accumulation in body and support body weight gain.

Effect of Treatment on Feed Efficiency

Averages of feed efficiency at every treatment during the experiment were presented at Table 2. Feed efficiency of chicken broiler experiment were about $0.391 \pm 0,07$ - $0.437 \pm 0,02$. This result was lower than Wahyu (1978) recommendation, namely 0.35 – 0.49. Analysis of variance showed that treatments significantly ($P < 0.01$) influence that feed efficiency. Further test evidence that feed efficiency of R0 were not significant different to R1, R2 and R3. Between R1 and R2 were not significant different, whereas between R1 and R3, R2 and R3 were significantly difference. This result show that WCFO in ration up to 10 % was affect chicken broiler

feed efficiency, and the best result was R2 (8%). Feed efficiency of R0 was lower than R1, R2 and R3; this was caused by body weight gain of chicken broiler that fed R0 ration was lower than body weight gain of R1, R2 and R3 treatment. This result was suit to Suprijatna *et al.* (2005), that feed efficiency was affected by consumption and body weight gain.

Feed efficiency was show the biology ability of an animal to result a product from feed. Feed efficiency value was the ratio between body weight gain average compare to ration consumption average for each animal during the experiment period. Feed efficiency of R0 was lower caused by the feed consumption was lower too, so the nutrient for body weight gain was lower too. According to Anggorodi (1985) that feed efficiency was affected by ration consumption, digestibility, and nutrients utility.

CONCLUSION

Waste Canned Fish Oil in chicken broiler ration up to 8% revealed the best result with the highest body weight gain and feed efficiency.

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Table 1. Compositions of Some Feedstuff, and Nutrient in The Experiment Ration

Ingredient	Treatment			
	R ₀	R ₁	R ₂	R ₃
Yellow corn	48	45	42	39
Rice bran	10	10	10.5	10.5
Soybean	13	14.5	15	16
Fish meal	15	15.5	16	16.5
Coconut meal	9	8	7.5	7
Premix	1	1	1	1
WCFO	4	6	8	10
Total	100	100	100	100
Crude Protein	21.63	22.05	22.22	22.55
Crude Fiber	5.36	5.32	5.35	5.34
Fat	8.67	9.9	11.12	12.36
Calcium	0.85	0.87	0.89	0.9
Phosphor	0.91	0.93	0.96	0.99
ME (kcal/k)	3070.73	3125.3	3181.16	3236.76

Note: Calculated adjustable with Table of Composition of Feedstuffs

Table 2. Averages of Feed Consumption, Body Weight Gain and Feed Efficiency

Variables	Treatments			
	R ₀	R ₁	R ₂	R ₃
Feed Consumption	106,3±2,26	104,81±1,87	104,70±1,64	104,44±1,10
Average Body weight Gain	41,57±0,69 ^a	43,81±1,61 ^b	45,75±0,46 ^c	44,32±0,59 ^{bc}
Feed efficiency	0,391±0,07 ^a	0,418±0,06 ^b	0,437±0,02 ^c	0,424±0,01 ^b

Means in the same row with different superscript differ significantly ($P < 0.05$)

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11. THE DYNAMICS OF INDIGENOUS LACTIC ACID BACTERIA PROBIOTICS ON CARCASS YIELD, ABDOMINAL FAT AND INTESTINAL MORPHOLOGY OF BROILERS

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Abstract

The study was carried out to investigate the dynamics of broiler chickens supplemented with a direct-fed of indigenous Lactic Acid Bacteria (LAB) probiotics relating to live body weight, carcass yield, weight of cut up meat parts, abdominal fat weight, abdominal fat percentage, and intestinal morphology such as villus height and width, crypt depth (μm) in duodenum, jejunum and ileum. One hundred and twenty broilers were assigned randomly into 4 treatment groups of LAB probiotics (mixed culture) orally supplementation consisted of (T0) one group of unsupplemented and three groups of supplemented chickens as much as 10^7 (T1), 10^8 (T2) and 10^9 CFU/ml/bird/day (T3) respectively. All of treatment groups were replicated into 6, with 5 chickens each. The antibiotic-free diet was formulated to meet the NRC recommendations and provided *ad libitum*. The data were analyzed using analysis of variance followed by Duncan New Multiple Range Test. The result evidenced that the live weight gain, carcass weight, breast portion weight, abdominal fat weight, abdominal fat percentage were significantly affected by the Lactic Acid Bacteria (LAB) probiotics ($P < 0.05$). The higher live weight were found in treatments T1 and T3 however the highest breast portion weight (573.57 ± 19.56 g/ bird) was found in the lowest dose of LAB probiotics (T1). The abdominal fat percentage of broiler chickens decreased by probiotics 2.00 ± 0.08 , 1.8 ± 0.08 , 1.67 ± 0.06 , and 1.43 ± 0.05 % in T0, T1, T2, and T3 respectively. LAB probiotics significantly increased villus height, villus width, crypt depth in duodenum, jejunum and ileum which indicated the improvement of the intestinal histology structure.

Key words : LAB probiotics, carcass yield, abdominal fat, intestinal morphology, broiler chicken.

INTRODUCTION

The efficiency of a poultry digestion depends on the microorganisms which live naturally in its digestive tract. Probiotics defined as living microorganism which given to animals, assist in the establishment of an intestinal population which is beneficial to the host and antagonistic to harmful microbes (Denly *et al.*2003), and improving its intestinal microbial balance and gut health resulting in greater intestinal enzyme activities and nutrient availability (Angel *et al.*, 2005; Willis and Reid,2008). Potential beneficial effects of probiotics for farm animals such as greater resistance to infectious diseases, increased growth rate, improved feed conversion, improved digestion, better absorption of nutrients, improved carcass quality and less contamination (Tannock, 1999). Reported by Santin *et al.*(2001), that probiotics induced to enhancement of the formation of short chain fatty acids. The short chain fatty acids which are by products of bacterial fermentation stimulate the proliferation of epithelial cells of the intestine (Chikawa *et al.*, 1999 in Gunal *et al.*, 2006). The main objective was to study the

dynamics of indigenous lactic acid bacteria probiotics on carcass yield, abdominal fat and intestinal morphology of broilers supplemented with a direct-fed of indigenous Lactic Acid Bacteria (LAB) probiotics. The carcass yield and abdominal fat consisted of live body weight, weight of cut up meat parts, abdominal fat weight, and abdominal fat percentage. The tissue morphology of the intestine such as villus height and width, crypt depth (μm) in duodenum, jejunum and ileum of broiler chickens.

MATERIALS AND METHODS

A total of 120 day old of male broiler chickens were used in this study conducted at Laboratory of Poultry Science, Faculty of Animal Science, Gadjah Mada University, Yogyakarta, Indonesia. Chicks were individually weighed and randomly divided into 4 treatment groups of Lactic Acid Bacteria probiotics mixture of *Lactobacillus murinus* (Ar3), *Streptococcus thermophilus* (Kd2), and *Pediococcus acidilactici* (Kp6) which were orally supplementation consisted of (T0) one group of unsupplemented and three groups of supplemented chickens as much as 10^7 (T1), 10^8 (T2) and 10^9 CFU/ml/bird/day (T3) respectively. All of treatment groups were replicated into 6, with 5 chickens each. The antibiotic-free diet was formulated to meet the NRC recommendations and provided *ad libitum*. To study the carcass yield and cut up meat part of birds, six birds from each treatment group were sacrificed randomly on 7 week of age. Weight of carcass and cut up meat parts were calculated separately. To study the intestinal morphology, 4 cm segments of duodenum (from the top of one side loop to distal), jejunum and ileum (from Mickel diverticulum to distal) were removed, rinsed, and placed into 10% buffered formalin until further processing (histological process followed haematoxylin-eosin stained). Pictures of villus height and width, crypt depth were obtained with Olympus BX 51 microscope replenished by Olympus DP 12 projector in magnification 40 x, and used the monitor of JVC TMH 1750C. The data were analyzed using analysis of variance in a Completely Randomized Design and means were compared for significant differences by Duncan New Multiple Range Test.

RESULTS AND DISCUSSION

Indigenous Lactic Acid Bacteria (LAB) probiotics supplementation to broiler orally as much as 10^7 (T1), 10^8 (T2) and 10^9 CFU/ml/bird/day (T3) respectively had significantly effect compared to unsupplementation birds (T0) on live weight, carcass yield, breast portion weight, abdominal fat weight, abdominal fat percentage but not in carcass percentage (Table 1.). The carcass percentage in this experiment as much as 68.26 ± 0.60 (T2), 68.66 ± 1.86 (T3) and 69.2 ± 0.90 (T1) respectively were lower compared to 70.40 resulted from the experiment which used of Lactina probiotic. However the results of live body weight by the supplementation of indigenous Lactic Acid Bacteria (LAB) probiotics 1824.5 ± 38.89 (T2), 1899 ± 43.50 (T3) and 1966.2 ± 38.05 (T1) respectively were higher compared to 1688.9 g of broiler supplemented with Lactina probiotic (Djouvinov *et al.*, 2005). It can partly be explained by different strain of microorganisms, amount of live and survivability of the microorganism, dietary nutrient levels used, and incidence of subclinical health problems prevalent in the testing facility (England *et al.*, 1996). Lactina is a probiotic preparation of freeze-dried pure cultures of *Lactobacillus bulgaricus*, *L. acidophilus*, *L. helveticus*, *L. lactis*, *Streptococcus thermophilus* and *Enterococcus faecium*. Each gram of Lactina contained 1×10^9 CFU. Angel *et al.*, (2005) reported that the addition of direct – fed microbial to broilers improved N, Ca and P retention. The other researchers found the increasing

nutrient utilization through the improvement of intestinal health that resulting in greater intestinal enzyme activities and nutrient availability (Gunal *et al.*, 2006). Base of those researcher statements, it has been guessed that lower carcass percentage in this experiment resulted greater yield of meat and bigger internal organs for human consumption (gizzard, liver and heart).

The total volatile fatty acids production of caeca increase between 7 and 42 days of age in broiler chickens (Fischer, 2003). These fatty acids were transferred to the blood and thus could contribute energy to the bird (Svihus *et al.*, 2013). It has been reported that under in vitro, probiotics increase the level of the short chain fatty acids (Gunal *et al.*, 2006). The lowest abdominal fat percentage (1.43 ± 0.05) resulted from the highest level of probiotics supplementation 10^9 CFU/ml/bird/day (T3) as showed in Table 1., it might be associated with utilization of the energy from the host and reducing the use of energy from the broiler diet.

After 28 days of probiotics supplementation to broilers the data of villi height, villi width, crypt depth of duodenum, jejunum and ileum of them were statistically significant ($P < 0.05$) from the control groups (unsupplementation probiotics) as presented in the Table 2.

These results confirm the fact that probiotics supplementation to the broiler chickens increase villus height and villus width. A similar result reported that villus height, villus width and crypt depth in jejunum, ileum, and caecum significantly increased in 28 and 42 day old broiler chicks fed a probiotic mixture of Protexin (Gunal *et al.*, 2006). The results of this experiment most probably due to enhanced short chain fatty acids formation induced by probiotics. The short chain fatty acids which are by products of bacterial fermentation stimulate the proliferation of epithelial of the bowel.

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Table 1. Effect of probiotics supplementation on live weight, carcass yield, cut up meat parts, abdominal fat weight, and abdominal fat percentage of 35th days of age

	T0	T1	T2	T3
Live weight (g)	1748.2±19.52 ^a	1966.2±38.05 ^d	1824.5±38.89 ^b	1899±43.50 ^c
Carcass yield (g)	1173.63±32.79 ^a	1360.78±40.81 ^d	1245.51±27.08 ^b	1304.41±59.42 ^c
Carcass (%)	67.6±1.46	69.2±0.90	68.26±0.60	68.66±1.86
Breast portion	504.24±20.61 ^a	573.57±19.56 ^c	523.26±16.33 ^a	551.85±13.22 ^b
Abdominal fat weight (g)	35.16±1.64 ^c	35.40±2.25 ^c	30.52±1.34 ^b	27.28±1.60 ^a
Abdominal fat (%)	2.00±0.08 ^d	1.8±0.08 ^c	1.67±0.06 ^b	1.43±0.05 ^a

abcd Means with different superscripts columnwise differ significantly at (P<0.05)

Table 2. Effect of probiotics supplementation on intestinal morphology of 35 th day of age

	T0	T1	T2	T3
Duodenum				
Villus height (mm)	497.23 ^a	697.20 ^b	713.87 ^b	688.87 ^b
Villus width (mm)	73.33 ^a	104.47 ^b	122.20 ^b	111.13 ^b
Crypt depth (mm)	90.53 ^a	141.70 ^b	125.03 ^b	134.46 ^b
Jejunum				
Villus height (mm)	558.33 ^a	811.32 ^b	791.66 ^b	775.56 ^b
Villus width (mm)	75.53 ^a	136.10 ^c	119.43 ^b	122.23 ^b
Crypt depth (mm)	92.80 ^a	113.90 ^b	120.57 ^b	114.43
Ileum				
Villus height (mm)	516.66 ^a	738.90 ^b	747.23 ^b	722.23 ^b
Villus width (mm)	69.97 ^a	132.20 ^b	113.90 ^b	121.67 ^b
Crypt depth (mm)	76.10	108.33	114.43	123.86

abcd Means with different superscripts columnwise differ significantly at (P<0.05)

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12. THE EFFECTS OF XYLANASE SUPPLEMENTATION ON MEAT QUALITY, CARCASS RECOVERY AND BLOOD CHOLESTEROL IN BROILERS FED ON WHEAT-BASED DIETS

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Abstract

This study was conducted to investigate the effects of xylanase supplementation on meat quality, carcass recovery and blood cholesterol in broilers fed on wheat-based diets. The experimental diets consisted of a wheat-based diet. Four isoenergetic and isonitrogenous diets consisting of control diet without enzyme and three test diets supplemented with xylanase at 0, 0.75, 1.5 or 2.25 g/kg enzyme preparation (xylanase activity was 60.30 U/g). The diets were fed between 14 and 42 days of age. Each diet was offered to 3 replicates of 5 Lohmann broiler chicks in a randomized one way design. Data was analyzed using SPSS for one-way design. Enzyme used in the study was semi-purified having xylanase activity 62.03 U/g. Xylanase supplementation had no significant effect ($p>0.05$) on carcass recovery and meat quality in broilers fed on wheat-based diets. The treatment increased ($P<0.01$) concentration of blood cholesterol contrast to control, and tended to increase ($P<0.1$) meat protein content and abdominal fat pad. In conclusion, enzyme supplementation contributed few responses in birds when supplemented at three levels.

Keywords: Xylanase, Broilers, carcass recovery, meat quality and blood cholesterol

INTRODUCTION

Enzyme use is well documented across different types of poultry diets. The possibility of using exogenous enzymes in non-ruminant diets is a very important tool to improve the extraction of nutrients from the feed, thereby decreasing feed costs, improving bird performance, and decreasing the environmental impact of manure application to land. Many commercial enzymes have been reported to be effective when added to poultry diets containing large amounts of Non-Starch Polysaccharides (NSP) such as wheat and sorghum, due to well digestion of soluble and insoluble NSP (Selle *et al.*, 2010). The addition of xylanase and β -glucanase, with the inclusion of rye instead of corn in a broiler chicken diet, increased zootechnical performance and lowered the apparent digestibility of protein and fat. This could be partly due to decreased bile acid deconjugation and increased the small intestine wall. Thus, addition of xylanase and β -glucanase will lead to an improvement in broiler chicken performance (Mathlouthi *et al.*, 2002).

Wheat is a common raw material in broiler diets because of its high availability, good price:quality ratio, and its lack of pigments. Therefore, it is common to use wheat as the only cereal in broiler diets with inclusion percentages of 55% where contributes up to 60 to 65% of the AMEn and 35 to 40% of the CP of the diet (Gutierrez del Alamo *et al.*, 2009). Because starch is the major energy yielding component inside cereals, attention should be paid to its digestion. The positive effect of the slowly digestible starch rate on chicken performance was suggested to result from better synchronization of energy to protein availability and to a more continuous

supply of glucose to the intestinal lumen. The rate of starch digestion could be reflected in plasma glucose levels and glycemic index (Englyst *et al.*, 1996). Wheat contains NSP that decrease the use of nutrients. Arabinoxylans are the major NSP in wheat that increase viscosity of digestive content in the small intestine and interfere with digestion and absorption of nutrients when fed to poultry (Gao *et al.*, 2008). Xylans are the principal NSP of wheat, and high levels of wheat in poultry diets can increase the viscosity of the gut contents, which impedes the circulation and absorption of nutrients, causing reduced feed intake, body weight gain, and feed efficiency (Annison and Choct, 1991). Bu and Ravindran (2004) reported that neither whole wheat inclusion nor xylanase supplementation influenced the relative weight and length of the small intestine, carcass recovery, breast muscle yield, and the relativeweight of abdominal fat pad. It is known that dietary factors can influence the development and the size of digestive organs, which in turn may be anticipated to influence saleable carcass yield. The effects of supplemental xylanase on blood parameters, carcass characteristics and meat quality in broilers fed on wheat-based diets are therefore of practical interest.

So we hypothesized that the effects of xylanase supplementation on carcass recovery and meat quality may be associated with changes in the blood parameters in broiler chickens fed wheat-based diets. Until now, few studies have been conducted to determine the effects of xylanase supplementation to wheat based diets on blood parameters of broilers. Therefore, the objectives of this study were to examine the effects of xylanase supplementation on concentration of blood cholesterol, carcass recovery and meat quality in broilers fed on wheat-based diets.

MATERIALS AND METHODS

Enzymes

Xylanase used in this study was produced from xylanolytic bacteria of previous study with tapioca flour as carrier of enzyme. Xylanase activity was 60.30 U_g⁻¹. One unit of xylanase is defined as that amount of enzyme that liberates 1 μmol of xylose in 1min at pH 6 and 50°C.

Experimental design and dietary treatments

The experimental design involved a one way arrangement of treatments with four levels of of xylanase (0, 0.75, 1.5 and 2.25 U kg⁻¹ diets).

Broiler starters were fed with commercial diets, one set of basal diet was used for broiler 14-42 days of age. The ingredient and nutrient composition analysis of the basal diets are shown in Table 1. The basal diets were formulated to meet or exceed the NRC (1994) recommendations for all nutrients. Diets were offered *ad-libitum* and water was available at all times. Diets were antibiotic-free and were provided in mash form.

General experimental procedures

Day-old male broiler (Lohmann) chicks were obtained from a commercial hatchery and randomly assigned to 12 pens (5 birds/ pen). All chicks were continuously provided with uniform at night. Room temperature was maintained at 33 32 ± 1°C during the first week and gradually decreased to a final temperature of 30°C. After the 14 d adjustment period, birds were fed their assigned diets until 42 d age.

Blood samples collection

A blood sample was collected from a wing vein with a heparinized syringe from 5 birds (2 birds per replicate) per group at the age of 41 days. Blood samples were

centrifuged, and plasma samples were collected to be measured the concentration of cholesterol using cholesterin as a standard (Plummer, 1978).

Table 1 Ingredient and nutrient composition of the basal diets (% DW basis)

Ingredients	Concentration
Composition	100.00
Wheat pollard	30.00
Soybean meal	12.50
Yellow corn	44.00
Fish meal	8.00
Vegetable oil	4.00
Limestone	1.35
Salt	0.15
Nutrient composition	
Metabolisable energy (Kcal kg ⁻¹)	2,746.16
Crude protein	20.09
Crude fiber	4.62
Calcium	0.81
Total phosphorus	0.72

Carcass measurements

On day 42, two birds (close to the mean pen weight) were selected, fasted for 6 h weighed and killed by cervical dislocation, followed by exsanguination. After the removal of feathers, viscera, shanks and neck, the weights of the eviscerated hot carcass and abdominal fat pad were measured. Analysis of meat compositions (dry matter, moisture, ash, protein, fat, cholesterol) were conducted to breast muscle using AOAC (2005). Subcutaneous back-fat samples were taken at 2 cm from the spine midline at the dorso-lumbar region (2x2 cm square) using a sharpened edge. In addition to skin, biopsies included the inner and outer layers of the subcutaneous back-fat. Fat extracts from biopsy samples were obtained by AOAC (2005) for crude lipid extraction.

Statistical analysis

Statistical analysis of all data was performed by one way design with SPSS 10 software. Values were expressed as the mean \pm S.E.M. All statements of significance were based on a probability of less than 0.05. The differences of mean value were analyzed by Duncan's new multiple range test (Rosner, 1990).

RESULTS AND DISCUSSION

The effect of xylanase supplementation on carcass characteristics and blood cholesterol is shown in Table 2. There were no significant main effects of xylanase supplementation (all responses $P > 0.1$) on carcass characteristics. However, abdominal fat pad tended to increase ($P < 0.085$) with the enzyme supplementation. The FCR was significantly increased in birds fed with xylanase supplementation ($P < 0.01$).

Effect of xylanase supplementation up to 2.25 g/kg did not show difference compare with control, and no significant effect was detected, except for meat protein tended to increase ($P < 0.083$).

Diets were formulated to meet or exceed the broiler's performance guidelines for energy, protein, calcium, and phosphorus. As such, the birds performed well in relation to these guides, considering the unit conditions were of academic rather than

commercial type. There were no effect of xylanase, which was consistent regardless of the diet employed in this study. This result was similar to the work of Masey O’Neill *et al.* (2012), who found no effect of xylanase for carcass yield and abdominal fat pad.

Table 2. Carcass characteristics (carcass recovery, abdominal fat pad, subcutaneous fat-pad) and blood cholesterol of male broilers at 42 d of age as influenced by xylanase supplementation

	Level of enzyme (g kg ⁻¹)			
	0	0.75	1.50	2.25
Carcass recovery, g	616.07 ± 43.63	630.60 ± 23.60	626.69 ± 22.00	652.91 ± 7.56
Kg BW ⁻¹				
Abdominal fat pad, g	12.03 ± 1.03	13.05 ± 2.87	17.35 ± 2.32	15.44 ± 2.70
Kg BW ⁻¹				
Subcutaneous fat-pad, %	50.03 ± 5.74	55.74 ± 1.94	53.63 ± 2.12	56.13 ± 0.85
Blood cholesterol, %	120.93 ^a ± 25.32	120.44 ^a ± 0.40	217.19 ^b ± 21.74	204.22 ^b ± 4.07

Means in the same column with different letters (a, b) are significantly different (P<0.01)

Table 3 Meat quality (% DW; dry matter, moisture, ash, protein, fat, cholesterol) of male broilers at 42 d of age as influenced by xylanase supplementation

	Level of enzyme (g kg ⁻¹)			
	0	0.75	1.50	2.25
Dry matter	26.13 ± 0.81	26.49 ± 0.61	25.90 ± 0.61	23.41 ± 3.06
Moisture	73.87 ± 0.81	73.52 ± 0.61	74.10 ± 0.61	76.6 ± 3.06
Ash	0.93 ± 0.33	0.82 ± 0.06	1.03 ± 0.29	1.30 ± 0.03
Protein	22.45 ± 0.34	22.41 ± 0.42	23.57 ± 0.79	23.23 ± 0.57
Fat	2.24 ± 0.66	2.09 ± 0.22	2.27 ± 0.93	2.91 ± 0.62
Cholesterol	53.82 ± 6.11	51.40 ± 8.47	55.61 ± 3.14	52.87 ± 18.26

The fat used in the current study was vegetable oil therefore relatively unsaturated. The choice of fat has been shown to affect the response to xylanase and in a recent holo-analysis of the factors that affect xylanase efficacy, the amount of animal fat in the diet was a significant factor (Masey O’Neill *et al.*, 2011). Tallow, compared with soy oil, can increase the response to xylanase, when used as the majority enzyme in an NSPase cocktail (Dänicke *et al.*, 1999; Preston *et al.*, 2001). Presumably this is because gastric retention with such a saturated fat is greater, increasing peptic digestion of protein. If the response to xylanase depends on the choice of fat, and one improves gain response over another, the choice of fat should also be considered in relation to tissue deposition. A greater level of saturation of dietary fat also increases the proportional weight of the abdominal fat pad (Crespo and Esteve Garcia, 2001). Additionally, in latter age periods, BW gain was increased with fat content due to increased abdominal deposition (Monfaredi *et al.*, 2011). The reason for such a difference in fat types was originally explained through the effect of viscosity on fat emulsification; that is, tallow is harder to emulsify than vegetable oil and therefore is most susceptible to changes in intestinal viscosity. However, more saturated fats can delay gastric emptying, which in amino acid-limiting diets may also result in improved

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performance. Another mechanism by which gastric retention is promoted by the use of xylanase was suggested by Bedford and Schulze (1998).

CONCLUSION

In conclusion, enzyme supplementation contributed few responses in birds when supplemented at three levels. The whole diet composition should be considered in conjunction with enzyme dose to achieve the best advantage.

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13. DIGESTIVE ORGAN'S GROWTH OF LOCAL DUCK FED HIGH DIETARY FIBER DURING POST-HATCH: EFFECT ON ALLOMETRIC MEASUREMENT

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Abstract

The research aimed to evaluate the effect of feeding high dietary fiber during post hatch on allometric growth of local duck's digestive organs. A total of 195 birds of day-old male ducks with initial body weight (BW) of 41.53 ± 4.69 g were placed in 15 cages. The experiment was assigned in completely randomized designed (CRD) with dietary fiber level of 5, 10 and 15% as the treatments ration. The ration was formulated to contain 17% crude protein and metabolisable energy of 2700 Kcal/kg diet. After 14 days feeding treatment, the ration was changed into commercial broiler diet fed up to 28 days of age. Variables observed weekly were feed consumption, allometric growth of proventriculus, ventriculus and small intestine as well as body weight (BW), body weight gain (BWG) and feed conversion ratio (FCR). Digestive organ allometric growth was measured as follows: ratio of organ weight at day n and at day 1 divided by the ratio of body weight at day n and at day 1. The result showed that feeding high fiber diet during 2 weeks post hatch did not affect feed consumption, allometric growth of digestive organs, except the small intestine. Feeding 10 and 15% dietary fiber for 2 weeks post hatch stimulated the growth of small intestine. However, replacing dietary treatments with commercial ration thereafter did not influence the growth of those 3 sections of digestive organs. The conclusion is that small intestine of the duck until 14 days post-hatch is much more responsive to the stimulating effects of the ration with 10 or 15% fiber without affecting its performances except the FCR value is improved starting on week 3.

Key words: local duck, digestive organs, dietary fiber, relative weight, allometric growth

INTRODUCTION

Digestive system development of some kinds of post hatched poultry have been investigated by many researchers, such as in chicken (Imondi and Bird, 1966; Baranyiova and Holman, 1976; Lim and Low, 1977; Nir *et al.*, 1993), in turkey (Sell *et al.*, 1991; Pinchasov and Noy, 1994), and in several strains of ducks (Gille *et al.*, 1990; Watkins *et al.*, 2004). Digestive system is the only organs that responsible on the up take of feed and/or nutrients in order to ensure and maintain the growth of the birds. Comparing the digestive organs development of broiler and native chicken at 3 weeks post hatched, Wahyuni (2001) reported that the pancreas and small intestine of both kinds of poultry given commercial ration were still growing about 3 times higher than their body weight.

Some authors have also stated that the function of post hatched digestive systems depended on the early accessed to exogenous feed in chicken (Moran, 1985; Noy and Sklan, 1999; Sklan and Noy, 2000; Uni *et al.*, 2002), and in young turkey (Noy

et al., 2001). Other investigations, for example fiber content of poultry ration (Scheideler *et al.*, 1998; Brenes *et al.*, 2002) or other nutrient such as amino acid (Batal and Parson, 2002) indicated the great influence on digestive organ development both in broiler and layer chicken. Svihus (2011) stated that structural feedstuff was required to stimulate gizzard development and function in poultry, and also Amerah *et al.* (2007) found that particle size of diet or grains mixed to the diet was intensely affected digestive organs development. Similarly, inclusion of fibrous feedstuff into the poultry ration may enhanced digestive system development, since it could represent as structural feedstuff due to larger particle size.

Digestive system of duck was more or less similar to that of the chicken. Research indicated that duckling could tolerate 14 % dietary fiber without affecting feed conversion ratio (Wizna, 1995). However, a contradictory finding was reported by Jamroz *et al.* (2001) that duck were less capable in digesting dietary fiber. Therefore, the present research was carried out to evaluate the influence of high dietary fiber fed right after hatching on the digestive organ's allometric growth and performance in male duck.

MATERIALS AND METHODS

Day old male local duck of 195 birds with an average body weight of 41.53 ± 4.69 g were grouped based on completely randomized design (CRD) arrangement, and were placed in 15 units of cages. Dietary treatment was formulated to contain 17 % crude protein and 2700 Kcal/kg of metabolizable energy with 3 different levels of dietary fiber, namely 5, 10 and 15%. Sawdust was included into the ration to increase fiber level. Composition of the experimental rations and nutritional contents was indicated in Table 1. Rations were provided in wet form through mixing it with water at the ratio of 2:1, and it started to be fed on day 1. After 14 days feeding treatment, the ration was changed into commercial broiler diet and was fed up to 28 days of age. One bird of duck from respective experimental unit was randomly decapitated weekly. The digestive organ was removed from the abdomen. Proventriculus, ventriculus and small intestine were separated, cleaned and weighed. The allometric growth was computed according to Nir *et al.* (1993) as follow: ratio of organ weight at day n and at day 1 divided by the ratio of body weight at day n and at day 1. Data were subjected to analysis of variance according to split in time and continued to Duncan Multiple Range Test (DMRT) to compare differences between treatments mean, when the treatments effect was significant.

RESULTS AND DISCUSSION

Feed Consumption and Body Weight

The average weekly feed consumption and body weight were presented in Table 2. There was no interaction of dietary fiber levels and ages on feed consumption and body weight. Feed consumption (Table 2) was almost similar at the first week (9 vs. 8 g/bird/day) and it was lower at the second week (20 vs. 25 g/bird/day) when compared to the average feed consumption of the Indonesian duck in general (Srigandono, 1997). Considering feed consumption is closely related to growth, it is therefore reliable that body weight (Table 2) during the first week was the same (72 vs. 75 g/bird/day) and the second week was lower (130 vs. 140 g/bird/day) as compared to the report of Srigandono (1997). On the other hand, feed consumption on week 3 (45 vs. 30 g/bird/day) and week 4 (63 vs. 40 g/bird/day) were higher as compared to those stated

by Srigandono (1997). The higher feed consumption had an intense contribution to the better growth (Table 2), this relationship could be proved thereafter, that body weights when compared to previous study (Srigandono, 1997) were higher on week 3 (317 vs. 225 g/bird/day) and on week 4 (524 vs. 325 g/bird/day). This phenomenon indicates that feeding high fiber diet during 2 week post hatched depressed feed consumption, but the birds can be categorized to achieve normal body weight. However, body weight was increased there after, since the feed provided has been changed into commercial diet.

Allometric Growth of Digestive Organs

Weekly allometric growth of proventrikulus, ventrikulus and small intestine of duck fed high dietary fiber 14 days post hatch were presented in Table 3. Only the allometric growth of the small intestine was significantly affected by the interaction between dietary fiber levels and ages. Dietary fiber levels did not influence the allometric growth of proventriculus and ventriculus, but ages significantly influence the allometric growth of these 2 digestive organ sections.

Treatments differences on small intestine allometric growth was analyzed based on separated respective factor, namely: the influence of dietary fiber levels at the same age and the influence of ages at the same dietary fiber level. When dietary fiber at the level of 10% was fed, the highest allometric growth of small intestine was indicated on week one and two. Feeding 10% dietary fiber stimulated the small intestinal growth by 54-59% as compared to feeding affect of 5% fiber which was achieved only 35-38% of the body basis. Shifting the treatments into commercial diet on week three and four, the effect of feeding dietary fiber seemed to be eliminated, since the allometric growth were more or less similar. Allometric growth of small intestine closely related to its size which could be an impact on the absorptive surface area. Jiang *et al.* (2012) found out that utilization of 9% alfalfa containing 5% dietary fiber level did not affect the allometric growth, but affected the intestinal morphology such as villus height and crypt dept. It would be obvious that morphological changes of the small intestine should have been influenced nutrients digestibility and availability.

Allometric growth of ducks' proventriculus decreased as the ages increased. High dietary fiber fed during 14 days post hatch did not affect proventriculus allometric growth even when the diet was changed into commercial ration. Proventriculus was actually function as a glandular stomach only that secreted pepsinogen and hydrochloric acid (Larbier and Leclercq, 1994). Since proventriculus was a small compartment located at the anterior site of the ventriculus, caused digesta retention time was very short. Therefore, it can be assumed that there was no effect of dietary treatment on proventriculus growth relative to the body weight.

However, data in Table 3 shows that the growth of ventriculus in all treatment at 14 days post-hatch was more pronounced compare to those at 28 days of age when treatment diet was shifted into commercial ration. When the fibrous diet was omitted the growth of ventriculus was still increased by 33 to 40% of the whole body weight. Therefore, it can be assumed that the effect was due to physical form of feedstuffs included in the previous treatment ration. Since sawdust was included to increase the fiber content and sand was also used as a filler to balance the nutrient content of the treatment ration (Table 1), it suggests that there was a residual effect of previous diet on ventriculus growth. The present finding was supported by the research conducted by Hetland *et al.* (2005) that ventriculus weight of layer increased up to 60% when fed

wheat diet with access to coarse sawdust. Larbier and Leclercq (1994) stated that the role of ventriculus which is essentially mechanical among the digestive organs, so that the presence of coarse material such as sawdust and sand are greatly possible to stimulate its growth (Svihus, 2011).

Even though small intestine of the ducks was the only digestive organ indicated highly response to high dietary fiber level, but nutrient digestibility would not be improved without the important contributed functions of proventriculus and ventriculus. The presence of coarse particle size of the feedstuffs enter the ventriculus enhanced the grinding activities which further stimulate enzyme and hormone secretions that responsible on nutrient digestibility process. The effect of course particle size could not be detected soon after feeding treatment, but the duck, via the functions of digestive organs, needed “time” to give response. The physiological aspect involving nutritional utilization due to the works of digestive organs could be indicated by the improved body weight starting on week 3 there after (Table 2) exceeded the growth of local Indonesian ducks in general, as have been discussed previously.

Duck's Performances

Average weekly body weight gain and feed conversion ratio were indicated in Table 4. It can be explained that there was no interaction between dietary level of fiber and ages on body weight gain and feed conversion ratio. But, ages exerted highly significant effect upon body weight gain and feed conversion ratio. Body weight gain improved as the ages increased, and the gain was the highest on week 4, however, the lowest feed conversion ratio was reached on week 3 (Table 4). This condition could be attributable to the improved function of the digestive organs, proventriculus, ventriculus and small intestine.

It has been discussed in the previous paragraph that high dietary fiber fed during 14 days post hatched only affected the allometric growth of small intestine, but such dietary treatment would also be predictable to stimulate the physiological functions of proventriculus and ventriculus in relation to the improvement of nutrients digestibility. As it has been mentioned previously that sawdust and sand were included into the ration to increase the dietary fiber and to balance nutritional content (Table 1), therefore, the particle size of the two added materials affected the mechanical function, especially ventriculus to be more effective in grinding feed. Svihus (2011) stated that nutrient availability in poultry would improve if at least 3% of coarse fiber was included into the diet, due to the beneficial effect of coarse feedstuffs on ventriculus growth and function, and further stimulate grinding activity to reduce dietary particle size. According to the initiation process of physiological mechanism as described above bring about the work of the small intestine to be easier for nutrients digestion and absorption.

CONCLUSION

It can be concluded that feeding 15% dietary fiber at 14 days post hatched has more pronounced effect in stimulating the growth of small intestine rather than proventriculus and ventriculus, without affecting its performances except the FCR value is improved starting on week 3.

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Table 1. Composition of Experimental Ration and Nutritional Content

Feedstuff	Dietary Fiber Level (%)			Comercial diet ^{**})
	5	10	15	
	----- % -----			
Corn	45.50	45.50	45.20	C
Rice bran	4.00	4.00	4.00	O
Water hyacinth leaf meal	1.40	0.60	0.20	M
Fish meal	8.00	8.00	8.00	M
Soy bean meal	20.00	20.00	20.00	E
Cassava by-product	0.50	1.00	1.00	R
Sludgedust	2.70	10.00	17.40	C
Sand	13.90	6.90	0.00	I
CaCO ₃	0.20	0.20	0.20	A
Premix	0.10	0.10	0.10	L
Vegetable oil	3.70	3.70	3.90	Diet
Total	100.00	100.00	100.00	
Nutrient Content				
Energy Metabolism [*]) (Kkal/kg)	2700.40	2701.00	2700.90	-
Crude Protein	17.03	16.90	16.96	21.00
Crude Fiber	5.01	9.93	14.93	5.00
Extract Eter	5.86	6.00	6.34	5.00
Ca	0.63	0.62	0.61	0.90
P	0.34	0.34	0.34	0.60
Methionine (%) [*])	0.32	0.32	0.32	-
Lysine (%) [*])	0.98	0.98	0.98	-
Energy Protein Ratio	15.88	15.88	15.88	-

^{*})Computed based on Hartadi *et al.*, (1997); ^{**}) Label of commercial diet (averages)

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Table 2. Weekly Relative Weight of Proventriculus, Ventriculus and Small Intestine of Local Duck Fed High Dietary Fiber at 14 Days Post-Hatch and Shifted to Commercial Ration up to 28 Days of Age.

Parameters	Age (week)	Dietary Fiber Level (%)			Average
		5	10	15	
Proventriculus	1	1.33	1.38	1.28	1.33 ^A
	2	1.26	1.31	1.30	1.29 ^{AB}
	3	1.24	1.27	1.25	1.25 ^B
	4	1.10	1.16	1.12	1.13 ^C
	Average	1.23	1.28	1.24	
Ventriculus	1	1.47	1.50	1.48	1.48 ^A
	2	1.52	1.56	1.52	1.53 ^A
	3	1.40	1.37	1.44	1.40 ^B
	4	1.34	1.33	1.40	1.36 ^B
	Average	1.43	1.44	1.46	
Small Intestine	1	1.38 ^{aP}	1.59 ^{bP}	1.48 ^{abP}	1.48
	2	1.35 ^{aP}	1.54 ^{bP}	1.52 ^{abP}	1.47
	3	1.32 ^{aP}	1.37 ^{aQ}	1.30 ^{aQ}	1.33
	4	1.20 ^{aQ}	1.24 ^{aR}	1.17 ^{aR}	1.20
	Average	1.31	1.44	1.37	

Different superscript in the collum of average value for each parameter showed significantly difference ($P \geq 0.05$)

^{abc} superscript in each row of small intestine parameter showed significantly difference ($P \geq 0.05$)

^{PQR} superscript in each collum of small intestine parameters showed highly significant difference ($P \geq 0.01$)

Table 3. Weekly Feed Consumption and Body Weight Gain of Local Duck Fed High Dietary Fiber at 14 Days Post-Hatch and Shifted to Commercial Ration up to 28 Days of Age

Parameters	Age (week)	Dietary Fiber Level (%)			Average
		5	10	15	
Feed Consumption	1	9.62	9.34	8.75	9.24 ^D
	2	20.82	19.71	19.95	20.16 ^C
	3	46.47	44.28	46.41	45.72 ^B
	4	64.13	63.12	63.85	63.70 ^A
	Average	35.26	34.11	34.74	
Body Weight	1	72.18	72.40	71.17	71.91 ^D
	2	134.98	127.23	128.48	130.23 ^C

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	3	329.92	311.06	312.20	317.73 ^B
	4	538.06	511.00	523.30	524.12 ^A
	Average				
Body Weight Gain	1	29.54	28.68	26.77	28.33 ^D
	2	63.48	55.00	57.41	58.63 ^C
	3	194.94	283.83	183.72	187.50 ^B
	4	208.14	199.94	211.10	206.39 ^A
	Average				
		124.03	116.86	119.75	

Different superscript in the collum of average value for each parameter showed highly significant difference ($P \geq 0.01$)

Table 4. Weekly Body Weight Gain And Feed Conversion Ratio of Local Duck Fed High Dietary Fiber at 14 Days Post-Hatch and Shifted to Commercial Ration up to 28 Days of Age

Parameters	Age (week)	Dietary Fiber Level (%)			Average
		5	10	15	
----- g/bird/day -----					
Body Weight Gain	1	29.54	28.68	26.77	28.33 ^D
	2	63.48	55.00	57.41	58.63 ^C
	3	194.94	283.83	183.72	187.50 ^B
	4	208.14	199.94	211.10	206.39 ^A
	Average				
		124.03	116.86	119.75	
Feed Conversion Ratio	1	1.96	1.96	2.05	1.99 ^{AB}
	2	2.00	1.99	2.06	2.02 ^{AB}
	3	1.67	1.74	1.78	1.73 ^B
	4	2.20	2.28	2.16	2.21 ^A
	Average				
		1.96	1.99	2.01	

Different superscript in the collum of average value for each parameter showed highly significant difference ($P \geq 0.01$)



The Role of Nutrition and Feed in Supporting Self Sufficient in Animal Products, Food Safety and Human Welfare”

FEED SCIENCE AND TECHNOLOGY, PASTURE AND RANGE LAND, NUTRITION AND REPRODUCTION, SOCIO-ECONOMIS OF FEED AND FOOD

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penetration capacity and optimum transfer of genetic material. All current work on semen analysis seeks to identify some kinetic, morphological or biochemical parameters indicating the status of the sperm cell at any given time, while at the same time correlating with fertility and ejaculate quality. In routine production, the test should be accurate, simple, fast and economical (Dahmani, 2009).

Yung *et. al.* (1994) reported that injection of bovine somatotropin could enhance reproductive performance of cattle. Bovine somatotropin (BSt) also known as bovine growth hormone, is a protein hormone produce by pituitary glands of cows. There are 20 amino acids that comprise the structure of all proteins and each protein has a unique sequence of amino acids are combined in specific sequences to form the more than 10.000 different proteins in the body (Bauman and Collier, 2010) and it cannot be fed to cows since it would be digested in the gastrointestinal tract into individual amino acids and peptide fragments which have no hormonal activity. As a result, bST has to be injected into the circulatory system (Etherton, 2006). The objectives of this study was to evaluate the effect of somatotropin hormon on semen quality of Simbrah bulls.

MATERIALS AND METHODS

Three Simbrah breed bulls had been subjected to the same managerial and environmental condition from birth, and age of bull did not different. The design used in this study were T test assuming a two-way where if $P < (+/-) 0.05$ is significantly different results, while if $P > (+/-) 0.05$ then there sultisnot significantly different. The experiment was conducted in July to September and consisted of a 2 week pretreatment period (regular injection of Vitamin A, D, E and K). Control bulls had been collected a semen quality every 7 days (session 1). Treated bulls were the same bulls after collecting semen on session 1, then administered by somatotropin hormone 500 mg after 7 days (session 2).

Collecting semen use anartificial vagina and teasing conducted 2-3times to ensure a good quality of semen during ejaculation. Collected semen immediately analyzed macroscopically and microscopically. Macroscopic analysis were volume, colour, consistency and pH of semen and microscopic analysis were mass movement, concentration, motility and abnormality. Electric microscope (optical lab) was performed to observe sperm mass movement, motility and abnormality. Spechtophotometer also performed to evaluate sperm concentration.

RESULTS AND DISCUSSION

1. Macroscopic Evaluation of Bulls Semen

Volume. The volume of semen produced by the bulls may vary depending on the race animals, large and heavy animals, the frequency of collecting, and etc. Twice-weekly collection of semen from week to week will remain good the quality and quantity condition will be maintained (Partohardjo, 1982). Semen volume will increase according to age, body size, food levels, changes in the state of reproductive health, the frequency of shelter and will decline after peak matures (Amin, 1998; Toelihere, 1993).

Treated bulls with somatotropin hormone were higher (5.3 ± 1.2 mL) than control (4.9 ± 1.1 mL) but not statistically significant effect on volume of semen ($P = 0.27$). It means, administrating of somatotropin hormone were not affect tthe volume of semen. Hafez (2000) and Turyan (2005) reported semen volume about 5-8 mL and 8.45 ± 4.45 mL, respectively. Enoket. al. (2001) also reported that the second ejaculation produce

more semen volume. According Toelihere (1985), cattle semen volume varies between 1-15mL, for European cattle (*Bostaurus*) like Limousin semen volume ejaculate dan average of 6-8mL. Toelihere suggested that the volume of semen obtained from the ejaculation did not indicate the quality of semen.

Color.

Semen color ranges from white transparent, milky white to cream and it correlates with the number of sperm cells contained in the cement. All collected semen shown the same color from control and treated bulls semen. Bearden and Fuquay (1984) and Susilawati et.al. (2003) reported that the color of semen from normal ejaculation are milky white and 10% colore dcream. Macroscopic evaluation data of cement color on control and treated bulls are shown on Table 1

Tabel 1. Macroscopic evaluation of bull semen

No	Bulls	R	Volume		Color		Consistency		pH	
			Before	After	Before	After	Before	After	Before	After
1.	Carlos	I	6	3.5	MW	MW	SV	SV	6.5	6.7
		II	6	6	MW	MW	SV	SV	6.5	6.8
		III	6	6	MW	MW	SV	SV	6.5	6.6
2.	Zitun	I	3.3	6	MW	MW	SV	SV	6.3	6.7
		II	3.3	3.7	MW	MW	SV	SV	6.3	6.8
		III	3.3	3.5	MW	MW	SV	SV	6.3	6.7
3.	Segentar	I	5.5	6	MW	MW	SV	SV	6.7	6.6
		II	5.5	6.9	MW	MW	SV	SV	6.7	6.6
		III	5.5	6.2	MW	MW	S	S	6.7	6.5
AVG±SD			4.9±1.1	5.3±1.2					6.5±0.16	6.6±0.09

R = replication; Color : C = cream, MW = milky white, WT = white transparent; Consistency: A = aqueous, SV = slightly viscous, V = viscous; AVG= Average, SD = standart deviation

Consistency.

Consistency is the degree of turbidity, the examination can be done by shaking beakers containing fresh semen. Enok et. al. (2001) reported the relationship between consistency, sperm concentration and color. The consistency of the relationship between sperm concentration and semen color. The evaluation results semen consistency were aqueous (A), slightly viscous (SV) and viscous (V). The more aqueous semen means the lower concentration of spermatozoa and cement color more transparent. Partodihardjo (1982) also reported that good cement viscosities almost the same with milk.

All collected semen shown the same turbidity from control and treated bulls semen (slightly viscous). It suggested that administration of somatotropin hormone did not affect the semen consistency.

pH. Acidity determine spermatozoa life status in the semen, the lower or the higher pH will make the sperm die faster and appropriate pH is normal pH (7). Changes in pH to a more acid occurs due to the accumulation of lactic acid that resulted from spermatozoa metabolism under anaerobic conditions (Toelihere, 1985).

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Table 2 shown that semen pH were higher after administration of somatotropin hormone (6.6±0.09). Although both control and treated bulls showing a little difference of pH range, but the results significantly different (P=0.011). Control bulls showing the lower pH (6.5±0.16), it suggested that somatotropin give effectiveness to changes semen pH.

2. Microscopic Evaluation of Bulls Semen

Mass Movement. Spermatozoa tendency to move together towards unity, so as to forma thick or thin wave, moving fastor slow depending on the concentration of spermatozoa that live in it. These movements determined the quality of the cattle semen. Toelihere (1993) has distinguished four criteria semen wave thickness, there are very good (+3) consisted large waves, bold and active, good (+2) consisted wave sare small, thin and sparse, moderate (+1) consisted active and progressive wave, bad (-0) consisted no individuall movement of spermatozoa.

Somatotropin hormone gives statistically significant (P=0.0005) on spermatozoa mass movement. According to Toelihere (1993), all spermatozoa mass movement of treated bulls categorized active and progressive wave (+1) but not gives more value for mass movement.

Table2. Microscopic evaluation of bulls semen

No	Bulls	R	Mass Mov.		Concentration ^a		Motility (%)		Abnormality (%)	
			before	After	Before	After	before	After	before	After
1.	Carlos	I	+1	+1	1000	1500	40	50	60	68
		II	-0	+1	-	1200	-	40	-	65
		III	-0	+1	-	1000	-	20	-	75
2.	Zaitan	I	+1	+1	800	800	20	20	60	31
		II	-0	+1	-	500	-	30	-	75
		III	-0	+1	-	500	-	55	-	78
3.	Segentar	I	+1	+1	1000	1000	30	30	60	38.1
		II	-0	+1	-	-	-	50	-	55
		III	-0	+1	-	1000	-	30	-	63
AVG±SD					933.3±94.2	937.5±315	30±8.1	36±12.4	60±0	60.9±15.6

R = replication; a= 10⁶/mL

Concentration.

Concentration of spermatozoa can be used to predict bulls fertility (Correa, Pace, 2002; Mottershead, 2000). Treated bulls shown higher spermatozoa concentration than control and statistically different (P=0.49). It indicated that somatotropin hormone increased spermatozoa concentration. Spermatozoa concentration are shown on Table 2.

Bovine spermatozoa concentration range between 800-2000 million/mL (Hafez, 2000).Spermatozoa concentration differences between male sallegedly caused by the genetic quality of eachbulls (Situmorang,2002). Concentration and percentage of motile spermatozoa is influenced by bulls age and have a tendency to increase with bulls age until 22 months (Mathevon et. al. 1998).

Motility.

Motility of spermatozoa was measured from individual movement. Toeliehere (1993) classifying the individual movement of spermatozoa into six group, there are (1) notmoving, (2) circular motionin place, (3) circularswingingmotion = <50% progressive move, no waves, (4) progressive move and generate waves = 50-80%, (5) agile progressive movements and wave form = 90% and (6) movementis very progressive, very fast wave = 100% active. Table 2 shown that treated bulls were higher (36±12.4) than control (30±8.1),but it statistically not significantly different (P=0.24).

According to Maxwell and Watson (1996) Decreased motility can also be caused by the thawing process. During thawing spermatozoa cells vulnerable to damageas a result of changes in osmotic pressuresuddenly that caused by the rapid melting. Susilawati et. al. (2003) reported that average spermatozoa motolity percentage before freezing and post thawing motility were 52±5.37% dan 41±3.16%, spermatozoa viability before freezing and post thawing were 94.28±1.74%, and spermatozoa concentration before freezing and post thawing motility were 143.5±10.1 million.

Abnormality.

Semenfrom different bulls containing some form of abnormal spermatozoa. This does not indicated low fertility until the number of abnormal spermatozoa more than 20%. Similarly, the types of abnormali tiesare not associated with infertility. Number of abnormal spermatozoa can be detected with the sample when calculating the percentage of spermatozo aviability (Pena et.al, 1998).This is in accordance with the opinion of Toeliehere (1993) which states that abnormality is less than 20% can still be used for insemination and bovine sperm abnormalitie spassed 30-35% indicates that bull infertility.

Collected semens from both control and treated bulls are shown very high abnormality, 60% and 60.9±15.6% respectively. Higher abnormality shown from treated bulls and its not significantly different from control. The spermatozoa abnormality shown on Table 2.

CONCLUSION

Based on theresults ofthe studyit can be concluded that administrating somatotropin hormone did not significantly increase Simbrah bulls semen quality both macroscopic and microscopic. However, the data resulting a significant results on pH, mass movement, concentration of semen not enough to guarantee bull fertility because of very high percentage of spermatozoa’s abnormality.

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02. EFFECT OF VEGETABLE AND ANIMAL-DERIVED INGREDIENTS ON THE PHYSICAL PELLET PROPERTIES OF EXTRUDED FEED

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Abstract

This research was designed to examine the effect of vegetable and animal-derived ingredients on the physical pellet properties of extruded feeds. Five experimental feeds formulated using various vegetables and animal-derived ingredient were extruded under identical extrusion variables (i.e. CLEXTRAL BC45 twin screw extruder; pressure 15 bars; temperature 112 °C). Representative sample with three replications were collected from each experimental extruded feed and then tested for the parameter of physical pellet properties namely bulk density (BD), pellet durability index (PDI), expansion ratio (ER), sinking velocity (SV) and percentage of sinking pellet (PSP). Based on analysis of variance (ANOVA), it was determined that all parameters were affected by feed ingredient ($P < 0.05$). The results of experiment showed that the values of BD ranged from 533.40 to 652.00 g/l; PDI ranged from 95.75 to 99.71%; ER in the range of 0.94 to 1.13 cm/cm; SV and PSP varied from 2.47 to 8.18 cm/s and 20.00 to 75.56% respectively. In conclusion, the use of vegetable-derived ingredients in feed formulation reduced BD, SV, and PSP but improve PDI and ER.

Key words: *physical pellet properties, vegetable-derived, extrusion*

INTRODUCTION

In intensive system, feed is one of main factors affecting performance. Giving sufficient quantity of high quality feed allows animal or fish to achieve their maximum growth potential. As a consequence of intensive farming system, the need for formulated feed in animal farms and aquaculture also increase. Following meat and fish production, global compound feed production for animal farm increased 2% a year for the period of 2002 through 2010 (FEFAC, 2010). Meanwhile, total feed used in aquaculture in the year of 1995 through 2010 increased from 7.6 million tonnes to 35.4 million tonnes, and total feed production in 2020 was predicted as much as 70.9 million tonnes (FAO, 2011).

Production of formulated feeds relies on the use of several ingredients of both marine (e.g. fish meal and fish oil) and vegetable (e.g. oilseeds, cereals) origin. The announced increase on feed production volumes will surely be conditioned by the market availability of such ingredients. For instance, supply of fish meal is highly dependent of capture of industrial fisheries and the reprocessing of fisheries by-products. This implies that feed production faces a sustainability challenge regarding the reliable and economical sourcing of ingredients. Hence, explorations of new feedstuff have been done as alternatives ingredients. For example the use of plants protein source for fish meal replacement.

It is now consensual that vegetable protein and oil sources are valid ingredients in fish feeds. Over recent years a great research effort has been devoted to the evaluation of various vegetable meals with crude protein content of 20 to 50 % and plant oils, as sustainable alternatives to fishmeal. Regarding fishmeal replacement, available

knowledge shows that sensible blending of different vegetable protein sources is necessary to balance the indispensable amino acid (IAA) profile. Furthermore, under high fishmeal replacement certain crystalline amino acids and inorganic phosphorus may have to be supplemented to fulfil the nutrient requirements of a given species (Furuya et al., 2004; Gómez-Requeni et al., 2004). There is also evidence that when including high levels of certain plant protein sources, the carbohydrate fraction and/or the presence of heat stable antinutritional factors may condition its nutritional value by altering digestion and nutrient utilization (Francis et al., 2001).

Some studies on feed formulation in animals and fish have attempted to mitigate the disadvantages of plant protein including enzyme supplementation (Morales et al., 2011; Selle and Ravindran, 2007), essential amino acid supplementation (Cheng et al., 2003), and probiotic incorporation (Baurhoo et al., 2008; Selle and Ravindran, 2007). Also, several studies assessed the benefits of using specific heat treatments (e.g. toasting) aiming at the inactivation of selected anti-nutrients of plant ingredients (Akinmutimi et al., 2008; Onimawo and Akpojobwo, 2006). Further, extrusion process is widely applied in manufacturing of pet animal as well as fish feed. (Amornthwaphat et al., 2005; Scott et al., 1991), and its high processing temperatures is an effective way to eliminate heat-labile anti-nutritional factors (e.g. trypsin inhibitors).

In previous decades, feed formulations for fish were based on marine-derived protein sources (Stickney and McVey, 2002), and it included relatively few ingredients. Recently, given the economic and market availability constraints, feed formulations are more dynamic and rapidly changing through time (Hardy, 1995) toward higher and broader inclusion of plant origin feed ingredients. Consequently, there will be large variations on pellet quality after processing as every feed ingredient processed has a contribution. The aim of this research was to study the effect of variable vegetable and animal-derived ingredients on the physical pellet properties of extruded fish feeds. Precisely, it was hypothesized that under the same extrusion variables, the higher inclusion levels of plant proteins would decrease bulk density, pellet durability, sinking velocity and percentage of sinking pellet, but increase expansion ratio.

MATERIAL AND METHODS

Experimental Feeds

Five experimental feeds were formulated for gilthead sea bream, *Sparusaurata* (Table 1). All feeds were formulated to be isonitrogenous (44% crude protein), isolipidic (16-17%) and isoenergetic (23 kJ/g DM). This experiment used two control feeds (i.e. CTRL1 and CTRL2), which were animal-derived feeds. The CTRL1 was a practical formulation with fishmeal and fish oil levels in accordance to commercial practices. In relation to the CTRL1, the CTRL2 diet had a 60% replacement of fishmeal by animal by-products such as feather meal hydrolysis and haemoglobin powder. Additionally this diet had also a concomitant 70% replacement of fish oil by poultry fat. The PP diet is a plant-protein rich formulation in which the fishmeal was replaced at 60% by soy and pea protein concentrates and wheat gluten. This PP diet was also supplemented with monocalcium phosphate (MCP) as a source of inorganic phosphorus. Additionally, in the PP diet, fish oil was replaced by a blend of vegetable oils (rapeseed, linseed and palm oils) and poultry fat.

The PP diet is a plant-protein rich formulation in which the fishmeal was replaced at 60% by soy and pea protein concentrates and wheat gluten. This PP diet was also supplemented with monocalcium phosphate (MCP) as a source of inorganic phosphorus. Additionally, in the PP diet, fish oil was replaced by a blend of vegetable oils (rapeseed, linseed and palm oils) and poultry fat.

The PHY and PPE are relatively similar to the PP formulation, differing only on the fact that the level of MCP was reduced and phytase was included in both PHY and PPE to ensure adequate phosphorus availability in those diets. In the PHY diet, phytase was applied post extrusion by vacuum coating in an oil emulsion, while in the PPE diet phytase had been used to pre-treated selected ingredients (corn-gluten, pea protein concentrate, rapeseed meal, pea starch) three-months prior to its use.

Extrusion Process

Feed manufacturing was done in SPAROS Lda. factory in Loule, Portugal. The mixture was extruded in a three-section twin screw extruder (CLEXTRAL BC45, screw length 75 cm and diameter 55.5 mm). The extrusion process for all types of experimental diet was done in the same extrusion variables (screw speed=267 rpm; water= 25%; pressure 15 bar; temperature at zone 1=28-30°C, at zone 3=112-117°C; feeder 90%). The pellets coming out from extruder was dried using an oven at 70 °C and then air cooled prior to coating. Oil ingredients were coated using vacuum coater (DINNISEN PG-10VCLAB)

Measurements

Bulk density

Bulk density was determined by filling a small plastic can (known volume of 1 litre) with the pellets sample. Then excess pellets were gently removed by pulling a scrape one time over the edge of the can. The sample was then weighted on an electronic scale. The measurement of bulk density was conducted in triplicate

Expansion ratio

Ten pellets of extruded feed from each experimental feed were measured for its diameter, using a plastic calliper. Expansion ratio was measured as the ratio of average diameter of dry pellet to the die hole diameter (Rout and Bandyopadhyay, 1999)

Pellet durability index

According to Thomas and Van der Poel(1996), pellet durability was tested using Holmen pellet tester (BorregaardLingotech, Hull, UK). A number of pellets sample were pre-sieved to remove fines, having clean pellet. Sub sample taken from pre-sieved pellets (100 g) were loaded into the pellet tester on 2.5 mm screen. Pellet testing was conducted in the duration of 30 second in triplicate. The pellet durability is expressed as pellet durability index (PDI) defined as the percentage of remaining pellet on the screen.

Sinking velocity and percentage of sinking pellet

The measurement of sinking velocity was referred to Chen et al (1999) with slightly modifications. A 45 cm length of 7 cm diameter transparent tube was marked every 5cm and placed in a vertical position. The measurement was conducted in artificial sea water by mixing tap water and commercial salt to have 32 ppt. Water was filled into the tube up to 40 cm high. Temperature was maintained at 23°C. Pellets were introduced at the water surface, and then the sinking velocity was determined by measuring the time of sinking pellet between two marks. The starting mark of the measurement was 5 cm below the surface giving the distance 35 cm. The amount of 30 pellets was used for

each measurement in triplicate. Next to the sinking velocity, the percentage of sinking pellet was also determined, which was measured as the number of sinking pellet over total pellets used in each measurement after introduce 30 seconds in water.

Data Analysis

To test the difference between feed formulations, all measurements results were analysed using one way ANOVA. Prior to ANOVA, data were checked for normality and homogeneity of variance using Kolmogorov and Levene’s test respectively. The level of significance was set at $\alpha = 0.05$. Means were compared using Duncan test. Statistical analysis was performed using SPSS 19.0.

RESULTS AND DISCUSSIONS

Bulk Density

Overall experimental results are presented in Table 2. Bulk density of extruded feed was significantly affected by types of formulation ($P < 0.05$). The values of bulk density ranged from 533.40 to 652.00 g/l. It appeared that vegetable rich ingredients feed had low bulk density compare to animal derived ingredients feed. CTRL2 diet showed the highest bulk density, followed by PHY, PPE, PP, and CTRL1. Inclusion of sunflower meal in CTRL1 results low bulk density. However, PHY, PPE, and PP which were plant-protein rich formulations showed similarity of bulk density.

This study showed that replacing fish meal by plant-protein and increasing the number of plant ingredient reduced bulk density of pellet. Piedecausa et al (2009) reported that bulk density of sea bream pellet was 1125.36 g/l (diameter 4.07mm) and 1069.85 (diameter 5.42mm) which was higher than the result of this study (533.40 g/l – 652.00 g/l). The difference in experimental results possibly because of measurement techniques, pellet diameter, and pellet length. Bulk density of the present experimental feed can be classified as slow sinking feed which has 540 – 580 g/l (Riaz, 2007). Common bulk density of sinking pellet for marine fish species is 525 g/l (SØRensen, 2012).

Bulk density is influenced by particle size (Thomas and van der Poel, 1996), type of ingredients and starch gelatinization (Thomas et al., 2001). In the present study, high bulk density of CTRL2 diet was considered due to smaller pellet sized compare to others (based on expansion ratio measurement). Even though both CTRL2 and CTRL1 were formulated based on animal derived protein, bulk density of CTRL1 was low, which can be linked to its sunflower meal content. Feed formulation plays a role in product density (Riaz, 2007). As feed formulation in this study elaborated various plant ingredients (PP, PHY, and PPE diet), it permits starch gelatinization to occur which has functional effect on bulk density. These data suggested that bulk density reduced by including vegetable ingredient.

Pellet Durability Index

PDI was significantly affected by type of formulation ($P < 0.05$). Vegetables feed was less durable compare to animal derived feed. CTRL2 which was animal protein rich diet has the highest PDI followed by PP, PHY, PPE, and CTRL1. It also appeared that PP, PHY, and PPE were the same.

In the present study, the PDI value (95.75 – 99.71%) of extruded fish feed was higher than the PDI value (80 – 92 %) reported by SØRensen (2012). High PDI resulted from extrusion of CTRL2 diet could be related to contribution of animal protein sources

i.e. fishmeal, feather meal hydrolysate and haemoglobin powder. However, high PDI of CTRL2 cannot be merely related to animal protein sources because it also had soybean meal content, which was higher than PP, PHE, and PHY. In addition, CTRL2 contained rapeseed meal which was higher than CTRL1. Under extrusion process, inclusion of soybean improved the physical quality of feed in terms of breaking force and durability, bulk density (Sørensen et al., 2009). Other existing studies reported that feather meal and haemoglobin powder promoted physical quality of pelleted feed *via* their good pellet binding capacity (Thomas, M., et al. 1998; Lee, K. and Bai, S.C. 1997). This finding also explained high PDI of CTRL2

Expansion Ratio

The types of formulation significantly affected expansion ratio ($P < 0.05$). It was observed that CTRL2 had lowest expansion ratio compare to CTRL1, PP, PHE, and PPY. Expansion ratio between CTRL1, PP, PHE, and PH were comparable. Interestingly, CTRL1 was feed containing fish meal as protein source and the only feed formulated using sunflower meal.

Data from this study appeared to meet expectation that extruded of plant rich ingredient would increase its expansion ratio. Bandyopadhyay and Rout (2001) reported that expansion ratio was affected by extrusion process, for example increasing screw speed reduced expansion ratio. However, since extrusion processes for all types of formulation were identical, the causative factors affecting expansion rate was addressed to feed ingredients. The value of expansion rate showed that PP, PPE, and PHY which were plant-protein rich diet have ability to expand beyond CTRL2, which was in turn with animal-protein rich diet. It is notable that expansion rate of CTRL1 (i.e. fish meal base protein diet) was comparable to PP, PPE, and PHY, which might be caused by sunflower meal.

Expansion ratio of extruded feed was related to degree of starch gelatinization (Arhaliass et al, 2009). In the present study, feed ingredient constituents containing relatively high starch were sunflower meal, wheat meal, and pea starch. Kraugerud et al. (2011) noticed that during extrusion, starch from sunflower meal was efficiently gelatinized and had high emulsifying property. The indication of starch gelatinization was visually observed, which can be distinguished based on pellet texture and the occurrence of vacuole-like forms.

Even though degree of starch gelatinization was not measured using specific techniques in this study, the inclusion of water during extrusion and temperature at section 3 of the screw extruder (i.e. 112 – 117 °C) ensured starch gelatinization to occur. Gelatinizing starch in the presence of water and heat is the common way to affect functional properties of starch (Thomas et al., 1998). Temperature of starch gelatinization varies and depends on ingredient. For example 81% starch is gelatinized at 70 °C in wheat and 66% starch was gelatinized at 75 °C in maize (Ratnayake and Jackson, 2008). Not only starch, protein content of vegetable ingredient was also reported to give positive effect on expansion rate *via* their ability to affect water distribution in the matrix and their macromolecular structure, which affected the extensional properties of extruded melts (Arhaliass et al, 2009). This role of protein might be also a possible explanation of high expansion rate for PP, PHE, and PHY diet.

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Sinking Velocity and Percentage of Sinking Pellet

Sinking velocity of extruded feed was significantly affected by the type of formulation ($P < 0.05$). The sinking velocity of CTRL2 was fast, giving approximately 50% faster than PP, PPE, and PHY. CTRL1 diet, which was rich in fish meal and was the only feed containing sunflower meal showed slow sinking velocity. Significant difference was observed in percentage of sinking pellet ($P < 0.05$). CTRL2 diet had higher amount of sinking pellets, being around 42% higher compare to CTRL1, and being around 54% higher than PP, PPE, or PHY. However, PP, PPE, and PHY appeared to have the same percentage of sinking pellet.

Sinking velocity is important because it determines the instantaneous feed availability to the fish which may influence competition for feed (Andrew et al., 2004). For 5 mm pellet diameter, sinking velocity of sea bream pellet was 8 – 11 cm/s which was measured at temperature of 23 °C and salinity of 36 ppt (Vassallo et al., 2006), faster than the result of this study (2.47 – 8.18 cm/s). The way of measurement (i.e. water salinity, temperature, and equipment) might affected the results.

Plant-protein rich formulation in the present study (PP, PPE, and PHY) appeared to have slow sinking velocity. It is interesting to notify that inclusion of sunflower meal CTRL1 formulation resulted slowest sinking velocity compare to others, indicating its functionality on sinking velocity, which was linked to starch content (Kraugerud et al., 2011). This study observed that bulk density was a determinant for sinking velocity. The relationship of sinking velocity and bulk density is depicted on Figure 1. It showed that increasing bulk density resulted high sinking velocity. Increasing one unit of bulk density will increase 0.0491 unit of sinking velocity.

Theoretically, sinking velocity is also related to expansion rate (Sørensen, 2012), in which high expansion rate will lower sinking velocity. Although the linear regression line followed the theoretical trend, the relationship between sinking velocity and expansion ratio in this experiment could not confidently confirm that high expansion ratio would result slow sinking velocity (linear regression not shown). This was possible caused by the method of expansion ratio measurement. There are several techniques to measure expansion ratio for example sectional and volumetric expansion ratio (Alvarez-Martinez, L. et al., 1988). In the present study measurement of expansion ratio relied on pellet diameter section and die hole diameter of extruder, and pellet length were not taken into account. Hence, variety in pellet length would affect measurement result. This study observed an inverse relationship between percentage of sinking and expansion ratio (Figure 2.). Given one unit expansion ratio increment, percentage of sinking will decrease 282.61 unit.

A shift in fish feed formulation, from high level of animal protein sources and few feed ingredients to the plant-protein source and wide variety of feed ingredients, have to be compensated with adequate data on both nutritional and processing aspects. This study enhanced information which is useful to predict physical quality of extruded fish feed. However, data generated from this experiment did not allow to distinguish contribution of single feed ingredient to the pellet properties of extruded feed. The physical pellet properties resulted from this experiment were concurrently contribution of feed ingredients constituents.

CONCLUSION

It is concluded that physical pellet properties were influenced by feed formulation. Under the same extrusion variables, the use of vegetable ingredients reduced bulk density, pellet durability index, sinking velocity and percentage of sinking, but increased expansion ratio. In order to examine specific contribution of single feed ingredient to pellet physical properties, feed formulation need to be created in such a way that the change in formulation is coming from distinctive feed ingredient.

It is recommended that starch gelatinization degree should be measured to examine its effects on expansion ratio in relation with pellet properties. It is also useful to define whether pellet quality is a *resultante* from extrusion and feed ingredient. Hence, effect of different extrusion variables and feed formulation are recommended to observe.

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Table 1. Formulation and composition of experimental feed

Ingredient, %	CTRL1	CTRL2	PP	PHY	PPE
Fishmeal 70 LT (%)	10.0	5.0	5.0	5.0	5.0
Fishmeal 65 (%)	20.0	7.0	7.0	7.0	7.0
Feather meal hydrolysate (%)		9.0			
Haemoblobin powder AP3 (%)		5.0			
Soy PC (Soycomil P) (%)			5.0	5.0	5.0
Pea PC (Lysamine GP) (%)			6.0	6.0	6.0
Wheat gluten (%)			5.0	5.0	5.0
Corn gluten (%)	11.0	11.0	12.0	12.0	12.0
Soybean meal (%)	16.0	15.0	13.5	13.5	13.5
Rapeseed meal (%)	7.0	10.0	10.0	10.0	10.0
Sunflower meal (%)	5.0				
Wheat meal (%)	6.0	5.0	4.0	4.5	4.5
Pea starch (%)	6.0	5.1	4.2	5.2	5.2
Fish oil (%)	15.0	5.0	5.0	5.0	5.0
Rapeseed oil (%)			0.5	0.5	0.5
Linseed oil (%)			2.5	2.5	2.5
Palm oil (%)			1.0	1.0	1.0
Poultry fat (%)		11.4	7.5	7.5	7.5
Vit& Min Premix (%)	1.0	1.0	1.0	1.0	1.0
Lutavit E50 (%)	0.1	0.1	0.1	0.1	0.1
Bilyses (%)	0.5	0.5	0.5	0.5	0.5
Betaine (%)	0.5	0.5	0.5	0.5	0.5
Soy lecithin (%)	0.5	0.5	0.5	0.5	0.5
Binder (%)	0.5	0.5	0.5	0.5	0.5
Antioxidant (%)	0.2	0.2	0.2	0.2	0.2
MCP (%)		2.2	2.2	0.7	0.7
Glycerol (%)		5.0	5.0	5.0	5.0
L-Lysine (%)	0.5	0.8	1.0	1.0	1.0
DL-Methionine (%)	0.2	0.2	0.3	0.3	0.3
TOTAL (%)	100.0	100.0	100.0	100.0	100.0
Composition*					
DM (%)	96.61	94.83	95.32	94.74	95.15
CP (% DM)	43.84	44.28	44.27	44.36	44.34
GE (kJ/kg DM)	22.40	23.11	23.22	23.40	23.39

* DM=dry matter; CP=crude protein; GE=gross protein. The chemical composition analysis of the diets was made using the following procedures: DM after drying at 105°C for 24 h; CP (N×6.25) by a flash combustion technique followed by a gas chromatographic separation and thermal conductivity detection (LECO FP428); GEbyadiabatic bomb calorimeter (IKA).

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Table 2. Physical pellet properties of experimental feeds extruded under identical extrusion.

Pellet properties	Type of feed				
	CTRL1	CTRL2	PP	PPE	PHY
Bulk density (g/l)	533.40±13.79 ^c	652.00±4.67 ^a	562.70±3.32 ^b	569.10±2.83 ^b	570.40±6.36 ^b
PDI (%)	95.75±0.06 ^c	99.71±0.06 ^a	98.57±0.15 ^b	98.18±0.12 ^b	98.54±0.15 ^b
Expansion ratio (cm/cm)	1.06±0.03 ^a	0.94±0.03 ^b	1.13±0.03 ^a	1.11±0.01 ^a	1.12±0.03 ^a
Sinking velocity (cm/s)	2.47±0.49 ^c	8.18±0.81 ^a	4.10±0.55 ^b	4.39±0.81 ^b	3.52±0.11 ^{bc}
Percentage of sinking (%)	33.28±2.75 ^b	75.56±6.90 ^a	20.00±6.67 ^c	21.11±1.92 ^c	21.11±3.84 ^c

Values are means ± S.D. Values in the same row with different superscript letters are significantly different (P<0.05).

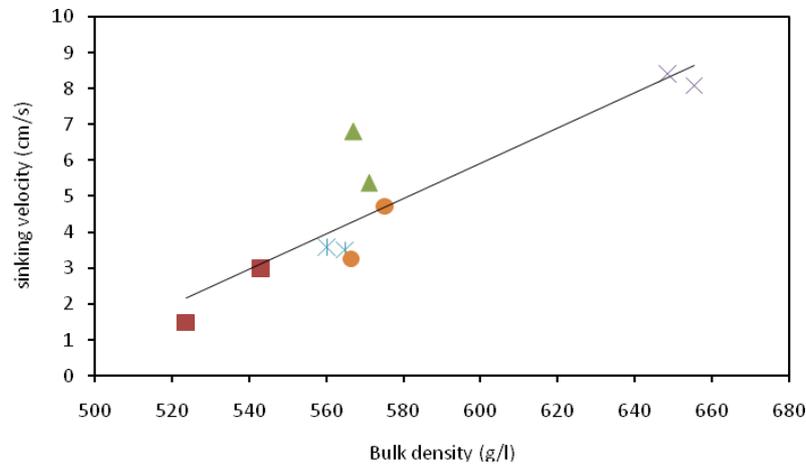


Figure 1. Linear regression between sinking velocity and bulk density of experimental feeds. $Y=0.049X - 23.54$; $R^2=0.80$; $N=10$. ■CTRL2; ▲PPE; ×CTRL1; * PP; ●PHY.

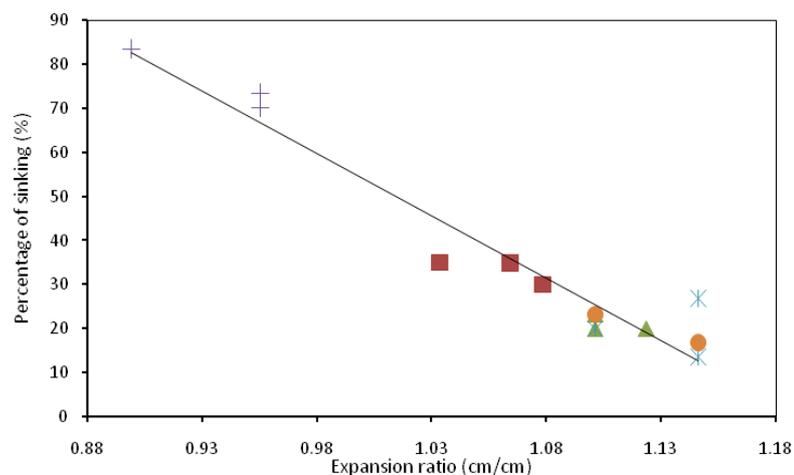


Figure 2. Linear regression between percentage of sinking pellet and expansion ratio of experimental feeds. $Y = -282.61X + 336.94$; $R^2 = 0.94$; $N=15$. ■CTRL1; ▲PPE; + CTRL2; * PP; ●PHY.

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03. ISOLATION AND IDENTIFICATION OF LACTIC ACID BACTERIA FROM FECEES OF YOUNG CALVES AS A POTENTIAL CANDIDATE OF PROBIOTIC

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Abstract

The purpose of this study was to isolate, select and characterize lactic acid bacteria (LAB) from a feces of young calves a potential candidate probiotic in a fesses of young calves. Selective isolation of LAB was performed using de Man Rogosa Sharpe medium. LAB isolates that potential as probiotic was screened. Selection was based on it's a bility to suppress the growth of pathogenic bacteria, bacterial resistance to accdic wass conditios anda bacterial resistance to bile salts (bile). Further characterization and identification conducted to determine the species. The results showed that two of the ten isolates potential to be developed as probiotic bacteria that have the ability to inhibit several pathogenic bacteria such as Eschericia coli ATCC 2922, Staphylococcus aureus ATCC 25923, and *Bacillus cereus*, able to grow at acidic condition and bile tolerance during the incubation for 24 hour. Based on the API test kit, the both of isolate identified as members of the species *Lactobacillus rhamnosus spp rhamnosus*.

Key word: lactic acid bacteria, isolation, identification, *Lactobacillus rhamnosus spp rhamnosus*

INTRODUCTION

Various attempts to improve livestock productivity have been done. Such efforts include the optimization of ruminant rumen fermentation, through the suppression of methane production by using sulphate and nitrate, orincreased production of propionate by using monensin, ovaparsin and other atibiotika. On the other hand modification of feed given to increase the brightness also hasa lot to do. An example is the chemical treatment is doneona high forage fiber roughnessor the addition of cellulose enzymeon forage silage making. Both approaches, both rumen ecosystem manipulation and treatment ofthe feed givento livestock have the same goal of increasing livestock productivity. Besides,various attempts were made to prevent disease and maintain health of livestock. To achieve these goals, such asprobiotics supplemental feedingis the right choice.

Probiotics are microbial feed supplement containing live that can providea beneficial effecton the cattle in a way to maintainand improve the microbial balance in the gastrointestinal tract (Fuller, 1992). Furthermore probioticsare definedaslive microorganisms which, when consumed by humans or animals in sufficient quantities, can provide health benefits to its host (FAO /WHO2002). FAO/WHO(2002) has issued guide lines for evaluating probiotics in food setscriteria:1) isolates had knownher identification, both feotipik and genotypicranging from genus to species and even to the degree galurnya, 2) characterization of the properties of probiotics, both in vitro as well as animal studies.

Probiotics have been used in the prevention of infectious diseases in developed countries, such as staphylococcal diarrhea in children (Marteau et al., 2001), atopy (Kalliomaki et al., 2003), abnormalities of the immune system (Isolauri et al., 2002), *Helicobacter pylori* infection, vaginosis (Reid et al., 2001), and colon cancer (Hirayama and Rafter, 1999).

Human or animal digestive tract normal flora is estimated to contain up to 10¹² bacteria per gram of gastrointestinal contents and consists of at least 500 species, mostly lactic acid bacteria (Drasar and Hiil, 1974 in Salminen and Wright, 1998; Gorbach, 2001). The newborn dairy calf born is consuming milk. Starting from the nature of it is very possible that in the fecal calf newborn dairy lactic acid bacteria can be used as a source of bacteria that have the potential to be developed for the manufacture of probiotics. It is therefore very important to do a study on the isolation and identification of lactic acid bacteria from feces of newborn dairy calf probiotic potential.

MATERI AND METHODS

Isolation of LAB strains from feces of New Dairy Calf Born A total of 10 g samples of feces of newborn dairy calf is taken from the village of dairy farmers Olean Sleman Yogyakarta Special Region is sterile. Isolation of LAB performed by spread plate method developed by Brashear et al. (2003) and Ray et al. (1997). A total of 1-10 g of feces of newborn dairy calf added to 10 ml of de Man Rogosa Sharpe (MRS) broth sterile and mixed until homogeneous. Suspension was then spread on MRS medium pH 5.5 plus 0.1% Na-azide which was added plus 1% calcium carbonate. Furthermore the petri vials were incubated at 37°C for 48 hours in an incubator in the anaerobic atmosphere. Single colonies were taken and each Petri dish and transferred into test tubes containing 10 ml of MRS broth. Then incubated at 37°C for 48 to 72 hours to obtain maximum growth of culture. Culture isolates were streaked on the back of the agar petri dishes and incubated at 37°C for 48 hours to obtain a single colony/pure culture. In this pure culture is done Gram staining for early identification. Lactic acid bacterial culture obtained is stored in freezing temperatures. Stock that will be used is prepared by growing bacterial isolates in MRS liquid medium and incubated at 37°C for 24-48 hours (Rahayu et al., 2004).

Inhibitory Power Test Isolates of Lactic Acid Bacteria against Bacterial Pathogens

LAB isolates were then tested for their anti-bacterial properties against microbial pathogens namely *Escherichia coli* 25922, *Bacillus cereus* and *Staphylococcus aureus* 25923 with methods wells (well diffusion assay). Each isolate was treated in the form of fermented supernatant containing extra cellular metabolites, obtained by inoculating liquid cultures of lactic acid bacteria isolates as much as 2% into the liquid medium de Man Rogosa Sharpe H6.5 and then incubated at 37°C for 96 hours (Bar et al., 1987). Upon incubation pH measurement, subsequent liquid culture centrifuged at 3500 rpm for 20 min. Supernatant obtained with bacterial filter sterilized (0.2 µm in porous diameter, Whatman) to obtain sterile extra cellular metabolites. Antibacterial test conducted by the method of diffusion wells (well diffusion) developed by Djaafar (1996) and modified by Sarkono et al. (2006), i.e. by planting of test bacteria of *Escherichia coli*, *Bacillus cereus*, and *Staphylococcus aureus* in different petri dishes with nutrient so that is solid, then added by soft nutrient in order soft on it. After cooling for 1 hour in a cooler room, given wells with a diameter of 0.7 mm and included isolates of

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bacterial supernatant, incubated at 37 ° C for 24-48 hours. Each of these isolates contained a clear zone diameter was measured.

Tolerance Test on Acid and Bile

LAB isolates were extensively screened inhibit the growth of pathogenic bacteriato acid and bile tolerance. Tolerance to acid test method Brashear et al. (2003). BAL culture sharvested fresh from MRS broth by centrifugation and the pellet sobtained were washed and resuspended in phosphate buffered saline (PBS) sterile. Each strainis added to 4 ml of sterile PBS and the pH adjusted to pH 2, 4, 5 and 7 (control) and incubated for 2, 4 and 24 hours in a water bath at 37 ° C.Each of the incubation period, the growth of strain can be determined by measuring the absorbance at 620 nm. Tolerans test against bileusing methods Gilliland et al. (1984). Fresh cultures of selected LAB isolates were inoculated into tubes containing 10 ml of MRS broth with levels of 0 (control), 0:05, 0:15, and 0.3% oxgall. Inoculation tubes were incubated at 37 ° C in a waterbath. Isola growth observed at 2, 4, 6, and 24 japed premises measuring the absorbance of 600 nm.

Early identification of isolates by API

Early identification made on LAB isolates that have growth inhibitory activity against *E.coli*, *B.cereus* and *S. Aureus* also their tolerance to acid and bile. Selected LAB isolates were identified through fermentation with the standard test pattern profile analysis 50 CHLAPI index with Kit (Biomerieux 2009).

RESULTS AND DISCUSSION

Selective isolation of Lactic Acid Bacteria from Dairy Cattle Feces new born calf

From the 10 colonies that gained insulationis suspected isolates of Lactic Acid Bacteria(LAB) because it produces a clear zone on the isolation medium.

(Picture 1),



Then do a reinforcement trial by regenerate on MRS media then plus CaCO₃ as much 1%. Confirmation of the test showed that the 10 isolates of LAB were able to grow well and produce a clear zone around the colony. Further characterization results proved that the 10 isolates allegedly a member of the lactic acid bacteria (Table 1).

Results obtained identification at the genus level that the four isolates were characterized is members of the genus *Lactobacillus*. The fourth isolate had phenotypic characters such as stem cells form long, lined cell structure resembles their own fences and scattered, Gram-positive reaction, not motile and do not form endospores (Sneath et al. 1986).

Figure1. Colonies are indicated as BAL with a clear zone around the colony.

Table1. Identifier character test results Lactic Acid Bacteria isolated from dairy cattle feces new born calf.

Isolates	Characteristics of lactic acid bacteria identifier				
	Shape of Cell	Gram	Catalase	Motility	Endo-spora
FSP1	Stem	Positive	Negative	Non motil	Negative
FSP2	Stem	Positive	Negative	Non motil	Negative
FSP3	Stem	Positive	Negative	Non motil	Negative
FSP4	Stem	Positive	Negative	Non motil	Negative
FSP5	Stem	Positive	Negative	Non motil	Negative
FSP6	Stem	Positive	Negative	Non motil	Negative
FSP7	Stem	Positive	Negative	Non motil	Negative
PBL1	Stem	Positive	Negative	Non motil	Negative
PBL2	Stem	Positive	Negative	Non motil	Negative
PBL3	Stem	Positive	Negative	Non motil	Negative

Inhibitory Power Test Isolates of Lactic Acid Bacteria against Bacterial Pathogens

Results of indicator bacteria growth inhibition test showed that seven out of ten isolates showed the ability to inhibit the growth of indicator bacteria, characterized by the formation of clear zones around the wells with varying sizes. Three isolates had the ability to inhibit three indicator bacteria isolates as well as the FSP3, FSP4 and PBL1. Three isolates were able to inhibit the growth of the indicator bacteria isolates two FSP5, FSP6 and FSP7. While it was only one isolate that is only able to inhibit the growth of the indicator bacteria isolates FSP1, whereas the other three FSP2, PBL2 and PBL3 not have any ability to inhibit bacterial indicators.

Based on the character of inhibition zones, ten isolates tested showed inhibition of different characters, but in general there is a showing inhibition zone with fuzzy fringe and some others showed clear inhibition zone with fringe. Blurred edges indicate that the active metabolites contained in the supernatant is bacteriostatic, which inhibits cell growth merely indicator bacteria but does not kill the cell. According Rahayu (2004), the inhibition zone escape probability is the action of acid and other anti bacterial components that are only bacteriostatic for most bacteria test (indicator) remain alive in the clear zone area, although with a very slow growth. While the barriers to suburban zones clearly indicate that isolates have the ability to produce metabolites that are bactericidal, which can kill bacterial cell metabolites indicator. This is one of the expected ability of probiotic bacteria so it can control the growth of pathogenic bacteria in the application.

Tolerance Test on Acid and Bile

Based on the test results on the growth of pathogenic bacteria kemampuan penghambatan at the research stage sebelumnya kemudian 2 isolates were selected that have the best inhibitory effects on the growth and then proceed with the test isolates in acid and bile. The data obtained from this test is data absorbance using a spectrophotometer. The addition of the absorbance values along with the addition of the incubation time showed a growth of LAB isolates tested. Both isolates of Lactic Acid Bacteria tested showed the ability to grow in acidic relatively equal. Isolates FSP4

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and PBL1 have excellent adaptability to acidic conditions, because an increase in grow that 3 pH levels in the 24-hour period. At pH 2 the two isolates did not grow, because the pH 2 is an extreme pH for petumbuhan microorganisms, including lactic acid bacteria are generally adapted to live inhabitats with a pH cukup rendah atmosphere. At pH 4, 5 and 7, both isolates were able to grow well, exponential growth occurred in 24 hours of obser vation time span because of the long incubation of 4 hours toget to 24 hours resulting in significant cell division. Isolates FPS 4 achieve the best growt hat pH 7,where as at pH 5 PBL 1 isolates. Lactic acid bacteria in the atmosphere generally prefer pH slightly below neutral pH for best growth (Axellson 1998).

The test results of the bile resistance isolates showed that the four isolates have the ability extremely well, due to an increase in the overall level of growth in bile concentrations (0.05%, 0.15% and 0.30%) in the period.

Ability to grow in the bile of both isola tes the PBL 1 FPS 4 and can not be distinguished from each other, it is presumably because the concentration of bile that is used is still very low. Resistance tobilettest this using a method developed by Gilliland et al. (1984) which uses bile concentration of 0.05%, 0.15% and 0.30%. As a comparison, other researchers (L jungh et al. 2002) tested the resistance of isolates of *Lactobacillus paracasei* sub sp. *Paracasei* F 19 on 20% bile and continue to show growth in the 2-hour incubation period.

Early identification of isolates by API

Biochemical tests with API Kit used untukmengetahui biochemical character of BALyang isolates tested so it can be used for the sake of identification. FPS4 isolates, and isolates PBL1. BAL tested is a member of the genus *Lactobacillus*, then just use the API Kit 50CHL whose contents are 49 types of sugars and their derivatives, coupled with the negative control so that overall there are 50 types of test (Biomerieux, 2009). Sugar-sugar fermentation test is one of the very important process of characterization in the genus *Lactobacillus* to know his character towards dentifikasi species diversity (Holt et al. 1994). Sugar-sugar test results with the API kit against 2 isolates isolated in the form of positive character () and negative (-) which in total amounted to 50 characters long, then analyzed by a computer program ApiwebTM Version 1.2.1 to identify the name of the species. The test results showed that isolates FPS4 after 48 hours of incubation gave a positive reaction on sugar numbers 5, 10, 11, 12, 13, 14, 16, 18, 19,21, 22, 23, 24, 25, 26, 27, 28, 31, 32, 34, 39,40, 41, 42 and 47 (Figure 2). PBL1 isolates after incubation of 48 jm gave positive reactions on sugar numbers 5,10,11,12,13,14,16, 18, 19, 21, 22,23, 24, 25, 26, 27 28, 31,32, 34, 39, 40, 41, 42, and 47 (Figure 3).

The second possible isolates derived from the same strain, at least a member of the same spesiesin the form ofttest results sugar fermentation profiles were an alyzed with the program A piweb TM version1.2.1, the result is that both the tested isolates are members of the same species, namely *Lactobacillus ramnosus* FPS 4 and .

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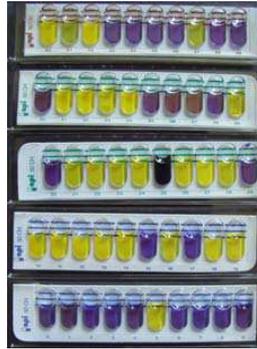


Figure 2. Identification of isolates FSP 4 results after 48 hours on the 50 CHL Fire Kit

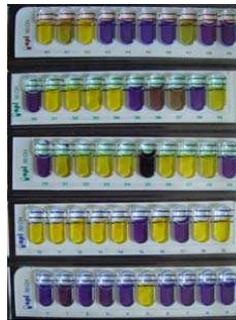


Figure 3. PBL1 isolates identification results after 48 hours on the AP I 50 CHL kit

CONCLUSION

A total often isolates of lactic acid bacteria isolated from stool holding new born dairy calf. After the selection obtained two isolates potentially remedy a probiotic that is FPS 4 and PBL 1. Both isolates have the ability to inhibit bacterial growth entero patogenik *Escherichia coli*, *Bacillus cereus* and *Staphylo coccus aureus* with inhibition zone varied widely, able to grow in acidic conditions and bile tolerant during the 24-hour incubation. Based on the API testkit and analyzed with soft ware 50 CHL Apiweb TM Version 1.2.1, the two isolates were identified as members of the *Lactobacillus* species *ramnosus*, FPS 4 and *Lactobcillus ramnosus*, PBL 1. Isolates of this research with the potential to be used as can didate probiotics in animal feedis expected to be further studied in order to know its potential in improving the quality of their fodder in vitro and in vivo, so it can be recommended as a probiotic bacterium especially in the field of animal husbandry in the future comes.

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04. EFFECT OF SHADE ON GROWTH AND PRODUCTIVITY OF TORBANGUN (*Coleus amboinicus*, Lour.)

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Abstract

Torbangun (*Coleus amboinicus*, Lour.) Is a plant that can increase milk production of animal. Cultivation of this plant is still limited. The purpose of this study was to analyze the effect of shade on the growth and productivity of Torbangun plants. The experimental design was completely randomized design with four treatments and six replications, namely: P0 (without shade), P25 (25% of shade), P50 (50% of shade) and P75 (75% of shade). Variables measured were the rate of plant height, leaves number, leaf width and plant biomass (leaves, stems, and roots). Analysis of the data using analysis of variance, if the real differently will be done Contrast Orthogonal Test. The rate of plant height, leaf number and plant biomass (leaves, stems, roots) showed significantly different, whereas leaf width showed no significantly different. The rate of plant height on P25 and P0 was significantly lower when compared with P50 and P75. Number of leaves and biomass (leaves, stems, roots) showed was not significantly difference between P0 and P25, but significantly higher when compared with P50 and P75. The best treatment is P25, although is not different from P0.

Keywords: *Coleus amboinicus*, Lour., Shade, forage

INTRODUCTION

Torbangun (*Coleus amboinicus*, Lour.) is a plant that can increase milk production of animal. The Active Compounds content of Torbangun are thymol (4.3%), Forskholin (1.5%), carvacrol (1.3%). According Acamovic and Brooker (2005), thymol is a promising alternative to antibiotics and can be used for livestock without negative effects on meat or milk produced. Carvacrol as a supplement in rations for swine lactation produce litter size, birth weight, dry matter, organic matter and protein digestibility were higher. Torbangun leaves contains more calcium, iron and total carotenoids (Mahmud *et al.*, 1990). Based on research was conducted by Rumetor *et al.* (2008) that leaves and Zn-vitamin E supplementation on Etawah Goat increased dry matter intake, DMD (dry matter digestability), OMD (organic matter digestability), VFA, TDN, and milk production about 67.22-98.65%. Treatment with any shade in *Curcuma* sp. increased some properties of plants, such as plant height, stem diameter, leaf length, leaf width, leaf number, number and length of roots, dry weight and the amount of canopy primary buds on the rhizome (Archita, 2005). Cultivation of Torbangun plant is still limited. Sunlight will affect the growth and production of crops due to the sunlight needed for photosynthesis process for the formation of carbohydrates. The objective of this study was to analyze the effect of shade on the growth and productivity of Torbangun plants.

MATERIALS AND METHODS

This research was conducted in Agrostology Laboratory, Faculty of Animal Science, Bogor Agricultural University. Planting torbangun plant used stem cuttings and shading used artificial net. The experimental design was completely randomized design with four treatments and six replications, namely: P0 (without shade), P25 (25% of shade), P50 (50% of shade) and P75 (75% of shade). The procedure of this research

consist of cutting selection, stage adaptation, building of shade, the measurement of growth, variables were measured, and Weighing dry biomass. Variables measured were the rate of plant height, leaves number, leaf width and plant biomass (leaves, stems, and roots). Analysis of the data using analysis of variance, if the significantly different will be done with DuncanTest..

RESULT AND DISCUSSION

Results of analysis of variance showed that the effect of shade indicates a very significantly different to the average rate of increase in plant height and number of leaves. Table 1 showed that the average rate of increase in plant height at P0 and P25 lower than the P50 and P75. P50 and P75 showed etiolation process due to lack of sunlight. Extension of stem may occur due to degradation of auxin (Salisbury and Ross, 1995). Table 1 showed that P0 dan P25 have the rate of leaves number were heigher than P50 and P75. Plants grown in full sunlight produced more biomass, tillers and leaves, and allocated a larger proportion of their total production to roots than plants grown in shade. The accumulation of root and shoot biomass over the first two months of seedling growth was primarily responsible for the larger size at harvest of plants grown in full sunlight (Elizabeth, 1990).

Table 1. The Effect of Shading to Rate of plant height and Leaves Number

Treatments	Rate of plant height (cm)	Leaves Number (unit)
P0	4.24 ^B ± 0.52	21 ^A ± 6.19
P25	4.86 ^B ± 0.63	23 ^A ± 5.87
P50	6.22 ^A ± 0.73	14 ^B ± 7.59
P75	5.35 ^A ± 1.13	6 ^C ± 3.14

Table 2. Effect of Shading to Leave Width

Treatmens	Leave width
P0	8.13 ± 0.67
P25	8.85 ± 1.30
P50	8.53 ± 3.51
P75	9.61 ± 1.62

Table 2 showed the effect of shade was not significantly affected leave width. P75 tended to increase the leaf width to get more sunlight. Philip *et al.*, (2013) said that Increasing shade was positively associated with specific leaf area, leaf length, leaf width and total chlorophyll content.

Table 3 showed the effect of shade were significantly decreased biomassa weight (leaves, stem and root). P0 dan P25 showed the biomassa were heigher than P50 and P75. Increased dry matter accumulation was also found in barley, millet, beetroot grown under continuous light. Homma *et al.*, (2009) reported that continuous irradiation by blue and especially red showed positive effects on growth of young tea

plants. Plant size, number of branches, leaf area, leaf dry mass, and stem dry mass decreased linearly with increasing shade in *Kalmia latifolia* (Brand, 1997). With increasing shading degree, the light compensation point, light saturation point, CO₂ compensation point, CO₂ saturation point, and carboxylation efficiency decreased, and made reducing of the biomass production (Zhang, 2009)

Table 3. Effect of Shading to Biomassa Weight

Treatmens	Weight (gram)		
	Leaves	Stem	Root
P0	70.1 A ± 17.5	55.6 A ± 7.9	6.2 A ± 1.5
P25	81.1 A ± 31.6	57.9 A ± 9.4	8.2 A ± 3.9
P50	43.3 B ± 26.6	39.5 B ± 21.7	3.9 B ± 2.6
P75	10.2 C ± 8.1	9.8 B ± 6.2	0.9 B ± 0.9
Mean	51.2 ± 20.9	40.7 ± 11.3	4.8 ± 1.8

CONCLUSION

The rate of plant height, leaf number and plant biomass (leaves, stems, roots) of P25 were similar with control. Torbangun can grow with good production until 25 % of shade

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05. EVALUATION OF AVAILABILITY AND QUALITY OF FORAGES AT LIMAU MANIS CAMPUS OF ANDALAS UNIVERSITY, PADANG, WEST SUMATRA

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Abstract

Ruminant livestock raised at teaching farm unit of Andalas University, West Sumatra is almost entirely dependent on forage feeds. Forages are often of poor quality, usually high in fiber and deficient on protein and minerals. The present research on assessment of availability and quality of forage sources was aimed to define appropriate stocking rate of beef cattle raised at teaching farm of the Andalas University of West Sumatra. Three areas of forage sources located at campus were selected as research sites, i.e.: teaching farm pasture, palm oil and teak wood plantations. Samples of forages in fresh form were collected in 5 different sampling points of each site by using quadrant of 0.5x0.5 m in size. The fresh samples were weighed and then sorted by plant species for identification of botanical composition. The samples were then remixed, dried and ground for chemical analysis. Parameter measured included forage mass yield, botanical composition, carrying capacity, DM and nutrient content of CP, CF, crude ash and macro minerals of Ca, P, Mg, K, Na and S. Results shown that production of forages mass both in fresh and dry form harvested from teaching farm pasture differed not significantly ($P>0.05$) with those from palm oil and teak wood plantations. The production of forages mass in DM form from the pasture of teaching farm, palm oil and teak wood plantation areas were found of about 24.1, 18,9 and 27.8 t/ha/year with carrying capacities of about 4.5, 3.5 and 5.1 animal unit/ha, respectively. The quality of forages from the pasture in term of CP content (11% DM) was equal to those of from teak wood plantation (11.5%), but significantly lower ($P<0.05$) than those of from palm oil plantation (18.2%). Ca content ranged between 7.0 to 7.7 g/kg. The highest mineral content was Na of about 11-14 g/kg, followed by Mg (8.7-9.5 g/kg), K (8.3-8.9 g/kg DM), while S content ranged between 3.8 to 4.5 g/kg DM. Mineral P was found the lowest, varied from 0.5 to 1.3 g/kg DM. Forages harvested at teak wood plantation showed the lowest P content of about 0.5 g/kg ($P<0.05$). Based on total wide of each sites, forages mass from pasture, palm oil and teak wood plantation could support feed for about 13.4, 69.9 and 20.6 AU, respectively, so that the stocking rate of beef cattle raised at the faculty teaching farm could be increased by about 7-8 times, if the potency of the forages available was optimally exploited.

Key words: forage mass production, forages quality, carrying capacity.

INTRODUCTION

The Faculty of Animal Science of Andalas University which is located eastern part Padang city of West Sumatra possesses a livestock teaching farm for raising different kinds of livestock animals. The teaching farm which is occupied of about 25 ha and located at upland area of Limau Manis of Pauh subdistrict ought to be an ideal location for raising ruminant livestock, especially beef cattle. The farm which was mainly assigned to serve education and research is potentially developed as a center for beef cattle production in West Sumatra. It will be not only as a potentially source of

income for the faculty, but also supports the government in achieving self-sufficiency program in meat in the year 2014.

The farm has been completely set up with stalls, animal handling facilities and pasture. In the fact, the number of beef cattle raised at present time is very limited in compare to the availability of stalls and other facilities. The present capacity of stalls are available for about 100 head of beef cattle, but the present number of cattle raised are only about 20 heads (Infitria, 2012). The main constraint in raising beef cattle is the limited availability of forages as the main feed of cattle. The farm possesses limited pasture, which is only 3 ha from the total of about 25 ha land area occupied by the farm (Infitria, 2012). Productivity of pasture and the quality of forages are also presumably low due to lack of management. The pasture was planted with two kinds of high productivity grass of *Pennisetum purpureum* and *Brachiaria decumbens*, but the grass grown not uniform and there were significant invasion of weeds.

The availability and quality of fodder feed might be enhanced by improving of pasture management and exploiting forages sources outside the farm within campus area. Most of campus area which occupied of about 479 ha (UNAND, 2007) are allocated as open green areas. These areas were planted with various perennial trees and crop plantations for several purposes, i.e. soil erosion control, land conservation, field laboratory for study of biodiversity and crop estate production and management. The inter row areas of these tree crops are covered with vegetation comprising of native grass, legumes, broadleaf species and ferns which can be utilized as forage for beef cattle production in order to increase stocking rate of teaching farm.

The most potential forages sources for teaching farm are palm oil (*Elaeis guineensis*) and teak wood (*Tectona grandis* L) plantations which occupied of about 20 and 4 ha, respectively. These plantation areas are located in adjacent to the teaching farm at the southern part of campus, a long road side of main entrance of the University. Such favorable site position and accessibility lighten in exploiting and utilizing the forages without and carry feeding system. In the plantations, plants that grow in the inter rows are considered weeds. Chemicals are used regularly to control weed growth so that the cost of weeding is quite substantial. Moreover, constant application of chemicals will alter the ecological profile of vegetation and soil, reduced biodiversity in the soil and plant growth can be affected. According Hassan & Sani (1991) there were about 60 to 70 plant species growing under the young plantation crops and the number decline to 20 to 30 species under older trees. Out of these, about 70% of these species are palatable for ruminant animals.

The present research was aimed to study the availability and quality of forage sources from three different sites of pasture, palm oil and teak wood plantations located at Limau Manis campus to define a potential stocking rate of beef cattle raised at livestock teaching farm of Andalas University.

MATERIALS & METHODS

Study Sites and Sampling Points

The study was initiated by collecting of forages samples from three different sites at Limau Manis campus area of Andalas University, Padang West Sumatra in September 2012. First was teaching farm pasture with total area of about 3 ha. The pasture which was managed by the Faculty of Animal Science consisted of 12 paddocks

with different sizes. The second was palm oil plantation with the total area of about 20 ha. The oil palm plantation was located at the southwestern part of campus in adjacent to the faculty teaching farm. The palm oil crops were about 4 years old with the total production of about 4 ton/month. The third was teak wood plantation with total area of about 4 ha and located at western campus in adjacent to palm oil plantation. The teak wood trees were about 10 year old with the mean canopy height of about 10 m.

Forage samples at pasture were collected at 8 paddocks from the total of 12 paddocks available due to relatively high variability in plant growth and land topography. At each paddock were determined 5 sampling points by dividing the paddock area proportionally into 5 blocks by considering land contour, plant condition and accessibility. Samples at palm oil and teak wood plantations were collected at 5 sampling points each. Sampling points were determined by dividing the areas proportionally into 5 blocks by considering land contour and accessibility.

Sample Collection and Analysis

Samples of forages were collected by using quadrats plate meter of 0.5 x 0.5 m in size which has been also used by Parlak et al. (2011) for estimation of forage availability. Plate meter was randomly placed at each sampling points. Plant materials in plate meter were cut at ground level and placed in individual plastic bag. The fresh samples were weighed and then separated into species and then weighed for determination of botanical composition. All samples of each sampling point were mixed and chopped. Representative samples of about 50 g were dried in a forced draught oven at 60°C for 24 hours and ground in meal form prior to analysis for dry matter (DM), crude ash, crude protein (CP), crude fiber (CF) and macro minerals of Ca, P, K, Na, Mg and S.

DM and nutrient contents of crude ash, CP and CF were determined using the procedure described by AOAC (2005). Samples for mineral analysis were prepared by wet digestion method using concentrated sulfuric acid and hydrogen peroxide. The concentration of Ca, K, Na, Mg, P and S were determined using an atomic absorption spectrophotometer (Fritz and Schenk, 1979). All analysis results were reported on DM basis.

Forage mass production in ton/ha/year was estimated using formula: $\{(fresh\ weigh\ per\ sampling/1000) \times 40\} \times (360:40)days$. Total forage mass production (ton/year) was calculated by multiplying forage mass production per hectare with total area in hectare. Daily forage mass production was calculated by dividing total forage mass production with 360 days, while forage mass production in DM was obtained by multiplying forage mass production in fresh form with DM content. Livestock carrying capacity was estimated using method described by Reksohadiprodo (1985) and Damry (2009). One animal unit (AU) is equal to one beef cattle with average body weight of 500 kg and daily feed requirement in DM form of about 3% body weight, so that carrying capacity was calculated by dividing daily forage mass production in DM form in kg with daily feed requirement of 15 kg DM.

Statistical Analysis

Data on forage mass production, carrying capacity and nutrient and mineral content were subjected to analysis of variance (ANOVA) in completely random design 3x5 consisting of 3 forage source sites and 5 sampling points as replicates. Duncan's

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Multiple Range (DMRT) was applied to separate means. Differences were considered significant at $P < 0.05$ (Steel et al., 1997).

RESULTS AND DISCUSSION

Biomass Production and Quality

The mean weight of samples in fresh form harvested from three forages sources at campus area of Andalas University ranged from 200 to 280 g/sampling, while forages mass production in DM form ranged from 19-28 tons/ha/year with the mean carrying capacity of about 3.5-5.1 AU/ha (Table 1). The biomass yields from these selected forage source sites at campus area were much higher than that from native pasture. Chen et al. (1991) reported that productivity of native pasture has been estimated conservatively to range from 3 tons/ha/year, while carrying capacity of nature grass land in Poso district of Central Sulawesi was about 0.61-0.65 AU/ha (Damry, 2009).

DM yield and carrying capacity of teaching farm pasture of about 24.1 tons/ha/year and 4.5 AU/ha, respectively, were found lower than that of teak wood plantation and not better than that of palm oil plantation, even though the data was statistically not significantly difference ($P > 0.05$). In term of crude protein and crude fiber content, the quality of forages from teaching farm pasture was equal to that from teak wood plantation, but the crude protein content of about 11% was found significantly lower ($P < 0.05$) that that from palm oil plantation (18.2%) (Table 2). According to Damry (2009) protein was very important nutrient in ruminant animals to support the optimal development and function of microbial activities in rumen. The grass grown not uniform and the pasture was severely invaded by weeds due to lacks of management. These facts were proved by the data of botanical composition (Table 3). As shown in Table 3, the kinds of plant species grown in the pasture were found the highest variation amongs the three research sites. In addition, the percentage of improved grass of *Brachiaria decumbens* was only 5% in compare to native species of *Cyperus rotundus* of about 19% (Table 3). Attempts should be undertaken to improve pasture productivity and yield quality through weed control, improvement of soil fertility grazing management and renovation grass species.

Teak wood plantation area produced the highest biomass yields in form both fresh and dry, followed by teaching farm pasture and palm oil plantation. On the other hand, the forages from teak wood plantation were found the lowest quality due to the highest content of crude fiber (45.5%) and crude ash (12.1%) (Table 1). Vegetation covered teak wood plantation area were mostly mature and more variety in species due to lack of weed control. There were 4 species dominantly grown in the teak wood plantation area: *Axonopus compressus* (40%), *Imperata cylindrica* (16%), *Cyperus rotundus* (19%) and *Melastoma malabatricum* (13%) (Table 3).

Crude fiber and crude ash content increased with maturation (Tolunay et al., 2009; Haddi et al., 2003; Mountousis et al., 2008), while protoplasm compound like crude protein decreases (Parissi et al., 2005). In addition, stem ratio increases more than leaf ratio over time with plant development (Frost et al., 2008). Crude fiber content of forages harvested at teak wood plantation was equal to the fiber content of grass from native grass land in Central Sulawesi as reported by (Damry, 2009). Feeding of the native grass on Bali cattle gave poor body weight gain of about 0.25-0.50 kg/head/day (Damry et al., 2008).

Forages from palm oil plantation showed biomass yield of about 19 tons DM/ha/year with mean carrying capacity of 3.5 AU/ha (Table 1). The availability of forages under palm oil crops managed by the Faculty of Agriculture and its carrying capacity were the lowest among the research sites ($P < 0.05$). In addition to lower biomass yield in fresh form (71.7 tons/ha/year), forages from palm oil contained lowest dry matter of about 25% (Table 1). The carrying capacity of forages harvested from the campus palm oil plantation was slightly higher than that reported from Malaysia. Research results in Malaysia reported by Chen et al. (1991) showed that in young palm oil, 3 steers/ha can be kept for two years and the stocking rate should be reduced to 2 and 1 when the canopy is closed. The higher forage mass production of palm oil plantation at Andalas University due to lack of weed control, especially at the plantation area located far from the main entrance road of the University.

In term of quality, forage mass from palm oil contained the highest in crude protein (18%) ($P < 0.05$) and lowest in crude fiber of 36.5% (Table 1). Nutritive values of forages from palm oil were higher than that of native grass. The crude protein content of native grasses on offer ranges from 6.7 to 11.4% (Lane & Mustapa, 1983). It is quite common that the animals through selective grazing may consume forages under palm oil crops with 16% crude protein or even higher, especially when grazing broadleaves and legumes (Chen et al. 1991). In addition, vegetation grown under palm oil trees were seen fresh and green as direct effect of regular application of fertilizer for tree crops. Kinds of plant species grown under palm oil were also found not so much as found under teak wood plantation. Botanical composition was dominated by relatively high palatable forages of *Axonopus compressus* and young *Imperata cylindrica* (Wong & Chin, 1998) with the mean percentages of about 32 and 50%, respectively (Table 3).

Macro mineral contents of forages were presented in Table 2. There were no statistically differences in mineral content of forages from different sources. Ca content ranged between 7 to 7.7 g/kg. The findings of present study revealed that Ca content of forages on campus were considered high, while optimum level of Ca in plants ranged from 4 to 6 g/kg (Georgievskii, 1982; Khan, et al., 2009). On the other hand, in compare to minimum level of Ca in cattle diet of about 35 g/kg to fulfill its maintenance and production requirement (NRC, 1996), the optimum Ca content of forages should range of 17 to 42 g/kg (Sultan et al., 2008).

As shown in Table 2, the highest mineral content was Na of about 11-14 g/kg, followed by Mg (8.7-9.5 g/kg) and K (8.3-8.9 g/kg DM). Mineral P was found the lowest, varied from 0.5 to 1.3 g/kg DM. This variation might be due to available P in the soil. DM. These results were coincided with the mean P content of tropical forages. The P content in tropical grasses varied from 0.2 to 0.6 g/kg of plant dry matter (Skerman & Riveros, 1990). Forages harvested at teak wood plantation showed the lowest P content of about 0.5 g/kg which was presumably due to P deficiency in the soil.

Stocking Rate

Based on total area of research sites, forages from teak wood plantation with total area of about 4 hectares were available about 403.6 tons/year in fresh form or about 309 kg/day in dry matter form. These forage mass might supply feed for about 20.6 AU or 20.6 heads of mature beef cattle. Teaching farm pasture with total area of about 3 hectares might produce forages of about 201 kg in dry matter from per day, which was

only enough to feed about 13.4 AU. This estimated carrying capacity was nearly coincided with unit number of cattle raised at the teaching farm during the research. There were about 20 heads of local beef cattle (Bali cattle) with different ages and body weight. The mean body weight was of about 300-350 kg/head.

Even though palm oil plantation gave the lowest yield of forage mass per hectare both in fresh (71.7 tons) and dry matter form (18.9 tons) (Table 1), the potential forage available at palm oil plantation with total area of about 20 hectares was estimated about 1434 tons in fresh form per year or 1048 kg/day in dry matter form. These forage mass might use to feed about 70 AU or 70 head of beef cattle per day. Therefore, if the potency of feed from the three forage sources of pasture, palm oil and teak wood plantations located at Limau Manis campus, the number of beef cattle raised at teaching farm of the Faculty of Animal Science of Andalas University might be increased to about 100 heads. These results were in line with the potential stocking rate of beef cattle raised at teaching farm of the Faculty of Animal Science, Andalas University, reported by Infitria (2012)

CONCLUSIONS

The availability of forages from teaching farm pasture was very limited and could only support about 13.4 AU. The stocking rate of the farm might be increased by using underutilized forage resources available at palm oil and teak wood plantations located in adjacent to the farm. Biomass yield of forage from teak wood plantation was higher and its quality was equal to that from teaching farm pasture, while the forages from palm oil showed the highest total biomass yield and the best quality. If the potency of the forages available in palm oil and teak wood plantations was optimally exploited, the stocking rate of beef cattle raised at the faculty teaching farm could be increased about 7-8 times from the present capacity.

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Table 1. Biomass production, carrying capacity and quality of three forage sources located at Limau Manis campus of Universitas Andalas

No.	Parameters	Teaching farm pasture	Palm oil plantation	Teak wood plantation
1.	Fresh sampling weight, g/sampling	222.7 ^{ab} ± 58.6	199.2 ^b ± 69,3	280.3 ^a ± 78.2
2.	Fresh forage mass yield, t/ha/year	80.2 ^{ab} ± 23.7	71.7 ^b ± 25.0	100.9 ^a ± 28.2
3.	DM content, %	29.2 ± 6.1	25.5 ± 4.5	27.7 ± 3.6
4.	DM yield, t/ha/year	24.1 ^{ab} ± 10.0	18.9 ^b ± 9.3	27.8 ^a ± 8.2
5.	Carrying capacity, AU/ha	4.5 ± 1.9	3.5 ± 1.7	5.1 ± 1.5
6.	Proximate analysis, % DM:			
	- Crude protein, %	11.1 ^b ± 4.3	18.2 ^a ± 4.2	11.5 ^b ± 1.4
	- Crude fiber, %	42.6 ± 3.2	36.5 ± 7.4	45.5 ± 6.2
	- Crude ash, %	8.8 ± 1.1	9.1 ± 3.5	12.1 ± 2.3

Note: Means in the same row with different superscript differ significantly (P<0.05).

Table 2. Macro mineral content of forages from three different sources located at campus area of Andalas University

No.	Minerals	Teaching farm pasture	Palm oil plantation	Teak wood plantation
1.	Ca	7.7 ± 1.3	7.0 ± 0.8	7.7 ± 1.1
2.	P	1.3 ^a ± 0.2	1.0 ^a ± 0.3	0.5 ^b ± 0.2
3.	Mg	9.5 ± 1.1	8.7 ± 1.4	9.0 ± 1.2
4.	K	8.8 ± 0.6	8.9 ± 1.3	8.3 ± 1.8
5.	S	4.3 ± 0.2	3.8 ± 0.3	4.5 ± 0.3
6.	Na	11.3 ± 1.8	14.8 ± 8.2	13.6 ± 7.9

Note: Means in the same row with different superscript differ significantly (P<0.05).

Table 3. The botanical composition of forages from three different locations at campus of Andalas University (%)

No.	Plant species (local name)	Teaching farm pasture	Palm oil plantation	Teak wood plantation
1.	<i>Pennisetum purpureum</i> (rumput gajah)	39.7	-	-
2.	<i>Brachiaria decumbens</i> (rumput bede)	5.3	-	-
3.	<i>Axonopus compressus</i> (rumput pahit)	8.6	31.9	40.1
4.	<i>Imperata cylindrica</i> (ilalang)	7.8	50.4	15.6
5.	<i>Cyperus rotundus</i> (teki)	18.9	8.5	19.0
6.	<i>Melastoma malabatricum</i> (sikaduduak)	0.5	2.9	12.9
7.	<i>Cyclosorus parathelyptens</i> (pakis)	5.6	-	8.1
8.	<i>Mimosa pudica</i> (putri malu)	3.7	-	2.9
9.	Others	9.9	6.3	1.4

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06. FEED INTAKE AND EFFICIENCY IN MICE (*MUS MUSCULUS*) GIVEN TREATED – *JATROPHA CURCAS L.* SEED MEAL

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Abstract

An experiment was carried out to study response of mice (*Mus musculus*) given detoxified *Jatropha curcas L.* seed meal (JCSM) in feed intake and efficiency. Before mixing in treatment diets, JCSM was treated with the following treatments : 1) washed four times with distilled water followed by autoclaving at 121 °C for 30 minutes (moist-heat treatment), 2) solubilising in methanol solution (methanol treatment) or 3) solubilising in 4% (w/v) NaOH solution (4% (w/v) NaOH treatment); the methanol and 4% NaOH treated JCSM were then washed four times with distilled water and autoclaved at 121 °C for 30 minutes. Five treatment rations were applied which were R1 (control diet without JCSM); R2 (95% R1 + 5% untreated JCSM); R3 (95% R1 + 5% moist heat-treated JCSM); R4 (95% R1 + 5% methanol- moist heat treated JCSM), and R5 (95% R1 + 5% NaOH (4%)- moist heat treated JCSM). A total of fifty sexual matured mice were used with the ratio of 1 : 1 between male and female. A factorial (2x2) – completely randomized design was applied; the first factor was treatment diets, and the second factor was sex of mice; five mice from each sex were used as replications. Variables measured were feed intake, nutrient digestibility, body weight gain, feed efficiency and mortality rate. The data were descriptively analysed because of high mortality rate occurred in male and female mice (80-100%) consuming R2 (untreated JCSM) and R3 (moist-heat treated JCSM). The results indicate that mice consuming R2 (untreated JCSM) or R3 (moist-heat treated JCSM) had low feed intake, nutrient digestibility, body weight, and feed efficiency, and had high mortality rate. On the other hand, given R4 (methanol-moist heat treated JCSM) and R5 (4% (w/v) NaOH-moist heat treated JCSM) can increase feed intake, nutrient digestibility, body weight gain and feed efficiency, and decrease mortality rate, especially in male mice. It is concluded that the use of 5% JCSM treated with methanol or 4% (w/v) NaOH followed by washing with aquadest four times and autoclaving at 121 °C for 30 minutes in the ration can improve feed intake and efficiency in mice.

Keywords: detoxification, feed intake, feed efficiency, *Jatropha curcas* seed meal, mice

INTRODUCTION

Jatropha curcas seed meal (JCSM) is the residue of oil extracted from *Jatropha curcas* seed. Production of JCSM has increased recently with the increase in *Jatropha* oil production; the *Jatropha* oil is used as biofuel or biodiesel. The potential production of JCSM has been estimated to be 1 ton/ha from 5 ton seed production/ha (Becker and Makkar, 2000). This production demonstrates potential use of JCSM as animal feed. This potential use is also supported by the nutrient content of JCSM. This JCSM contains 7.71% ash, 37.56% crude protein (CP), 35.02% ether extract (EE), 7.23% crude fibre (CF), and 12.47% nitrogen free extract, NFE, (Tjakradidjaja *et al.*, 2007). However, the CP (24.28%) and EE (15.99%) contents are reduced, but the CF (38.49%) and NFE (16.06%) contents are increased when the seed husks are included in oil extraction (Tjakradidjaja *et al.*, 2007); the inclusion of seed husks in oil extraction is required to increase the amount of oil extracted from the seed. These nutrient contents indicate that JCSM can be used as protein or energy supplements.

However, its use as animal feed can be limited by high contents of CF. The other limitation for potential use of JCSM is the presence of antinutrients, such as trypsin inhibitor, saponin, phytic acid, tannin, lectin or curcin and phorbol ester (Makkar *et al.*, 1997; Makkar *et al.*, 1998; Aderibigbe *et al.*, 1997; Stirpe *et al.*, 1976; Adolf *et al.*, 1984). The presence of curcin and phorbol ester become important antinutrients although its concentrations vary among provenances of *Jatropha curcas* (Makkar *et al.*, 1998). The use of JCSM causes toxicity to mice (Adam, 1974). JCSM can be used in mice diet as much as 5%, the increase in JCSM amount to 7.5 and 10% had caused high mortality rate, respectively, at 50% in 40 days and 100% in 29 days (Siagian *et al.*, 2007; Siagian *et al.*, 2008).

Detoxification of curcin and phorbol ester is important to increase the use of JCSM. Physical, chemical and biological treatments have been applied to detoxify curcin, phorbol ester and other antinutrients (Aderibigbe *et al.*, 1997; Martinez-Herrera *et al.*, 2006; Aregheore *et al.*, 2003; Tjakradidjaja *et al.*, 2007). Extraction with 90% ethanol + 0.07% NaHCO₃ at 121 °C for 25 min has reduced curcin and phorbol ester levels in *J. curcas* (Martinez-Herrera *et al.*, 2006). Several detoxification methods or procedures have been conducted by Aregheore *et al.* (2003), such as treatments with methanol, NaOH, or NaOCl which is combined with washing and autoclaving at 121 °C for 30 min. The present experiment is developed based on the treatments that have been done by Aregheore *et al.* (2003). JCSM was treated with 1) washing four times with distilled water followed by autoclaving at 121 °C for 30 min, 2) methanol or 3) 4% NaOH; the last two treatments were then washed and autoclaved at 121 °C for 30 min. The treated JCSM is then used in ration and the effects are evaluated on mice. Therefore, this experiment is carried out to study response of mice (*Mus musculus*) given detoxified *Jatropha curcas* L. seed meal (JCSM) in feed intake and efficiency.

MATERIALS AND METHODS

General procedure:

Before mixing in treatment diets, JCSM was treated with the treatments that are described above. The treatments were applied to decrease the negative effects of curcin and phorbol ester. The moist-heat treated JCSM was prepared by washing 1 kg JCSM four times with distilled water which was then autoclaved at 121 °C for 30 min. The methanol-treated JCSM was conducted by solubilising JCSM in methanol solution followed by washing four times with distilled water and autoclaving at 121 °C for 30 min. The 4% NaOH-treated JCSM was carried out by solubilising JCSM in 4% NaOH solution which was then washed four times with distilled water and autoclaved at 121 °C for 30 min. All treated-JCSM was then oven-dried at 60 °C for 24-72h. Since there was a limitation in the facilities and methods for analyzing the antinutrient contents, curcin and phorbol ester contents were not analysed. The proximate analysis was conducted for determining nutrient contents of JCSM, treatment diet and feces.

These treated-JCSM and untreated-JCSM were used to substitute a commercial diet that was utilized as control diet. The diets were then made up as pellet diets. In this experiment, there were five treatment diets used. These were R1 (control diet without JCSM); R2 (95% R1 + 5% untreated JCSM); R3 (95% R1 + 5% heat-treated JCSM); R4 (95% R1 + 5% methanol-treated JCSM), and R5 (95% R1 + 5% 4% NaOH-treated JCSM).

A total of fifty sexual matured mice were used in this experiment; these mice consisted of twenty-five males and twenty-five females. Each mouse was kept in an individual box.

The experiment was conducted for one week preliminary period which was followed with six weeks of collecting period. Each mouse was weighed in the beginning of experimental period, and in each week of collecting period. This weighing procedure was applied to determine the body weight gain in each week. Feed and drink were given *ad libitum* and feed intake was measured every week. To determine nutrient digestibility, mouse feces from each treatment were collected, separated from feed or other materials, and sun-dried for 72-96h followed with oven-dried at 60 °C for 24-48h. The composite-dried feces were ground and the samples together with feed samples were analysed with proximate analysis.

Statistical analysis :

This experiment was conducted in a factorial (5x2) – completely randomized design; the first factor was treatment diets, and the second factor was sex of mice; five mice from each sex were used as replications (Steel and Torrie, 1993). Variables measured were feed intake, nutrient digestibility, body weight gain, feed efficiency and mortality rate. Since high mortality rates occurred in this experiment, the data were analysed descriptively.

RESULTS AND DISCUSSIONS

Nutrient composition of untreated- and treated JCSM :

The results indicate that there are effects of treatment JCSM on nutrient composition (Table 1). Untreated JCSM are high in dry matter (DM), ash, CP, EE, NFE and phosphor (P) contents, but low in CF content. In comparison to untreated JCSM, all treatments reduce DM, ash, CP, EE, and P contents, but increase CF contents. The reduction in CP content and increase in CF are significant in JCSM treated with 4% NaOH- moist heat treated. Ether extract is increased when JCSM was moist heat treated, but it is reduced significantly in methanol- and 4% NaOH- moist heat treated JCSM. These results are similar to that obtained for calcium (Ca). The energy content of untreated JCSM is similar to that of methanol- moist heat treated JCSM. However, these energy contents are lower than that of 4% NaOH- moist heat treated JCSM which does not differ significantly from that of moist heat treated.

Treatment has altered nutrient compositions of JCSM. The reductions in DM contents in all treated JCSM are due to the inclusion of solution, i.e. water, methanol or 4% NaOH for solubilising JCSM, and the use of distilled water for washing treated JCSM. Heat-treatment (ie autoclaving at 121 °C; 30 min) denatured the protein of JCSM (Schum, 1993). Since curcumin is a protein base compound and a heat labile, denaturation is expected to reduce curcumin contents significantly; this reduction was more effective by applying moist heat treatment than dry heat treatment (Aderibigbe *et al.*, 1997). Heat-treatment also increases EE and it is also expected that phorbol ester is still found in EE compound; phorbol ester is heat stable compound (Aregheore *et al.*, 2003; Wakangara *et al.*, 2013); the presence of oil in JCSM may prevent effectivity of heat treatment in inactivating antinutrients (Aderibigbe *et al.*, 1997). In addition, the EE is not solubilised and discarded by washing procedure.

Table 1. Nutrient composition of untreated- and treated- JCSM

Nutrient (%DM)	JCSM ¹			
	Untreated	Moist – heattreated	Methanol- moist heat treated	4% NaOH - moist heat treated
Dry matter (%)	87.19	13.05	27.64	13.49
Ash	6.17	3.31	3.41	4.13
Crudeprotein	21.14	19.63	19.14	4.63
Ether extract	9.93	12.42	1.30	2.31
Crude fibre	26.40	32.46	37.15	53.22
NFE	36.36	32.18	39.00	35.71
Ca	0.58	0.74	0.51	0.37
P	0.86	0.42	0.49	0.11
Gross energy (cal/g) ²	3881	3968	3869	3943

¹ Analysed by Laboratorium Sumber Daya Hayati & Bioteknologi

² Analysed by Laboratorium Ilmu & Teknologi Pakan, Fakultas Peternakan IPB

Methanol treatment does not cause reduction in CP content. The reduction in CP content to a level that is similar to heat treatment on JCSM shows that the reduction is due to heat treatment. Methanol treatment has significant effects on reductions of EE indicating that methanol is able to extract almost all EE compound, including phorbol ester compound. These compounds, then, can be solubilised and discarded by washing with distilled water; therefore, it is expected that the treated JCSM may have low phorbol ester and curcumin contents. 4% NaOH treatment also produces the same effects as methanol treatment on EE. However, this 4% NaOH has also bound part of the protein, i.e. the hydroxyl compound, and denatured the protein (Winarno, 1992; Schum, 1993) including curcumin that cause a greater reduction in CP and curcumin contents. The low level of CP can decrease JCSM utilisation as protein supplement. This treatment also increases CF content significantly which is twice that of heat and methanol treatments. These show that NaOH treatment has also broken down binding between cellulose and hemicellulose with lignin increasing CF fraction availability. The changes in NFE contents by all treatments may indicate that the treatments change the structure of NFE to more simple forms. This study, therefore, demonstrates that curcumin and phorbol ester contents can be reduced by treating JCSM with methanol- or 4% NaOH followed by washing with distilled water and autoclaving (121 °C; 30 min).

Nutrient composition of treatment diets :

Variations in nutrient contents are not significant among diets containing control, untreated and all treated JCSM, except for the contents of ash, EE, CF and GE (Table 2). Ash contents of rations containing methanol- and 4% NaOH- moist heat treated JCSM are lower than those of other treatments. Rations containing moist heat treated, methanol- and 4% NaOH- moist heat treated JCSM reduce EE contents compared to those of control and untreated JCSM. Utilisation of JCSM products has increased CF and GE contents of treatment rations to that is found in control diet. Ca in rations containing moist heat treated and methanol- moist heat treated JCSM are slightly greater than those of the other rations. Lower P contents are observed in rations containing untreated, methanol- and 4% NaOH- moist heat treated JCSM compared to those found in control and moist heat- treated JCSM. These results indicate that nutrient composition of treatment rations are not affected by the changes in nutrient

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composition of all treated JCSM. This means that all treatment diets are in iso-nitrogenous and iso-caloric levels. It is, therefore, expected that the effects of treatment diets can be due to type of treated JCSM.

Table 2. Nutrient composition of treatment diets

Nutrient (%DM)	Treatment diets ¹				
	Control	Untreated JCSM	Moist heat- treated JCSM	Methanol- moist heat treated JCSM	4% NaOH - moist heat treated JCSM
Dry matter (%)	90.72	90.45	91.50	89.58	91.20
Ash	6.46	6.35	6.25	5.95	5.20
Crudeprotein	21.85	21.08	20.10	21.37	20.11
Ether extract	7.02	7.37	6.70	6.12	5.82
Crude fibre	2.94	3.95	4.95	5.24	6.20
NFE	61.73	61.32	62.36	61.32	62.67
CP	1.17	1.01	1.44	1.31	1.10
EE	0.64	0.54	0.68	0.57	0.55
Gross energy(cal/g) ²	3874	3929	3935	3970	3968

¹ Analysed by the Laboratorium Sumber Daya Hayati & Biotechnologi, PAU Hayati IPB

² Analysed by the Laboratorium Ilmu & Teknologi Pakan, Fakultas Peternakan IPB

DM and nutrient intakes :

Dry matter (DM) intake of mice consuming control diet are not different from those of eating methanol- and % NaOH- moist heat treated JCSM (Table 3). In contrast, the DM intakes of mice that are given untreated JCSM and moist heat treated JCSM are about half of those of other treatments. There are only slightly different of DM intakes of male from those of female mice; however, both sex of mice given control diet and rations containing methanol- and NaOH- moist heat treated JCSM have greater DM intakes than mice consuming other rations.

Intakes of OM, CP, EE, CF, NFE and GE showed similar patterns to those obtained in DM intakes. Greater intakes of those nutrients are also observed in both sex of mice fed with control, methanol- and 4% NaOH- heat treated diets than those given untreated and moist heat treated JCSM diets. CF intakes are lower in mice consuming untreated and moist heat treated JCSM diets than other treatments. Slightly differences in nutrient intakes of male from those of female mice are observed (Table 3).

The results in DM and nutrient intakes indicate a poor utilisation of untreated JCSM on feed intakes. Treating JCSM with moist heat treatment does not improve JCSM utilisation. On the other hand, treating JCSM with methanol and 4% NaOH combined with moist heat treatment can increase DM and nutrient intakes that do not differ from control diet. These demonstrate that both treatments combined with moist heat treatment have been capable of reducing curcumin and phorbol ester contents (Aregheore *et al.*, 2003), and washing the treated JCSM may solubilise and discard the two compounds. Aregheore *et al.* (2003) indicated that treating JCSM with 3.5% NaOH has reduced, respectively, curcumin or lectin from 102 to nil mg/ml, and phorbol ester from 1.78 to 0.18 mg/g phorbol-12 myristate-13-acetate equivalent. These reductions have led to the increase of intakes of mice consuming methanol- and 4% NaOH- moist heat treated JCSM to similar level to that of control diet. Despal *et al.* (2009) also found that daily and total feed intakes were observed in mice consuming

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ration containing JCSM treated with 4% NaOH + 10% NaOCl followed with heat treatment. This again shows that combined treatment between chemical and moist heat treatment has reduced antinutrient/toxic compounds of JCSM with the mechanism has been described above. However, washing treatment applied in this experiment may help to discard those antinutrient/toxic compounds, especially the phorbol ester, of JCSM after chemical treatments. Slightly greater DM and nutrient intakes in male mice than in female mice demonstrate differences among both sexes which may be associated with differences in capacity of digestive tract.

Nutrient digestibility :

Table 4 demonstrates that average DM digestibility of control diet is 93.12%. This DM digestibility is reduced when untreated JCSM and moist heat - treated JCSM were used in the rations. The use of methanol- and 4% NaOH- moist heat treated JCSM in rations can improve digestibility to a similar level to that of control ration in both sexes. DM digestibilities of all diets tend to be greater in female mice than in male mice. Digestibilities of OM, CP, EE, CF, NFE and energy (Table 4) of all diets follow the same trends as those that are found in DM digestibility. Male mice have lower nutrient digestibilities in all diets than female mice.

The effects of treatments on nutrient digestibilities are similar to those obtained in feed intakes. These means that untreated and moist heat treated JCSM are difficult to be digested by the mice. Although feed intakes of diets containing both JCSM are small, difficulties in digestion could be due to effects of curcumin and phorbol ester contents.

Improvement in nutrient digestibilities of rations containing methanol- and 4% NaOH- moist heat treated JCSM indicates that the products can be accepted by the mice without producing adverse effects; these can be due to reduction and exclusion of curcumin (curcumin) and phorbol ester contents (Aregheore *et al.*, 2003). The lower nutrient digestibilities in male mice than in female mice is due to higher nutrient intakes in male mice. High intakes in male mice may cause faster rate and low retention time of nutrients in digestive tract; as a result, the nutrients are not all digested by the enzymes secreted in digestive tract which increase nutrients discarded in feces (McDonald *et al.*, 2002).

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Table 3. Dry matter and nutrient intakes of treatment rations

Nutrient intake (g/head/day)	Sex	Control	Untreated JCSM ¹	Moist heat treated JCSM ²	Methanol - moist heat treated JCSM	NaOH - moist heat treated JCSM	Average
Dry matter	Male	3.03	1.16	1.23	3.26	3.28	2.39
		± 0.12	± 0.38	± 0.56	± 0.34	± 0.27	± 0.10
	Female	2.38	1.34	1.00	2.79	2.92	2.09
		± 0.70	± 0.39	± 0.18	± 0.33	± 0.31	± 0.87
	Average	2.71	1.25	1.11	3.03	3.10	2.24
		± 0.46	± 0.13	± 0.16	± 0.33	± 0.25	± 0.98
Organic matter	Male	2.58	0.98	1.05	2.88	2.28	2.07
		± 0.10	± 0.32	± 0.47	± 1.53	± 0.23	± 0.97
	Female	2.00	1.20	0.86	2.33	2.51	1.78
		± 0.59	± 0.36	± 0.16	± 0.28	± 0.26	± 0.72
	Average	2.29	1.09	0.96	2.61	2.70	1.93
		± 0.41	± 0.16	± 0.13	± 0.39	± 0.26	± 0.84
Crude protein	Male	0.66	0.24	0.24	0.68	0.66	0.50
		± 0.03	± 0.08	± 0.08	± 0.10	± 0.05	± 0.23
	Female	0.52	0.20	0.20	0.60	0.59	0.44
		± 0.15	± 0.04	± 0.04	± 0.07	± 0.06	± 0.18
	Average	0.59	0.22	0.22	0.64	0.63	0.47
		± 0.01	± 0.03	± 0.03	± 0.06	± 0.05	± 0.21
Ether extract	Male	0.21	0.09	0.08	0.19	0.19	0.15
		± 0.01	± 0.03	± 0.04	± 0.03	± 0.02	± 0.06
	Female	0.17	0.10	0.04	0.17	0.17	0.13
		± 0.05	± 0.03	± 0.01	± 0.02	± 0.01	± 0.06
	Average	0.19	0.10	0.06	0.18	0.18	0.14
		± 0.03	± 0.01	± 0.03	± 0.01	± 0.01	± 0.06
Crude fibre	Male	0.09	0.05	0.06	0.18	0.20	0.12
		± 0.04	± 0.02	± 0.03	± 0.10	± 0.02	± 0.07
	Female	0.07	0.06	0.05	0.15	0.18	0.10
		± 0.02	± 0.02	± 0.01	± 0.02	± 0.02	± 0.06
	Average	0.08	0.06	0.06	0.17	0.19	0.11
		± 0.01	± 0.01	± 0.01	± 0.02	± 0.01	± 0.06
NFE	Male	1.87	0.71	0.77	1.94	2.06	1.47
		± 0.07	± 0.23	± 0.35	± 0.29	± 0.17	± 0.67
	Female	1.86	0.88	0.39	1.71	2.19	1.41
		± 1.15	± 0.27	± 0.12	± 0.21	± 0.23	± 0.74
	Average	1.87	0.80	0.58	1.83	2.13	1.44
		± 0.01	± 0.12	± 0.27	± 0.16	± 0.09	± 0.70
Energy (cal/head/day)	Male	117.48	45.72	48.59	125.72	130.28	93.56
		± 4.64	± 14.97	± 22.15	± 18.53	± 10.79	± 42.62
	Female	92.05	56.26	39.65	110.75	115.89	82.92
		± 27.20	± 16.91	± 7.16	± 13.47	± 12.20	± 33.64
	Average	104.77	50.99	44.12	118.24	123.09	88.24
		± 17.98	± 7.45	± 6.32	± 10.59	± 10.18	± 37.82

Data are obtained up to week 5th for male mice and week 4th for female mice

Data are obtained up to week 5th for male mice and female mice

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Table 4. Digestibilities of DM and nutrients of treatment rations

Nutrient digestibilities (%)	Sex	Control	Untreated JCSM ¹	Moist heat - treated JCSM ²	Methanol - moist treated JCSM	NaOH - moist treated JCSM	Average
Dry matter	Male	93.20	74.10	78.29	91.99	92.36	85.99
		+ 0.44	+ 27.45	+ 14.09	+ 2.48	+ 1.61	+ 9.07
	Female	93.03	90.66	87.23	94.98	94.63	92.11
	+ 1.32	+ 3.04	+ 4.29	+ 0.29	+ 3.14	+ 3.32	
Average		93.12	82.38	82.76	93.49	93.50	89.05
		+ 0.12	+ 11.71	+ 6.32	+ 2.12	+ 1.60	+ 5.92
Organic matter	Male	96.36	87.91	86.68	94.91	95.07	92.19
		+ 0.24	+ 12.81	+ 8.64	+ 1.57	+ 1.04	+ 4.52
	Female	96.50	96.87	93.65	98.21	96.57	96.36
	+ 0.67	+ 1.04	+ 2.13	+ 0.11	+ 0.44	+ 1.66	
Average		92.39	92.39	90.17	96.56	95.82	94.27
		+ 6.34	+ 6.34	+ 4.93	+ 2.33	+ 1.06	+ 2.86
Crude protein	Male	93.82	75.52	78.96	92.26	92.67	86.65
		+ 0.40	+ 25.93	+ 13.65	+ 2.39	+ 1.55	+ 8.69
	Female	93.73	90.22	84.75	94.14	94.32	91.43
	+ 1.19	+ 3.25	+ 5.12	+ 0.34	+ 0.74	+ 4.09	
Average		93.78	82.87	81.86	93.20	93.49	89.04
		+ 0.07	+ 10.39	+ 4.09	+ 1.33	+ 1.17	+ 6.11
Ether extract	Male	95.56	85.00	90.18	96.29	96.25	92.86
		+ 0.22	+ 15.90	+ 6.37	+ 1.14	+ 0.79	+ 5.15
	Female	96.35	95.24	87.54	97.44	97.55	94.82
	+ 0.69	+ 1.58	+ 34.98	+ 0.11	+ 0.32	+ 4.18	
Average		94.46	90.12	88.86	96.87	96.90	93.84
		+ 0.15	+ 7.24	+ 1.87	+ 0.81	+ 0.92	+ 4.00
Crude fibre	Male	65.36	-9.58	28.42	71.77	75.45	46.29
		+ 2.26	+ 116.13	+ 46.44	+ 8.73	+ 5.19	+ 36.42
	Female	62.49	64.53	59.20	75.65	79.04	68.18
	+ 7.13	+ 11.79	+ 13.70	+ 1.43	+ 2.72	+ 8.66	
Average		63.93	27.48	43.81	73.71	77.25	57.23
		+ 2.03	+ 52.40	+ 21.76	+ 2.74	+ 2.53	+ 21.11
NFE	Male	93.53	75.13	81.19	92.43	93.68	87.19
		+ 0.42	+ 26.35	+ 12.20	+ 2.33	+ 1.34	+ 8.53
	Female	93.57	92.13	88.41	95.22	95.15	92.90
	+ 1.22	+ 2.62	+ 3.89	+ 0.28	+ 0.63	+ 2.82	
Average		93.55	83.63	84.80	93.83	94.42	90.05
		+ 0.03	+ 12.02	+ 5.10	+ 1.97	+ 1.04	+ 5.35
Energy	Male	93.53	75.13	81.19	92.43	93.68	87.19
		+ 0.42	+ 26.35	+ 12.20	+ 2.33	+ 1.34	+ 8.53
	Female	93.57	92.13	88.41	95.22	95.15	92.90
	+ 1.22	+ 2.62	+ 3.89	+ 0.28	+ 0.63	+ 2.82	
Average		93.55	83.63	84.80	93.83	94.42	90.05
		+ 0.03	+ 12.02	+ 5.10	+ 1.97	+ 1.04	+ 5.35

Data are obtained up to week 5th for male mice and week 4th for female mice

Data are obtained up to week 5th for male mice and female mice

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Body weight gain and feed efficiency :

Data in Table 5 indicates that male and female mice consuming control diet have positive body weight gain (BWG). Positive BWG is also obtained in male and female mice consuming methanol- and 4% NaOH- moist heat treated JCSM. In all these treatments, BWG of male mice is greater than that of female mice. The data in BWG of all mice given untreated JCSM and moist heat treated JCSM are negative. These BWG and feed efficiency are obtained up to the last week of the last number of mice death.

Feed efficiency of male mice consuming control ration does not differ from that of consuming 4% NaOH- moist heat treated JCSM; however, these are lesser than that of eating methanol moist heat treated JCSM (Table 5). Feed efficiency of female mice given methanol- and 4% NaOH- moist heat treated JCSM are greater than those of eating control diet. Feed efficiency of both male and female mice consuming untreated JCSM and moist heat-treated JCSM are negative and the lowest.

Good effects of control, methanol- and 4% NaOH- moist heat treated JCSM on BWG and feed efficiency demonstrate that the consumed nutrients can be digested without adverse effects by the mice, and the digested nutrients can be used to increase mice BWG more efficient compared to other treatments. These mean that treating JCSM with methanol- or 4% NaOH- followed by washing with distilled water and autoclaving (121 °C; 30 min) have been able to decrease curcin and phorbol ester content. In contrast, the presence of those antinutrients has been responsible for the low nutrient intakes and digestibilities of rations containing untreated and moist heat treated JCSM; these cause low BWG leading to death to occur (Aregheore *et al.*, 2003; Despal *et al.*, 2009).

Table 5. Body weight gain and feed efficiency

Variables	Sex	Control	Untreated JCSM ¹	Moist heat treated JCSM ²	Methanol - moist heat treated JCSM	NaOH - moist heat treated JCSM	Average
Body weight gain (g/head/day)	Male	0.22	-0.16	-0.09	0.25	0.24	0.09
		± 0.11	± 0.04	± 0.11	± 0.10	± 0.12	± 0.20
	Female	0.08	-0.19	-0.16	0.12	0.15	0.00
		± 0.14	± 0.07	± 0.08	± 0.04	± 0.07	± 0.16
	Average	0.15	-0.18	-0.13	0.19	0.20	0.05
		± 0.10	± 0.02	± 0.05	± 0.09	± 0.06	± 0.16
Feed efficiency³ (%)	Male	7.38	-14.88	-37.53	13.68	7.39	-4.79
		± 3.50	± 7.72	± 11.70	± 14.65	± 3.51	± 21.27
	Female	2.13	-16.34	-15.56	4.46	5.15	-4.03
		± 7.78	± 7.84	± 5.70	± 1.46	± 2.38	± 10.94
	Average	4.67	-15.61	-26.55	9.07	6.27	-4.41
		± 3.71	± 1.03	± 15.54	± 6.52	± 1.58	± 15.77

Data are obtained up to week 5th for male mice and week 4th for female mice

Data are obtained up to week 5th for male mice and female mice

Feed efficiency (%) = ([g body weight gain/g feed intake] x 100%)

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Mortality rates :

High mortality rates occur in this experiment (Table 7). For male mice consuming untreated, moist heattreated and methanol- moist heat treated JCSM, the mortality rates, respectively, are 100, 80, and 20%. The mortality rates, respectively, are 20, 100 and 100% for female mice eating control diet, untreated-, and moist heattreated JCSM. The highest mortality rates (100%) occur in both sexes when the male and female mice consumed untreated- JCSM diet. These high mortality rates have led to analyse the data descriptively.

The occurrence of death differs among treatments diets and sex of mice. Death occurs in female mice consuming control diet at week 6th which was due to low individual health condition of the mice. For male and female mice eating untreated JCSM diet, death occurs starting at week 3rd with the highest numbers are found in male than female. Death for male mice consuming moist heat treated JCSM starts at week 2nd, and those that ate methanol- moist heat treated JCSM ration occurs at week 6th. No death occurs for female consuming methanol- and 4% NaOH- moist heat treated JCSM rations, and for male eating 4% NaOH - moist heat treated JCSM ration.

Table 6. Mortality rates of mice during the experiment

Mice sex	Treatment diet	Number of death mice on week						Total number (head)	Total (%)
		1	2	3	4	5	6		
Male	Control	0	0	0	0	0	0	0	0
	Untreated JCSM	0	0	3	1	1	0	5	100
	Moist heat treated JCSM	0	1	1	1	1	0	4	80
	Methanol moist heat treated JCSM	0	0	0	0	0	1	1	20
	4% NaOH moist heat treated JCSM	0	0	0	0	0	0	0	0
	Total(head)	0	1	4	2	2	1	10	40
Female	Control	0	0	0	0	0	1	1	20
	Untreated JCSM	0	0	1	4	0	0	5	100
	Moist heat treated JCSM	0	0	0	3	2	0	5	100
	Methanol moist heat treated JCSM	0	0	0	0	0	0	0	0
	4% NaOH moist heat treated JCSM	0	0	0	0	0	0	0	0
	Total (head)	0	0	1	7	2	1	11	44
Total (head)	0	1	5	9	4	2	21	84	
Average	0	0.5	2.5	4.5	2	1	10.5	42	

High mortality rate in mice consuming untreated and treated JCSM was also observed by other researchers. Tjakradidjaja *et al.* (2009^a) show that high mortality rate (50 - 100%) was found when the mice were given *Rhizopus (R.) oryzae* treated JCSM at levels of 5 - 10%; death occurred in male faster (at the 2nd week) than in female (at the 3rd week) when the mice consumed ration containing 10% *R. oryzae* treated JCSM. High mortality rate (80 - 100%) in male mice also occurred when the mice were fed with untreated- and treated- JCSM with various moulds; however, time of death occurrence depended on mould species (Tjakradidjaja *et al.*, 2009^b). High mortality of

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mice occur due to toxicity of curcin and phorbol ester, and the effects are similar to those obtained by Siagian *et al.* (2007), Siagian *et al.* (2008), Despal *et al.* (2009), Adam (1974), and Adam and Magzoub (1975). However, the present experiment demonstrates that combined treatments between methanol or 4% NaOH followed by washing with distilled water and moist heat treatment have been successful to reduce mortality rate by decreasing curcin and phorbol ester contents (Aregheore *et al.*, 2003).

CONCLUSIONS

It is concluded that the use of 5% JCSM treated with methanol or 4% NaOH followed by washing with distilled water four times and autoclaving at 121 °C for 30 minutes in the diet can improve feed intake and efficiency in mice, and reduce mortality rate. It is necessary to increase the amount of methanol or 4% NaOH treated JCSM in the diet in the next experiment.

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07. EFFECT OF THE EXTRACTED CINNAMON STICK AND GROUND CINNAMON STICK ON THE RANCIDITY OF PALM OIL DECANter MEAL

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Abstract

The aim of this present study was to evaluate the effects of different levels of methanol-extracted cinnamon bark (MCB) and ground cinnamon bark (GCB) on the rancidity value of palm oil decanter meal (PODM). Cinnamon bark (CB) was originally imported from West Sumatra, Indonesia. The CB was extracted by using methanol and some of those was grinded. Fresh PODM sample was collected from a palm oil plant in Kemaman, Trengganu, Malaysia. PODM samples were treated with three levels of GCB (0.05, 0.075, 0.1% (w/w) for GCB1, GCB2, GCB3 respectively), three level of MCB (0.010, 0.015, 0.020 % (w/w) for MCB1, MCB2, MCB3 respectively), BHT (B) at 0.01% (w/w) and Vitamin E (E) at 0.01% (w/w). Peroxide value (PV) and Thiobarbituric acid (TBA) were determined. The Completely Randomized Design in factorial arrangement with nine treatments (C, MCB₁, MCB₂, MCB₃, GCB₁, GCB₂, GCB₃, B and V), four times of data collection (day 3, 5, 7 and 10) and three replications was applied to this experiment. Anova followed by Duncan test were applied to analyze the mean of FA, PV and TBA during these studies. There is no significantly different ($P > 0.05$) effect of interaction between treatment and time on PV. There is significant different ($P < 0.05$) effect of treatments on PV. There was significant different ($P < 0.05$) the effect of treatment on TBA value of PODM. GCB₃, 5.81 mg/kg, was the lowest TBA value of PODM among the treatments. It was significantly lower ($P < 0.05$) than control and not significantly different ($P > 0.05$) from B and V, the synthetic antioxidant. OM, ash, CP, EE and NDF but not significantly ($P > 0.05$) different on those of ADF. The use of 0.1% of GCB gave the best result to reduce rancidity of PODM. It could reduce PV (29.77%) and TBA (22.16%) in comparison with control. GCB was better than MCB. Increasing the level of GCB to preserve PODM was recommended.

Keywords: cinnamon bark, palm oil decanter meal, rancidity,

INTRODUCTION

Palm oil decanter meal (PODM) is easy to turn rancid when it is kept standing in the open air for a few days (Afdal *et al.*, 2012). This might be possibly caused by the oxidation of oleic acid (Berger, 1994), the major component of FA, in the PODM. It is a major problem of PODM so that this by-product is not optimally utilized and considered as waste. Only few PODMs are used as organic fertilizers. Mahyuddin and Praharani (2010) reported that PODM is still left untouched on the field and there are only very few farmers, who live around the palm oil factory, using this by-product as feed supplement for their livestock. BPS (2002) reported that the production of PODM in Indonesia is around 460,000 ton per year. The production of PODM may also be plentiful in Malaysia and in other palm oil producing countries. Therefore this is one of hot issues faced by the palm oil factory presently.

Cinnamon (*Cinnamomun burmanni* (Nees and T.Nees) Blume) bark (CB) is a natural product which has been used to preserve food or as food spice. This might be due to the content of active compounds, like cinnamaldehyde, cinnamal acetate,

cynnzcylanol, cynnzcylanine, phenylpropyl acetate, tannin and safrole, within CB (Hariana, 2008). Some efforts were done to extract these compounds using solvents like methanol, ethanol, hexane and ethylacetate. Parekh *et al.*, (2005) reported that methanol is the good solvent and more active than the aqueous extracts. Methanol was also successfully used to extract CB in comparison with hexane and ethyl acetate (Afdal unpublished data). Methanol extracted CB (MCB) was used to preserve food (Smith-Palmer *et al.*, 2001; Gutiérrez *et al.*, 2009). Traditionally, ground CB (GCB) is also a good potential in preserving food. Therefore MCB or GCB along with the antioxidant content could also apply to delay, inhibit or prevent the oxidation process in food so it protects food from being rancid. With the above in mind, MCB or GCB could be possibly applied to preserve PODM. The aim of this present study was to evaluate the effects of different levels of MCB and GCB on the rancidity value of PODM.

MATERIAL AND METHOD

2.1. Grinding and extracting of cinnamon stick

CB was originally imported from West Sumatra, Indonesia. It was then coarsely ground in a grinder machine (Retsch SM 100, Retsch GmbH & Co KG, Germany) and sieved in 1 mm sieve at the Factory Unit, Universiti Putra Malaysia. The CB was extracted according to the method created by Mancini-Filho *et al.* (1998). Around 3 kg of air dried powder of CB was placed into a 10 L bucket and then around 7.5 L of methanol was poured, followed by 1 l of 6 M HCl solution and manually mixed for 20 minutes. The mixture was kept to stand overnight. The following day the mixture was filtered using filter paper (Whatman No.1, England) and taken to dry using vacuum Rotary Evaporator (Buchii, Switzerland) at 40 °C set up with the vacuum pump. The recovery sample was then freeze-dried and kept in a plastic container pending to be used.

2.2. Sample preparation and procedure

Fresh PODM sample was collected from a palm oil plant in Kemaman, Trengganu, Malaysia and transported to the laboratory of Animal Nutrition, Universiti Putra Malaysia. PODM samples were treated with three levels of GCB (0.05, 0.075, 0.1% (w/w) for GCB₁, GCB₂, GCB₃ respectively), three level of MCB (0.010, 0.015, 0.020 % (w/w) for MCB₁, MCB₂, MCB₃ respectively), BHT (B) at 0.01% (w/w) and Vitamin E (E) at 0.01% (w/w). Each single sample was mixed by using a blender machine and kept standing at a room temperature prior to the data collection. Then samples were collected on day 3, 5, 7 and 10 and subsequently transferred into a fridge at -20 °C prior to analysis.

2.3. Chemical analysis

Proximate analysis, including DM, ash, CP and EE was done according to the procedure of AOAC (1990). Analysis of fiber content, including NDF and ADF, was done following the procedure of Van Soest (1963). Mineral analysis for Cr, Fe, Mn, Ni and Cu were carried out using atomic absorption spectrometry (AAS).

2.4. Rancidity analysis

2.4.1. Peroxide value

Peroxide value (PV) was determined according to the procedure of Vanhanen and Savage (2006) with few modifications. Approximately 5 g of PODM samples were placed into a beaker glass and 30 ml of mixture of acetic acids and chloroform (ratio of 2:2) were added, shaken by hand and kept standing on a table for 1 h. The mixture was filtered using filter paper (Whatman 125 mm Ø Cat No 1001 125, Whatman

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International Ltd Maidstone, England) into an Erlenmeyer flask and treated with 1.5 ml of saturated potassium iodide. The solution was kept on the table for a while and shaken by hand, after which 30 ml distilled water and one or two drops of an indicator (starch solutions) were added. The solution was subsequently titrated with sodium thiosulphate (0.1 N Na₂S₂O₃) until the purple color disappeared. The blank sample was also done under the same condition as PV determination itself. PV was calculated according to the formula:

$$PV = (A \times N \times 1000)/S$$

Where, PV (meq kg⁻¹) is peroxide value, A is ml amount of sodium thiosulphate titrated, N is normality of sodium thiosulphate and S the sample weight.

2.4.2. Thiobarbituric acid

Thiobarbituric acid (TBA) number was determined according to the procedure of Tadmors et al., (1960). 10 g of PODM sample were mixed for 2 min with 50 ml distilled water using a blender. The mixture was then poured into a distillation flask and washed with 47.5 ml of distilled water. It was afterward treated with 2.5 ml of 4 N hydrochloric-acid together with an anti foam agent and marble. The flask was heated for about 10 min and 50 ml of distillate was collected. 5 ml of this distillate was pipeted into a 15-ml-glass stopper tube and 5 ml of 0.2883% (w/v) TBA solution in 90% glacial acetic acid was added. The tubes were capped, shaken and then heated to the boiling point in a water bath for 35 min. A blank tube preparation was made in the same way using 5 ml of distilled water and 5 ml of the reagent. Both sample and blank were cooled in tapped water for 10min, and the absorbance (D) was recorded against the blank at 538 nm using 1.5 ml cuvette. The TBA number was calculated in mg of malondialdehyde kg⁻¹ of samples, which was equal to 7.8 times using the formula:

$$TBA = D \times 7.8$$

Where, TBA is thiobarbituric number and D is absorbance.

2.5. Experimental design and statistical analysis

The Completely Randomized Design in factorial arrangement with nine treatments (C, MCB₁, MCB₂, MCB₃, GCB₁, GCB₂, GCB₃, B and V), four times of data collection (day 3, 5, 7 and 10) and three replications was applied to this experiment. Anova followed by Duncan test were applied to analyze the mean of FA, PV and TBA during these studies. All statistical tests were conducted at 95% confidence level. Analysis of correlation was done for time of sampling, rancidity and FA content using Microsoft SAS 9 (SAS, 2008).

RESULTS AND DISCUSSION

3.1. Rancidity

Rancidity occurred as a process of the [oxidation](#) of [fats](#), [oils](#) and other [lipids](#). The value of rancidity is expressed as PV and TBA.

3.1.1. Peroxide value

The peroxide value is generally expressed as miliequivalents (meq) of peroxide and hydroperoxide per kilogram of sample and is a valuable measure of the early stages of rancidity occurring under ambient conditions (Robards *et al*, 1988). PV of PODM in this experiment was determined and the result can be seen in Table 1 and Figure 1.

There is no significantly different ($P>0.05$) effect of interaction between treatment and time on PV. There is significant different ($P<0.05$) effect of treatments on PV. GCB₃ shows the lowest PV of 10.24 meq/kg. This value was lower than those of synthetic antioxidant (treatment B and V). The PV of all MCBs was not significantly different ($P>0.05$) from control but significantly lower than those of GCBs and synthetic antioxidant of B and V. This result indicated that 0.1% of GCB was the best level to slow PV and also shown that GCB is better than MCB during storage of 10 days (see Figure 1). This level could reduce PV up to 29.77% (see Table 1). In terms of time, there was significant different ($P<0.05$) on the time of sample collected.

The result illustrates that the ability of bioactive compound existing in GCB is better than that of MCB in reducing PV. The content of bioactive compound within GCB might be relatively more available than those of within MCB. Karpińska et al (2001) stated that natural antioxidant was better than synthetic additives. Eugenol, a phenolic group, might play as an important compound in the GCB. This is due to that GCB only got grounded so there was not many active compounds evaporated or came out from GCB. Meanwhile MCB underwent some treatments such as soaking into methanol, heating when evaporating thus this caused the reduction of the active compound availability in it. Vijesekera (1991) reported that the quality of extract is affected with the kinds of machine used to extract as well as the procedure of extraction, including material size, solvent used to extract, solvent concentration, ratio between materials and the solvent, temperature, the length of extraction, evaporation, purification and drying of extract. Sangani *et al* (2005) studied on cumin plant reporting that the particle sizes of ground plant influence the content of essential oil available from plant. Sowbhagya *et al* (2008) also added that the yields and the quality of spice oil were significantly affected by the methods of grinding of the plant material.

Based on Figure 1, the interesting picture shows PV rate flattened after day 7. This may initiate the declining of PV from this time.

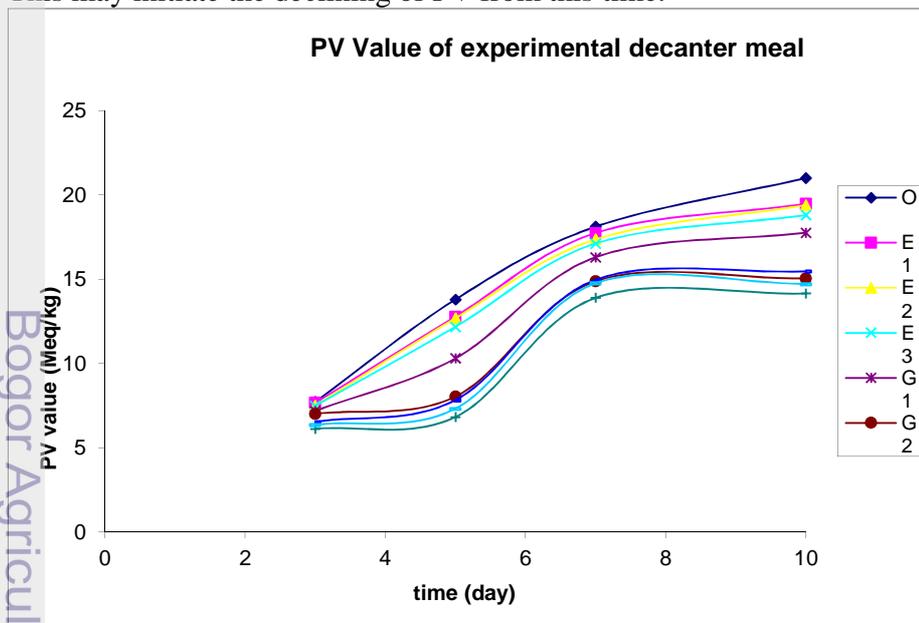


Figure 1. Peroxide value of experimental PODM for ten days

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Table 1. The value and percentage of the decrease of TBA and PV of PODM

Treatment	PV		TBA	
	Value (meq/kg)	Decrease (%)	Value (mg/kg)*	Decrease (%)
C	15.28 ^a	0.00 ^a	10.84 ^a	0.00 ^c
MCB ₁	14.66 ^a	2.98 ^{ab}	8.09 ^b	1.34 ^c
MCB ₂	14.07 ^a	5.11 ^{abc}	7.13 ^{bc}	3.26 ^{bc}
MCB ₃	14.05 ^a	6.92 ^{abc}	6.49 ^{cd}	5.43 ^{bc}
GCB ₁	13.12 ^{ab}	12.63 ^{abc}	10.12 ^a	3.17 ^{bc}
GCB ₂	11.24 ^{bc}	22.94 ^{abc}	6.32 ^{cd}	11.27 ^{abc}
GCB ₃	10.24 ^c	29.77 ^c	5.81 ^d	22.16 ^{ab}
B	11.50 ^{bc}	28.55 ^{bc}	6.26 ^{cd}	17.97 ^{abc}
V	11.10 ^{bc}	26.59 ^{bc}	6.29 ^d	24.83 ^a
Time				
	7.15 ^z	8.45 ^y	5.67 ^w	11.00
	9.67 ^y	27.00 ^x	6.98 ^x	10.59
	16.13 ^x	9.15 ^y	8.04 ^y	9.71
	17.35 ^x	17.88 ^{xy}	8.81 ^z	8.48
Time (Tm)	*	*	*	N
Treatment (T)	*	*	*	*
Tm*T	N	N	n	N

% is the decrease of value from control

All values are means of 3 replications and standard error of the mean

Means with different superscript within the same column are significantly different (P<0.05)

a, b, c and d for antioxidant source effect, w, x, y and z for time effect *mg of Malonaldehyde/kg.

MCB₁₋₃: Methanol extracted cinnamon bark level of 0.010, 0.015 and 0.020 % (w/w), GCB₁₋₃: Ground cinnamon bark level of 0.05, 0.075 and 0.100 % (w/w), B: BHT (0.01 % (w/w)) and V: Vitamin E (0.01 % (w/w))

3.1.2. Thiobarbituric acid

TBA test is also a common method used for detecting oxidation of lipids (Asakawa dan Matsushita, 1979). In general, TBA test is a reaction between thiobarbituric acid and some aldehydes contained in the secondary products formed from lipid peroxides. TBA is defined as the quantity of mg malonaldehyde in one kg sample. TBA value is an index of lipid oxidation measuring malonaldehyde content. The method is based on the spectrophotometric quantification of the pink complex formed at an absorbancy of 532 nm after reaction of one molecule of malonaldehyde with two molecules of thiobarbituric acid (Tadlargis, *et al*, 1960; Zhang *et al*, 2010). Bernheim *et al* (1952) reported that of the unsaturated FA tested, linolenic acid gives

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the most color with TBA and oleic acid the least. Table 1 and Figure 2 expose the TBA value of experimental PODM.

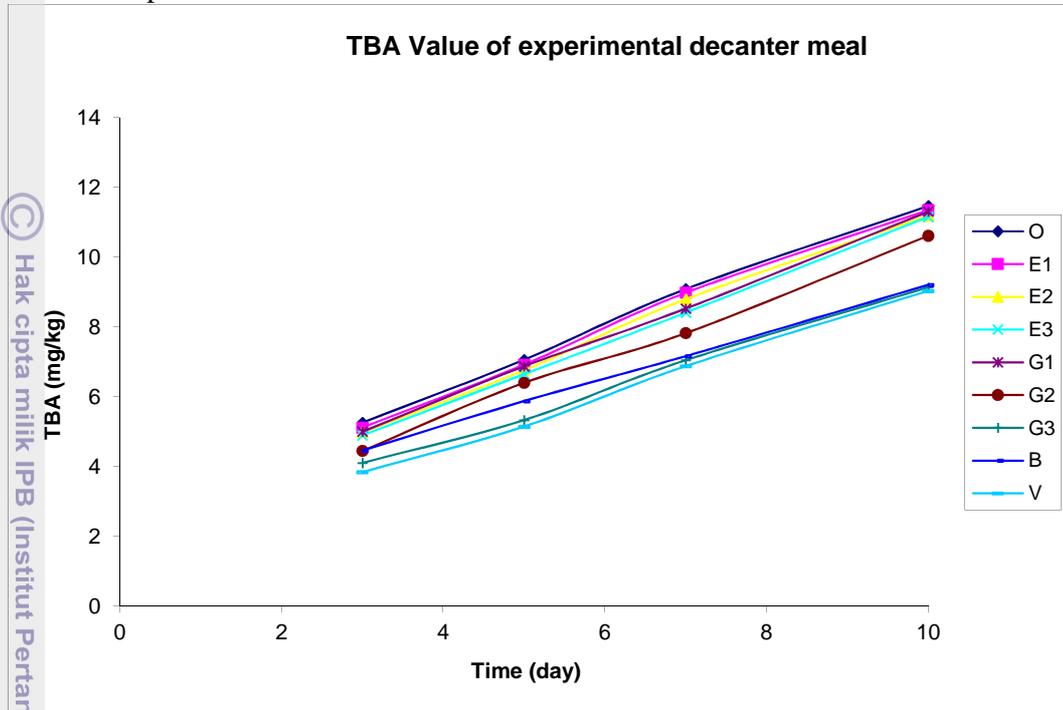


Figure 2. Thiobarbituric acids number of experimental TDM up to ten days

Table 1 shows TBA value of PODM. There is no significantly different ($P > 0.05$) the effect of interaction between treatment and time on TBA. There was significant different ($P < 0.05$) the effect of treatment on TBA value of PODM. GCB₃, 5.81 mg/kg, was the lowest TBA value of PODM among the treatments. It was significantly lower ($P < 0.05$) than control and not significantly different ($P > 0.05$) from B and V, the synthetic antioxidant. The same trend with PV, using 0.1% of GCB proved to be the best treatment to slow the TBA value of PODM and also discovered that GCB was better than MCB extracted during storage of ten days (see Figure 2) 0.1% of GCB in PODM could slow the TBA up to 22.16%.

TBA value of experimented PODM looks the same trend with PV. GCB₃, 0.1% of ground CB, exposed the best level to inhibit the formation of TBA value of PODM. This indicates that GCB probably contained more complete active compounds than that of MCB. Therefore, the ability of GCB₃ to slow down the formation of malonaldehyde was also stronger than that of E. This is a general phenomenon that the bioactive compound is completely available in the material before being extracted. The relationship between the value of TBA and PV appears strong with the correlation coefficient (R^2) of 0.5424 (see Table 3). Asakawa and Matsushita (1979) reported that there was a relationship between TBA value and PV.

2. Chemical composition of oil palm decanter meal

The composition of PODM may be influenced by the rancidity. Frankel (1984) reported that the problems related to the lipid oxidation have got much interest as they are related to flavor deterioration, loss of nutritional value and safety, biological damage, ageing, functional property changes and environmental pollution. Rancidity could lower both the nutrients and palatability of PODM. However, Frankel (1996)

mentioned that antioxidant can interrupt lipid autooxidation by interfering with either the chain propagation or the decomposition processes. Treating of PODM with antioxidant from CB was studied on the PODM chemical composition.

Table 2 shows the chemical composition of experimented PODM. There was significant different ($P < 0.05$) the effect of the interaction between the treatment and time on DM, OM and EE but not significantly different ($P > 0.05$) those on ash, CP, NDF and ADF. The treatment effect was significantly different ($P < 0.05$) on the composition of DM,

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Table 2: DM, OM, ash, CP, EE, NDF and ADF of DM treated with CBE and GCB (g/kg)

	DM	OM	Ash	CP	EE	NDF	ADF
Antioxidant sources							
C	378 ^a ±0.21	912 ^b ±0.31	88 ^a ±0.22	133 ^{ab} ±0.44	16 ^d ±0.52	774 ^a ±0.22	587±0.77
MCB ₁	332 ^b ±0.53	935 ^a ±0.93	65 ^b ±0.26	128 ^{ab} ±0.46	16 ^d ±0.58	687 ^b ±0.25	550±0.38
MCB ₂	336 ^b ±0.39	939 ^a ±0.48	61 ^b ±0.36	125 ^{ab} ±0.48	27 ^c ±0.56	680 ^b ±0.29	540±0.82
MCB ₃	331 ^b ±0.22	939 ^a ±0.56	61 ^b ±0.36	131 ^{ab} ±0.51	28 ^c ±0.64	690 ^b ±0.45	632±0.63
GCB ₁	336 ^b ±0.71	936 ^a ±0.55	64 ^b ±0.37	124 ^b ±0.58	34 ^b ±0.69	673 ^b ±0.65	600±0.74
GCB ₂	338 ^b ±0.62	933 ^a ±0.54	67 ^b ±0.38	124 ^b ±0.64	36 ^b ±0.74	668 ^b ±0.67	627±0.65
GCB ₃	341 ^b ±0.27	933 ^a ±0.42	67 ^b ±0.43	124 ^b ±0.66	44 ^a ±0.66	666 ^b ±0.73	618±0.78
B	337 ^b ±0.29	934 ^a ±0.34	66 ^b ±0.54	130 ^{ab} ±0.69	31 ^b ±0.55	682 ^b ±0.68	603±0.49
V	335 ^b ±0.31	933 ^a ±0.25	68 ^b ±0.61	138 ^a ±0.44	34 ^b ±0.58	665 ^b ±0.66	626±0.48
Time (day)							
3	339±0.84	934±0.68	66±0.36	124 ^x ±0.33	40 ^x ±0.65	686±0.55	599±0.55
5	337±0.45	933±0.72	67±0.66	129 ^{xy} ±0.81	28 ^y ±0.84	682±0.63	592±0.58
7	344±0.26	932±0.63	68±0.71	132 ^y ±0.92	37 ^x ±0.42	693±0.68	603±0.56
10	346±0.53	932±0.65	69±0.84	134 ^y ±0.47	36 ^x ±0.47	696±0.76	603±0.71
Antioxidant sources(AS)	*	*	*	N	*	*	n
Time (T)	n	n	n	N	*	n	n
AS*T	*	n	n	N	*	n	n

The value of 3 replications and standard error of the mean

n : not significant (P>0.05) * : significant (P<0.05)

Means with different superscript within the same coulomb are significant (P<0.05), a, b, c and d for antioxidant source effect, x and y for time effect

Treatment included C: control (no treatment), MCB₁₋₃: Methanol extracted cinnamon bark level of 0.010, 0.015 and 0.020 % (w/w) respectively, GCB₁₋₃: Ground cinnamon bark level of 0.05, 0.075 and 0.100 % (w/w), B: BHT (0.01 % (w/w)) and V: Vitamin E (0.01 % (w/w))

OM, ash, CP, EE and NDF but not significantly ($P>0.05$) different on those of ADF. The differences occurred was between control and the treatment except for EE. Rancidity influences the chemical composition of PODM. It is normally caused by lipid oxidation (Ladikos and Lougovois, 1990). However, the treatment effect to PODM is unclear on the composition of DM, OM, ash, CP, ADF and NDF except for EE. It was observed that the rancidity within PODM only decomposed FA that influences the composition of EE. Using treatment of whether MCB or GCB could protect PODM and its chemical composition. The capability of both MCB and GCB seemed the same as that of synthetic antioxidant (vitamin E and BHT) on protect nutrient.

CONCLUSION

The use of 0.1% of GCB gave the best result to reduce rancidity of PODM. It could reduce PV (29.77%) and TBA (22.16%) in comparison with control. GCB was better than MCB. So this proves that MCB did not give better result. It was found that there was no increase of PV but there was still the increase of TBA after day 7. Increasing the level of GCB to preserve PODM was recommended.

ACKNOWLEDGMENT

Very deep appreciation was addressed to Ladang Rakyat Trengganu Sdn BHD for providing PODM samples.

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08. FEED INTAKE, NUTRIENT DIGESTIBILITY AND BLOOD GLUCOSE OF SHEEP SUPPLEMENTED WITH ORGANIC CHROMIUM FROM FUNGI *GANODERMA LUCIDUM*

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Abstract

An experiment was carried out to evaluate the use of organic chromium from *Ganoderma lucidum* on feed intake, nutrient digestibility, chromium (Cr) intake and blood glucose of sheep. Randomized block design with four treatments were used in this experiment. Sixteen sheep were fed basal diet contained 13% crude protein and 67% TDN (Total Digestible Nutrient). The first treatment was control (A) = basal diet, while the other three treatments were supplementation of Cr: B= basal diet + 3 ppm inorganic Cr in CrCl₃; C= basal diet + 3 ppm organic Cr in fermented rice straw; D= basal diet + 3 ppm organic Cr in fermented palm fiber+ palm empty fruit bunches. The result showed that supplementation of organic chromium significantly increased (P<0.01) Cr intake, but treatments had no effect on dry matter and organic matter intake. Nutrienst digestibility were also not significant different (P>0.05). Blood glucose was decrease with Cr supplementation. The lowest blood glucose was 46.67 mg/dl in D treatment, while in control was 55.24 mg/dl. It can be concluded that supplementation of 3 ppm organic Cr from *G. lucidum* in basal diet maintained dry matter and organic matter intake, nutrients digestibility, increased chromium intake and decreased blood glucose.

Key words: chromium, Ganoderma lucidum, digestibility, blood glucose

INTRODUCTION

Chromium is an essential micro nutrient for animals (NRC, 1997) in maintaining the metabolism of other nutrients at the cellular level (Pechova & Paclata, 2007; Vincent, 2000). On carbohydrate metabolism, Cr role in increasing the activity of the hormone insulin, is through increasing insulin receptor response. Insulin is the key to the entry of glucose into the cells (Anderson & Kozlovsky, 1985; Mertz, 1993; Vincent & Davis, 1997; Vincent, 2000; Pechova & Pavlata, 2007), so that it stimulates the flow of glucose from the blood into the cells (Stipanuk, 2000). Chromium also plays a role in the immune system (Burton, 1995; Pechova & Pavlata, 2007; Toghyani *e tal.*, 2007).

The role of Cr in fat metabolism is to increase fat synthesis in adipose tissue. Sufficient availability of Cr needed for further work on improving insulin in fatty acid synthesis, facilitates the entry of glucose into adipocytes. Glucose in these cells can be used for glycerol synthesis and inhibits the breakdown of fat in adipose tissue by inhibiting the intracellular lipase enzyme, which hydrolyze triglycerides to release fatty acid. Chromium in the protein metabolism plays a role in increasing uptake of amino acids through its role in improving insulin activity. Rapid utilization of blood glucose for energy needs will reduce protein

catabolism for energy thus raising the efficiency of protein utilization. The rise in the efficiency of protein utilization is expected to increase protein synthesis.

Chromium in the organic form is more soluble, is absorbed easily and is not toxic than inorganic Cr (NRC, 1997; Vincent & Davis, 1997; Stipanuk, 2000; Pollard, 2001; Pechova & Pavlata, 2007). Cr in the form of inorganic elements have absorption rates of only 2-3% (Anderson and Kozlovsky, 1985). The active role of Cr can be enhanced by converting them into the form of an organic complex (Zetic *et al.*, 2001). Inorganic chromium in $CrCl_3 \cdot 6H_2O$ form can be incorporated into *G. lucidum* using paddy straw substrate (Agustin *et al.*, 2013). And palm fiber (Agustin *et al.*, 2010). This is possible due to *G. lucidum* species of fungi which is able to degrade lignin (Chang & Miles, 2004). Additionally mycelium and fruiting bodies of fungi are capable of producing the active compound polysaccharide (Bao *et al.*, 2002) which serves as an immune modulator (Lin and Zang, 2005; Gao *et al.*, 2005) and can improve immune function (Gao *et al.*, 2005) as well as antioxidants (Chen *et al.*, 2005, Sun *et al.*, 2004; Zao *et al.*, 2004).

The ability of *G. lucidum* to incorporate inorganic components into their mycelium and fruit body needs to be supported by a review of the nutritional benefits of the incorporation products. Cr element in the product of the incorporation by *G. lucidum* is expected to be used and play an active role in the animal body. In connection with this study, an experiment was designed to evaluate the use of organic chromium from *Ganoderma lucidum* on performance, nutrient digestibility, chromium (Cr) intake and blood glucose of sheep.

MATERIALS AND METHODS

Animal, diet and experimental design

Sixteen sheep with body weight 27.69 ± 1.27 kg were divided into four groups. Randomized block design (Gomez & Gomez, 1995) with four treatments were used in this experiment. All of sheep were fed basal diet contained 13% crude protein and 67% TDN (Total Digestible Nutrient) (NRC, 1985). The first treatment was control (A) = basal diet, while the other three treatments were supplementation of Cr: B = basal diet + 3 ppm inorganic Cr in $CrCl_3$; C = basal diet + 3 ppm organic Cr in fermented rice straw (RS Cr); D = basal diet + 3 ppm organic Cr in fermented palm fiber + palm empty fruit bunches (EFB Cr). Organic Cr supplement is the mixture of mycelium *G. lucidum* and media from the addition of 3000 ppm $CrCl_3 \cdot 6H_2O$ into rice straw (Agustin *et al.*, 2013) or palm fiber (Agustin *et al.* 2010). The sheep were fed twice a day according to body weight, while drinking water was available *ad libitum*. Dry matter and organic matter intake, nutrient digestibility, Cr intake, blood glucose and average daily gain were determined.

The experiment consisted of an adaptation period (4 weeks), the preliminary period (3 weeks) and the collecting period (5 weeks). In the adaptation period, sheep was injected with Kalbazen (5 ml). Sheep was allocated in metabolic cages. The amount of feed given is calculated based on body weight. Calculated daily feed intake and body weight of lambs is done every week.

Sampling and Analysis

During the first week of the collecting period, the rest of the feed and feces were weighted everyday. Individual feces samples were collected 10% /day or proportional to the amount of feces. Samples were dried in the sun. All of feed residues and daily feces samples each individual lamb were mixed at the end of the study for analysis. Blood samples were collected from each sheep before

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preliminary period and at the end of collecting period for blood glucose analysis. Blood samples were immediately put into a container officebox.

Dry matter and organic matter analysis of feed and feces were carried out according to proximate analyses procedure (AOAC, 2000). The samples to analyses of Cr content in feed were ashed according to the method of Restz *et al* (1960) and Cr content of solution were determined by atomic absorption spectrophotometer (AAS).

The data collected were analyzed by analysis of variance (ANOVA) and continued with Duncan’s Multiple Range Test (Gomez & Gomez, 1995).

RESULTS AND DISCUSSION

Dry Matter and Organic Matter Intake

There were no significant differences in dry matter intake (DMI) and organic matter intake (OMI) of sheep supplemented with organic Cr in fermented rice straw and palm fiber + palm empty fruit bunches (EFB Cr) with fungi *G. lucidum* (Table 1). Dry matter intake of sheep based on % body weight were $2.75 \pm 0.40\%$ in D treatment D up to $3.11 \pm 0.11\%$ in C treatment. Supplementation inorganic and organic chromium significantly increased ($P < 0.01$) Cr intake, the highest Cr intake was $8.33 \pm 0.45 \text{ mg}$ in C treatment. Blood glucose was decrease with Cr supplementation with the lowest value was $46.67 \pm 4.27 \text{ mg/dl}$ in D treatment. This illustrates that there is an organic Cr role in increasing the flow of nutrients from the blood into the cells and the cells will be changing the nutrients (glucose) into energy that is used as an energy source for cell metabolism, protein synthesis, cell maintenance and tissue growth and fertility (Stipanuk, 2000; D’Mello, 2003). Associated with the hormone insulin, chromium also increases amino acid transport and uptake by cells for synthesis of proteins as amino acid uptake by cells is influenced by the hormone insulin (Sipanuk, 2000; D’Mello, 2003). With the fulfillment of energy requirements for cell metabolism, utilization of amino acids and fatty acids can be more efficient, so it does not happen reshuffle body protein or amino acid utilization as an energy source through the process of metabolism . The same thing also happened on the fatty acid. An essential fatty acid derived from corn oil in the ration can be used both for the integrity of the cell membrane structure and for the synthesis of fat in order to improve the use of energy efficiently due to the energy that was used for the extension of fatty acids can be used to other metabolic processes.

The active form of chromium in GTF (*Glucose Tolerance Factor*) which is a complex of chromium nicotinic acid with two and three amino acids glycine , glutamate and cysteine is a liver component which soluble in water, blood plasma, and cell biological extracts (Linder, 1992). Currently known as chrpmodulin which is a low molecular weight oligopeptides ± 1500 Dalton that binds chromium (Vincent, 2000).

Nutrient Didestibility and Blood Glucose

Supplementation of organic Cr (in fermented rice straw and palm fiber + palm empty fruit bunches) from fungi *G. lucidum* in basal diet had no significant effect ($P > 0.05$) on dry matter and organic matter digestibility of sheep. This means that organic Cr supplementation from fungi *G. lucidum* did not work in improving digestibility. Digestibility is largely determined by the physical and chemical composition of the ration. In this study, all animals were given a diet with the same chemical composition in the form of basal ration.

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Table 1. Dry matter (DMI) and organic matter intake (OMI), chromium intake and blood glucose in sheep offered diet supplemented with inorganic, organic chromium from *Ganoderma lucidum*

Parameter	Treatment			
	Control	Inorg. Cr	Org. Cr Rice Straw	Org. Cr Palm fiber+ EFB
Intakei :				
DMI (g/day)	727.52±18.96	716.08±106.41	809.15±57.00	703.18±102.81
DMI (% body weight)	2.86±0.23	2.83±0.37	3.11±0.11	2.75±0.40
OMI (g/day)	532.92±14.11	527.96±83.31	593.71±42.43	515.39±75.30
Cr intake (mg)	5.00±0.13 ^a	7.07±1.05 ^b	8.33±0.45 ^c	6.94±1.01 ^b
Blood glucose (mg/dl)	55.24±4.65	50.04±16.04	48.91±2.60	46.67±4.27

Amount of digested nutrients determined the amount of absorbed nutrients, including Cr, which will determine the amount of body weight gain of sheep . The amount of digested dry matter and d organic matter ranged between 460.42 - 521.12 gram/day and 393-436.48 gram/day respectively. The role of Cr associated with the hormone insulin in increasing the flow rate of blood glucose into cells.

Blood glucose was decrease with Cr supplementation with the lowest value was 46.67±4.27 mg/dl in D treatment. Supplementation with organic Cr from fungi *G. lucidum* decreased blood glucose levels compared to controls by 15.5 % (46.67 mg / dl vs. 55.24 mg / dl). This illustrates the increased uptake of glucose from the blood into the cells as a result of the increased number of insulin receptors and activity in the presence of organic Cr supplementation. Transport of glucose from blood into cells occurs through a transporter (glucose transporter), which is a cell membrane protein. Transport of glucose from the blood into the cells depend on the hormone insulin, which occurring in the muscle cells and adipose tissue (Stipanuk, 2000). Insulin receptor is a second messenger that is activated by Cr. With the active insulin receptor by Cr, insulin facilitates the entry of glucose from the blood into muscle cells (Stipanuk , 2000). Glucose into the cells were used by cells as a source of cell energy metabolism, as material for the glycogen synthesis , for the fat synthesis (energy reserves), for the NADPH and ribose 5 phosphate synthesis (Stipanuk, 2000).

Balance between glucose oxidation, glucose biosynthesis and stored glucose depend on hormones and nutritional status of the cells and the whole network. Inorganic chromium in the form of $CrCl_3 \cdot 6H_2O$ gave effect in lowering blood glucose levels was 9.41 %. This suggests that the amount of Cr is less available to improve insulin activity. The Lack of Cr availability due to the low level of its absorption.

Table 2. Nutrients digestibility in sheep offered diet supplemented with inorganic, organic chromium from *Ganoderma lucidum*

Parameter	Treatment			
	Control	Inorg. Cr	Org. Cr Rice Straw	Org. Cr Palm fiber+ EFB
Digestibility:				
Dry matter (%)	68.11±2.34	64.27±1.50	66.46±1.90	67.5±2.51
Organic matter (%)	76.41±1.81	74.51±2.36	76.55±2.53	77.15±1.31
Digested Nutrient:				
Dry matter (g/day)	495.64±24.19	460.42±72.00	521.12±19.93	475.22±75.84
Organic matter (g/day)	407.31±17.27	393.99±69.36	454.48±35.68	398.28±63.12

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CONCLUSION

Supplementation of 3 ppm organic Cr from *G. lucidum* in basal diet maintained dry matter and organic matter intake, nutrients digestibility, increased chromium intake and decreased blood glucose. The lowest blood glucose was 46.67 mg/dl in organic Cr supplementation (D treatment), while in control was 55.24 mg/dl. Dry matter intake of sheep based on % body weight were $2.75 \pm 0.40\%$ up to $3.11 \pm 0.11\%$.

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09. ANALISYS PERFORMANCES OF EGG POULTRY INDUSTRIES IN 50 KOTA REGENCY AS A BASED SECTOR OF WEST SUMATRA

Rahmi, E. W. Sartika. Unand, West-Sumatra

Abstract

The research was purposed to (1) identified potency egg poultry industries in 50 Kota Regency as a based sector in West Sumatera, and (2) determined performances egg poultry industries in 50 Kota Regency. The research was conducted on December 2012. The research method that was used, was survey method. Analsys was conducted, were (1) LQ index (*location quotient*), (2) Localization Indexs (LI) , (3) Specialization Indexs and, (4) SCP (*structure – conduct – performance*) analisys. The result of research showed were : (1) Egg poultry industries in 50 Kota Regency had LQ index was 1,471, it was mean 50 Kota Regents had market potencial to be was based sector and had a big part of livestock sector nationally. And automatically, this regency according in categories production region. The result of Localization Index (LI) was 0,196. This index only one commodity that had positive index, and it was mean, 50 Kota Regency was potencial region to developing egg poultry industries in West Sumatera. Localization index described relative size certain activity concentrate at certain region. Specialization Index (SI) was 0,227, this index the most near 1 (one) than others, it was mean there were specialization and the region showed that had egg poultry industries developed better than others commodities. And, (2) SCP analisys identified that suply and acces of input production to was critical problem, specially feed and day old chick (availability and high price). To developed egg poultry insudtries that had competitive advantaged had to accelerate developing agribusiness based, specially feed industry. Indonesia rich of feed resources and feed scientific, there for had to give more attention for feed indutsry that had domestic resources based, that was very important to was priority.

Keyword : Egg Poultry Industries, Based Sector, LQ index, LI, SI, SCP

INTRODUCTION

Businesses poultry in Indonesia has become an industry that has a full component of upstream to downstream. The development of this business to contribute to the development and economic growth. Contribution of gross domestic product (GDP) of the livestock sub-sector of agriculture is 12 per cent (at current prices), while for the agricultural sector to national GDP is 17 percent. This suggests that the role of the livestock sub-sector of the agricultural development is significant, where the poultry industry is the main driver of business growth in the livestock sub-sector (Ministry of Agriculture, 2009). Poultry industry has a strategic value in the areas of animal protein to meet domestic demand and export opportunities, in addition to its role in exploiting employment opportunities. In West Sumatra showed that increasing statistic of poultry every year, such as population of layer increased 5,03%/year, population of broiler 7,3 %/year, production meat of chicken 5,6 %/year, production of egg 13,7%/year, consumption meat of chicken 17,5%/year, and consumption of egg 7,7%/year. Commodities poultry (more than 90 percent is the contribution of chicken) topped the first commodities to Indonesia, namely meat consumption by 56 percent. Effort in terms of chicken produki have been able to take advantage of market opportunities that exist. Market development and commodity prices for

poultry products broiler, broiler and laying both very volatile depending on the willingness of input supply and out put (Saragih, 2000).

Poultry farm policy geared towards empowering vision of breeder farms and agribusiness, increase value-added and competitiveness with a mission to encourage the development of poultry farming and sustainable tough. One of the necessary policies and influence effectively achieve this vision is expanding and improving production base through increased private investment, the government and the public, as well as policy regional commodity. Development of poultry farming and sustainable tough were based production/regional commodity. Livestock development should integrated the development process between sectors and between regions. Development is done with reference to the support of the potential of existing resources in a particular location, infra structure and economic relations between regions that support each other through the agro-ecosystem approach, so that a region is expected to exploit its potential more efficiently.

From the data poultry in West Sumatra were in 50 Kota District, 124 km from provincial capital, data showed that broiler were 40% of total population province and layer were 61% of total population province in 50 Kota District (Animal Husbandry Extension of West Sumatera, 2012). Problems of layer on the layer farm include inconsistencies in the structure, behavior, and relationships between actors in the industry, include inconsistencies price of input and output. And had a complex institutional characteristics Low government supporting. So, that the dynamics between economic agents believed would greatly affect the performance of an industry and, should integrate the development process between sectors and between regions. Development is done with reference to the support of the potential of existing resources in a particular location, infrastructure and economic relations between regions that support each other through the agro-ecosystem approach, so that a region is expected to exploit its potential more efficiently. The Purposed of The Research were (1) identified potency of layer industries in 50 Kota District as a based sector in West Sumatera, and (2) determined performances of layer industries in 50 Kota District.

MATERIALS AND METHODS

The research was conducted on December 2012. The research method that was used, was survey method. Data that used were secondary data (production of layer) and primary data. Analsys of data were :

(1) LQ index (location quotient), was a comparison of the magnitude of the role of the sector in an area of the size of the role of the sector nationally. This analysis can also be used to identify categorization for production are as, whether sectors including base or non-base in a region.

$$LQ = \frac{Si/S}{Ni/N}$$

Interpretation of the results of the calculation were LQ following:

- If $LQ > 1$, meaning that the area has the potential to be the basis of commodity markets analyzed
- If $LQ = 1$, meaning that the area has been self-sufficient for the commodities analyzed
- If $LQ < 1$, meaning that the area has a tendency to import commodity analysis

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(2) Localization Index (LI), was a measure of the relative concentration of certain activities in an area compared to larger areas with a certain scale. This analysis is used to determine which areas have potential to develop a commodity

$$\alpha I = \frac{\sum (S_i/N_i) - (S/N)}{S/N} \times 100\%$$

Having obtained the calculation of the results of calculations which is positive only aggregated commodities investigated in line with the criteria:

- If $0 < \alpha < 1$, meaning that the commodity or livestock enterprises tend to spread by partial
- If $\alpha = 1$, meaning that the commodity concentration in a region.

(3) Specialization Index (SI), This analysis is used to describe the distribution areas based on economic activities. It means that a specific location would be central to the development of certain commodities

$$\beta I = \frac{\sum (S_i/S) - (N_i/N)}{N_i/N} \times 100\%$$

If the results of a study SI district close to 0 (zero), means that the district has no peculiarities (specialization). In other words, have no activities (farm) is distinctive and relatively prominent kecamatan development compared to other regions. And if the SI values close to 1 (one), meaning there are all the typical late (specialization). It means that the observed districts have activity (farming) development typical relatively more prominent than the other kecamatan region.

(4) SCP (Structure – Conduct – Performance) Analysis.

This analysis will be descriptive-qualitative approach to SCP (structure - conduct-performance). The basic premise underlying the SCP is that the components of the structure (the number of farmers, traders, the number and composition of the market as well as the quality and quantity of infrastructure support) will directly affect the behavior within an industry (patterns of production, marketing and pricing) and then will affect the performance of the industry overall (market price, quantity traded and income distribution in the industry). Based on this premise, there are many components in the laying chicken industry that can affect the overall performance.

RESULTS AND DISCUSSION

Laying industries in 50 Kota District had LQ index was 1,471, it was mean 50 Kota Regency had market potential to be based sector and had a big part of livestock sector nationally. And automatically, this district according in categories production region. Planners region must have the ability to analyze the economic potential of the region, it is associated with real sectors that need to be developed so that the regional economy grew faster and were able to identify the factors that make the potential of certain sectors and determine priorities for policy. There are several analytical tools that can be used to determine the relative economic potential of an area, among other things, of comparative advantage, location quotient (LQ), and shift-share analysis (Tarigan, 2009).

Localization Index (LI) was 0,196. This index only one commodity that had positive index, and it was mean, 50 Kota District was potential region to developing layer industries in West Sumatera. Localization index described relative size certain activity concentrate at certain region. Djaenudin, et al (2002), states that the zoning of agricultural commodities in accordance with the carrying capacity of the land intended for the cultivated land productivity reaches optimal. In

support of agribusiness activities , understanding the productivity of land intended for a particular type of land use (Land Utilization Types) . From the economic aspect , the commodities produced must have a market opportunity , both as a domestic and export commodity . To achieve these objectives, the commodity must be developed on the land and the most appropriate areas , so it will have comparative and competitive advantages

Specialization Index (SI) was 0,227, this index the most near 1 (one) than others, it was mean there were specialization and the region showed that had layer industries developed better than others commodities. The maintargetis the development of human beings and their activities (social activities and economic activity). Isard (1975) revealed that in fact, regional science appears to improve the frame work to think about the social and economic order, so it can be prepared a more comprehensive general theory that in corporates patial dimensions and time, humans adapt in their activities and interact with the physical environment. During its development, (Rustiadi, et al, 2009) regional science is not merely concerning the social and economic aspects, but also many things about the interactions between the components of the region, namely, (1) geophysical, (2) economic, (3) institutional, (4) political, in aspace (space).

Data from animal husbandry extention 50 Kota district, showed that for the year 2012 there were about 4, 7 million layers were raised by the community spread over 13 districts, which are able to produce eggs about 3, 7 million eggs per day. Most chicken farms are in sub-district: Guguak were around 1,573,000 heads, Mungka were 1, 040.354 heads, Payakumbuh were 985 770 heads, Situjuah Limo Nagari were 925 000 heads, Akabiluru were 485.500 heads, Lareh Lago Halaban were 317.350 heads. Scienceis a vehicle forthe development ofcross-disciplinaryareathatincludes a variety oftheoreticalandapplied sciences, namely geography, economics, sociology, mathematics, statistics, political science, regional planning, environmental science, and so on(Budiharsono, 2001)

SCP analisys identified that supply and acces of input to was critical problem, specially feed.Structural components like number of farmer, number of merchant, composition market, quality and quantity infrastructure support directly influence behavior in the industry like production patterns, marketing, determination price, and affect the overall performance of the industry like market prices, amount transacted and distribution income in the industry.

Of the 3.7 million eggs, 500,000 chicken eggs to meet local needs that are marketed to various cities and counties in the province of West Sumatra. While the remaining 3, 2 million eggs it to meet the market demand to neighboring regions such as Riau and Jambi. Output Performace showed that prices were very fluctuated and tends to drop, and this were showed from the figure below.

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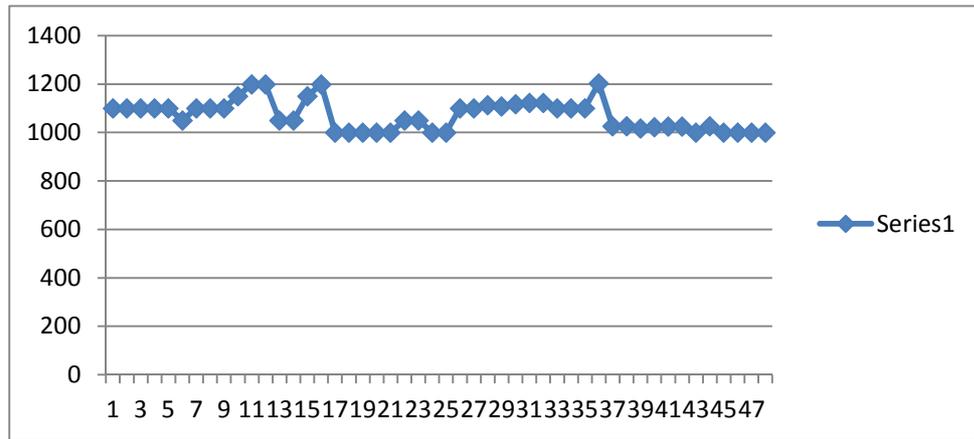


Figure 1. Price of Egg in West Sumatra, 2012

And very was contradicted with input performance, specially feed, like corn and concentrate, there were many problem about this input. Corn showed that the quantity were not fulfilled and prices were not stable. And this were showed from the figure below.

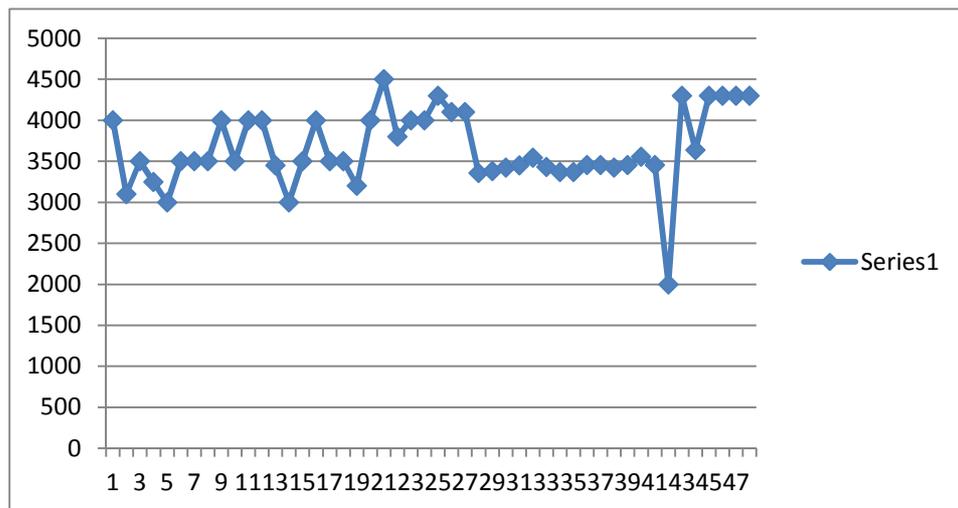


Figure 2. Price of Corn in West Sumatra, 2012

There were many problem about corn, like quantity were not fulfilled, corn production of West Sumatra 2012 were 495.497 tons or 1.358 ton/day, and need of corn by layer farmers 354,5 ton/day, but the real this were not fulfilled and difficult to obtain, because corn bough by feed manufacture. Corn availability is still an issue that haunts the livestock sector. This requires inter-agency sinergistas, by eliminating sectoral ego (Chairman Synchronizers Farmers Movement in West Sumatra, 2012). The use of feed resources is often hampered free trade issue that was not fair. Contributing factor because large agricultural businesses involve dealing with smallfarmers. It takes political and economic stability, infrastructure improvements, especially respect and understanding of the rural poor (Prof. E.R. Orskov, 2011).

Concentrate showed that prices higher and content of the concentrate was not listed on label. Concentrate were written content of 36-38 percents label, but when

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taken to the lab, Unand and Medion Labor, apparently content of concentrates only were 32-33 percents.

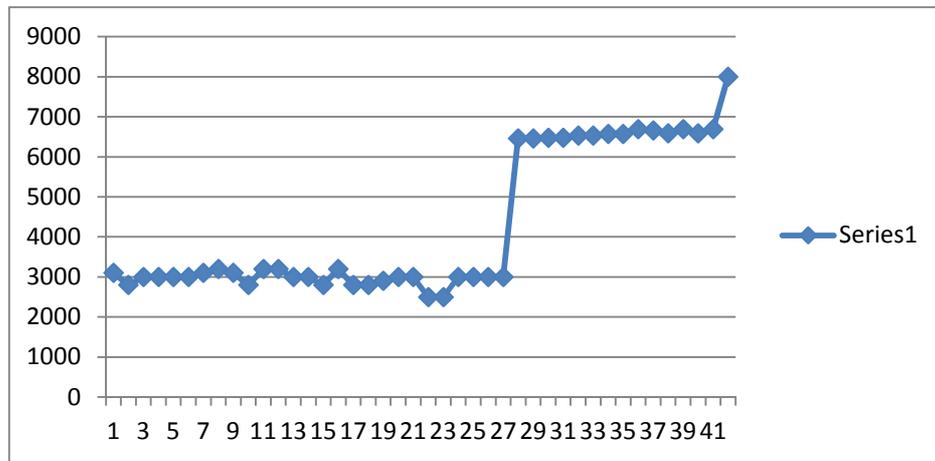


Figure 3. Price of Concentrate in West Sumatra, 2012

False bran is also available in the market. Made from flour mixed with chaff (apioca, Sometimes, there are also fine bran mixed. If it had made false bran farmers as chicken feed, chicken egg production will immediately decrease.

Government Statement were willpublish ‘Ranpergub’ about poultry quotas, so the chicken stock in West Sumatra was known, and the farmers with the manufacturer, can be mutually, and animal health provides a free Labor in Unand and Baso, through PPUI (Indonesian Poultry Farmers Association).

CONCLUSIONS

50 Kota District was production centre in West Sumatera and as based sector on regional economy. Performance of layer industries was supply and acces of input to was critical problem, specially feed. Suggestion that could be recommend were had to support from the government to be consistent with the concept of commodity-based farm development area or region. There should be an agreement that we make with manufacturers, related animal feed prices. So, prices remained stable, both in times expensive eggs, or eggs when the price dropped, Issued regulations concerning the standardization of the price of corn. Indonesia rich of feed resources and feed scientific, there for had to give more attention for feed indutstry that had domestic resources based, that was very important to was be priority.

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10. COMPARISON OF CORN STOVER NUTRIENT CONTENT IN LOWER AND UPPERLAND AREAS IN WEST SUMATRA

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ABSTRACT

The nutrient content of corn stover in the lower land versus upper land areas of West Sumatra had been studied. The lower land area was areas in which they are less than 200 m above sea level, while the upper land area was areas in which they are 200 m or higher above the sea level. The corn stover was collected from four districts of the lower land areas (Padang Pariaman, Pesisir Selatan, Pasaman Barat and Dharmasraya), and from four districts of the upper land areas (Agam, 50 Kota, Tanah Datar and Solok). There were eight corn stover samples from the lower land areas, and eight samples from the upper land areas. These corn stover samples were analyzed their nutrient contents (crude protein, crude fiber, ether extract, Ca, P, amino acids, fatty acids and carotenoids), fiber fractions (NDF, ADF, cellulose, hemicellulose and lignin), and anti-nutrient content (phytic acid). These nutrient contents (crude protein, crude fiber, ether extract, Ca, P, NDF, ADF, cellulose, hemi-cellulose and lignin) of corn stover in the lower land areas were statistically compared with those in the upper land area by using the student's t test, while the amino acids, fatty acids, β -carotene and phytic acid contents were numerically compared between lower land and upper land areas. Results of this study indicated that the crude protein content of corn stover in the upper land areas tended to be higher ($P < 0.10$) than that in the lower land areas, and hemicellulose in upper land areas was significantly higher ($P < 0.05$) than those in the lower land areas. However, crude fiber, P, ADF and lignin were significantly higher ($P < 0.05$) in the lower land than those in the upper land areas. Meanwhile, the ether extract, Ca, NDF and cellulose contents of the corn stover in the lower land areas did not differ ($P > 0.05$) from those in the upper land areas. The limiting amino acids (methionine and lysine), essential fatty acid (linoleic acid), β -carotene and phytic acid contents of corn stover indicated no significant difference numerically between lower and upper land areas. In conclusion, based on its crude protein content, the corn stover in the upper land areas tended to be better than that in the lower land areas of West Sumatra.

Keywords: corn stover, upper and lower land, crude protein content and West Sumatera

INTRODUCTION

Corn stover is a by-product of harvesting corn. It is a potential source of animal feed because of its ample amount available every year. According to Indonesia Statistics (2007), the plantation area of corn in West Sumatra was approximately 43,010 Ha with the corn production of 202,298 tonnes. Sudjana *et al.* (1991) reported that 20% of total corn production was the corn stover, so that it was estimated that the corn stover production in 2007 in West Sumatra was approximately 40,459.6 tonnes.

Iraslina (2004) found the chemical composition of the corn stover as follows: crude protein 3.03%, crude fiber 36.32%, ether extract 2.14%, nitrogen-

free extract 56.56%, and ash 1.95%. According to Preston (2006) the corn stover contained crude protein 3.0%, crude fiber 36.0%, ADF 39.0%, NDF 88.0%, ether extract 0.5%, ash 2.0%, Ca 0.12%, P 0.04%, K 0.8%, S 0.4% and Zn 5 ppm. While, Rince (2007) reported that the chemical composition of corn stover were crude protein 4.60%, crude fiber 46.90%, ether extract 2.38%, and ash 1.23%. The utilization of corn stover as an animal feed is still limited because of the high in its crude fiber content and the low in its crude protein content.

According to West Sumatra Statistics (2011), the corn production in West Sumatra was scattered in several areas (districts) with the three districts of higher production were Pasaman Barat, Pesisir Selatan and Tanah Datar. It means that those three districts also have higher production of corn stover. The districts in West Sumatra are located in the different elevation ranging from 0 to >1000 m above the sea level. The corn plantation in West Sumatra is grown in two areas of different elevation (from 0 to <200 m above the sea level and ≥ 200 m above the sea level). The difference in elevation will bring up to the difference in temperature of the areas which in turn will affect the growth and development of plants in those areas. Swenson (1970) reported that the area at the higher elevation has the low temperature when compare to the area at the lower elevation, in which increases the elevation of the area every 100 m above the sea level, decreases the temperature of that area approximately 0.65 °C.

Morecroft and Woodward (1996) found that elevation affected the crude protein content of the leaf of *Alchemilla alpine* in which it was higher in the high elevation compared with the low elevation, and Mountousis *et al.* (2006a and 2006b) also found that the crude protein content of rangelands was higher in upper land than that in lower land areas in Northern and North-Western Greece. Meanwhile, the crude protein content of grass was higher in the lower land areas than those in the upper land areas (Aoetpah, 2002). According to Alam *et al.* (2010) the sugar content of cacao was higher in the lower land areas than those in the upper land areas. Mountousis *et al.* (2006a) reported that the crude fiber content of rangelands in lower land was higher than that in the upper land areas. The fiber fractions of rangelands (ADF, cellulose and lignin), except for NDF in lower land were higher than those in the upper land areas (Mountousis *et al.*, 2006b). Further, Mountousis *et al.* (2006b) found that ether extract and Ca contents of rangelands in upper land were higher than that in the lower land areas. Yuliasari (2011) found that the growth and generative development of Rose cultivar Tequila Sunrise was better in the upper land areas when they were compared with that in the lower land areas. Less information available on the altitudinal effects on the nutrient contents, fiber fractions, and anti-nutrition content of corn stover.

MATERIALS AND METHODS

The study of the comparison of corn-stover nutrient content between the lower and the upper land areas in West Sumatra has been conducted. Eight districts were chosen as samples based on their height position in West Sumatra. Four districts were selected from the lower land area (Padang Pariaman, Pesisir Selatan, Pasaman Barat and Dharmasraya) in which they are <200 m above the sea level. Meanwhile, four districts were selected from the upper land area (Agam, 50 Kota, Tanah Datar and Solok) in which they are ≥ 200 m above the sea

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level. Nine corn-stover samples were collected from those four districts in the lower land, and nine samples from those four districts in the upper land areas. The nutrient contents (crude protein, crude fiber, ether extract, Ca, P, amino acids, fatty acids and carotenoids), fiber fractions (NDF, ADF, cellulose, hemicelluloses and lignin), and anti-nutrient (phytic acid) were measured from each district. The crude protein content was determined according to the Kjeldahl procedure. The crude fiber, ether extract, Ca and P were analyzed according to AOAC (2002). The fiber fractions were measured according to Goering and van Soest (1970). Amino acids were measured by using HPLC according to AOAC (2002). Fatty acids were determined according to the procedure by Garces and Mancha (1993). Phytic acid was analyzed based on the colorimetric method developed by Gao *et al.* (2007). β -carotene was determined according to Mathiasson *et al.* (2002). The data of nutrient contents and fiber fractions obtained from the corn stover in the lower land versus upper land areas were compared by using a student's t test (Steel and Torrie, 1980), while the data on amino acids, fatty acids, phytic acid and β -carotene were compared numerically.

RESULTS AND DISCUSSION

Nutrient Content of Corn-Stover in Lower Land versus Upper Land of West Sumatra.

The nutrient content of corn stover in the lower land versus upper land areas of West Sumatra is depicted in Table 1. It is shown in this table that the crude protein content of corn stover in the upper land areas was tended to be higher ($P < 0.10$) than that in the lower land areas. The crude fiber and P contents in the lower land were significantly higher ($P < 0.05$) than those in the upper land areas. The ether extract and Ca contents of this corn stover were not differed ($P > 0.05$) in both areas. The elevation of the area affected crude protein content of plants, in which in the higher area the content the crude protein content was higher than that in the lower area (Morecroft *et al.*, 1996; and Mountousis *et al.*, 2006). NRC (1982) found that the corn stover contained 8% crude protein and 1.3% ether extract. In addition, NRC (1982) also reported that the corn stover content of N, P₂O₅, and Ca were 18.8, 4.1, and 10.8 pound/ton successively. Crude fiber content in corn stover was 25% reported by Preston (2006). According to Koerner (1989), with the increasing elevation, low soil temperature associated with low rates of soil microbial activity were repeatedly considered as the main limiting factor for plant growth and nutrient supply. Meanwhile Koehler *et al.* (2006) documented the decrease of foliar N, P, K and Ca concentration with increasing elevation in several plant species in Canary Island. Macek *et al.* (2012) found that plant nutrient content does not simply increase with elevation under the extreme environmental conditions of Ladakh, NW Himalaya.

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Table 1. Nutrient content of corn stover in lower land versus upper land of West Sumatra.

Nutrient Contents	LowerLand	UpperLand
Crude Protein (%)	4.07 ^b	5.01 ^a
Crude Fiber (%)	40.46 ^a	37.97 ^b
Ether Extract (%)	0.64	0.68
Calcium (%)	0.41	0.42
Phosphor (%)	0.04 ^a	0.01 ^b

a, b Means with different superscripts at the same row are significantly differed (P<0.05), except for crude protein is tended to be differed (P<0.10).

2. Fiber Profile of Corn-Stover in LowerLand versus Upper Land of West Sumatra.

The fiber profile of corn stover in the lower versus upper land areas of West Sumatra is figured out in Table 2. The NDF and cellulose contents of corn stover in the lower land and in the upper land of West Sumatra did not differ significantly (P>0.05). However, the ADF and lignin contents of corn stover in the lower land were significantly higher (P<0.05) than that in the upper land of West Sumatra. Meanwhile, the hemicelluloses content of corn stover in the lower land was significantly lower (P<0.05) than that of the upper land of West Sumatra. Lignification in plants was affected by the temperature, day-length, light and plant stress (van Soest, 1982). High temperature in the lower land areas will increase the rate of plant maturity. Mature plants contain more lignin than the immature plant of the same age. Onim *et al.* (2012) reported that altitude influenced the lignin content of grasses in which every 20 m reduction in elevation increased the lignin content of grasses 1%. Mountousis *et al.* (2006b) also reported that the NDF content of rangelands was not affected by altitude, but the ADF, cellulose and lignin contents of rangelands in lower land was higher than those in the upper land areas.

Table 2. Fiber profile of stover in lower land versus upper land of West Sumatra.

Fiber Components	Lower Land	Upper Land
NDF (%)	71.12	73.70
ADF (%)	49.44 ^a	44.94 ^b
Cellulose (%)	33.76	33.28
Hemicellulose (%)	21.67 ^b	28.76 ^a
Lignin (%)	10.23 ^a	8.64 ^b

a, b Means with different superscripts at the same row were significantly differed (P<0.05).

3. Amino Acid Profile of Corn-Stover in LowerLand versus UpperLand of West Sumatra.

Table 3 shows that the profile of amino acids of corn stover in the lower versus the upper land areas of West Sumatra. Some of the amino acids content of

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Table 3. Amino acid profile of stover in lower land versus upper land of West Sumatra.

Amino Acids	LowerLand	UpperLand
Aspartic acid (%)	0.50	0.58
Glutamic acid (%)	0.58	0.65
Serine (%)	0.25	0.25
Histidine (%)	0.09	0.09
Glycine (%)	0.22	0.21
Threonine (%)	0.21	0.21
Arginine (%)	0.22	0.21
Alanine (%)	0.33	0.35
Tyrosine (%)	0.15	0.18
Methionine (%)	0.05	0.05
Valine (%)	0.30	0.33
Phenylalanine (%)	0.43	0.29
Isoleucine (%)	0.24	0.26
Leucine (%)	0.43	0.47
Lysine (%)	0.27	0.24

corn stover (serine, histidine, glycine, threonine, arginine and methionine) were not different in the lower land areas versus upper land areas of West Sumatra. However, some amino acids (aspartic acid, glutamic acid, alanine, tyrosine, valine, iso-leucine and leucine) were higher in the upper land areas than those in the lower land areas. Meanwhile, some amino acids (phenylalanine and lysine) were lower in upper land areas than those in the lower land areas. According to Nautiyal (1984), the amino acid content in some plants in the high altitude was higher than that in the lower one, while in other plants, the amino acid content in some plants in the high altitude was lower than that in the lower one.

4. Fatty Acid Profile of Corn-Stover in LowerLand versus UpperLand of West Sumatra.

The profile of fatty acids of the corn stover in the lower land versus the upper land areas of West Sumatra is illustrated in Table 4. Saturated fatty acids (lauric acid, palmitic acid, arachidic acid, behenic acid and lignoceric acid) were numerically higher in lower land areas than those in upper land areas, while saturated fatty acids (myristic acid and stearic acid) were numerically higher in upper land areas than those in lower land areas. The unsaturated fatty acids (myristoleic acid and eicosapentaenoic acid) in lower land areas were higher than those in upper land areas, whereas unsaturated fatty acids (palmitoleic acid, oleic acid, linoleic acid, linolenic acid, eicosanoic acid, eicosadienoic acid, erucic acid and docosadienoic acid) in lower land areas were numerically lower than those in upper land areas. Thus, most of saturated fatty acids were numerically higher in lower land areas than those in upper land areas, while most of unsaturated fatty acids were numerically higher in upper land areas than those in lower land areas. These findings were in accordance with the study by Linder (2000) who also found that the unsaturated fatty acids of plants were higher in upper land than those in lower land areas.

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Table 4. Fatty acid profile of stover in lower land versus upper land of West Sumatra.

Fatty Acids	LowerLand	UpperLand
Lauric acid, C12:0 (%)	0.56	0.49
Myristic acid, C14:0 (%)	0.56	0.62
Myristoleic acid, C14:1 (%)	0.11	0.07
Pentadecanoic acid, C15:0 (%)	0.11	0.15
Palmitic acid, C16:0 (%)	7.00	6.90
Palmitoleic acid, C16:1 (%)	0.16	0.19
Heptadecanoic acid, C17:0 (%)	0.22	0.25
Cis-10-Heptadecanoic acid, C17:1 (%)	0.03	0.05
Stearic acid, C18:0 (%)	2.83	3.71
Elaidic acid, C18:1n9t (%)	nd	0.04
Oleic acid, C18:1n9c (%)	2.63	4.03
Linoleic acid, C18:2n6c (%)	0.03	0.04
Arachidic acid, C20:0 (%)	5.44	5.37
Cis-11-Eicosanoic acid, C20:1 (%)	1.16	1.28
Linolenic acid, C18:3ω3 (%)	nd	0.08
Heneicosanoic acid, C21:0 (%)	3.71	1.63
Cis-11, 14-Eicosadienoic acid, C20:2 (%)	0.09	0.11
Behenic acid, C22:0 (%)	0.12	0.03
Erucic acid, C22:1n9 (%)	1.16	1.45
Tricosanoic acid, C23:0 (%)	0.09	0.20
Cis-13, 16-Docosadienoic acid, C22:2 (%)	nd	0.03
Stigmasterol, C24:0 (%)	0.90	0.60
Cis-5,8,11,14,17-Eicosapentaenoic acid, C20:5ω3 (%)	0.05	0.03

5. β-Carotene and Phytic Acid of Corn-Stover in LowerLand versus Upper Land of West Sumatra.

The β-carotene and phytic acid contents of the corn stover in the lower land versus upper land areas of West Sumatra is presented in Table 5. The β-carotene and phytic acid contents of the corn stover in the both areas were not numerically different.

Table 5. β-carotene and phytic acid of stover in lower land versus upper land of West Sumatra.

Chemical Composition	LowerLand	UpperLand
β-carotene (ppm)	120,9	120,6
Phytic acid (ppm)	6400	6400

CONCLUSION

Results of this study indicated that the crude protein and hemicelluloses contents in upper land areas were higher than those in the lower land areas, but crude fiber, P, ADF and lignin contents were higher in the lower land than those in the upper land areas. Meanwhile, the ether extract, calcium, NDF and cellulose contents of the corn stover in the lower land areas did not differ from those in the upper land areas. The limiting amino acids (methionine and lysine), essential fatty acid (linoleic acid), β-carotene and phytic acid contents of corn stover indicated no significant difference numerically between lower and upper land areas. Based

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on the height in crude protein content, it is concluded that the corn stover in the upper land areas was better than that in the lower land areas of West Sumatra.

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11. NUTRIENTS INTAKE AND THEIR RELATION TO MILK PRODUCTION AND QUALITIES UNDER TRADITIONAL AND SMALL SCALE INDONESIAN DAIRY FARMS ENTERPRISES

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Abstract

Relationship between nutrient intakes and milk production and qualities has been investigated in two different types of Indonesian dairy farming systems. Traditional dairy farming in Lembang keeping 5 cows in average and small scale dairy farm enterprise in Kunak Cibungbulang, Bogor with an average of 12 cows were compared for their feeding practices and cow's productions performance in two different seasons. Fifty farmers from both locations have been interviewed. In total, 246 heads of 419.6 ± 37.8 kg average body weight lactating cows have been observed twice (rainy and drought seasons). The amount and type of feeds offered were measured and recorded. Nutrient contents of each feed has been analysed in laboratory. Milk produced from morning and afternoon milking were recorded and scanned to get milk compositions data. The results showed that the average cow's body weight in Lembang (437 ± 36.2 kg) was higher than in Cibungbulang (402.1 ± 39.4 kg). Farmer in Cibungbulang used more type of concentrate than farmer in Lembang, but the opposite was happen for forage. During dry season, farmer in Cibungbulang provide low quality agricultural by-product such as rice straw and corn Stover, while farmer in Lembang used field grass and high quality agricultural by-product. The amounts of feed offered were higher in Lembang (23 kg DM) in compare to Cibungbulang (15.5 kg DM). Proportion of forage offered in Lembang was not influence by season, but farmer in Kunak reduce forage proportion when the forage shortage during drought season occurred. The average milk production in Lembang during rainy and drought seasons (17.3 l and 16.9 l) was higher than in Cibungbulang (10.8 l and 9.4 l). Milk total solid was higher in drought season in both locations lead by increasing milk fat content. In both type of dairy farming systems, deposit nutrient in cow's body were lower (BCS < 3), although higher in Lembang than in Cibungbulang (2.6 vs. 2.5). With this result, it can be concluded that traditional dairy farming system with small number of cows such as in Lembang could provide better nutrition for their cows, resulted higher milk production. In both systems, nutrient provided for the cows could not support a long term persistency of high milk production.

Keywords: dairy, forage type, milk production, small scale, traditional

INTRODUCTION

Cow's milk is the most consumed milk in Indonesia (Abdullah, 2006). Insecurity milk supply in Indonesia is caused by low dairy cattle population, productivity and persistency. Annually, not less than 1.15 million ton of milk was imported to fulfil 80% of national demand (Dirjennak, 2010). The volume of imported milk will continuously increase in line with increasing Indonesian economic growth and population and in contrast to decreasing number of cattle population after massive slaughter of cattle as impact of higher beef price.

One of the major reasons for the low dairy cattle performance is lack of balanced nutrient intake. Lack of local feeds availability (quantity, quality and continuity) that could support cattle requirements, lack accurate information, available but irrelevant feeding table and cattle requirement are among the major cause for the low feed efficiency utilization which lead to low animal performances. This condition is caused by the lack of capacity (technically and economically) of dairy farmer in evaluating feeds and feeding practice.

Most of Indonesian dairy farmer are less educated (67.7% junior high school or elementary school), smallholder (6.07 AU) and traditional farmer (14.8 years of experience) who mainly depend on dairy farming as main income source for their family (76.3%). The farmer occupied limited land (0.44 ha) that could support only 62.7% of forage requirements. Lack of land occupation (35%) and slow growth of plant during drought season were among the reasons for the lack of forage supply reported by dairy farmer (National Dairy Survey, 2012). Improving feed availability and information at traditional and small scale dairy enterprises will improve Indonesian milk security.

The objectives of this study was to identify type of feed used, to analyse of nutrient contents in the feed, to measure animal performance, to calculate dairy nutrient requirement at different type of farming system and at different seasons. Based on the feed type used and their nutrient contents, cows’ performance and their nutrients requirements, a balance ration will be formulated.

MATERIALS AND METHODS

The study was conducted using interview, field observation and laboratory analyses methods. The amounts of 50 farmers (30 farmers from KPSBU Lembang; 20 farmers from KUNAK) have been interviewed to get basic information about the farmer identities, feeding managements and cows’ performances. In total 494 cows have been observed and measured for the amount of daily feeds offered, milk production, body weight and body condition score. The observations were also aimed at confronting data from interview. The survey (interview and field observations were conducted twice (rainy and drought seasons) from July 2012 until June 2013. Laboratory analyses were conducted to determine nutrient contents of feeds used and milk compositions.

Interviews were conducted by 4 trained enumerators with guidance of a questioner. The amount of feeds offered were measured using 5000 ± 0.1 g digital scale for concentrate and 50 ± 0.1 kg balance for forage. Milk productions were measured in liter and convert to kilograms. Cows’ body weights were calculated using Schoorl’s formula. Body conditions were scored according to five scales according to Penn State University (2004) procedure. Proximate analyses followed Naumann and Bassler (1997) procedures, while Ca and P sample preparation followed Reitzel *al.* (1987) procedure. Determination of Ca sample concentration followed AOAC(2003) procedure and P sample concentration determined using Taussky & Shorr (1953). Milk protein, lactose, fat and SNF were scanned using Lactoscan type S_L.

The collected data were analyzed using T-test to compare the effect of seasons. Correlations between parameters were made prior to regression analysis to estimate the milk production and quality yields per unit of nutrient offered.

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RESULTS AND DISCUSSION

The types of feeds used by farmer in the two locations were different. The farmer strategies in coping lack of feeds during drought season were also different. The types of feeds used during rainy and drought seasons in both location were shown in Figure 1.

There was no effect of seasons on type of concentrate used but forage. Concentrate types used by farmer in Kunak more vary than farmer in Lembang. Lower quality of cooperative concentrate and the capacity of farmer in Kunak to provide cash to buy concentrate from other suppliers were among the reasons for the situation. Higher degree of Kunak’s farmer education might also cause for the variety of concentrate used. A farmer in Kunak even formulated his own used concentrate.

The types of forage used in Lembang were more vary than in Kunak. In contrast to concentrate types used, types of forage used were affected by the season. In both locations, Napiergrass was the main forage sources. More farmers in Lembang used rice straw and local field grass when corn stover and banana leaves become less available during drought season. Meanwhile, in Kunak more farmers utilized rice straw and corn stover during drought season which were more commercially transported when Napier grass growth slower. Ratio of forage to concentrate offered by farmer in Lembang was 0.46 to 0.54. This ratio was not different in both seasons. In Kunak, however, the farmer offered ratio forage to concentrate 0.47 to 0.53 during rainy season and dropped to 0.33 to 0.67. Dairy farming in Lembang which are surrounded by horticultural land provided variety of agricultural by-product that could support dairy production in Lembang especially during drought season. This advantage resulted more resilient of forage supply during drought season compare to farmer in Kunak which should reduce forage proportion when drought season come.

The amount of nutrient offered to the cows in both dairy farming systems were shown in Figure 2.

The amounts of nutrients offered to the cows were affected by the seasons. Figure 2 showed that nutrients offered were more stable in Lembang then in Kunak especially for DM, TDN and CP. Variety of forage available during drought season in Lembang could supply the same amount of nutrient that in the rainy season. In Kunak, however, the amount of nutrients offered were increase during drought season. It might be caused by the same of amount of feed offered in both seasons although in drought season the DM content was higher. In the case of Ca and P, in both locations there were fluctuations of supply but in contradictions pattern. Farmers in Lembang offered less Ca and P during drought season but in Kunak more Ca and P were available for the cows.

Comparing both locations, farmers in Lembang offered more nutrients to their cows than farmers in Kunak. Imbalance Ca to P ratio was occurred in daily ration of Lembang’s cows where farmers offered more P than Ca. To support maintenance, production and reproduction requirements, cow should be fed balance ration with ratio Ca to P about 2 to 1 (NRC, 2001).

Consequences to the feeding practice could be observed from cows’ performances. Daily milk production and quality was shown in Figure 3.

The higher feeds offered by Lembang farmer resulted higher milk production. Although the amount of feeds and nutrients offered by farmer in

Kunak were higher during drought season, however the cows’ production were lower. Decreasing feed digestibility might cause for the situation which lead to lower precursor available for milk synthesis. In both locations, total solid milks were increase during drought season. The condition was led by increasing milk fat content. During drought seasons, increasing crude fibre content in forages led to higher acetate proportion in the rumen and more available as milk fat precursor synthesis.

Cows’ body condition score (BCS) in both locations were lower than they should be. There were slightly increase of BCS during drought seasons as a mid-term effect of higher feeds and nutrients offered during rainy season in Lembang. In Kunak, decreasing BCS was even occurred. BCS cows in Lembang were higher than in Kunak (2.66 vs. 2.55 in rainy and 2.67 vs. 2.53 in drought seasons).

In both locations the amount of feeds offered were higher than they were required (Table 1). The amounts of nutrients offered were also above their requirements except for Ca. In rainy season, P offered to the cows in Kunak was also below their requirements. Although the amounts of macro nutrients offered were higher than their required, but they would not lead to furthers increasing milk production. Deficiencies and imbalance of micro nutrients such as Ca and P offered might explain the fact. There is a need to help farmer in formulating a balance ration. The excessive offered feed and nutrients by the farmer may harmful the environment and lead to inefficiencies of feed utilization. Feeding practice of long cut Napier grass in low feeding frequencies might be the cause of this problem. Many of the feeds offered didn’t come into intake but fall down in the herd floor. There is a need for improving feeding practice to reduce feeds leftover.

CONCLUSION

Forage availabilities were affected by season. Farmer in Lembang provided higher nutrients with more stable quality than farmer in Kunak. Excessive DM, energy and protein offered but deficiencies and imbalance minerals Ca and P in both dairy farming systems hinder increasing milk production and body conditions score. Traditional dairy farming system with small number of cows such as in Lembang could provide better nutrition for their cows resulted better cows performances than in Kunak. During drought season, decreasing milk production but increasing milk total solid occurred in both location which driven by increasing fat contents. In both systems, nutrients provided for the cows could not support a long term persistency of high milk production.

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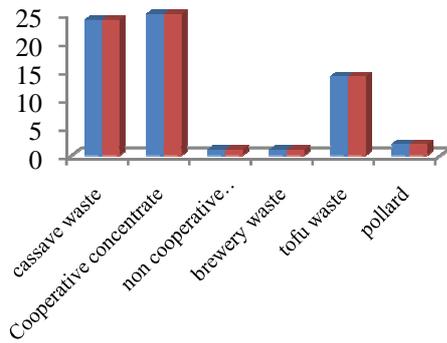
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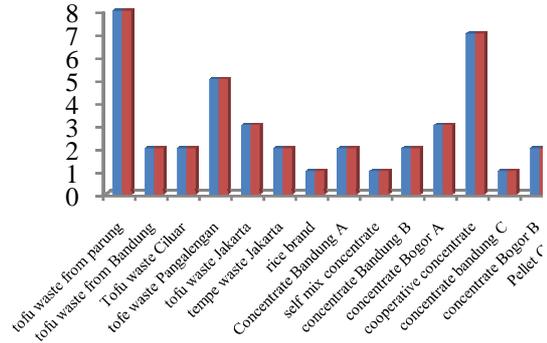
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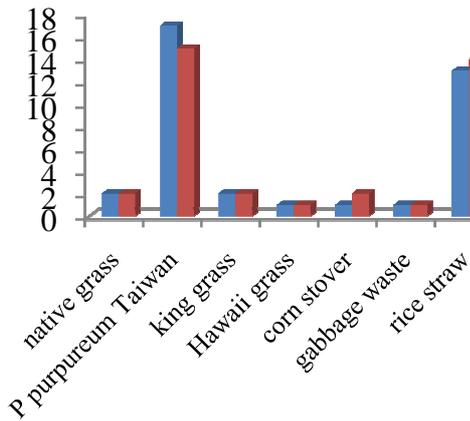


(a) Concentrate used in

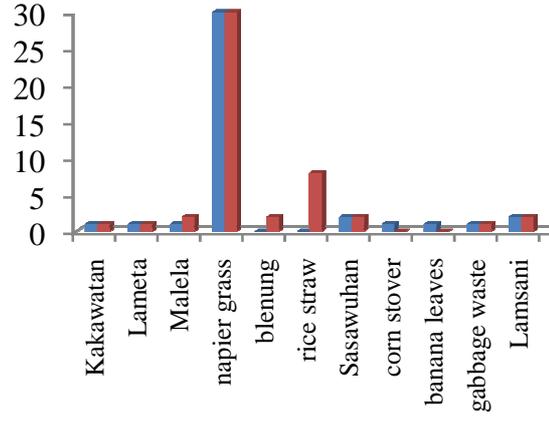
Lembang



(b) Concentrate used in Kunak



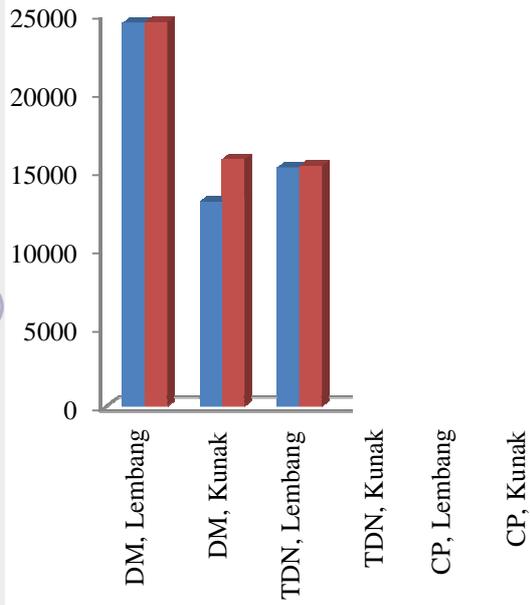
(c) Forage used in Kunak



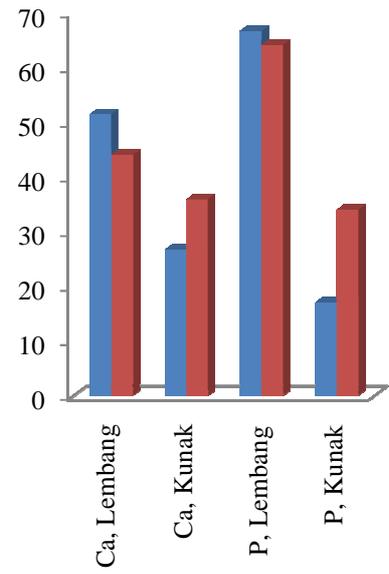
(d) Forage Used in Lembang

Figure 1: Types of feeds used in Lembang and Kunak (■ = Rainy; ■ = drought)

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(a) DM, TDN and CP offered



(b) Ca and P offered

Figure 2: The amounts of nutrient offered (■ = Rainy; ■ = drought)

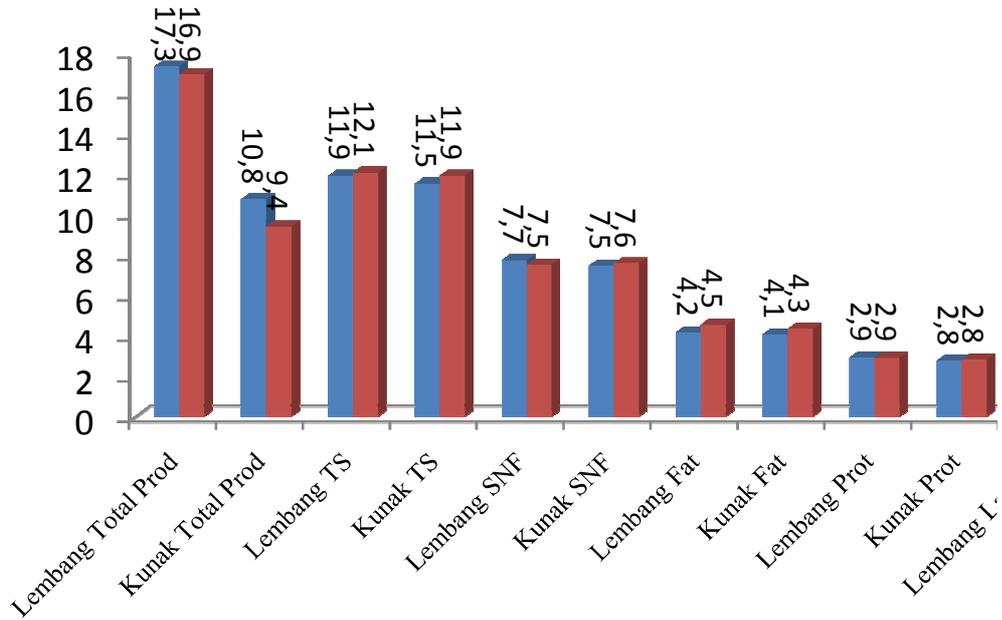


Figure 3: Milk production and quality (■= Rainy; ■= drought)

Table 1: Feeds and nutrients surplus above requirements

Season	Nutrients	Rainy				Drought			
		Lembang		Kunak		Lembang		Kunak	
		kg	%	kg	%	kg	%	Kg	%
Rainy	DM	9.90	68.13	0.89	7.330	9.94	68.36	3.99	33.99
	TDN	5.81	61.81	4.05	108.3	5.75	60.15	3.52	52.15
	CP	0.95	46.80	0.40	28.78	0.92	44.44	0.88	68.75
	Ca	-0.03	-37.50	-0.02	-40.00	-0.04	-50.00	-0.01	-20.00
	P	0.02	40.00	-0.02	-50.00	0.01	20.00	0.02	200.0

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12. UTILIZATION ONGGOK ENRICHED WITH EGG POWDER TO MAKING NUTRITIOUS INSTANT FOOD

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Abstract

This study aimstoget NutritiousInstant Food formulations are accept able to people. Thisstudy is expected tobe able to find alternative sources of efficient foodas a source of carbohydrate besides rice and instant noodles. This research also have goal to supporting the government program on food security. This research was conducted with an experimental method using a completely randomized design (CRD) 2 treatments and 3 replications. The treatments are Food formulation (A) to sufficient50 g proteinand2200kcalof energy, formulation (B) to sufficient40g fat content and the amount ofenergy 2200 kcal. Both treatments are equally good to be consumed in there commended amounts to as much as 442.33g for formulation A and512.82 g for formulation B/person/day. A is the best formulation because ithas higher nutritional than Bper100g of material. In terms ofefficiency, with a small amount of A already fulfill daily needs of individuals per day. Palatability tests showed the preferred formula in terms of aroma and flavor. For suggestion, there must be improve the Food design so it can be acceptable by the public.

Key words : Onggok, Nutritious Instant Food, Protein, Fat, Calories.

INTRODUCTION

For this time Onggok known as waste of tapioca starch factory that has quite high nutritious but low economic value and not useable maximally. On 100 Onggok has 1.4 g protein, 0.9 g fat and 86.5 g carbohydrate and containing 359 Kcal of energy (Food Plant Marketing and Process Directory, 2003). On the other hands every 100g rice containing 6.8g protein, 0.7g fat, 78.9g carbohydrates and 360 Kcal of energy.

In some of area in West Java to be exact at Cirendeudeu village in Cimahi have used onggok as their source of their energy that proceed to be traditional food called Rasi. Unfortunately the nutrition contain rasi still below of rice. That is the reason why we must improve the nutrition by adding egg powder.

By adding egg powder nutritious instant rasi can increase radically to cover all human nutrition needs. Food Plant Marketing and Process Directory (2003) said at least every human needs 2200 Kcal, 40 g Fat and 50 g Protein every days. It need good formula to make combination between onggok and egg powder to get requirement food for human.

MATERIAL AND METHOD

The material was used for this research was cassava, egg, water. This research was conducted with random experimental design with 2 treatments and 3 repetitions. Which is for Formulation A sufficient 50 g protein and 2200 Kcal energy, for Formulation B sufficient 40 g fat and 2200 Kcal energy. The formulation would known based on amount of energy contain, protein and fat. The formulation would get by simplification this equality.

$$(X) \text{ amount of onggok energy} + (Y) \text{ amount egg powder energy} = 2200 \dots (1)$$

$$(X) \text{ Onggok protein contain} + (Y) \text{ egg powder protein contain} = 50 \dots (2)$$

$$(X) \text{ Onggok fat contain} + (Y) \text{ egg powder fat contain} = 40 \dots (3)$$

Simplification equality (1) and (2) would get Formulation A, Simplification equality (1) and (3) would get formulation B

RESULT AND DISCUSSIONS

Egg Powder and Ongkok Analysis.

The composition nutrition of egg powder and onggok found were

Table 1 : Nutrient contain and energy on 100 g egg powder and onggok

No.	Nutrition	Egg Powder	Onggok
1.	Fat (g)	49,36	0,72
2.	Protein (g)	47,55	1,77
3.	Water Contain (g)	2,17	8,30
4.	Energy (Kcal)	681,21	401,3

Egg powder was made has soft texture, yellow and a little a bit rank. Fat and protein contain on egg powder found were 49.36% and 47.55% slightly close with egg powder analyzing by Agricultural Research Program (USDA, 1994) were it 40.95% and 47.35%. Around 681.21 Kcal energy contained on this egg powder and on other hands egg powder was produced has low water contain (0.17%)

The onggok were made has coarse typical, white and specific cassia flavour. Fat, protein and water contain on onggok were 0.72%, 1.77% and 8,30%. This lowness cause cassava as onggok material have low nutrition too. Just like Suprpti (2002) said that fat and protein contain on cassava around 0.3% and 1.6%.

Formulation Material Nutritious Instant Food.

Mixture formulation known based on fat, protein and energy contain on the egg powder and onggok. The formulation got from this simplicity of equalization.

$$401,3 X + 681,21 Y = 2200 \dots\dots\dots (1)$$

$$1,77 X + 47,55 Y = 50 \dots\dots\dots (2)$$

$$0,72 X + 49,36 Y = 40 \dots\dots\dots (3)$$

For Formulasi A

$$\begin{array}{rcl} 1,77 X + 47,55 Y & = 50 & /401,3/ 710,3 X + 19081,815 Y & = 20065 \\ 401,3 X + 681,21 Y = 2200 & /1,77/ & 710,3 X + 1205,7417 Y & = 3894 - \\ & & 17876,07 Y & = 16171 \\ & & Y & = 0,90 \end{array}$$

$$\begin{array}{rcl} 1,77 X + 47,55 Y & = 50 \\ 1,77 X + 47,55 (0.90) & = 50 \\ 1,77 X & = 7,205 \\ X & = 4,07 \end{array}$$

For Formulasi B

$$\begin{array}{rcl} 0,72 X + 49,36 Y & = 40 & /401,3/ 288,9 X + 19808 Y & = 16052 \\ 401,3 X + 681,21 Y = 2200 & /0,72/ & 288,9 X + 490,4712 Y & = 1584 - \\ & & 19.317,69 Y & = 14468 \\ & & Y & = 0,75 \end{array}$$

$$\begin{array}{rcl} 0,72 X + 49,36 (0.75) & = 40 \\ 0,72 X & = 3,03 \\ X & = 4,21 \end{array}$$

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X = Amount of Onggok need
 Y = Amount Egg Powder need
 Formulation A need 407 g onggok (X) and 90 g egg powder (Y).
 Formulation B need 421 g onggok (X) and 75 g egg powder (Y).

Physic, Chemists and Rendemen

Table 2 : Physicals, Chemists Characteristic and rendemen Nutritious Instan Food A Formula and B Formula per 100 g Sampel

No.	Komponen	Formulasi A	Formulasi B
1.	Rendemen (%)	89	82.3
2.	Lemak (%)	8.89	7.80
3.	Protein (%)	13.54	9.79
4.	Air (%)	6.04	6.60
5.	Abu (%)	1.49	1.33
6.	Daya Serap Air (%)	52.17	59.50
7.	Jumlah Energi (kkal)	485.65	449.23

The rendemen for formulation A was 89%, meanwhile form formulation B was 82.3%. The reduction was happened to formulation B cause the egg powder was add slightly little than Formulation A that led to sticky typical not as good as formulation A. Winarno and Koswara (2002) explained albumin has ovomucin that make insoluble film and act like binding agent.

Fat and protein to formulation A was 8.89% and 13.54% as well as 485.65 Kcal/100g energy. To fulfil daily energy need, per person should eat formulation A for 442.33 g that will supply 39.29 g fat, 59.85 g protein and 2146.57 Kcal energy. On the other hand fat and protein to formulation B was 7.8% and 9.79% as well as 449.23 Kcal. To complete daily energy need per person require eat formulation B for 512.82 g. By eating that much consumer will get 39.99 g fat, 50.20 g protein and 2307 Kcal.

Ash content was representative of mineral content onto food (Tejasari, 2005). Winarno (1991) also explained mineral beneficial for building agent to body. Ash content to nutritious instant food was 1.49% for formulation A and 1.33% for formulation B. These ashes assume come from egg powder as material. As we see formulation A containing ash more than formulation B because egg powder add more than formulation B. Ash content on whole egg reach 11% (North and Bell, 1990).

Water absorbs capacity for formulation A and Formulation B were 52.17% and 59.50%. Water absorbs capacity related with texture nutritious instant food when it would consume. Formulation A water absorbs capacity worse than formulation B cause formulation A adding more fat on it. As we know fat and water are antagonistic agent. Same opinion also said by Winarno (1991) that explained water and fat have different berat jenis.

Palatability Test

Nutritious Instant Food was made on pellet form with old brown. At this test, panellist asked to express about their personal responds about the sample. Result of this palatability test can see on table 3.

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Table 3 : Nutritious Instant Food panellist level based on formulation

No.	Formulasi	Average Aroma	Average Taste	Average Texture	Averages Colour
1.	Formulasi A	4,25	3,55	2,65	2,05
2.	Formulasi B	4,00	3,60	2,90	2,45

Nb : 5 = very like, 4 = like, 3 = slightly like, 2 = slightly dislike, 1 = dislike.

Based on table 2 for aroma and taste most of panellists prefer like for Nutritious Instant Food. The panellists like the Nutritious Instant Food cause fat content on it. Same opinion also said by Wolke (2006) the material having high fat content will having *savoir vivre*.

On the other hands panellist slightly like into Nutritious Instant Food texture. We assume this is caused unbalance size of Nutritious Instant Food granule that led to smaller granule dry faster than the bigger one. For colour, panellist slightly dislike cause it become pale when it on process would deserve to panellist.

CONCLUSION

To sum up, this research used egg powder contain 49.36% fat, 47.55% protein 6.17% water content and 681.21 Kcal energy per 100g in the meantime onggok contain 0.72% fat, 0.72% protein, 8.30% water content and 401.3 Kcal energy per 100g. For the best treatment on this research is formulation A (mixture 90 g egg powder and 407 g onggok) that recommended amount for eat 442.33 g. Actually both of formulation as good as formulation to eat, just only per 100 g formulation A have better nutrition than formulation B and more efficient to consume by people.

As suggestion for next research, the nutritious instant food should be design with better form than now. It could be on flakes, porridge or noodle form. Better design can increase appetite consumer just by see it.

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13. STUDY ONIN VITRO DIGESTIBILITY OF SOAKED OIL PALM FIBER BY FILTRATED OIL PALM FRUIT BUNCH ASH

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Abstract

Oil palm fiber has potency as feed fiber source for ruminant, but it contains high lignin and causes limited digestibility. This research was aimed to find out the effect of soaking oil palm fiber filtrated oil palm fruit bunch ash (FOPBA) on *in vitro* digestibility. This experiment used a completely randomized design that repeated 4 times. Oil palm fruit bunch ash was mixed in water and was entered into the container during 24 hours with concentrations of 50, 100, 150 and 200g/L, furthermore, this filtrate was used to soak palm oil fiber for 3 hours. Processed products were analyzed for level of lignin and crude fiber and *in vitro* test was used to measure digestibility. Results indicated that soaking in filtrated oil palm fruit bunch ash were significant effect ($P < 0.05$) to decrease level of lignin and crude fiber, and to improve ($P < 0.05$) digestibility of dry and organic matter compared to control treatment (no soaking). Treatment by filtrated oil palm fruit bunch ash at 150 g/L and 200 g/L produced level of low lignin ($P < 0.05$), that were 17.25 and 18.53%, nevertheless, concentration at 150g/L was higher level of crude fiber than 200 g/L (46.04 vs 43.87%), whereas digestibility of dry and organic matter showed the same results ($P > 0.05$), each of 23.48 and 24.12% as well as 16.70 and 17.06 %. The conclusion, that soaked oil palm fiber by 150g/L concentration of filtrated oil palm fruit bunch ash was more effective in improving digestibility.

Keywords : ash, crude fiber, digestibility, lignin, and oil palm

INTRODUCTION

Processing of oil palm fruit becomes crude palm oil (CPO), will produce oil palm fiber as feed ruminant. According to Jalaludin *et al.* (1991) in 1 ton of oil palm fruit produced 180-260 oil palm fiber. This amount was equal to 5.060 oil palm fiber for every hectare per one year. Oil palm fiber contained a high fiber, but a low protein, that was each of 46.1 and 6.2% (Mathius, *et al.* 2003). However, oil palm fiber contained lignin as high as 12.91% - 21.92% (Irawadi *et al.* 1996; Suharto, 2004). Lignin bounded cellulose and hemicellulose that was difficult to be broken, until level of digestibility was low (Komar, 1984).

An effort to break bounding of lignocellulose and lignohemicellulose was conducted with alkali compound (Komar, 1984). Alkali potency to process oil palm fiber can be obtained from ash that dissolved with water. Filtrate of cacao ash was reported to improve organic matter digestibility of corn straw on goat (Adebowale, 1985).

Oil palm fruit bunch ash yields abundant. Currently, it has been exploited for fertilization in oil palm plantation. Mineral composition of oil palm fruit bunch ash were Kalium (K) 25.8%; Natrium (Na) 0.03%; Calcium (Ca) 2.7%; Magnesium (Mg) 2.8%; Chlor (Cl) 4.9%; Carbonate (CO₃) 9.2%; Phosfat (P) 0.2%; Silica (SiO₂) 19.1% (Zahrina, 2007). It can be seen that oil palm fruit

bunch ash have level of high alkali. In consequence, if dissolved in water can be used to process oil palm fiber, and expected to improve digestibility.

MATERIALS AND METHODS

Oil palm fruit bunch ash was each of 5, 10, 15, and 20 g, entered into container and mixed with water 100 mL. Mixture precipitated for 24 hours and filtered with Whatman No. 41 paper and obtained filtrated oil palm fruit bunch ash/FOPBA (% g/mL). Then, 100 g oil palm fiber were mixed with FOPBA in accordance with treatment. After that, they entered into plastic bag and tied at mouth part for soaking about 3 hours at room temperature. Hydrolyzed result was dried to test level of crude fiber (AOAC, 1991), lignin (Van Soest, 1967) and *in vitro* digestibility (Tilley and Terry 1963). Experiment used a completely randomized design and collected data was analyzed with Duncan's test (Steel and Torie, 1983).

RESULTS AND DISCUSSIONS

Results indicated that processing of oil palm fiber with FOPBA degraded level of lignin. FOPBA had level of high alkalinity at pH 9.73 – 9.84. Principally alkali works : (1) to break the bounding between cellulose and hemicellulose with lignin and silica, (2) to esterify of acetyl group to form uronic acid, and (3) to break cell wall structure, by developing of fiber network, which turn to facilitate penetration by microorganism enzyme molecule (Komar, 1984). Processing of dry *Sorghum plumosum* grass var. Timorensis was soaked by filtrate of rice hull ash at concentration 15% for 1.5 hours decreased level of lignin as high as 20.28% (Dato, 1998).

Tabel. 1. The Effect of Soaked by FOPBA on Fiber Component and Digestibility

Variables	T0	T1	T2	T3	T4
Lignin (%)	22.90 ^c	21.87 ^{bc}	20.35 ^b	17.25 ^a	18.53 ^a
Crude Fiber (%)	49.68 ^c	46.74 ^b	46.72 ^b	46.04 ^b	43.87 ^a
Dry Matter Digestibility (%)	19.60 ^a	21.77 ^b	23.18 ^c	23.48 ^c	24.12 ^c
Organic Matter Digestibility (%)	12.22 ^a	14.95 ^b	15.72 ^c	16.70 ^d	17.06 ^d

Notes : Subscript indicates significant differences (P<0,05); T0 = No treatment, T1 = FOPBA 5%, T2 = FOPBA 10%, T3 = FOPBA 15%, T4 = FOPBA 20%

The same result was indicated at oil palm fiber was lower crude fiber than after soaking with FOPBA. This condition was caused by the existence of decreasing level of lignin compound. Lignin is crude fiber component and decreasing lignin level which cause to degrade level of crude fiber at oil palm fiber. In other hand, other crude fiber component like cellulose and hemicellulose lose due to dissolve in fats. According to Anggorodi (1984), crude fiber was cellulose and hemicellulose and they were insoluble in water but dissolve in watery alkali and break in watery acid. Suwandystuti *et al.* (1984) reported that damping of rice straw with solution NaOH 3% and used filtrate of rice hull ash 10% that were enriched with 4% urea, 0.2% brimstone, 1.8% salt, and 1% lime can degrade level of crude fiber of rice straw as high as 1.7% and 2%.

At the same table, indicated that processing with FOPBA can increase digestibility of dry and organic matter, with the highest result was obtained at treatment T3 and T4, that is 23.48 vs 24.12% and 16.70% vs 17.60%. Hydrolysis

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activity by FOPBA can break its bounding which was marked by decreasing level of lignin, as a consequence enzyme cellulase and hemicellulase produced by bacterial rumen can degrade cellulose and hemicellulose, which in turn it can improve digestibility. Sutrisno *et al.* (1986) reported that processing of rice straw by using filtrate of rice hull ash can increase digestibility coefficient. Ismadi (1990) conducted a research on bagasse that was splashed with filtrate of rice hull ash 20 %, then covered tightly and soaked for 21 days showed there was degradation of fiber composition, except silica and improvement of its digestibility compared to control.

CONCLUSION

Soaked oil palm fiber by 150g/L concentration of filtrated oil palm fruit bunch ash was more effective to increase digestibility.

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14. PRODUCTIVITY OF BALI CATTLE BASED ON SCROTUM SIZE, BODY WEIGHT AND FEED QUALITY

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Abstract

Bali cattle is a national assets in agriculture sector so that it needs an increase of cattle population and productivity to raise beef production in Indonesia. The effort of raising domestic cattle population, i.e. through Artificial Insemination (AI) program and genetic potency but the domestic production of beef has not been able to cover country's need of beef and alive cows import. The purpose of this research is: To provide solution of the low productivity problem and to increase genetic potency and productivity of Bali Cattle. The research uses Thirty (30) Bali cattles from three Regencies in West Nusa Tenggara (NTB) Province (Lombok Tengah, Lombok Barat and Lombok Utara Regency), on the average 3 years old, divided into three groups size of scrotum and body weight, i.e. K1 = average scrotum size of Bali Cattle to average standard deviation (sd)+1; K2 = scrotum size from average sd +1 to sd +2; K3 = scrotum size from average sd +2 to sd +3. The quality of the cattle feed material, it is analysed the proximate of the ration (dry matter, organic matter, crude protein, grease and fiber, TDN). Variables observed: Scrotum size (length and diameter), Body weight and quality of the Bali cattle feed. The results shows that the highest size either on scrotum with the average scrotum length/SL (cm) = 18.36 ± 2.76 and scrotum diameter/SD (cm) = 10.09 ± 2.08 or on body weight/BW (kg) = 459.6 ± 77.96 , is showed by the group of Bali cattle in Lombok Barat location. So does the result of score on the average SL and SD, primarily on K3 (height size) with SL(cm) = 20.38 ± 2.19 and SD (cm) = 10.33 ± 2.05 , though there is no difference on the feed treatment but the air temperature and the humidity affects the productivity of Bali cattle. It is concluded: The correlation of BW with the scrotum size (length and diameter). Feed and climate (air temperature and the humidity) also affects the productivity of the cattle.

Key words: Bali Cattle, Productivity, Scrotum Size and Feed Quality.

INTRODUCTION

Bali cattle is one of livestock commodities which has important roles as the producer of service and product that are useful for the purpose of human life; it is a national assets in agriculture sector so that its existence need to be conserved, develop and increased its population and productivity as the effort of beef sufficiency in 2014.

The Government has conducted various attempts to increase domestic beef production, such as by *grading upward* the local beef cows with the overseas ones, through Artificial Insemination (AI) technology even through genetic quality of livestock improvement.

The domestic production of beef has not been able to cover country's need of beef. In fact, in 2011, Indonesia constantly imported alive cows and frozen beef in number 23,670 of cows which was priced at US \$ 16,714,000 and

US \$ 14,345,000 for 2,844 tons of beef (Anonim, 2011a). While, in 2012 the target was increasing up to 282,000 of cows at cost US \$ 199,114,000 and beef up to 34,000 tons at cost US \$ 171,525,000 (Anonim, 2011b). The effort of raising domestic cattle population to increase the slaughtered cows supply as the source of beef production was expected to be able to reduce the number of either beef or alive cows import.

There are some efforts that can be done to increase Bali cattle population, one of them is by improving its cattle productivity through the selection of cattle that will be a stud. General criteria commonly used in the selection of stud candidate is scrotum size that is also included as the criteria of assessment on the cattle contest (Ismaya. 1991; Ismaya. 1993).

Some resesarches noted that the raising of scrotum diameter size, will also improve the quality of spermatozoa (Latif et al. 2009; Sajjad et al. 2007; Sarder 2005). Crossbreed cows with scrotum diameter size > 30 cm, produce good quality of cement (Latif et al. 2009). According to Soeroso & Duma (2006) in every 1 cm scrotum diameter size increase then the sperm concentration raise up to 0.15×10^9 /ml. While, the cattle studs as the couple have around 19.5 – 34 cm scrotum diameter size (Lindsay et al., 1982). For the Bali cattle ranges on that figure. According to Torres-Júnior dan Henry (2005), scrotum diameter size linearly increased with the age and body weight.

Another effort done in increasing cattle productivity and reproductivity is that through Artificial Insemination (AI) programme (Salisbury and Van Demark, 1985). The success of AI is determined by the quality of spermatozoa correlates to scrotum diameter size. But today, AI implementation is frequently failed and the local government's in ability in finding a correct and effective solution to overcome this problem, cause the need of strategic steps to implement the effort of productivity and cattle population improvement, that is by increasing Bali cattle productivity based on the scrotum size and the cattle's feed quality.

MATERIAL AND METHOD

This research consists of field and laboratory observation which are sequentially conducted.

Research Material

30 male Bali cattles (they are taken from Lombok Tengah, Lombok Barat and Lombok Utara regencies where each regency is taken 10 cows), it is measured scrotum diameter and is scored the result of the measurement; their weight weighing.

Research Method

The research used thirty (30) Bali cattle from three Regencies in West Nusa Tenggara (NTB) Province (Lombok Tengah Regency, Lombok Barat Regency and Lombok Utara Regency), average 3 years old, divide into three groups size of scrotum, i.e. K1 = average scrotum size of Bali Cattle to average standard deviation (sd) +1; K2 = Bali cattle which has bigger scrotum size from average standard deviation (sd) +1 to average standard deviation (sd) +2; K3 = Bali cattle which has bigger scrotum size from average standard deviation (sd) +2 to average standard deviation (sd) +3. The observation and measurement (length and diameter) the scrotum of Bali cattle, is obtained by measuring the length and diameter of scrotum with tape measure/calipers.

Body weight is measured with an *electric* cattle scales i.e Ruudweight brand, in capacity 1000 kg and accuracy 0,10 kg. The quality of feed material composing the cattle ration, is analysed the rations proximate (dry matter, organic matter, crude protein, grease and fiber, TDN).

Then the data obtained is analysed descriptively.

RESULT AND DISCUSSION

The result of body weight measurement and scrotum of Bali cattle in these three locations, are shown in Table 1 below.

Table 1. The average of scrotum size and body weight of Bali cattle

Location	The average of measure		
	SL (cm)	SD (cm)	BW (kg)
Lombok Tengah Regency	17.48 ± 3.06	7.22 ± 1.36	233.6 ± 40.69
Lombok Utara Regency	16.70 ± 1.89	7.96 ± 0.66	243.9 ± 29.02
Lombok Barat Regency	18.36 ± 2.76	10.09 ± 2.08	459.6 ± 77.96

Note : SL = Scrotum length
SD = Scrotum diameter
BW = Body weight

On the Table 1. It is seen that the highest size of Bali cattle either on the scrotum or body weight was found in the group of Bali cattle that were in Lombok Barat location, with the average SL (cm) = 18.36 ± 2.76, SD (cm) = 10.09 ± 2.08 and BW (kg) = 459.6 ± 77.96. This is in line with Torres-Júnior dan Henry’s opinion (2005), the scrotum size increases linearly with the age and body weight, while the scrotum size increase, also will improve spermatozoa quality (Latif et al. 2009; Sajjad et al. 2007; Sarder 2005).

While the result of observation toward the scrotum size based on the average of the highest to the lowest score from each group of Bali cattle in those three research locations, listed in table 2 below.

Table 2. The average of scrotum size of Bali cattle in three groups

Groups	Lombok Tengah Regency		Lombok Utara Regency		Lombok Barat Regency	
	PS (cm)	DS (cm)	PS (cm)	DS (cm)	PS (cm)	DS (cm)
K1(Low)	14.93 ± 1.25	6.00 ± 1.34	14.67 ± 0.58	6.90 ± 0.00	15.60 ± 1.51	0
K2(Medium)	16.75 ± 0.59	7.67 ± 0.21	16.50 ± 0.58	7.58 ± 0.39	17.45 ± 0.07	7.90 ± 0.00
K3 (High)	20.00 ± 3.28	8.40 ± 0.44	19.00 ± 1.00	8.48 ± 0.29	20.38 ± 2.19	10.33 ± 2.05

Note : Score of PS (cm) : High = 18 - ≥ 20 Score of DS (cm) : High = 8 - ≥ 10
Medium = 16 - 17,9 Medium = 7 - 7,9 Low = 13 - 15,9, Low = 4 - 6,9

Based on the Tabel 2. above, the highest scrotum size was indicated by the Bali cattle in Lombok Barat location, both on K3 with SL (cm) = 20.38 ± 2.19 and SD (cm) = 10.33 ± 2.05; then Lombok Tengah location was not too different on SL (cm) = 20.00 ± 3.28 and SD (cm) = 8.40 ± 0.44; the last Lombok Utara on SL (cm) = 19.00 ± 1.00 and SD (cm) = 8.48 ± 0.29, also in K2 dan K1 group. But on th Bali cattle in Lombok Barat location, based on the average of calculated score, it did not have low scrotum size (K1). This was probably caused by the conditions (temperature and air humidity) where Bali cattle breded, as shown in Table 3 and 4, whereas the feeding treatment was not different (Tabel 5), because the Bali cattle in those three locations were breded in the same field and treatment.

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Table 3. The temperature of research locations (°C)

Observation hours	In-situ		Ex-situ	
	KLU+LOTENG	LOBAR	KLU+LOTENG	LOBAR
00	23.8±1.2 ^{abz}	21.5±0.9 ^{ay}	23.0±1.4 ^{bz}	21.6±1.1 ^{by}
02	22.0±2.3 ^a	21.5±0.8 ^a	20.1±2.3 ^a	19.9±2.2 ^a
04	22.7±1.1 ^{abz}	21.3±0.7 ^a	21.8±1.1 ^{ab}	20.3±3.3 ^a
06	24.9±2.2 ^{bz}	21.4±0.9 ^{ay}	25.1±2.5 ^{cz}	22.5±1.8 ^{bcy}
08	27.6±1.6 ^c	26.6±0.8 ^c	28.0±2.2 ^{dez}	23.2±4.0 ^{cy}
10	28.0±1.0 ^c	26.6±0.7 ^c	29.7±1.1 ^{ef}	28.9±2.5 ^e
12	30.6±1.2 ^{dz}	27.7±0.5 ^{cy}	30.5±1.0 ^f	29.4±4.0 ^e
14	31.9±1.4 ^{dz}	27.7±0.6 ^{cy}	30.4±1.3 ^f	29.2±3.1 ^e
16	27.0±0.8 ^{cz}	23.9±0.5 ^{by}	26.5±1.0 ^{cd}	26.5±7.4 ^d
18	24.7±2.5 ^b	23.9±0.5 ^b	24.0±2.3 ^{bz}	21.2±0.3 ^{by}
20	24.8±0.7 ^{bz}	22.3±1.3 ^{ay}	23.7±0.8 ^{bcz}	20.9±0.1 ^{aby}
22	22.6±2.3 ^a	22.2±2.3 ^a	21.6±2.4 ^a	20.2±0.7 ^a

^{a, b, c, d, e, f} Means superscript on the same column differ significantly (P<0,05)

^{y, z} Means same superscript in the same locations differ significantly (P<0,05)

Table 4. The humidity of the research locations (%)

In Situ		Hours					
		06	10	14	18	22	02
KLU	+	82.9	63.7	66.2	84.1	83.3	85.4
LOTENG							
LOBAR		87.7	70.1	69.5	89.1	93.7	91.2
Ex Situ							
KLU	+	79.4	49.7	60.9	87.5	92.5	94.2
LOTENG							
LOBAR		82.2	59.7	63.4	90.5	94.5	94.0

The result of proximate analysis in each feed material consumed by the Bali cattle encountered in the reasearch locations as apparently shown in Tabel 5. The nutrient contentof each feed material, is shown in the following table.

Table5. The quality of feed nutriens (%)

Feed	Condition	DM	OM	CP	Fat	CF	TDN
Elephant grass	Fresh	11.9	77.9	8.4	1.6	29.4	56.3
Field grass	Fresh	20.7	83.5	5.2	2.3	30.9	55.9
Rice straw	Fresh	41.1	67.0	6.8	1.5	23.4	45.6
Rice straw	Dry	61.6	67.0	6.3	0.3	30.4	69.9
Corn straw	Fresh	19.8	82.5	15.7	1.3	22.8	64.1
Corn straw	Dry	36.5	79.9	10.5	0.6	29.9	62.7
Cassava waste	Fresh	11.5	82.7	20.2	2.6	19.9	65.5
Soybean straw	Dry	90.1	83.8	4.2	1.9	45.4	49.7

DM = Dry Matter, OM = Organic Matter, CP = Crude Protein , CF = Crude Fiber,

TDN = Total Digestible Nutrients

CONCLUSIONS

From the result of the research, it can be concluded that the correlation of body weight (BW) with the scrotum size (lengthand diameter). Feed and climate (air temperature and the humidity) also affects the productivity of the cattle.

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15. QUALITY OF RENNET FROM RABBIT STOMACH DURING COLD AND FROZEN STORAGE

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Abstract

Problems in cheese production in Indonesia is the use of milk coagulating enzymes (rennet) for the curd formation, which is still imported. Stomach rabbit waste of rabbit slaughtering can be used as an alternative in the cheese production. The purpose of this study was to determine the quality of rabbits stomach rennet during cold and frozen storage temperatures. The rennet quality include the milk coagulating ability, curd yield percentage, pH and proteolytic activity of rennet. Rennet quality data were analyzed statistically using a two-way ANOVA, with three times replication. The results showed that the average of milk coagulation time, curd yield and pH were not significantly different between the rennet stored at cold and frozen temperatures. However, during storage 0; 15 and 30 days occurred significant difference ($P < 0.05$) on milk coagulation time, curd yield and rennet pH, a started 0; 15 and 30 days: the average coagulation time was 3.35; 3.91 and 4.63 minutes, the average yield of curd (rendemen) was 11.07; 8.93 and 7.47%, and the average of rennet pH was 5.60; 5.88 and 5.94, respectively. The proteolytic activity of rabbit stomach rennet stored at frozen temperatures was higher ($P < 0.05$) than the rennet stored at cold temperatures. There was a significant change in proteolytic activity of rennet during storage. At storage 0; 15 and 30 days, the average of proteolytic activity of rennet was 25.87; 24.79, and 24.43 mg / ml / min, respectively. In conclusion, the rabbit stomach rennet stored at cold and frozen temperatures has the same quality in its ability to milk coagulate, curd yield and pH value. However, there were slight changes in the quality of rabbit stomach rennet during 30 days of storage at cold and frozen temperatures, which is an increase in the milk coagulating time, decreasing in curd yield, increasing the pH value and decreasing of proteolytic activity of rennet.

Key words: Rennet Quality, Rabbit Stomach, Storage.

INTRODUCTION

The digestibility of organic matter of feeds is lower in rabbits than in other herbivorous animals, primarily due to lower digestion of crude fibre. The degradation of organic matter in the digestive tract of rabbits involves a number of hydrolytic reactions which are catalysed by enzymes of endogenous and/or microbial origin. It is generally assumed that hydrolytic activity and digestive volume correlate positively with the digestion efficiency. Part of the digestive tract of rabbit i.e starting from stomach, small and large intestine and also caecum known to contain proteinase activity, and in the stomach having the highest proteinase activity (Marounek and Vovk, 1995).

The availability of milk clotting enzyme in Indonesia is still a problem for cheese production, so that it still imports. Various sources of plant-protease can be used to produce cheese but usually tasted bitter. Rabbit stomach is an animal

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rennet, which is a waste of rabbit slaughtering can be used as an alternative to milk clotting enzyme. Enzymes are proteins that are easily damaged by a variety of factors, such as pH and temperature. Freeze-thawing or freeze-drying has been widely used for the preservation of various kinds of biological materials, in particular, for maintaining their biological activities over a long period of time. It is known, however, that to a certain extent the biological activities suffer damage under certain conditions of freeze-thawing or freeze-drying (Hanafusa, 1967).

Therefore, for sustainability of availability of milk clotting enzyme from rabbit stomach, proper handling and storage required to maintain their enzyme activities. The purpose of this study was to determine the quality of rennet from rabbit stomach during cold and frozen storage temperatures.

MATERIALS AND METHODS

Materials

Materials in this study includes rabbit stomach, acetic acid solution 1.5%, NaCl 5%, NaOH 1 N for rennet extraction. For proteolytic assay using rennet extract, hydrolysate casein, aquadest, tyrosin, HCl, phosphate buffer pH 7 and trichloroacetic acid (TCA) 5%.

Methods

Rennet extraction of rabbit stomach

Rennet extraction was performed according to Utama (1985) method. After removing the internal contents, rabbit stomach was washed with tap water internally while their veins and fat contents were removed externally, cut in small size and weighed. Solution for extraction was prepared by using mixture of acetic acid solution 1.5% and NaCl 5%. The pieces of stomach was mixed with extractor solution, covered and stirred overnight at room temperature. After stirring, the mixed was filtered with cheese cloth, and measured the pH value using digital pH-meter. NaOH 1N was added, when pH of rennet extract less than 5.6 until reaches pH 5.6. Rennet was stored in refrigerator and freezer for 30 days. At the day 0; 15 and 30 the rennet quality was assessed which includes milk clotting and rennet evaluation, pH, and proteolytic activity.

Evaluation of milk clotting activity and rennet

Milk clotting and rennet evaluation were performed according to Scott (1981) with slight modification. Tubes filled pasteurized milk as much as 5 ml, added 10% rennet extract and incubated in waterbath at 40°C. As the flocculation started the time was noted. Coagulated milk (curd) filtered and weighed.

Measuring pH of rennet

Value of pH was measured by digital pH-meter (HANNA –S 487092) which has been calibrated with a buffer standard.

Proteolytic activity assay

The proteolytic assay of rennet extract (enzyme) from rabbit stomach was assayed by using standard curve of tyrosine solution according to Whitaker (1972) with slight modification. Tyrosine was diluted in small volume of HCl and added with phosphate buffer pH 7.0 until 100 ml. Casein powder (Hammersten) 0.2 g diluted in small volume of NaOH 0.1 N and added phosphate buffer pH 7.0 until 100 ml as a substrate (S) for enzyme. Mixture of 3 ml substrate and 1 ml enzyme (E) incubate in shaker waterbath at 40 °C for 30 minutes. Enzyme activity was stopped with 5 ml TCA 5%. This mixture of substrate, enzyme and TCA was allowed to stand at room temperature for 60 minutes, and then centrifuged

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(Eppendorf centrifug 5804R) merk)at 3000 rpm for 15 minutes at 4oC. Supernatant was measured on Spectrophotometer 280 nm (UV-1601PC, UV-VISIBLE Spectrophotometer, SHIMADZU). The control of enzyme activity was prepared with the blank (without enzyme).

The proteolytic enzyme activity =

$$(\text{volume S} + \text{volume E} + \text{Vol TCA}) \times (\% \text{ tyrosine}/100) \times 1/t \times 10^3$$

$$\mu\text{g/ml/minute}$$

Note: (S: Substrat, E: Enzyme, t: incubation time)

Statistical analysis

The results of this study was analyzed statistically by two way ANOVA, and difference between the means was analyzed by DMRT (Duncan’s Multiple Ranges Test).

RESULTS AND DISCUSSION

Milk coagulation (clotting) time

Coagulation time in this study showed that how much time it takes rennet to coagulate milk. There was no significantly different in coagulation time of rennet after storage at cold and frozen temperature, whereas the increased storage time it will increase the milk coagulation time (P<0.05) (Table1). The time required of rennet at cold or frozen stored for 30 day to reach the flocculation of cow milk was longer than that of rennet stored 0 or 15 day.

Table 1. The average of coagulation time (minute) of rennet **from rabbit stomach during cold and frozen storage**

Storage	Storage time (day)			Average ^{ns}
	0	15	30	
Cold	3.35	4.09	4.51	3.99
Frozen	3.35	3.73	4.76	3.95
Average	3.35 ^a	3.91 ^b	4.63 ^c	3.97

ns : not significant

a,b,c: different letter in the same row indicated significantly different (P<0.05)

According to Ahmed et al. (2013), milk clotting activity of gastric enzyme Camel was influenced by the pH of the milk at the renneting stage, and the flocculation time increase with the age of the Camel. The pH of the milk for rapid flocculation is very important during cheese making since the acidification by the lactic acid bacteria helps the enzyme activity in which the enzyme is a protease having an optimum activity around pH 5.5. This contributes to the destabilization of the casein micelles.

Curd yield (rendemen)

Curd yield (rendemen) obtained by coagulating milk with fresh rennet (0 day) was highest (11.07%) followed by rennet that storage 5 days (8.93%) and 30 days (7.47%) (Table 2). Curd yield (rendemen) obtained by coagulating milk with fresh rennet (0 day) was highest (11.07%) followed by rennet that storage 5 days (8.93%) and 30 days (7.47%) (Table 2). Curd yield in this study was lower than curd yield in the previous study obtained by coagulating buffalo milk with laboratory made rennet derived from buffalo calves abomasum (fresh and stored rennet) and commercial rennet. The mean values (32.2%±0.24%) of curd obtained from fresh rennet coagulated milk was highest followed by commercial rennet coagulated milk (29.87%±0.11%) and stored rennet (3 month) coagulated milk

(28.24 %±0.13%) (Ahmed et al., 2013). The difference of curd yield maybe caused by different sources of milk and types rennet. In this study, using rennet from rabbits stomach, whereas in the previous study by Ahmed et al. (2013) using buffalo calves abomasum.

Table 2. The average of curd rendement(%) produced by rennet coagulation

Storage	Storage time (day)			Average ^{ns}
	0	15	30	
Cold	11.07	9.13	7.53	9.24
Frozen	11.07	8.73	7.4	9.07
Average	11.07 ^a	8.93 ^b	7.47 ^c	9.16

^{ns} : not significant

^{a,b,c}: different letter in the same row indicated significantly different (P<0.05)

According to Mona et al. (2011), cheese yield is affected by many factors including milk composition, amount and genetic variants of casein, milk quality, somatic cell count (SCC) in milk, milk pasteurization, coagulant type, vat design, curd firmness at cutting, and manufacturing parameters (Mona et al., 2011). As compared to cow milk, buffalo milk is richer in fat, lactose, protein, total solids, vitamins and minerals, such as calcium, magnesium and inorganic phosphate (Murtaza et al., 2008). The high protein content of buffalo milk and total solids helped in developing high viscosity curd (Ghadge et al., 2008).

It is a very important parameter: the higher the recovered percentage of solids, the greater the amount of cheese obtained and therefore gain in economic terms. In the cheesemaking process, therefore, it is very important to obtain the maximum possible recovery of substances from milk. Equally important is the calculation of the effects that each milk component, and in particular, fat and casein, can have on cheese yield, in order to adopt a milk quality payment system that could remunerate each parameter for its actual value (Paolo et al., 2008). Cheese yield is affected by many factors including milk composition, amount and genetic variants of casein, milk quality, somatic cell count (SCC) in milk, milk pasteurization, coagulant type, vat design, curd firmness at cutting, and manufacturing parameters (Mona et al., 2011).

The pH value of rennet

There was no significantly different between the pH of rennet derived from rabbit stomach after cold and frozen storage (Table 3). However, the pH of fresh rennet (0 day) was lower (5.60) (P<0.05) than rennet stored at 15 days (5.88) or 30 days (5.94).

Table 3. The average of pH value of rennet during cold and frozen storage

Storage	Storage time (day)			Average ^{ns}
	0	15	30	
Cold	5.6	5.92	5.97	5.83
Frozen	5.6	5.84	5.91	5.78
Average	5.6 ^a	5.88 ^b	5.94 ^b	5.81

^{ns} : not significant

^{a,b} : different letter in the same row indicated significantly different (P<0.05)

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The pH of rennet in this study similar to the previous study (Ahmed et al., 2013), showed that the mean pH value of stored rennet (5.75±0.02) was noted greater than that of fresh rennet (5.47±0.02). The clotting activity of rennet decreases as its pH turns towards alkalinity (Ahmed et al., 2013).

Milk clotting activity was influenced by the pH of the milk at the renneting stage. All enzyme preparations exhibited almost a linear curve with an increased pH from 5.8 to 6.6. The optimum pH for clotting camel milk for gastric enzyme Camel was at 5.8, and the flocculation time increased with the age of the Camels. The pH of the milk for rapid flocculation is very important during cheese making since the acidification by the lactic acid bacteria helps the enzyme activity in which the enzyme is a protease having an optimum activity around pH 5.5. This contributes to the destabilization of the casein micelles. In regards to bovine milk, the optimum pH for gastric enzyme camel 6.0 (Saliha et al., 2011).

Proteolytic activity

As shown in Table 4, the average proteolytic activity of rennet derived from rabbit stomach after frozen stored was higher (P<0.05) than rennet after cold storage. The longer in storage time showed the lower activity of rennet (P<0.05). The other study showed that total of gastric proteinase activity of 3-month-old rabbits was 4322 (expressed as mg azocasein decomposed/h), whereas total proteinase activity of 4-week-old was 1550. The gastric proteolytic activity represented 46.3% of the total proteolytic activity of the digestive tract (Marounek and Vovk, 1995).

Table 4. The average of proteolytic activity (µg/ml/minute) of rennet during cold and frozen storage

Storage	Storage time (day)			Average
	0	15	30	
Cold	25.87	24.45	24.23	24.85 ^p
Frozen	25.87	25.15	24.65	25.22 ^q
Average	25.87 ^a	24.79 ^b	24.44 ^c	25.04

a,b, c : different letter in the same row indicated significantly different (P<0.05)

p,q : different letter in the same column indicated significantly different (P<0.05)

According to the previous study, shelf life of rennet derived from buffalo calves abomasum that soaked in 12% NaCl solution and added 1% sodium benzoate solution reflected that all samples were active up to three months storage period, while after six months evaluation 15% rennet samples were found inactive (Ahmed et al., 2013).

Proteins can undergo degradation by many mechanisms. However, the primary mechanism of concern with frozen storage is aggregation. The freezing process, however, subjects proteins to other stresses as a consequence of the removal of water as ice. The resulting cryoconcentration and desiccation of protein can be classified as osmotic stresses. Protein structure changes that occur as a consequence of such stresses have a greater probability of being irreversible, and are classified as freeze denaturation (Singh et al., 2009). Upon the fast freezing (e.g., when the freezing rate >20°C/min), small ice crystals and a relatively large surface area of ice–liquid interface are formed, which increases the exposure of protein molecules to the ice–liquid interface and hence increases the damage to the proteins. During thawing, additional damage to proteins is

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caused by recrystallization process. Recrystallization exerts additional interfacial tension or shear on the entrapped proteins and hence causes additional damage to the latter (Cao et al., 2003). When the freezing process was varied so as to achieve different freezing rates, the slowest freezing rate caused the highest enzyme leakage (Nilsson and Ekstrand, 1993).

This low-temperature effect (“chill”) is distinct from the effect on protein structure that comes from the actual freezing (e.g., cryoconcentration, phase changes, and ice surface denaturation). A more precise thermodynamic explanation for cold denaturation comes from considering the free energy of protein unfolding. Cold-induced unfolding (cold denaturation) is a physical consequence of the temperature sensitivity of noncovalent electrostatic and hydrophobic interactions, which become weaker at lower temperatures. However, chill-induced unfolding probably makes a molecule more susceptible to freeze-induced stresses, leading to aggregate formation and/or loss of structure (Singh et al., 2009).

CONCLUSION

Milk clotting enzyme extracts derived from rabbit stomach has the same quality in cold storage and frozen for 30 days, but there was a slight decrease in the proteolytic activity of rennet are kept cool. The longer it is stored there is little loss of quality, namely an increase in rennet coagulation time, curd yield reduction, increased pH and decreased proteolytic activity.

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16. *IN VITRO* CULTURE FOR THE SUPPLY OF MATERIAL GENETIC TRANSFORMATION ON DWARF NAPIERGRASS (*Pennisetum purpureum* cv. Schumach)

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Abstract

We have established a plant regeneration system via somatic embryogenesis formation from apical meristem of dwarf napiergrass (*Pennisetum purpureum* cv. Schumach). Apical meristem as initial explants were isolated from aseptically shoot-tillers in the field, and cultured *in vitro*. The most effective treatments for embryogenic calli formation was 2.0 mgL⁻¹ 2,4-dichlorophenoxy acetic acid (2,4-D) plus 0.5 mgL⁻¹ benzyl amino purine (BAP). Induced calli were transferred to the same fresh Murashige Skoog (MS) medium, supplemented with 50 µM CuSO₄. Plant regeneration from somatic embryos was achieved by culturing on solid MS medium containing 2.0 mgL⁻¹ BAP. All regenerants were successfully grown up uniform in soil. Based from this result, calli from proliferation can be used as regenerable target tissue for genetic transformation and breeding program of *Pennisetum purpureum* cv Schumach using particle bombardment.

Keyword: In Vitro Culture, Dwarf Napiergrass

INTRODUCTION

Napiergrass (*Pennisetum purpureum* cv. Schumach) is an important forage in the tropics and subtropics, valued for its high biomass, perennial nature and pest resistant. Napiergrass tolerates a wide range of soil conditions ranging from low fertility acid soils to slightly alkaline conditions and has good drought tolerance, high photosynthetic and water use efficiencies. In the leafy stage, this grass is highly nutritious, is selectively chosen as a feed and is very palatable to cattle. Furthermore, napiergrass produces more dry matter per unit time than other grasses or legumes. Because of its rapid growth and easily degradable biomass, napiergrass also has a potential for conversion to alcohol or use in methane production (Anderson *et al.* 2008).

Napiergrass is an allotetraploid (2n=4x=28) and has the genome formula of A'A' BB, where A'A' is homologous to the AA genome of pearl millet (2n=2x=14) (*Pennisetum glaucum* (L.) R. Br.). Because the species exhibits broad morphological variation and cross-pollinates, napiergrass is a valuable source of genetic variation for pearl millet. Although napiergrass forms hybrids with pearl millet, the hybrids are sterile (2n=3x=21) and must be vegetatively propagated (Hanna and Monson, 1988).

A breeding programme in Tifton, Georgia released *Pennisetum purpureum* cultivar Merkeron, derived from an intraspecific cross between a high yielding clone and a dwarf leafy clone, that has improved yield and disease resistance. A selection from the selfed progeny ‘Merkeron’ resulted in Dwarf Tift N75/ Mott, a dwarf leafy type. As a forage crop, dwarf types are preferable to normal types as normal types can become stemmy and unpalatable (Hanna and Monson, 1988 and Harris *et al.* 2009). Furthermore, dwarf napiergrass facilitates hand-harvesting by farmers and is assessed to be more suitable for grazing than normal varieties

(Mukhtar *et al.* 2003). In another *Pennisetum purpureum* breeding program, improvement of napiergrass began with the development of strains resistant to eyespot disease caused by the fungus *Helminthosporium ocellum* faris (Harris *et al.* 2009).

Plant transformation can contribute to *Pennisetum purpureum* breeding because new characteristics can be introduced into the species using genetic engineering techniques. These techniques offer opportunities to improve *Pennisetum purpureum*. In addition, a combination of a transformation system and crossbreeding with this grass will play a role in increasing the gene pool of *Pennisetum purpureum*.

Establishment of an efficient and reproducible *in vitro* plant regeneration system is essential for the successful genetic engineering of plants (Vasilet *et al.* 1990). Callus and multiple-shoot clumps induction are the crucial steps for knowing the suitability of each genotype for tissue culture and for plant improvement. Most tissue culture research with forage grasses has been conducted in temperate species (Ishigaki *et al.* 2009). Efficient plant regeneration systems for warm-season grasses have been developed via somatic embryogenesis (Bovo & Mroginski 1989; Akashi & Adachi 1991, 1992; Akashi *et al.* 1993; Gondo *et al.* 2007 and Ishigaki *et al.* 2009). *Pennisetum purpureum* has been previously regenerated via somatic embryogenesis (Vasil *et al.* 1983); however, no data have been published about the frequency of embryogenic callus formation and plant regeneration. In addition, there are no reports about plant regeneration from multiple-shoot clumps from *Pennisetum purpureum*.

In this study, we used dwarf variety of late-heading type napiergrass (dwarf napiergrass) for examining somatic embryogenesis formation and plant regeneration capacities. The objective of this study was to establish a suitable plant regeneration system for dwarf napiergrass using apical meristems from shoot-tillers as the initial explants.

MATERIALS AND METHODS

Explant sterilisation

Shoot-tillers of dwarf variety of late-heading type napiergrass (dwarf napiergrass) to be used as explants were collected from the Kibana Field, University of Miyazaki. The shoot-tillers were washed with running tap water to remove sand and dust particles. The shoot tiller were sterilized by immersing them in 70% (v/v) ethanol for 2 minutes, followed by immersion in a 2% (v/v) sodium hypochloride solution. The solution containing the shoot tips was agitated for 15 minutes, followed by three washes with sterilized water for 2 minutes. After sterilisation, the older leaves were removed from the primary explant stalk. Explants of equal size were placed in tubes containing Murashige Skoog (MS) media (Murashige and Skoog 1962) supplemented with various concentrations of 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzylaminopurine (BAP).

Embryogenic calli induction

The initial explants were cultured on MS medium containing 3% sucrose, 0.3% phytigel and 0.1% (v/v) Preservative for Plant Tissue Culture Media (PPM) supplemented with 2 mgL⁻¹ 2,4-D and 0, 0.01, 0.1 and 0.5 mgL⁻¹ BAP. After 30 days in culture, primary calli were individually removed and transferred to fresh

solidified MS medium. To proliferate embryogenic calli, MS media containing the optimum combination of 2,4-D and BAP with 0, 5 or 50 μM CuSO_4 were used.

Plant regeneration conditions

Embryogenic calli were transferred onto regeneration medium containing MS basal media containing 3% sucrose and 0.3% phytigel supplemented with 2 mgL^{-1} α -naphthalene acetic acid (NAA) in combination with 0, 0.01, 0.1 and 0.5 mgL^{-1} BAP, or hormone-free media as the control. After 2 weeks, the plant regeneration percentage was calculated. Elongated shoots were isolated and transferred to fresh $\frac{1}{2}$ MS medium under light conditions to induce root development. Rooted plants from embryogenic calli were transferred to soil and maintained in the greenhouse until maturation. All media were adjusted to pH 5.6-5.8 prior to being autoclaved at 121°C for 15 min. The cultures were incubated under fluorescent lights of 3500 lux for 16 hours at 27°C. The transformed data were analyzed by analysis of variance (ANOVA) and Tukey’s test using SPSS 10 software.

RESULTS AND DISCUSSION

Embryogenic callus formation and plant regeneration

Figures 1a.1-1a.3 show cultures of apical meristems from shoot tillers on MS containing 2 mgL^{-1} 2,4-D and 0.1 mgL^{-1} BAP after 30 days in culture. The apical meristem develops into primarily embryogenic calli (Figure 1a.4). Table 1 shows the percentage of cultures that formed embryogenic calli under different hormone concentrations. Although only media with BAP and 2,4-D formed calli, the frequency of embryogenic callus induction differed among the combinations of tested hormones with a range of 8.3 – 25%. The highest frequency of embryogenic calli were produced in the 2 mgL^{-1} 2,4-D plus 0.1 mgL^{-1} BAP treatment. Although embryogenic calli developed into scutella after 14 days of culture on MS medium, they did not germinate completely on the medium (Figures 1b.1 – 1b.4). Therefore, embryogenic calli were transferred to MS medium containing 2 mgL^{-1} 2,4-D, 0.5 mgL^{-1} BAP and (0, 5 and 50 μM) CuSO_4 . As a result, scutella developed into coleoptiles on the medium containing 50 μM CuSO_4 (Figures 1b.5 – 1b.8). Within a few days, numerous green plantlets were evident (Figures 1c.1 – 1c.4). The percentage of calli producing embryos on the most effective medium (2 mgL^{-1} 2,4-D, 0.5 mgL^{-1} BAP and 50 μM CuSO_4) was 41.46% (Table 2). The frequency of plant regeneration from embryogenic calli after 30 days in culture was influenced by hormone concentration and ranged from 11.1 – 30.1% (Table 3). Although there were no significant differences among media on the percentage of embryo formation, the highest levels of regeneration occurred on a medium containing 2 mgL^{-1} BAP. Regenerants grew vigorously (Figure 1d.1) and rooted well (Figure 1d.2).

In a previous report, embryogenic calli of several tropical grasses formed in the presence of high concentrations of 2,4-D (Akashi and Adachi 1991, 1992) Akashi *et al.* (1993) Gondo *et al.* (2007) and Ishigaki *et al.* (2009). Ishigaki *et al.* (2009) reported that a high 2,4-D concentration in combination with a low BAP concentration was very effective for embryogenic calli formation in *Brachiaria brizantha* and *Brachiaria ruziziensis*. In this study, embryogenic calli of dwarf napiergrass also developed on MS medium containing a high concentration of 2,4-D (2 mgL^{-1}) and a low concentration of BAP (0.5 mgL^{-1}) with the addition of 50 μM CuSO_4 . Gondo *et al.* (2004) reported that the addition of BAP and CuSO_4 was effective for proliferating highly regenerative embryogenic calli of bahia grass

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without the appearance of albino plants during long term culture. The same effect was found in this study. No albino regenerated plants developed from embryogenic calli on the proliferation medium (MS medium contain 2 mgL^{-1} 2,4-D and 0.5 mgL^{-1} BAP) containing $50 \mu\text{M}$ CuSO_4 . The same effects of CuSO_4 were also found in other crops (Cho *et al.* 1998, 1999). Yang *et al.* (1999) reported that the supplementation of CuSO_4 in the proliferation medium of *indica* rice increased the number of regenerated plants. Embryogenic calli from proliferation medium can be used as regenerable target tissues for genetic transformation and breeding programs for dwarf napiergrass using particle bombardment. Shenoy and Vasil (1992) reported that embryogenic calli of *Pennisetum purpureum* cv Schum from inflorescences and leaves have the genetic uniformity of plants regenerated from somatic embryos and highlighted their value both for clonal propagation and for genetic transformation.

In another previous reports, *in vitro* plant regeneration systems via multiple-shoot clumps facilitated the production of many fertile transgenic plants in maize (Zhong *et al.* 1996) and oats (Zhang *et al.* 1999). Other researchers have investigated plant regeneration of *Pennisetum purpureum* cv Schum from protoplasts (Vasil *et al.* 1983), from somatic embryos derived from the inflorescence (Wang and Vasil 1982), and from somatic embryos derived from leaf tissue and anthers (Haydu and Vasil, 1981). Most of these reports showed that plant regeneration from callus occurred at low frequencies. Nirwan and Kothari (2003) reported that high copper levels improve callus induction and plant regeneration in *Sorghum bicolor*. In this study, we supplemented CuSO_4 in the proliferation medium, and the efficiency of regenerated plants from embryogenic calli on rooting media form roots (88%). Copper ions are well known to be essential for plant growth and development; however, it is still difficult to explain why a high concentration of copper improves callus proliferation and stimulates plant regeneration from callus cultures (Yang *et al.* 1999; Nirwan and Kothari 2003).

Additionally, in this study we used apical meristems from shoot-tillers not from seeds or immature inflorescences as explants because dwarf napiergrass does not produce inflorescences or seeds. Flowering in dwarf napiergrass is restricted because their flowers are very small, and the pollen is short lived, resulting in a low level of seed formation (Pontongkan 2006). For that reason, we used apical meristems from shoot-tillers to induce somatic embryogenesis and multiple-shoot clump formation. Our enhanced protocol decreases the difficulties faced in *Pennisetum purpureum* tissue culturing and establishes an efficient and highly effective tissue culture system.

CONCLUSIONS

Our results indicate that the apical meristem from shoot tillers can be induced in embryogenic calli induction medium; with 0.1 mgL^{-1} 2,4-D and 2 mgL^{-1} BAP being the best concentrations of phytohormones for embryogenic calli induction in this 30 day study. Addition of $50 \mu\text{M}$ CuSO_4 to the medium induced the proliferation of embryogenic calli promoted growth on rooting medium. For the next investigation, we plan to study the genome size of regenerated plants by using flow cytometry (FCM) analysis to confirm that this protocol can be used as a regeneration system for dwarf napiergrass. Our success in achieving high levels of

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regeneration from embryogenic calli is very important for the development of a efficient transformation system and for ensuring a steady supply of novel plant material to use in future breeding programs.

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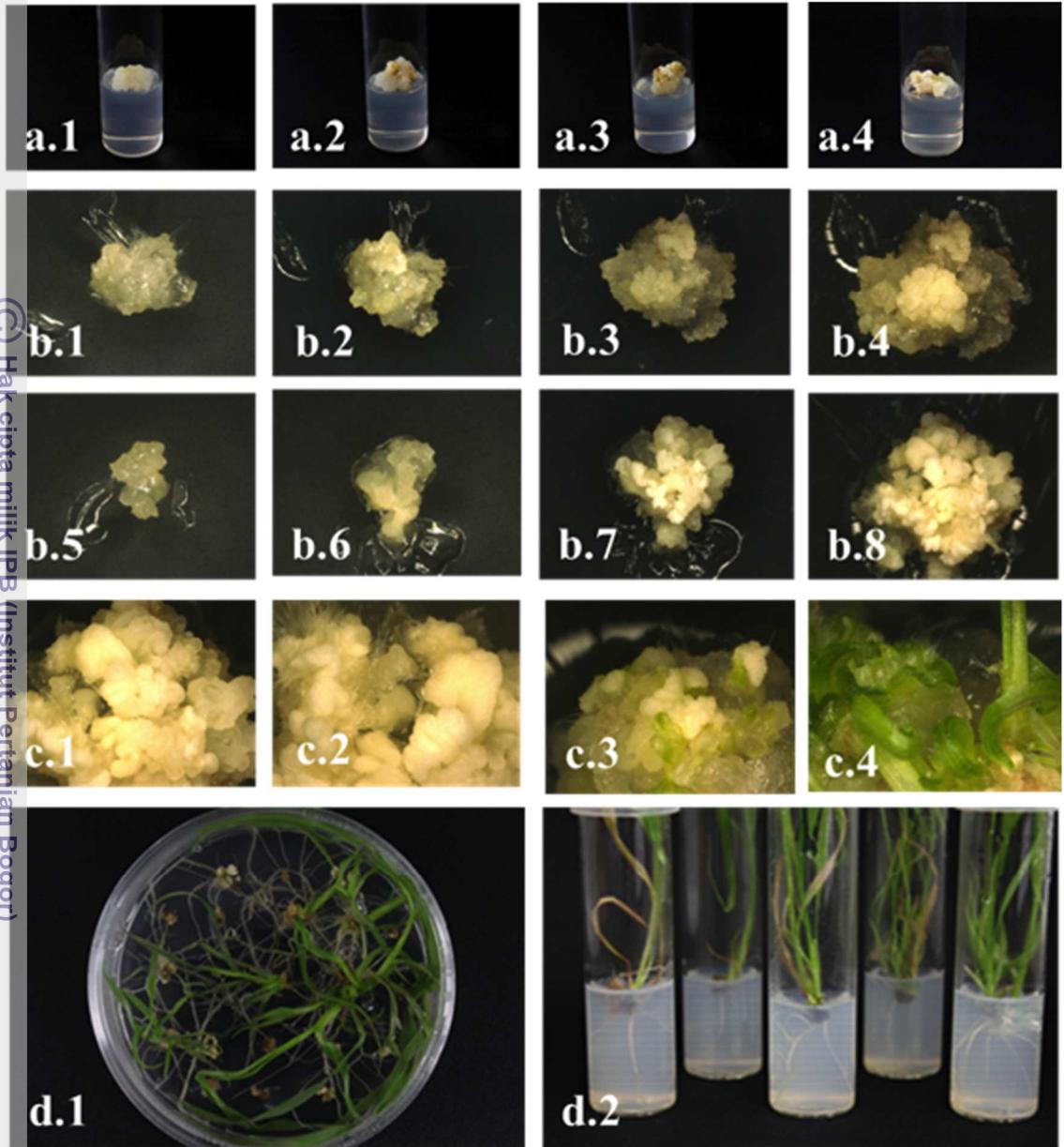
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Umami Figure 1

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17. EGGS RENDANG CHARACTERISTIC BY ADDITION OF GAMBIER CATECHIN ANTIOXIDANT

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Abstract

Egg Rendang has been a traditional food of West Sumatra. The use of coconut milk in the manufacture of large amounts causes the product has been easily oxidized. Gambier catechin contains natural antioxidants that can be used to prevent rancidity that occurs in the eggs rendang. The research objective was to determine the effect of the addition of the antioxidant gambier catechin to fat content, water content and shelf life of eggs rendang. The material of this study using chicken egg Isa Brown strain 40 grains a day old, weighing about 55-60 g obtained from the farm Mount Farm Nago, Ulu Gadut Padang, 1% of total catechins rendang kalio. This study used a randomized block design with 5 treatments and 4 groups, with the group as replicates. Provision of such treatment was the percentage of catechins in the manufacture of egg rendang was : (A) 0% or control, (B) 0.5%, (C) 1%, (D) 1.5% and (E) 2% total of rendang kalio. Furthermore, the data were analyzed by variance and differences between treatments were tested with Duncan's Multiple Range Test. Variables measured after the control foul was a fat content, moisture content and shelf life. The results have real impact on levels of fat, moisture content and shelf life. From the research results could be concluded that the addition of the antioxidant gambier catechin after storage for 19 days at room temperature and open container with a concentration of 1% was effective with a fat content of 53.68%, 1.30% water content and shelf life of 25 days.

Key words: antioxidant, gambier catechin, eggs rendang, shelf life and kalio.

INTRODUCTION

Egg Rendang was been a special food made from eggs and coconut milk from the region of West Sumatra Payakumbuh. Eggs, flour, and rendang spices processed in such a way as to produce eggs rendang was so crispy, crunchy and creamy. Eggs rendang contain relatively high vegetable fat that was equal to 16.2%. On a fat-containing foods such as eggs rendang are relatively high, the damage that may occur was the oxidation of fat so the food becomes rancid. The process of fat breakdown would lead to free radicals. Free radicals were very dangerous when taken with excessive amounts of food because it would cause some dangerous diseases. This could be overcome by the addition of antioxidants. Antioxidants were substances in very small amounts can inhibit or suppress the occurrence of oxidation processes on materials easily oxidized and could be long shelf life.

Catechin has been a natural antioxidant compound. Catechins found in most plant gambier (*Uncaria gambier*). Super quality gambier contains catechins 73.3% (Kasim, 2010). Whereas tea catechins in about 30-40% (Barus, 2009). Catechins were polyphenolic compounds that have the potential as an antioxidant and antibacterial properties (Arakawa, Masako, Robuyusi and Miyazaki, 2004) as well as safe use in food processing so as to extend the shelf life of eggs rendang.

Materials and Methods

Materials and Equipment

This study uses eggs isa brown strain of 40 items derived from livestock Nago Mountain Farm, Ulu Gadut. The materials used were 1% catechins from rendang kalio, 40% tapioca starch, 1% garlic and 0.5% ginger by weight of the eggs. For thick coconut milk sauce was used as much as 500% of the weight of the eggs and the spices were ginger 1%, 20% ground red pepper, 0.6% onion, 0.4% lemon grass, 0.1% bay leaves and 0.2% citrus leaves of the amount of concentrated milk.

Chemicals used were ethyl acetate, distilled water and benzene. The equipment used was a stove, teflon, spoons, pots, stirring bar, distillation equipment, rotary evaporator, filter flask, measuring cups, bowls petridish, glass cup, glass Erlenmeyer, thermometer, balance of power, and the desiccator.

Draft Research

The research was implemented using the experimental method with Random Design Group, consists of 5 treatments and 4 groups, with the group as replicates. The treatment was the percentage of catechins in making egg rendang was : (A) 0%, (B) 0.5%, (C) 1%, (D) 1.5% and (E) 2% based on the number kalio rendang.

Preparation of catechins extract

Preparation of catechins extract using modified maceration technique Novia and Kasim (2010) with the working procedures as follows: gambier to be extracted with ethyl acetate before crushed into powder, then add the solvent of ethyl acetate. Materials and ethyl acetate used with magnetic stirring for 2 hours stirrer then macerated for 24 hours at room temperature and stirring was performed at least three times. After 24 hours, the solution was separated (filtered) by using filter paper, the waste is macerated for 24 hours and filtered with filter paper, test performed three times. Filtrate first, second, and third combined and evaporated with a rotary vacuum evaporator. Dry extract obtained is then used as an antioxidant according to the treatment.

Preparation of Eggs Rendang

Making research eggs rendang (Modified Sugiati (2010) for a one-time test is as follows: a) eggs as many as 10 grains that have been discarded shell was inserted into the container, then put tapioca starch 40%, 1% garlic, and ginger 0.5% of weight of eggs that have been milled and stirred to form a dough. b) The batter on the griddle at the teflon that has been smeared as much as 2 cc of oil and 0.2 cm thick pancakes cooked at a temperature of 80⁰ C for 2 minutes until the colour changes to golden brown. c) Omelet which has been cooked to form parallelogram cut 2 x 3 cm with a thickness of 0.2 cm. Then omelet is divided into five sections. d) We have already prepared rendang sauce, which was 3 liters of concentrated coconut milk with spices rendang; 20% ground red chili pepper, minced garlic 1%, 1% ground onion, ginger 0.5%, 0.5% minced galangal, lemon grass stems 0.4%, 0.1 % bay leaves, lime leaves and 0.2% of the amount of coconut milk (all ground spices) cooked in coconut milk, stirring until thickened and remove the oil for about 60 minutes. e) rendang sauce was divided into 5 sections which were then randomly divided into 5 treatment. Antioxidants were added according to the addition of catechin treatment A. 0% (0 g), B. 0.5% (1.5 g), C. 1% (3 g), D. 1.5% (4.5 g) and E. 2% (6 g) based on the amount of rendang (300 g per treatment) and then stirred until homogeneous. f) Then enter omelet into the appropriate treatment rendang sauce was cooked over low heat

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(80° C) and stirred for 30 minutes until the omelet harden and become crisp and dry rendang bran brownish yellow, and cause the smell rendang. g) Eggs rendang was removed and cooled. h) Eggs rendang stored at room temperature with an open packaging to control damage. Once the damaged control eggs were analyzed according to the parameters rendang research. Above procedure done 4 times.

The Observations

The observations made on eggs rendang after left openat room temperature for 19 days (defective control) was the fat content, water content and shelf life. Fat content by the method of soxlet and moisture content by oven method. Shelf life of a product was determined by changes in physical, chemical and especially the growth of mold.

RESULTS AND DISCUSSION

Content Fat and Moisture Eggs Rendang

on the results obtained that a decrease in fat content and increased water content of eggs rendang plus catech in. The study average levels of fat and water content of eggs rendang can be seen in Table 1.

In Table 1. can be seen that the average of the highest levels of fat found in eggs rendang A treatment that was 57.40% and the average of the lowest fat content found in egg rendang E treatment was 52.98%. The analysis of variance showed that catechins provide significant effect ($P < 0.05$) on fat content of eggs rendang. This means that catechins affect the fat content of eggs rendang.

The test results with in the multiple Duncan's showed that the fat content of eggs rendang between treatments C, D, and E differ from each other are not significant ($P < 0.05$), but all three treatments showed fat content significantly ($P > 0.05$) lower than the levels of fat eggs rendang in treatment B. These results indicate that catechins in the percentage addition of 1% was effective at inhibiting the absorption of fat in the egg rendang.

Based on Table 1 above can be seen that the average moisture content of the highest eggs rendang found in treatment E was 1.54% and the average moisture content of lowest eggs rendang found in treatment A was 1.02%. The analysis of variance showed that the treatment gives a very real effect ($P < 0.01$) on the water content eggs rendang. This suggests that catechins as antioxidants significantly affect the moisture content of eggs rendang.

The test results with in the multiple Duncan's showed that the moisture content of eggs rendang in treatment C (1.28%) differed not significantly ($P > 0.05$) with treatment B (1.19%) and treatment D (1.32%), but both show the moisture content different highly significant ($P < 0.01$) higher than the moisture content of eggs rendang in treatment A (1.02%). This suggests that the higher the concentration of catechins were given increasing levels of water eggs rendang. This was because water as a product of their action that occurs in order to prevent the formation of hydroperoxides which could cause acidity in eggs rendang.

Increased moisture content of eggs rendang along with the addition of the higher levels of catechins, this was due to catechin can bind oxygen well and make the water as a product of the reaction, so that the moisture content in the eggs rendang be increased. As shown in this study, the treatment B (1.19%), treatment C (1.28%), treatment D (1.32%) and treatment E (1.54%). In accordance with the opinion Winarsi (2007) that antioxidants play a role in the decomposition reaction of hydrogen peroxide into oxygen and water. Antioxidants were able to oxidize one molecule of

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hydrogen peroxide into oxygen. Later, simultaneously reducing antioxidant second molecule of hydrogen peroxide into water.

Moisture content eggs rendang was also influenced by fat content egg rendang. Moisture content was proportional to the fat content which decreases the fat content of the lower moisture content and otherwise.

This shows that the addition of catechin with the percentage of 0.5% after left open at room temperature for 19 days, was very effective at inhibiting the formation of peroxides. Foods that contain fat without antioxidants would be damaged by the formation of peroxides.

Shelf Life

Based on the research results obtained eggs rendang an increased shelf life. Each treatment produces a different shelf as shown in Table 2.

TABLE 2. The Average Value of Shelf Life Eggs Rendang

Treatment	Shelf Life (days)	
A (0% catechin /control)	19.00	c
B (0.5% catechin)	23.00	b
C (1.0% catechin)	25.00	a b
D (1.5% catechin)	27.25	a
E (2.0% catechin)	27.75	a
SE(%)	3.96	

Note : Different Superscript indicates a real difference (P <0.05)

In Table 2 could be seen that the average shelf life of the highest eggs rendang found in treatment E was 27.75 days and the lowest in treatment A was 19 days. Diversity of the results showed that the treatment gives significant effect (P <0.05) to the shelf life of eggs rendang. This means that the addition of catechin affect the shelf life of eggs rendang.

The results of Duncan's multiple range test showed that the shelf life of eggs in treatment C eggs rendang not significantly different (P>0.05) with treatment B, D and E, while the shelf life in treatment eggs rendang B and A showed significantly different (P <0, 05). This suggest that the higher the addition of catechin was given it will extend the shelf life of eggs rendang.

Rendang shelf life of eggs in treatment B and C showed no significantly different (P>0.05) while treatments C, D and E also showed different effects were not significant (P>0.05) due the catechin were antioxidants and antibacterial properties that could maintain in the quality of the eggs rendang damage.

Conclusions

From the results of the study concluded that the addition of gambier catechins an antioxidant effect on fat content, water content and shelf life of eggs rendang. From the research results could be concluded that the addition of the antioxidant gambier catechin after storage for 19 days at room temperature and open container with a concentration of 1% was effective with a fat content of 53.68%, 1.30% moisture content and shelf life of 25 days.

Acknowledgments

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TABLE 1. The average value of Fat and Moisture Content Egg Rendang

Treatment	Fat Content (%)		Moisture Content (%)	
A (0% catechin/control)	57.40	a	1.02	d
B (0.5% catechin)	54.45	b	1.19	c
C (1.0% catechin)	53.68	c	1.30	b c
D (1.5% catechin)	53.18	c	1.39	b
E (2.0% catechin)	52.98	c	1.54	a
SE(%)	6.39		5.31	

Note : Different Superscript indicates a real difference (P <0.05)

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Table 1 Effect of hormone concentration on embryogenic callus formation from apical meristems of tiller shoots of dwarf napier grass

Medium	Hormone concentration (mg L ⁻¹)		No. of inoculated shoot apices	No. of calli formed (%)	No. of embryogenic calli formed (%)
	2,4- D	BAP			
D0B0	0	0	60	40 (66.7)	0 (0) ^a
D2B0	2	0	60	49 (81.7)	0 (0) ^a
D2B0.01	2	0.01	60	56 (93.3)	5 (8.3) ^a
D2B0.1	2	0.1	60	52 (86.7)	12 (20.0) ^b
D2B0.5	2	0.5	60	54 (90.0)	15 (25.0) ^b

^b: different letters following each value within a column indicate a significant difference by Tukey’s Test (P<0.05).

Table 2 Effects of hormone and CuSO₄ on proliferation of callus from embryogenic calli of dwarf napiergrass

Medium	Hormone concentration (mgL ⁻¹)		CuSO ₄ (μM)	No. of inoculated calli	No. of proliferated calli ^{ns}	No of embryogenic calli formed ^{ns}	% of embryogenic calli formed ^{ns}
	2,4-D	BAP					
D2B0.5	2	0.5	0	63	43	8	18.60
D2B0.5CL	2	0.5	5	63	42	13	30.95
D2B0.5CH	2	0.5	50	63	41	17	41.46

Table 3 Effects of hormone concentration on plant regeneration from embryogenic calli of dwarf napier grass

Medium	Hormone concentration (mg L ⁻¹)		No. of inoculated calli	No. of regenerated calli (%) ^{ns}	No. of shoots formed	No. of shoot/ regenerated calli
	NAA	BAP				
NOB0	0	0	63	13 (20.6)	26	2
NOB2	0	2	63	19 (30.1)	38	2
NO.01B2	0.01	2	63	7 (11.1)	16	2.28
NO.1B2	0.1	2	63	15 (25.0)	29	1.93

^{ns}: Non significant.

Figure 1. Somatic embryogenesis in dwarf napiergrass. a) callus induction from shoot apical meristems in different induction media (a.1) 2 mgL⁻¹ 2,4-D and 0 mgL⁻¹ BAP; (a.2) 2 mgL⁻¹ 2,4-D and 0.01 mgL⁻¹ BAP; (a.3) 2 mgL⁻¹ 2,4-D and 0.1 mgL⁻¹ BAP and (a.4) 2 mgL⁻¹ 2,4-D and 0.5 mgL⁻¹ BAP. b) Developmental stages of embryogenic callus in MS medium containing 2 mgL⁻¹ 2,4-D and 0.5 mgL⁻¹ BAP with different concentrations of CuSO₄. (b.1-b.4) 0 μM CuSO₄ and (b.5-b.8) 50 μM CuSO₄; (b.1,b.5) 0 day; (b.2,b.6) 3 days; (b.3,b.7) 7 days and (b.4,b.8) 14 days. c) the regeneration stage of embryogenic calli on a medium containing 2 mgL⁻¹ 2,4-D, 0.5 mgL⁻¹ BAP and 50 μM CuSO₄. (c.1) mature embryo; (c.2) germinated embryo; (c.3) shoot germination with scutellum formation and (c.4) shoot elongation. (d.1) regenerated plants from embryogenic calli on MS medium containing 0.01 mgL⁻¹ NAA and 2 mgL⁻¹ BAP. (d.2) regenerated plants on rooting medium containing half-strength, hormone-free MS medium.

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P-01. THE EFFECT OF CONCENTRATE RATION BASED ON PALM KERNEL CAKE ON pH, VFA AND NH₃ IN *IN-VITRO* RUMEN

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Abstract

The aim of the research was conducted to determine the quality of concentrate ration based on palm kernel cake. The parameters measure were by rumen's fluid characteristics (pH, VFA and NH₃) according to *in-vitro* analysis. The matter experiment using palm kernel cake which be formulated in a concentrate ration with different composition of palm kernel cake (PKC), that is 40%, 50%, 60% and 70% PKC in design of Completely Randomized with 4 treatments and 5 replications. Substrate of concentrate ration will be evaluated through *in-vitro* analysis (Tilley and Terry, 1969). Variable measured was the characteristics of rumen's fluid including pH, VFA and NH₃ was 48 hours of incubation using Micro Diffusy Conway (NH₃) and destilation techniques (VFA). The results of experiment showed that the used for palm kernel cake in a concentrate ration produced the characteristic of rumen's liquid is same to standard, that pH : 6.57 – 6.84, NH₃ : 9.73 – 12.00 mg/100 ml and VFA : 85.00 – 11.25 mM. There was no significant difference (P>0.05) between treatments in NH₃ rumen fluid and significant difference (P<0.05) on pH and NH₃. From the whole parameter above, it can be concluded that the palm kernel cake can be used as feedstuffs and have big potential to replace some of conventional feeding.

Key words : Concentrate ration, palm kernel cake, rumen fluid, *in-vitro* analysis

INTRODUCTION

Palm plantation with the main product crude palm oil (CPO) is one of Indonesia's main export commodity. In 2012, Indonesia is the largest palm oil producer in the world with CPO production of 1 27 million tons / year, well ahead of Malaysia, as the country's second largest producer with production of 16.9 million tonnes (Wihardandi, 2012). The area of oil palm plantations in Indonesia in 2012 reached 11.5 million hectares and continues to increase with increasing average each year to reach 12% (Agriculture Departement, 2012). Improved land and palm oil production will increase the production of palm oil waste because from overall production of palm oil, 60% of which is waste / byproducts as a potential alternative feedstuff, one of which is palm kernel cake (PKC) (Matthew, 2004) .

Liwang (2003) states that each hectare of oil palm can produce 4 tons of CPO / year obtained from 16 tons of fresh fruit bunches (FFB) (Jalaluddin et al., 1991). Furthermore every 1 ton FFB produce 294 kg of palm oil sludge, 35 kg of palm kernel cake and 180 kg of fiber. Those, amount can be synchronized with 1.132 kg of palm oil sludge, 514 kg of palm kernel cake, 2681 kg and 3389 kg of fiber noticed palm bunches per hectare / year. Wan Zahari et al. (2003) adds that the produced by products derived from plants or derived from palm fruit are potential as animal feedstuff for ruminants. Utomo et al. (1999) further explained that one of the byproducts of the palm oil industry potential as animal feed that is Palm Kernel Cake (PKC).

General overview indicates that the content and quality of nutrients in particular palm oil byproducts is quite low due to high crude fiber , low

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digestibility and protein content. High crude fiber content on the palm by products causes the limited use of by-products as animal feedstuffs (Wan Zahari et al, 2003). Some researchers assert that PKC has been used widely in animal feed as a protein substitute to replace conventional protein sources at different levels (Onwudike, 2006). Loh et al (2002) reported that using of 15-25% PKC did not give significantly different effects on growth, consumption and animal weight. Furthermore, Orunmuyi et al (2006) stated that the PKC can be used to replace 30% of the corn and soybean meal rations without adverse effects on animal performance. Arief et al (2012) found that PKC can be used in the concentrate ration to 40% without affecting the production performance of etawah goats.

Palm kernel cake has a higher nutritional value than other waste of palm oil processing industry with 15% crude protein content and gross energy 4,230 kcal / kg so that it can act as an amplifier feed (Ketaren, 1996). African researchers also reported that the PKC can be used in livestock rations of sheep in Africa to 35% and give satisfactory results. As an additional feed to supplement energy and nutrients, molasses and corn was added (Ukanwoko and Ibeawuchi, 2009; Ahamefule et al, 2007).

Farm development in the future, especially ruminants, will depend heavily on the use of local resources-based feed ingredients for agricultural waste agro waste such as palm kernel cake. Therefore, the use of palm oil processing industry byproducts such as animal feed will have the dual benefit of improving the productivity of livestock and prevent environmental pollution.

The objectives of this research were to determine the quality of the concentrate ration based on palm kernel cake as measured through the rumen fluid characteristics (pH, VFA and NH₃) according to In-vitro analysis.

MATERIALS AND METHODS

In this research, Palm Kernel Cake (PKC) was formulated in concentrate ration which is then followed by in-vitro testing. Others feedstuff used were corn, rice bran, coconut cake and minerals. The research objective is to get the best formulation of PKC based concentrate ration as measured by rumen fluid characteristics (pH, VFA and NH₃) according to *In-vitro* analysis.

This experiment used completely randomized design (CRD) with 4 treatments concentrate ration formulation based on PKC with different levels with 5 replications. Composition and nutrient content of the ration treatments can be seen in Table 1:

Implementation of In-vitro studies was using the method of Tilley and Terry (1969). *In-vitro* studies conducted through the stages of preparation of Mc Dougalls solution, rumen fluid preparation and in-vitro evaluation. Fermentation is done using a shaker water bath at a temperature of 39°C and incubation carried out for 48 hours. After the incubation period is complete, the pH was measured using a pH meter which has been standardized with buffer solutions at pH 4 and 7, after which the samples are centrifuged with a speed of 1200 rpm for 30 minutes. Supernatant was taken and then analyzed for content of VFA and NH₃. VFA measurements made using steam distillation technique while NH₃ rumen contents were measured using the technique of micro diffusy Conway (Conway cup method).

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Table 1. Composition and Nutrients Contents of Ration

Feedstuff	Formulation of Ration Treatment (%)			
	A	B	C	D
Palm Kernel Cake	40	50	60	70
Corn	25	20	15	10
Rice bran	19	19	19	19
Coconut Meal	15	10	5	0
Mineral	1	1	1	1
Percent (%)	100.00	100.00	100.00	100.00
Nutrien Contents				
Crude Protein (%)				
FDN (%)	14.23	14.32	14.40	14.48
Crude Fat (%)	64.68	67.98	68.52	70.05
	3.35	3.77	3.50	3.17

Data were analyzed using analysis of variance (ANOVA) according to Steel and Torrie (1991) , while the differences between treatments were tested by Duncan 's Multiple Range Test (DMRT).

RESULTS AND DISCUSSION

Influence Treatment on Rumen Fluid Characteristics

The effect of rumen fluid characteristics can be seen in Table 2.

Table 2. Effects of Treatment on Rumen Fluid Characteristics

No.	Rumen Fluid Characteristics	Treatments			
		A	B	C	D
1.	pH	6.57 ^b	6.59 ^b	6.72 ^{ab}	6.84 ^a
2.	VFA (mM)	85.00 ^b	98.75 ^{ab}	98.75 ^{ab}	111.25 ^a
3.	NH3 (mg/100 ml)	10.31	9.73	11.03	12.00

Different superscrib showed significant differences ($P < 0.05$).

pH (degree of acidity) of Rumen Fluid

Degree of acidity of rumen fluid is very important to support the bioprocess of rumen microbes and its very influential on the rumen microbial population . The activity of selulolitic rumen bacteria will be blocked if rumen pH of fluid pH less than 6 , and his work will increase as the increase of pH above 6 . It means also that selulolitic bacteria has very important role in the process of feed digestion and is very sensitive to genuine transformation of pH rumen..

Results of statistical analysis of rumen pH in Table 2 above shows that the ration formulation produces a significant difference ($P > 0.05$) in the pH of rumen fluid and the pH produced is still in the normal range . According to Leng (1990) normal pH for microbial life and the sidelines of a good fermentation in the rumen ranged from 5.5 to 7.

The results of research indicate that rumen microbial activity in digestive system was uninterrupted feed and rumen microbe's can reform well feed digestion process will be interrupted if the pH of the rumen fluid is under 6 (Chanjula , 2004) . Further explained that at pH 5 and 6, the activity of rumen microbes to digest the feed will be hampered even stops while according to Orskov (1992) at a pH of less than 6.2 will inhibit significantly the growth of rumen microbes.

PH values obtained from the above studies indicate that rumen pH is good category for rumen microbial activity where the normal average rumen pH range between 6-7 (France and Siddon , 1993) whereas the ideal pH for fiber digestion is 6.4 - 6.8 . Suitability of pH can help and bacterial colonization and push activity of cellulolytic bacteria .

If associated with of VFA of rumen fluid, pH of rumen fluid was positively correlated with increasing VFA, in which increasing pH will in turn increasing VFA production . In other words, increasing the VFA production will rise about rumen fluid acidity . As described by Benchaar et al (2007) that there is a positive relationship between rumen pH and VFA . Thalib (2002) reported that the pH of the rumen fluid can be influenced by the content of VFA , NH₃ and lactic acid , the pH value of genuine transformation rumen fluid can be assumed as a result of the influence of VFA and NH₃ content . Further explained by Sugoro et al (2005) that the pH value proportional to the VFA and was influenced by rumen liquid ammonia which tends to increase pH if concentrations were high.

VFA Content of Rumen Fluid

Result of statistical analysis shows that the treatment gave significantly different ($P < 0.05$) in the rumen fluid VFA content in which treatment D was significantly ($P < 0.05$) higher than other treatments .

High concentrations of VFA on D treatment was caused by the increase fermentation due to the increasing of Total Digestible Nutrients (TDN) ration . As is known, TDN is the sum of Organic Materials (BO) feed that will serve as a good energy source energy for rumen microbial growth as well as energy for the body of livestock in the form of ATP (Tillman et al , 1998) . Furthermore , the content of organic material (BO) feed will be degraded into simple carbohydrate then suffered glycolysis into pyruvate acid which is then converted for both VFA . This is consistent with the statement of Soebarinoto et al (1991) that the final product of carbohydrate digestion and soluble starch is particularly source of VFA especially propionate . Further explained that the VFA formed is very much influenced by the type of feed (feed composition , comparative protein forage and concentrate feed) , the type of treatment (ground , pellets or heating) and feeding.

Results obtained in this study are also in line with the increasing availability of NH₃ in rumen fluid until the microbe 's activity can grow and with the end result which is the availability of VFA source of energy for microbes. The average result of VFA concentration on four treatment ration is 85.00 - 111.25 mM . According to Mc Donal et al (2002) the normal concentrations of total VFA in the rumen fluid optimal for microbial growth is 80-160 mM , whereas according to Preston and Leng (1989) the minimum amount of VFA in the rumen fluid for microbial survival is 50 mM . Mayangsari study (2011) using the unified feed with PK 13 % and 68 % TDN produce VFA between 122.25 - 158.75mM.

NH₃ Content of Rumen Fluid

Ammonia (NH₃) is the main nitrogen source used for the synthesis of microbial protein . NH₃ concentration in rumen fluid also determine the efficiency of microbial protein synthesis which will ultimately affect the outcome of fermentation of organic material feed in the form of volatile fatty acid (VFA) , which is the main source of animal energy for ruminants.

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Overall results of the research show that the availability of N- NH₃ in rumen fluid ranged between 9.73 - 12:00 mg/100 ml (non significant , P > 0.05), which is the normal condition of the availability of NH₃ in the rumen fluid and are at above the minimum concentration threshold required for the NH₃ growth and optimal bacterial activity which is 3:57 to 7:14 mM (Rahmadi et al , 2010) .

The relatively similar, NH₃ production between treatment was thought to be caused by relatively similar protein ration content. In the rumen , protein feed will hydrolysed by proteolytic enzymes of rumen's microbes which produced oligopeptida and will be further digested into peptides. Partial of hydrolysis products escaped degradation in the rumen and partly hydrolyzed into amino acids then be deaminationed into α keto acid and NH₃ (Widodo , F et al , 2012) . The more amount of NH₃ in rumen fluid showed the more easily degraded feed protein in the rumen . Increased availability of ammonia will balance nitrogen and good energy needed for rumen microbes growth .

According to Erwanto et al (1993) NH₃ concentration in rumen fluid also determine the efficiency of microbial protein synthesis which will ultimately affect the result of fermentation of organic material feed in a form of volatile fatty acid (VFA), which is the main source of energy for animal ruminants. Added by Winugroho (1999) that at concentrations above 12 mM of NH₃ , conversion process of NH₃ will be disturbed and if NH₃ was less than 4 mM (condition of low protein ration) degradation process will be disturbed

CONCLUSION

From the description above it can be concluded that palm kernel cake can be used as animal feed and has a great potential to replace conventional feed ingredients.

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P-02. INTEGRATED FARMING SYSTEM WITH EMPOWERMENT OF CATTLE FARMERS GROUP IN VILLAGE KINOMALIGAN

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Abstract

Farmer groups in villages such Kinomaligan cattle farmer groups Bulawan Jaya with the main program is farming rice and corn. In 2007, the group received help cattle by the government. The issue of land owned by the land has not been used as a forage planting fodder. Cattle still consume agricultural waste and low quality grass field. Based on these problems has been done for the IbM activities to empower members of the group. Empowerment has been done to increase revenue so that the welfare of the group members and their families increased. Cattle farming in the village Kinomaligan in general is still cultivated extensively. Cattle removed only in groups, grazing farms. This causes low productivity of cattle. The application of science and technology through IbM's cattle farmers group Bulawan Jaya I and Bulawan Jaya II have been successful. The products produced in the form of the land area of 0.3 ha planted with grass quality (dwarf), silage, amoniasi of rice straw and maize straw, compost and biogas. The advice given is necessary accompaniment by the universities to members of the group can be independent. If the application of science and technology can be carried out by members of the group continue the concept of integrated farming system can be implemented to the maximum.

Keywords: Empowerment, group, cattle

INTRODUCTION

Bolaang Mongondow is one of the districts in the province of North Sulawesi with a population of 223 485 inhabitants, has an area of 3506.24 km² which is divided into 12 districts and 152 villages (PEMDA Bolaang Mongondow, 2011). The agricultural sector is a source of livelihood Bolaang Mongondow society. Contributed by agriculture, plantation, animal husbandry, forestry and fishery of 52.08% at current prices.

Bolaang Mongondow as a food basis for the North Sulawesi province. It can be seen from some potential commodities that can be developed in an attempt to support the potential for increased food security in the province of North Sulawesi, which is illustrated by the achievements of commodity production. One of the favored commodity is rice.

Districts Dumoga West is one of the districts in Bolaang Mongondow potential for cattle development (Elly, 2008). In the District of West Dumoga (including Kinomaligan village) there are several farmer groups. Formation of the group is a government program based on joint decision of the Minister of Interior and Minister of Agriculture, No. 54 of 1996 and No. 304/KPTS/LP120/4/96, on Guidelines for the Implementation of Agricultural Extension. The program is expected to develop farmer groups in accordance with local conditions and potential resources, and considering strategic environment that influence (Department of Animal Husbandry, 1998).

In the village of Kinomaligan have formed farmer groups, ie groups of cattle farmers Bulawan Jaya who were divided into 2 groups on the initiative of

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members of the group and Team Faculty of Animal Husbandry Department of Social Economics UNSRAT. Group of Bulawan Jaya was originally formed in 2004 with the main program is paddy and maize fields. The group was formed at the initiative of 13 people and in 2005 by 10 members so that the number of members to 33 people. In 2006 the group "Bulawan Jaya" gets a flood that damage crops residents. Group nearly broke up because not bound by the rules are written. In 2007 the group became active again. Now the cattle belonging to the group "Bulawan Jaya" totaled 32 head and has obtained donations of some 18 heads of cattle. Managed land area of 15 hectares.

The issue of available land has not been used as a land for cattle development. Problems faced by cattle farmers is cattle business management is not as it should be. Cattle still consume agricultural waste and low quality grass field. This causes low productivity of cattle. To increase production and productivity of cattle depending on the feed consumed. Members of both groups have not been able to utilize the available land for planting forage quality. Dry land and belonging to the group can be integrated with planted forage crops.

Based on the above reasoning and problem then formulated the following priority issues: (i) lack of knowledge of the group concerning quality feed and are available continuously, thus needs to introduction of cattle food quality. (ii) lack of knowledge of the group on the use of rice straw and corn and forage preservation. (iii) lack of knowledge of the group members use cattle manure as fertilizer and biogas.

MATERIALS AND METHODS

The application of science and technology have been made since July to October 2012 at the cattle farming group Bulawan Jaya in Kinomaligan village of West Dumoga District. Application of science and technology that have been made in the form of empowerment for members of the group by using two methods: extension and training methods.

Extension for members of the group "BULAWAN JAYA" with the aim of changing the behavior of resource group members toward better (Pambudy, 1999). Some of the philosophy of extension is: (1) extension program relying on farmers' needs, (2) extension is basically the process of education for adults who are in formal. The goal is to teach farmers, improve their lives by his own efforts, as well as teaching farmers to use natural resources wisely, and (3) extension in collaboration with other organizations to develop individuals, groups and nations. Material presented is concerning cattle business management, the benefits of compost and biogas benefits. Having conducted illumination to members of the group, further training for the member of the cattle farmer group "BULAWAN JAYA". Training is a practical application of the technology is the introduction of a dwarf grass, silage making, manufacture of ammoniation, composting and biogas production.

RESULTS AND DISCUSSION

Cattle farming is one of the mainstay of rural households in improving their welfare. Cattle are genetic potential and has a high adaptability to the tropical environment. Cattle have a role to food sources (meat), as savings, sources of income, sources of labor, organic fertilizer sources and alternative energy sources (Elly, 2008., Elly et al, 2008).

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Cattle maintenance considerations by looking at its role in society in general increased revenue and earnings cattle farmer groups "Bulawan Jaya" in the village Kinomaligan in particular. Characteristics of the groups showed that 100% of the members were farmers.

Age group members influence the production process cattle farming. Age group members ranged from 30-52 years. The average age for members of the group "Bulawan Jaya I" is 42.36 years. The average age of members of the group "Bulawan Jaya II" 39,20 years. This condition indicates that the age of the group members still in the productive age. The indication that the group members are more productive in applying science and technology is being introduced. This is apparent at the extension that has been done over at 20.00 pm, was attended by members of the group to finish. According Kiswanto et al (2004), the higher the age of the farmer, to a certain extent, the ability to work will increase thus increasing productivity.

The level of education also affects the acceptance of the application of science and technology group members. Educational level of the lowest member of the group is the primary school level. Highest level of education is high school level. Education level group members "Bulawan Jaya I" for elementary schools and high schools each ranging from 27.27%, junior high school level is of the largest ranges 45.46%. Education level group members "Bulawan Jaya II" for elementary schools and high schools each ranging from 10.0%, junior high school level is the largest around 80%. This condition shows that the education level is still considered low for both members of the group. The indication in the training of some members of the group are inactive. This is supported by Kiswanto et al (2004) that the higher the level of formal education of farmers, the more rational mindset and the power of reason.

Number of cattle owned by members of the group at the time of application of science and technology as much as 62 tails. 28 tail belongs to member "Bulawan Jaya I" and 34 tails belong to members of "Bulawan Jaya II". Cattle belonging to the group are managed individually. This is as reported Fagi et al (2004) that in general members of the group of beef cattle farmers "Karang Endah" in Central Lampung managing individual cattle.

Cattle farming in the village Kinomaligan generally still cultivated extensively. This condition as proposed Bamualim et al (2004) that cattle generally maintained extensively. Cattle removed only in groups, grazing pastures, in rainfed areas and in other open land. Members of the group "Bulawan Jaya" also maintain the grazing cattle farms. This causes low productivity of cattle.

Feed is one of the critical elements of a successful cattle farming (Elly, 2008). Kardiyanto (2009) argues that in livestock farming, livestock require food substances that contain protein and energy. The low productivity of cattle belonging to members of the group caused by the feed consumed just a field of grass and agricultural waste. Based on this, it has made the introduction of forage, the dwarf grass. This introduction is very response by members of the group due to meet the feed requirements of cattle moved from one land to another farm. Planting grass on land owned by members of the group covering 0.3 ha. Type of grass that is planted dwarf grass (*Pennisetum purpureum* cv Mott). This grass quality is better than grass or king grass.

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Members of the group had been trained to preserve the grass, in the form of silage. The application of science and technology to anticipate when the grass is planted there is over production. Silage processed with utilizing local resources. Ingredients such as fresh grass (derived from BPTP Kalasey) and rice bran. Harvest fresh grass cut 2-5 cm, then put in an airtight plastic bag. Every 15 cm of fresh grass sprinkled with rice bran. Then, the grass filled up solid and firmly closed (tied plastic bag). The process for 21 days and after it opened to smell fragrant and slightly sour. It is also addressed by members of the group.

The main farming developed by members of the group are rice and corn. It is a show that wastes enough rice available but not utilized by the group members. According Djayanegara and Ismail (2004), the use of agricultural waste to feed to reduce reliance on land to feed, and the funding needs time to mengarit grass foreclosure. Based on this phenomenon, the waste rice and corn waste has been used as ammoniation. Members of the group have been trained to make ammoniation of rice straw and corn straw.

The process of making rice straw ammoniation dry (water content of about 60%) was cut into pieces 2-5 cm. Straw that has been cut into pieces stacked in a plastic bag, and then trampled to solid. Then the straw is stacked in a plastic bag sprinkled with probiotics (SB) and urea in the ratio of each 6 kg for every ton of rice straw. To develop probiotic then sprinkled water to 60% the moisture, which is indicated by the hand-wringing that has straw. When seen, the water in the palm of the hand as if it's about to drip. That is, the water will be enough. Method was repeated again with a pile of 15 cm to a plastic bag full. After a closed plastic bag and left tied up for 21 days in a protected area of rain and direct sunlight. After 21 days, fermented ready to be given to the cattle. The same process has been done for corn straw ammoniation.

Members of the group have been trained to utilize cattle feces as compost. The results showed that cattle manure is left scattered on farms. This can cause pollution if left lying cattle manure on farms and even in the streets of the garden. Cow manure that is left can lead to increased greenhouse gas emissions. To prevent the increase in the cattle manure compost is made.

Compost is an organic fertilizer derived from crop residues and cattle dung that has undergone a process of decomposition or weathering. Benefits of compost to improve soil fertility is owned by members of the group. According Salendu (2012), in the production process that integrates all existing waste utilized the principle of zero waste. In this case, there is no waste remains, the waste can be processed to generate income for farmers and their families. Composting has done is above the soil surface were originally made of container and bamboo beams measuring 150 x 100 x 90 cm.

Composting procedure is rice straw was dried stacked in the container. The number of rice straw as high as 15 cm in the container provided, then put the dried cattle feces while being trampled so solid. Then sown EM4 (starter bacteria) that has been mixed with water and sugar. So forth step is repeated until the container is full. Once the container is full, open beams and bamboo, then a pile of straw and cattle feces covered with a tarp and tied. A week later screened compost and reversal process is done every week for 4 weeks.

Group members are also trained utilizing waste of cattle as biogas. Biogas production has been done with the aim of preventing environmental pollution

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resulting from cattle feces. In addition, the price of kerosene is expensive and increasingly scarce, requiring the development and deployment of non-fuel energy technologies that are environmentally friendly (Elly, 2012). Furthermore, according to Elly (2012), the policy is primarily intended for rural households whose incomes are relatively low. One of the energy technologies that comply with these requirements is biogas technology. Fuel biogas from cow manure can substitute kerosene increasingly expensive and scarce.

Biogas is a renewable energy source that can address the need for energy, as well as can provide soil nutrient needs in a sustainable agricultural system. Utilization of cattle waste into biogas to support the application of the concept of zero waste to sustainable agriculture and eco-friendly can be achieved. Srisertpol et al (2010) suggested that biogas is a type of energy and sustainable development that are essential to energy and environmental planning.

Elly (2012) suggested that cattle waste produces methane gas (CH₄) is increasing greenhouse gas emissions (GHG). According to Masse et al (2003), methane is one of the greenhouse gases accumulate in the atmosphere due to human activities. This is why farms claimed to cause global warming.

Biogas production procedure by following these steps. The first member of the group set up a biogas reactor made of drums, which are designed in such a way as to accommodate the cattle feces. Two other drums are provided for the gas reservoir. Then the cattle manure mixed with water, a ratio of 1: 1, stirring until dissolved then inserted into biogas reactor. A large drum with a capacity of 200 liters is supplied to the gas container filled with water, the drum controller function as gas formation. Then, a small drum with a capacity of 120 liters filled into a large drum that had been filled with water. Four weeks later the gas was formed, the small drum will be lifted. Then the stove ignited and produces heat which is used for cooking. The more a mixture of cattle dung, it means a capacity greater biogas reactor will produce a bigger fire, hotter and longer used for cooking.

CONCLUSION

The application of science and technology through IBM in cattle farmer groups "Bulawan Jaya I" and "Bulawan Jaya II" has been successful. The products produced in the form of the land area of 0.3 hectares planted quality grass (dwarf), silage, ammoniation of rice straw and maize straw, compost and biogas. Suggestions submitted are necessary guidance by the College to members of the group can be independent. If the application of science and technology can be carried out by members of the group konttinyu the concept of integrated farming system can be implemented to the maximum.

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P-03. THE EFFECT BIOPROCESS OF BANANA PEELS WITH THE DIFFERENT OF INCUBATION LENGTH AND THE SOURCE OF LOCAL MICROORGANISMS (MOL) ON CRUDE PROTEIN, CRUDE FIBER AND LIGNIN CONTENT AS A FEEDING

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Abstract

Local micro-organism (Mol) is a potential sources of bacteria and fungi decomposer of organic matter. The liquid mol are indicated have many kind of microbe (bacteria, fungi). Mol will be wanted to be the same of function with commercial probiotic to increasing the nutrient content. This researched aimed to see The Effect Bioprocess of Banana Peels With The Different of incubation time and the source of Localmicroorganisms(MOL)on Crude Protein, Crude fiber and Lignin content as a Feeding. Factorial Randomized Complete design 2x3, with 3 replication each treatment. Factor A was incubation time (7 days , 14 days) and factor B was the kind of local micro-organism was used in this study. The results showed that there was no significantly effects among treatments on crude protein and crude fiber, but significant effect on contain of lignin.

Key words : local micro-organism, banana peels, feed.

INTRODUCTION

To resulting the high livestock production are required the nutrients for maintenance and a variety of production. The one of Factor that should be considered is amount of feed be given. The production will be increasing when they get the balance feeding. The important factor to improve the productivity of livestock when the feed ingredients are cheap, available, as well as and continuous quality.

Banana is the largest of horticultural production as compared to other fruits production in Indonesia. Banana production about 5,755,073 tones in Indonesia and about 48 443 tons on Jambi in 2010 (BPS Indonesia, 2011). Third part of banana whole is the peels (Sumarsih et al., 2009). So, It can be predicted the production of banana peels about 1,918,358 tones in Indonesia and 16,148 in Jambi.

Banana peels can be used as an energy source for livestock feed. The results of Kurniati's research (2011) the composition of the nutritional content of a banana peels were TDN 59.1%, 19.4% BK, PK 10.91%, 10.60% crude fiber, ash 24.10%, 34.5% BETN, fat 19.90%, 29.42% lignin and 3.81% silica. Base of that banana peel (*Musa brachyarpa*) is a potential material that can be used as animal feed ingredients but its use is still very limited, but it needs the treatment to reduce the lignin content, early.

The treatment of agricultural wastes can be done physically by cutting, steaming, and with a chemical treatment using lye to loosen the lignocelluloses, or with biological treatment using microorganisms like of fungi or bacteria, that are all aimed at improving the nutritional value of the feed material. Kurniati's research (2011) has conducted treatment to banana peel (*Musa brachyarpa*) with the steaming, ammoniating, and silage fermentation method. It could reduce the content of NDF, ADF, cellulose, hemicelluloses, lignin and silica. The best

treatment was fermentation methods. Local Microorganisms (MOL) is a solution, the result of fermented that –base on from various resources available . It was containing the bacteria and fungi with potential as a decomposer of organic matter. The advantage of MOL was easy to use because of cheap, and base on ingredients from fruits and vegetables wasted, animal waste or household waste, that easy in the manufacturing process and applicability. Because of It was important to study aimed to see the effect of bioprocess MOL on a banana peels on the content of crude protein (CP), crude fiber (CF) and Lignin as a feeding.

MATERIALS AND METHODES

Material of this research was using banana peels, slaughterhouse waste, and waste vegetable market. Procedures of research was conducted in 3 stages of :

Collecting the sample

Banana peel as research material be collected from industrial waste household, cutting and dried. In addition a small portion of the banana peel was be used as material to make a solution of Mol. Because of the sample base on banana peel, so it be expected the optimal resource of MOL will get from a banana peel.

Preparation as a Source of Mol

The source of Mol (rumen ingest, banana peels, vegetable waste) Materials required were :

- rumen ingest 5 kg
- banana peels 5kg
- vegetable waste 5 kg
- water of coconut 10 liter
- Sugar 1 kg (3x 1 kg)

Procedure :

Mol source material (rumen ingest, banana peels, vegetable waste), respectively until finely ground, then put into 3 different plastic buckets, then each ingredient was mixed with 5 liters of coconut water and 1 kg of sugar as a carbohydrate source that needs to living microorganisms. Bucket have to be sealed, so there was no possibility of incoming air (anaerobic fermentation). The top of bucket be holed in the middle and be connected to the bottle (which was sealed) containing the aquades using a small tube (diameter ± 1 cm). The aquades in the bottle serves to capture the gas produced during fermentation. (Figure 1.). Each source of Mol would be incubated for 10 days, and then the solution as a source of Mol were ready to be filtered and use to ferment banana peel.



Figure 1. The procedure made a source of local microorganisms (MOL)

fermentation of Banana skinwithMol

The Banana peel have dried , weighted @ 250 grams and put it to 18 bags. All of Samples and the equipment that will be used in the preparation of fermented have to be cleaned and be sterilized by autoclave for 30 minutes at a temperature of 110oC. The samples was cooled , then mix about 250 ml each Mol : 6 bags with a mol of rumen ingest, 6 bags with a banana peel and 6 bags with a solution of vegetable waste of Mol. All of samples were Incubation for 7 days and 14 days at different laminar that have been sterilized and sealed. It was ready to be analyzed.

The study was conducted experimentally by using Factorial Randomized complete Design of 2x3 with 3 replication each treatment (Steel and Terrie, 1991). Factor A was incubation length: A1= 7 days (1 week),A2 = 14 days (2 weeks). Factor B was the source of MOL: B1 = Rumen ingest,B2 = banana peels, B3 = Vegetable waste

The parameters were the content of : crude protein, crude fiber and lignin.

RESULTS AND DISCUSSION

The content of Dry matter (DM), Organic Matter (OM), Crude Protein (CP), Crude Fiber (CF) and lignin banana peels before treatment bioprocess with MOL can be seen in Table 1, and the average content of CP, CF and lignin after bioprocess with MOL are presented in Table 2.

Table 1. The content of Dry matter (DM), Organic Matter (OM), Crude Protein (CP), Crude Fiber (CF) and lignin banana peels before treatment bioprocess with MOL

The content	%
Dry matter	96,11
Organic Matter	81.92
Crude Protein	8.38
Crude Fiber	13.08
Lignin	25.68

Note: Laboratory of ruminant nutrition Animal Science, andalas University, 2012

The Results of analysis of variance showed that interaction between the incubation length with different sources of MOL showed that the effect was not significantly ($p > 0.05$) on the content of CP, CF of banana peel. The average content of CP banana peels were incubated 7 days showed the different effect with the average content of the CP have been incubation for 2 weeks (14 days). When doing the tested by Duncan's Multiple Rate Test (DMRT) that content of CP 9.69% (1 week) was not different with 11.96% (2 weeks). When the result of treatment be compared with the control, that showed an increase about 24.58% - 37.11% in average crude protein content of a banana peel after be fermented bioprocess with Mol.

During the fermentation could be cause reduce of carbohydrate and be followed by an increase in CP so It would be suspected that there was a change in proportion of organic matter. As long as the fermentation process happen an overhaul carbohydrate by the fungi, the first were easy overhaul such as cellulose and then followed by a poorly digested carbohydrates.

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Table 2. the average content of CP, CF and lignin after bioprocess with MOL (% DM)

Factor A (incubation length)	Factor B (source of MOL)			average	SE
	B1	B2	B3		
Crude Protein					
A1	9.76	10.29	9.04	9.69 ^A	0.81
A2	11.33	12.70	11.84	11.96 ^A	
Average	10.54	11.49	10.44		
Crude Fiber					
A1	13.81	13.06	11.43	12.77	1.1
A2	14.55	13.55	11.80	13.31	
Average	14.18	13.31	11.62		
Lignin					
A1	23.06 ^{Bb}	28.40 ^{aB}	22.73 ^{aA}	24.73	0.52
A2	20.28 ^{cA}	22.00 ^{aA}	24.86 ^{bB}	22.38	
Average	21.67	25.20	23.79		

Note: The average with be followed by the superscript (A, B) were different in columns and (a, b, c) and the as same as row indicate significantly different (p <0.05)

The data contained in Table 1, and Table 2, showed that the average content of CP increased after having bioprocess with MOL. Based on the above data illustrated that all of treatment bioprocess with MOL could increase the content of CP. Astuti's Research (2008) that the crude protein content of the passion hull about 29.37% after fermentation with *Aspergillus niger* and 42.62% after fermentation with *Trichoderma harzianum*. The fungi could increase the CP. Supriyati and Kompang (2002) conducted research on cassava peel with fermented and unfermented, that showed an increase in the content of the PK results of 4.8% to 28% and this increase was due to the contribution of proteins from fungi. Base on research of Asriyanti,(2011) that the identification of a solution Mol from banana weevil and get the identified *Bacillus sp.*, *Aeromonas sp.* and *Aspergillus niger*. Fermentation could increase the protein content because of the material the body of fungi contains 19-38% protein (Jamarun et al., 2000; Jamarun and Agustin, 1999; Jamarun and Nur, 1999; Nurhaita et al., 2012)

The results of this study show there was no interaction effect between incubation length and resources of MOL solution on contain of CF, but there was reduce (13.68% Vs 11.62) with the vegetable waste of mol when compared before treatment (control).

This was probably due to the MOL vegetables waste could overhaul the cellulose and hemicelluloses by cellulose enzymes that produced by microorganisms in the MOL solution. Moore and Lendecker (1996), microbial cellulose and hemicelluloses could overhaul the contained on the substrate to produce energy, so that the coarse fibers of the substrate to be down. Enari (1983) the source of breaker cellulose enzyme could be obtained from cellulolytic microbes such as fungi of the genus *Trichoderma* and *Aspergillus*

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There interaction was significantly effect ($P < 0.05$) between the incubation length with sources of MOL on lignin content of banana peel. Based on Table 2, the lowest (20:28%) lignin content were visible on the banana peels treated with MOL bioprocess with incubation as long as 14 days. When compared with the control decreased the lignin of 21,06% (controls 25.68%) with the rumen ingest of MOL, the highest with 7 days old. Based on that suspected the source of solution mol could produce enzymes that capable to changing the structure of lignin in lignocelluloses.

Nelson and Suparjo (2011), the amount of lignin content influenced fermentation, that could be loss of lignin content during fermentation ranged from 29.09 to 38.61%. Lignin is a component of plant cell walls that had been developed after the plants undergo a process of maturation. Banana peel as old plants, which have cell walls lignified advanced stage. Lignin of banana peel was high at around 29.42% (Kurniati, 2011). The average lignin content of banana peels before was 25.68% (Table 1).

A part of ligninolitik fungi didn't have the ability to use lignin as source of carbon and energy and depends on the easily digestible polysaccharides in the substrate (Maheshwari, 2005). The case in the processing of lignocelluloses materials by microorganisms was a loss of organic matter substrates utilized by microorganisms as a source of nutrients in the bioconversion process. Microorganisms are ideal in the bioconversion of lignocelluloses decompose lignin but low power degradation of the cellulose and hemicelluloses.

CONCLUSIONS

Banana peel that had bioprocess of MOL with different f incubation length and sources MOL showed no interaction ($p > 0.05$) on the content of CF, and CP, but there were different interactions significantly ($p < 0.05$) on lignin content. Mol base on rumen ingest with incubation of 14 days is the best treatment in this study. It needs Further research to identify the types of microbes present in the solution mol.

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P-04. THE CONTENT OF PHYTOCHEMICAL AND ANTIBACTERIAL ACTIVITY OF CINNAMON LEAF (*Cinnamomum burmanii*) AND NONI FRUIT AND LEAF (*Morinda citrifolia L*) MIXTURE EXTRACT TO REPLACE ANTIBIOTIC

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Abstract

This research was conducted to determine the best extraction method to produce cinnamon leaf (*Cinnamomum burmanii*) and noni fruit and leaf (*Morinda citrifolia L*) mixture extract as source of phytochemical compound to replace the role of antibiotic in broiler production. The mixture extract was named as 'Cinnamononi extract'. There were four different extraction methods in this experiments, i.e.: Type A: maceration extraction method with aquadestilic solvent, Type B: Maceration extraction method with metanolic solvent, Type C: Modified of Reflux extraction, and Type D: Combination of reflux and masseration extract. Two experiments were conducted to evaluate phytochemical of four types cinnamononi extract and to examine antibacterial activity in these extracts. The antibacterial activity of these exrcacts against *Escherichia Coli* and *Salmonella typhimurium* were determined using agar ditch diffusion method. The experiment 2 was designed as completely randomized design (CRD) with 5 times replication. There were 13 Treatments in this experiment, i.e.:T = antibiotic tetraciline 0.02 g, A1 = cinnamononi extract Type A without dilution (0,2 g), A2=cinnamononi extract Type A with dilution concentration1 g/ml, A3 = cinnamononi extract Type Awith dilution concentration 0,1 g/ml, B1 = cinnamononi extract Type B without dilution (0,2 g), B2=cinnamononi extract Type B with dilution concentration1 g/ml, B3 = cinnamononi extract Type Bwith dilution concentration0,1 g/ml, C1 = cinnamononi extract Type C without dilution (0,2 g), C2=cinnamononi extract Type C with dilution concentration1 g/ml, C3 = cinnamononi extract Type Cwith dilution concentration0,1 g/ml, D1 = cinnamononi extract Type D without dilution (0,2 g), D2=cinnamononi extract Type D with dilution concentration1 g/ml, D3 = cinnamononi extract Type Dwith dilution concentration0,1 g/ml. Variable in this experiment waszone of inhibition (clear zone) produced after incubation. The zone's diameter was measured in milimeter. In conclusion, cinnamononi extract type A and C had the best activity to inhibit *Escheria coli* bacterial, but only cinnamononi extract type C which have the best activity to inhibit *Salmonella sp*.

Key word: antibacterial activity, mixture extract, phytochemical content, *Cinnamomum burmanii*, *Morinda citrifolia L*.

INTRODUCTION

The shift in the public interest to consume food that was free from chemical additive (particularly antibiotics), causes organic broiler carcass (free from antibiotic residues) to be increased. Therefore, organic farm system was a promising future business (Yuniza dan Kusnadi, 2010). Organic farm system has not been widely applied by breeders in Indonesia. At the organic farm, the use of antibiotics should be avoided, so it needs to look for the replacement of the role of antibiotics and other additives that were safe for health. In this case, cinnamon and noni leaves could be an alternative to antibiotics, due to their rich and various

phytochemical content. They were useful as antibacterial and could boost immunity

Yuniza and Kusnadi (2010) reported that the use of mixture of cinnamon leaves, noni leaves, and natural grass 8% to 12 % in ration, besides can improve immunity but can also reduce abdominal fat, fat thigh meat and thigh cholesterol. However, the use of cinnamon and noni leaves has not showed an increase in weight and even tended to decrease the weight of the chicken (although not significantly). This was due to the use of the forage causes coarse fiber content of the ration to rise to chicken’s limit to receive coarse fiber. It was the existence of the high coarse fiber that causes the limited amount of the forage (not more than 8 % in ration).

The limited usage amount of cinnamon and noni leaves in ration causes the limited amount of phytochemical intake of the forage. Therefore, to make use the role of phytochemical of cinnamon and noni leaves to the fullest, extraction technique of the cinnamon and noni leaves mixture is needed, so that natural feed additive and feed supplement to replace the role of antibiotics and growth promoter can be obtained. These Cinnamon leaves and noni fruit and leaves mixture are called ‘Cinnamonini Extract’. In Extracting the mixture of cinnamon leaves and noni fruit and leaves, the solvent and method used determine the yield and phytochemical compound in the resulting extract.

Based on the explanation above, this research needs to be conducted in order to find the appropriate extraction method or technique so that phytochemicals needed of cinnamon leaves and noni fruit and leaves mixture were not damaged and reduced in number. In addition, the research also conducted to determine the ability of cinnamononi extract generated, as a substitute for the role of antibiotics in inhibiting pathogenetic bacteria.

MATERIAL AND METHOD

There were two experiments in this study that is an Experimental Phase 1 to determine the phytochemical contents of 4 types of cinnamononi extract produced by four different extraction method, and an Experimental Phase II to determine the antibacterial activity of these four cinnamononi extracts against *Escherichia coli* and *Salmonella sp.*

Material required in the Experimental Phase 1 were: fresh cinnamon leaves, fresh noni leaves, and fresh ripe noni fruit (yellow), methanol solvent, and aquadest. Experimental Phase II took DKMM extracts from Experimental Phase 1 and bacteria stock of *Escherichia coli* and *Salmonella sp*, alcohol 70 %, *Nutrient broth* (NB) flour, agar, and tetracyclin.

Reseach Design. Experimental Phase 1 was conducted to determine the phytochemical compound of these four cinnamononi extracts qualitatively. This qualitative test result will be expressed in negative statement or absent (-), weakly positive or low (+), positive or medium (++), strongly positive (+++), and very strongly positive or high (++++) . Experimental Phase II was conducted to test the antibacterial activity of the 4 types of cinnamononi extracts through Completely randomized design (RAL), with five replications. There were 13 treatments of the combination of 4 DKMM extracts with 3 dose levels used. The treatments were as follow: T = Tetracycline Antibiotics with 0,002 g dose, as a control.

A1= Cinnamononi Extract type A, without dilution

A2= Cinnamononi Extract type A, dilution concentration of 1 g/ml

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- A3= Cinnamononi Extract type A, dilution concentration of 0,1 g/ml
- B1= Cinnamononi Extract type B, without silution
- B2= Cinnamononi Extract type B, dilution concentration of 1 g/ml
- B3= Cinnamononi Extract type B, dilution concentration of 0,1 g/ml
- C1= Cinnamononi Extract type C, without dilution
- C2= Cinnamononi Extract type C, dilution concentration of 1 g/ml
- C3= Cinnamononi Extract type C, dilution concentration of 0,1 g/ml
- D1= Cinnamononi Extract type D, without dilution
- D2= Cinnamononi Extract type D, dilution concentration of 1 g/ml
- D3= Cinnamononi Extract type D, dilution concentration of 0,1 g/ml

The measured variable was the clear zone formed on the agar medium used as bacteria inhibiting zone.

The Making of Extract of Cinnamon Leaves and Noni Fruit and Leaves Mixture (Cinnamononi Extract)

There were 4 types of extraction methods to obtain the cinnamononi extract, they are: method A, B, C, and D. Methods A, B, and C took noni leaves and dried noni fruits, while method D took fresh raw materials. The ratio of cinnamon leaves and noni fruit used was 1 : 2 : 1 based on dry material.

Type A Method. Stratified Maceration Extraction Method by Aquadest Solvent. Dried *DKMM* mixture of 50 gram was dissolved in 250 aquadest (ratio 1:5), then it was let for 24 hours in shaker, it was filtered with filter paper (first extraction) afterward. The filtration residue was dissolved again by aquadest and second extraction was done. Filtrate obtained from the second extraction was heated with 50°C temperature to form a paste which is referred to as cinnamononi extract type A

Type B Method. Stratified Maceration Extraction Method by Methanol Solvent. Dried *DKMM* mixture of 50 gram was dissolved in 250 ml methanol (ratio 1:5), then it was let for 24 hours in shaker, afterward it was filtered with filter paper (first extraction). The filtration residue was re-dissolved by methanol and the second extraction was done. Filtrate obtain from the second extraction was evaporated to vaporize methanol with *rotary evaporator* 50°C machine with speed of 80 rpm. The paste form is referred as cinnamononi extract type B.

Type C Method. Modification of Reflux Extraction Method (distillation method). *DKMM* mixture of 50 gram was included in the distillation flask, then it was aquadest of 250 ml was added (1:5). Distillation process was conducted later for 3-3,5 hours to produce essential oil as an entrained component, and phytochemical compound of dried filtrate in an oven with 50°C temperature. The paste formed was referred to as cinnamononi extract type C.

Metode Type D. Combination of Maceration Extraction Method with Reflux Method between Cinnamon Leaves and Fresh Noni Fruit. 100 gram of fresh cinnamon leaves were included in the distillation flask, by then aquadest of 400 ml (1:4) was added. The distillation process, thereafter, took 3 – 3,5 hours to produce essential oil as an entrained component, and liquid which contain several phytochemical compound as the extract of cinnamon leaves. The next step was the extraction of noni leaves and fruits in the following ways: 485 gram of fresh noni leaves plus 432 gram of fresh noni fruits with filtrate of fresh cinnamon from

destillation process. They were macerated for 2 hours, then were filtered. The filtration residue was dissolved by aquadest of 100 ml, they were macerated again, and filtered. The filtrate of the first and second filtration were gathered and essential oil of the destillation of fresh cinnamon leaves. The filtrate was heated at 50°C to form paste which is referred to as cinnamononi extract type D.

Antibacterial Activity Test

Making Media for Bacteria. Liquid medium prepared was made of 8 g dissolved NB flour in 1 litre aquadest, then it was heated and stirring homogenously. Solid medium was made of 8 g of NB flour and 20 g agar dissolved in 1 litre aquadest, it was heated and stirring up homogenously. All media were sterlized in autoclave at temperature of 121 °C and pressure of 2 atm for 15 minutes.

Bacteria Regeneration. Bacteria stocks (*EscherichiaColi*, *Salmonella typhimurium*) on slant agar culture were taken in one loop and they were inoculated on erlenmeyer containing 10 ml of sterilized liquid medium. Erlenmeyer then incubated in shaker at 37 °C for 24 hours.

Determination of Antibacterial Activity Using Agar-Ditch Diffusion Method

Determination of bacteria *E. coli* activity took 20 petri dishes containing 20 ml agar media and bacterial inoculum *E. coli* with density of 10⁶ CFU/ml. Petri dishes then shook up until medium and inoculum were homogenous, and let them to be condense. After they bacame solid, a well with perforator in diameter of 5 mm. Each well were filled with cinnamononi extract of the four types of 20 µl with 3 concentration levels: without dilution (equivalent to 0,2 g), 1 g/ml (equivalent to 0,02 g), and 0,1 g/ml (equivalent to 0,002 g). As a positive control tetracycline of 0,002 g was used. Every treatment was repeated 5 times. It was, then, incubated at 37 °C for 24 hours and measured the inhibitory zone which indicates the antibacterial activity of *E. Coli*.The same ways were done on *Salmonella sp* bacterial test, by adding inoculum bacteria of *Salmonella sp* with density of 10⁶ CFU/ml on liquid medium.

RESULT AND DISCUSSION

Analysis of Phytochemical of Cinnamononi Extract

The use of solvent and different methods in extracting cinnamon and noni leaves mixture produce the following yield: extract A = 11,29%, extract B = 8,39%, extract C = 6,56 %, and extract D = 11,94%

Qualitative test result of these four cinnamononi extract showed that each of the extracts contain phenolic, alkaloids, saponins, and tripernoids compound, but with different levels, meanwhile the levels of flavonoids only exist in cinnamononi extract type A, C, and D(Table 1).

Flavonoid compound was not found in extract B. It indicated that flavonoid compound in cinnamon and noni leaves were flavone glycosides which tend to dissolve in polar solvent. The solvent used to obtain cinnamononi extract type B was methanhe ol with polarity lower than water, so that the flavone glycosides could be seen in extract A, C, and D which used aquadest a solvent and had higher polarity than that of water. Flavonoid compound was, generally, easy to dissolve in water, particularly in the form of its glycosides

Table 1. Phytochemical Compound of Four Types of Cinnamononi Extract

Compound	Types of Cinnamononi Extract			
	Type A	Type B	Type C	Type D
Phenolic	+++	++	+++	+++
Saponins	+	+	+	++
Alkaloids	++	+	+	++
Triterpenoids	+	+++	+	+
Flavonoids	++	-	++	++

Notes : (-) = negative or absent

(+) = weakly positive or low

(++) = positive or medium

(+++)= strongly positive or strong enough

Antimicrobial potential in Cinnamononi extract Type A, C, and D was determined by the combination of strong phenol content, medium alkaloids, and present of saponins and triterneoid. Meanwhile in Type B, it was prominently determined by the strong triterpenoid, and was supported by medium phenolic, less saponins and alkaloids contents.

Cinnamononi Extract Activity against Bacteria *E. coli* dan *Salmonella sp*

Measurement result of mean of inhibiting zone of the growth of bacteria *E. coli* and *Salmonella sp* of four cinnamononi extract types is presented in Table 2.

Table 2. Mean of Inhibition Zone Distribution of *E coli* and *Salmonella sp*

Treatment	<i>E coli</i> (mm)	<i>Salmonella sp</i> (mm)
T = Tetracycline, 0,002g,	21,2 ^{ef}	9,6 ^f
A1= Type A, without dilution	25,8 ^b	25,2 ^b
A2= Type A, 1 mg/ml	24,6 ^{bc}	24 ^b
A3= Type A, 0,1 mg/ml	13 ^g	8,8 ^f
B1= Type B, without dilution	23,2 ^{cde}	25 ^b
B2= Type B, 1 mg/ml	21,4 ^{def}	21,2 ^c
B3= Type B, 0,1 mg/ml	10,4 ^h	9,8 ^f
C1= Type C, without dilution	28,2 ^a	28,4 ^a
C2= Type C, 1 mg/ml	23,8 ^{bcd}	23,4 ^{bc}
C3= Type C, 0,1 mg/ml	11,8 ^{gh}	13,4 ^e
D1= Type D, without dilution	25,4 ^{bc}	25,4 ^b
D2= Type D, 1 mg/ml	19,2 ^f	20,6 ^d
D3= Type D, 0,1 mg/ml	11,2 ^{gh}	11,2 ^f

The result of variance analysis indicated that treatments were significant ($P < 0,01$) against diameter of inhibiting zone of *E. coli*, and *Salmonella sp*. After further DMRT test conducted, it could be seen that treatment A1, C1, and D1 as well as A2 and C2, gave significantly greater inhibiting zone of *E. coli* ($P < 0,05$) than of tetracycline, whereas treatments B1, B2 and D2 produced non-significant inhibiting zone ($P > 0,05$) with tetracycline. The data also showed that treatments A2 and C2, were able to match diameter of inhibiting zone of A1, B1, and D1. DMRT test showed that treatments A3, B3, C3, and D3 with a very low dose (dilution of 0,1 mg/ml) produced diameter of inhibiting zone significantly lower than ($P < 0,05$) than of tetracycline.

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The data indicated that by using lower dose, that is 1 mg/ml, the four types of cinnamononi extracts have been able to match and even exceeded (i.e. A2 dan C2) tetracycline ability in inhibiting the growth of *E. coli*, but their ability became lower if tetracycline dose is reduced to 0,1 mg/ml.

Against *Salmonella sp.*, DMRT test showed that treatments A1, A2, B1, B2, C1, C2, D1, D2, and C3 provided inhibiting zone of bacterial growth of *Salmonella sp* which were significantly greater than tetracycline, whereas treatments A3, B3 and D3 produced nonsignificantly inhibiting ($P>0,05$) with tetracycline. There was no treatment produced inhibiting zone diameter of *Salmonella sp* lower than tetracycline. DMRT results showed that cinnamononi extract given with a very low dose, that is 0,1 mg/ml, were able to match and even exceeded (i.e. C3) tetracycline ability in inhibiting the growth of *Salmonella sp.*

Antibacterial activity of cinnamononi extract types A, C, and D was due to its high phenolic and was supported by the present of alkaloids, saponins, and flavonoids, whereas type B antibacterial potential given by the high content of terpenoid. The role of phenol as an antibacterial was by denaturing bacterial protein through absorption involving hydrogen bonds. At the high level, phenol causes protein coagulation and membrane cells undergo lysis, thereby change the permeability of the bacterial membrane (Siswandono and Soekardjo, 2000). Antibacterial properties of phenol also were also investigated by Pambayun, Gardjito, Sudarmadji, dan Kuswanto (2007) which stated that the phenolic compound in the gambier extract play in inhibiting the growth of *S. aureus* and *B.*

High level of terpenoid in cinnamononi extract type B gave antibacterial potential on the extract. Terpenoids can be an antibacterial by damaging bacterial cell membrane (Cowan, 1999). Phytadiene terpenoids and 1,2 seco-cladiellans containing in stonebreaker herb (*Phyllanthus niruri* Linn) proved to be active against *S. aureus* and *E. coli* (Gunawan, Bawa, Sutrisnayanti, 2008). Kaurenoic acid terpenoids of *Pseudognaphalium vira vira* can damage cell membranes of *S. aureus* through hydrogen bonds kurenoic acid carboxylic group with phosphoryloxygenatom of cell membrane i.e. $\text{CO}_2\text{H}-\text{O}=\text{P}$ with a distance of 1,91Å (Urzúa, Rezende, Mascayano, and Vásquez, 2008).

CONCLUSION

Based on the data, it can be inferred that cinnamononi extract type A, B, C, and D has the ability to inhibit the growth of *E. coli* dan *Salmonella sp.* Cinnamononi extracts with the most excellent antibacterial potential to inhibit *E. coli* were type A (macerated and stratified with water solvent), and Type C (modification of reflux extraction method), but the most excellent antibacterial potential to inhibit *Salmonella sp.* was type C.

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P-05. THE EFFECT OF SUPPLEMENTATION LEUCAENA LEUCOCEPHALA BASED ON AMONIATED RICE STRAW RATIO ON IN-VITRO RUMEN CHARACTERISTICS.

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Abstract

Rice straw has been used as feed for ruminant in many of Asian countries. They have low in nutritive value and digestibility. This experiment aim to study to improve the utilization of ammoniated rice straw supplemented with lamtoro (*Leucaena leucocephala*) as source of protein and protein by-pass. The parameters measure were *in-vitro* rumen characteristics and digestibility. Rice straw was previously ammoniated with 3% urea. This experiment was arranged in Completely Randomized Design with 5 treatments and 4 replications and differences among treatment means were examined using a Duncan multiple range test. The treatments consist of 50% Ammoniated Rice Straw + 50% Concentrate as a control diet (A), B = A + 5% Lamtoro, C = A + 10 % Lamtoro, D = A + 15 % Lamtoro and E = A + 20 % Lamtoro. Variables measured were rumen characteristics : pH, Ammonia (NH₃) and Volatile Fatty Acid (VFA) as fermentability indicators, and *In-Vitro* Dry Matter digestibility (IVDMD) and *In-Vitro* Organic Matter Digestibility (IVOMD) as degradability indicators, The results of this research indicated that fermentability and digestibility of treatment D and E were significantly higher than treatments A, B, and C on *in-vitro* rumen characteristics (pH, VFA and N-NH₃) and IVDMD and IVOMD, but no significantly different between treatment D and E. Supplementation of Lamtoro (*Leucaena leucocephala*) were able to improve fermentability and digestibility of ammoniated rice straw. It can be concluded that using ammoniated rice straw as based ration in ruminant feed must be supplemented with Lamtoro (*Leucaena leucocephala*) to improve its digestibility, fermentability and utilization.

Key Words: Ammoniated rice straw, *leucaena leucocephala*, *in-vitro* rumen digestibility

INTRODUCTION

Rice straw is agricultural by-products that have poor nutritional value because of their low nitrogen and high fiber content (Tang *et al*, 1995). Low productivity rates of animal production are observed in cattle with use this diet in tropical areas such of Indonesia. Therefore, the objective of our experiment was to determine the effect of supplementation of lamtoro (*leucaena leucocephala*) on digestibility, fermentability and utilization of rice straw as feed of animal. Siregar and Prawiradiputra (1978) stated that lamtoro has been used as animal feed for a long time due to high in protein content and source of protein by pass. Bamualim (1985) in his research indicated that 60 % of lamtoro has degraded in rumen and 40 % of dried lamtoro has been fed to goat and sheep as source of protein died. The results of Herawati *et al*, 2013 experiment indicated that supplementation of

Saccharomyces cerevisiae and *leucaena leucocephala* have improve fiber digestibility and animal production.

METHODS AND MATERIALS

Rice straw as main source of feed was previously ammoniated with 3% urea. The treatments consist of 50% Ammoniated Rice Straw + 50% Concentrate as a control diet (A), B = A + 5% Lamtoro, C = A + 10 % Lamtoro, D = A + 15 % Lamtoro and E = A + 20 % Lamtoro. This experiment was arranged in a Completely Randomized Design with 5 treatments and 4 replications and differences among treatment means were examined using a Duncan multiple range test. Variables measured were rumen characteristics : pH, Ammonia (NH₃-N) and Volatile Fatty Acid (VFA) as fermentability indicators, and *In-Vitro* Dry Matter digestibility (IVDMD) and *In-Vitro* Organic Matter Digestibility (IVOMD). *In vitro* fermentability and degradability of nutrients were determined following the first stage of the Tilley and Terry procedure (1969). Rumen fluid was obtained from a slaughter house. Fermentation tubes contained of 10 ml of ruminal fluid and 40 ml of McDougall buffer solution. Samples were incubated in duplicate in 100 ml polyethylene tubes in 39 °C in a shaken water bath for 48 h. Two fermentation tubes that did not contain diets were also incubated and used as blanks. Fermentation was terminated at 48 h by injecting the tubes with 1 ml of HgCl₂. Tubes were then centrifuged at 2000 x g for 15 min and the supernatant was removed. Tubes with residue were dried at 60 °C for 48 h and weighed and the data were used for degradability determination. These residues were also analyzed for its DM, and OM by using standard procedures (AOAC, 2007 and Goering and van Soest, 1970)). Supernatants were used in order to determine NH₃-N concentration (microdiffusion Conway method), total VFA concentration (Gas chromatography) and rumen fluid pH. Data were analyzed by ANOVA using the GLM procedure and differences between the control treatment and lamtoro supplementation treatment were analyzed by Duncan multiple range test (DMRT) (Steel and Torrie, 1981).

RESULTS AND DISCUSSIONS

Table 1 shows results of *Leucaena leucocephala* supplementation on fermentation in the rumen and in vitro dry matter and organic matter degradability of feeds.

Results of supplementation of Lamtoro on rice straw ammoniated on pH, VFA and NH₃-N IVDMD and IVOMD can be shown in table 1. The anova indicated that supplementation of lamtoro has significantly different (P<0,01) on NH₃-NH₃, VFA, IVDMD and IVOMD and non significantly different (P>0,01) on rumen pH.

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Table 1. Effect of *Leucaena leucocephala* supplementation on fermentation in the rumen and *in-vitro* dry matter and organic matter digestibility.

Variables	Treatments				
	A (0)	B (5)	C (10)	D (15)	E (20)
pH	6,81 ^a	6,80 ^a	6,84 ^a	6,79 ^a	6,84 ^a
Total VFA (mg/100ml)	122,25 ^A	126,00 ^A	129,25 ^A	138,75 ^B	143,24 ^B
N-NH ₃ (mM)	23,91 ^A	32,83 ^B	35,46 ^B	24,99 ^A	25,33 ^A
IVDMD (%)	50,52 ^{aA}	61,06 ^{bB}	63,75 ^{cC}	65,04 ^{dC}	65,59 ^{cdC}
IVOMD (%)	53,35 ^{aA}	62,80 ^{bB}	63,80 ^{bBC}	65,03 ^{cC}	65,11 ^{cC}

Notes: Superscript in capital letter within rows are highly significantly different ($P < 0,01$) and in normal letter are significantly different ($P < 0,05$) among treatment means.

Lamtoro supplementation were not significantly effect ($P > 0,05$) on pH rumen fluid. Ph condition are normal vary from 6,79 – 6,84, as mean that lamtoro supplementation could give normal condition of rumen for microbial growth as mention by Church (1988) and Leng (1991). Lamtoro supplementation were highly significantly effect ($P < 0,01$) on VFA production in rumen. Increased lamtoro supplementation from 0% to 20% affected on VFA production from 122,25 to 143,25 mg/100ml. Ensminger (1990) stated that microbes enzyme will digest carbohydrate to VFA for source of energy by animals. According to Van Soest (1982), the level of VFA for growth of rumen microorganisms were from 80–160 mg/100ml. Lamtoro supplementation were highly significantly effect ($P < 0,01$) on rumen N-NH₃. The values of N-NH₃ at lamtoro treatments from 5%, 10% were 32,82 and 35,46 mM that significantly higher than treatments of 15% and 20% were 24,99 and 25,33 mM. The low level of N-NH₃ at level lamtoro supplementation of 15% and 20% caused by tannin content in lamtoro that protect digestible protein in the rumen. Leng (1991) stated that protein protection can be occur due to tannin content in lamtoro. According to McDonald *et al* (2002) level N-NH₃ optimum for rumen microorganisms activities were 20-300 mM,

Lamtoro supplementation on basic feed of rice straw highly significantly effect ($P < 0,01$) on *in-vitro* dry matter and organic matter digestibility. Supplementation of lamtoro increased dry matter digestibility from 50,52% to 65,59% and organic matter digestibility from 53,35% to 65,11%. Crampton dan Harris (1969) stated that nutrients digestibility of feed depend on rumen microbes activities that affected by pH and availability of nutrients in feeds. Supplementation of lamtoro 15% (treatment D) and 20% (treatment E) were not significantly different ($P > 0,05$) between treatments means on dry matter and organic matter digestibility due level of tannin. Makkar (2003) stated that tannin can reduce digestibility and utilization of feeds.

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P-06. DRY MATTER AND ORGANIC MATTER DIGESTIBILITY OF JAVA WOOD (*Lannea Coramandelic*) LEAVES BY USE IN SACCO

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Abstract

The aims of this research is to determine the dry and organic matter digestibility of java wood (*Lannea coramandelic*) leaves by used in sacco methods. The materials were used fresh leaves and dry leaves. The data observed were dry matter and organic matter digestibility. The procedure applied was by taken 2 g of each sample of leaves and put into nylon bags and incubated in the rumen of PO cattle with fistula. The samples was used 5 replicates. The nylon bags were incubated for 2, 4, 8, 12, 16, 24 and 48 hours. The results of in sacco methods was found dry matter digestibility of 60.06% in fresh samples and 43.80% in dry samples. While the results of organics matter digestibility was identified of 59.12% in fresh samples and 44.17% in dry samples. Characteristics value of fresh samples were 13.90%, 64.91%, and 0.12% per hour respectively for fractions a, b and c, and the value of the dried samples were 29.15%, 33, 17%, and 0.003% per hour respectively for fractions a, b and c. Results of statistical analysis was shown significant differences ($P < 0.05$) of dry matter and organics matter digestibility in fresh and dry leaves samples. Conclusion from this research was found the fresh of java wood (*L. coramandelic*) leaves showed more high digestibility than the dried samples by used in sacco methods.

Keywords: *Lannea coramandelic* leaves, dry matter, organic matter, in sacco

INTRODUCTION

Central Sulawesi, in particular Palu City is one of Indonesian provinces that has extreme climatic conditions (high temperature and low rainfall) especially in the dry season, so the shortage of feed ingredients is a major problem for the development of animal husbandry, especially for the supply of forage. Additional feed that should be used during the dry season in general have limitations in the number and quality. This can be seen in the low appearance of livestock. Various measures that can be implemented among other things, by utilizing forage source that the availability is relatively continuous during the dry season, for example, java wood (*L. coramandelic*) leaves, as an alternative feed ingredient that is relatively inexpensive, easy to obtain with good nutrition. However, this has not been done a lot considering study on java wood leaves especially about the level of degradation has not been much published, which resulted in the sheep farmers were still hesitant to use it. Based on the study results of Lowry *et al.* (1992) in (Amar, 2002) that the crude protein of *L. coramandelic* leaves was 11%, while according to Arief *et al.* (2008) was 4.84% with 50.95% crude fiber content. There

is a difference in the crude protein content as a result of the differences of the methods used to analyze, of the samples taken, and also of the location.

This experiment aims to determine the values of dry matter (DM) and organic matter (OM) digestibilities of java wood (*L.coramandelic*) leaves by using in sacco method, so the results of this study are expected as information material for use as an alternative animal feed, especially in the dry season.

MATERIALS AND METHODS

This experiment was conducted to determine the values of DM and OM digestibility *in-sacco* in the experiment cage and the chemical analyses were conducted in the Nutrition and Animal Feed Laboratory. Feed ingredients used in the experiment were java wood (*L. coramandelic*) leaves which grows wild in Mantikulore Village, Tondo Subdistrict of Palu City, Central Sulawesi.

Sample preparation

The preparation was done by following the instructions of Soejono *et al.* (2000), the used foliage was java wood (*L. coramandelic*) leaves, the taken parts as edible portions were the leaves, leaf shafts and stem parts that can be still chewed by livestock (goats). Fresh foliage samples (FJW) and sun dried foliage (SDJW) (for a day) were dried using oven 55°C, then the samples were milled using a Wiley mill with a 3 mm diameter sieve. Nylon / polyester bags incubated in the rumen have porosity 46 µm, size 6 × 11 cm and on their three sides were glued by using heaters, labeled / marked according to the livestock, incubation time and repetition for rumen incubation. Then the bags were oven-dried at temperatures 55°C until their weights constant and the empty bags were weighed. Then, 5 g sample was put into a bag for rumen incubation and the bag closed with a rubber.

Incubation period

In this experiment, the sample is put into the rumen for incubation for 2, 4, 8, 12, 16, 24, and 48 hours. Incubation period of 0 hour means that the sample is not put into the rumen, but only to be washed with a washing machine for five minutes to remove dissolved particles, then dried at 60 ° C for 18-24 hours. Taking the bag from the rumen, washing and drying are done by: (1). Remove the nylon bag with rope from the rumen and put it into a bucket of warm water to keep the fermentation and to wash food particles that are outside of the bag, (2). Remove the nylon bag from the strap with scissors; (3). Wash the nylon bag with water (washing machine) until the water is clear; (4). Dry the nylon bag at 60 ° C for 18-24 hours or until the weight is constant; (5). Weigh the nylon bag with feed material, record the weight of sample (*c* g). Put the obtained data of degradation values of DM and OM nutrients into the equation: $p = a + b(1 - e^{-ct})$ with *p* as the cumulative amount degraded at time *t*, *a* is the fraction that is rapidly dissolved / degraded (%), *b* is the fraction that is potentially degraded in rumen (%), *c* is the

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degradation rate of fraction b (% / h), and t is the time (hours). Data for degradation in rumen is tested by test.

RESULTS AND DISCUSSION

Degradation Value of Dry Matter

The average value of the fraction a, b, and c, and the DT value of fresh java wood (FJW) and of sun-dried java wood (SDJW) in sacco are presented in Table 1.

Based on the results of T-test showed that there were significant differences in DT value of DM between fresh java wood and sun-dried java wood ($P < 0,05$). The DT value of dry matter of fresh java wood was higher than that of DM and OM of sun-dried java wood, i.e. 60.06% and 43.80% and DT OM 59.12% and 44.17% (Table 1).

Table 1. Degradation parameter values of java wood (*Lannea coramandelica*) DM *in-sacco*.

Treatment	Degradation parameter			DT (%)
	a (%)	b (%)	c (%)	
Fresh Java Wood	13,90	64,91	0,12	60,06 ^a
Sun-DriedJava Wood	29,15	33,17	0,003	43,80 ^b

b, Different superscripts in the same column indicate that there are significant differences ($P < 0.05$)

It is visible that the degradation level of java wood leaves in the fresh form is better used as ruminant feed ingredients than in the dry form. It is also visible on the parts that can be degraded potentially, ie for fresh java wood and sun-dried Java wood 64.91% and 33.17% respectively. In terms of the degradation rate, digestibility value of fresh java wood (FJW) leaves ($c = 0.12$) is higher than that of sun-dried java wood leaves ($c = 0.003\%$). The height of dry matter digestibility that occurs in fresh java wood leaves is also expected that the high absorption of nutrients occurs in post-rumen compared with dry java wood. According to Orskov (1980), one of the factors that affects degradation characteristics are physical properties of the feed ingredients and the rumen environment. The properties in question include the solubility of feed ingredients, feed outflow rate or digestion in the rumen, the level of consumption, the availability of fermentation substrate, microbial population, particle size, physical shape, and rumen pH. Drying treatment is one of the physical property factors that can cause a decrease of soluble carbohydrate content and increase dry matter of the other components (Norton and Ahn, 1997), while Rusdi et al. (2012) reported that the process of drying leaf turi can reduce degradation in the rumen, affect the degradation characteristics but furthermore it is expected to increase the protein

components that escape from degradation, so that more protein is available in the small intestine (post-rumen).

Degradation of organic matter

Based on the T-test results of DT OM values indicate significant differences ($P < 0.05$) (Table 2). The value of soluble fraction (a) and potentially degradable fraction (b) and the rate of potentially degradable fraction (c) and DT of *L. coramandelic*a leaves both in the form of fresh and sun-dried are presented in Table 2.

Table 2. Degradation parameter values of java wood (*Lannea coramandelic*a) OM *in-sacco*.

Treatment	Degradation parameter			DT (%)
	a (%)	b (%)	c (%)	
Fresh Java Wood	13,89	64,85	0,12	59, 12 ^a
Sun-Dried Java Wood	29,14	33,15	0,003	44,17 ^b

a, b, superscripts in the same column indicate that there are significant differences ($P < 0.05$)

Judging from the DT values are visible that there are value differences of a, b, c, and DT between the two treatments that can be caused by differences in the availability of nutrients. Thus, it is visible that the percentage of OM loss in feed ingredients of *L. coramandelic*a in fresh form is always higher than that of foliage feed ingredients of *L. coramandelic*a in the dry form. The long retention time in the rumen will result in increased contact time between the feed with rumen microbes, it will allow greater rumen microbial activities to degrade feed. Linkages of the two can minimize the value of the degradation rate of potentially degradable fraction. The differences of potentially soluble fraction and degradation rate of potentially degradable fraction are influenced by the nutrient composition of the feed, the retention time length of feed in the rumen and also the availability of substrate for microbial activity to degrade the feed in the rumen. Greenery of *L. coramandelic*a is a tree plant that has a content of strong enough lignin and can bind to cellulose and hemicellulose. The parts which have woody tissue of the plant such as corncob, hard skin, seeds, parts of coarse fibers, roots, stems, and leaves contain a complex substance that can not be digested and called lignin. On young plants this matrix layer is composed of cellulose and hemicellulose, but on old plants the matrix is coated by lignin and other polysaccharides (Hartadi et al., 2008). This is consistent with the statement of Thomaszewska *et al.* (1993) that nutrient compositions both between species and within the same variety of plants have content of cell wall proportions (cellulose, hemicellulose, and lignin) that are different. Maximum degradation (a + b) of 78.81% is still higher than that of *L. coramandelic*a in the dry form that is 62.32%. Although it is still lower than the study results of Hadi *et al.* (2011) that

the (a + b) value of type of the studied trees in the form of legume has the maximum degradation rate at 77, 86% for gamal and 88.34% for white turi. These results indicate that the maximum degradation of *L.coramandelica* leaves in dry form has not occurred yet because it takes time > 48 hours for the maximum degradation.

CONCLUSION

Based on the results and discussion, it is concluded that the DT value in sacco of dry matter in fresh java wood (FJW) 60.06% and in sun-dried java wood (SDJW) 43.80% with the degradation characteristic values of fresh java wood are 13.90%, 64.91%, and 0.12% per hour respectively for fractions a, b and c, while the value of the sun-dried leaves of java wood 29.15%, 33.17%, and 0.003% per hour respectively for fractions a, b and c. While the DT OM of FJW is 59.12% and of SDJW 44.17%, with the degradation characteristic values of fresh java wood are 13.89%, 64.85%, and 0.12% per hour respectively for fractions a, b and c, while the value of the sun-dried leaves of java wood 29.14%, 33.15%, and 0.003% per hour respectively for fractions a, b and c. The statistical test results show that there are significant differences in both DMD and OMD ($P < 0.05$) between the FJW and SDJW. Concluded that the rates of both degradation and analyzability of dry matter and organic matter of fresh java wood leaves are higher than those of sun-dried java wood leaves.

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P-07.IMPROVING THE QUALITY OF SOYBEAN MILK WASTE THROUGH FERMENTATION BY *NEUROSPORA CRASSA* AS POULTRY RATION

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Abstract

The aim of this research was to improve the quality of soybean milk waste (SMW) through fermentation by combination with various substrat composition, standard mineral and humic acid. The experiment used complete randomized design (CRD) with seven treatments and tree replications. The Treatments were (1) 100% SMW, (2) 70% SMW, + 30 % rice brand (3) 70% SMW, + 30% rice brand + standart mineral (4) 70% SMW, + 30 % rice brand + humic acid, (5) 70 SMW, + 30% corn (6) 70% SMW, + 30% corn + standart mineral dan (7) 70% SMW, + 30% corn + humic acid. The parameters were dry matter, crude protein, crude fiber and crude lipid of soybean milk waste fermented by *Neurospora crassa*. The results of study showed that there were highly significant ($P < 0,01$) affect to dry matter, crude protein, crude fiber and crude lipid of soybean milk waste fermented by *Neurospora crassa*. The conclusion that soybean milk waste which was fermented by *Neurospora crassa* with composition 70% SMW + 30% rice brand + humic acid 600ppm had better nutrient content. This condition can be seen in dry matter 85,92%, crude protein 32,64%, crude fiber 14,88% and crude lipid 9,29.

Keywords: Fermentation, *Neurospora crassa*, Soybean milk waste, Poultry, Ration

INTRODUCTION

One of the waste that can be used in poultry rations is soybean milk waste. The availability of soybean milk waste at the moment is very high along with the proliferation of home industrial soybean milk production due to high public awareness of healthy living. Besides, people has already know the benefits of soybean milk waste contains high protein and isoflavones that can lower blood cholesterol levels (Koswara, 2006). Along with the increasing demand of soybean milk in the form of waste is also increasing, if it is not utilized it will have an impact on the environment, so it should be used as a source of animal feed especially for poultry feed.

Nutritional content of soymilk waste was quite high with 27.62% crude protein, 2.95% crude lipid, 13.81% crude fiber and 2.96% ash, 0.09% Ca, 0.04% P. But it is utilization is still low with only 6.2% in broiler rations (Mirnawati, 2012). This is due to the low palatay and quality which can be seen from the low nitrogen retention (40%). Because of that, there are needed to have a treatment by fermentation so that the benefits can be increased.

Based on previous studies, soybean milk waste fermented by *Rhizopus oligosporus* give better results compared to than the mold *Penicillium* and *A. niger* viewing from its highest crude protein (34.76%), dry matter (81.18%), crude fiber (14.91%) and nitrogen retention (58,69%) content and the high amino acid content before fermentation. But the use of it in broiler rations can only replace up to 75% soybean meal (Muis, 2010).

Therefore in this study, the mold *Neurospora crassa* was used which has advantages compared to other fungi for its complete enzyme activity such as the enzyme amylase, protease and lipase, and others, for its high content of β -carotene

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(Saono and Budiman, 1981) . This fungus is easily spread and multiply quickly so that fermentation by *Neurospora sp* can increase nutrients and produce karatenoid pigments that act as pro-vitamin A. The results found by Nuraini *et al.* (2006) is with fermented feed (*Neurospora crassa*) β -carotene rich as much as 80.00 mg / kg in the diet can lower cholesterol eggs as much as 33%. Furthermore Nuraini *et al.* (2008) explained feeding of β -carotene-rich fermentation as much as 95.09 mg/ kg in the ration can lower cholesterol eggs by 43%

Based on the above framework it is necessary to do a research to improve the quality of soy bean milk waste by using a *Neurospora crassa* to yield a product for poultry feed ingredients that can lower the cholesterol content of poultry meat and eggs. In fermentation need additional minerals such as Brooketaltoenhancemicrobial growth. usedinthis studybecause it also contains humic acid minerals such micro Cu, Zn and Mn (Tan 1998). The addition of humic acid also provides the component such as N, P and S into land and energy for microorganism activity (Stevonson, 1994). So the processing PKC has better quality.

Biside that substrat composition was really needed by microorganism for developmental and growth. Microbe will be growth well in a substrat that enough energy and nutrient for its growth. So this research will be to improve the quality of soybean milk waste (SMW) throughfermentationby combination with various substrat compositision, mineral and humic acid.

MATERIALS AND METHODS

This experiment aims to determine the optimum composition of the substrate that can improve the quality of soybean milk waste (SMW), with the addition of standard mineral and humic acid. This experiment was conducted with the experimental method. The design used was a completely randomized design (CRD) with 7 treatments and 3 replications (Steel and Torrie, 1991). Treatment is the composition of the substrate, namely: (A) 100% ASK, (B) 70% ASK+30% rice bran, (C) 70% ASK+30% rice bran+mineral standard (D) 70% ASK+30% rice bran+ Humic Acid, (E) 70% ASK +30% corn (F) 70% ASK +30% corn + standard mineral and(G) 70% ASK +30% corn + humic acid. The variables measured were: dry matter, crude protein, crude fiber and crude lipid fermented by *Neurospora crassa* of soybean milk waste.

RESULTS AND DISCUSSION

The effect of substrat composition withthe addition ofstandard mineral and humic acid on dry matter, crude protein, crude fiber, crude lipid of soybean milk waste (SMW) fermented with *Neurospora crassa* was shown inTable1.

Statistical analysis showed that each treatment effect is highly significant ($P < 0.01$) on dry matter, crude protein content, crude fiber, crude fat of soybean milk fermented by *Neurospora crassa*. From the above data it turns out that the dry matter of soybean milk fermented by *Neurospora crassa* on A treatment is much higher than the other treatments, but with the addition of bran (30%) and corn (30%) in the treatment of B and E the lower dry matter was obtained compared with treatment A. Even with the addition of standard mineral and humic acid on treatment C, D, F and G much lower dry matter content was obtained. The low dry matter in these treatment is because fungi growth more with the

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Table 1. Mean of dry matter, crude protein, crude fiber, crude fat of soybean milk waste fermented by *Neurospora crassa* (%)

Treatmens	Dry Matter	Crude Protein	Crude Fiber	Crude fat
A	96.74 ^A	30.14 ^D	20.66 ^A	11.92 ^A
B	94.96 ^B	31.36 ^{BC}	17.50 ^B	10.57 ^B
C	87.18 ^C	32.79 ^A	16.92 ^{BC}	9.44 ^{CD}
D	85.92 ^C	32.64 ^A	14.88 ^D	9.29 ^{CD}
F	94.63 ^B	31.33 ^C	17.85 ^B	10.28 ^{BC}
F	85.89 ^C	32.42 ^{AB}	15.28 ^{CD}	9.10 ^D
G	87.24 ^C	32.41 ^{ABC}	14.61 ^D	9.03 ^D
SE	0.82	0.63	0.82	0.49

Note: Different superscript indicate very significant effect (P<0.01)

addition of nutrients, minerals and humic acid on the substrate so that the microbes will flourish more. The more microbes growth more the metabolic process occurs in which the metabolic process will produce water. The more water produced, the lower the dry ingredients at the end of fermentation obtained. This is consistent with the results obtained by Mirnawati *et al.* (2012) that the addition of nutrient sources will be able to improve the nutritional content of food products after fermentation. Also the addition of humic acid in the fermentation will be able to activate microbes that can improve the quality of food substances after fermentation (Mirnawati *et al.*, 2010 and Kucukersan *et al* 2005).

The treatment C, D, F and G that is treated with the addition of rice bran and corn as well as the addition of standard mineral and humic acid so the crude protein content will increase, while crude fiber and crude fat decreased. Increasing of crude protein was resulted from fertile growth of mold. This is consistent with the statement Pepler (1973) that the addition of a nutrient source materials into the fermentation media can support and stimulate the growth of mold. The more amount of molds growth, the more enzyme produced, the more of dry matter content loss during fermentation. This is in accordance with the opinion of Wang *et al.* (1979) which stated that the increase in protein during fermentation was due to the increase in cell mass mold and the loss of dry matter during fermentation progress (Halid, 1991).

The decrease the crude fiber and crude fat in treatment C, D, F and G, which was treated with the addition of rice bran, corn and standard minerals and humic acid causes mold to grow more fertile. The more fertile molds, the more cellulose and lipase enzyme produced, which will result on a decrease of crude fiber and crude fat of soybean milk waste. This is consistent with the statement Pepler (1973) which stated that the addition of a nutrient source materials into the fermentation media can support and stimulate the growth of mold. The more mold growing, the more cellulose enzyme produced. This is consistent with the opinion Kasim *et al.* (1985) which states that there is a positive relationship between growth and cellulase enzyme production, with the fertile growth of *A. niger* the more cellulase enzymes produced to break down cellulose into glucose consequently resulted on declining of crude fiber at the end of fermentation. This is consistent with findings obtained by Nuraini and Susilawati (2006) and Mirnawati *et al.* (2010) which stated that there is a decrease in crude fiber of

Palm Kernel Cake fermented by *Neurospora crassa* and *A. niger* mold. There is also a decrease of crude fat and the end of fermentation. This is consistent with the opinion Falcony *et al.* (2006) that fungal was known as good lipase producing microorganism, one of it is *Neurospora crassa* (Kundu *et al.*, 1987).

CONCLUSION

The result of this research, it can be concluded that the substrate composition of 70% ASK + 30% rice brand + 100 ppm humic acid can increase the crude protein content up to 32.64%, and decrease crude fiber (14.88%), crude fat (9:29%) of soybean milk waste fermented by *Neurospora crassa*.

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P-08.EFFECT OF TOTAL TIME MARKETING ON MICROORGANISMS IN CATTLE MEAT MARKETED IN PASAR RAYA PADANG, WEST SUMATERA

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Abstract

Research to determine the time limit marketing to the amount of bacteria that can be tolerated up to the amount of bacteria that can be tolerated for consumption and types of microorganisms that contaminate beef products were sold in Pasar Raya Padang. The usefulness of this study to be able to provide information about the number and types of microorganisms that contaminate beef products were sold in Pasar Raya Padang at intervals varying and is expected to be used as consideration for entrepreneurs, traders, and consumer-related agencies in the setting meat that can be tolerated to be sold and consumed. Research using 6 types of beef products that sampled from Pasar Raya Padang is the meat derived from skeletal muscle tissue, liver, spleen, heart, intestine and rumen. The data were processed with a randomized block design consisting of three treatments, namely marketing time 0 hours, 4 hours and 8 hours with 6 groups. The measured variables are the number of bacterial colonies and identification of bacterial species. The results were obtained (a) The average number of colonies of bacteria to treatment 0 hours, 4 hours and 8 hours respectively is 7.73×10^5 CFU/gram, 7.47×10^5 CFU/gram and 52.95×10^5 CFU/gram. The data were processed with statistics showing a long time marketing was highly significant ($P < 0.01$) in the number of bacteria. In addition, the types of beef products was highly significant ($P < 0.01$) in the number of bacteria. Identification of bacterial species from cattle products are marketed in Pasar Raya Padang is *Staphylococcus* sp., *Bacillus* sp., *Enterobacter* sp., *Proteus* sp. and *Serratia* sp.

Key words : Marketing time, cattle product, *Staphylococcus* sp., *Bacillus* sp., *Enterobacter* sp., *Proteus* sp., *Serratia* sp.

INTRODUCTION

Foods such as beef and beef products, can contain harmful bacteria. These beef and beef products can be made safe by preventing the contaminant such as bacteria. However, the harmful bacteria on these foods can be spread to cooked or ready-to-eat foods either by direct contact or via people and objects. Meat is animal body parts are used as food as the nutritional value of food is a complete protein, containing all essential amino acids for the human body (Webster, 2013). Livestock products that contain bacteria, antibiotics, hormones, dioxins and other toxins that can host causes that lead to serious health problems in humans. Microorganisms that spoil the meat can come from infection and contamination of meat and live animals postmortem. Meat or carcass surface contamination may have occurred since the time of slaughter livestock to meat consumed. At the abattoir, the source of contamination or infection can come from the surrounding soil, the skin (dirt on the skin), the contents of the digestive tract, the water, the tools used during the process of preparing the carcass, such as knives, saws, pulleys and hooks and tools place, dirt, air and workers (Soeparno, 1994). Furthermore Davies (1998) stated that the slaughtering and butchering of food animals provide bacteria with an opportunity to colonize beef surface. A wide range of microorganism coming from different sources is introduced to surfaces

which contain abundant nutrients and which have high water availability. Only a few contaminants will be able to initiate growth, and only some of these will eventually spoil the beef by means of their biochemical attributes. Potter and Hotchkiss (1995), the practices of resting animals before slaughter can help delay bacterial spoilage of beef. Ray (2004) stated that the normal tendency of a microbial cell when it comes in contact with a solid surface is to attach it self to the surface in an effort to compete efficiently with other microbial cells for space and nutrient supply and to resist any unfavorable environmental conditions. The research objective was to determine the time limit marketing up to the amount of bacteria that can be tolerated for consumption and types of microorganisms that contaminate beef products were sold in Pasar Raya Padang. One of methods for bacterial identification used a kit API-20E from Bio Merieux. The API-20E test kit for the identification of enteric bacteria provides an easy way to inoculate and read tests relevant to members of the Family Enterobacteriaceae and associated organisms.

MATERIALS AND METHODS

Preparing of meat samples

Meat samples taken from Pasar Raya Padang put into sterile plastic bags and taken using a thermos filled with ice, then analyzed in laboratory. Types of beef products analyzed were skeletal muscle, liver, spleen, heart, intestine and rumen. Treatment of each of the products in accordance with the treatment interval is long marketing 0 hours, 4 hours and 8 hours.

Bacterial count of meat products

Counting the number of bacteria in each sample using standard methods Plate Count (Harley and Prescott, 1993). A total of 5 grams weighed and crushed in a blender by adding as much as 45 ml of sterile distilled water for 5 minutes. It gives a 1/10 dilution. Dilution is done to 10^{-6} as indicated in figure 1. Use the wax pencil, the petri plate was labeled and aseptically a liquid was pipetted from the dilution blanks to the petri plates. Melt the plate count agar pours in a water bath and cool to about 48 – 50 °C. The cooled PCA agar added to the plates. Gently swirl on a flat surface in a figure-eight motion and allow to hardening. The plates were incubated in an inverted position for 24 hours at 37 °C. The average number of bacteria per gram sample was calculated by formulation:

$$\text{Number bacteria/gram}$$

$$= \text{number of colony} \times \frac{1}{\text{dilution factor}} \times \frac{1}{\text{weight of sample}}$$

Bacterial identification

Single colonies derived from counting bacteria are inoculated into Nutrient broth medium solution and incubated for 24 hours at 37°C. Identification of bacteria isolated from each beef product (skeletal muscle, liver, spleen, heart, intestine and rumen) were identified using conventional methods and confirmed by using the API 20 E kit from Bio Merieux, Inc., Hazelwood.

RESULTS AND DISCUSSION

Effect of long time beef products are marketed in Pasar Raya Padang on the development of the average number of bacteria can be seen in the table below.

Tabel 1. The number of bacteria on beef products marketed in the Pasar Raya Padang with different time intervals ($\times 10^5$ CFU/gram)

Time intervals	Groups						Total	Averages
	1	2	3	4	5	6		
0 hours	2,7	3,1	2,6	10,2	7,5	20,3	46,4	7,73
4 hours	8,1	9,0	7,0	22,5	19,8	38,4	104,8	17,47
8 hours	43,0	39,0	24,0	72,0	42,0	94,0	314,0	52,33

Looking at the average number of initial bacterial contamination prior to marketing (0 hours) is 7.73×10^5 CFU/gram of meat. When compared with Adhar study (1992) that the initial number of bacterial contamination of meat is 0.23×10^5 CFU/gram of meat where there is a difference. This difference is caused by management factor before slaughtering and carcass handling and cleaning of carcasses at slaughter and also due to the contamination of the product during transport to the slaughter house marketing, in this case the Pasar Raya Padang, where the research of Adhar, this is not the case. Besides, it is also in accordance with the opinion Hendrasah (1987) that the number and type of bacteria that contaminate the meat is determined by the administration of pre-slaughter and carcass clean up. In addition to the Slaughter house, the meat can be contaminated with microorganisms when brought from the abattoir to the sale of meat (Ressang, 1982), so that the contamination that occurred on the products in this study was greater. When viewed the average number of bacteria at the 4th hour of marketing, which is 17.47×10^5 CFU / gram of meat, it can roughly be the multiplication of bacteria was 2.3 times of the average amount at time 0 hours. This means that with increasing time to marketing and open market conditions, the bacteria continue to evolve and outside contamination of beef products (market environment) continue to occur. This is in accordance to Ressang opinion (1982) that contamination by pathogenic bacteria or decay also occurs when meat is marketed or in companies that can be entered flesh flies freely. Time is a factor which greatly influenced the development of bacteria. This opinion presented in accordance with Eckles(1957), that at room temperature the bacteria will grow and divide every half-hour, this division takes place according to the geometric (geometric progression) of a single cell will split into two, two into four, and four cells become eight bacterial cell sand so on.

At next 8th hours marketing, the average number of bacteria is 52.33×10^5 CFU/gram of meat. This means that there has been a multiplication by 7 times of the average number of bacteria at 0 hours. Thus the number of bacteria that contaminate beef products in this study were within bounds to be tolerated bias in consumption and yet there are mucus and foul odor. This is in accordance with the opinion of Buckle *et al.* (1987), where the number of 10^7 - 10^8 CFU/gram of meat will be visible in the form of mucus and foul smelling, damaged or unsuitable for sale (consumed). The same thing also expressed by POM(1989) on the maximum limit microbial contamination in food where bacteria can still be tolerated in the carcasses was 10^7 CFU/gram of meat.

Bacterial population increased gradually at intervals of 4 hours. However this does not present anormal growth pattern, but is apart of the normal growth

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curve. Accordance with the opinion Soeparno (1992) that the logarithmic or exponential growth phase, which at this stage the number of microorganisms increase and grow with the growth rate up to environmental factors become limiting. To find out how much influence intervals varying the number of bacterial colonies that beef products sold in Pasar Raya Padang, analysis of variance performed.

Variance analysis shows that there is insignificant influence between the treatment of the development of the average number of bacteria ($P < 0,01$). This means that the interval will vary and increase the number of bacteria affect beef products were old in Pasar Raya Padang. Growing bacteria from contamination at slaughter, debarking and cleaning of carcasses in Slaughter house. Further contamination occurs when transporting cattle to market products and for marketing. Bacteria that contaminate the meat has the potential to grow at room temperature, the amount increases with time. This means that the bacteria entered development backed by the nutritional value of the meat, the meat temperature and physical state. This is in accordance with the opinion of Forrest *et.al.* (2005) that the factors affecting the growth of microorganisms on or in the flesh is the nutritional value of meat and water content as well as intrinsic factors of temperature, humidity, oxygen and physical state of the meat as external or extrinsic factors besides time.

To know the differences between each treatment carried out further LSD test. Here among the treatments showed significantly different results, with each measurement interval (4 hours) gave a significant influence ($P < 0.01$) on the number of bacteria on beef products were sold in Pasar Raya Padang. Long time 8 hours significantly different with long time 0 hours to the total bacteria ($P < 0.01$) and highly significant for a long long time with time 0 hours to total bacteria ($P < 0,01$). Based on the maximum limit of microbial contamination in carcasses established by the Director General of Food and Drug Administration (1989) is 10^7 CFU/gram of meat. The results showed the average number of bacteria is below the maximum limit.

The number of bacteria at the 8th hour is still in the tolerable limit and the quality of meat is still in the category of good, though not excellent at 0 hours as marketing, because there has been a break down of complex molecules of organic substances into simple units as food by bacteria so that the meat will lose some of the nutrient value and they contain metabolites and replaced with materials that are secreted by microorganisms that contaminate meat, in accordance with the opinion of Buckle *et al.* (1987) that the complex molecules of organic substances such as polysaccharides, lipids and proteins must be resolved prior to a simple unit, beginning of solving this could occur as a result of excretion of extracellular enzymes (a trait that is closely related to food spoilage).

From the analysis of variance indicated that there is a very significant difference between the beef products to the development of bacterial counts ($P < 0,01$). This means that different products affect multiplication. To know the differences between each of the test products beef up LSD. Here obtained significantly different results and different is not real, in which group I (muscle) did not differ significantly with group II (liver) ($P > 0.05$), and significantly different with group IV (heart), V (colon) and VI (rumen) ($P < 0,01$). Group III (spleen) did not differ significantly with group I (muscle), II (liver) ($P > 0.05$) and highly significant with

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group IV (heart), V(colon) and VI (rumen) ($P < 0,01$). Group IV(heart) significantly different with groupVI (rumen) ($P < 0.01$) and groupVI(rumen) significantly different with group I (muscle), II (liver), III (spleen), IV(cardiac) and V (intestine) ($P < 0.01$).

Bacterial identification

From the observations obtained 6 kinds of bacteria in growth media. Form colonies, and his reaction to the Gram stain can be seen in Table2.

Table 2. Form colonies that grow on the medium growth and reaction to Gram staining

No.	Colonies		Microscopic form		Gram staining	Endo Agar
	Forms	Colors	Form	Color		
1.	Coccus	Turbid white	Coccus	Purple	(+)	-
2.	Coccus	Yellowish white	Coccus	Purple	(+)	-
3.	Coccus	Turbid white	Rod	Red	(-)	Red
4.	Spherical spreading	Turbid white	Sporulating rods	Purple	(+)	-
5.	Spherical spreading	Turbid white	Rod	Red	(-)	Shadowy
6.	Spherical spreading	Yellowish white	Rod	Red	(-)	Pink

From the results of staining colony, numbers 1, 2 and 4 are Gram positive, regularly rounded cell shape and like grapes. Because of the shape and distribution can be matched with the morphology of bacteria, then based on these results is known that bacteria are *Staphylococcus sp.* In accordance with the opinion of Merchant and Packer (1961) and Volk and Wheeler(1988) that *Staphylococcus sp.* In Gram stain will form round the group Grampositive colored purple like grapes. Upper pigments made, *Staphylococcus sp.* Divided in several species, namely white colored colonies are *Staphylococcus albus* and yellowish colored is *Staphylococcus aureus*. Colony number 4 rod-shaped, Gram-positive and spore-forming, then by this bacterium is *Bacillus sp.* This is in accordance with Hadioetomo (1990) that colonies of *Bacillus sp.* Grown on nutrient agar are round or irregular surfaces bleak and Gram-positiverod-shaped and formed endospora which became his trademark.

On colony numbers 3, 5 and 6 of the results obtained Gram stain Gram-negative bacteria. Effect of Gram staining is caused by the response to the staining procedure, cells initially characterized by Gram staining procedure. By using a biochemical test to differentiate Gram-negative bacteria, it is known that the colony numbers 3, 5 and 6 in a row is a bacterium *Enterobacter sp.*, *Proteus sp.* And *Serratia sp.* To find out the results of biochemical tests can be found in Appendix 1.

To confirm identification followed by using kit API 20-E produced by Bio Meriaux Inc. such as the following figure 1.



Figure 1. Bacterial identification by using API 20-E

To determine the type of bacteria on beef products were sold in Pasar Raya Padang with different time intervals can be seen in Table 3.

Table 3. Types of bacteria on beef products were sold in Pasar Raya Padang

No.	Time intervals		
	0 hours	4 hours	8 hours
1.	<i>Staphylococcus sp.</i> <i>Bacillus sp.</i>	<i>Staphylococcus sp.</i> <i>Serratia sp.</i>	<i>Staphylococcus sp.</i>
2.	<i>Staphylococcus sp.</i>	<i>Staphylococcus sp.</i>	<i>Staphylococcus sp.</i> <i>Enterobacter sp.</i>
3.	<i>Staphylococcus sp.</i>	<i>Staphylococcus sp.</i>	<i>Staphylococcus sp.</i>
4.	<i>Staphylococcus sp.</i> <i>Bacillus sp.</i>	<i>Staphylococcus sp.</i> <i>Enterobacter sp.</i> <i>Bacillus sp.</i> <i>Proteus sp.</i>	<i>Proteus sp.</i> <i>Enterobacter sp.</i> <i>Bacillus sp.</i>
5.	<i>Staphylococcus sp.</i> <i>Bacillus sp.</i>	<i>Staphylococcus sp.</i> <i>Serratia sp.</i> <i>Proteus sp.</i>	<i>Staphylococcus sp.</i> <i>Enterobacter sp.</i>
6.	<i>Staphylococcus sp.</i> <i>Proteus sp.</i>	<i>Enterobacter sp.</i> <i>Bacillus sp.</i> <i>Proteus sp.</i>	<i>Staphylococcus sp.</i> <i>Enterobacter sp.</i>

From the table above, it can be seen that in general that contaminate beef products in this study were *Staphylococcus sp.*, *Bacillus sp.*, *Enterobacter sp.*, *Proteus sp.* and *Serratia sp.*

Staphylococcus sp. This bacteria is a common bacteria found in beef product originating from the surrounding environment, especially coming from people who handle when cutting and cleaning products as well as the transportation of carcasses. Accordance with the opinion Suriawiria (1986) that these bacteria come from the surrounding environment, particularly air and water during processing, can also come from the pork bellies. According to Pelczar and Chan (1988) which states that this organism can come from the people who handle food are suffering from infectious or infection.

Food poisoning by bacteria *Staphylococcus sp.* Caused infection that it produces enterotoxin, enterotoxin which is very heat resistant and becomes inactive at temperatures of 121°C for 30 minutes, causing dizziness, vomiting and diarrhea as well as abdominal cramping, rarely cause death and symptoms will be cured. This is in accordance with the opinion of Buckle *et al.* (1987) and Soeparno (1992) that the poisoning symptoms will appear within 1-8 hours after ingestion of toxins and healing quite fast and generally a day.

To prevent contamination of beef products in the Slaughter House (RPH) should operate environmental hygiene officers and employees. Accordance with the opinion Pelczar and Chan (1988) that the best means of prevention are the ones

who handle food should not have festering sores and a type of transmitting these bacteria in the environment and most importantly, RPH should really be kept clean. *Bacillus* sp. These bacteria are widely distributed in soil and water. Until recently this class of organisms is not classified as pathogenic, but a number of food poisonings due to contaminated food discovered by *Bacillus* sp. Ability to form spores enables the organism to stay alive in the processing and heating. Symptoms of poisoning are abdominal pain, diarrhea and dizziness during the 10-12 hours after eating contaminated food by these bacteria. Accordance with Soeparno (1992) that the symptoms of food poisoning is not known whether a food poisoning that is intoxication or infection.

Enterobacter sp. These bacteria from cow feces and can live in soil, water and waste. Accordance with the opinion Pelczar and Chan (1988) that the bacterium *Enterobacter* sp. Found in human and animal feces, sewage, soil and some waters that frequently contaminate food.

Proteus sp. These bacteria from water, soil and human digestive tract contents and livestock. Contamination occurs due to circumstances RPH, when transporting and marketing of products. This is in accordance with the opinion of Kelly and Hite (2005), that these organisms are widely distributed in soil, dust and water, usually found in the human digestive tract contents and livestock. Bacterium *Proteus* sp. Can cause rot in the flesh. This is in accordance with the opinion of Buckle *et al.* (1987) that the genus *Proteus* sp. Of ten cause spoilage of food proteins. Species of *Proteus* sp. Can cause intestinal disorders because of its urinary tract infection that can hydrolyze urea.

Serratia sp. is a group of bacteria commonly found enterobacteriaceae in soil and water as well as causing pigmen redness on the surface of food, according to the opinion of Buckle *et al.* (1987), several groups of bacteria enterobacteriaceae important for public health because it raises the issue of food poisoning and infectious diseases are transmitted through food.

Enterobacter sp., *Proteus* sp., *Serratia* sp. are family enterobacteriaceae, Gram-negative bacteria, facultative anaerobic, spore-forming rod-shaped cells, derived from the human gastrointestinal tract, the animal itself and the air, soil and water. From the obtained results, the average group of products that serve beef cattle contaminated by bacteria treated this family. It can be caused due at the time of cutting is still happening contamination with feces from a cow's stomach contents. Subsequent contamination occurred during the transport of the product from the slaughter house to the market. In the transport to the place of sale in the market in a way that does not carry workers can be guaranteed health. Contamination increases during marketing as laid out in an open space and can be a source of contamination carrier flies. Accordance with the opinion Hendarsih (1987) that the number and type of bacteria that contaminate the meat is determined by management prior to slaughter, and levels hygienic carried on slaughtering and cleaning. Source of contamination after it is during transport and marketing.

CONCLUSION

Number of bacterial colonies beef products marketed in Pasar Raya Padang at 0 hours was 7.73×10^5 , 4 hours and 8 hours respectively is 17.47×10^5 and 52.33×10^5 CFU per gram of sample. Types of bacteria found in beef products were sold in Pasar Raya Padang is *Staphylococcus* sp., *Bacillus* sp., *Enterobacter* sp., *Proteus* sp., and *Serratia* sp. Types of beef products very significant effect on the

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number of colonies and the product have different capabilities in supporting the proliferation of bacteria.

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one important part of South Minahasa people's lives, in addition to agriculture. Agricultural products such as maize, cassava, grasses, agricultural waste can be used as animal feed so that there is value added. In this case, it can be said indirectly that needs feed (forage and concentrate) for livestock can be met. In addition, livestock manure as waste organic indispensable as a source for plants. Useful organic fertilizers for soil fertility. Utilization of organic fertilizer from animal waste can increase agricultural productivity. Thus, livestock population increases, automatic meat production increased. Increased meat production is that farmers are directly perceived increase in income and welfare. Agriculture in this area is dominated by coconut plantations, while still dominated by farm livestock. Land under coconut trees used for food crops and forage. Crop waste is a source of cattle feed, cattle manure instead be used to increase soil fertility.

Sinonsayang districts is one of the districts in the South Minahasa are agropolitan. Commodity area is coconut with the highest cattle population (BAPPEDA South Minahasa Regency, 2006). In this district there are several farmer groups including cattle farmer group.

The groups that have been formed in the district are Sinonsayang crop farmer groups, pig farmer and cattle farmers groups. Ongkaw village is one of the villages in the district Sinonsayang, have formed farmer groups: the group of cattle farmers Maesa and LM3 Bahtera. LM3 Bahtera group formed in 2007 with the group's main program is seeding rice and corn farming. Maesa Group was formed in 2010 with the group program is composting. The second group was originally formed from the group The Bahtera Church Fathers. Maesa cattle farmer groups have been formed for the development of compost. The group has received financial from the central government for composting and in February 2011 has been building for composting.

LM3 Bahtera cattle farmer groups is a group who had received financial from the government to farm field activities undertaken are fattening and breeding. Cattle population in 2007 amounted to 25 heads have been increased to 44 head in 2011. Cattle farmer groups LM3 Bahtera started to form in 2007 with meetings every week on sunday, gathering and cattle operations. Farmer group Maesa started to form in 2010 and has received aid in the form of cattle some 30 tails.

The problem from LM3 Bahtera and Maesa cattle farmer groups in the village Ongkaw not have an understanding and knowledge of cattle development an integrated between farm crops and coconut. System integration is very profitable cattle farmer group members of LM3 Bahtera and Maesa. Straw and waste can be utilized by members of the group as a nutrient-rich feed. Straw and waste are very useful as a feed stock in the dry season. The problem straw / agriculture waste has not been used by members of the group. So in the dry season the group members have to find grass in the distant farmland. That group members do not have knowledge of handling waste and surplus when the harvest.

Based on the ideas and issues that have defined the priority issues that need to be handled by a members of LM3 Bahtera and Maesa cattle farmer group, as follows: (1) lack of knowledge of the group members on the provision of feed (forage) is continuous and quality, (2) lack of group members' knowledge about the use of hay and forage preservation, (3) lack of knowledge of the group members use the manure as fertilizer.

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MATERIALS AND METHODS

The application of science and technology has done for LM3 Bahtera and Maesa cattle farmer groups from July to October 2012. The application of science and technology activities have been carried out based on priority issues LM3 Bahtera and Maesa cattle farmer groups. The application of science and technology have been made through the empowerment of the group by using two methods:

1. Extension

In order to develop beef cattle farms in particular, extension has a very important role for the group. According to Abdullah (2008) extension particularly instrumental in strengthening farmer groups and an increase in the adoption of farm technology to farmers. Extension made to the cattle farmers in the village Ongkaw (group of LM3 Bahtera and Maesa) with the aim of changing the behavior of livestock farmers towards a better (Pambudy, 1999). Some of the philosophy of extension is: (1) extension program relying on farmers' needs, (2) extension is basically the process of education for adults who are non-formal. The goal is to teach farmers, improve their lives by his own efforts, as well as teaching farmers to use natural resources wisely, and (3) extension in collaboration with other organizations to develop individuals, groups and nations. Extension materials about forage management, maize and legume integration, preparation of rations, biogas production, business analysis, institutional strengthening and making recordings. While the media used are brochures and LCD.

Training

Having conducted extension to members of the group, further training for members of farmer group LM3 Bahtera and Maesa. Training is a practical application of the technology is in the form of introduction of grass dwarf, composting, silage making and ammoniation. Planting grass is integrated with coconut plantations.

RESULTS AND DISCUSSION

Development of agriculture and animal husbandry in South Minahasa mutually supportive and beneficial, so that integrated farming systems provide great benefits for both. Development of sustainable farming can be done by developing a model of integration of coconut-cattle (Salendu and Elly, 2011). Further high integration of crops and livestock is often considered as a step forward (Rota and Sperandini, 2010). Integrated farming systems by Ahmed et al (2011) is the best farm system in terms of resources, efficiency, productivity, production and supply of food.

Livestock and crops are the main source of rural households. A nutrient-poor soils, high rainfall and lack of irrigation water, the region has a comparative advantage for the production of livestock. Cattle business is one of the reliable business cattle farmer groups LM3 Bahtera and Maesa in Ongkaw village. Cattle productivity can be improved by involving members of the group and the government. In connection with the above ideas have empowered the group members by providing extension and training. Extension materials on the integration of livestock and crops as well as matters relating to the management of cattle business. Educational events responded well by members of the group.

LM3 Bahtera and Maesa cattle farmer groups cattle production process is integrated with crops of corn and coconut. Some research indicates that the

application of farming systems CLS (Crop-Livestock System) increase production profits higher than the non-CLS. According Channabasavanna et al (2009) that the integrated farming system is very productive and profitable. This indicates that the integration of cattle with crop can benefit for the animals and plants. Elly (2008) and Salendu et al (2012) suggested that the income of the business is greater than the integrated the cattle-coconut businesses that are not integrated.

Cattle business success is determined by three elements are interrelated, ie seed, feed and management. The success of these efforts depends on the characteristics of the group members the cattle LM3 Bahtera and Maesa. Characteristics of the group members see their age and level of education.

Cattle business success is determined by the age of the group members. Age group members ranged from 30-69 years. The average age of the group members Maesa was 44.43 years. While the average age of the group members LM3 Bahtera was 45.10 years. This condition indicates that the age of members of the two groups is still considered productive. They have a strong enough physical ability to carry out farming activities. According Kiswanto et al (2004), the adoption of technology is closely related to business productivity. Further stated that age is a factor that can affect the productivity of beef cattle fattening.

The level of education ranged from primary school to high school level. Average education level Maesa group members are as much as 21.43% elementary school, junior high school level as much as 42.86% and 35.71% as much as the high school level. The average level of education for members of the group LM3 Bahtera are elementary level as much as 20%, 40% junior high and high school levels as much as 30%. This condition shows that the education level of the group members still considered low. It can be seen from the support home composting since being founded in 2010 by members of the group have not responded. Prior to any application of science and technology by the universities, members of the group have not produced compost. It means that the level of education influence the adoption of technology by the group members. According Kiswanto et al (2004), the higher the level of education that allows to change attitudes and behavior to act more rationally. This action provides an opportunity to be more successful in managing the farm.

Cattle owned by members of the group Maesa numbered 40 tail, while the members of the group belong to as many as 32 head LM3 Noah. Problems in the development of the cattle that forage is not available. Cattle consume grass field and agricultural waste to meet the forage needs. Alternatively, the cattle grazing in coconut plantations or other dry land and allowed to consume grass that grows wild. According to Muslim and Nurasa (2011) introduction of superior forage has actually been carried out by the government. In this condition the group members do not take advantage of the enclosure has been built with the help of the government (Ministry of Agriculture). Their reasons for the unavailability of forage fodder.

Standards / norms forage needs of livestock feed per cattle per day by Animal Unit under the Ministry of Agriculture (2010) are : adult cattle (1 AU) require as much as 35 kg of forage, heifers (0.50 AU) of 15-17.5 kg and calf (0.25 AU) of 7.5-9 kg / head / day. To meet the needs of the group members had to prepare the land for cattle forages.

Application of science and technology that has been done is the introduction of quality forage in the lands that belong to each member of the group and the group owned land covering an area of 0.3 ha. A land area of 0.3 hectares of land is under a coconut tree planted dwarf grass (*Pennisetum purpureum cv mott*). Planting grass is very response by members of the group. It is evident from the grass that has been planted in an area of 0.3 ha and in the land of each member of the group.

If the land is under a coconut tree that used to grow quality grass then the income will be higher (Salendu, 2012 and Salendu et al, 2012). Land under coconut for forage also serves as a cover crop. Cover crops is an act of conservation at the time instead of the growing season (Rahim, 2006).

Group members have also been trained to weed preservation, namely silage. This is done to anticipate when there is excess production and can be used the dry season. The existence of silage, cattle feed requirements can be met. Method for production of silage is: dwarf grass harvesting fresh cut 2-5 cm by group members using the cooper. The grass then put in an airtight plastic bag. Every 15 cm of fresh grass sprinkled with rice bran, and so forth until plastic bags filled and solid. Once the grass is filled solid, tightly closed plastic bag (a plastic bag tied). Making process for 21 days and after it opened to smell fragrant and slightly sour. Silage making this very response by members of the group.

Agricultural residues can also be used as a source of forage such as rice straw, leaves and cobs of corn, and others. Rice straw has a high fiber content and low energy levels so that its low digestibility values. This requires a treatment that easily digested by the fermentation process (Kardiyanto, 2009). Method of making rice straw ammoniation is dry (water content of about 60%) was cut into pieces 2-5 cm. Straw that has been cut into pieces stacked in a plastic bag, and then trampled to solid. Then the straw is stacked in a plastic bag sprinkled with probiotics (SB) and urea in the ratio of each 6 kg for every ton of rice straw. To grow the probiotic then sprinkled water to 60% the moisture, which is indicated by the hand-wringing straw, then seen that the water in the palm of the hand as if he was about to drip means the water is enough. Stage was repeated again with a pile of 15 cm to a plastic bag full. After a closed plastic bag and tied then left for 21 days in a place protected from rain and direct sunlight. After 21 days of fermentation ready to be given to the cattle.

Maesa Group has obtained support house for composting. Cattle manure can be used as fertilizer for paddy fields. According to Kelvin (2005), the use of manure inputs by 10%, assuming *ceteris paribus* can increase production by 1.25%. Further stated that the manure can improve and maintain the diversity of living organisms and soil. The indication that the manure is needed to improve soil fertility. According Kariyasa and Pasandaran (2004), the use of anorganic fertilizers continuously and tend to cause a lot of excess agricultural land in Indonesia is not fertile conditions. Under this condition, the manure as organic fertilizer started to be used to substitute anorganic fertilizers. Manure of cattle by Menegristek (2000), is a source of nutrients that can improve soil structure so that it becomes more loose and fertile. Prasetyo and Suriadikarta (2006) states that the provision of organic matter from manure and crop residues to improve soil physical properties.

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Members of the group have been trained to make compost by using cattle feces that is the stable. Composting process has been carried out in the house compost. Composting method is as follows: at first made of beams and bamboo container measuring 2 x 1 x 1 m. Then dried rice straw stacked in the container 15 cm then put the dried cattle feces while being trampled so solid. EM4 then sown that has been mixed with water and sugar. Stages are repeated until the container is full and solid. Once the container is full, open beams and bamboo, then covered with a tarp and tied. A week later screened compost and this is done for 4 weeks.

After 4 weeks of composting has been opened and aerated. Good compost is already quite experienced weathering. It is characterized by a different color is the color of its constituent materials, odorless, low water levels and corresponding room temperature. Group members are preparing the ground for planting corn by utilizing compost. According to Elly et al (2008), cattle serve as a source of meat, as well as producers of fertilizer or compost to increase crop production. However, the weather does not currently support the planting of corn. In addition to corn crops, compost can be used as a source of income for the group members.

CONCLUSION

Members of both groups (LM3 Bahtera and Maesa) have responded well to the activities applying science and technology through extension and training. This is evident from the availability of grass in the fields each member of the group and the land in the coconut plantation area of 0.3 ha. Another product that is produced, the silage and ammoniation and compost. The application of science and technology can be continued if there is guidance from college.

Suggestion given is that the concept of integrated farming system can be implemented to the maximum, then the members of the group were trained to make biogas not only in the form of extension. In developing compost needed intervention from the government, relating to the care and preparation of printing and packing machines.

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P-10. PERFORMANCES AND HEMATOLOGICAL PROFILE OF BROILER UNDER HEAT STRESS FED DIET CONTAINING

Carica papaya L. LEAF MEAL AND Curcoma domestica val

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Abstract

An experiment was conducted to evaluate the effect of supplementation of combination Carica papaya Leaf meal and Curcuma domestica meal in diet broiler on the performances and hematological profile of broiler under heat stress. Heat stress happened on the third weeks on temperature 34°C and take care of it for six weeks. Two hundred doc broiler used this experiment and they reared for six weeks. The diet contained metabolizable energy 2900 kcal/kg and crude protein 21 %. Treatments were control diet (R1), 99,98 % control diet and 0.02 % bambarmycin (R2), 98 % control diet and 1 % Carica papaya L Leaf and 1 % Curcuma domestica val (R3), 97 % control diet 1,5 % Carica papaya val leaf meal and 1,5 % Curcuma domestica (R4), 96 % control diet and 2 % carica papaya L leaf meal and 2 % Curcuma domestica val meal. Feed consumption, weight gain, final body weight, feed conversion ratio (FCR) and abdominal fat were examined. Hematological profile as erythrocyte, hemoglobine, heterophyle, and lymphocyte were observed. Data were analyzed by completely randomized design with 5 treatments and 4 replicated. Ten chicks each replication. The result show that supplementation of combination Carica papaya leaf meal and Curcuma domestica were significantly ($P < 0.01$) decreased feed consumption, body weight gain, final body weight, abdominal fat and increased feed conversion ratio compared with control diet and bambarmycin diet. The treatments were not significantly on erythrocyte, hemoglobine, heterophyle but significantly ($P < 0.01$) increased lymphocyte and hematocrite compared control diet and bambarmycine diet. The conclusion that supplementation of combination Carica papaya meal and Curcuma domestica each 1-2 % not yet prevented heat stress (34°C on 3-6 weeks reared) on broiler compared antibiotic treatment.

Key word. heat stress, Carica papaya leaf meal, Curcuma domestica, performance of broiler and hematological profile.

INTRODUCTION

Heat stress is one of the most important environmental stressors challenging poultry production worldwide (Lara and Rostagno, 2013). The broilers chickens require a comfortable temperature in order to produce a good performance. The main problem in the raising of broilers in the tropics is high ambient temperatures lead to decreased productivity and increased mortality. This may increase water consumption, decrease feed intake and it turn, decrease production level. The decrease of feed intake and body weight gain and final body weight was higher in broiler chickens kept at a temperature of 32°C-35°C compared to 21°C-25°C temperature (Kusnadi, 2004; Al-Fatahtah (2007). The neutral temperature zone is comfortable for the raising of broiler chickens $\pm 19-27^\circ\text{C}$ where chickens can regulate the release of heat, while at temperatures $< 32^\circ\text{C}$ chicken should improve heat dissipation and temperature $< 15^\circ\text{C}$ broiler chicken must increase heat production. The efficiency of broiler chickens can be improved is mainly used various supplements and vitamins. The variety of herbal

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can be given a broiler chickens at hot environmental temperatures like antanan (*Centella asiatica*) meal in the diet, extract of beluntas leaf (*Pluchea indica* Less) in water drinking, etc. The antanan (*Centella asiatica*) meal used as 10% can reduce diet intake and decreased body weight gain due to increased environmental temperature of 21°C to 35°C (Kusnadi, 2004). The extract of beluntas leaf (*Pluchea indica*) can reduce heat stress at 10% level in the diets of broiler chickens as an antistress drug (Setiaji and Sudarman, 2005). The use of *Curcuma domestica* meal and *Carica papaya* leaf meal contained bioactive substances that could be expected to reduce the decline in feed intake and body weight gain of broiler chickens kept at high ambient temperatures. Bioactive substances *Curcuma domestica* (curcumin and essential oil) as well as bioactive substances *Carica papaya* (papain enzyme) can increase appetite.

MATERIALS AND METHODS

Animal and Diet

Two hundred unsexed day old broiler chickens were randomly assigned to four pens. Each pen housed ten birds. Birds were given a diet from 0-6 weeks of age. Formula and composition of experimental diets are shown in table 1. Diets were contained 2900 kcal metabolizable energy and 21% crude protein and were formulated based on the composition of the ingredients to NRC (1994). Experimental diets used *Curcuma domestica* meal and *papaya* leaf meal mixed 1% until 2%. *Curcuma domestica* meal made of *Curcuma domestica* tubers are washed thoroughly, thinly sliced then dried with oven temperature of 60°C (for 24 hours) and made flour. *Carica Papaya* leaf meal (old leaves) sliced all the way and then dried using an oven with a temperature of 60°C and made flour.

Experimental Design

As shown in table 1., experimental diets were formulated contain control diet (R0), diet contain 0.02% bambarmycine (R2), diet contain mixture of 1% *Curcuma domestica* meal and 1% *Carica papaya* leaf meal (R3), diet contain mixture of 1.5% *Curcuma domestica* meal and 1.5% *Carica papaya* leaf meal (R4), and diet contain mixture of 2% *Curcuma domestica* meal and 2% *Carica papaya* leaf meal). Feed intake, weight gain, feed conversion ratio (FCR), carcass weight, abdominal fat were examined. Feed intake and body weight were recorded weekly and mortality was recorded. Hematological profile as erythrocyte, hemoglobine, heterophyle, and lymphocyte were observed from blood serum taken from 6 weeks old chicken. Chicken blood sample from vein in neck was used syringe. The broiler chickens kept at heat stress conditions at high environmental temperature (24°C-35°C) on the third until sixth weeks by created turning a 60-watt light bulb for 24 hours straight. Data were analyzed by completely randomized design with 5 treatments and 4 replicated.

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Table 1. Formula and composition of experimental diets

Ingredients	Experimental diets (0-6 weeks)(%)				
	R1	R2	R3	R4	R5
Yellow corn	53	53	53	53	53
Rice bran	7	6,98	5	4	3
Soybean meal	26	26	26	26	26
Fish meal	9	9	9	9	9
Dicalcium Phosphat	2	2	2	2	2
Palm Oil	2,5	2.5	2.5	2.5	2.5
Premix	0.5	0.5	0.5	0.5	2.5
Bambermycin	-	0.02	-	-	-
<i>Curcoma domestica</i> meal	-	-	1	1.5	2
<i>Carica papaya</i> leaf meal	-	-	1	1.5	2
Total	100	100	100	100	100
Analysis proximate composition					
Dry matter (%)	86.94	86.94	85.16	85.60	85.52
Ash (%)	6.45	6.45	6.52	6.63	7.08
Crude protein (%)	20.34	20.34	21.83	21.91	21.75
Crude fiber (%)	4.56	4.56	4.17	3.82	3.62
Ether extract(%)	4.29	4.29	3.95	3.93	3.90
NFE (%)	51.30	51.30	48.69	49.31	48.76
Gross energy (kcal/kg)	4116	4116	4030	4073	4069
Methionine (%)*	0.42	0.42	0.42	0.41	0.41
Lycine (%)*	1.28	1.28	1.27	1.23	1.26

Premix contain : vitamin A 4.000.000 IU, vitamin D3 800.000 IU, Vitamin E 4.500 mg, vitamin B 450 mg, vitamin B2 1.350 mg, vitamin B6 480 mg, vitamin B12 6 mg, Ca-d-panthotenat 2.400 mg, folic acid 270 mg, nicotinic acid 7200 mg, cholin chlorida 28000mh, DL-metionin 28000mg, L-Lysin 50.000 mg, Copper 700 mg, Mangannase 18.500 mg, Zinc 14.000 mg, Cobalt 50 mg, Iodin 70 mg, Selenium 35 mg, Antioksidan and carrier 1 kg

Table 2. The environmental temperature of broiler chickens pen

Week	Morning		Aftenoon		Evening	
	Temperatur e (°C)	Humidit y (%)	Temperatur e (°C)	Humidit y (%)	Temperatur e (°C)	Humidit y (%)
1	25.00	94.00	34.14	72.29	30.43	81.14
2	25.64	90.71	34.07	78.43	30.00	78.71
3	24.43	96.43	34.36	74.86	29.36	82.71
4	24.29	93.14	35.14	72.00	30.35	78.00

RESULTS AND DISCUSSION

Performaces of Broiler Chickens

Growth performance are shown in table 3. Birds fed *Curcoma domestica* meal and *Caricapapaya* leaf meal 1% until 2 % in a broiler diets was resulted

significant ($P < 0.01$) lower feed intake and weight gain compared to bird fed bambermycine diet and control diet. There was no difference in carcass weight but abdominal fat was significant differences ($P < 0.01$). Conditions of heat stress during third weeks to sixth week (table 2) can be tolerated by broiler chickens. The feed intake of broiler chickens control did not decreased compared to bambermycine diets. But the chickens are given a mixture of *Curcuma domestica* and *Carica papaya* leaf meal was produced lower feed intake compared with the control diet and bambermycine diet. Decline in feed intake of broiler chickens were given *Curcuma domestica* and *Carica papaya* is not caused by heat stress because of the low ration palatability. Unknown bioactive substances of *Curcuma domestica* meal and *Carica papaya* leaf meal causes feed intake reduced. The decrease of feed intake causing nutrients into the body decreases so that the body weight gain lower and feed/gain increased. Lack of energy into the body causes the body's energy reserves was used to generate heat that for metabolic body so that the formation of abdominal fat decreased. This is evident from the abdominal fat content was lower ($P < 0.01$) in chickens fed a mixture of *Curcuma domestica* meal and *Carica papaya* meal compared with control diet and bambermycine diet. The same chick mortality in chickens given the control diet with bambermycine diet as long as reared in chicken on heat stress conditions. It was showed that under conditions of stress (24°C - 35°C) during the third week can be tolerated by chickens. But a mixture of *Curcuma domestica* and *Carica papaya* at 1% and 2% treatment higher mortality rate. The high mortality rate of chickens caused by low feed intake that decreased immune system, but in contrast to the treatment of a mixture of 1.5% *Curcuma domestica* and *Carica papaya* obtained the lowest mortality rate than other treatments. Require further study, does the composition of both herbal bioactive optimum composition for increasing immunity.

Table 3. Feed intake, weight gain, feed/gain, carcass weight, abdominal fat of broiler chickens fed experimental diets

Variable	Control (R1)	Bambermycine (R2)	<i>Curcuma domestica</i> meal and <i>Carica papaya</i> leaf meal		
			1 %	1.5%	2 %
Feed intake (%)	2296.18 $\pm 166.57^A$	2237.13 $\pm 136.16^A$	1897.60 $\pm 98.86^B$	1966.70 $\pm 161.67^B$	1575.55 $\pm 115.65^C$
Weight gain (g/ekor)	1012.59 $\pm 88.89^A$	1041.58 $\pm 78.73^A$	708.49 $\pm 47.82^B$	777.73 $\pm 62.42^B$	689.17 $\pm 53.13^B$
Feed/gain	2.27 $\pm 0.12^{ab}$	2.16 $\pm 0.18^b$	2.69 $\pm 0.26^a$	2.53 $\pm 0.13^{ab}$	2.29 $\pm 0.09^{ab}$
Mortality (%)	2	2	2.5	1	3.5
Carcass weight (%)	67.68 ± 2.60	66.19 ± 1.42	65.13 ± 2.05	66.95 ± 1.54	66.95 ± 1.13
Abdominal fat (%)	1.31 $\pm 0.15^A$	1.23 $\pm 0.08^B$	0.81 $\pm 0.03^C$	0.80 $\pm 0.04^C$	0.82 $\pm 0.04^C$

Means in the same row with different superscript differ significantly ^{AB} statistical ($P < 0.01$) and ^{ab} statistical ($P < 0.05$)

Hematological profile of Blood Serum

Profile of blood serum are shown in table 4. Profile of blood serum to describe the condition of broiler heat stress. Broiler chickens exposed heat stress

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will be increased the number of leucocytes because of the body's attempt to cope with stress. The result showed erythrocyte, hemoglobin and leucocyte values are not influenced by heat stress were performed in this study despite the decreased in feed consumption in chickens fed a mixture of *Curcuma domestica* meal and *Carica papaya* leaf meal compared to control and bambermycine diets. But value of hematocrit, lymphocyte and heterophil significantly different ($P < 0.05$). Hematocrit of *Curcuma domestica* meal and *Carica papaya* leaf meal 1% diet had higher than bambermycine diet but its same with control diet. The control diet had value of lymphocyte highest but value of heterophil lowest than other treatment diets. Leucocyte values chickens fed control diet did not differ significantly with other treatments showed that heat stress conditions in this study can be tolerated by chicken. Leucocyte value this research ($(22-31 \times 10^3 \text{ mm}^3)$) lower than result from Setiaji dan Sudarman (2005). Result showed Setiaji and Sudarman (2005) that chickens are reared without being vitastress had value of leucocyte was $40.10 \times 10^3 \text{ mm}^3$. Heterophil and lymphocyte ratio in chickens fed the control diet low in comparison to other treatments (0,86). When compared with the results of research Setiaji and Sudarman (2008) that lymphocyte and heterophil ratio had a value was not significantly different (0.30 - 0.55). But the ratio of heterophil and lymphocyte in chickens fed bambermycine and mixture of *Curcuma domestica* meal and *Carica papaya* leaf meal were higher (1.82 to 2.34). The increased ratio of heterophil and lymphocyte due to heat stress, number of heterophil increased and number of lymphocyte decreased is an indicator of the high levels of stress. Reduced number of lymphocyte between judgments due to the effects of the hormone corticosterone is increased during stress. Due to heat stress experienced by broiler chickens, will stimulate the central nervous system, especially the hypothalamus to produce CRH (*Corticotropin releasing hormone*) (whose existence will increase anterior pituitary to release ACTH (*Adrenocorticotropic hormone*) which will stimulate the adrenal cortex to produce steroid hormones that is corticosteroids. The existence of these corticosteroids cause the body to produce more leukocytes. Value of blood profile variable in this experiment still normal range as 22-35 % hematocrit, $20-30 \times 10^3 \text{ mm}^3$ leucocyte and 7-13 g/100ml hemoglobine (Swenson, 1984). Sugito (2007) had reported this experiment at normal condition that broiler chicken had $2,46 \pm 0,21 (\times 10^6 \text{ mm}^3)$ erythrocyt; $8,73 \pm 0,64 \text{ g \%}$ hemoglobine, $27,17 \pm 2,89$ hematocrite (%) and $15,47 \pm 6,29 \times 10^3 \text{ mm}^3$ leucocyte.

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Table 4. Haematological profile of blood serum broiler

Haematological parameters	Control (R1)	Bambermycine (R2)	Mixture of <i>Curcuma domestica</i> meal and <i>Carica papaya</i> leaf meal		
			1 %	1.5%	2 %
Erythrocyte ($\times 10^6$ mm ³)	2.31 \pm 017	2.23 \pm 0.14	2.40 \pm 0.22	2.27 \pm 0.08	2.49 \pm 0.19
Haemoglobine (g%)	6.95 \pm 0.25	6.90 \pm 0.58	8.10 \pm 1.32	7.75 \pm 1.18	7.85 \pm 0.87
Hematocrite (%)	28.59 \pm 1.36 ^{ab}	25.61 \pm 0.23 ^b	30.41 \pm 2.26 ^a	29.50 \pm 2.46 ^{ab}	28.88 \pm 2.03 ^{ab}
Leucocyte ($\times 10^3$ mm ³)	22 \pm 3.48	27.90 \pm 10.37	28.35 \pm 3.99	31.05 \pm 3.19	24 \pm 1.63
Limphosit (%)	53 \pm 12.78 ^a	29.75 \pm 13.6 ^b	33 \pm 5.72 ^{ab}	33.25 \pm 9.67 ^{ab}	35 \pm 5.83 ^{ab}
Heterophil (%)	45.50 \pm 11.85 ^b	69.50 \pm 14.06 ^a	66.25 \pm 5.68 ^{ab}	65.50 \pm 9.04 ^{ab}	63.75 \pm 4.92 ^{ab}
Heterophil/limphosit	0.86 \pm 0.59	2.34 \pm 3.17	2.01 \pm 0.52	1.97 \pm 1.04	1.82 \pm 0.50

Means in the same row with different superscript differ significantly (P<0.05)

CONCLUSION

Broiler chickens fed 1 % to 2 % mixed *Curcuma domestica* meal and *Carica papaya* leaf meal in the diets had not been able to replace antibiotics (bambermycine) to cope with heat stress (24-35°C) in the third weeks to sixth week. Decrease in feed intake and body weight gain and increased feed conversion occurred in chickens fed treatment diets.

Broiler Chickens fed bambermycine diet produced lower hematocrit and limphosit but highest heterophil of blood serum compared than treatment diets and control diet on heat stress conditions.

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P-11. MIX OF LINGZHI (*Ganoderma lucidum*), ORGANIC CHROMIUM AND ROASTED SOYBEAN EVALUATED AS FEED SUPPLEMENT FOR LAYING HEN

Tania Perdana Putri¹, Dwierra Evvyernie¹, Dwi Margi Suci¹ and Muhammad Lukmannulhakim¹

Abstract

The objective of this study was to determine effect of supplement which consists of lingzhi mushroom (*Ganoderma lucidum*), organic chromium and roasted soybean as source of CLA (*conjugated linoleic acid*) on laying hens and egg. Hundred and twenty Lohmann Brown line 26 weeks of age layer were consumed four treatments of supplement, P1 Control (basic ration), P2 (control + 0.01% BW of Lingzhi + 3 ppm organic Cr + 1% fat diet of roasted soybean), P3 (control + 0.01% BW of Lingzhi + roasted soybean 1% fat diet), P4 (control + 3 ppm organic Cr + 1% fat diet of roasted soybean). Completely randomized design with three replications and analysis of variance (ANOVA) were applied in this research. The finding result showed that the contents of cholesterol, LDL and HDL in the serum of hens and egg were not influenced by the supplement, but there was indication reducing of blood cholesterol in lingzhi addition (P2 and P3= 119.56 mg/100 ml and 112.65 mg/100 ml vs. P1= 138.88 mg/100 ml). The highest Chromium content in egg was P2 (2.27 ppm) and the lowest was P1 (1.87 ppm). CLA content increased from control even in small amount. Egg weight, Haugh unit, eggshell weight and eggshell thickness, yolk color, hen day, and feed consumption were not changed. However, P3 showed the highest egg production at 76.33% and the lowest generated by P2 at 66.66%, while the control was 68.57% hen day. The egg weight of P3 were 49.83 grams and control showed the largest one (52.22 grams). As conclusion the supplementation of feed with *G. lucidum*, organic Cr and roasted soybeans were not influence the egg performance. However, addition of chromium as well as CLA in ration containing lingzhi (*G. lucidum*) increased the content of chromium or CLA in the egg, and reduced the blood cholesterol concentration.

Keywords: *cholesterol, egg performance, Ganoderma lucidum, organic chromium, roasted soybean*

INTRODUCTION

Egg is the most available and the cheapest farming product. The quality of an egg is not only assured from its nutrition, but also from its performance including its weight, yolk colour, albumin, and its shell. Egg performance indicates the physical status of the egg which is one of consumer interest. It will effect the egg demand. Therefore, egg's quality should be increased. One of best efforts which can be done is manipulating the feed or adding the feed supplement for laying hens.

Lingzhi (*Ganoderma lucidum*) is one of a commercial medical mushroom from China. This herb contains polysaccharides, germanium, ganoderic acids that function as immune modulator, anti bacteria, reducing cholesterol level in human and animal (Jiang *et al.*, 2004; Paterson, 2006; Evvyernie *et al.*, 2002). Combination using of lingzhi with other mineral or substances which support high animal production should be more investigated. From many researches, trivalent chromium (Cr) likes Chromium picolinate is one of essential micronutrient for human and animal. Chromium has potential role on carbohydrate metabolism. Addition of Cr enhancing the action of insulin as a critical hormone in metabolism

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and storage of carbohydrate, fat, and protein in the body. Chromium was identified as GTF (glucose tolerance factor), an active ingredient that interact to insulin and cell receptor, induce glucose enter cell, change to energy and then used for protein synthesis, maintaining cell and growth of tissue in the body (Vincent and Davies, 1997), maintaining the normal level of glucose in the blood (Yesilbag and Eren, 2009). Other important substance should be added to the animal ration is Conjugated Linoleic Acid (CLA). Dairy lactation that consumed CLA from roasted soybean showed increasing in milk production, fat and protein around 15%, 11,4% and 18,5%, respectively (Adawiah, 2006). Supplementation of CLA in diet of human reduced body fat, colon and breast cancer (Kelly, 2001; Riserus *et al.*, 2004), in ration of laying hen increased the proportions of myristic, palmitic, stearic, CLA (9-cis, 11-trans CLA and 10-trans, 12-cis CLA isomers), and unidentified fatty acids in egg yolk lipids, and decreased palmitoleic, oleic, linoleic, linolenic, arachidonic, and docosahexaenoic acid (Du *et al.*, 1999).

Based on those researches, in this research we applied all of the substances to be mix supplement containing lingzhi, organic chromium and roasted soy bean CLA to laying hen. The aim of this research was to study the addition of mix supplement to blood cholesterol and the quality of egg performances.

MATERIALS AND METHODS

Completely randomized design with three repetition was conducted in this research, in which 120 Lohmann Brown line 26-week-old layers were treated by four diets i.e. P1/Control (basic ration), P2 (control + 100 ppm Lingzhi + 3 ppm organic Cr + roasted soybean 1% of total ration fat), P3 (control + 100 ppm Lingzhi + roasted soybean 1% of total ration fat), and P4 (control + 3 ppm organic Cr + roasted soybean 1% of total ration fat). Several parameters were evaluated in this research including blood cholesterol level of laying hens, egg cholesterol content, and quality performance of the eggs.

The mix supplements contained fruiting body of *Ganoderma lucidum* (GL), organic Chromium (orgCr), and roasted soybean as source of CLA (*conjugated linoleic acid*) were used in this experiment. To produce orgCr, an-organic Chromium was incorporated into rice straw as a substrate in fermentation process using *Ganoderma lucidum*, and source of CLA was obtained by dry frying the soybean in 15 minutes. We used 120 Lohmann Brown line 26-week-old layers with average of body weight $1,67 \text{ kg} \pm 0,135 \text{ kg}$. There were four rations i.e. P1/Control (basic ration), P2 (control + Lingzhi + organic Cr), P3 (control + Lingzhi + CLA), and P4 (control + organic Cr + CLA). Doses of supplement substances were 100 ppm/day *G. lucidum*, 3 ppm/kg DM ration orgCr, and roasted soybean 1% of total ration fat as CLA. The composition and the nutrition of basic ration were showed on Table 1.

Completely randomized design was used in this experiment with four types of ration/supplement and three repetition consist of 10 laying hen. Several parameters were evaluated in this research such as consumption, egg weight, eggshell weight and thickness, egg yolk, haugh unit, hen day, egg cholesterol content, egg CLA, serum and egg chromium. The results data of experiment were analyzed by ANOVA and Duncan's test (Steel and Torrie, 1993).

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RESULTS AND DISCUSSION

Chromium, CLA and Cholesterol Content in Serum and Egg

Table 2 presented serum and eggs cholesterol, chromium and CLA in laying hens. Result showed that during feeding with lingzhi mushroom, roasted soybeans and organic chromium, blood cholesterol level of laying hens were not significantly different. According to Swenson (1984), normal level of blood cholesterol in hen is 125-200 mg/100ml. The supplementation feeding resulted blood cholesterol level of 112,65-138,88 mg/100ml which is lower than the above statement. This study showed that there was reduction in blood cholesterol level in laying hens which were fed by either P2, P3 or P4 diets.

Based on the results of variance, feeding a mixture of the three supplements showed no significant difference in blood cholesterol level either P2 (Lingzhi, roasted soybeans, organic chromium), P3 (Lingzhi, roasted soybeans) or P4 (roasted soybeans, organic chromium). According to Swenson (1984), normal cholesterol level of hen blood ranged from 125 to 200 mg/100ml. The feed supplementation using a mixture of Lingzhi mushroom, roasted soybeans and organic chromium produced cholesterol level of hen blood serum ranged from 112.65 to 138.88 mg/100ml which is lower than the above statement. This study showed a decrease in cholesterol level in blood serum of laying hens after feeding by three supplementation diets (P2, P3 and P4), although the results showed no significant difference.

The group of laying hens given P2 and P3 diets, which were supplemented with lingzhi, showed lower cholesterol level compared to the other groups given P1 (control) and P4 diets. Supplementation using lingzhi showed a higher decrease in cholesterol level of 16.4% compared to research conducted by Nisa (2005) which showed decrease in cholesterol level of 3.8% after feed supplementation using *Sambilo* flour.

The study conducted by Irene (2001) showed body weight decrease in broiler chickens which were supplemented with lingzhi mushroom. It means that there was a decrease in body fat of the broilers which was allegedly due to reduction of cholesterol level. Lingzhi itself has several components such as adenosine and terpenoids which are known to be able to lower cholesterol level (Susanto 1998).

Based on the results of variance, feeding a mixture of the three supplements showed no significant difference in LDL level either P2 (Lingzhi, roasted soybeans, organic chromium), P3 (Lingzhi, roasted soybeans) or P4 (roasted soybeans, organic chromium). Mean LDL level in blood serum of laying hens fed by those three diets ranged from 52.64 to 58.67 mg/100ml. It showed a lower decrease in LDL level of 0.15% compared to research conducted by Nisa (2005) which showed LDL level 64.789 to 84.193 mg/100ml or 7.8% decrease after supplementation using *Sambilo* flour.

The risk of Atherosclerosis will be high if the ratio between HDL and LDL is less than 0.25. The results of this study showed that the ratio of HDL and LDL of all treatments were more than 0.25, which means lower risk of atherosclerosis in the layers. It also means the products are safe and healthy.

Based on the results of variance, feeding a mixture of the three supplements showed no significant difference in HDL level either P2 (Lingzhi, roasted soybeans, organic chromium), P3 (Lingzhi, roasted soybeans) or P4

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(roasted soybeans, organic chromium). Mean HDL level in blood serum of laying hens fed by those three diets ranged from 33.79 to 40.64 mg/100ml. Increased content of HDL in the blood negatively correlated to the risk of suffering Atherosclerosis.

Starich and Blincoe (1983) stated that organic chromium can be absorbed 20 to 30 times more efficient than inorganic one. Chromium compounds such as Cr₂O₃ has been recognized as a marker to determine feed intake (FI), digestibility of nutrients and eliminated minerals. Organic chromium has several benefits that are more soluble, easily absorbed, non-toxic compared to inorganic Cr.

Table 2 shows the presence of increasing chromium content in the eggs. Highest increase of 21.4% performed in the eggs produced by P2 fed layers, while P4 diet increased the chromium content of the eggs to 12.8%. The chromium content in the blood serum of laying hens declined after feeding by either P2, P3 or P4 diets. This suggested that chromium is rapidly secreted so that the risk of excess or chromium poisoning are rare.

The content of CLA in eggs slightly increased in each treatment (P2, P3, and P4) compared to control. It might happen because the given doses of roasted soybeans were low. The cholesterol content in eggs did not show any significant reduction in accordance with the terms of the CLA content of the eggs. It is because the greater the amount of unsaturated fatty acids the lower the cholesterol content. This happened because the fatty acids which cause high cholesterol are saturated fatty acids. Soy bean might be protected from rumen microbes degradation, due to roasted process, in turn it was absorbed in the digestive tract post rumen and transfer the nutrients for milk synthesise (Adawiah, 2006).

Table 3 showed egg production when laying hen consumed treated diet. Egg production at the age of 26 weeks should be able to reach 92% (Weaver and Bell, 2002). Even though the result in this study showed that providing the control diet followed by combination of those three supplements have not been able to reach that number, P3 diet could provide the greatest egg production compared to control diet. The low chromium transfer into the egg appears to be due to the prevention mechanism of the excessive accumulated absorption of this mineral in the eggs (Piva et al., 2002). Egg might be facilitated with certain mechanisms that prevent harmful nutrients toward the embryo, so that the chromium was absorbed in the limit that may be given. Chromium complex could be expected to interact with the catalyst oxygen to produce hydroxyl radicals (Vincent, 2000) and damage all types of macromolecules that could interfere with survival of the embryo, but it may decrease the concentration of cholesterol in egg yolk. Kim et al. (1997) stated that administration of chromium at a dose of 400 ppb picolinat reduces egg cholesterol content. This may be because chromium responds and cooperates in the activities of lipid metabolism.

Roasted soybeans supplementation at a dose of 1% of total fat ration into P2, P3 and P4 diet can not promote the egg production compared with control diet (P1) which was 68.57%. However, the average shows that P3 diet could potentially have a higher egg production of 76.33% than the control. Combination of lingzhi mushroom and roasted soybeans appeared to have positive collaboration in increasing egg production. It is probably caused by the potential of lingzhi in enhancing the immunity of livestock, especially in reducing stress level in hens during egg-laying period.

Effect of supplement treatment on physical quality of eggs

Table 4 showed physical quality of treated eggs. The eggs weight shows inversely proportional to the egg production. Therefore, the higher the egg weight the less the number of eggs and vice versa. Although the results in the analysis of variance was not significantly different, but it can be seen that P3 diet generates the lowest egg weight and the highest egg production. The weight of eggs produced in this study ranged from 49.94 to 52.22 grams, whereas 26-week-old laying hens should have produced eggs in weight of 56.4 grams (Weaver and Bell 2002). The analysis of variance showed that supplementation of these three supplements did not affect the weight of produced eggs. This is presumably because the protein and amino acids contained in the eggs that used for protein synthesis are in the same relative amount. Protein requirement in laying hens is associated with eggs size and its production. Reduction in the provision of amino acids or protein in the rations influences eggs weight and its production, depending on the level of supplementation. Slight decrease in methionine intake may affect the weight of eggs (Weaver and Bell 2002). Eggs weight is closely associated with albumin weight because the relative weight of albumin reached 50.8%; so if the albumin weight is high, the eggs weight is also high (Romanoff and Romanoff 1949).

Supplementation of organic chromium contained in the P2 and P4 diets did not affect the value of Haugh unit. Piva et al. (2001) reported that supplementation of chromium yeast on the rations of laying hens at a dose of 21.11 ppm and 4.1 ppm chromium did not affect Haugh unit. The same result was reported in the supplementation of chromium combined with folic acid (Eseceli et al., 2010). However, the Haugh unit value at P1, P2 and P4 treated group showed that the eggs can be categorized in AA class. While the P3 treated group is still categorized as A class. P3 treated group produced eggs that are smaller than the other treated groups and resulted smaller Haugh unit as well. The combination lingzhi and roasted soybeans seems produces lower fatty acids in the egg so that the eggs weight is low. Furthermore the protein in lingzhi and roasted soybeans have not been able to significantly improve the albumin weight. Haugh unit is influenced by the albumin viscosity, whereas a more specific protein that contribute to the quality of the albumin depicted on the albumin viscosity is lysosomes. High viscosity albumin is formed by the electrostatical interaction between β -ovomusin and lysosomes supported by calcium and magnesium ions (Yuwanta, 2009). Providing CLA has negative influence on the quality of eggs, but by combining it with 2% of another fat will reduce the rate of change in the eggs performance (Kim et al., 2007).

The analysis showed that all four treated group have a standar ration of egg yolk. The value of 7 on a scale of Roche means 43 micrograms beta-caroten and 27.5 milligrams xantophil per gram of egg yolk (North and Bell, 1990). Bright egg yolk (platinum) can be obtained from the provision of white maize, sorghum, wheat or barley (Jacob et al., 2000). The given rations in this study only contained about 520 g yellow corn per kg ration, so that it did not affect the color of egg yolk. Piva et al. (2003) stated that providing 540 g/kg of yellow maize, 30 g/kg alfalfa even 20 g/kg glutenized corn did not affect the reddish color of the eggs. Chromium influences a lot more on the glucose concentration in the cells. It controls the transportation of glucose in and out of the cells which commonly

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known as GTF (Glucose Tolerance Factor). The absorption and metabolism of chromium does not related to concentration of xantopil which gives color in egg yolk, so that it will not affect the color of egg yolks. Suksombat et al. (2006) reported that rations without CLA supplementation produced better color in egg yolk than the addition of CLA in the level of 0.82% -4.03%.

The highest eggshell weight was generated by the control rations (P1). This is likely due to the lack dose of chromium in supplements which is only 3 ppm. Amatullah et al. (1999) reported that administration of 250-500 mg/kg hexavalent chromium can increase eggshell thickness. Piva et al. (2002) reported that the addition of chromium supplementation has no effect on eggshell weight. Eseceli et al. (2010) reported that the addition of chromium did not affect eggshell quality, however, it can improve the quality of albumin and egg yolk.

Roasted soybeans supplementation did not give effect to the weight and eggshell thickness. The factors causing poor quality of eggshell includes the lack of phosphorus contained in the ration which may increase mortality on caged chickens (North and Bell, 1990). Fatty acid content in roasted soybean seems to only be utilized in the metabolism of fat, while eggshell forming mineral Calcium can not be helped to deposit to eggs; or in other words, CLA has opposite effect on chickens (Yeung et al., 2000). Highest eggshell weight was generated by control group (P1). Although the content of phosphorus in lingzhi is quite high compared to other minerals that is 41.50 mg/g (Parjimo and Soenanto, 2008), lingzhi supplementaion in P2 and P3 diets could not affect the eggshell thickness. It may be caused by the phosphorus contained in those diets has not been able to absorbed by the eggs.

CONCLUSION

Feed supplementation with lingzhi mushroom (*G. Lucidum*), organic chromium, and roasted soybeans had no effect on the overall performance of chicken eggs. The given dose of lingzhi, organic Cr, and roasted soybeans did not seem to give any effect associated with egg physical performance. Highest egg production was generated by P3 treated group, however, it has low Haugh unit and also the lowest egg weight. Therefore P3 rations is good choice if the main objective is promoting the quantity of eggs. Whereas P1 and P3 rations are good alternative to produce better quality of eggs.

The supplementation of those three supplements is also not able to lower the cholesterol content in eggs, but the addition of lingzhi on feed can lower blood cholesterol level in laying hens. There was also an increase of chromium content in the egg on the addition of the supplements.

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Tabel 1. The basic ration composition and nutrition

Feed	Amount (%)	Nutrient	Amount
Corn	52	Metabolic Energy(kcal/kg) ¹	2851,48
Rice bran	16	Crude Protein (%) ²	17,44
MBM	7	Crude Fat (%) ²	5,35
Soybean meal	10	Crude Fibre (%) ²	5,28
Fish meal	5,75	Ca (%) ²	3,44
Coconut oil	1,75	P (%) ²	0,44
Premix	0,5	Lysine (%) ¹	1,0
DCP	1	Methionine (%) ¹	0,4
CaCO ₃	6,5		
Total	100		

Notes: 1) Feed composition according to Leeson and Summer (2005). 2) Proximate analysis from Laboratory of Science and Feed Technology (2009).

Table 2. Serum and eggs cholesterol, chromium and CLA in laying hens

Parameters	P1	P2	P3	P4
Serum Cholesterol (mg/100ml)	138,88±41,72	119,56±17,71	112,65±22,86	129,92±37,88
LDL (mg/100ml)	55,28±12,51	54,28±29,99	58,67±28,57	52,64±26,16
HDL (mg/100ml)	36,90±10,31	40,55±1,90	38,19±6,10	41,47±10,80
Cholesterol in egg (mg%)	2,107	2,29	2,323	2,073
Cr serum (ppm)	0,0526±0,03	0,0361±0,004	0,0181±0,02	0,0121±0,01
Cr eggs (ppm)	1,87±0,22	2,27±0,31	1,97±0,20	2,11±0,18
CLA in egg (%)	0,42	0,48	0,5	0,44

Notes: P1 = Control (without supplement); P2 = P1 + Lingzhi + org Cr + roasted Soybean; P3 = P1 + Lingzhi + roasted Soybean ;P4 = P1 + roasted soybean + org Cr

Table 3. The influence of supplement to egg production

Week	Ration of Treatment			
	P1	P2	P3	P4
	-----%-----			
	--			
1	70,95±8,12	59,52±12,48	61,67±35,50	72,38±6,44
2	72,38±2,97	69,52±9,72	84,28±6,06	74,76±7,87
3	74,76±4,36	74,76±2,97	65,71±0,00	73,81±6,60
4	63,81±4,36	67,14±6,23	85,71±4,04	61,43±11,43
5	60,96±5,77	62,38±6,75	84,28±2,01	65,71±5,71
Number	342,86	333,32	381,65	348,09
Mean ±sd	68,57±5.90	66,66±5.99	76,33±11.64	69,62± 5.79

Notes: P1 = Control (without supplement); P2 = P1 + Lingzhi + organic Cr + Roasted Soybeans; P3 = P1 + Lingzhi + Roasted Soybeans; P4 = P1 + roasted soybean + organic Cr

Table 4. Physical quality of treated eggs

Variables	Rations of Treatment			
	P1	P2	P3	P4
Egg weight (g / grain)	52,22±4,28	51,67±3,65	49,83±4,04	50,83±4,22
Haugh Unit	73,88±27,07	73,02±30,67	68,95±28,14	73,22±27,70
Egg Yolk Color	7,44±1,54	7,53±1,28	7,56±1,44	7,53±1,18
Eggshell weight (g / grain)	6,43±0,79	6,36±0,43	6,17±0,65	6,41±0,88
Eggshell thickness (mm)	0,41±0,03	0,42±0,03	0,42±0,03	0,42±0,03

Notes: P1 = Control (without supplement); P2 = P1 + Lingzhi + organic Cr + Roasted Soybeans; P3 = P1 + Lingzhi + Roasted Soybeans; P4 = P1 + roasted soybean + organic Cr

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P-12. FERMENTABILITY AND DEGRADATION OF CONCENTRATE CONTENTS DRY CARBOXYLATE SALT OR METHYL ESTER IN RUMEN LIQUID

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Abstract

High unsaturated Fatty Acid n-3 (HUFAs n-3) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has been reported to exert beneficial effect on cardiofascular health, and their deficiency was positively associated with dyslexia. These fatty acids were contained in ruminants product such as milk and beef. The dry carboxylate salt(DCS) and dry methyl ester (DME) was oil fish processing product. It was a feed supplement as EPA and DHA. The experiment was design to evaluate to effect of DCS's and DME's concentrate in ruminal fermentation. It used completely randomized design with 5 treatments (Concentrate 14% Crude Protein and 70% TDN with 0, 15, 30, 45, 60 gkg⁻¹ of DCS and DME). Rumen liquid was used to fermentation from slaughter house. Variable experiment were ammonia and totak VFA concentration and dry matter degradation. The Result Showed that 30 to 60 gkg⁻¹ of DCS in concentrate decreased ammonia concentration ($6,2 \pm 0,1^b$, $5,3 \pm 0,3^c$ and $4,3 \pm 0,1^d$ vs $8,0 \pm 0,2^a$), but level 15 to 60 gkg⁻¹ DCS increased total VFA concentration ($61,05 \pm 0,1^b$, $61, 2 \pm 0,1^{ab}$, $61,5 \pm 0,1^a$, and $61,7 \pm 0,1^a$ vs $60,4 \pm 0,4^c$) whereas it was decreased dry matter degradation ($60,67 \pm 0,1^b$, $61, 2 \pm 0,2^b$, $61,0 \pm 0,3^b$, and $51,6 \pm 0,5^b$ vs $64,3 \pm 0,0^a$). Level 15 to 30 gkg⁻¹ DME in concentrate increased ammonia concentration ($9,6 \pm 0,2^a$, $8,9 \pm 0,1^b$ vs $8,0 \pm 0,2^c$) but 15-60 gkg⁻¹ DME concentrate increase total VFA concentration ($61,3 \pm 0,1^b$, $61, 6 \pm 0,3^a$, $61,3 \pm 0,0^{ab}$, and $61,2 \pm 0,3^a$ vs $60,4 \pm 0,4^c$) whereas it was decreased dry matter degradation ($55,8 \pm 0,3^c$, $54,7 6 \pm 0,2^c$, $61,1 \pm 0,4^b$, and $54,9 \pm 0,3^d$ vs $64,3 \pm 0,0^a$). Although that, it was still in the range normal concentration. The conclusion of this experiment that concentrate with 15, 30,45, and 60 gkg⁻¹ DCS's and DME's could be used to ruminant dietary.

Keywords: Fermentability, Degradation, Dry Carboxylate Salt, Methyl Ester, Rumen Liquid.

INTRODUCTION

The dry carboxylate salt (DCS) was result of drying a mixture carboxylate salt with tapioca. Whereas dry methyl ester (DME) was result of drying a mixture of methyl ester with tapioca. These product were experted to a feed supplement as source omega 3 EPA (C_{20:5n3}) and DHA (C_{22:6n3}). Before it was to ruminant dietary.

High unsaturated fatty acid n-3 (HUFA_{5n-3}) such as eicosapentaenoit acid (EPA) and docosahexaenoic acid (DHA) has been reported to exert beneficial effect on cardiovascular health and their deficiency was positively associated with dyslexia. These fatty acids were containing in ruminant product such as milk and meat.

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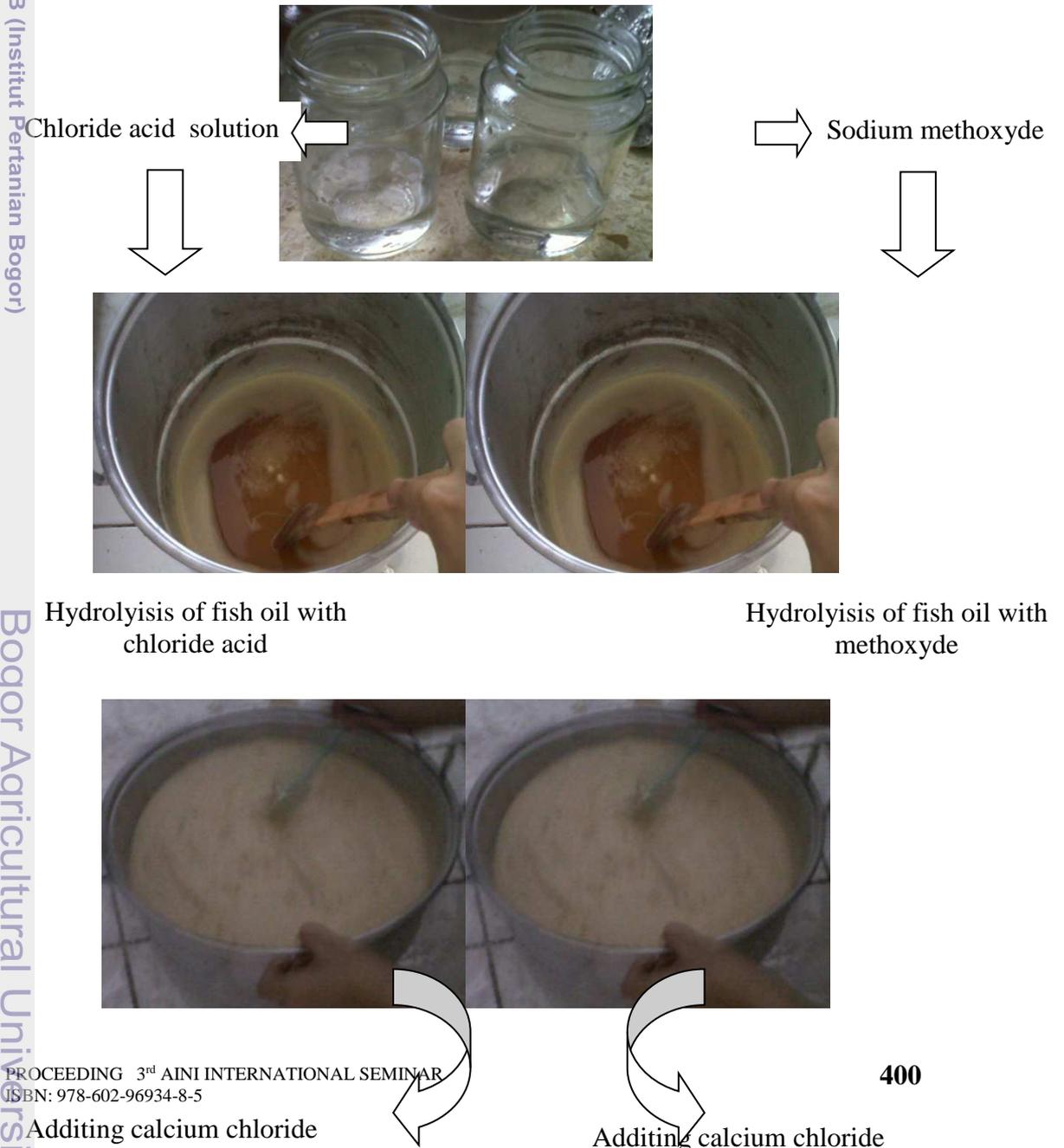
One of requirement to feed supplement, could maintenance or did not disturb to digestion in ruminal was indicated by ammonia (NH₃) and total VFA concentration, and degradability of dry matter. These components were in vitro variables.

Laboratory in vitro method was conducted to evaluate the fermentability and the digestibility of feedstuffs. Feedstuffs are digested by preparation of microorganism or enzymes which are similar function to these present in the digestive tract of the ruminant.

Ammonia was product of protein fermentation digestibility of dry matter was total nutrient which was degradation in rumen. The present research was aimed to evaluate nutrient fermentability and dry matter digestibility of dry carboxylate salt and dry methyl ester supplement which was formulated in concentrates.

MATERIALS AND METHODS

Dry Caboxylate Salt (DCS's) and Dry Methyl Ester (DME's)





Mixing carboxylate salt with tapioca

Mixing methyl ester with tapioca



Dry carboxylate salt

Dry methyl ester

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Treatment

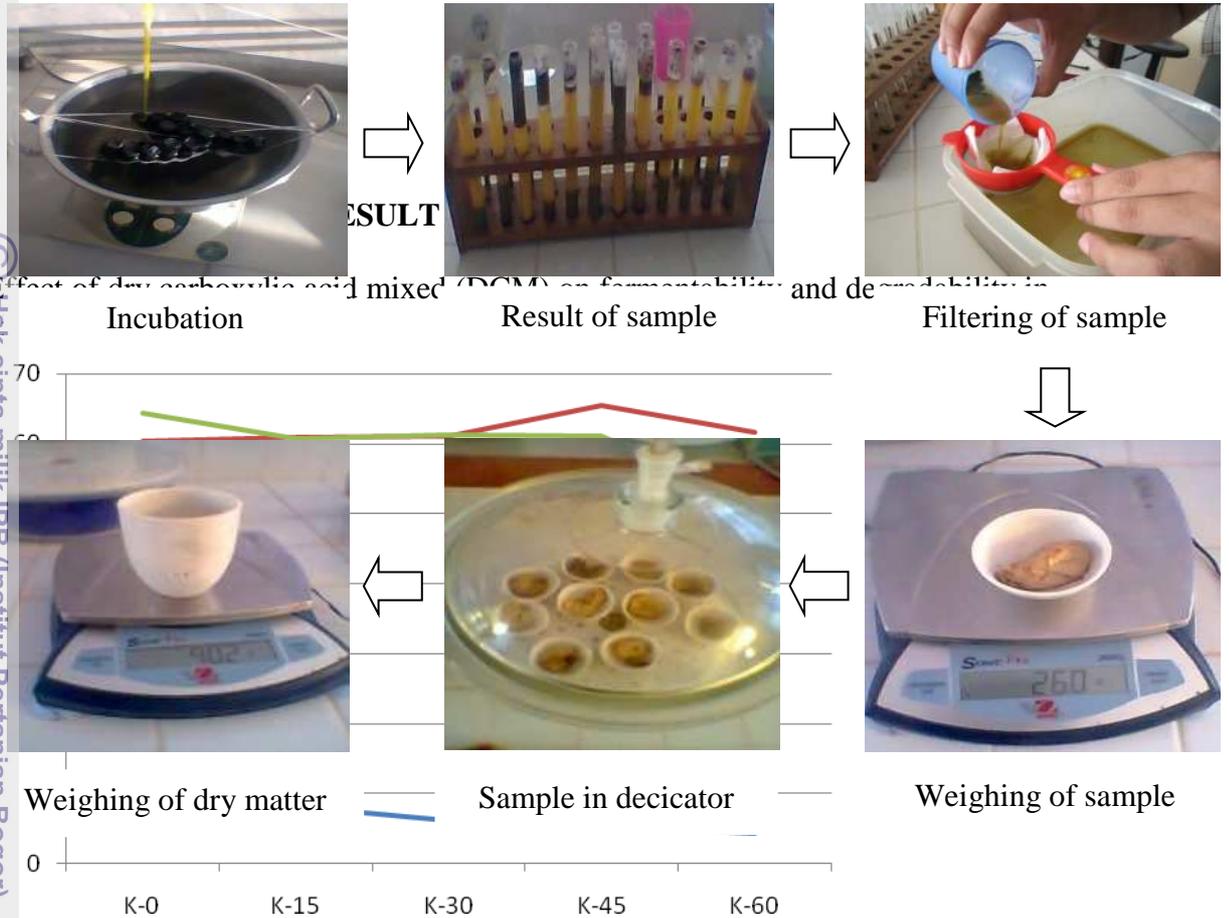
Dry Caboxylate Salt (DCS's)

- C-0 = Concentrate with 12% Crude Protein, 17% TDN
- C-15 = C-0 + 15 gkg⁻¹ DCS
- C-30 = C-0 + 30 gkg⁻¹ DCS
- C-45 = C-0 + 45 gkg⁻¹ DCS
- C-60 = C-0 + 60 gkg⁻¹ DCS

Dry Methyl Ester (DME's)

- M-0 = Concentrate with 12% Crude Protein, 70% TDN
- M-15 = M-0 + 15 gkg⁻¹ DCS
- M-30 = M-0 + 30 gkg⁻¹ DCS
- M-45 = M-0 + 45 gkg⁻¹ DCS
- M-60 = M-0 + 60 gkg⁻¹ DCS

In Vitro Test



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Picture 1. Effect of dry carboxylic acid mixed (DCM) on fermentability and degradability in vitro

The range of ammonia concentrations of C-0 to C-60 were 4,3 to 8,1 mM in the normal range 4 to 12. The range of total VFA concentration of C-0 to C-60 were 60,4 to 61,5 mM in the lower than 80 to 160 mM. The range of degradability of C-0 to C-60 were 51,6% to 64,3% in the normal range.

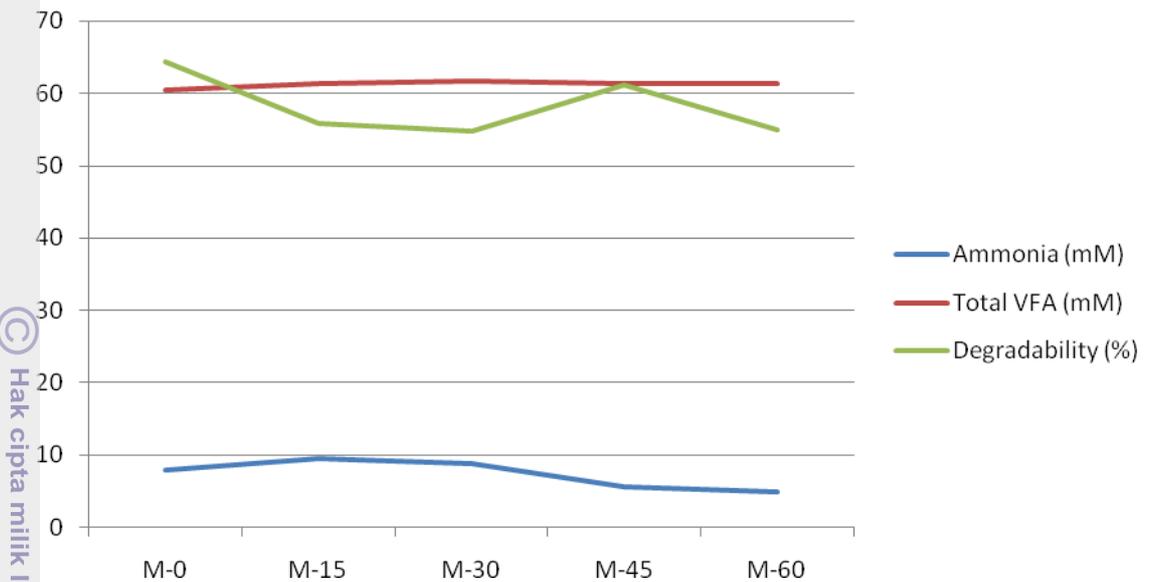
Effect of dry methyl ester mixed (DMM) fermentability degradability in vitro

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Picture 2. Effect of dry methyl ester mixed (DMM) fermentability degradability in vitro

The range of ammonia concentration of M-0 to M-60 were 5,0 to 9,0 mM in the normal range 4 to 12 mM. The range of total VFA concentration of M-0 to M-60 were 60,4 to 61,6 mM lower than 80 to 160 mM. The range of degradability of M-0 to M-60 were 54,6% to 64,3%.

CONCLUSION

The concentrate with 15, 30, 45 and 60 gkg⁻¹ dry carboxylate salt (DCS's) and dry methyl ester (DME's) could be used to ruminant dietary.

ACKNOWLEDGMENTS

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P-13. COMPARATIVE ANALYSIS OF NUTRIENT COMPOSITION OF DIFFERENT SORGHUM VARIETIES AFTER ENSILAGE PROCESSES

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Abstract

This research was conducted to observe the nutrient composition of some many varieties of sorghum silages, before and after ensiling treatment. The whole sorghum plant was a product of the sorghum bioethanol project among LIPI and Sorghum Japan. Sorghum was maintained for 90 days, however napier grass (*Pennisetum purpureum*) was cut at 75 days. Plants were cut into pieces of 2 cm and then mixed with other ingredients such as rice bran, molasses, water, and salt and then fermented for 21 days. Silage was harvested and analyzed proximate completed. Proximate analysis variables including the percentage of moisture, ash, crude protein, crude fiber, ether extract content, and nitrogen free extract (NFE). There were significant differences ($P < 0,05$) on moisture, dry matter, ash, crude protein, crude fiber, ether extract and NFE of all varieties before ensiling. There were significant differences ($P < 0,05$) on ash, crude protein, crude fiber, and NFE after ensilage. Based on the results of this research, we conclude that ensilage processes was significantly influenced on nutrient composition consisted of ash, crude protein, crude fiber, and NFE. There were no differences of different varieties of sorghum on nutrient composition when sorghum was ensilaged, however for ash, crude protein, crude fiber, and NFE composition significant differences on sorghum varieties.

Keywords: Sorghum, Proximate Analysis, Silage

INTRODUCTION

Ensilage technology is the forages processing technology for the preservation purpose (Erowati, 2000; Aganga, *et al.*, 2005; Sofyan, 2010). The limitation stock of forages in dry season imply serious problem for ruminant productivity especially dairy cattle. Several forages were used as silages for ruminant feed. The application of napier grass (*Pennisetum purpureum*), rice straw, corn stalk, nor the vegetables residue from traditional market as silages had ever been reported (Muwakhid, *et al.*, 2007; Sofyan, 2010; Sofyan *et al.*, 2007), and even a silage production of sorghum (*Sorghum bicolor*). The cultivation of sorghum for silage production has become an increasingly common practice justified by its agronomic characteristics and chemical composition (Oliveira *et al.*, 2007).

Whole sorghum plant has a good potency for silage production. Jansman (1993) stated that polyphenols found in sorghum as a condensed tannins. The presence of tannins during silage fermentation gave an advantage because of their forage proteins guarding from degradation (Kondo *et al.*, 2004). Many researcher concern explore sweet sorghum as bio-ethanol. Due to sweet sorghum (*Sorghum bicolor* (L.) Moench) which have a high concentration of soluble sugars in the plant sap or juice. This crop is attractive because of the easy accessibility of readily fermentable sugars combined with very high yields of green biomass

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(Vermerris *et al.*, 2011). Sweet sorghum is a domesticated grass containing a sugar rich juice can be readily utilized for ethanol production (Jia *et al.*, 2013).

However, study of nutrient composition of sweet sorghum after ensilage process is still limited in scientific publication. The comparative study of nutrient composition between some varieties of sorghum was needed, to determine a good silage product quality of sorghum, and to evaluate the effect of variety and ensilage treatment on nutrient composition neither whole sorghum nor silage.

MATERIALS AND METHODS

The Time and Place of Study

An experiment was conducted on January-June 2013 in the Laboratory of Animal Feed Processing and Chemical Analysis Laboratory, in Research Unit for Processes Development and Chemical Engineering (UPT. BPPTK), Indonesian Institute of Sciences (LIPI), Yogyakarta. Forages (sorghum and napier grass) were sampled from field laboratory UPT BPPTK LIPI, Yogyakarta. Sorghum was harvested at 90 days from each variety (B.6, B.16, B.19, B.2, B.62, B.7), however napier grass was cut at 75 days. Complementary materials such as rice bran and molasses were purchased from the local feed shop.

Ensilage Processes

Sorghum silage was processed with withering whole plant of B.6, B.16, B.19, B.2, B.62, and B.7 varieties for 24 hours. Whole plant is a mixture of stems, leaves, flowers, and seeds and chopped in a size of ± 2 cm. Whole plant was chopped and mixed with other additives materials to the composition according to Table 1.

Table 1. Composition of Sorghum Silage in Different Varieties

Ingredients	Volume
Chopped sorghum	19-21 kg
Rice bran	1,9-2,1 kg
Molases	200-225 ml
Water	4-5 liter
Napier grass (<i>Pennisetum purpureum</i>)	180-220 gram

All of ingredients was mixed homogenously and added by water up to moisture content 40-60%. Treatment (six variety) was arranged on completely randomized design (CRD) with two replications for each treatment. Mixture was compressed into a thick plastic and tightly bound. Then, mixed material was stored in polybag and incubated during 21 days in room temperature (25-30 °C).

Proximate Analysis

Moisture and non-organic compound (ash) content were evaluated using thermogravimetry. Crude protein content was analyzed according to Kjeldahl method. Ether extract content of the sample was measured by extraction in a Soxhlet apparatus with petroleum ether. The chemical method (NaOH and H₂SO₄) was done for crude fiber content analysis. Ash content by weighing residual mineral combustion at temperatures around 550°C (AOAC, 1990; Apriyantono *et al.*, 1988).

Data Analysis

Data from sorghum or ensilage sorghum nutrient composition consisted of dry matter (DM), crude protein (CP), crude fiber (CF), ether extract (EE) and nitrogen free extract (NFE) were analysed using analysis of variance (ANOVA) with Duncan Multiple Range Test (DMRT) for comparing between treatment

mean. Statistical analysis was performed using CoSTAT® Statistical Software (LIPI License).

RESULTS AND DISCUSSION

Nutrient composition of sorghum before ensilage was indicated by Table 2. Based on the data, chemical composition of each variety of sorghum seem varied means. Moisture, dry matter, crude protein, ash, and fat the highest shown by sorghum B.2 varieties ($P < 0.05$). It also has the highest glucose content (Table 4). Sugar content (Brix value) in a sorghum is related to the NFE value in proximate analysis. Nitrogen free extract (NFE) is parameter indicated the content of non-structural carbohydrate (starch). In the ensilage processes, starchy materials used by microorganism especially lactic acid bacteria to produce lactic acid (McDonald *et al.*, 1991).

Sugar content or Brix values in sorghum averaged about 14.8% (Vermerris *et al.*, 2011). Previously, McDonald *et al.* (1991) reported that sorghum contained 65-176 g/kg DM (16.5-17.6%) of total sugar and 53-72 g/kg DM (5.3-7.2%) of starch when it harvested early milky dough stage. Differences sugar content of sorghum caused by maturity, variety, and environment factor (soil fertility, temperature etc).

Table 2. Composition and Nutrient Content (DM Basis) of The Various Sorghum

Nutrient Content	Variety of Sorghum					
	B.6	B.16	B.19	B.2	B.62	B.7
Moisture (%)	5.44 ± 0.36 ^{bc}	5.57 ± 0.25 ^b	5.38 ± 0.21 ^{bc}	6.44 ± 0.30 ^a	4.95 ± 0.10 ^c	4.97 ± 0.32 ^c
Dry Matter (%)	94.56 ± 0.36 ^{ab}	94.43 ± 0.25 ^b	94.62 ± 0.21 ^{ab}	93.56 ± 0.30 ^c	95.05 ± 0.10 ^a	95.03 ± 0.32 ^a
Ash (%)	6.55 ± 0.27 ^c	7.66 ± 0.64 ^b	8.94 ± 0.30 ^a	8.64 ± 0.54 ^a	6.36 ± 0.14 ^c	6.77 ± 0.24 ^c
Crude Protein (%)	6.74 ± 0.40 ^c	7.38 ± 0.28 ^b	7.26 ± 0.37 ^b	7.09 ± 0.05 ^{bc}	7.56 ± 0.30 ^b	8.18 ± 0.07 ^a
Crude Fiber (%)	29.43 ± 1.06 ^c	34.64 ± 0.72 ^a	35.07 ± 0.24 ^a	32.32 ± 0.79 ^b	27.43 ± 0.10 ^c	29.17 ± 0.74 ^c
Ether extract (%)	3.44 ± 0.38 ^a	3.65 ± 0.28 ^a	3.82 ± 0.51 ^a	1.72 ± 0.09 ^b	3.58 ± 0.22 ^a	3.39 ± 0.33 ^a
NFE (%)	53.84 ± 0.98 ^{ab}	46.67 ± 1.05 ^c	44.91 ± 0.67 ^d	50.22 ± 0.86 ^c	55.07 ± 0.34 ^a	52.49 ± 0.34 ^b

Note: ^{a,b,c} means in the same row with different superscript differ significantly ($P < 0.05$)

Nutrient composition of sorghum after ensilage is indicated by Table 3. There were no significant differences ($P > 0.05$) on moisture, dry matter and ether extract in different sorghum varieties. In other side, crude protein, ether extract, crude fiber and nitrogen free extract significantly influenced ($P < 0.05$) by ensilage processes.

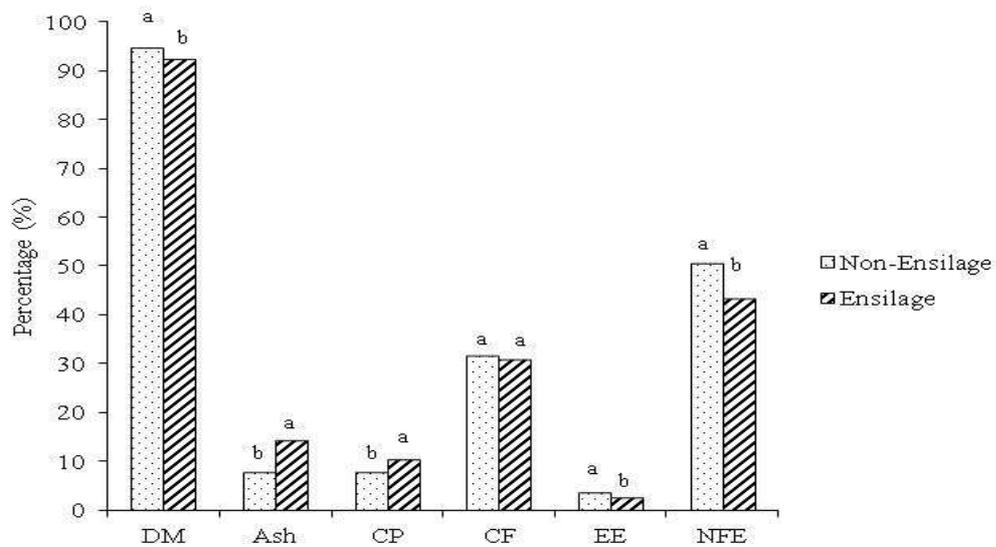
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(Note: DM: dry matter, CP: crude protein, CF: crude fiber, EE: ether extract, NFE: nitrogen free extract, Superscript with different letter at the same variable indicated significant difference (P<0.05))

Figure 1. Comparative analysis of sorghum nutrient composition influenced by ensilage processes)

Meanwhile, the composition of the ash, crude protein, crude fiber, and NFE showed significant variation between varieties. Sorghum contained 165-176 g/kg DM of total sugar and 53-72 g/kg DM of starch when it harvested early milky stage (McDonald *et al.*, 1991).

Table 3. Composition and Nutrient Content (DM Basis) of The Various Sorghum Silages

Nutrient Content	Variety of Sorghum					
	B.6	B.16	B.19	B.2	B.62	B.7
Moisture (%)	7.87 ± 0.61 ^a	7.32 ± 0.68 ^a	7.83 ± 0.98 ^a	7.79 ± 1.41 ^a	7.14 ± 0.54 ^a	7.48 ± 0.85 ^a
Dry Matter (%)	92.13 ± 0.61 ^a	92.68 ± 0.68 ^a	92.17 ± 0.98 ^a	92.21 ± 1.41 ^a	92.86 ± 0.54 ^a	92.52 ± 0.85 ^a
Ash (%)	16.78 ± 0.61 ^a	11.99 ± 1.97 ^b	16.04 ± 0.79 ^{ab}	13.04 ± 2.62 ^{ab}	13.24 ± 3.01 ^{ab}	12.08 ± 2.27 ^b
Crude Protein (%)	11.38 ± 0.35 ^a	10.36 ± 1.11 ^{ab}	9.59 ± 0.80 ^{ab}	11.29 ± 1.66 ^a	8.90 ± 0.41 ^b	9.25 ± 1.11 ^b
Crude Fiber (%)	30.74 ± 1.32 ^{abc}	27.56 ± 2.46 ^c	33.84 ± 0.33 ^a	30.99 ± 2.68 ^{abc}	31.84 ± 3.18 ^{ab}	29.57 ± 0.68 ^{bc}
Ether extract (%)	1.64 ± 0.07 ^a	2.28 ± 0.83 ^a	1.90 ± 0.67 ^a	1.94 ± 0.16 ^a	2.99 ± 1.99 ^a	2.19 ± 1.97 ^a
NFE (%)	39.46 ± 1.86 ^c	47.81 ± 3.06 ^a	38.63 ± 0.87 ^c	42.73 ± 2.75 ^{bc}	43.03 ± 2.16 ^{bc}	46.91 ± 3.13 ^{ab}

(Note: ^{a,b,c} means in the same row with different superscript differ significantly (P<0.05))

Total sugar of different varieties of sorghum before ensilage processes is showed by Table 4. Sugar content of B.2 (14.1%) variety was the highest value followed by B62, B16, B6, B7, and B19. Sweet sorghum juice usually contain 16-18% of fermentable sugar which are mainly comprised of sucrose, glucose, and fructose (Jia *et al.*, 2013).

Based on the data, there was no correlation between sugar content and alteration nutrient composition. It was indicated by B16 ensilaged-sorghum variety was the best nutritional composition. NFE content, crude protein seem to be higher when crude fiber was lower than other variety.

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Table 4. Total sugar of different varieties of sorghum before ensilage processes*

Variety of Sorghum	%
B.2	14.1
B.6	8.4
B.62	11.1
B.7	7.6
B.16	9.1
B.19	3.6

*Reffered from Analysis Result of Sorghum Japan Company – UPT. BPPTK-LIPI Research Project 2013

Although NFE value was closely related with the sugar content, in this experiment resulted different phenomenon. It might be due to the part of sample was analyzed and it need to be continued to replicate by several sample. In silage fermentation, microbial activity was influenced by nutrient composition and water activity. Sweet sorghum as known as high sugar content was supported ensilage successfully.

CONCLUSION

Ensilage processes was significantly influenced on nutrient composition consisted of moisture, dry matter, and ether extract. There were no differences of different varieties of sorghum on nutrient composition when sorghum was ensilaged, however for ash, crude protein, crude fiber, and nitrogen free extract (NFE) composition significant differences on sorghum varieties. Future analysis is necessary to conduct for comparing total sugar and nutrient composition of silage.

ACKNOWLEDGEMENT

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P-14. VIABILITY OF LACTIC ACID BACTERIA ISOLATED FROM RUMEN LIQUOR ON MOLASSES MIXTURE MEDIUM

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Abstract

An experiment was conducted to study the effect of molasses level and storage temperature on the viability of lactic acid bacteria as well as to determine the most optimal treatment in improving the shelf life of the viability of lactic acid bacteria. The research had been performed using a completely randomized design (CRD) which consisted of two factors. The first factor was concentration of molasses with three levels (2%, 1.5%, and 1%). The second factor was storage temperature with two levels (cold temperature (4°C) and room temperature (28°C)). Physiological and biochemical parameters were observed, the calculation of the number of bacteria using Total Plate Count method, measurement of acidity (pH) using pH meter, total reducing sugars using DNS method and identification of isolates using API 50 CHL (API bio Mérieux). Data collected were analyzed using ANOVA followed by DMRT. The results showed that the MRSB treatment and storage temperature influence in increasing the viability of lactic acid bacteria. International Dairy Federation (IDF) provided a standard minimum number of live probiotic as a reference is 10⁶ cfu/ml in the final product. The most optimal treatment in improving the shelf life of the viability of lactic acid bacteria is 2% molasses and 1.5% molasses concentration at cold temperature (4°C) during 8 weeks, whereas at room temperature (28°C) is molasses 2%, molasses 1.5%, and molasses 1% for 3 weeks. Identification of lactic acid bacteria isolates showed that the *Lactobacillus brevis* (98.1%).

Keywords: LAB, probiotic, viability, molasses

INTRODUCTION

Lactic acid bacteria are bacteria that can ferment sugars or carbohydrates in food into lactic acid. At this time the use of lactic acid bacteria not only as microorganisms that play a role in food fermentation processes, but also can be used as a functional purpose such as the development of probiotic products. Several genera of lactic acid bacteria is *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* (Khalid, 2011).

Cell damage caused by lactic acid bacteria for preservation treatment and storage can lead to malfunction of the product such as probiotics. On the other hand in a fresh culture of safety cannot be done in the long term. Thus need a method of preservation of lactic acid bacteria that can maintain viability. Storage environment affects the damage of cell culture. Storage temperatures will affect the resistance of the cell culture. Encapsulation of *Lactobacillus acidophilus* R0052 can produce cell viability from 9.1 x 10⁹ CFU to 5.3 x 10⁹ on day 50 at 4 °C storage (Siuta-Cruce and Goulet, 2001).

Molasses is a medium which is already known as a bacterial growth substrate. Molasses contains about 52% sugar in the form of sucrose, glucose and fructose. Glucose levels in molasses with 5000X dilution giving absorbance of 0.514 were 159.95 ppm at a wavelength of 490 nm. Further with reference glucose levels commonly used in the fermentation medium (2%) corresponding to the optimum growth of bacteria. The use of relatively low levels of molasses has a specific reason, namely because it forms a thick molasses will increase pressure on the osmoses making it less efficient substrate for bacterial growth (Kusmiati et al., 2007). Moreover, probiotics can be produced cheaply and convenient storage at 28 °C and longer storage at 4 °C.

MATERIALS AND METHODS

Materials

Materials were used consisted of lactic acid bacteria (LAB) isolated from rumen liquor (code RS2), MRS (*de Mann Rogosa Sharpe*) broth (Oxoid, CM0359), MRS Agar (Oxoid, CM0361), molasses, 3.5 dinitrosalisilat, NaOH, sodium metabisulphite, Na-K tartat, phenol, distilled water, HCl, glucose-d- monohydrate, API 50 CHL kit (bioMérieux) were obtained from the Laboratory of Microbiology, UPT BPPTK LIPI Yogyakarta.

Methods

Bacterial inoculation

Lactic acid bacteria isolated from rumen liquor that have been regenerated as much as 20 mL of media were inoculated into 400 mL molasses concentration and incubated at 37 °C for 24 hours.

Medium Containing Molasses Preparation

Medium *de Mann Rogosa Sharpe Broth* (MRSB) as much as 52 grams homogenized in 1 liter of distilled water. Glucose levels in the 5000X dilution of molasses with reference to glucose levels commonly used in the fermentation medium (2%) suitable for optimum growth of bacteria (Kusmiati et al., 2007). Molasses dilution was calculated by the formula:

$$V1 \times M1 = V2 \times M2$$

Description:

V1 = initial volume of molasses

M1 = initial molar of molasses

V2 = required volume of molasses

M2 = required molar of molasses

After molasses and MRSB homogeneous, 500 mL erlenmeyer was prepared and bacterial growth media filled 400 mL with the composition according to Table 1.

Table 1. Optimal concentrations of molasses media concentration

Concentration Molasses (%)	Composition Medium	
	Molasses 2% (mL)	MRSB (mL)
2.0	400	0
1.5	300	100
1.0	200	200

A variation in different temperature storage was performed at a temperature of 4 °C cold and room temperature 28 °C and stored during 0, 1, 2, 3, 4, 8, and 12 weeks.

Bacterial Enumeration and Reducing Sugars Measurement

Lactic acid bacterial (RS₂isolates) that had been grown in medium diluted MRSA, condensed as much as 100 mL with spread plate method and then incubated for 24 hours at 37 °C. Counting the number of bacterial colonies that meet the standard plate count was numbered 30-300 colonies in a petri dish.

Reducing sugars was measured according to DNS methods (Miller, 1959). Reducing sugar testing DNS using a standard curve by entering 1 mL samples of molasses mixed media and MRSB into a test tube, then added 3 mL of DNS reagent. The solution is placed in boiling water for 5 minutes. Left to cool at room temperature. Then measured the absorbance at 550 nm wavelength.

Identification of isolates

Biochemical tests performed using API 50 CHL kit (bioMérieux). How to use it is by dissolving media isolates of MRSA results streak plate incubation at 37 °C for 24 hours by ± 2 use to the API 50 CHB / E medium. Each API 50 CHL strips inoculated with 100 μ L of the suspension and covered with 2 drops of mineral oil in order to create anaerobic atmosphere (Ozgun and Hasibe, 2011). Incubated for 24-48 hours. Said test results positive (+), when the 49 types of sugar that the color changes from red to yellow. The result is a biochemical profile that is used to identify the species of BAL using identification software that is APIWEB (bioMérieux).

Data Analysis

Data were got analyzed using ANOVA (*One Way Completely Randomized*) to determine the effect of treatment on the variable being measured. If there is a significant difference between treatments followed by *Duncan Multiple Range Test* (DMRT) at the level of 95% which is operated by CoSTAT[®] *Statistical Software* (LIPI License).

RESULTS AND DISCUSSION

Total Lactic Acid Bacteria

Increase in the number of cells affected by the bacterial cell adaptation period to the environment in the form of nutrients. Table 2 indicated that the total plate count results greatly in the storage room temperature (28 °C) compared to media stored for 1 week at a cold temperature (4 °C). According Widyani and Suciaty (2008) when the bacteria were stored at 4 °C of microbial activity will decrease, however, it is note to stop its metabolic activity. Bacteria just need to do a bit of nutrition metabolism. A number of bacteria in different medium and time of incubation showed on Table 2.

On the 2nd week storage, there is a decline in the viability of lactic acid bacteria. This is due partly because it reduced the nutrients found in the substrates. However, bacteria can still survive until certain storage. It means good quality probiotic products if the number of bacterial cells in which more than 10⁶ cfu/ml. At cold temperatures (4 °C) lactic acid bacteria which can last up to 8 weeks of storage, with a logarithmic decline in various media were 6.45 log cfu/ml (2% molasses) and 6.00 log cfu/ml (molasses 1,5%).

Table 2. Total bacteria on molassesmedia at different incubated temperature

Temp. (°C)	Molasses (%)	Incubation time (weeks)						
		0	1	2	3	4	8	12
----- (log cfu/mL) -----								
4 (cold)	2							3.56±0.1
	1.5	7.37±0.31	7.89±0.36	7.35±0.57	7.52±0.35	7.41	6.45	9
	1	8.29±0.37	8.34±0.36	7.26	7.19	7.47	6.00	2.08
28 (room)	2	8.45±0.35	8.18±0.11	7.26	7.27	6.72	5.77	5.86
	1.5	7.50±0.26	8.09±0.01	6.88	6.06±1.44	4.48±0.1	7	3.30
	1.5	8.45±0.32	8.35±0.07	6.99	6.43	5.72	3.00	1.00
	1	8.47±0.03	8.56±0.25	7.14	6.62	3.94±0.4	1	4.30

Description: good quality probiotic products if the number of bacterial cells in which more than 10⁶CFU/ mL

While at room temperature (28 °C) lactic acid bacteria can survive up to 3 weeks of storage, with a logarithmic decline in various media was 6.06 ± 1.44 log cfu/ml (2% molasses), 6.43 log cfl/mL (molasses 1.5%), and 6.62 log cfu/ml (1% molasses). However, viability is still relatively stable because the viability of the bacteria is still quite high in the 8th week of storage to cold temperatures and 3rd week at room temperature. *International Dairy Federation* (IDF) provides a standard minimum number of live probiotic as a reference is 10⁶ cfu/ml in the final product (Indratingsihet *et al.*, 2004).

Degree of Acidity (pH) value

Changes in pH can affect the decrease in the number of cells that grow in the next phase (Usmiati and Juniawati, 2011). Table 3 shows the decrease in pH value of molasses concentration on the storage media at room temperature (28 °C) and cold temperature (4 °C).

Table 3. pH value of media concentration on molasses and storage temperature

Temperature (°C)	Concentration Molasses (%)	pH value (Week)						
		0	1	2	3	4	8	12
4°C (cold)	2	4.37	4.33	4.28	4.28	4.21	4.16	4.1
	1.5	4.02	4.17	4.08	4.16	4.06	4.07	4.05
	1	4.02	4.15	4.03	4.05	4.01	4.02	3.96
28°C (room)	2	4.31	4.16	3.92	3.86	3.73	3.63	3.76
	1.5	4.01	3.92	3.85	3.91	3.83	3.87	4.02
	1	4.01	3.86	3.74	3.85	3.74	3.75	3.73

Lowest pH value of cold temperature storage (4 °C) found at 1% molasses concentration during the study (except 0 hours). While the lowest pH value of storage at room temperature (28 °C) contained in molasses concentration of 1% at week 1, 2, and 3. pH value during storage at room temperature (28 °C) was inversely related to total lactic acid bacteria. Therefore, the substrate contains more MRSB mixed with yeast extract and peptone composition which is a source of amino acids or nitrogen which serves to stimulate the growth of lactic acid bacteria (Jenie *et al.*, 2000).

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Degree of acidity (pH) value of media concentration molasses stored at cold temperatures (4 °C) during 12 weeks had a similar phenomenon with storage at room temperature (28°C), the longer the product is stored, the pH value down. Acidity increases as long as the number of bacteria saves more and more so that lactic acid is formed up and causes the condition to become acidic condition (Usmiati and Juniawati, 2011). Compared with the pH value on the storage media at room temperature (28 ° C), then the decrease in pH during storage at cold temperatures (4 °C) is relatively smaller. This is due to the metabolic activity of LAB at cold temperatures less than optimal, such as shown by the low pH impairment. According to Widodo (2003), BAL activity tends to be faster at room temperature than at low temperature. Increasing toxic metabolites and decreasing the amount of nutrients due to decreased pH value can cause the death phase of bacteria. It means that 4th week of storage at room temperature 28 °C pH value of 9.73 lactic acid bacteria viability was decrease (4.48 ± 0.17).

Sugar Reduction

The amount of sugar reducing sugars (glucose and fructose) measurable resulted carbohydrate hydrolysis is used to perform cell metabolism during storage. At the storage temperature of the cold (4 °C) and room temperature (28 °C) during 4 -8 week, the amount of reducing sugars increased in various concentration of molasses (2%, 1.5%, 1%) for solving oligosaccharides to sugars a simple majority is reducing sugars, such as glucose and fructose (Figure 1).

At 12 weeks of storage levels decreased reducing sugar because the sugar has been widely used for bacterial metabolism during 3 months of storage and lactic acid bacteria can degrade reducing sugar (glucose and fructose) in to organic acids, malic acid, butyric acid, and acid-other acids (Yuliana, 2007). Decreased value in reducing sugar levels caused by the use of sugars for cell activity. The amount of the use of sugar in each treatment showed differences due to the different mix of nutrients in the medium concentration (Damayanti and Sumarno, 2006).

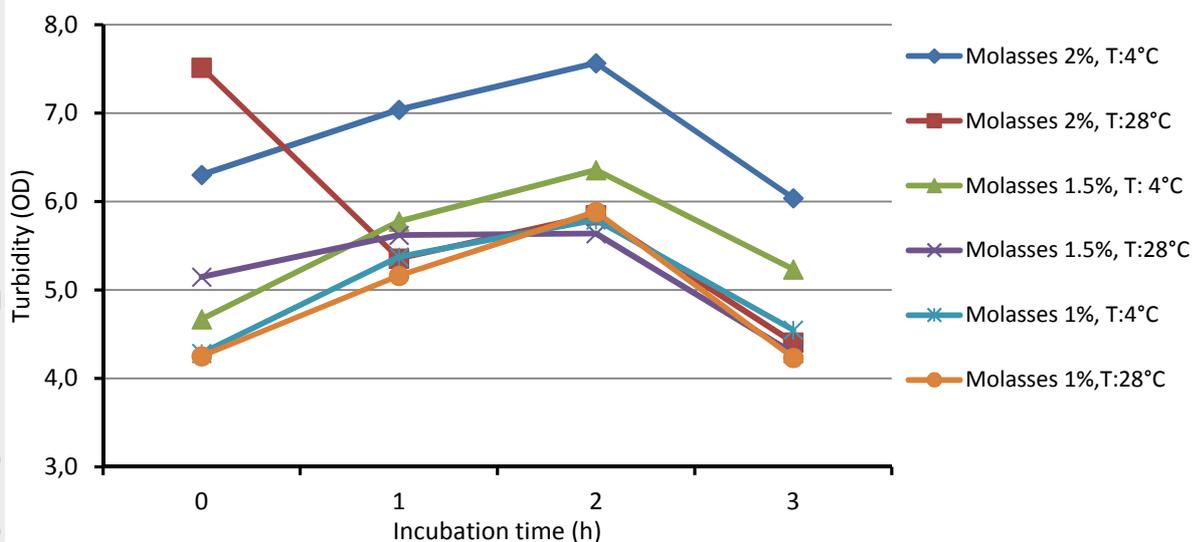


Figure 1. Changes in reducing sugar content in molasses concentration during storage

Identification of Lactic Acid Bacteria Isolates

Characterization of LAB isolates with RS₂ kit bioMérieux API CHL 50 are shown in Table 4. Based on the results of the test with a reading of LAB

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isolates using software APIWEB, LAB isolates were identified with both the identification category (98.1%), as *Lactobacillus brevis*.

Lactobacillus brevis is a lactic acid bacteria are rod-shaped. These bacteria live in the singular or short chains, diameter 0.7-1.0 µm and length 2.0-4.0 µm, is non-motil. *Lactobacillus brevis* are obligatory heterofermentative, can produce lactic acid, CO₂ and ethanol from carbohydrates. *Lactobacillus brevis* able to grow resistant to lactic acid at a pH as 3.7 (ICMFS, 1980).

CONCLUSION

The combination of media concentration molasses (2%, 1.5%, 1%) and storage temperature (4 °C and 28 °C) affected the viability of lactic acid bacteria.

The most optimal treatment at cold storage temperatures (4 °C) was found from 2% molasses media concentration (6.45 log cfu/ml) and 1.5% molasses concentration (6.00 log cfu/ml) was able to maintain the viability of lactic acid bacteria for 8 weeks.

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Table 4. Characterization of LAB isolates (RS2) using API 50 CHL bioMérieux

No	Type of Substrate Test	Reaction	No.	Type of Substrate Test	Reaction
0	Control	-	25	Esculin & ferric citrat	+
1	Glycerol	+	26	Salicin	+
2	Erythritol	-	27	D-cellobiose	+
3	D-arabinose	-	28	D-maltose	-
4	L-arabinose	+	29	D-lactose (bovin origin)	+
5	D-ribose	+	30	D-melibiose	-
6	D-xylose	+	31	D-saccharose (sucrose)	-
7	L-xylose	-	32	D-trehalose	+
8	D-adonitol	-	33	Inulin	-
9	Methyl-βD-xylopyranoside	-	34	D-melezitose	-
10	D-galactose	+	35	D-raffinose	-
11	D-glucose	+	36	Amidon (starch)	-
12	D-fructose	+	37	Glycogen	-
13	D-mannose	+	38	Xylitol	-
14	L-sorbose	-	39	Gentibiose	+
15	L-rhamnase	-	40	D-turanose	-
16	Dulcitol	-	41	D-lyxose	-
17	Inolitol	-	42	D-tagatose	+
18	D-mannitol	-	43	D-fucose	-
19	D-sorbitol	-	44	L-fucose	-
20	Methyl-αD-mannopyranoside	-	45	D-arabitol	-
21	Methyl-αD-glucopyranoside	-	46	L-arabitol	-
22	N-acetyl glucosamine	+	47	Potassium gluconate	+
23	Amygdalin	+	48	Potassium 2 celogluconate	-
24	Arbutin	+	49	Potassium 5 celogluconate	+

Description:

- No reaction (color remains red)

+ Positive reaction (yellow color)

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P-15. EFFECT OF FORMIC ACIDS IN SILAGE PROCESSING FROM SHRIMP HEAD WASTE AS ANIMAL FEED

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Abstract

The shrimp industry in Indonesia has grown significantly. Much waste is generated by this industry because of the high percentage of shrimp heads, exoskeletons, and soluble components lost during processing. The experiment was conducted to examine the effect of ensilage processing of shrimp heads waste (SHW) on nutritional quality. The objectives of this research were to determine whether the effect and interactions of formic acid concentration and length of ensilage in the shrimp heads waste on the improvement of its nutritive value. The experiment was designed in Completely Randomized Design, using factorial (3 x 3) with three replication. The first factors was concentration of formic acid (a1 = 3 %/kg SHW; a2 = 5 %/kg SHW; and a3 = 7 %/kg SHW), and the second factors was length of ensilage processing (b1 = 5 days; b2 = 8 days; and b3 = 11 days). The results showed that there was no interaction effects ($P > 0.05$) between concentration of formic acid and length of ensilage processing to crude protein (CP), crude fiber (CF), lipid, chitin and the pH of SHW silage, but concentration of formic acid and length ensilage factors was significant ($P < 0.05$) crude protein, crude fiber, lipid, chitin contents and pH of SHW silage. Based on nutritive value can be concluded that the best treatment was concentration of formic acid 7 % and length of ensiling process for 11 days.

Key word: Shrimp head waste, ensilage, nutrient quality, chitin

INTRODUCTION

Indonesia is one of the four bigger shrimp exporter country in the world. According Bureau Statistics of Indonesia (BPS, 2013), the rate prediction about 109 010 ton of shrimp head waste are generated annually in Indonesia and increase 14 % per year. Generally, shrimp is exported from Indonesia in frozen shrimp without exoskeleton and head, or it sell at local market either with or without exoskeleton. The shell and head from shrimp frozen industry (cold storage) is abundance as shrimp head waste (SHW) and it is dumped or unpleasant smell in environment. Waste that is associated with shrimp (prawns) that are being harvested and processed for human consumption. Shrimp head waste consist of head, shell and meat portions of shrimp that are being processing for human consumption. Approximately 70 % of shrimp landing becomes waste, so there is a tremendous tonnage of shrimp waste produced. Shrimp waste from the processing of black-tiger shrimp consist of 38 % heads, 7 % shell and 55 % meat (Piangchai, 1994). A portion of the crude protein that is contained in SHW meals is in the form of chitin, which is not readily digestible as a protein source for poultry diets (Mirzah, 2013).

The high protein content in SHW is potentially as protein sources for poultry diet. Some researchers found variation content of crude protein in SHW, the level from 39 to 52 %, depend on the strain of shrimp (Mirzah, 1990; Gernat, 2001; Fanimo *et al.*, 2004; Okaye *et al.*, 2005; Khempaka *et al.*, 2006; Kobayashi

et al. (2006). The shrimp waste contains several bioactive compounds such as chitin, pigments, crude protein, amino acids, and fatty acids. These bioactive compounds have a wide range of applications including medical, therapies, cosmetics, biotechnology, and feed applications.

The development of feed industry particularly poultry diets is hampered by supply of feedstuffs a specially fish meal, which is scarce and expensive for poultry. This has stimulated the evaluation of a variety of alternative dietary protein sources with the objective of partially or totally replacing fish meal protein in poultry diets. A possibility is the use of shrimp head waste meal, which contains high levels of protein with excellent amino acid profile comparable to that of fish meal (Meyers, 1986). But the utilization of available protein in shrimp head waste meal by fishes and poultry diets is limited by the presence substantial quantity exoskeleton chitin and ash (Bhuiyan, 1989; Mirzah, 1990; Mirzah *et al.*, 2010). The need for improvement in the quality of shrimp head waste protein has attracted the application of different processing methods (Fox, *et al.* 1994; Mirzah, 1997). Degradation of chitin into glucose requires the enzymes specific (chitinase) and very expensive (Begum and Aubert, 1994; Bhat and Bhat, 1997; Mahata, 2007) while sun-drying is frequently carried out under unhygienic condition leading to meals with high microbial loading (Wood, 1982; Mirzah, 1990). Soaking by organic acids and continued with steam heat pressure can improve the nutritional quality and feedstuffs value of SHW meals (Mirzah, 1990; 1997; Wahyuni and Budiastuti, 1992). And residue of mineral acid to be neutralized before use in animal feeding.

Continued production of the shrimp head waste without corresponding development of technology utilizing the waste has resulted in waste collection, disposal and pollution problems using of these wastes into poultry feed production part from minimizing the cost s of poultry production would serve as an excellent means of sanitizing the environment. Based on the estimated economic benefits and nutrient utilization indicate that SHW meal by processing can effectively and replace fish meal up to 30 % in diet (Nwana *et al.*, 2003). Ramasubburayan *et al.* (2013), concludes that acid fish silage prepared from the processing wastes could effectively be utilized as fish feed stuff and indicates its potential means of minimizing fishmeal and reducing possible environmental pollution. Moreover, the present findings clearly pointed out the successful replacement of basal diet with 2% formic acid silage product significantly augmented weight gain and SGR of the experimental fish *C. carpio* fingerlings. Formic acid (organic acid) is the best choice for the preparation of chemical silage, the silages made using formic acid are not excessively acidic and therefore do not require neutralization before being used (Oetterer, 2002). Whether increasing concentration of formic acid can be decreased length of ensiling process. This project was conducted to evaluate the effect increasing of formic acid concentration (3 ; 5 ; 7 %) and length of ensilage in silage preparation of the SHW on the improvement of its the nutritive value. Specific objectives include evaluating the chemical quality of SHW silage meals.

MATERIAL AND METHODS

Sample Collection: Fresh shrimp head waste (comprising mainly heads of *Panaeus meriensis* and *Panaeus renenbergi*) were collected from Tanah Kongsu local market in Padang –West Sumatra and its was stored at 0 °C prior to processing in Feed Technology Laboratory at Faculty of Animal Science Andalas University Padang. Three

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different concentration was 3 ; 5 ; 7 % (w/v) of formic acid prepared and were purchased from Sari Kimia Chemical Store Padang.

Silage Preparation: Twenty seven (27) kilogram of sample were aseptically collected from local market and thoroughly rinsed in fresh water before chopped into 2 cm, after minced using a wet grinder into pasta for silage. Acid silage prepared by acidifying 1kg of SHW paste with three different concentration was 3 ; 5 ; 7 % (w/v) of formic acid. Simultaneously triplicates were maintained in each concentration. Ensilage process was then aided by incubating the material in the bottle containers (2 L capacity) at ambient room temperature ($28 \pm 2 ^\circ\text{C}$) up to 11 days. The silages were stirred daily in the morning aseptically and samples of known volume were drawn from the respective containers at definite intervals of 5, 8, and 11 days duration. During each sampling interval, ensilages were evaluated for physicochemical parameters like dry matter (DM), pH and biochemical constituents like protein, fiber, lipid and chitin contents (AOAC, 1990). All of the sample were immediately sun-dried ($30 - 32 ^\circ\text{C}$) and stored in a freezer at $20 ^\circ\text{C}$ prior to analyzed for proximate analysis. At the end of the ensiling process (11th day), the amino acid contents of silage were determined (AOAC, 1990).

General analytical procedures: The moisture content was measured by drying the sample in an oven at $105 ^\circ\text{C}$ for 24 h. Ash content was determined by burning the sample in crucible at $600 ^\circ\text{C}$. Crude protein and fiber was determined by proximate analysis and chitin content determined by demineralization and deproteinization methods as reported by Hong (1989), while amino acid of crude protein determined by methods Vidotti *et al.*(2003).

Experimental design

The experiment was designed in Completely Randomized Design, using factorial (3×3) with three replication. The first factors was concentration of formic acid ($a_1 = 3 \text{ %/kg SHW}$; $a_2 = 5 \text{ %/kg SHW}$; and $a_3 = 7 \text{ %/kg SHW}$), and the second factors was length of ensilage processing ($b_1 = 5$ days; $b_2 = 8$ days; and $b_3 = 11$ days). The data were analyzed statistically by two-way analysis of variance to determine the main and interaction effects of formic acid concentration and length of ensiling process. Significant differences among treatments were determined by Duncans multiple range test (Steel and Torrie, 1995).

RESULTS AND DISCUSSION

Previous studies has shown that proximate analysis composition of SHW to be variable : CP ranged from 35 % (Mirzah, 1990) to 62 % (Rosenfeld *et al.*, 1997), crude ash from 12 % (Islam *et al.*, 1994) to 23 % (Gernat, 2001), crude fiber from 11 % (Rosenfeld *et al.*, 1997) to 16 % (Gernat, 2001), crude fat from 4.8 % (Odugawa *et al.*, 2004) to 7.7 % (Rosenfeld *et al.*, 1997) and chitin content from 9 - 13 % (Mirzah, 1997). This variation among studies may be due to the difference in shrimp species, source and/or processing methods, as these can affect the nutritional values of SHW (Meyers *et al.*, 1973; Ngoan *et al.*, 2000). SHW used in the present study was characterized by a high fiber and ash content, and a low CP content (Table 1). This SHW was made from headless shells, which contained less CP and fat, and more fiber and ash than shrimp heads (Mirzah, 1997) compared the soybean meals.

The proximate, mineral composition and amino acid critics of SHW meal is presented in Table 1 shown that CP of 39.62 %, fiber content of 21.29, lipid content of 5.43, Calcium content of 15.88 %, Phosphor content of 1.90 % and Chitin content of 15.24 %. The high fiber and chitin contents and the low CP content are indications that the meal may be lowest digestible than the soybean meals. In addition, amino acid profiles revealed that SHW had lower concentration of methionine, lysine, tryptophane than soybean meal (Table 1). The values of proximate analysis and amino acid profile of SHW meal are marginally lower than commercial fish meals and only comparable to the commercial fish meals in many developing countries (Tacon, 1993). While the quality of

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nutrition such as nitrogen retention, metabolisable energy and protein digestibility also had lower than soybean meals, and record as 55.23%, 1984.87 kcal/kg and 52.00% respectively.

Table 1. Nutritional composition of SHW compared soybean meals (dry matter)

Component	Feedstuffs	
	Shrimp Head Waste (%)	Soybean meal (%) ¹
Moisture	8.96	12.00
Dry matter	91.04	88.00
Crude protein	39.62 ²	42.67
Crude fat	5.43	4.53
Crude fiber	21.29 ³	6.86
Ash	30.82	6.68
Calcium	15.88	0.47
Total Phosphorus	1.90	0.20
Chitin	15.24	-
Methionine	1.16	2.92
Lysine	2.02	6.14
Tryptophane	0.53	1.36
Nitrogen Retention	55.23	-
M E (kcal/kg)	1984.87	2240.00
Digestibility of CP (in-vitro)	52.00	88.00

1. Standard tables of feed composition in Indonesia (2004)
2. Corrected crude protein = (total N – chitin N) x 6.25
3. Crude fiber is mainly in form of chitin

Maintenance of acidity in SHW has the added advantage of keeping the product more hygiene and safety by inhibiting the growth of pathogenic organisms. In the present study, the pH of the acid ensilages and the proximate analysis, mineral composition and amino acid after ensilage process of SHW meals was presented in Table 2 and Table 3. The results showed that there was no interaction effects ($P > 0.05$) between concentration of formic acid and length of ensilage processing to the pH silage. The average pH of the acid ensilages decreased significantly ($P < 0.05$) from the initial pH of 3.95 to 3.32 with respect to progressive increase in concentration of 3 to 7 % formic acid (Figure 1). At the end of the experimental duration (11th day), the pH level of silages was considerably reduced and recorded as 3.29, 3.13 and 2.91 at 3, 5 and 7% formic acid ensilages respectively (Table 2). The value of pH obtained in the present study was in consonance with Hasan (2003), who observed the reduction of pH from 6.5 to 3.38 in 3% formic acid acidified fish mince of *Rastreliger brachysoma*. According to Yeoh (1979), the result on pH profile obtained in the present study is within the recommended range of successful fermentation.

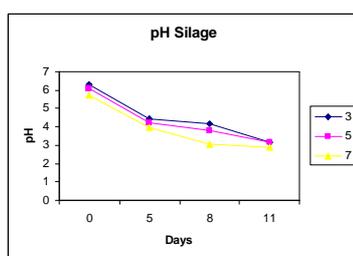


Figure 1. Aerobic plate count of different concentrations of formic acid silages at various fermentation days interval.

In the present study, the dry matter (DM) composition of silages decreased gradually from the first phase of experiment. It was noticed that at the beginning of the experiment, the dry matter recorded in 3% formic acid ensilage was 37.49%, but it decreased to 33.80% at the end of the experiment. Maximum dry matter of 39.88% was observed in 7% acid ensilage during initial day. When the ensilage process duration increased, a constant decrease in dry matter was noticed till the end of the experiment and attained 35.80, 34.18 and 35.97% in 3, 5 and 7% acid ensilages, respectively (Table 2). In agreement with the present findings, Hammoumi *et al.* (1998) stated that decrease in dry matter in the silage product relative to the initial raw material and also it may be due to hydrolysis of protein content by enzyme or microorganisms. In contrary, Hasan (2003) found only 6.7% reduction of dry matter content in 3% formic acid ensilage of mackerel *R. brachysoma* at the end of 30 days of ensilation process.

The proximate biochemical constituents such as protein, lipid and crude fiber contents of the ensilages were studied (Table 2). The results showed that there was no interaction effects ($P > 0.05$) between concentration of formic acid and length of ensilage processing to the protein of silage. But each of concentration of formic acid and length of ensilage processing is significant ($P < 0.05$). Result displayed a significant ($P < 0.05$) decrease of crude protein content of silages with respect to increase in experimental duration, however increase with respect to increase in concentration of acid level. For instance, during the initial day (5th day) of the experiment, the crude protein content recorded with 36.32, 41.17 and 43.28% in 3, 5 and 7% formic acid ensilages, respectively. The increasing of the crude protein content SWH silage caused increase level of formic acid from 3 to 7%. In agreement with the present study, Mairizal (2005) also emphasized that level of formic acid can be increase protein contents of offal fish silages with 39.55%. A similar phenomenon of increase was reported during formic acid ensilage of fish silage meal (Kompiang and Ilyas). This little increase of protein content of SHW in the present study may be due to increasing bacheri lactic acid with increase of level formic acid, so that single cell protein to be increased. But at the end of the experiment, a fall in protein level was noticed with considerable level of 35.06, 37.28 and 38.26% in 3, 5 and 7% formic acid ensilages, respectively. Reduction of protein content in the ensilage may be due to break down / hydrolysis of protein (FAO, 2007). Vidotti *et al.* (2002) observed a reduction in crude protein level in combined (2% each of formic acid and sulfuric acid) fermented silage of tilapia filleting residue when compared to non-fermented tilapia filleting residue. Similarly decrease of crude protein level in the combined (1:1 ratio of formic and sulfuric acids) ensilage of tilapia filleting residue was noticed after 180 days of storage (Geron *et al.*, 2007).

The crude fiber obtained from the present study are low when compared with raw SHW ones. It has 15.71, 13.41 and 11.74 % in 3, 5 and 7% formic acid ensilages on the first five days, respectively. At the end of the experimental duration (11 days), the crude fiber contents was considerably reduce and recorded as 13.36, 12.56 and 10.15% at 3, 5 and 7% formic acid ensilages respectively. The decreasing of the crude fiber content SWH silage caused increase level of formic acid. There were significant difference ($P < 0.05$) in the crude fiber values for each level of formic acid, and so there were significant differences ($P < 0.05$) with length of ensilage processing from 5, 8 and 11 day respectively. These results agreement with the present study, Mairizal (2005) who observed better quality of crude fiber when level of formic acid is increase, and the best quality of crude fiber of offal fish silage with the formic acid reduce as 27.33% of crude fiber contents, from initial crude fiber of 20.56 to 14.93% with respect to progressive increase in concentration of 3 to 7% formic acid. The crude fiber of SHW will be counted as chitin. So that, roughly 21.42% of the fiber content of chitin is approximately 17.59% (Watkins, *et al.* 1982).

Table 2. The proximate analysis parameter of different concentration of formic acid at various ensilaging days interval

Parameters	Ensilaging Days	Acid Silage (%)			
		3	5	7	Average
pH	5	4.41	4.23	3.98	4.21
	8	4.16	3.79	3.07	3.67
	11	3.29	3.13	2.91	3.11
	Average	3.95 ^a	3.72 ^b	3.32 ^c	3.66
Dry matter	5	37.49	38.90	39.88	38.75
	8	35.56	35.98	37.93	36.49
	11	33.80	34.18	35.97	34.65
	Average	35.62 ^c	36.36 ^b	37.93 ^a	36.63
Crude protein	5	36.32	41.17	43.28	40.26 ^a
	8	35.74	40.87	41.90	39.50 ^a
	11	35.06	37.28	38.26	36.87 ^b
	Average	35.71 ^c	39.77 ^b	41.15 ^a	38.87
Crude fiber	5	15.71	13.41	11.74	13.63 ^a
	8	14.24	13.03	10.40	12.56 ^b
	11	13.36	12.56	10.15	12.02 ^c
	Average	14.43 ^a	13.00 ^b	10.76 ^c	12.73
Lipid	5	5.16	5.14	5.30	5.20
	8	4.13	4.68	4.18	4.33
	11	4.04	4.19	4.05	4.09
	Average	4.44 ^b	4.67 ^a	4.51 ^a	4.54
Chitin	5	8.32	7.98	6.07	7.46 ^a
	8	7.16	6.82	5.95	6.64 ^a
	11	6.08	5.43	5.22	5.58 ^b
	Average	7.19 ^a	6.74 ^a	5.75 ^b	6.56

abc : Means within a column with different superscripts are significantly different (P < 0.05)

The lipid content in the present study revealed that at the first five of the experiment (5th day), it was between 5.16 and 5.30% in all the tested concentrations of formic acid silages. But when the experimental days continued (11th day), it was between 4.04 to 4.05%. The average lipid content significantly (P<0.05) increased at the end of the experiment, the increase in lipid content was 4.44, 4.67 and 4.51% respectively in the three formic acid silages. But no significant (P > 0.05) effect of length of ensilage processing to lipid content of SHW meal. This result also agreed with present findings, Mirzah (1997), stated that no effect length for SHW processing by steam heat pressure use autoclaving. The finding of lipid profile in the present study was supported by the work of Hasan (2003), who found a continuous increase (11.2 to 19.23%) of lipid content in 3% formic acid silage of *R. brachysoma* during the ensilage process up to 60 days. In the present study the continuous increase in lipid content from 2.58 to 3.97% in 2 -3% formic acid silage with respect to increase in storage period may be due to release of fats from the viscera of raw material used. In accordance to the present study, Dapkevicius *et al.* (1998) reported 3.6% increase in lipid content from 11.3 to 14.9% from initial to final stage (15 days) of storage of 3% formic acid ensilage of blue whiting.

The chitin content obtained from the present study are lower when compared with raw ones. It has 8.32, 7.98 and 6.07% in 3, 5 and 7% formic acid ensilages on the 5th day, respectively. At the end of the experimental duration (11 days) has with 6.08, 5.43 and 5.22%. There are no interaction (P > 0.05) between concentration of formic

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acid and length of ensilage processing to the chitin content of silages. But each of concentration of formic acid and length of ensilage processing is significantly. Result displayed a significant ($P < 0.05$) decrease of chitin contents of silages with respect to increase in experimental duration as well as decrease in concentration of acid level. The average chitin content significant ($P < 0.05$) decreased at the end of the experiment, the decrease in chitin content was 7.19, 6.74 and 5.75% respectively in the three formic acid silages as well as there were significant differences ($P < 0.05$) decreased from 7.46 to 5.58 with respect to progressive length of ensilage processing from 5, 8 and 11 day respectively. These results agreement with the present study, Mairizal (2005) who observed better quality of chitin when level of formic acid is increase, and the best quality of chitin of offal fish silage with the formic acid reduce as 27.74% of chitin contents, from initial crude fiber of 34.06 to 24.61% with respect to progressive increase in concentration of 3 to 7% formic acid. The crude fiber of SHW will be counted as chitin. So that, roughly 21.42% of the fiber content of chitin is approximately 17.59% (Watkins, *et al.* 1982). Considering practical used of SHW, its adverse effects are of great concern nutritionist. High levels of chitin and/or calcium in SHW are possible factors involved in the decreased growth performance of poultry. Chitin content was rich in SHW (Table 2) and may decrease digestibility, because of a non-digestible amino polysaccharide, although chickens given diets containing chitin at 3% (Razdan and Petersson, 1994) and 5% (Kobayashi and Itoh, 1991) did not show decreased performance. These results are in agree with present study, Mirzah (1997) and Filawati *et al.* (2003) that time factors are significantly affect to chitin content of SHW. Chitin prepared from the shell residue of formic acid had good quality compared to other (Santhosh, *et al.* 2007).

The nutritive value of protein of any ingredient depends mainly on the protein's capacity to fulfill the needs of organism with respect to essential amino acids (Vidotti *et al.*, 2003). In the present study the proximate amino acid profile of the silages at the end of the ensiling process evidenced a significant increase in level of amino acids in 2% acid ensilage than the other respective ensilages. The description of amino acids trend increasingly level of formic acid. In the present study, considering the amino acid profile of the ensilages, cystine, tyrosine and histidine levels were lower than the other identified amino acids (Table 3). In accordance with these, Dapkevicius *et al.* (1998) also stated that acid hydrolysis method used for silage preparation lead to partial destruction of cystine. Similarly Arason (1994) indicated relatively a similar report that tryptophan is unstable in acid medium; therefore it becomes the first limiting amino acid in fish acid silage. In the present study, followed to serine, arginine, threonine, isoleusine, cystine, tyrosine and histidine recorded lower level in all the acid silages. Dapkevicius *et al.* (1998) recorded lower levels of methionine, histidine and proline in 3% formic acid ensilage of Blue whiting (*M. poutassou* Risso). Thus the reduction of amino acid contents noticed in increasing concentrations of acid ensilages in the present study might have occurred due to some chemical reactions between α amino and aldehyde groups present the amino acid during ensilation process (Johnson *et al.*, 1985).

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Table 3. Amino acid content of different concentrations of formic acid silages after 11th days of ensilage

Amino acids (g/100g)	Acid silages (%)		
	3%	5%	7%
Asparagine	1.38	1.57	1.70
Serine	0.54	0.61	0.66
Glutamine	1.24	1.41	1.52
Glycine	0.63	0.71	0.77
Threonine	0.57	0.65	0.70
Alanine	0.69	0.79	0.85
Arginine	0.58	0.66	0.71
Cystine	0.40	0.45	0.49
Tyrosine	0.44	0.50	0.54
Histidine	0.26	0.29	0.32
Valine	0.66	0.75	0.80
Methionine	0.92	1.04	1.12
Isoleucine	0.52	0.59	0.64
Phenylalanine	0.62	0.70	0.76
Leucine	0.92	1.04	1.12
Lysine	1.31	1.52	1.64
Proline	0.71	0.81	0.88
Tryptophan	0.90	1.02	1.11
Taurine	1.85	3.03	1.87

CONCLUSION

Based on the results obtained in the present study, it could be attributed that properly processed to improving quality of SHW can be use in as a protein source in poultry diet and reducing possible environmental pollution. Based on nutritive value can be concluded that the best treatment was concentration of formic acid 7 % and length of ensiling process for 11th day.

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P-16. EFFECTS OF SAGA LEAVES AND YELLOW LEAVES ON RUMEN MICROBES AND IN VITRO DIGESTIBILITY

Dwierra Evvyernie^{1,2,4}, Heri Ahmad Sukria^{1,2}, Eva Harlina^{1,3}, Eka Rachmi², Astri Winarni² and Uray Nurjannah²

Abstract

Saga leaves and yellow leaves are herbs that are rich in antioxidants and anti-bacterial properties, so they potential for anti mastitis and anti parasite. The objective of this initial research was to study the extent of leaf extracts saga and yellow leaves can be added to the ration so that does not inhibit the growth of rumen protozoa and bacteria as well as in vitro digestibility. Testing the growth of microbes (protozoa and total bacteria) was performed according to the method Ogimoto and Imai (1981), and testing in vitro digestibility *i.e.* dry matter and organic matter digestibility's (DMD and OMD) were performed according to methods of Tilley and Terry (1963). Four treatments were tested: P0 (control)= basal rations for lactating dairy goats (60% grass + 40% concentrate; contain 55% TDN and 12% crude protein) without addition of extract; P1= P0 with addition of saga leaves extract; P2= P0 with addition of yellow leaves extract; P3= P0 with addition of mix of both leaf extracts. The leaf extract levels were: 0, 4, 8, 12 and 16%. The results showed that the addition of leaf extract treatments reduced the growth of rumen microbes and digestibility. Growth of protozoa, total bacteria, and digestibility increased with increasing the concentration levels of the leaf and blends up to 12%. The addition of ofsaga leavesextract gave morepositiveeffecton the growthanddigestibilitythanother treatments. As conclusion, the extract of saga leaves, yellow leaves or blends of both extracts can be added a maximum of 12% in the ration of lactating dairy goats.

Keywords: digestibility, in vitro, rumen microbes, saga leaves, yellow leaves

INTRODUCTION

The requirement of milk in 2012 was 3.5 million tons and it will expected to increase until 4.2 million tons in 2015 (Zuhri, 2012). Unfortunately, those expectation are not able to fulfill due to lack of milk production from the dairy farm in Indonesia, for example in 2011, only 690,000 tons fresh milk was provided from 3.2 million tons required. As consequently, the government decided to import about 75% of milk powder. Actually, in 2020 was declared by the Government as Milk Self Sufficient Year. To support that purpose, population of dairy cattle should be increased around 2.3 million, which should be followed by the sufficiently of forages (grass and legume) about 27 million tons with an area of approximately 111 thousand hectares of crops, and also should be supported by the availability of about 4 million tons of concentrate (Wijaya, 2012). Sudarwanto *et al.* (2008) reported around 80% of dairy cows in Indonesia suffer from subclinical mastitis which is disease caused by attack of bacteria *Streptococcus sp.*, *Staphylococcus aureus*, where impact to decrease milk production up to 40%. Weak immune system of livestock and poor sanitation might trigger the development of subclinical mastitis disease. Some supplement and also feed additive become the alternative solution to avoid and cure subclinical mastitis. In previous research, as much 100 ppm fungal fruit body of *Ganoderma lucidum* in concentrate diet can reduce somatic cells (indicator of subclinical mastitis) in udder gland of dairy cows lactation up to 50-60%, beside that also detected that the cows immunity relatively more stable compared with

the control (Evvyernie *et al.*, 2008; 2009). Some herbs also potential as an anti mastitis and also acts as a prebiotic such as saga leaves (*Abrus precatorius* Linn) and kemuning leaves (*Murraya paniculata* [L.] Jack) due to their active compounds such as glisirhizin, precatorine, coumarine, cadinene, etc. Both leaves extracts with 50% concentration have capability inhibited the growth of *Staphylococcus aureus*, and increased the growth of non-pathogenic bacteria like *Lactobacillus rhamnosus*, *L. agilis* and *L. amyliophilus* (Rahminiwati *et al.*, 2010).

Saga and kemuning leaves contain a phytochemical compound like saponin. In the rumen, this compound will perform soap or undergo saponification effects that interfere the growth of rumen protozoa (defaunation). Defaunation partial of protozoa in the rumen do not cause negative impacts on the rumen ecosystem otherwise decreasing methane production (Guo *et al.*, 2008). The resultant effect of other phytochemical compounds also might decreasing rumen bacteria, where impact to death of the animal host because microbes are the most protein source for ruminant. Therefore the aim of this study was to examine the use of saga and kemuning leaves extracts as feed additives for ruminant that safe for rumen microbes and function as an anti mastitis and antihelminthic.

MATERIALS AND METHODS

Materials

Basic ration of dairy goats containing 60% napier grass + 40% concentrate; with 55% TDN and 12% crude protein (NRC, 2001) was used in this experiment like presented in Table 1. As inoculums, fresh dairy goat rumen fluid was used beside other materials such as: distilled water, BHI Powder, glucose, starch, CMC, resazurin, hemin, cysteine, TBFS solution, a McDougall buffer solution, CO₂ gas, Whatman filter paper No. 41, and HgCl₂ saturated.

The Design of Experiments

There were two steps in this experiment *i.e.*, counting population of bacteria and protozoa and measuring dry matter and organic matter digestibility (DMD and OMD). In the first step, description analysis was used; in the second step, factorial randomized block design 3 x 5 with 3 periods of rumen fluid collection as a block was used. Factors observed consist of two factors, Factor A was three kinds of extract: saga leaves extract, kemuning leaves extract, and Mixed extracts from 50% saga and 50% kemuning leaves; Factor B was concentration of extracts given to basic ration: 0, 4% , 8% , 12% , and 16% of concentrate. Four treatments were: P0 (control)= basic ration for lactating dairy goats (60% grass + 40% concentrate; contain 55% TDN and 12% crude protein); P1= P0 + saga leaves extract; P2= P0 + kemuning leaves extract; P3= P0 + mix of saga and kemuning leaves extract.

Parameter

Parameter measured in this in vitro studies were: total bacteria and protozoa populations, according to Ogimoto and Imai method (1981); dry matter digestibility (DMD) and organic matter digestibility (OMD), according to Tilley and Terry method (1963).

RESULTS AND DISCUSSION

Table 2 showed effect of different concentration of extract that given in the ration by measured surviving of bacteria and protozoa in the rumen. There were very significantly differences ($P < 0.01$) on total bacteria of the extracts from sage leaves, kemuning leaves, and mixtures both of leaves. The highest bacterial

population was achieved by addition of saga leaves extract, while the lowest was occurred by addition of kemuning leaves extract. If compared than control (R0) increasing level of saga leaves extract affected the bacteria as well as protozoa population. However, in case of kemuning leaves extract increasing level concentration caused decreasing bacteria and protozoa population. Kemuning leaves have pharmacologic effects include anesthesia (numbing), sedatives (tranquilizers), anti-inflammatory, anti swelling, and facilitating blood circulation, antibacterial (in the presence of coumarin), antioxidant, anticancer and stimulate the enzyme (Adfa *et al.*, 1999). Based on those researches, active compound of kemuning leaves extract especially antibacterial coumarin might suppressed bacteria viability of some species. There was a fluctuation amount of bacteria when the level leaves extract increased, it might caused by the role of other active compounds that function like a 'growth promoter' for some bacteria species, because kemuning leaves extract have potency to support the viability of bacteria *Lactobacillus* spp (Rahminiwati *et al.*, 2010).

Based on statistical results, there were no significantly different among type or concentration of extract and among types of extract, and no interaction between them in protozoa populations. There were decreasing in the treatment of saga leaves, kemuning leaves, and a mixture of both leaves extract compared than control. It might active compound like saponin and other phytochemical substances in saga and kemuning leaves extract suppressed protozoa population thereby increasing the fiber degrading bacteria population and providing significant effect in improving the DMD and OMD.

Table 3 showed the digestibility of rations contain leaves extracts with different concentrations. There were very significantly differences ($P < 0.01$) on dry matter digestibility (DMD) of the extracts from sage leaves, kemuning leaves, and mixtures both of leaves. Level of treatment given did not show differences and also there was no interaction effect on types and concentration level of extract. In case of OMD, there was no significantly different and also no interaction detected. The highest DMD as well as OMD were achieved by giving saga leaves extract, while the lowest DMD and OMD were achieved by giving kemuning leaves extract. In this experiment, range of DMD and OMD were up to 55%, that means digestibility were still in normal conditions and able to support the animal host production (Sutardi, 1979; Muhtarudin and Liman, 2006). Results of counting microbe population were similar trend to results of digestibility. Increasing concentration level of extract caused decreasing of bacteria population as well as digestibility and higher decreased on protozoa especially when used kemuning leaves extract in the ration. In previous research, Rahminiwati *et al.* (2010) reported that saga and kemuning leaves extract inhibited the growth of bacteria *Staphylococcus* spp, but supported the growth of *Lactobacillus* spp. In vitro apparatus like closed fermentation system, end product of fermentation will accumulated, then pH decreased and make toxic of fermentor environment. *Lactobacillus* spp is one type of bacteria produce lactate acid, if their growth high supported by saga and kemuning leaves extract, medium pH will decrease rapidly, other bacteria like cellulolytic bacteria become die and finally decreasing the digestibility.

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CONCLUSION

Addition of saga, kemuning and mix of both leaves extracts in the ration affected growth and digestibility of rumen microbes. Bacteria were more tolerance than protozoa when leaves extract added to the ration. Kemuning leaves extract was the most potential substance to decrease rumen microbes. The pattern of microbe population was similar to pattern of digestibility. The highest of digestibility was achieved by ration containing saga leaves extract.

Based on those results, the use of saga leaves extract in the ration of dairy lactation goat until 12% of concentrate were still support the fermentation process in the rumen, and the use of kemuning leaves extract could not more than 4% of concentrate, caused of high phytochemical restriction.

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Table 1 . Composition and Nutrition of Dairy Goat Basic Ration

Ingredients	(%)
Cassava waste	11,63
Tempe waste	34,88
Rice Bran	23,26
Coconut Meal	23,26
CPO	6,97
Crude Protein	12
TDN	55

Source: NRC 2001

Table 2. Counting of Total Bacteria and Protozoa Population of rations contain leaves extract with different concentration (log cell/ml)

Ration	Total Bacteria			Average	Protozoa			Average
	Saga	Kemuning	Mix		Saga	Kemuning	Mix	
R0	8.63±2.27	8.63±2.27	8.63±2.27	8.63±2.27	4.80±0.22	4.80±0.22	4.80±0.22	4.80±0.22
R1	7.50±1.09	6.92±0.28	7.95±1.72	7.46±0.52	4.50±0.38	4.69±0.09	4.36±0.07	4.52±0.18
R2	8.21±0.98	7.31±0.56	7.50±0.66	7.67±0.47	4.50±0.20	4.69±0.10	4.45±0.30	4.54±0.20
R3	6.05±5.37	7.25±0.10	7.05±0.84	6.78±0.64	4.34±0.47	4.52±0.26	4.57±0.26	4.48±0.33
R4	8.16±1.76	6.89±0.65	8.62±1.14	7.89±0.90	4.67±0.25	4.52±0.32	4.43±0.06	4.54±0.21
Average	7.48±1.00 ^a	7.09±0.22 ^b	7.78±0.67 ^a		4.50±0.32	4.61±0.19	4.45±0.17	

Notes: Mean in the different column with different superscript differ very significantly (P<0,01). R0: control ration; R1: R0 + 4% extract; R2: R0 + 8% extract; R3: R0 + 12% extract; R4: R0 + 16% extract

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Table 3. In vitro digestibility of rations contain leaves extract with different concentration (%)

Ration	DMD			Average	OMD			Average
	Saga	Kemuning	Mix		Saga	Kemuning	Mix	
R0	59.8±8.9	59.8±8.9	59.8±8.9	59.8±8.9	63.7±16.5	63.7±16.5	63.7±16.5	63.7±16.5
R1	66.6±6.5	59.1±3.7	59.8±7.4	61.8±4.1	64.0±7.4	60.4±3.4	63.6±7.3	62.7±2.0
R2	60.6±0.7	60.7±1.3	62.0±7.2	61.1±0.7	61.0±0.9	62.8±1.6	62.5±7.4	62.1±1.0
R3	64.3±3.1	57.4±6.3	61.3±3.2	61.0±3.4	65.1±3.2	57.0±6.3	61.9±3.6	61.3±4.1
R4	64.3±0.5	56.2±4.4	63.0±0.5	61.2±4.3	66.9±0.9	57.4±4.7	63.9±2.0	62.7±4.9
Average	63.9±3.8 ^a	58.8±4.1 ^b	61.5±4.8 ^{ab}		64.1±2.1	59.4±4.5	63.1±0.9	

Notes: Mean in the different column with different superscript differ very significantly ($P < 0,01$). R0: control ration; R1: R0 + 4% extract; R2: R0 + 8% extract; R3: R0 + 12% extract; R4: R0 + 16% extract. DMD = dry matter digestibility, OMD = organic matter digestibility.

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P-17. FLUSHING WITH DIFFERENT SOURCES OF ENERGY QUALITY RATION ON REPRODUCTIVE PERFORMANCE LOCAL SHEEP

L. Khotijah, K.G. Wiryawan, K.B.Satoto, D. Diapari, N.B.Sitepu and N.E.K.Santi

Abstract

The study aimed was to compare the reproductive performance of local sheep receiving rations flushing with different quality of energy sources. 15 local female sheep with average initial weight of 16.1 ± 1.17 kg used in the study. Experimental design used was completely random design (CRD) 3x5, with a ration treatment of different types of energy sources. The treatment are Pj = corn, Po = cassava, Pjo = corn+cassava. The variables measured were dry matter intake pre breeding, pregnant and lactation (kg / head); percentage of pregnancy, number of embryos, litter size, mortality of embryo, average daily gain. Dry matter intake (DMI) were analyzed using ANOVA, while for mains reproductive performance tested descriptively. The results showed no significant difference for dry matter intake during pre-breeding, pregnant or lactating. For the overall reproductive performance of sheep consumed diets with the cassava of energy source tend to be better than the rations with corn of energy sources as well as a combination of both. It can be concluded that in general cassava as a source of energy ration providing reproductive performance ewe better than rations containing only corn or a combination of both, but did not give a different effect on the appearance of the ewes.

Key words :energy, flushing,performance, reproductive, sheep

INTRODUCTION

Nutrition is one of the major environmental factors that contribute to the reproductive success of sheep, both the people and the industrial scale. Deficiency of nutrient substances in the reproductive phase will inhibit the growth and maturation of follicles, delayed estrus and ovulation (Chillo, 1992; Randel, 1990). Freer and Dove (2002), states that directly provide nutrients glucose, amino acids, vitamins, and essential chemical elements. Indirectly, the nutrients can modify the hormonal functions, improve egg maturation, ovulation, embryo development, fetal growth, and the endurance of children born. Intake of food is very important in the process of ovulation and steroid hormone synthesis for the maintenance of pregnancy, then during post partum mother's nutritional status will affect the immunity level of children born to weaning.

Some researchers in developed countries make improvements in nutritional status of livestock mother goat during the breeding season until the birth with Flushing program, the addition of energy sources in the form of grains (Acuero, 2000; Kusina et al, 2001; Abu EL-Ella, 2006; Camero et al., 2008; Sabra and Hasan, 2008), with one of the results showed an increase in the percentage of multiple births in goats receiving flushing, compared to the control. Generally for flushing program assessment are emphasized in a given energy level, Schillo (1992) states that the energy source for the reproduction of the form of energy which is a precursor substrate glukogenik (propionate), fats (fatty acids) or protein (amino acids alanine, glutamine) , this suggests that in addition to the levels, should also be assessed the quality or type of energy source suitable for use as an energy source in the ration reproduction.

Corn and cassava meal are energy sources that are commonly used as constituent rations for all types of livestock. Both the feed contains high

carbohydrates such as starch, but there are differences in structure. Form of cassava starch amylopectin, whereas corn starch in the form of amylose (Richana and Suarni, 2010). In the form of amylopectin starch is easier to digest than in the form of amylose (Tisnadjaja, 1996). The content of cassava starch is more easily digested than the starch content in corn (Thang *et al.*, 2010). The nature and structure of the different starches from the two materials will have an effect on the supply of energy in the form of glucose required for reproduction. The extent to which differences in the quality of the energy source can affect reproductive performance, the reproductive performance observed ewe with coconut meal-based ration combined with corn or cassava.

MATERIAL AND METHOD

The experiment was conducted in Laboratory Nutrition Meat and Work Livestock, Department of Nutrition and Feed Technology, Faculty of Animal Science Bogor Agricultural University. The study used 15 local ewe with initial body weight of an average of 16.1 ± 1.17 kg, and 1 male Garut sheep. Animals are caged individually.

Rations were given during the study consists of native grass and concentrate with a ratio of 30:70 giving. The composition of feed ingredients and nutrients ration study are presented in Table 1.

Table 1. Feed and Nutrient Composition of Treatment

Feed	Treatment		
	Pj	Po	Pjo
	----- (%) -----		
Native grass	30,50	29,50	30,10
Corn	20,62	-	8,77
Cassava	-	17,67	8,25
Copra meal	46,00	50,55	51,60
CaCO ₃	2,60	2,00	1,00
Salt	0,14	0,14	0,14
Premix	0,14	0,14	0,14
Dry matter	67,83	68,96	68,18
Ash	6,45	7,54	6,86
Crude Protein	16,01	15,95	16,50
Ether Ektrak	6,25	6,26	6,07
Crude fiber	21,27	22,15	22,25
Beta-N	50,02	48,10	48,32
Ca	1,65	1,72	1,71
P	0,42	0,42	0,44
FDN**	65,37	65,52	66,16

Note: *) Results Analysis Laboratory Animal Feed Science and Technology, IPB (2010). **) The results of the calculation according to Hartadi *et al.* (1997). Pj: ration with corn energi source, Po: ration energy sources, cassava, Pjo: ration with corn and cassava energy source

The Design of Experiment and Data Analysis

Research using completely randomized design with 3 treatments and 5 replications. Treatment is provided rations with different energy sources, ie Pj = corn, Po = cassava meal and , Pjo=corn+cassava meal. The variables observed were dry matter consumption ewe, the ewe body weight gain, percentage of

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pregnancy, number of embryos (litter size), the sex ratio of lambs, twins and single ratio, child birth weight, mortality at birth, weight shrinkage parent and pre-weaning growth of lambs. Data were analyzed using analysis of variance (Analyses of Variance, ANOVA) (Steel and Torrie, 1993) for dry matter consumption of ewe and growth of lamb pre weaning, while the reproductive variables were tested with descriptive analysis.

Estrus Detection and Mating

Estrus detection is conducted by observing the behavior and coupled with a male in the cage group. Females which desire would marry naturally.

Pregnancy and the number of embryos

The success of pregnancy and ovulation rate (number of embryos) was detected using ultrasound through transrectal conducted approximately one month after ewe mated (Bearden, 2004).

RESULTS AND DISCUSSION

The dry Matter Intake

The dry Matter consumption of ewes since pre mating, pregnant and lactation were presented in Table 2

Table 2. The dry matter consumption of ewes pre matting, pregnancy and lactation

Ration	Dry matter intake (g/head/days)			
	Pre matting	Early pregnancy	late pregnancy	Lactation
P0	477.70 n=5	518.98, n=2	457.75 n=1	516.77 n=1
P1	497.66 n=5	542.02, n=5	482.00 n=5	549.03 n=5
Pjo	477.76 n=5	537.12, n=3	507.95 n=3	549.91 n=3

Statistically, rations containing corn, cassava and the combination did not affect the level of consumption of dry matter a sheep, from pre-mating, pregnant until lactation. It illustrates that the three rations containing feed material with the same level of palatability. These results are consistent with the results of Thang *et al.* (2010) and Browne *et al.* (2005), that the use of corn and cassava meal as a source of energy in ruminant feed having the same effect on energy consumption. Even though not statistically significantly different, look no reduction in the consumption pattern of dry matter of the parent at the end of pregnancy, which then increased again during lactation. Decrease in dry matter intake in late pregnancy are more likely due to the increase in size and number of fetal abdominal cavity resulting in full, so that the less is available to accommodate the feed (Robinson, 1986), an increase in dry matter intake is high enough during lactation, according to the statement Forbes (2007), that increased consumption due to stem postpartum high milk production and a larger volume of the stomach in the absence of fetus.

Increase body weight of ewe

Ewe body weight gain in early pregnancy, birth and two months postpartum, were presented in Table 3.

Table 3. Body weight of ewe early pregnancy, birth and Weight depreciation of postpartum

Ration	Body weight (kg/head)				
	Early pregnancy	A moment after childbirth	difference (Δ^a)	Weaning	difference (Δ^b)
Pj	19,00 ± 0,00	22,00 ± 0,00	3,00	23,00 ± 0,00	1.00
Po	19,25 ± 1,32	23,00 ± 2,35	3,75	22,38 ± 1,89	-0.63
Pjo	20,17 ± 1,15	25,17 ± 1,04	5,00	24,57 ± 1,21	-0.60

Description: The difference (Δ^a) = Weight of the parent after the birth - initial weight; (Δ^b) = weight two months post partum - the weight after giving birth

In all treatments, it appears there was an increase in body weight of the mother from early pregnancy until birth and 2 months postpartum. Difference in body weight at birth and early pregnancy for the mother sheep consuming rations with corn and cassava meal to 5 kg, whereas rations containing corn or the cassava meal each 3 kg and 3.75 kg. Depreciation of sheep body weight two months after giving birth parent that consume energy sources rations with corn, cassava meal and combinations respectively 1 kg, 0.63 kg and 0.6 kg, it appears that the decline of the parent body weight gain ration energy sources, corn and cassava meal, have shrinkage small compared to other treatments. The amount of depreciation body weight after giving birth to 2 months postpartum is important to note, because it has to do to preparedness of the parent to be married again.

The combination of energy sources such as corn and cassava meal are able to provide energy to parent a faster recovery compared to only cassava meal or the corn alone. For cassava meal seems better as well in providing energy than corn. This is consistent with the value of the energy digestibility of rations with corn and cassava meal, it is significantly higher than any rations containing cassava meal or the corn alone (Karolita, 2011).

The Ewe Reproduction performances

Performances reproduction ewewithration containing corn, cassava meal, and combination of both are presented in Table 4.

The Pj, Po and Pjo percentage of pregnancy each 60, 100 and 60%. The success rate of pregnancy on Po ration better than both the other rations. This is might be connected with the parent at the consumption of before mating, where Po rations consumed more than Pj and Pjo rations. Parent before mating feed intake can affect ovulation rate as described (Docic and Bilkei, 2001), the consumption ration which affects ovulation rate before feeding (flushing) or after mating may increase ovulation and fertility, can improve the growth of follicles (Docic and Bilkei, 2001), to growth of follicles increased the more it will increase

Table 4. Performance reproduction of ewes

Variables	Treatment			Average
	Pj	Po	Pjo	
The number of initial ewe (head)	5	5	5	5
The number of pregnancy ewe (head)	3	5	3	3.66
The presentation of pregnancy (%)	60	100	60	73.3
The number of embryo (USG)	6	9	6	7
Litter size (%)	33.33	120	100	90,00
Embryo mortality (%)	83,33	33,33	50	55,56

the rate of ovulation, ovulation to a high level of success the pregnancy would be high (Sumaryadi and Wasmen, 1996). Energy content of to different sources in the treatment is thought to have an impact on the success of the parent pregnancy. Pregnancy success Po parent compares favorably to other treatments. The nature of cassava meal starch which easily degraded, may be able to produce the form of VFA propionate, so availability may be a precursor of glucose as a primary energy source in the process of egg maturation, glucose derived from propionate is the main energy source for the hypothalamus of work in the process of egg maturation, through regulation of FSH and LH to ovulation and formation of corpus luteum (Hess, 2005).

The number of embryos and the litter size

Based on the results of ultrasound, the average number of embryos Pj, Po and Pjo are respectively 6.00, 9.00 and 6.00 tails. While the numbers of litter size each 1, 6 and 3 tailed (3:33 average tail). The number of Po embryos more than Pj and Pjo treatment, so with the number of children born. It was probably has to do with the high consumption of Po in the moments before a pregnant and early pregnancy compared to other treatments.

The parent consumption before pregnant is 497.66 g / head / day, whereas the early pregnancy is 542.02 g / head / day. The high consumption of will affect the availability of the energy needed in the process of ovulation. These results are consistent with several studies that try flushing program on small ruminants showed that to flushing program, the rate of ovulation and laying fetus in the uterus can be repaired (Kusina *et al*, 2001; Acurero, 2000). Sabra and Hasan (2008), in his research to obtain the result that the flushing program conducted before cattle breeding can markedly increase estrus, estrus cycles shorten, increasing the percentage of lambingrate.

Embryonic mortality

The mortality rate was calculated by comparing the number of embryos which lamb birth with the number of embryos successfully ultrasound results. There is a considerable difference between the number of embryos ultrasound results to the number of lamb was born. Embryo mortality of ewe that consumes Pj, Po and Pjo respectively are 83.33%, 33.33% and 50%. The greatest mortality are on rations with carbohydrate derived from corn. Mortality of embryo is higher than that reported Dixon *et al*. (2007), that death can occur in embryonic or fetal stage is 19.9%. High embryonic mortality suggests that parent sheep are not able to maintain pregnancy. This could happen due to the lack of compounds required to maintain pregnancy, such as hormone progesterone, or alternatively, due to the high protein derived from coconut as a source of protein ration, in the form of NH₃ that cannot be exploited well, when combined with corn and cassava meal. But when carbohydrates are cassava meal, embryo mortality is lower than both other treatments. This relates to the nature of cassava meal that are easily available in the rumen, making available more carbon framework to capture NH₃ derived from coconut, corn while non-structural carbohydrates slowly degraded in the rumen contains little C framework that can capture the NH₃. NH₃ possibility be accumulated and impact on embryo mortality.

Litter size

The litter size is the percentage of the number of lamb born per pregnant ewe. Litter size on Pj, Po and P+jo each 33.33%, 120% and 100% to an average of

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90%. The litter size of ewe which consume ration with cassava meal as energy sources better than both the other rations (Pj and Pjo). These results are in line with the number of embryos produced. The average litter size generated was low, when compared to the results of research Frimawaty (1998) on the thin tail sheep was improved presentation of litter size of feed (average of 120%). Litter size obtained values are still lower than Ismoyo research (2011) that provides flushing rations to different energy levels, resulting litter size of 150%.

The ratio of births (twins: Single)

Type of birth consists of a single type and twin births. A parent could produce one, two, even three in one birth. The parent ratio of twins that consume carbohydrate ration to cassava meal (Po) is more than the others. This is consistent to the number of embryos produced Po consume rations that also mean more number of embryos which amounts twins. The average of single and twin children ratio in this study are quite high 83:17. The number of twins produced different, to same energy level rations, illustrates that in addition to the energy levels of such statements Camero *et al.* (2008), which states that a high level of energy in the ration of the number of children born twins produce more than to energy content ration lower energy. Quality of resources energy will also possibility affect the number of multiple births.

CONCLUSION

Based on the pregnancy rate, number of embryos, number of litter size, presentation of litter size and the number of twins produced shows that the quality of ration energy sources can affect the performance reproductive of local sheep. Generally cassava meal as a source of carbohydrate ration providing reproductive performance better than rations containing only corn or a combination of both. Ration energy sources, cassava, corn and combinations provide pre-weaning lamb performance of local sheep the same well.

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P-18. THE USE OF *Trichoderma harzianum* IN THE FERMENTATION OF TOFU WASTE PRODUCT

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Abstract

A study on the use of *Trichoderma harzianum* in the fermentation of tofu waste product was conducted from 23 May to 30 June 2012 at Chemistry Laboratory, Djuanda University, Bogor. The study was aimed at assessing the quality of tofu waste product fermented with *T. harzianum* in different fermentation periods. *T. harzianum* was used by 5, 10, and 15%. Fermentation was done within 1, 2, 3, 4, 5, 6, and 7 days. Water content, fiber content, pH, total acid, color, aroma, and texture of the resulted fermented product were measured. Data were subjected to an analysis of variance and a Duncan multiple range test.

The fermentation was found to run well as indicated by the stable pH of 4 found during the fermentation process from day 1 to 7. Water content of the fermented tofu waste product was not different ($P>0.05$). The lowest fibre content (5.68%) was found in the product fermented with *T. harzianum* in 3 days ($P<0.01$). The product fermented with *T. harzianum* in 2 days was found to have the highest total acid content (0.56%). Fermentation with 15% *T. harzianum* any days of fermentation period gave the best fermented product with bright color, acidic aroma, and soft texture with no mucous found.

It was concluded that the use of 15% *T. harzianum* gave the best fermented tofu waste product. A further study to assess the effect of this fermented tofu waste product on the performance of poultry and other animals was suggested.

Keywords: tofu waste product, fermentation, *T. harzianum*.

INTRODUCTION

Tofu waste product is a solid waste of tofu production. This waste product is reasonably nutritious as it contains 22.64% crude protein and 4010 kcal/kg gross energy (Tanwiriah *et al.*, 2007). Tofu waste product has been used as feed in ruminants and poultry. However, the use of it as poultry feed is restricted by its high fiber content. High water content is another constrain this feed has making it difficult to store for pending use. Fermentation of tofu waste product with *Trichoderma harzianum* process is expected to degrade its fiber and improve its nutritive value so that more proportion of it can be used in poultry ration. In addition, fermentation is also expected to reduce the water content of tofu waste product so that it can be stored for longer time.

Fungi of the genus *Trichoderma* are abundantly found in soil all over the world. They have been studied for various applications and are proved to be successful colonizer of their habitats and tough fighter to their competitors. These fungi are found as potential decomposer of various substrates (Schuster and Schmoll, 2010). *T. harzianum* is used in the fermentation process for its ability to produce cellulase enzyme. It has been used to improve nutrient quality of forages and agroindustrial waste products including cassava root (Muindi and Hansen, 1981), wheat bran, rice bran, soybean husk, tofu waste product (Noviati, 2002),

banana peel (Sankar *et al.*, 2011), salvinia weed (Herawaty and Latief, 2009), azolla (Noferdiman, 2012).

The study was conducted in order to produce quality and low-cost feed for poultry. The specific aim of this study was to determine the optimal condition and the most appropriate fermentation duration to produce the best fermented tofu waste product by using *T. harzianum*.

MATERIALS AND METHODS

The study was conducted from 23 May to 30 June 2012 at Chemistry Laboratory, Djuanda University, Bogor. *Trichoderma harzianum* inoculum was obtained from Biotechnology Research Center of the Indonesian Institute of Science (LIPI), Cibinong, Bogor. Tofu waste product was purchased from a local tofu industry in Tajur, Bogor.

Fermentation was conducted according to the following procedures. One-hundred grams fresh tofu waste product was put into plastic bags. To avoid leaking double plastic bag was used. *T. harzianum* inoculum was added in the concentrations of 5, 10, and 15%. Fermentation was done within 1, 2, 3, 4, 5, 6, and 7 days. Two replicates were allocated into each treatment. These procedures are depicted in the following flow chart in Figure 1.

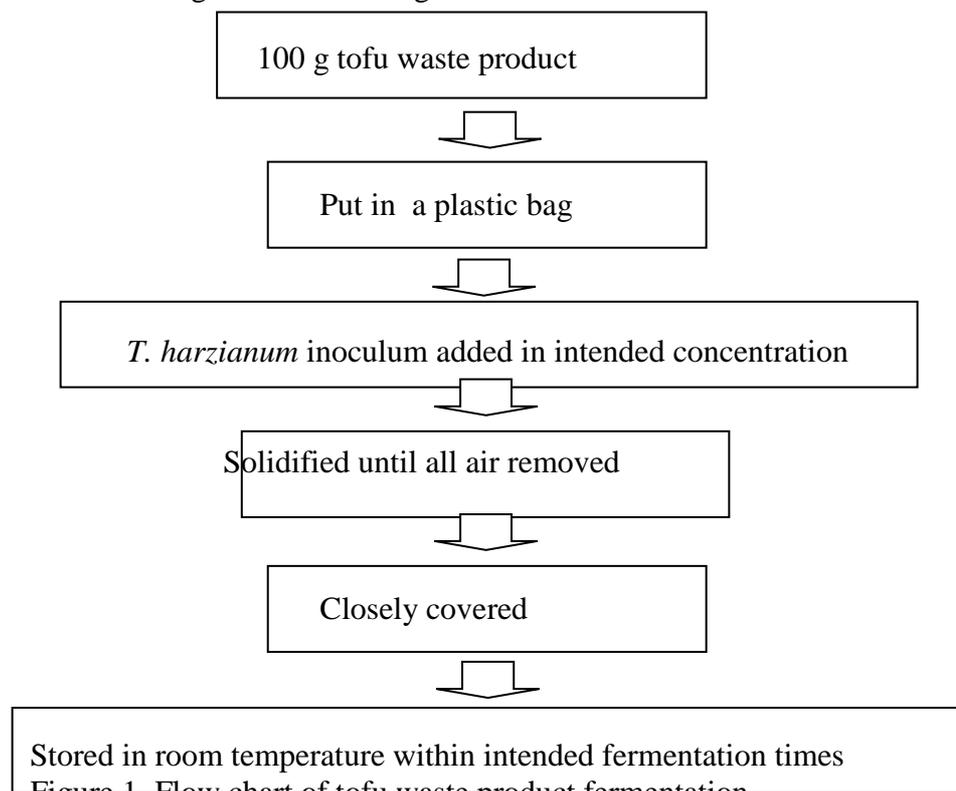


Figure 1. Flow chart of tofu waste product fermentation

Fermented tofu waste product was analyzed for its water and fiber contents by using a proximate analysis (AOAC, 1998). Acidity level (pH), total acid content, and physical properties including texture, color, and aroma of the fermented tofu waste were measured. pH was determined by using litmus paper. Total acid content was measured by using a titration method developed by Hadiwijoto (1994).

A completely randomized factorial design was used. Data were subjected to an analysis of variance and a Duncan multiple Range Test (Gomez and Gomez, 1995). Regression analysis was conducted by using Minitab 14 application.

RESULTS AND DISCUSSION

Acidity level (pH)

Acidity level (pH) is an important measure to indicate good fermentation process. The acidity level (pH) in this study was found decrease from 5 when the fermentation was initiated to 4 during the fermentation process from day 1 to 7. This indicated that the fermentation process ran well as the pH value was within the range of 4.0-4.5, a range of pH values of good fermentation process for substances containing high water content (Kung and Shaver, 2001). However, somehow different pH values might be obtained if the measurement were taken by using a more precise pH meter device than litmus paper used in this study. pH values obtained during the fermentation process are presented in Table 1.

Table 1. Acidity level (pH) of tofu waste product fermented with *T. harzianum*

Concentration of <i>T. harzianum</i> (%)	Day of fermentation							
	0 (control)	1	2	3	4	5	6	7
(control)	5	4	4	4	4	4	4	4
5	5	4	4	4	4	4	4	4
5	5	4	4	4	4	4	4	4
5	5	4	4	4	4	4	4	4

Water content

Water content offermented tofu waste product was given in Table 2. No interaction effect of day of fermentation and concentration of *T. harzianum* was found. Fresh tofu waste product used in this study had a mean water content of 90.27%. Neither day of fermentation nor concentration of *T. harzianum* used affected the water content of fermented tofu waste product. High water content was not preferable as high water content in fermented products would not allow the products to be stored in longer time. Therefore, a treatment such as mixing tofu waste product with a substance with low water content such as dry sugarcane leaves (Hernaman *et al.*, 2005) deserves a consideration.

High water content found in fermented products is expected. Microorganisms used in the fermentation process will use the energy resulted from the degradation of carbohydrate contained in the substrate. In addition to energy, carbohydrate degradation results in water and carbon dioxide (Fardiaz, 1988).

Crude Fiber Content

Crude fibercontent offermented tofu waste product was given in Table 2. No interaction effect of day of fermentation and concentration of *T. harzianum* was found. Although no significant effect was detected, there was a tendency (P=0.098) that higher concentration of *T. harzianum* reduced the fiber content of fermented product. The lowest fibre content (35.68%) was found in the product fermented with *T. harzianum* in 3 days(P<0.01).

Tabel 2. Water, crude fiber, and total acid contents of tofu waste product fermented with *T. harzianum*

Day of fermentation	Parameters		
	Water (%)	Crude Fiber (%)	Total Acid (%)
1	88.27±5.78	39.92±3.08 ^{AB}	0.43±0.04 ^C
2	90.63±0.43	44.52±6.44 ^B	0.56±0.08 ^D
3	90.34±0.83	35.68±2.86 ^A	0.10±0.02 ^A
4	90.95±0.88	36.85±3.03 ^A	0.18±0.02 ^{AB}
5	91.02±0.68	38.96±4.59 ^{AB}	0.17±0.02 ^{AB}
6	89.15±5.02	41.07±3.53 ^{AB}	0.26±0.11 ^B
7	91.15±0.57	36.42±4.66 ^A	0.21±0.04 ^B
Concentration of <i>T. harzianum</i> (%)			
(control)	90.27±0.83	40.52±7.63	0.26±0.13
	89.38±3.20	40.94±4.60	0.26±0.13
	89.60±4.23	37.84±4.20	0.28±0.18
	91.64±0.50	37.67±3.73	0.29±0.20

Note: different superscripts in the same column indicate significant difference (P<0.01)

In the early stage of fermentation process, fungi require lot of glucose for their growth and enzyme production. This results in higher glucose and lower cellulase amount (Herawaty and Latief, 2009).

Results of this study were different from those obtained by Novianti (2002). She found a decrease of crude fiber content by 51.08% in tofu waste product fermented with only 2% *T. harzianum* in 6 days.

Total Acid

Acid content is a measure of the quality of a fermentation process of a substrate. Good fermentation process results in fermented product with high acid content. The total acid content of fermented tofu waste product in this study is listed in Table 2. No interaction effect of day of fermentation and concentration of *T. harzianum* was found. Concentrations of *T. harzianum* were found to give no significant effect on total acid content of the fermented tofu waste product. On the other hand, longer fermentation time significantly (P<0.01) reduced the acid content. The highest total acid content (0.56%) was found in tofu waste product fermented in 2 days.

It was shown in Figure 2 that the relationship between total acid content (TA) and day of fermentation (DF) in fermented tofu waste product was quadratic (TA = 0.6769 – 0.1992 DF + 0.0196 DF²). After a significant increase of total acid in day 2 of fermentation, there was a sharp drop of in day 3. Then, it gradually rebounded from day 4 to 7 of fermentation. This phenomenon might be explained by the notion that fermentation process is a dynamic process and can be divided into several phases (Seglar, 2003). In silage fermentation, ensiling process occurs extensively in phase 2 starting on day 2. In this phase, significant amount of volatile fatty acids including acetic, lactic, and propionic acid is produced. This phase lasts no longer than 24-72 hours. In phase 3, heat dissipates from the fermented substrate and pH decreases. This results in the inhibition of lactic acid production. As pH gets more stabilized, in phase 4, lactic acid production starts to increase (Seglar, 2003). In this study, phase 2 seemed to

occur in day 2 and lasted for about 24 hours until day 3 when phase 3 started to take place.

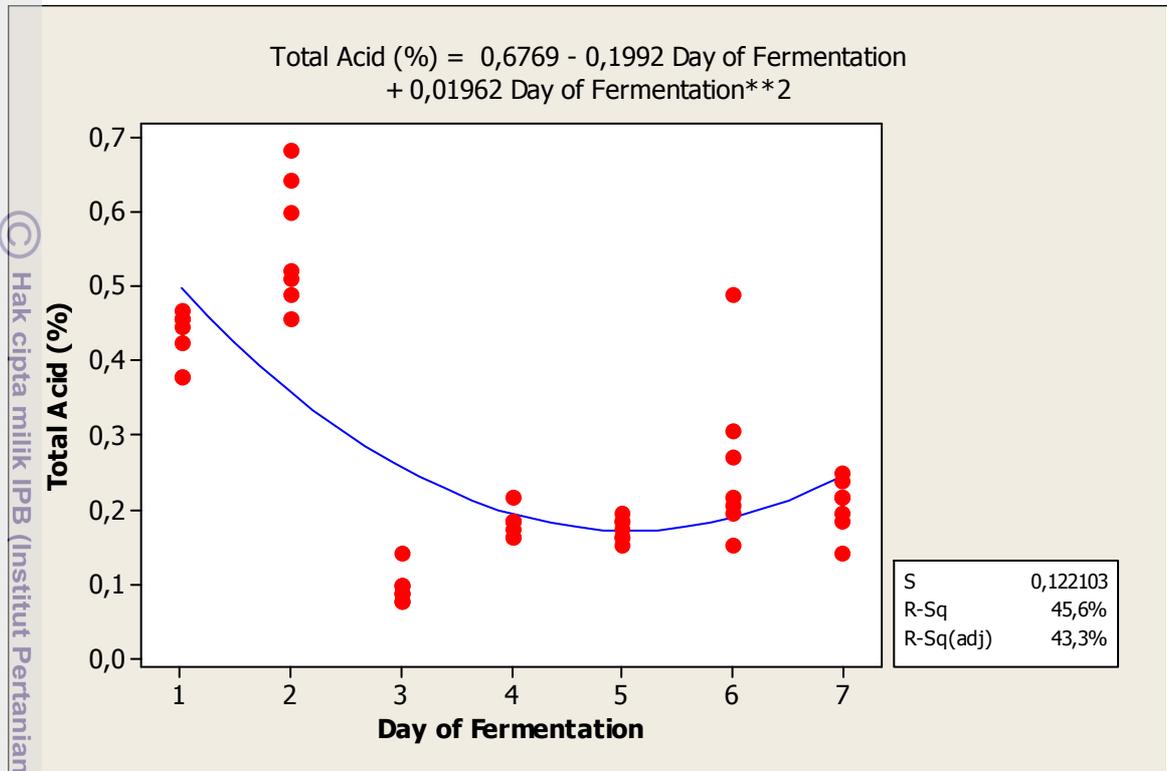


Figure 2. Relationship between total acid and day of fermentation in tofu waste product fermented with *T. harzianum*.

Physical Properties

Physical properties of fermented tofu waste product assessed in this study included color, aroma, and texture. They are presented in Table 3.

Color

It is shown in Table 3 that there were changes in color during fermentation period. Clear change in color was found in untreated tofu waste product. The color of untreated product changed from dark white on day 1 to cloudy yellow on day 7 indicating that this untreated product underwent a putrefying process.

The use of 5% *T. harzianum* seemed to be unable to preserve the freshness of tofu waste product. This was indicated by the formation of cloudy white color in the substrate. Meanwhile, the use of *T. harzianum* of 10 and 15% was found to be effective in maintaining the freshness of tofu waste product. This was shown by the formation of brighter white color in the fermented product.

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Table 3. Physical properties of tofu waste product fermented with *T. Harzianum*

Day of fermentation	Concentration of <i>T. harzianum</i> (%)			
	0 (control)	5	10	15
Color				
1	Dark white	Cloudy white	White	White
2	Cloudy white	Cloudy white	White	White
3	Cloudy white	Dark white	White	White
4	Dark white	Cloudy white	White	White
5	Cloudy white	Cloudy white	Cloudy white	Bright white
	Cloudy white	Cloudy white	White	White
	Cloudy yellow	Cloudy white	Bright white	Bright white
Aroma				
	Rather putrefied	Acidic	Acidic	No aroma
	Rather putrefied	Little acidic	Little acidic	Little acidic
	Putrefied	Acidic	Acidic	Acidic
	Putrefied	Acidic	Acidic	Acidic
	Putrefied	Acidic	Acidic	Acidic
	Putrefied	Little putrefied	Little acidic	Little acidic
	Putrefied	Rather putrefied, acidic	Rather putrefied, acidic	Acidic
Texture				
1	Soft, no mucous	Soft, rather moist	Soft, rather moist	Soft, rather moist
2	Soft, no mucous	Soft, rather moist	Soft, rather moist	Soft, rather moist
3	Soft, no mucous	Soft, no mucous	Soft, no mucous	Soft, no mucous
4	Soft, no mucous	Soft, no mucous	Soft, no mucous	Soft, no mucous
5	Soft, no mucous, moist	Soft, no mucous, moist	Soft, no mucous, moist	Soft, no mucous, moist
	Soft, rather dry	Soft, no mucous	Soft, no mucous, moist	Soft, no mucous, moist
	Coarse, rather dry, moldy	Soft, no mucous	Soft, no mucous	Soft, no mucous

Aroma

Aroma is an important measure of good fermentation process. As fermentation produces significant amount of volatile fatty acids, acidic aroma is

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expected to be found in well fermented product. Untreated tofu waste product immediately underwent a putrefying process since day 3. This was indicated by putrefied aroma emitted by unfermented tofu waste product. In contrast, treated tofu waste product was found to have strong acidic aroma. However, tofu waste product fermented with *T. harzianum* of 5 and 10% started to emit putrefied aroma on day 7 of fermentation. This was not found in the product fermented with 15% *T. harzianum* which still strongly smelled acidic.

Texture

All treated tofu waste product were found to have soft texture with no mucous. Compared to untreated product, fermented tofu waste product had moist texture. This was caused by increased water content as a result of degradation activities of enzymes produced by microorganisms during the fermentation process (Fardiaz, 1992). Moulds were found in the untreated tofu waste product while all fermented products were found to be free from mould.

CONCLUSIONS

The use of *T. harzianum* resulted in well fermented tofu waste product. The use of 15% *T. harzianum* gave the best fermented tofu waste product with bright white color, acidic aroma, soft texture and no mucous. The good result of fermentation of waste tofu product with 15% *T. harzianum* was consistent from day 1 to 7. A further study to assess the effect of this fermented tofu waste product on the performance of poultry and other animals was suggested.

ACKNOWLEDGEMENT

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P-19. *INDIGOFERA ZOLLINGERIANA* ADAPTATION TO DROUGHT STRESS AND MYCORRHIZA INOCULATION

Simel Sowmen^{a)}, L. Abdullah^{b)}, P.D.M.H. Karti^{b)}, D. Soepandi^{c)}

Abstract

The study was conducted to recognize adaptation mechanisms of *Indigofera zollingeriana* inoculated with arbuscular mycorrhiza fungi (AMF) and investigate effect of mycorrhizal inoculation to legume adaptability to overcome drought stress. This experiment was arranged in factorial completely randomized design with three replicates, the first factor was AMF inoculation (no mycorrhiza, with mycorrhiza), the second factor was drought (optimum watering, drought stress). Physiological and agronomical parameters consisted of soil water content (swc), leaf water potential (lwp), leaf relative water content (lrwc), leaf proline, leaf water soluble carbohydrate (lwsc), and plant dry weight were observed. Data were analyzed by ANOVA and differences between treatments were tested by LSMEAN. The result showed that drought stress significantly reduced ($P < 0.05$) swc, lwp, lrwc, plant dry weight, and increased proline content of *Indigofera zollingeriana*. There was no significant interaction ($P > 0.05$) between AMF inoculation and drought treatments in all parameters. *Indigofera zollingeriana* showed more tolerance to drought condition.

Keywords: drought stress, legume, arbuscular mycorrhiza fungi, proline, *Indigofera zollingeriana*

INTRODUCTION

Indigofera zollingeriana is a potential tree legume to be used as feed crop. *Indigofera* having a crude protein content of 27 to 31% (Abdullah, 2010). Forage availability is strongly influenced by the seasons especially the dry season when there is a shortage of soil water availability. Water stress in plants reduces the plant-cells water potential and turgor, which elevate the solutes concentrations in the cytosol and extracellular matrices. As a result, cell enlargement decreases leading to growth inhibition and reproductive failure (Lisar *et al.*, 2012). Plants can respond to drought stress at morphological, anatomical and cellular levels with modifications that allow the plant to avoid the stress or to increase its tolerance (Ruiz-Lozano, 2003). Utilization of mycorrhizae may assist plants to overcome drought stress. Therefore, the purpose of this work was to assess the effect of drought treatment and inoculation of mycorrhizae on physiological and morphological adaptation of *Indigofera zollingeriana* and to investigate effect of mycorrhizal inoculation to *Indigofera zollingeriana* adaptability to overcome drought stress.

MATERIALS AND METHODS

AMF inoculation used Mycofer that contains *Gigaspora margarita* and *Glomus manihotis*, commercial product produced by Laboratory of Forest Biotechnology, PAU, Bogor Agricultural University. Five kg pot capacity used in this study was filled with soil-manure in ratio of 9:1. Young *Indigofera zollingeriana* (\pm one month) planted to 16 pot (8 pot without mycorrhiza and 8 pot inoculation with mycorrhiza) and growth for one month. Before drought treatment started, all the pots watered until saturated conditions are created.

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Variables

Leaf RWC (Slatyer & Barrs, 1965) was measured every 4 d during treatment. Leaf Prolin content was analyzed using Bates *et al.*, 1973 every 8 d during treatment. Leaf Water Potential was tested every 4 d using Dewpoint Potentio Meter WP4. Water soluble carbohydrate was analyzed using Dubois *et al* (1956) method and modified according Buysse and Merckx (1993). Plant biomass dry weight measurement were performed at harvesting time. Shoot, root and stem were dried up in the oven 70°C for 48 h.

Experimental Design

was arranged in factorial completely randomized design with three replicates, the first factor was AMF inoculation (no mycorrhiza, with mycorrhiza), the second factor was drought (optimum watering, drought stress). Data were analyzed by ANOVA and differences between treatments were tested by LSMEAN.

RESULT

Drought stress significantly reduced ($P < 0.05$) soil water content (swc), leaf water potential (lwp), leaf relative water content (lrwc), plant dry weight, and increased prolin content of *Indigofera zollingeriana*. There was no significant interaction ($P > 0.05$) between AMF inoculation and drought treatments in all parameters.

Soil water content and leaf relative water content decreased significantly with increasing drought duration (Figure 1). In 20 d of drought stress treatment had lower RWC values than 8 d.

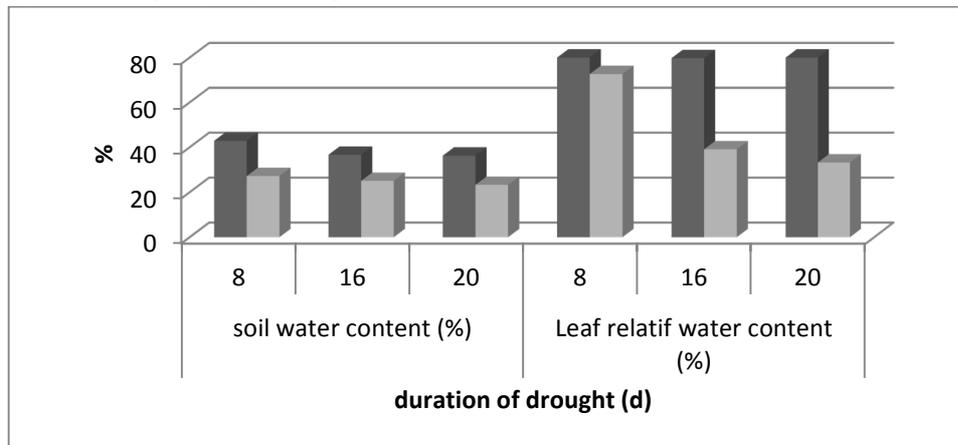


Figure 1. Effect of drought treatments to soil water content and leaf relative water content of *Indigofera zollingeriana*. ■ = watering, □ = drought stress.

Leaf water potential decreased during observation (Figure 2). Leaf water potential in 20 d were lower than 8 d drought stress treatment. This suggests that drought stress lowered leaf water potential inline with duration of drought.

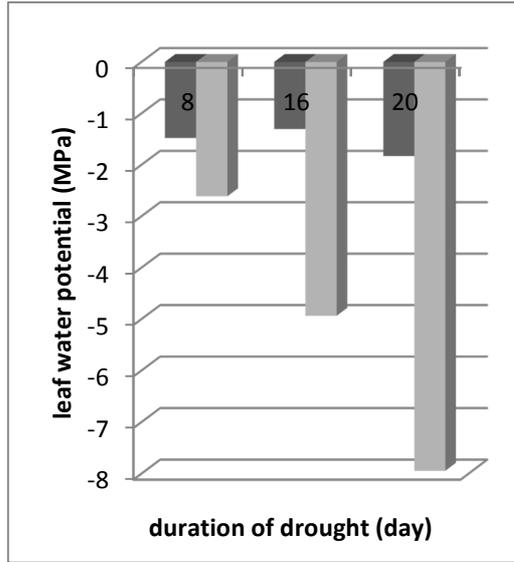


Figure 2. Effect of drought treatments to leaf water potential of *Indigofera zollingeriana*. ■ = watering, □ = drought stress.

Leaf proline content increased due to drought stress (Figure 3) and the increase was not influenced by the drought stress level. Highest leaf proline content occurred on the 16th day of drought stress.

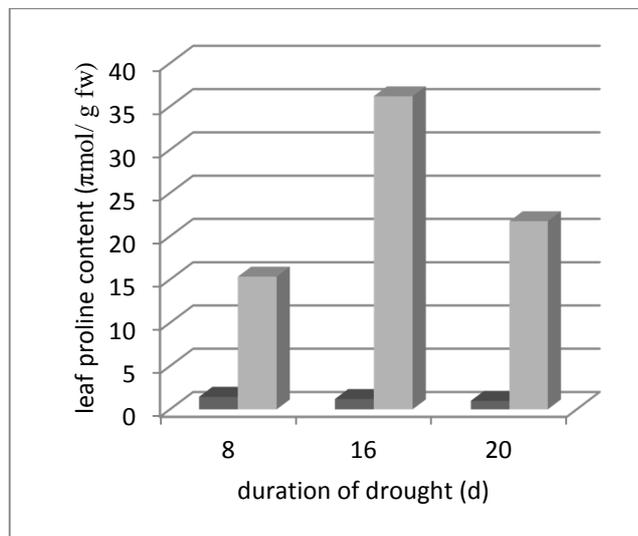


Figure 3. Effect of drought treatments to leaf proline content of *Indigofera zollingeriana*. ■ = watering, □ = drought stress.

Indigofera zollingeriana were not developed increasing water soluble carbohydrate mechanism when drought stress (Figure 4). Water soluble carbohydrate value in watered treatment were higher than drought stress plants.

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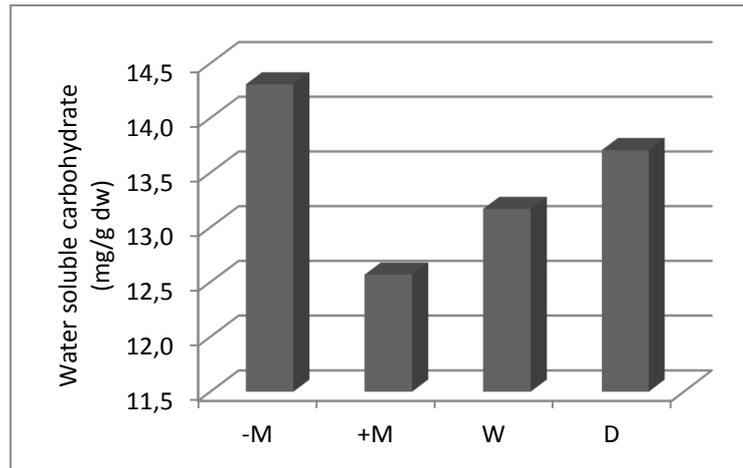


Figure 4. Effect of drought treatments to water soluble carbohydrate of *Indigofera zollingeriana*. -M = without mycorrhiza, +M = with mycorrhiza, W = watering, D = drought stress.

Drought stress significantly ($P < 0.05$) decreased plant biomass dry weight, and mycorrhiza inoculation increased plant biomass dry weight of *Indigofera zollingeriana* (Figure 5).

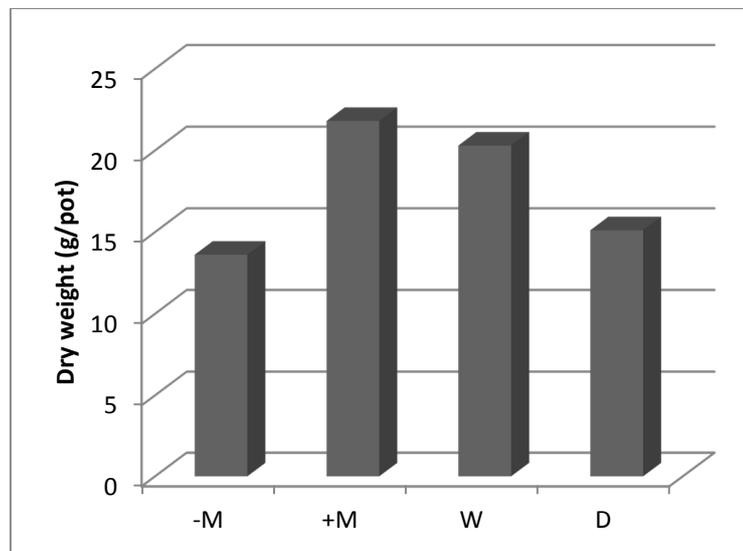


Figure 5. Effect of drought treatments to plant biomass dry weight of *Indigofera zollingeriana*. -M = without mycorrhiza, +M = with mycorrhiza, W = watering, D = drought stress.

CONCLUSIONS

Early growing phase of *Indigofera zollingeriana*. was more effective inoculated with mycorrhizal when exposed to drought stress. One mechanism of drought resistance in *Indigofera zollingeriana* is the accumulation of leaf proline. Drought stress does not always increase leaf water soluble carbohydrate content in plants.

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P-20. EFFECT OF COCOA POD AND COCOA LEAF ON *IN VITRO* FERMENTATION AND NUTRIENT DIGESTIBILITY

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Abstract

Indonesia has most cocoa byproduct such as pod cocoa and cocoa leaf that can be used as feed for ruminant. The aim of this experiment was to study the level of pod cocoa and cocoa leaf on *in vitro* digestibility and fermentation. Cocoa pod and cocoa leaf were previously treated with 4% urea. The four treatments consisted of native grass as control and three levels of cocoa by products were, A native grass (control), B = 75% pod cocoa + 25% cocoa leaf, C = 50% cocoa pod + 50% cocoa leaf, and D = 25% cocoa pod + 75% cocoa leaf. The formulated feeds were evaluated adopting Tilley and Terry method. Variables measured were Ammonia (NH₃) and Volatile Fatty Acid (VFA) concentrations, as fermentation indicators, as well as digestibility indicators including digestibility of dry matter (DM), and organic matter (OM). The results indicated that increasing use cocoa leaf in diet caused higher fermentation and digestibility of cocoa byproduct diet. Treatment D gave the similar result with treatment A. Further animal trials are need to confirm the optimal level of it use.

Key Words: cocoa pod, cocoa leaf, digestibility, fermentation, ammoniated

INTRODUCTION

Indonesia, a mayor cocoa (*Theobroma cacao*) producing country, generated vast quantities of cocoa by-product annually. Although a small fraction of it is converted into useful products, most of it is not utilized. The co-products/by-products from cocoa viz; cocoa leaf, cocoa bean shell and cocoa pod (constituting disposal problem at the factory sites and/or farmsteads in producing countries) have great potentials as feed ingredients, owing to their abundant availability, non-consumption by humans and richness in certain nutritional compounds (Hamzat *et. al.*, 2005; Nworgu, *et. al.*, 2003).

Marsetyo *et al.* (2008) showed that cocoa-pods, a by-product of the cocoa industry, could potentially be used as a feed resource for ruminants in areas where cocoa plantations exist. It may be a useful feed resource when fed at low levels of inclusion in the diet. The present study was conducted to evaluated the effect of different combination of the two cocoa by product (cocoa pod and cocoa leaf) on the *in vitro* fermentation and degradability in rumen.

MATERIALS AND METHODS

This research was conducted in the Ruminant Nutrition Laboratory, Faculty of Animal Science Andalas University during June to August 2013. The experiment was arranged in a Completely Randomized Design consisting of four treatments, each with four replication. The treatment diets consisted of native grass as control (A), and three combinations of cocoa by products viz B = 75% cocoa pod + 25% cocoa leaf, C = 50% cocoa pod + 50% cocoa leaf, and D = 25% cocoa pod + 75% cocoa leaf. Cocoa pod and cocoa leaf used was previously treated with 5% urea, as per

The fermentability and degradability of nutriens determined used the first stage of Tilley and Terry procedure (1963). Ruminal fluid was taken from a cannulated

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steer. Fermentation tubes contained of 0.5 gram samples and 40 ml of McDougall buffer solution and 10 ml of ruminal fluid and were incubated in 100 ml polyethylene tubes in 39°C for 48h in a water bath shaker. Treatments were replicated four times within an experiment and the experiment was repeated twice. The tubes that did not contain diets were also incubated and used as blanks.

After 48h h, fermentation was terminated by injecting the tubes with 1 ml of HgCl₂. Tubes were then centrifuged at 14000 x g for 15 min and the supernatant was collected and stored. The residue was dried for 48h at 60°C and weighed and the data were used for degradability determination. These residues were also analyzed for their DM, OM, and Nitrogen by using standardized procedures (1990). The NDF, ADF and cellulose of residues were determined as per method of [Goering and Van Soest \(1970\)](#). Supernatants were used to determine the total VFA concentration (by distillation), rumen fluid pH and NH₃ concentration (microdiffusion Conway method).

Data were analyzed by ANOVA using the GLM procedure. Differences between the control treatment and level of palm oil byproduct were analyzed by the Duncan multiple range test (DMRT) ([Steel and Torrie, 1980](#)).

RESULTS AND DISCUSSION

The average of digestibility and data on fermentation are presented in Table 1. The result showed that digestibility and fermentation of four treatments were significantly (P<0.05) different. Increased proportion of OPF in the diet caused higher digestibility and fermentation of nutrients in the rumen but it is lower than control treatment.

Table 1: Effect of various combination of cocoa by-products on *in vitro* fermentation and digestibility of nutrients

Parameters	Treatments			
	A	B	C	D
pH	6.79	6.80	6.82	6.94
VFA (mM)	122 ^a	90 ^b	97 ^b	105 ^b
NH ₃ (mg/dl)	8.5 ^b	9.25 ^b	11.759 ^{ab}	13.25 ^a
In vitro digestibility (%)				
DM	54,83 ^a	39,94 ^b	39,73 ^b	42,18 ^b
OM	58,44 ^a	38,05 ^b	37,08 ^b	41,85 ^b

Means in the same row with different superscript significantly (P<0.05)

There was no significant difference (p>0.05) effects of oil palm by-product combination on ruminal fluid pH. The pH range observed in this study (Table 2) was within normal ranges (6.0-7,0) which have been reported as optimal for microbial digestion of protein and fiber ([Van Soest, 1994](#)).

The concentration of NH₃-N in rumen of treatments were significantly (P<0.05). Further, when compare with control (A), the NH₃-N levels were significantly (P<0.05) increased when cocoa by-product were used in the diet irrespective of combination. Nonetheless, the observed, ruminal NH₃-N levels could be considered as optimum for microbial protein synthesis. The optimal NH₃-N concentration in ruminal fluid for maximum microbial growth or microbial protein synthesis is reported to be in the range of 5-8 mg/dl ([Satter and Tyler, 1974](#); [Pisulewski et al., 1981](#)).

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All the data on total VFA concentration in the rumen were significantly different ($P < 0.05$) among treatments following the same trend as that of $\text{NH}_3\text{-N}$. The VFA concentration was shows a clear decreasing when the level of cocoa pod in the diet was increased. High silica content and the slow rate of fermentation of fiber, could have been the cause for such a trend. The role of end products of fiber digestion in relation to the overall efficiency of energy was reported by [Mertenz, 2009](#));

Digestibility of DM, OM were significantly ($P > 0.05$) different among the treatments. The increasing use of the cocoa pod level in the diets decreased in nutrient digestibility. This could be related to the increasing of lignin content with higher levels of OPF incorporation that limit digestibility in ruminants.

CONCLUSION

Results of the study showed that cocoa by product has the potential to be used as ruminant feed. From the results using combination of 25% cocoa pod and 75% cocoa leaf, may be considered to give acceptable ranges of performance. However, further trials using target animals should be conducted to delineate optimum levels of its inclusion in a ruminant feed.

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P-21. EFFECT OF VITAMIN E SUPPLEMENTATION IN THE EXTENDER ON FROZEN – THAWED SEMEN PRESERVATION OF PESISIR CATTLE

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Abstract

The objective of this experiment to know the effect of vitamin E supplementation on semen extender to semen quality of frozen – thawed of Pesisir cattle . Vitamin E was added at concentration of 0.0; 0.1;0.15; 0.2; 0.25 and 0.3 gr/100ml to semen cryopreservation medium. Semen was collection from cauda epididymis of Pesisir cattle in slaughterhouse in Padang. The result showed that the sperm quality were highly significant ($P<0.01$) increased with supplementation of vitamin E. The percentage of sperm motility and live sperm in the extender supplemented vitamin E 0.3 gr/100ml was significant higher than other concentration ($P<0.05$) and the percentage of sperm abnormality was significant ($P<0.05$). The sperm motility and live sperm in the extender supplemented with vitamin E 0.2 gr/100ml and 0.25 gr/100ml were not significant ($P>0.05$). the extender supplemented with vitamin E 0.1 and 0.15 GR/100 ml exhibit significant ($P>0.05$) improve semen quality compare to control (0.0 gr/100ml). In conclusion of this research was vitamin E supplementation in the extender improve the semen quality during freezing – thawing.

Key words: semen,extender,vitamin E,frozen, Pesisir Cattle

INTRODUCTION

Pesisir cattle is one as a native cattle in west Sumatera as a beef production. In bovine industry artificial insemination is being used in breeding program in this country. Application of AI in Pesisir Cattle needs to semen cryopreservation for mating many cow. Poor reproductive rates are largely due to the limited number of bull in capacity, few opportunities for natural breeding and low semen quality after thawed and can prevent successful fertilization or conception. The population of Pesisir bull in rural farm is very small, this condition is caused high demand for meat production.

Frozen semen caused activity of sperm decrease , it relative with cold shock. The semen quality tend decrease during freezing – thawing. The problem of semen cryopreservation decrease the fertility (Hammersted,1990). During the procedure of frozen semen production sperm cells are exposed to oxygen and visible light radiation. This can lead to the formation of reative oxygen species (ROS), which damage sperm motility and genomic integrity (Foote and Hare,2000). Hu et al. (2011 reported that bovine semen cryopreservation allows the widespread dissemination of valuable genetic material. However the over production of reaction oxygen species (ROS) causes sperm membrane structural daaaaaamage and result in loss of 40 % to 50% of viable sperm during freeze – thawing. As the primary component of the sperm antioxidant system, vitamin E is major membrane protectant against ROS. Additions of various antioxidants to spermatozoa have been investigated in frozen – thawed semen of bulls (Beconi et al. 1993)

Bovine sperm cell contain a high proportion of polyunsaturated fatty acids and are therefore susceptible to peroxidative damage, speccially during cryopreservasi with a subsequent loss in membrane integrity and decrease sperm motility. According to Hu et. Al. (2011) that the vitamin E supplementation in the extender resulted in positive effect on bovine sperm motility and movement characteristic acrosom and membrane integrity and antioxidant activity. Supplementation vitamin E (0.3 gram α – tokoferol/ 100 ml extender) on freezing of FH cattle increased semen quality (Widiastuti,2001). However there are limited limited data of Pesisir cattle as a native cattle regarding the effect of

vitamin E in different extenders on sperm quality. Therefore the objective of this research was to know the effect of vitamin E supplementation on semen quality during freeze – thawed.

MATERIAL and METHODS

Semen collection

Semen was carried out from four cauda epididymis of Pesisir cattle at domestic slaughter house in Padang. Semen samples were collected by aspirating methods from cauda epididymis in 0.5 ml Talp medium and evaluation both fresh and frozen semen.

Extender preparation The extender for this treatment group used was composed in table 1 follows;

Table 1. extender composition

Bahan	Volume
Buffer	
- Natrium sitrat (gr)	2,9
- Fructose (gr)	1
Krioprotectance	
- Egg yolk (ml)	20
- Glycerol (ml)	6
Antibiotic	
- Penicillin (iu/ml)	1.000
- Streptomisin (mg/ml)	1.000
Distilwater (ml)	100

This extender supplemented with vitamin E at 0.1 ; 0.15; 0.2; 0.25 and 0.3 gr/ 100ml. The extender for control samples was not supplemented with vitamin E.

Semen processing

After the evaluation of semen quality, pooled semen was divided into six fraction . one fraction as a control treatment and five fraction as a treatment with vitamin E. Semen was cooled in 5 °C during four hours in refrigerator for equilibration stage. After that semen sealed in straw 0.25 cm and sealed and seeding to frozen and store to liquid nitrogen tank at -196°C. After 2 week before thawed evaluation the quality such as : live sperm percentage. Motility and sperm abnormality.

Statistical analysis

Data analysis was used analysis of variance in randomized block design . the mean values of live sperm, motility and abnormality were compared using Duncan's multiple range test.

RESULT AND DISCUSSION

Characteristic of fresh semen of cauda epididymis of Pesisir Cattle

The semen quality of Pesisir cattle was evaluated as macroscopic and microscopic shown in table 2. The average of semen volume range from 0.3 ml to 0.5 ml.

The characterization of macroscopic and microscopic evaluation of Pesisir cattle was relatively normal. This result is similar to Toelihere (1993) that semen volume of cauda epididymis ranges from 0.15 – 0.6 ml , Ph 6.4 – 7.8. According to Garner and Hafez (2000) that sperm concentration per ml range from 800 – 2000 millions/ml. High sperm concentration is related to sperm colour (Rouge,2003). Campbell et. Al. (2003) added that sperm motility ranges from 70 – 80 % and sperm abnormality 5 – 15 %.

Table 2. The averages characteristic of Fresh Semen from Cauda Epididymis of Pesisir Cattle

Parameters	Rataan
Volume (ml)	0.37
Warna	Creemy
Bau	Normal
pH	7
Motility (%)	70
Concentration (10 ⁷)	152.5
Live sperm (%)	78.37
Abnormality (%)	13.5

Semen quality frozen – thawed

Sperm motility

The effect of vitamin E supplementation in extender on frozen – thawed of Pesisir cattle are shown in table 2. The motility of sperm were improved (P<0.01) in presence of vitamin E compare to control. The highest motility percentage was 60 % in supplemented 0.3 vitamin E and the lowest was 37.5 % in control (without vitamin E). In this result shown that supplemented vitamin E 0.1 and 0.15 was no significant different (P>0.05) to control (without vitamin E). There is no significant different (P>0.05) between 0.2 gr/ml and 0.25 gr/ml, but tend to increased. Cryopreservation decreased viability and number of motile cells but supplemented vitamin E improved sperm motility.

Table 2. averages of sperm motility, live sperm and sperm abnormality in extender supplemented with vitamin E.

Parameter	Vitamin E (gr/ml)					
	0.0	0.1	0.15	0.2	0.25	0.3
Sperm Motility (%)	37.5	40	42.5	50.0	52.5	60.0
Live sperm (%)	64.2	65.4	67.2	68.95	70.83	73.21
Sperm Abnormality (%)	23.22	22.33	21.79	20.70	19.94	17.79

In this result showed that there was positive correlation between supplemented vitamin E with sperm motility. Supplementation vitamin E in lower concentration in extender can improved sperm motility in 0.1 gr/ml compare to control. This result similar with reported by Foote and Hare (2000); Widyastuty (2001) and Hu, et. Al. (2011). Supplementation vitamin E in extender as antioxidant and as major membrane protective against ROS and LPO, caused increased the movement activity of sperm.

Live Sperm of frozen – thawed semen

The average of live sperm is tend to increase (P<0.01) by supplementing vitamin E in extender. The higher live sperm percentage was 73. 21 in supplemented vitamin E 0.3 gr/ml and the lower was 64.2 % in no added vitamin E in semen extender. There is no significant different (P>0.05) between control, 0.1 and 0.15 gr /ml supplemented vitamin E, but significant different with 0.25 gr/ml supplemented vitamin E in extender. This result is similar in reported by Foote and Hare (2000); and Wydiastuti (2001). Hu et. Al. (2011) reported that vitamin E supplementation in extender reduced the lipid peroxidation potential and improved semen quality during freezing – thawing .

Sperm Abnormality of frozen – thawed semen

The averages of sperm abnormality is tend to decreased the sperm abnormality, this indicate that supplemented vitamin E in extender can improve (P<0.01) the cells damage during freezing and thawing processing. The higher sperm abnormality was 23.22 % in control treatment or no supplementation vitamin E and the lower was 17.79 %

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in supplemented vitamin E 0.3 gr/ml in extender. There is significant lower ($P < 0.05$) between supplemented 0.2 gr/ml vitamin E compare to 0.15 gr/ml and 0.2 gr/ml supplemented vitamin E in extender, but not significant different between 0,2 gr/ml supplemented vitamin E with 0.25 gr/ml supplemented vitamin E in extender. According to Asadpour et al. (2011) that vitamin C and vitamin E are very efficient antioxidant in citrate – egg yolk (CEY) extender.

CONCLUSION

Supplementation vitamin E in extender resulted positive effect on Pesisir cattle sperm motility, live sperm and sperm abnormality. The best result was founded in supplemented vitamin E 0.3 gr/ml with motility 60.0 %; live sperm was 73.21 % and sperm abnormality was 17.79 %.

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P-22. GREEN LIVESTOCK DEVELOPMENT: THE ROLE OF LACTIC BACTERIA FOR IMPROVEMENT AND UTILISATION OF TOTAL MIXTURE FORAGE

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Abstract

The Objective of this study was utilisation of lactic acid bacteria as inoculant for improvement and utilisation of total mixture forages (TMF) as good and complete ration in establishing of green livestock production. Value Km Analysis of *Lactobacillus Plantarum*, *L. Bulgaricus* and *L. Casei* in define medium with glucose limitation was done. The lowest Km of those microbes with glucose utilisation as carbon sources then is used as inoculant for total mixture forages (TMF-SILAGE). Three kind of different molasses (0.2%, 0.6% and 1.0%) were added at TMF-SILAGE with *L.Plantarum* as inoculant at 14 and 21 days incubation. The result of study showed that Km of those microbes as follow *L.plantarum* 0,2 g/100 ml, *L.bulgaricus* 3.6 g/100ml and *L.Casei* 71.7 g/100ml. The lowest pH of TMF-SILAGE with *L.Plantarum* as inoculant was obtained at 1.0% molasses addition 4.8 (14 days incubation) and 4,51 (21 days incubation). While at 21 days incubation the highest lactate content of TMF-SILAGE *L.Plantarum* as inoculant was obtained at 0.6 % molasses addition was 1031 mg/100 g and at 1.0 % molasses addition was 1210 mg/100 g. The Acetate content TMF-SILAGE *L.Plantarum* as inoculant at 21 days incubation was obtained as follow 4,06 (0.2% molasses addition), 3,83 (0.6% molasses addition) and 3.73 (1.0% molasses addition). The conclusion of this study was the lowest pH and the highest lactate content of TMF-SILAGE with *L.Plantarum* as inoculant was obtained either 0,6% and 1.0% molasses addition.

Key word: lactic acid bacteria, Km and Total Mixture Forage-SILAGE

INTRODUCTION

Intensification of livestock production has been done by utilisation of high input, while it produces by products that caused problem of environment. High utilisation of *feed additive* such as anti-biotic or synthetic one in livestock production will give improper condition either will be accumulated in their body or be excreted trough faces and urine.

Silage is the end product of fermenting a high moisture crop (40-80% water) and storing the product is called ensiling. Ensiling fodder has been around a long time and now contributes over 50% of the nutrients for beef and dairy cattle production. The process requires consideration of a wide variety of factors including plant growth, harvest, storage and feeding practices. Feed conservation by ensilage should be conducted in all countries especially for ruminant production. Lactic acid bacteria (LAB) as inoculant have benefit in silage production. They produce lactic acid in decreasing their pH and some important organic acid like bacteriocin and other. Therefore silage production need a culture

that potential for lactic acid production as well as other organic production to improve animal performance, especially for immunity condition.

Ruminant animal like sheep should be fed the highest quality ensiled forages and grains possible for maximum meat production since fermented feeds can exceed 50% of the total dry-matter ration.

By product of food production is abundant, to optimisation of their utilisation; those by product were fed in mixture composition. It is necessary for application of those as silage feed.

The objective of present study was utilisation of lactic acid bacteria as inoculant for improvement and utilisation of total mixture forage (TMF) as good and complete ration in establishing of green livestock production particularly for sheep production.

MATERIAL AND METHODS

Inoculation of LAB at Agar Medium

Lactobacillus plantarum, *Lactobacillus bulgaricus*, and *Lactobacillus casei* were inoculated at agar medium using MRS broth and agar (Doncheva *et al.*, 2002; Fenster *et al.*, 2003; Parente *et al.*, 2010) and incubated at 30°C for *L. plantarum* (Ratnakomala *et al.*, 2006; Parente *et al.*, 2010), while incubated at 37°C for *L. bulgaricus* and *L. casei* (Doncheva *et al.*, 2002; Fenster *et al.*, 2003;).

Inoculation of LAB at Liquid Medium

LABs at agar medium were inoculated at liquid MRS broth, at 37°C incubation for 12 hour.

Analysis of Km

First LABs were growth at liquid MRS broth, and then were inoculated at defined medium with glucose as carbon sources. This analysis used 6 kind of medium with different of glucose addition as follow 0,1; 0,2; 0,4; 0,6; 0,8; and 1%. Each medium has 3 replications. LAB were inoculated at those medium and monitored of optical density at 660 nm every hour.

Analysis of pH

Every medium of LAB was determined pH at beginning and the end of incubation Analysis of lactic acid, acetic acid, propionic acid and butyric acid.

Medium sample of LABs were centrifuged at 10.000 rpm and supernatant sample were determined of lactic acid using method of Baker and Summerson (Hawk *et al.*, 1954).

Total Mixture Forage (TMF) Silage

TMF Silage was composing of mixture grasses and legume with 12% crude protein content. TMF then was inoculated with LAB for 14 and 21 days with different molasses addition as carbon sources. Molasses addition based on Km analysis. Since Km of *L. Plantarum* bacteria was the lowest compare to others, TMF silage was used *L. Plantarum*. After 14 and 21 days fermentation, that silage was determined for pH, lactic acid, acetic acid, propionic acid and butyric acid. Aerobisitas test of TMF silage was done at 3 and 6 days **after harvesting of silage.**

RESULT AND DICUSSION

Based on the data of growth rate as response to different glucose content in medium as carbon sources, it can be determined the value of Km for *L. Plantarum*, *L. Bulgaricus* and *L. Casei*. The Km value of those bacteria as follow: *L. Plantarum* is 0,2 g/100 mL. *Bulgaricus* is 0,36 g/100ml and *L. Casei* is 6,96

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g/100ml. While this study found that the highest production of lactate (mg/100 g) was produced at *L. Plantarum* 164,53, and follow by *L. Casei* 152,27 and *L. Bulgaricus* 133,33. However the lowest pH was founded at *L. Casei*, and follow by *L. Plantarum* and *L. Bulgaricu*, the value of pH as follow: 3,68, 3,96 and 4,10 respectfully. Therefore the most affinity between LAB and glucose utilization as energy sources was showed by *L. Plantarum*. According to Wahyudiat *al* (2012) said that *L. Plantarum* FG1 has been known well as homo-fermentative LAB species, rod-shaped, isolated from plant that has important role in silage preparation. While Cai *et al.* (1998, 1999a, 1999b) cited by Wahyudi (2012), showed that LAB homo-fermentatif inoculants could improve silage quality. The LAB was used as inoculant for next experiment of silage fermentation. The lowest Km value of glucose means that microbe can utilize efficiently glucose as carbon sources compare with others.

The result of *TMFS* with *L. Plantarum* as inoculant of LAB at 14 days incubation showed that lowest pH value was 1% molasses addition treatment (pH 4.76 and 4.91). However, at 21 days incubation, *TMFS* with the same LAB showed that the best result of the pH were achieved at 0.6% and 1.0% molasses addition, the pH value as follow 4,14 dan 4,88 (for 0,6% molasses addition) serta 4,76 and 4,91 (1.0 % molasses addition), while lactate content of those *TMFS* with different incubation was fluctuating.

CONCLUSION

Km value of *L. Plantarum* was the lowest compare to other LAB, it means that *L. Plantarum* utilize glucose as carbon and energy sources most effectively compare other. The best value pH was founded at 1.0% molasses addition at 14 days incubation, but at 21 days incubation lowest pH was achieved at 0.6 % and 1.0% molasses addition

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P-23. THE EFFECTS OF ROASTED CORIANDER SEEDS IN THE DIET ON CARCASS TRAIT AND CHOLESTEROL CONTENT OF BROILER

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Abstract

Coriander (*Coriandrum sativum* L.) seed is one of spices which have potential to replace antibiotic in poultry diet. This experiment was conducted to study the effects of roasted coriander seeds in the diet on carcass trait and cholesterol content of broiler. The experiment used 90 day-old CP 707 broiler chicks (unsexed) which were reared for 5 weeks. This experiment used completely randomized design with 3 dietary treatments and 3 replications (10 birds/replication). The coriander seeds were roasted within 5 min. The dietary treatments were: T0 = basal diet as negative control, T1 = basal diet with 2% raw coriander seeds, T2 = basal diet with 2% roasted coriander seeds. Water and feed were provided *ad libitum*. At the end of feeding trial (5 weeks of age), 3 birds each treatment were sacrificed to measure the parameters. There were no significant difference on carcass yield, breast, thigh, wing and back percentage of broiler due to dietary treatments. However, birds were fed roasted coriander seeds (T2) significantly ($p < 0.05$) decreased carcass weight, abdominal fat and cholesterol content of thigh muscle. In conclusion, heat treatment (roasted) of coriander seeds has negative effect on carcass and body weight although cholesterol content of broiler decreased.

Key words: abdominal fat, broiler, carcass, coriander seed, cholesterol

INTRODUCTION

Herbs and spices become popular recently as natural feed additive to replace synthetic growth promoter. To avoid the residual effect of antibiotic in poultry products, there were some research had been done. It has been reported that herbs contain some active phytochemical including the flavonoid, terpenoids, linalool and essential oil (Hernandez, 2003).

Coriander (*Coriandrum sativum* L.) is one of spices from umbeliferae family. The seed contain an essential oil up to 1% and linalool was the main component of essential oil. Bioactive component of coriander seed was potential as antibacterial (Burn, 2004; Kubo et al., 2004), antioxidant (Wangenteen et al., 2004) and stimulatory effects in the digestion process (Cabuk et al., 2003).

Our previous experiment showed that feeding 2% raw coriander seed to broiler improved body weight gain and feed efficiency. However, we do not know yet the effects if the coriander seed was roasted. Therefore, the aim of the present experiment was to study the effects of roasted coriander seed on carcass traits and cholesterol content of broiler.

MATERIALS AND METHODS

Bird and Housing

This experimental was conducted at Laboratory of Poultry Nutrition, Faculty of Animal Science, Bogor Agricultural University. Ninety day-old CP 707 broiler chick (unsexed) were randomly assigned to three dietary treatments with three replication (10 birds/replication). The chicks were reared on deep litter system in open side house with standard management conditions throughout the experiment

period of 5 weeks. Feed and water were provided *ad libitum*. The chicks already vaccinated against New Castle and Gumboro diseases from breeding farm.

Experimental diet

Basal diet was formulated to met broiler requirement according to Lesson and Summer (2005) recommendation. The ingredient and nutrient composition are presented in Table 1. The coriander seeds were purchased from local market. These seeds were roasted within 5 minute and then grind to pass a 1 mm screen. The experimental diets are T0 = as negative control (diet without coriander seeds), T1 =basal diet contain 2% raw coriander seeds, T2= basal diet contain 2% roasted coriander seeds.

Chemical analysis

Proximate analysis of coriander seeds were conducted according to AOAC (1984). Carcass cholesterol from thigh muscle were determined according to Diebermen Burchard method (Kleiner and Dotti, 1962)

Data collection

At 5 weeks of age, three birds each treatment randomly selected and sacrificed for measure carcass trait, abdominal fat and carcass cholesterol. Carcass yield, breast, wing, thigh and back were calculated as percentage from live weight. Feed intake was recorded on a weekly basis.

Statistical analysis

This experiment used completely randomized design with three dietary treatments and three replications. All data were subjected to analysis of variance using the GLM procedure of SAS. Significant treatment means were separate by Least Significant Different (LSD) (Mattjik and Sumertajaya, 2000).

RESULTS AND DISCUSSION

The effects of feeding roasted coriander seed on carcass trait and cholesterol content of thigh muscle are presented in Table 2. There were no significant effects of experimental diet on carcass percentage, breast percentage, thigh, drum, back and wing percentage. However, chicks were fed roasted coriander seed significantly ($P < 0.05$) had lower body weight, and carcass weight as compared to other treatment. The lower body weight of chick fed roasted coriander seed due to decreasing feed intake. Heat treatment (roasted) of coriander seed destroy the essential oil and other nutrient. In contrast, feeding raw coriander seed significantly ($P < 0.05$) increased body weight and carcass weight. The effect of linalool of coriander seed were seen in this treatment. As reported by other researcher, the phytochemical of coriander seed will stimulate digestion process through increasing digestion enzyme production. Moreover, the coriander seed has antibacterial properties (Cabuk et al., 2003) so the chicken will be healthier than other treatment.

Abdominal fat and cholesterol content of chick fed roasted coriander seed significantly ($P < 0.05$) lower than other treatment. It was due to lower feed intake so fat intake will also decreased.

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Table 1. Composition of experimental diet (*as fed*)

Ingredient (%)	Starter			Finisher		
	T0*	T1	T2	T0	T1	T2
Corn	54.14	53.68	53.68	60.41	59.61	59.61
Rice bran	6.00	4.85	4.85	5.17	4.30	4.30
Soybean meal	28.00	28.00	28.00	19.46	19.19	19.19
Fish meal	6.05	5.93	5.93	9.39	9.52	9.52
CPO	3.61	3.34	3.34	3.37	3.18	3.18
Coriander	0.00	2.00	2.00	0.00	2.00	2.00
CaCO ₃	1.00	1.00	1.00	1.00	1.00	1.00
DCP	0.50	0.50	0.50	0.50	0.50	0.50
Premix	0.50	0.50	0.50	0.50	0.50	0.50
Lysine	0.10	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00	100.00
Nutrient composition (calculated)						
ME (kkal.kg)	3050	3050	3050	3100	3100	3100
CP (%)	22	22	22	20	20	20
CF (%)	6.19	6.08	6.18	6.17	6.15	6.28
Ca (%)	0.96	0.97	0.98	1.16	1.19	1.20
P _{aval} (%)	0.53	0.53	0.52	0.62	0.62	0.62

* T0 = control diet; T1= diet contain 2% raw coriander seed; T2= diet contain 2% roasted coriander seed

In conclusion, feeding roasted coriander seed had negative effects on body weight and carcass weight although cholesterol content and abdominal fat of broiler decreased.

Table 2. Effects of roasted coriander seeds on carcass trait and cholesterol content of broiler (5 week of age)

Parameters	T0	T1	T2
Body weight (g)	1183.33 ± 35.16 ^a	1285.00 ± 16.88 ^a	908.66 ± 50.20 ^b
Carcass weight (g)	791.66 ± 51.07 ^a	840.33 ± 81.93 ^a	596.33 ± 15.18 ^b
Carcass percentage (%)	66.86 ± 2.52	65.36 ± 0.46	65.70 ± 2.05
Breast percentage (%)	30.54 ± 1.50	31.59 ± 1.51	31.94 ± 2.21
Thight percentage (%)	16.92 ± 0.81	17.44 ± 1.89	17.95 ± 0.47
Drum stick (%)	15.71 ± 0.28	15.07 ± 0.26	15.25 ± 0.43
Wing percentage (%)	12.09 ± 1.62	12,45 ± 1.14	13.56 ± 0.29
Back percentage (%)	24.51 ± 1.56	26.57 ± 3.98	21.20 ± 2.32
Abdominal fat (%)	1.58 ± 0.28 ^b	1.78 ± 0.19 ^b	0.99 ± 0.34 ^a
Carcass cholesterol (mg/100g)	112.89 ± 21.41 ^a	163.50 ± 6.98 ^a	56.92 ± 28.92 ^b
Feed intake (g)	2199.04 ± 99.16 ^a	2223.51 ± 163.01 ^a	1872.55 ± 129.70 ^b

Means in the same row with different superscript are significantly different ($p < 0.05$)

T0 = diet without coriander

T1= diet contain 2% raw coriander

T2 = diet contain 2% roasted coriader

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