



# PROCEEDINGS

VOLUME II

The 2<sup>nd</sup> International Conference  
on Animal Nutrition and Environment  
(ANI-NUE2017)

*“Towards the Betterment of Animal  
Productivity, Conserving Resources  
and Environment”*

November 1-4,  
**2017**

Pullman Raja Orchid Hotel,  
Khon Kaen, Thailand



**EDITORS:** Cherdthong, A., Foiklang, S., Mapato, C., Pilajun, R., Kang, SC. and Wanapat, M.

**ISBN:** 978-616-438-084-4

Organized by

Tropical Feed Resources Research and Development Center (TROFREC), Department of Animal Science,  
Faculty of Agriculture, Khon Kaen University





**In Recognition for**

**Professor Dr. Metha Wanapat**

**“The Khon Kaen University Distinguished  
Research Professor”**

**for**

**His 37 years of continuous serving in  
teaching, research, education, technology  
development, and transfer in Animal  
Science nationally and internationally.**

**The 2<sup>nd</sup> International Conference on Animal Nutrition and  
Environment (ANI-NUE2017) Organizing Committee,**

**November 1, 2017**

**PROCEEDINGS**  
*of*  
**The 2<sup>nd</sup> International Conference on**  
**Animal Nutrition and Environment**  
**(ANI-NUE2017)**  
**Volume II**

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**November 1-4, 2017**

**Pullman Raja Orchid Hotel, Khon Kaen, Thailand**



## Welcome Remarks

by

**Professor Dr. Metha Wanapat**



Dear Distinguished Scientists, Friends, Ladies and Gentlemen, Sawaddee Krub!

It is my great pleasure and honor to warmly welcome and to express my most appreciation and sincerity to all participants and supporters of The 2<sup>nd</sup> ANI-NUE2017 International Conference at Pullman Raja Orchid Hotel, Khon Kaen, Thailand, Nov 1-4, 2017, with the theme of “Towards the betterment of animal productivity, conserving resources and environment”.

To refresh the memories, the 1<sup>st</sup> ANI-NUE2012 was organized here with full participation and great achievements. It is the prime time now for the 2<sup>nd</sup> ANI-NUE to be organized with great interest, enthusiasm and participation of both senior and young animal scientists from around the world.

With the rapid growth of global population and climate changes, it is highly inevitable that animal agriculture and the development of highly-qualified animal scientists would be in great demand, consequently.

I am confident that the gathering of all participants at the 2<sup>nd</sup> ANI-NUE would be prosperous, knowledge-enriching, collaborative-connecting and interactive-entertaining. The great successful of the conference in all dimensions is from the hard grit and determination of all organizing committee members under the chairman, Assist. Prof. Dr. Anusorn Cherdthong. The efforts of mutual implementations will certainly prosper their future undertakings and to promote the animal agriculture. The mindful attention and generous support of universities, institutions, organization, companies and all personal are gratefully acknowledged.

I wish to express my personal gratitude and willingness to all participants both nationally and internationally for the deep understanding and well-cooperation, as well as their contributions making the 2<sup>nd</sup> ANI-NUE successfully-unique conference. Beyond the 2<sup>nd</sup> ANI-NUE, I would encourage all participants to continue their keen deliberations through the future ANI-NUE to support the research and development of animal agriculture.

Ladies and gentlemen, may I alert you that the success and achievement of the 2<sup>nd</sup> ANI-NUE is from two pillars – one is from gaining knowledge and experience-sharing and another one is the happiness, socially-interactive exchanges among all ages of animal scientists, which are now already initiating and shining throughout the conference.

Ladies and gentlemen, friends of all ages, I truly believe that “Youth has no age”, as it has been inspired and brightened by our Professor Dr. Charan Chanthalakhana, who has been showing and reiterating the importance of the interactions and continuation of hard work. This confidence is one of the kinds that connect all generations together to help promote and develop the “Animal Agriculture”. Please enjoy yourself and be enriched with happiness, wealthiest, prosperity and the continuation beyond!

## II

Finally, as the President of the 2<sup>nd</sup>. ANI-NUE Int. Conf. I wish to express my heart-felt gratitude to Khon Kaen University, and all the Co-host Institutions as well as supporters, donors and friends. Special thanks are extended to the Organizing Committee Members, led by Assistant Professor Dr. Anusorn Cherdthong including all current and former students who have been putting enormous efforts for the success of the conference.

With my warm greetings, hospitality and best wishes for all your deliberations.

Thank you very much! Sawaddee Krub!



Professor Dr. Metha Wanapat

President, the 2<sup>nd</sup> ANI-NUE2017 International Conference

## *Message from the Chairman of ANI-NUE2017*



Dear Participants,

On behalf of the Organizing Committee, it is my great pleasure to present to you the organization of the 2<sup>nd</sup> International Conference on Animal Nutrition and Environment (ANI-NUE2017) held at Khon Kaen, Thailand, during November 1- 4, 2017.

The 2<sup>nd</sup> ANI-NUE Int. Conf. 2017 brought together leading experts specializing in animal nutrition and environment to discuss the current frontier issues in this area, and to promote awareness of ongoing research achievements. With a wide range of topics covering multiple aspects of animal nutrition and environment, this conference offered participants the most up-to-date information on advances of the researches of animal nutrition and environment to promote and develop animal agriculture.

May I report that this week, there are about 150 participants represented from more than 20 countries are here to attend the 2<sup>nd</sup> ANI-NUE2017 and present their research findings and ideas for promotion of “Towards the Betterment of Animal Productivity, Conserving Resources and Environment”. There are 99 oral presentations at this conference including invited speakers (Key-note, Plenary, Lead speakers). In addition, a special forum for young scientists on “How to improve writing papers to submit to International Journal with high Impact factor” and exhibition booths are organized. I would like to thank all of the researchers for submitting their works and all members of the Organizing Committee for their co-operation and hence making in the conference a great success.

I am grateful to Prof. Dr. Metha Wanapat, ANI-NUE2017 President, and all the members of the International Advisory Committee for the valuable advices and support during the preparation of this conference. My sincere thanks are due to all the co-host institutions, and sponsors without the support of whom a Conference of this scale would not have been possible.

In addition, this is a remarkable occasion in recognition for Professor Dr. Metha Wanapat “The Khon Kaen University Distinguished Research Professor” for his Outstanding Long-Term Contribution and Dedication in Education, Research and Development in Animal Science especially in Ruminant Nutrition, both nationally and internationally. Despite his reaching 65 years of age, he is still strong, physiology and mentally in serving the academic arena in the future to come. We all wish to congratulate him for the high dedication and inspiration to develop human researchers especially the young scientists.

On behalf of the Organizing Committee members and the Advisory Board, we wish you all the best, great health and prosperity. Please enjoy your stay in Khon Kaen and have a successful Conference. Again, thank you for your kind cooperation.

With my best wishes!

Assistant Professor Dr. Anusorn Cherdthong

Chairman, the 2<sup>nd</sup> ANI-NUE2017 International Conference

## Message from the editorial board

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Dear Participants,

On behalf of the Chair, Academic Committee, we would like to welcome you to The 2<sup>nd</sup> International Conference on ANI-NUE2017, 1-4 Nov 2017, Pullman Raja Orchid Hotel, Khon Kaen, Thailand. Your participation is important to make the event become meaningful and successful. This conference provides not only an opportunity to discuss and exchange experience and information with people who have the same interests from the different regions of the world but also a good environment to meet people and build up friendship among nations. The primary objective of ANI-NUE2017 is to provide a venue for animal nutritionists, agriculturists or environmentalists (academicians, researchers, administrators and livestock producers), to share their experiences and develop collaborations and to enhance development of animal nutrition, environmental friendly concerns in their respective countries. We also welcome our colleagues from the related fields to contribute to the above objectives.

This week, there are about 150 participants from more 20 countries are here to attend the 2<sup>nd</sup> ANI-NUE2017 and present their research findings and ideas for promotion of “Towards the Betterment of Animal Productivity, Conserving Resources and Environment”. There are 100 oral presentations at this conference including invited speakers (Key-note, Plenary, Lead speakers). In addition, a special forum for young scientists on “How to improve writing papers to submit to International Journal with high Impact factor” and exhibition booths are organized.

The overall conference, it includes Key-note, Plenary, invited papers, scientific abstract papers, full papers in CD- ROM, field trip, exhibition booths and a full social program.

We wish to thank the members of local organizing committee, the international advisory committee (IAC), international scientific committee, reviewers, all co-hosts, KKU graduate students, all ANI-NUE2017 committees and all individuals who have contributed in so many ways to make this conference a great success and enjoyable.

With best wishes!

On behalf of editorial board, the 2<sup>nd</sup> ANI-NUE2017 International Conference



## *International Advisory Committee*

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Prof. Dr. Charan Chanthalakhana	Thailand
Mr. Suthep Vongreun	Thailand
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Prof. Dr. Vu Chi Cuong	Vietnam
Asst. Prof. Dr. Anusorn Cherdthong	Thailand, Chairman, the 2 <sup>nd</sup> ANI-NUE2017 Int. Conf.
Prof. Dr. Metha Wanapat	Thailand, President, the 2 <sup>nd</sup> ANI-NUE2017 Int. Conf.

## *Organizing Committee: ANI-NUE2017*

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**President:** Professor Dr. Metha Wanapat

**Chairman:** Assistant Professor Dr. Anusorn Cherdthong

**Scientific:** Assistant Professor Dr. Anusorn Cherdthong

**Fund-raising/Finance:** Dr. Kampanat Phesatcha

**Public Relation:** Mr. Chaowarit Mapato

**Accommodations/Venue/Protocol:** Dr. Suban Foiklang

**Transportation/Tour:** Assistant Professor Dr. Nirawan Gunun

**Exhibition:** Assistant Professor Dr. Anusorn Cherdthong

**Culture and Recreation/Souvenir:** Associate Professor Dr. Opart Pimpa

**List of Major Reviewers:**

Prof. Dr. Metha Wanapat	<i>Khon Kaen University, Thailand</i>
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Dr. Suban Foiklang	<i>Maejo University, Thailand</i>
Mr. Chaowarit Mapato	<i>Khon Kaen University, Thailand</i>
Others	



**ANI-NUE2017 Proceedings of the 2<sup>nd</sup> International Conference on Animal Nutrition and Environment, “Towards the betterment of animal productivity, conserving resources and environment”:  
November 1-4, 2017, Pullman Raja Orchid Hotel, Khon Kaen, Thailand.**

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<b>Conference Program</b>	
<p>The 2<sup>nd</sup> International Conference on Animal Nutrition and Environment (ANI-NUE2017)            “Towards the Betterment of Animal Productivity, Conserving Resources and Environment”            November 1-4, 2017, Pullman Raja Orchid Hotel, Khon Kaen, Thailand</p>	
<b>October 31, 2017</b>	
04:00 pm-20:00 pm	Arrival of participants / Pre-Registration at Orchid ballroom I
<b>November 1, 2017</b>	
<b>Orchid ballroom I</b>	
<b>Registration</b>	
<b>Opening Ceremony</b>	
08:00 am-09:00 am	<i>Coffee/ tea break</i>
09:00 am-10:15 am	<b>Chairperson:</b> Prof. Dr. Metha Wanapat-Thailand
10:15 am-10:30 am	<b>Key-note speaker:</b> Prof. Dr. Charan Chanthalakhana-Thailand
10:30 am-11:15 am	<b>“Sufficiency Economy Philosophy as a Framework for the Promotion of Sustainable Agriculture and Better Life Quality”</b>
11:15 am-12:00 am	<b>Key-note speaker:</b> Prof. Dr. Junichi Takahashi-Japan
12:00 am-01:00 pm	<b>“Prospect on Mitigation of GHG and Renewable Energy Towards Sustainable Animal Agriculture”</b>
<i>Lunch</i>	

Session 1-Orchid ballroom 1		Session 2-Arawan 1	
01:00 pm-01:30 pm	<p><b>Chairperson:</b> Prof. Dr. Federico Infascelli-Italy</p> <p><b>Co-Chairperson:</b> Assist. Prof. Dr. Sineenart Polyorach-Thailand</p> <p><b>Invited speaker:</b> Assoc. Prof. Dr. Suneerat Aiumlamai-Thailand</p> <p><b>“Thai Dairy Productivity: Milk Quality and Days Open”</b></p> <p><b>ANN-01-0006: “Metabolizable Protein Requirements of Lactating Buffaloes (<i>Bubalus bubalis</i>) Fed on Silage Based Diet”</b> <i>Umesh Balaji Sontakke, Shivlal Singh Kundu, Sonali prusty, Gautam Mondal, Vijay Kumar Sharma and Muneendra Kumar-India</i></p> <p><b>ANN-01-0093: “Supplementation of mangosteen peel and banana flower pellet (MABAP) to improve ruminal fermentation and milk production in dairy cows”</b> <i>Thiwakorn Ampapon and Metha Wanapat-Thailand</i></p> <p><b>ANN-01-0015: “Energy Utilization, VFA, and A/P Ratio of Kacang Goat Fed Total Mixed Ration Containing Different Treatments of Soybean Meal”</b> <i>Kustantinah, I Gede Suparta Budisatria, Rusman and Retno Adhwinarti-Indonesia</i></p> <p><b>ANN-01-0063: “Feed intake and digestibility of dairy cows affected by mao (<i>Antidesma thwaitesianum</i> Muell. Arg.) pomace meal supplementation”</b> <i>Pongsatorn Gunun, Nirawan Gunun, Thanaporn Oupparamong, Srisuda Sirilaophaisan, Anusorn Cherdthong, Kessara Ampaporn, Patwan Punyakaew, Metha Wanapat and Sineenart Polyorach-Thailand</i></p> <p><b>ANN-01-0030: “Performance and Physiological Status of Kids Milking by Milk Replacer Containing Cricket Meal”</b> <i>Dewi Apri Astuti, Lilis Khotijah and Rika Damanik-Indonesia</i></p> <p><b>ANN-01-0080: “Variations in milk composition between morning and afternoon milking in dairy cow”</b> <i>Channanwit Promkot, Pitukpol Porn-aneek, and Lerdcchai Phu-oob-Thailand</i></p>	<p><b>Chairperson:</b> Prof. Dr. Junichi Takahashi-Japan</p> <p><b>Co-Chairperson:</b> Assist. Prof. Dr. Nirawan Gunun-Thailand</p> <p><b>Invited speaker:</b> Prof. Dr. Vu Dinh Ton-Vietnam</p> <p><b>“Current Non-ruminant Production and Future Prospects in Vietnam”</b></p> <p><b>ANN-01-0052: “Feeding POAB at Different Level of Amino Acids in The Diet of Broiler Chickens”</b> <i>C.H. Goh, T. C. Loh, H.L. Foo, and N. Frisco-Malaysia</i></p> <p><b>ANN-01-0089: “Effects of purple glutinous rice residue meal in concentrate diets on growth performance in growing pigs”</b> <i>Walailuck Kaewwongsa and Attapong Piladang-Thailand</i></p> <p><b>ANN-01-0010: “Effect of Diet Containing Dragon Fruit Peel Meal Fermentation for Productivity of Kampung Chickens”</b> <i>Gusti A.M.Kristina Dewi, I M. Nuriyasa, and I Wayan Wijana-Indonesia</i></p> <p><b>ANN-01-0103: “Effect of rice wine by-product as alternative protein source on growth performance of broiler chickens”</b> <i>Benya Saenmahayak, Smerjai Bureenok, Chalermpon Yuangklang, Sasiphan Wongsuthavas, and Kraisit Vasupen-Thailand</i></p> <p><b>ANN-01-0035: “A Comparison of Fat-Soluble Antioxidants in Wild and Farm-Reared Chukar Partridges (<i>Alectoris Chukar</i>)”</b> <i>Filiz Karadasa, Anders Pape Møller, and Mehmet Resit Karageçili-Turkey</i></p> <p><b>ANN-01-0101: “Used dried cassava leaves with enzymes from fermented tomato pomace with <i>Aspergillus niger</i> in laying duck diet on nutrient digestibility”</b> <i>Kraisit Vasupen, Savang Soykhammy, Sasiphan Wongsuthavas, Chalermpon Yuangklang, Smerjai Bureenok, Benya Saenmahayak-Thailand</i></p>	
01:30 pm-01:42 pm			
01:42 pm-01:54 pm			
02:54 pm-02:06 pm			
02:06 pm-02:18 pm			
02:18 pm-02:30 pm			
02:30 pm-02:42 pm			
02:42 pm-03:00 pm	<b>Coffee/ tea break</b>		

Session 3-Orchid ballroom I		Session 4-Arawan I	
	<b>Chairperson:</b> Prof. Dr. Effendi Abustam-Indonesia	<b>Chairperson:</b> Prof. Dr. Agung Purnomoadi- Indonesia	
	<b>Co-Chairperson:</b> Dr. Thitima Norrapoke -Thailand	<b>Co-Chairperson:</b> Dr. Ratchataporn Lunsin -Thailand	
03:00 pm-03:12 pm	<b>ANN-01-0001:</b> “Effect of Increasing Energy and Protein Ration on Nutrient Digestibility and Performance of Bali Heifer Calves” <i>Ni Nyoman Suryani, I Wayan Suarna, I Gede Mahardika and Ni Putu Sarini-Indonesia</i>	<b>ANN-01-0012:</b> “Use of <i>Bacillus subtilis</i> to Produce Feather Meal for Animal Feeds and Organic Fertilizers” <i>Chi-Chu Lo, Liang-Yi Lin, and Shu-Chuan Chen-Taiwan</i>	
03:12 pm-03:24 pm	<b>ANN-01-0003:</b> “Metabolizable Energy of Cassava Pulp for Thai Native Beef Cattle” <i>Ornvimol Keaokiang, Tomoyuki Kawashima, Wanna Anghong, Tomoyuki Suzuki, and Ramphrai Narmseelee-Japan</i>	<b>ANN-01-0032:</b> “Assessment of Metabolizable Energy, Nutrients Digestibility and Fatty Acids Composition of Fat Crystals Derived from Crude Palm Oil in Chickens” <i>Sarawat Treetat, Sonthaya Incharoen, Rangsun Charoensook, Wandee Tartrakoon, Papichaya Incharoen, and Tossaporn Incharoen-Thailand</i>	
03:24 pm-03:36 pm	<b>ANN-01-0008:</b> “Application of Pressurized Heating in Production Process of Bali Cattle Fur Meal to Its Nutrient” <i>Muhammad Irfan Said, Farida Nur Yulianti, Muhammad Zain Mide, Wempie Pakiding and Hamri-Indonesia</i>	<b>ANN-01-0034:</b> “Effect of Dietary Supplementation of Cinnamon and Curcumin on Performance, Humoral immune Responses, and Blood lipid Profile in Rabbits” <i>Hassan Zewei, Soliman Zahran, Mohamed Ahmed, Yasmin El-Gindy and Nagat Khoshera-Egypt</i>	
03:36 pm-03:48 pm	<b>ANN-01-0073:</b> “Effect of $\beta$ -glucan supplementation on feed intake, digestibility and rumen fermentation in Thai native beef cattle” <i>Anusorn Cherdthong and Anuthida Seankamsorn-Thailand</i>	<b>ANN-01-0033:</b> “Study on The Growth Performance, Meat Quality and Bone Breaking Strength of Broilers fed Dietary Rice Hull Silicon” <i>Sarawoot Nakhool, Sonthaya Numthum, Rangsun Charoensook, Wandee Tartrakoon, Papichaya Incharoen, and Tossaporn Incharoen-Thailand</i>	
03:48 pm-04:00 pm	<b>ANN-01-0040:</b> “Ruminal Nitrogen Release from Limestone-Urea Mixture” <i>M. Ainsyar Harahap, Limbang K. Niswantara, Eko Pangestu, Fajar Wahyono and Joetal Achmadi-Indonesia</i>	<b>ANN-01-0064:</b> “The effects of organic corn level decreasing in organic laying hen diets on egg production and egg quality” <i>KANN-01Ikar Hamprakorn, Buaream Maneewal, Tonglian Burjoom and Sukit Khantaprab-Thailand</i>	
04:00 pm-04:12 pm	<b>ANN-01-0074:</b> “Effect of sulfur levels supplementation in fermented total mixed ration containing fresh cassava root using F gas production technique” <i>Chanadal Supapong and Anusorn Cherdthong -Thailand</i>	<b>ANN-01-0067:</b> “Factors Effecting on Rabies Immunity Titer in Canine” <i>Suraphong Wongsuthawart, Bundit Nuansrichay, Ratchaneekorn Vitoonpong, Kongkul Kaskosol, Lamul Molee and Sasiphan Wongsuthavav-Thailand</i>	
04:12 pm-04:24 pm	<b>ANN-01-0042:</b> “The Performance of Extension Agent in Improving Adoption The Technology Beef Cattle Feed” <i>Agustina Abdullah, Jamila, and A. Amrullah-Indonesia</i>	<b>ANN-01-0011:</b> “Antibacterial activity of <i>Phaleria macrocarpa</i> fruit extracts: an <i>In vitro</i> study” <i>Niati Ningsih, Bambang Ariyadi, and Zuprizal-Indonesia</i>	
<b>Welcome party-Orchid ballroom I</b>			
07:00 pm-10:30 pm			

November 2, 2017	
Orchid ballroom I	
Registration	
08:00 am-09:00 am	Chairperson: Prof. Dr. Ermias Kebreab-U.S.A.
09:00 am-09:40 am	Plenary speaker: Prof. Dr. Metha Wanapat-Thailand “Diversity of Feed Resources and their Potential to Improve Ruminant Production and Mitigate Enteric Methane”
09:40 am-10:20 am	Plenary speaker: Prof. Dr. Peter Rowlinson-U.K. “Strategies to Increase The Efficiency of Nutrient Utilization”
10:20 am - 10.30 am	Coffee/tea break
	Session 5-Orchid ballroom I
10:30 am-11:00 am	Chairperson: Assist. Prof. Dr. Chalermpon Yuangklang-Thailand Co-Chairperson: Dr. Umesh Balaji Sontakke-India Invited speaker: Dr. Pattaya Napisirth and Dr. Viengsakoun Napasirth-Lao, PDR. “Ruminant Production in Lao, PDR.”
11:00 am - 11:12 am	ANN-01-0050: “Development strategies for dairy cattle production system and milk products in northeast of Thailand: Policy framework and challenges” <i>Theerachai Haitook, Samruay Ninking, Phruethinun Chukasem, Wuttikom Srakaew and Naritsara Suayroop-Thailand</i>
11:12 am - 11:24 am	ANN-01-0058: “Beef Cattle Feeding Management of Smallholder Farmers in Kon Tum City, Vietnam” <i>Ratchataporn Luansin, Thai Thi Bich Van, Truong Thi Tu Trinh and Somporn Daunyai-Thailand</i>
11:24 am - 11:36 am	ANN-01-0037: “The effect of bull ( <i>Bos indicus</i> ) and extender medium to additional antioxidant $\alpha$ -tocopherol of cryopreservation sperm post-thawing to minimize of repeat breeding” <i>ANN-01a Farhana, Ismaya, and Nono Ngadiyono-Indonesia</i>
	Session 6-Arawan I
	Chairperson: Prof. Dr. Vu Dinh Ton -Vietnam Co-Chairperson: Assist. Prof. Dr. Sasiphan Wongsuthavas-Thailand Invited speaker: Assoc. Prof. Dr. Monchai Duangjinda-Thailand “Tropical Animal Genetic and Environment Impact”
	ANN-01-0005: “Genetic Polymorphisms of Alphas1-Casein (CSN1S1) Gene In Indonesian Local Goat Population Reared In South Sulawesi Province” <i>Muhammad Ihsan Andi Dagoang, Lellah Rahim, RR Sri Rachma Aprilita Bugiwati, Magfirah Nur, and Nurul Purnomo-Indonesia</i>
	ANN-01-0079: “Induction of follicular growth and atresia: Expression of aromatase mRNA in the ovary of <i>Bos Indicus</i> ” <i>Nattavut Kogaram, Vilaivan Khanthusaeng, Surapong Tongrueng, Thuanya Bumma and Chainarong Navanukraw-Thailand</i>

11:36 am - 11:48 am	ANN-01-0056: “Effects of different tropical grasses on feed intake and blood metabolite of goats” <i>Pin Chanjula, Rawee Chiarawipa and Phantip Panklang-Thailand</i>	ANN-01-0083: “Early embryonic development, corpus luteum and metabolite of PGF in lactating dairy cows supplemented with palm or sunflower oil” <i>Aree Kraisoon, Jarunwan Kaekejon, Wiroon Inthamonee, and Chainarong Navanukraw-Thailand</i>
11:48 am - 12:00 am	ANN-01-0004: “Application of Tunnel-Ventilated Barn in Tropical Dairy Industry: A review” <i>Aan Andri Yano, Adiarto, and Diah Tri Widayati-Indonesia</i>	ANN-01-0086: “Development of corpus luteum in goats: interaction between progesterone concentration and luteal cell proliferation” <i>Thaiva Bunna, Chainarong Navanukraw, Vilaivan Khanthusaeng, Aree Kraisoon and Nattavut Kogram-Thailand</i>
12:00 am-01:00 pm	<b>Lunch</b>	
	<b>Session 7-Orchid ballroom I</b>	
01:00 pm-01:30 pm	Chairperson: Assoc. Prof. Dr. Pramote Paengkoum-Thailand Co-Chairperson: Dr. Onanong Pongchompu-Thailand Invited speaker: Assist. Prof. Dr. Anusorn Cherdthong-Thailand “Potential use of Alternative Source of Protein in Ruminant Feeding”	Chairperson: Prof. Dr. Gusti Ayu Mayani Kristina Dewi-Indonesia Co-Chairperson: Dr. Viengsakou Napsirith-Lao, PDR. Invited speaker: Dr. Kalaya Boonyanuwat-Thailand “The Influence of Climate Change and the Strategy for Producing Tropical Animals”
01:30 pm-01:42 pm	ANN-01-0018: “Optimization Total Digestible Nutrients - Protein Ratio To Achieve Good Feed Conversion Ratio In Indonesian Native Beef Cattle” <i>Nadlirotun Luthfi, Edy Rianto and Agung Purnomoadi-Indonesia</i>	ANN-01-0017: “The Effect of Liquid Smoke on Methane Emission from Faces” <i>Vita Restitrisnani, Khanza Syahira Dhia, Tegar Wicaksono, Edy Rianto, and Agung Purnomoadi-Indonesia</i>
01:42 pm-01:54 pm	ANN-01-0049: “Dietary Protein Requirement for Maintenance and Growth of Southern Thai Indigenous Cattle” <i>O. Pimpa, B. Khamseekhiew, B. Pimpa and S. Ruengsuwan-Thailand</i>	ANN-01-0057: “Effects of sunflower oil and nitrate supplementation on methane production and rumen fermentation by using in vitro gas production technique” <i>Jiravan Khotsakdee, Chalermpon Yuangklang, Siwaporn Paengkoum and Pramote Paengkoum-Thailand</i>
02:54 pm-02:06 pm	ANN-01-0019: “The effect of dietary protein intake on body protein growth in Thin Tailed Lambs” <i>Ari Prima, Nadlirotun Luthfi, Edy Rianto, Endang Purbowati and Agung Purnomoadi-Indonesia</i>	ANN-01-0043: “In Vitro Rumen Microbial Population and Fermentation with The Addition of <i>Sapindus rarak</i> Extract and Sesame/Canola Oils Microencapsulation” <i>Sri Suharti, Isma Firlitani, and Komang Gede Wiryawan-Indonesia</i>
02:06 pm-02:18 pm	ANN-01-0092: “Effect of yeast fermented cassava pulp (YFCP) supplementation on feed intake, digestibility and rumen fermentation in beef cattle” <i>Sukruthai Sommai, Metha Wanapat, Chaowarit Mapato and Pajaree Totakul-Thailand</i>	ANN-01-0097: “Effect of Dragon fruit ( <i>Hylocercus undatus</i> ) peel powder and roughage to concentration ratio on gas production kinetics, digestibility, and fermentation using in vitro gas production technique” <i>Maharach Maiza, Metha Wanapat, Suban Foiklang, Chaowarit Mapato, Thiwakorn Ampapong, Bounmaxay Viennasay and Ahkarapon Nunoia-Thailand</i>



02:18 pm-02:30 pm	ANN-01-0095: “Influences of Yeast fermented potato peel and cassava peel on gas kinetics and digestibility using in vitro gas technique” <i>Suban Foiklang, Risa Japanya, Phanuphong Ounpon, Metha Wanapat, Anusorn Cherdthong, Thittima Norrapoke, and Kampanat Phesatcha-Thailand</i>	ANN-01-0066: “Hematology and physiological responses as indicator of heat tolerance” <i>Pinkapol Porn-aneek, Chamnanvit Promkot, and Thaweechai Phuad-Thailand</i>
02:30 pm-02:42 pm	ANN-01-0098: “Effect of yeast fermented dehulled rice (YEFEDER) levels with different kind of roughage on gas production and in vitro degradability using in vitro gas production technique” <i>Pataree Totakul, Metha Wanapat, Suban Foiklang, Chaowarit Mapato, and Sukruthai Sommai-Thailand</i>	ANN-01-0071: “Effects of supplementation of Piper sarmentosum leaves powder in concentrates on feed efficiency, rumen fermentation and protozoa in Thai native beef cattle” <i>Anusorn Cherdthong and Benjamad Khonkhaeng-Thailand</i>
02:42 pm-03:00 pm	<b>Coffee/ tea break</b>	
	<b>Session 9-Orchid ballroom I</b>	
	Chairperson: Assoc. Prof. Dr. Filiz Karadas-Turkey	Chairperson: Dr. Kalaya Boonyanuwat-Thailand
	Co-Chairperson: Dr. Siwaporn Paengkoum-Thailand	Co-Chairperson: Dr. Muhammad Ihsan Andi Dagong- Indonesia
03:00 pm-03:12 pm	ANN-01-0061: “Determination of level protein intake to control fat and protein in carcass of fattened lambs” <i>Rizky Choirunnisa, Mukh Arifin, Robert Kussetyawan, Febrian Rhamadya, Ari Prima, Vita Restitrismani, Nadlirotun Lulhfi, Sutaryo and Agung Purnomoadi-Indonesia</i>	ANN-01-0013: “Use of Body Measurements to Predict Intermuscular Fat in Thin-tailed Lambs” <i>Ulita Renfelia Baysi, Ari Prima, Farah Nabila, Pradhipta Hersandika, Endang Purbowati, Christina Maria Sri Lestari, and Agung Purnomoadi-Indonesia</i>
03:12 pm-03:24 pm	ANN-01-0044: “Effect of yeast-fermented cassava pulp levels on growth performance of growing goats fed Napier Pakchong 1 grass” <i>Saran Parisuthikul, Tichakorn Thumtong, Theerasant Phohee, Panuwat Teebklang, Wanchai Inthiseang, Wichan Kaewluan and Ruangyote Pilajun-Thailand</i>	ANN-01-0099: “Effect of inclusion of enzyme from fermented tomato pomace with <i>Aspergillus niger</i> (FETPAN) in total mixed ration on feed intake and growth performance in beef cattle” <i>Chalermporn Yuangklang, Jiravan Khotsakdee, and Krisit Vasupen-Thailand</i>
03:24 pm-03:36 pm	ANN-01-0102: “Effect of mangosteen peel liquid protected soybean meal on methanogen and microbial population using in vitro gas fermentation technique” <i>Kampanat Phesatcha, Burarat Phesatcha, Suban Foiklang, Metha Wanapat-Thailand</i>	ANN-01-0100: “Influence of Tropical Roughages Combined with Urea and Bamboo Grass ( <i>Tillacora Triandra</i> , Diels) Supplementation on Gas Production and <i>In Vitro</i> Degradability” <i>Chaichana Suriyapha, Metha Wanapat and Chinda Wann-Thailand</i>
03:36 pm-03:48 pm	ANN-01-0082: “Fermentation quality and <i>in vitro</i> digestibility of fermented total mix ration with difference roughage and fermentation period” <i>Pichad Khejornsart, Teerayoot Jantanam and Metha Wanapat-Thailand</i>	ANN-01-0087: “Effect of Bamboo grass ( <i>Tillacora Triandra</i> ) pellet supplementation on feed intake, nutrient digestibility and rumen microbial population in Thai native beef” <i>Chinda Wann, Metha Wanapat, Chaowarit Mapato and Chaichana Suriyapha-Thailand</i>

03:48 pm-04:00 pm	ANN-01-0076: “Effect of replacement soybean meal by yeast waste on feed intake and rumen ecology in Thai native beef cattle” <i>Anusorn Cherdthong, Phussorn Sumadong, Suban Foiklang, and Nipa Milintawisamai-Thailand</i>	ANN-01-0105: “ <i>In Vitro</i> Fermentation and Methane Production Influenced by Leucaena Silage and Mangosteen Peel Powder” <i>Sungchhang Kang, Metha Wanapat, Ratana Khun, Laiheang Chhen, Piseth Try, Kimsan Sok, Ratha Long and Vannak Un-Cambodia</i>
04:00 pm-04:12 pm	ANN-01-0077: “Chemical composition and In vitro gas production of the local Thai and India Moringa ( <i>Moringa oleifera</i> Lam.) for ruminant” <i>Chaivawan Wattanachant, Nattha Rattanakosol, Chaermpun Yuangklang, and Apichat Loopachr-Thailand</i>	ANN-01-0106: “Influence of Bamboo Leaf Meal Supplementation on <i>In Vitro</i> Gas Production and Digestibility” <i>Sungchhang Kang and Metha Wanapat-Cambodia</i>
04:12 pm-05:30 pm	Chairperson: Prof. Dr. Metha Wanapat-Thailand Invited speaker: Prof. Dr. Peter Rowlinson-U.K. “Young Scientist Forum-How to Prepare Well for Publication in the International Journals with High Impact Factor”	Orchid ballroom I
November 3, 2017		
08:00 am-09:00 am	Chairperson: Prof. Dr. Peter Rowlinson-U.K. Plenary speaker: Prof. Dr. Ermias Kebreab-U.S.A. “Sustainable Intensification of Animal Systems in Emerging Economies”	Orchid ballroom I
09:00 am-09:40 am	Plenary speaker: Prof. Dr. K. Sarjan Reddy-India	Registration
09:40 am-10:20 am	“Climate Smart Livestock Production Systems (CSLPS)- A Novel Approach to Balance the Changing Climate”	
10:20 am - 10:30 am		Coffee/ tea break
	Chairperson: Assist. Prof. Dr. Anusorn Cherdthong-Thailand Co-Chairperson: Dr. Ni Nyoman Suryani- Indonesia	Session 11-Orchid ballroom I
10:30 am-10:42 am	ANN-01-0075: “Effect of Banana flower powder contained in high quality feed block and roughage to concentrate ratio on in vitro gas production kinetics, digestibility and fermentation” <i>Suban Foiklang, Metha Wanapat, Bounmaxay Viennasay, and Thitima Norrapoke-Thailand</i>	ANN-01-0072: “Inclusion of yeast waste as protein source to replace soybean meal in concentrate diet on ruminal fermentation and kinetics of gas using a gas production technique” <i>Anusorn Cherdthong, Rittikeard Prachumchai, Chanadol Supapong, Metha Wanapat, Suban Foiklang, and Nipa Milintawisamai-Thailand</i>
		Session 12-Arawan I
		Chairperson: Dr. Pakapun Skunmun-Thailand Co-Chairperson: Dr. Goh Chong Hau-Malaysia

10:42 am-10:54 am	ANN-01-0047: “Feed intake and blood metabolite of goats fed urea-calcium hydroxide treated oil palm frond” <i>Pin Chanjula, Suradech phetarvui, and Anusorn Cherdthong-Thailand</i>	ANN-01-0055: “Using of Urea and Molasses Fermented Cassava Pulp on Rumen Fermentation and Methane Production” <i>Thitima Norrapoke and Tanitpan Phongchongmit-Thailand</i>
10:54 am-11:06 am	ANN-01-0070: “Blood chemistries of dairy cow during pre-calving, at calving and post-calving period” <i>Chamtanwit Promkol, Pitukpol Porn-aneke, and Teerawat Srinukool -Thailand</i>	ANN-01-0046: “The Meat Quality of Bali Beef fed with Supplement Blocks with Different Liquid Smoke Levels as Antioxidant and Binder” <i>Effendi Abustam, Muhammad Irfan Said, and Muhammad Yusuf-Indonesia</i>
11:06 am - 11:18 am	ANN-01-0060: “The study on determination of feed digestibility using frequency of defecation on Thin Tailed lamb” <i>Febriantio Dwi Nugroho, Ari Prima, Edy Rianto, Agung Purnomoadi-Indonesia</i>	ANN-01-0094: “Effect of Flemingia ( <i>Flemingia macrophylla</i> ) as a protein replacement of soybean meal on feed intake, digestibility of nutrients and microbial population in Thai native beef cattle” <i>Burarat Phesatcha and Metha Wanapat-Thailand</i>
11:18 am - 11:30 am	ANN-01-0062: “Effect of <i>Terminalia Chebula</i> RETZ. meal on nutrient intake, digestibility and microbial population of goats” <i>Nirawan Gunuu, Pongsatorn Gunun, Anusorn Cherdthong, Sineenart Polyorach and Metha Wanapat-Thailand</i>	ANN-01-0068: “Nutrient utilization and rumen ecology of Thai indigenous cattle given hay and sago palm pith with different levels of soybean meal” <i>Bunseelarp Wiyada and Ngampongsai Wanwisa-Thailand</i>
11:30 am - 11:42 am	ANN-01-0090: “Effect of bamboo grass pellet (Bamboo-cass) levels on gas production kinetics and in vitro degradability” <i>Bounnaxay Viennasay and Metha Wanapat-Thailand</i>	ANN-01-0085: “Comparison between Hay and Silage of <i>Pennisetum purpurium</i> cv. Maharakham feeding on feed intake, nutrient digestibility, and rumen fermentation in Thai native beef bulls” <i>Chaowarit Mapato and Metha Wanapat-Thailand</i>
11:43 am - 11:54 am	ANN-01-0084: “Effect of fresh cassava root with feed block containing high sulfur on gas kinetics and rumen fermentation using <i>in vitro</i> gas production technique” <i>Ganonmas Dageay and Anusorn Cherdthong-Thailand</i>	ANN-01-0078: “Increasing Productive Performance of Native Chickens by Herbs in Rural Community” <i>Narumon Somkuna, Eakkasit Somkuna, Jarous Sawangtap and Phinithi Ratchwicha-Thailand</i>
11:55 am-01:00 pm	<b>Lunch</b>	

Orchid ballroom I	
	<p><b>Chairperson:</b> Prof. Dr. K. Sarjan Reddy</p> <p><b>Plenary speaker:</b> Prof. Dr. Luigi Zicarelli-Italy</p> <p><b>“The Role of Ruminants on Environmental Pollution”</b></p> <p><b>Plenary speaker:</b> Prof. Dr. Federico Infascelli-Italy</p> <p><b>“Milk fatty acid profile: influence of feeding model”</b></p> <p><b>Plenary speaker:</b> Prof. Dr. Huang Bizhi-P.R. China</p> <p><b>“Development of MBY in Yunnan and Diverse of Grass Resources in P.R. China”</b></p> <p><b>Plenary speaker:</b> Mr. John W. Long-JARVIS China, President</p> <p><b>“Advances in Meat Processing Technology, Slaughtering House Facility and Development: JARVIS”</b></p>
01:00 pm-01:40 pm	
01:40 pm-02:20 pm	
02:20 pm-3:00 pm	
03:00 pm-03:40 pm	
03:40 pm-04:00 pm	
<i>Coffee/ tea break</i>	
<b>Session 13-Orchid ballroom I</b>	
	<p><b>Chairperson:</b> Assoc. Prof. Dr. Pin Chanjula-Thailand</p> <p><b>Co-Chairperson:</b> Dr. Agustina Abdullah-Indonesia</p> <p><b>ANN-01-0059: “Regression Models for Estimating Fat Carcass Percentage Using Chest Measurement in Thin Tailed Lambs”</b> <i>Farah Nabila, Pradhipta Hersandika, Ari Prima, Vita Restitrisnani, Nadirotun Luthfi, Endang Purbowati, Sutaryo and Agung Purnomoadi-Indonesia</i></p>
04:00 pm-04:12 pm	
	<p><b>Chairperson:</b> Assist. Prof. Dr. Ruangyot Pilajun-Thailand</p> <p><b>Co-Chairperson:</b> Dr. Sri Suharti- Indonesia</p> <p><b>ANN-01-0088: “Effects of Microflora-treated rice straw on rumen fermentation and digestibility using In vitro gas production technique”</b> <i>S. Polyorach, M. Wanapat, C. Promkot, P. Gunun, S. Kang, A. Cherdthong, N. Gunun, and C. Mapato-Thailand</i></p>
04:12 pm-04:24 pm	
	<p><b>ANN-01-0091: “Effects of Fresh Purple Napier Grass (<i>Pennisetum Purpureum</i> ‘Prince’) and silage on Ruminant Gas Production In Vitro Study”</b> <i>Narawich Onjai-uea, Anan Petlum and Pramote. Paengkoum-Thailand</i></p>

04:24 pm-04:36 pm	ANN-01-0014: "Effect of Hydroponic Maize Fodder Supplementation on Production Performance in Graded Murrah Buffaloes of Scarce Rainfall Zone" <i>Atturi Krishna Murthy, Dhanalakshmi Guduru, Y.G.Prasad, and Sarjan Reddy Kapa-India</i>	ANN-01-0096: "Effect of Thapra Stylo silage treated with dried Mao pomace and lactic acid bacteria on feed intake and digestibility of goats" <i>Smerjai Bureenok, Chalermpon Yuangklang, Kraisit Yasupen, Benya Saenmahayak, Nitaya Pitwittayakul-Thailand</i>
04:36 pm-04:48 pm	ANN-01-0081: "Effect of fermented total mixed ration with microbial culture on fermentation quality and <i>in vitro</i> digestibility" <i>Pichad Khejornsart and Metha Wanapat-Thailand</i>	ANN-01-0036: "In Vitro and In Vivo Evaluation of Malic Acid on Methane Mitigation in Paddy Straw Based Complete Diet for Sustainable Animal Production in Dairy Cattle" <i>A. Bharathidhasan and R. Karunakaran-India</i>
04:48 pm-05:00 pm	ANN-01-0104: "Effect of Addition of Siamese Neem Foliage on pH and Number of Lactic Acid Bacteria in Napier Grass Silage" <i>Anan Petlum, Sukanya Kamphayae, Pramote Paengkoum, Walailuck Kaewwongsa, Smerjai Bureenok, Tanaporn Plong-uan, and Thanyalak Theppaw-Thailand</i>	ANN-01-0039: "In Vitro Gas Production Technique (IVGPT) on Evolving Methane Reduction by Malic Acid Supplementation in Forage Based Diet for Ruminants" <i>A. Bharathidhasan-India</i>
05:00 pm-06:00 pm	<b>Orchid ballroom I</b>	
07:00 pm-10:30 pm	Presentation award & Closing Ceremony: - Prof. Dr. Metha Wanapat - Assist. Prof. Dr. Anusorn Cherdthong - Prof. Dr. Peter Rowlinson	
07:30 am-08:00 am	Sarathai room I	
08:00 am-04:00 pm	<u>Farewell Party with Loy Kra-Tong Festival</u> November 4, 2017	
	Registration	
	Field trips	
	Dairy farming, DPO, temples	

## Contents

	Page
<i>Welcome Remarks</i>	I
<i>Message from the Chairman of ANI-NUE2017</i>	III
<i>Message from the editorial board</i>	IV
<i>International Advisory Committee</i>	V
<i>Organizing Committee: ANI-NUE2017</i>	VI
<i>Conference Program</i>	VIII
<b>Key-note, Plenary &amp; Lead Papers</b>	
<b>“Sufficiency Economy Philosophy as a Framework for the Promotion of Sustainable Agriculture and Life Quality”</b> <i>Charan Chantalakhana-Thailand</i>	1
<b>“Global Perspective on Mitigation of GHG and Renewable Energy Towards Sustainable Animal Agriculture”</b> <i>Junichi Takahashi-Japan</i>	2
<b>“Feeding Strategies on Farms to Improve Livestock Productivity and Reduce Methane Production”</b> <i>Metha Wanapat, Subun Foiklang, Thiwakorn Ampapon, Chaowarit Mapato, and Anusorn Cherdthong-Thailand</i>	14
<b>“Strategies to Increase the Efficiency of Nutrient Utilization”</b> <i>Peter Rowlinson-UK.</i>	30
<b>“Sustainable Intensification of Animal Systems in Emerging Economies”</b> <i>Ermias Kebreab-USA.</i>	44
<b>“Climate Smart Livestock Production Systems (CSLPS)- A Novel Approach to Balance the Changing Climate”</b> <i>K. Sarjan Reddy-India</i>	52
<b>“The Role of Ruminants on Environmental Pollution”</b> <i>Luigi Zicarelli-Italy</i>	76
<b>“Milk Fatty Acid Profile: Influence of Feeding Model”</b> <i>Federico Infascelli, Raffaella Tudisco, and Pietro Lombardi-Italy</i>	81
<b>“Development of MBY in Yunnan and Diverse of Grass Resources in P.R. China”</b> <i>Huang Bizhi-P.R. China</i>	88
<b>“Advances in Meat Processing Technology, Slaughtering House Facility and Development: JARVIS”</b> <i>John W. Long- JARVIS China, President</i>	89
<b>“Thai Dairy Productivity: Milk Quality and Days Open”</b> <i>Suneerat Aiumlamai-Thailand</i>	90
<b>“Current Non-ruminant Production and Future Prospects in Vietnam”</b> <i>Vu Dinh Ton-Vietnam</i>	98
<b>“Ruminant Production in Lao, PDR.”</b> <i>Pattaya Napsirth and Viengsakoun Napsirth-Loa, PDR.</i>	107
<b>“Tropical Animal Genetic and Environment Impact”</b> <i>Monchai Duangjinda-Thailand</i>	113
<b>“Potential use of Alternative Source of Protein in Ruminant Feeding”</b> <i>Anusorn Cherdthong-Thailand</i>	114
<b>“The Influence of Climate Change and the Strategy for Producing Tropical Animals”</b> <i>Kalaya Boonyanuwat-Thailand</i>	124

## Contents (Continued)

Oral presentation	Page
<b>Session 1</b>	
<b>ANN-01-0006: “Metabolizable Protein Requirements of Lactating Buffaloes (<i>Bubalus bubalis</i>) Fed on Silage Based Diet”</b> <i>Umesh Balaji Sontakke , Shivlal Singh Kundu , Sonali prusty, Gautam Mondal, Vijay Kumar Sharma and Muneendra Kumar-India</i>	136
<b>ANN-01-0093: “Supplementation of mangosteen peel and banana flower pellet (MABAP) to improve ruminal fermentation and milk production in dairy cows”</b> <i>Thiwakorn Ampapon and Metha Wanapat-Thailand</i>	142
<b>ANN-01-0015: “Energy Utilization, VFA, and A/P Ratio of Kacang Goat Fed Total Mixed Ration Containing Different Treatments of Soybean Meal”</b> <i>Kustantinah, I Gede Suparta Budisatria, Rusman and Retno Adiwiniarti-Indonesia</i>	148
<b>ANN-01-0063: “Feed intake and digestibility of dairy cows affected by mao (<i>Antidesma thwaitesianum</i> Muell. Arg.) pomace meal supplementation”</b> <i>Pongsatorn Gunun, Nirawan Gunun, Thanaporn Ouppamong, Srisuda Sirilaophaisan, Anusorn Cherdthong, Kessara Ampaporn, Paiwan Punyakaew, Metha Wanapat and Sineenart Polyorach-Thailand</i>	153
<b>ANN-01-0030: “Performance and Physiological Status of Kids Milking by Milk Replacer Containing Cricket Meal”</b> <i>Dewi Apri Astuti, Lilis Khotijah and Rika Damanik-Indonesia</i>	158
<b>ANN-01-0080: “Variations in milk composition between morning and afternoon milking in dairy cow”</b> <i>Chamnanwit Promkot, Pitukpol Porn-anek, and Lerdchai Phu-oob-Thailand</i>	163
<b>Session 2</b>	
<b>ANN-01-0052: “Feeding POAB at Different Level of Amino Acids in The Diet of Broiler Chickens”</b> <i>C.H. Goh, T.C. Loh, H.L. Foo, and N. Frisco-Malaysia</i>	167
<b>ANN-01-0089: “Effects of purple glutinous rice residue meal in concentrate diets on growth performance in growing pigs”</b> <i>Walailuck Kaewwongsa, Pattaya Napisirth, Nirawan gunun, Viboon Pensuk and Attapong Piladang-Thailand</i>	179
<b>ANN-01-0010: “Effect of Diet Containing Dragon Fruit Peel Meal Fermentation for Productivity of Kampung Chickens”</b> <i>Gusti A.M.Kristina Dewi, I M. Nuriyasa, and I Wayan Wijana-Indonesia</i>	183
<b>ANN-01-0103: “Effect of rice wine by-product as alternative protein source on growth performance of broiler chickens”</b> <i>Benya Saenmahayak, Smerjai Bureenok, Chalermpon Yuangklang, Sasiphan Wongsuthavas, and Kraisit Vasupen-Thailand</i>	189
<b>ANN-01-0035: “A Comparison of Fat-Soluble Antioxidants in Wild and Farm-Reared Chukar Partridges (<i>Alectoris Chukar</i>)”</b> <i>Filiz Karadasa, Anders Pape Møllerb , and Mehmet Reşit Karageçili-Turkey</i>	193
<b>ANN-01-0101: “Used dried cassava leaves with enzymes from fermented tomato pomace with <i>Aspergillus niger</i> in laying duck diet on nutrient digestibility”</b> <i>Kraisit Vasupen, Savang Saykhammy, Sasiphan Wongsuthavas, Chalermpon Yuangklang, Smerjai Bureenok, Benya Saenmahayak-Thailand</i>	194

### Contents (Continued)

<b>Session 3</b>	
<b>ANN-01-0001: “Effect of Increasing Energy and Protein Ration on Nutrient Digestibility and Performance of Bali Heifer Calves”</b> <i>Ni Nyoman Suryani, I Wayan Suarna, I Gede Mahardika and Ni Putu Sarini-Indonesia</i>	197
<b>ANN-01-0003: “Metabolizable Energy of Cassava Pulp for Thai Native Beef Cattle”</b> <i>Ornvimol Keaokliang, Tomoyuki Kawashima, Wanna Anghong, Tomoyuki Suzuki, and Ramphrai Narmseelee-Japan</i>	204
<b>ANN-01-0008: “Application of Pressurized Heating in Production Process of Bali Cattle Fur Meal to Its Nutrient”</b> <i>Muhammad Irfan Said, Farida Nur Yuliaty, Muhammad Zain Mide, Wempie Pakiding and Hamri-Indonesia</i>	205
<b>ANN-01-0073: “Effect of <math>\beta</math>-glucan supplementation on feed intake, digestibility and rumen fermentation in Thai native beef cattle”</b> <i>Anusorn Cherdthong and Anuthida Seankamsorn-Thailand</i>	210
<b>ANN-01-0040: “Ruminal Nitrogen Release from Limestone-Urea Mixture”</b> <i>M. Ainsyar Harahap, Limbang K. Nuswantara, Eko Pangestu, Fajar Wahyono and Joelal Achmadi-Indonesia</i>	217
<b>ANN-01-0074: “Effect of sulfur levels supplementation in fermented total mixed ration containing fresh cassava root using F gas production technique”</b> <i>Chanadol Supamong and Anusorn Cherdthong -Thailand</i>	222
<b>ANN-01-0042: “The Performance of Extension Agent in Improving Adoption The Technology Beef Cattle Feed”</b> <i>Agustina Abdullah, Jamila, and A. Amrullah-Indonesia</i>	228
<b>Session 4</b>	
<b>ANN-01-0012: “Use of <i>Bacillus subtilis</i> to Produce Feather Meal for Animal Feeds and Organic Fertilizers”</b> <i>Chi-Chu Lo, Liang-Yi Lin, and Shu-Chuan Chen-Taiwan</i>	235
<b>ANN-01-0032: “Assessment of Metabolizable Energy, Nutrients Digestibility and Fatty Acids Composition of Fat Crystals Derived from Crude Palm Oil in Chickens”</b> <i>Sarawut Treetan, Sonthaya Numthuam, Rangsun Charoensook, Wandee Tartrakoon, Papichaya Incharoen, and Tossaporn Incharoen-Thailand</i>	241
<b>ANN-01-0034: “Effect of Dietary Supplementation of Cinnamon and Curcumin on Performance, Humoral immune Responses, and Blood lipid Profile in Rabbits”</b> <i>Hassan Zeweil, Soliman Zahran, Mohamed Ahmed, Yasmin El-Gindy and Nagat Khoshera-Egypt</i>	247
<b>ANN-01-0033: “Study on The Growth Performance, Meat Quality and Bone Breaking Strength of Broilers fed Dietary Rice Hull Silicon”</b> <i>Sarawoot Nakhon, Sonthaya Numthuam, Rangsun Charoensook, Wandee Tartrakoon, Papichaya Incharoen, and Tossaporn Incharoen-Thailand</i>	253
<b>ANN-01-0064: “The effects of organic corn level decreasing in organic laying hen diets on egg production and egg quality”</b> <i>Kannikar Hamprakorn, Buaream Maneewan, Tonglian Buwjoom and Sukit Khantaprab-Thailand</i>	259



## Contents (Continued)

<b>ANN-01-0067: “Factors Effecting on Rabies Immunity Titer in Canine”</b> <i>Suraphong Wongsutthawart, Bundit Nuansrichay, Ratchaneekorn Vitoonpong, Kongkul Kaskosol, Lamul Molee and Sasiphan Wongsuthavas-Thailand</i>	264
<b>ANN-01-0011: “Antibacterial activity of <i>Phaleria macrocarpa</i> fruit extracts: an <i>In vitro</i> study”</b> <i>Niati Ningsih, Bambang Ariyadi, and Zuprizal-Indonesia</i>	270
<b>Session 5</b>	
<b>ANN-01-0050: “Development strategies for dairy cattle production system and milk products in northeast of Thailand: Policy framework and challenges”</b> <i>Theerachai Haitook, Samruay Ninking, Phruetthinun Chukasem, Wuttikorn Srakaew and Naritsara Suayroop-Thailand</i>	275
<b>ANN-01-0058: “Beef Cattle Feeding Management of Smallholder Farmers in Kon Tum City, Vietnam”</b> <i>Ratchataporn Lunsin, Thai Thi Bich Van, Truong Thi Tu Trinh and Somporn Daunyai-Thailand</i>	280
<b>ANN-01-0016: “Growth, Carcass Production, and Chevon Quality of Kacang Goat Fed Formaldehyde Protected Soybean Meal”</b> <i>Retno Adiwiniarti, I Gede Suparta Budisatria, Kustantinah, and Rusman, and Edwin Indarto-Indonesia</i>	286
<b>ANN-01-0056: “Effects of different tropical grasses on feed intake and blood metabolite of goats”</b> <i>Pin Chanjula, Rawee Chiarawipa and Phantip Panklang-Thailand</i>	291
<b>ANN-01-0004: “Application of Tunnel-Ventilated Barn in Tropical Dairy Industry: A review”</b> <i>Aan Andri Yano, Adiarto, and Diah Tri Widayati-Indonesia</i>	296
<b>Session 6</b>	
<b>ANN-01-0005: “Genetic Polymorphisms of Alphas1-Casein (CSN1S1) Gene In Indonesian Local Goat Population Reared In South Sulawesi Province”</b> <i>Muhammad Ihsan Andi Dagon, Lellah Rahim, RR Sri Rachma Aprilita Bugiwati, Magfirah Nur, and Nurul Purnomo-Indonesia</i>	302
<b>ANN-01-0079: “Induction of follicular growth and atresia: Expression of aromatase mRNA in the ovary of <i>Bos Indicus</i>”</b> <i>Nattawut Kogram, Vilaivan Khanthusaeng, Surapong Tongrueng, Thunya Bunma and Chainarong Navanukraw-Thailand</i>	307
<b>ANN-01-0037: “The effect of bull (<i>Bos indicus</i>) and extender medium to additional antioxidant <math>\alpha</math>-tocopherol of cryopreservation sperm post-thawing to minimize of repeat breeding”</b> <i>Anna Farhana, Ismaya, and Nono Ngadiyono-Indonesia</i>	312
<b>ANN-01-0083: “Early embryonic development, corpus luteum and metabolite of PGF in lactating dairy cows supplemented with palm or sunflower oil”</b> <i>Aree Kraison, Jaruwan Kaokejon, Wiroon Inthamonee, and Chainarong Navanukraw-Thailand</i>	319
<b>ANN-01-0086: “Development of corpus luteum in goats: interaction between progesterone concentration and luteal cell proliferation”</b> <i>Thanya Bunma, Chainarong Navanukraw, Vilaivan Khanthusaeng, Aree Kraison and Nattawut Kogram-Thailand</i>	325

## Contents (Continued)

<b>Session 7</b>	
<b>ANN-01-0018: “Optimation Total Digestible Nutrients - Protein Ratio To Achieve Good Feed Conversion Ratio In Indonesian Native Beef Cattle”</b> <i>Nadlirotun Luthfi, Edy Rianto and Agung Purnomoadi-Indonesia</i>	331
<b>ANN-01-0049: “Dietary Protein Requirement for Maintenance and Growth of Southern Thai Indigenous Cattle”</b> <i>O. Pimpa, B. Khamseekhiew, B. Pimpa and S. Ruengsuwan-Thailand</i>	336
<b>ANN-01-0019: “The effect of dietary protein intake on body protein growth in Thin Tailed Lambs”</b> <i>Ari Prima, Nadlirotun Luthfi, Edy Rianto, Endang Purbowati and Agung Purnomoadi-Indonesia</i>	341
<b>ANN-01-0092: “Effect of yeast fermented cassava pulp (YFCP) supplementation on feed intake, digestibility and rumen fermentation in beef cattle”</b> <i>Sukruthai Sommai, Metha Wanapat, Chaowarit Mapato and Pajaree Totakul-Thailand</i>	345
<b>ANN-01-0095: “Influences of Yeast fermented potato peel and cassava peel on gas kinetics and digestibility using in vitro gas technique”</b> <i>Suban Foiklang, Risa Japanya, Phanuphong Ounpon, Metha Wanapat, Anusorn Cherdthong, Thitima Norrapoke, and Kampanat Phesatcha-Thailand</i>	352
<b>ANN-01-0098: “Effect of yeast fermented dehulled rice (YEFEDER) levels with different kind of roughage on gas production and in vitro degradability using in vitro gas production technique”</b> <i>Pajaree Totakul, Metha Wanapat, Suban Foiklang, Chaowarit Mapato, and Sukruthai Sommai-Thailand</i>	356
<b>Session 8</b>	
<b>ANN-01-0017: “The Effect of Liquid Smoke on Methane Emission from Faces”</b> <i>Vita Restitrisnani, Khanza Syahira Dhia, Tegar Wicaksono, Edy Rianto, and Agung Purnomoadi-Indonesia</i>	361
<b>ANN-01-0057: “Effects of sunflower oil and nitrate supplementation on methane production and rumen fermentation by using in vitro gas production technique”</b> <i>Jiravan Khotsakdee, Chalermpon Yuangklang, Siwaporn Paengkoum and Pramote Paengkoum-Thailand</i>	366
<b>ANN-01-0043: “In Vitro Rumen Microbial Population and Fermentation with The Addition of <i>Sapindus rarak</i> Extract and Sesame/Canola Oils Microencapsulation”</b> <i>Sri Suharti, Isma Firliani, and Komang Gede Wiryawan-Indonesia</i>	374
<b>ANN-01-0097: “Effect of Dragon fruit (<i>Hylocercus undatus</i>) peel powder and roughage to concentration ratio on gas production kinetics, digestibility, and fermentation using in vitro gas production technique”</b> <i>Maharach Matra, Metha Wanapat, Suban Foiklang, Chaowarit Mapato, Thiwakorn Ampapong, Bounnaxay Viennasay and Ahkarapon Nunoia-Thailand</i>	381
<b>ANN-01-0066: “Hematology and physiological responses as indicator of heat tolerance”</b> <i>Pitukpol Porn-anek, Chamnanwit Promkot, and Thaweechai Phuard-Thailand</i>	388

## Contents (Continued)

<b>ANN-01-0071: “Effects of supplementation of Piper sarmentosum leaves powder in concentrates on feed efficiency, rumen fermentation and protozoa in Thai native beef cattle”</b> <i>Anusorn Cherdthong and Benjamad Khonkhaeng-Thailand</i>	400
<b>Session 9</b>	
<b>ANN-01-0061: “Determination of level protein intake to control fat and protein in carcass of fattened lambs”</b> <i>Rizky Choirunnisa, Mukh Arifin, Robert Kusetyawan, Febrian Rhamadya, Ari Prima, Vita Restitrisnani, Nadlirotun Luthfi, Sutaryo and Agung Purnomoadi-Indonesia</i>	408
<b>ANN-01-0044: “Effect of yeast-fermented cassava pulp levels on growth performance of growing goats fed Napier Pakchong 1 grass”</b> <i>Saran Parisuthikul, Tichakorn Thumtong, Theerasant Phothee, Panuwat Teebklang, Wunchai Inthiseang, Wichan Kaewluan and Ruangyote Pilajun-Thailand</i>	415
<b>ANN-01-0102: “Effect of mangosteen peel liquid protected soybean meal on methonogen and microbial population using in in vitro gas fermentation technique”</b> <i>Kampanat Phesatcha, Burarat Phesatcha, Suban Foiklang, Metha Wanapat-Thailand</i>	422
<b>ANN-01-0082: “Fermentation quality and in vitro digestibility of fermented total mix ration with difference roughage and fermentation period”</b> <i>Pichad Khejornart, Teerayoot Jantanam and Metha Wanapat-Thailand</i>	429
<b>ANN-01-0076: “Effect of replacement soybean meal by yeast waste on feed intake and rumen ecology in Thai native beef cattle”</b> <i>Anusorn Cherdthong, Phussorn Sumadong, Suban Foiklang, and Nipa Milintawisamai-Thailand</i>	436
<b>ANN-01-0077: “Chemical composition and In vitro gas production of the local Thai and India Moringa (<i>Moringa oleifera</i> Lam.) for ruminant”</b> <i>Chaiyawan Wattanachant, Nattha Rattanacosol, Chaermpon Yuangklang, and Apichat Loopachr-Thailand</i>	441
<b>Session 10</b>	
<b>ANN-01-0013: “Use of Body Measurements to Predict Intermuscular Fat in Thin-tailed Lambs”</b> <i>Ulia Renfelia Baysi, Ari Prima, Farah Nabila, Pradhipta Hersandika, Endang Purbowati, Christina Maria Sri Lestari, and Agung Purnomoadi-Indonesia</i>	447
<b>ANN-01-0099: “Effect of inclusion of enzyme from fermented tomato pomace with <i>Aspergillus niger</i> (FETPAN) in total mixed ration on feed intake and growth performance in beef cattle”</b> <i>Chalermpon Yuangklang, Jiravan Khotsakdee, and Krisit Vasupen-Thailand</i>	452
<b>ANN-01-0100: “Influence of Tropical Roughages Combined with Urea and Bamboo Grass (<i>Tiliacora Triandra</i>, Diels) Supplementation on Gas Production and In Vitro Degradability”</b> <i>Chaichana Suriyapha, Metha Wanapat and Chinda Wann-Thailand</i>	457

## Contents (Continued)

ANN-01-0087: “Effect of Bamboo grass ( <i>Tiliacora Triandra</i> ) pellet supplementation on feed intake, nutrient digestibility and rumen microbial population in Thai native beef” <i>Chinda Wann, Metha Wanapat, Choawarit Mapato and Chaichana Suriyapha-Thailand</i>	463
ANN-01-0105: “ <i>In Vitro</i> Fermentation and Methane Production Influenced by Leucaena Silage and Mangosteen Peel Powder” <i>Sungchhang Kang, Metha Wanapat, Ratana Khun, Laiheang Chhen, Piseth Try, Kimsan Sok, Ratha Long and Vannak Un-Cambodia</i>	469
ANN-01-0106: “Influence of Bamboo Leaf Meal Supplementation on <i>In Vitro</i> Gas Production and Digestibility” <i>Sungchhang Kang and Metha Wanapat-Cambodia</i>	475
<b>Session 11</b>	
ANN-01-0075: “Effect of Banana flower powder contained in high quality feed block and roughage to concentrate ratio on in vitro gas production kinetics, digestibility and fermentation” <i>Suban Foiklang, Metha Wanapat, Bounnaxay Viennasay, and Thitima Norrapoke-Thailand</i>	481
ANN-01-0047: “Feed intake and blood metabolite of goats fed urea-calcium hydroxide treated oil palm frond” <i>Pin Chanjula, Suradech phetarwut, and Anusorn Cherdthong-Thailand</i>	486
ANN-01-0070: “Blood chemistries of dairy cow during pre-calving, at calving and post-calving period” <i>Chamnanwit Promkot, Pitukpol Porn-aneke, and Teerawat Srinukool-Thailand</i>	491
ANN-01-0060: “The study on determination of feed digestibility using frequency of defecation on Thin Tailed lamb” <i>Febrianto Dwi Nugroho, Ari Prima, Edy Rianto, Agung Purnomoadi-Indonesia</i>	494
ANN-01-0062: “Effect of <i>Terminalia Chebula</i> RETZ. meal on nutrient intake, digestibility and microbial population of goats” <i>Nirawan Gunun, Pongsatorn Gunun, Anusorn Cherdthong, Sineenart Polyorach and Metha Wanapat-Thailand</i>	497
ANN-01-0090: “Effect of bamboo grass pellet (Bamboo-cass) levels on gas production kinetics and in vitro degradability” <i>Bounnaxay Viennasay and Metha Wanapat-Thailand</i>	500
ANN-01-0084: “Effect of fresh cassava root with feed block containing high sulfur on gas kinetics and rumen fermentation using <i>in vitro</i> gas production technique” <i>Gamonmas Dageaw and Anusorn Cherdthong-Thailand</i>	507
<b>Session 12</b>	
ANN-01-0072: “Inclusion of yeast waste as protein source to replace soybean meal in concentrate diet on ruminal fermentation and kinetics of gas using a gas production technique” <i>Anusorn Cherdthong, Rittikeard Prachumchai, Chanadol Supapong, Metha Wanapat, Suban Foiklang, and Nipa Milintawisamai-Thailand</i>	514

## Contents (Continued)

<p><b>ANN-01-0055: “Using of Urea and Molasses Fermented Cassava Pulp on Rumen Fermentation and Methane Production”</b>  <i>Thitima Norrapoke and Tanitpan Phongchongmit-Thailand</i></p>	519
<p><b>ANN-01-0046: “The Meat Quality of Bali Beef fed with Supplement Blocks with Different Liquid Smoke Levels as Antioxidant and Binder”</b>  <i>Effendi Abustam, Muhammad Irfan Said, and Muhammad Yusuf-Indonesia</i></p>	526
<p><b>ANN-01-0094: “Effect of Flemingia (<i>Flemingia macrophylla</i>) as a protein replacement of soybean meal on feed intake, digestibility of nutrients and microbial population in Thai native beef cattle”</b>  <i>Burarat Phesatcha and Metha Wanapat-Thailand</i></p>	532
<p><b>ANN-01-0068: “Nutrient utilization and rumen ecology of Thai indigenous cattle given hay and sago palm pith with different levels of soybean meal”</b>  <i>Bunseelarp Wiyada and Ngampongsai Wanwisa-Thailand</i></p>	540
<p><b>ANN-01-0085: “Comparison between Hay and Silage of <i>Pennisetum purpurium</i> cv. Mahasarakham feeding on feed intake, nutrient digestibility, and rumen fermentation in Thai native beef bulls”</b>  <i>Chaowarit Mapato and Metha Wanapat-Thailand</i></p>	545
<p><b>ANN-01-0078: “Increasing Productive Performance of Native Chickens by Herbs in Rural Community”</b>  <i>Narumon Somkuna, Eakkasit Somkuna, Jarous Sawangtap and Phinithi Ratchwicha-Thailand</i></p>	549
<b>Session 13</b>	
<p><b>ANN-01-0059: “Regression Models for Estimating Fat Carcass Percentage Using Chest Measurement in Thin Tailed Lambs”</b>  <i>Farah Nabila, Pradhipta Hersandika, Ari Prima, Vita Restitrisnani, Nadlirotun Luthfi, Endang Purbowati, Sutaryo and Agung Purnomoadi-Indonesia</i></p>	553
<p><b>ANN-01-0065: “Development of Near Infrared Spectroscopy for nondestructive and rapid measurement of chemical compositions and somatic cell counts in raw milk”</b>  <i>Onanong Pongchompu, Metha Wanapat, Chaluntorn Vichasilp, Yaungyote Jindatajak and Pongsagorn Pongchompu-Thailand</i></p>	558
<p><b>ANN-01-0014: “Effect of Hydroponic Maize Fodder Supplementation on Production Performance in Graded Murrah Buffaloes of Scarce Rainfall Zone”</b>  <i>Atturi Krishna Murthy, Dhanalakshmi Guduru, Y.G.Prasad, and Sarjan Reddy Kapa-India</i></p>	563
<p><b>ANN-01-0081: “Effect of fermented total mixed ration with microbial culture on fermentation quality and <i>in vitro</i> digestibility”</b>  <i>Pichad Khejornsart and Metha Wanapat-Thailand</i></p>	567
<p><b>ANN-01-0104- Effect of Addition of Siamese Neem Foliage on pH and Number of Lactic Acid Bacteria in Napier Grass Silage</b>  <i>Anan Petlum, Sukanya Kamphayae, Pramote Paengkoum, Walailuck Kaewwongsa, Smerjai Bureenok, Tanaporn Plong-uan, and Thanyalak Theppaw-Thailand</i></p>	574

## Contents (Continued)

<b>Session 14</b>	
<b>ANN-01-0088: “Effects of Microflora-treated rice straw on rumen fermentation and digestibility using In vitro gas production technique”</b> <i>S. Polyorach, M. Wanapat, C. Promkot, P. Gunun, S. Kang, A. Cherdthong, N. Gunun, and C. Mapato-Thailand</i>	578
<b>ANN-01-0091: “Effects of Fresh Purple Napier Grass (<i>Pennisetum Purpureum</i> ‘Prince’) and silage on Ruminant Gas Production In Vitro Study”</b> <i>Narawich Onjai-uea, Anan Petlum and Pramote. Paengkoum-Thailand</i>	584
<b>ANN-01-0096: “Effect of Thapra Stylo silage treated with dried Mao pomace and lactic acid bacteria on feed intake and digestibility of goats”</b> <i>Smerjai Bureenok, Chalermpon Yuangklang, Kraisit Vasupen, Benya Saenmahayak, Nittaya Pitiwittayakul-Thailand</i>	589
<b>ANN-01-0036: “In Vitro and In Vivo Evaluation of Malic Acid on Methane Mitigation in Paddy Straw Based Complete Diet for Sustainable Animal Production in Dairy Cattle”</b> <i>A. Bharathidhasan and R. Karunakaran-India</i>	593
<b>ANN-01-0039: “In Vitro Gas Production Technique (IVGPT) on Evolving Methane Reduction by Malic Acid Supplementation in Forage Based Diet for Ruminants”</b> <i>A. Bharathidhasan-India</i>	599



## ***Key-note, Plenary and Lead Paper***

# **Sufficiency Economy Philosophy as a Framework for the Promotion of Sustainable Agriculture and Life Quality**

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## **Abstract**

During the past fifty years, for most Southeast Asian countries it has been evident that due to materialistic and capitalistic economic development policies, many adverse impacts on various aspects such as economic aspects, social qualities, natural resources and environment, agricultural sustainability as well as national stability and security, have been clearly evident.

It was purposed that the sufficiency economy philosophy (SEP), as initiated and experimented by the late King of Thailand (Rama IX), should be considered as an alternative for a development framework in order to contain many of the past negative impacts. The detailed principles and methods of SEP were discussed. The Gross Domestic Happiness (GDH) was purposed to be used as the indicators of the development process instead of the Gross Domestic Products (GDP) alone, as the GDH includes not only the GDP but also the values of non-marketable household products, as well as human and community happiness.

Some important recommendations relevant to the promotion of sustainable agriculture and the farmer life quality within the SEP framework including farmer education and the research and development in agro- and bio-processing of farmer products, in order to diversity the demand and uses of agricultural products have been discussed in details.

**Keywords:** sustainable agriculture, sufficiency economy philosophy, gross domestic happiness, life quality



## Global Perspective on Mitigation of GHG and Renewable Energy Towards Sustainable Animal Agriculture

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### Abstract

The prophylactic effects of probiotics, prebiotics and miscellaneous to mitigate rumen methanogenesis have been developed instead of antibiotics ionophores such as monensin, lasalocid. Nitrate with L-cysteine will successfully suppress rumen methanogenesis without intoxication. The *in vitro* and *in vivo* trials have been conducted to clarify the prophylactic effects of L-cysteine, some strains of lactic acid bacteria and yeast and/or  $\beta$ 1-4 galactooligosaccharide (GOS) on nitrate-nitrite intoxication and methanogenesis. For prebiotics, the nisin which is a bacteriocin produced by *Lactococcus lactis* subsp. *lactis* has been demonstrated to abate rumen methanogenesis in the same manner of monensin. A protein resistant anti-microbes (PRA) has been isolated from *Lactobacillus plantarum* as a manipulator to mitigate rumen methanogenesis. Hydrogen peroxide was identified as a part of the manipulating effect of PRA to rumen methanogenesis. The mitigating effects of secondary metabolites from plants such as saponins and tannins on rumen methanogenesis have been examined. Especially, *yucca schidigera* extract, sarsaponin (steroidal glycosides) can suppress rumen methanogenesis improving protein utilization efficiency. The mechanism for accreditation of manipulators must be established to mitigate global methane (CH<sub>4</sub>) emission. Biogas plant has been widely spread over the world as one of the renewable energies generated from anaerobic CH<sub>4</sub> fermentation of bio-wastes recycling such as livestock manures. Furthermore, in advanced new biogas system, the ammonia stripping from digested slurry of livestock manure in biogas plant was examined to apply to nitrogen recycling-options mitigating nitrous oxide (N<sub>2</sub>O) emission. In addition, hydroponic culture system using CO<sub>2</sub> exhausted from the biogas system and the digested slurry as a culture solution was proposed for not only GHG mitigation, but saving water and nutrient resources for hydroponic culture system in semi-arid and arid areas. Alternatively, microbial removal of ammonia nitrogen in waste water contaminated with piggery manure into atmospheric pared N<sub>2</sub> was examined using *Alcaligenes faecalis* strain No.4 with heterotrophic nitrification and denitrification, because excess NH<sub>4</sub><sup>+</sup>-N might be derived from atmospheric N<sub>2</sub> using Haber-Bosch process for manufacturing synthetic fertilizers. Thus, animal agriculture has contribute to global emission of CH<sub>4</sub> and N<sub>2</sub>O, whereas livestock have come to be exposed to the bilateral impact of heat and oxidative stresses followed by climate change due to abruptly increased GHGs. In an attempt to seek the mitigation option of heat stress in lactating dairy cow exposed to hot environment, mitigating effect of rumen mechanical stimulating brush (Rumenfibe) was examined in pasture-based lactating cows in Australian spring and summer. Consequently, relatively higher value of biological antioxidant potential (BAP) in Rumenfibe administered cows.

**Keywords:** methane, nitrous oxide, biogas plant, heat stress, biological antioxidant potential





## Introduction

	Kyoto Protocol		Paris Agreement
	The first commitment period 2008-2012	The second commitment period 2013-2020	2020- Evaluation every 5 years
EU 27 Australia Other European countries	With targeted reduction obligation of GHG emission	With a targeted reduction of GHG emission	New framework which every countries can participate (accepted plan until 2015)
Japan Russia New Zealand		Without targeted reduction obligation of GHG emission (Non-participation to Kyoto Protocol)	
Canada	Secession		
Developing countries China India Others	Without reduction obligation of GHG emission (Participation to Kyoto Protocol)		
USA	Non-participation to Kyoto Protocol		Declaration of secession

**Fig 1.** New framework of the Post-Kyoto Protocol

Paris climate agreement based on pledge and review system was adopted at 21<sup>st</sup> conference of parties of the UNFCCC in 2016 as a global treaty instead of Kyoto Protocol (Fig.1). The mitigation of anthropogenic six greenhouse gases such as carbon dioxide (CO<sub>2</sub>), methane (CH<sub>4</sub>), N<sub>2</sub>O, sulphur hexafluoride (SF<sub>6</sub>), hydrofluorocarbons (HFCs) and perfluorocarbons (PFCs) have been established as legally binding the first commitments in The Kyoto Protocol (IPCC, 1996). Additionally, nitrogen trifluoride (NF<sub>3</sub>) was added as GHG in the second commitment period in the Kyoto Protocol (IPCC, 2007). The most important GHGs attributed to animal agriculture are CH<sub>4</sub> and N<sub>2</sub>O. In the second commitment period, when the greenhouse effect of carbon dioxide is set to 1, the impacts of the greenhouse effect of CH<sub>4</sub> and N<sub>2</sub>O have been changed 21 to 25-fold and 310 to 298-fold higher than CO<sub>2</sub> respectively (Table 1).

**Table 1.** Greenhouse gases (GHG) dealt in the first and the second commitment periods of Kyoto Protocol and their global warming potentials (GWP)

GHG	Global Warming Potential (GWP)	
	The First Commitment Period	The Second Commitment Period
CO <sub>2</sub>	1	1
CH <sub>4</sub>	21	25
N <sub>2</sub> O	310	298
HFCs	1,300	1,430
PFCs	6,500	7,390
SF <sub>6</sub>	23,900	22,800
NF <sub>3</sub>	—	17,200



Rumen fermentation of ruminant livestock and anaerobic fermentation of organic wastes including animal manures are major contributors of methane emission as anthropogenic sources (Moss, 1993). The total contribution of belching methane derived from rumen fermentation and the manure to global methane emission might be accounted for nearly 18-20%. Thus, the development of mitigation methods of rumen methane emission is the most significant issue in the world ruminant livestock production sector. For a solution of the mitigation of rumen methanogenesis, biological ionophores such as monensin and salinomycin which have been widely used as feed additives for growth promoter of ruminant livestock have a potential to abate rumen methane production. However, the efficacy may continue not only in the alimentary canal, but also manure (Table 2), and continuous use of the feed additives may put rumen flora at emergent risk of resistant bacteria associated with the antibiotics *i.e.*, uncontrolled antibiotics in the organic fertilizer will be spread over the soil environment (Mwenya et al., 2006). Therefore, manipulators to abate rumen methanogenesis must be safe to the environment as well as animals and humans.

**Table 2.** Progressive methane yield (L/g volatile solids fed (VS<sub>f</sub>)) in anaerobic digesters fed manure from steers supplemented with or without monensin

Retention time (day)	Control	Monensin	P-value
10	0.187 <sup>a</sup>	0.061 <sup>b</sup>	0.001
20	0.230 <sup>a</sup>	0.091 <sup>b</sup>	0.01
30	0.252 <sup>a</sup>	0.145 <sup>b</sup>	0.034
40	0.266	0.174	0.156
50	0.275	0.185	0.197

<sup>ab</sup>Means within a row with different superscripts differ by the corresponding P-value

The prompt increase of atmospheric N<sub>2</sub>O since last century is closely related to abrupt expansion of human and animal population after an innovation of Haber-Bosch process. Severe environmental pollutions were caused at the same time though the reactive nitrogen withdrawn from atmosphere as stable paired nitrogen brought about prosperous food production. Anthropogenic increases in these GHGs have been affected with continuous expansion of the chemical nitrogen fertilizer consumption (Takahashi, 2006). Accurate assessment of the impact of inorganic and organic nitrogen on greenhouse effect may be useful to make up an inventory and the mitigation strategies. To secure food production preventing environmental catalyses by global warming sustainable development of animal agriculture should be sought in not only developed nations but also newly industrialized and developing countries as an alternative way. Inventories of emitters and their abatements should be accurately assessed in both GHGs. The key element of these recycling must be low-input for sustainable livestock production. Carbon and nitrogen recycling in the agricultural biomass as alternative feeds, renewable energy and nitrogen resources might contribute to the mitigation of CH<sub>4</sub> and N<sub>2</sub>O (Takahashi et al., 2003). The mechanism for accreditation of manipulators must be established to mitigate global CH<sub>4</sub> and N<sub>2</sub>O emission.



Recent climate change has amplified a risk to expose dairy cattle to hot environment (Nidumolu et al., 2013). In consequence, milk production and reproductive proficiency of dairy cows suffer from heat stress due to their strong stress sensitivity (West, 2003). Heat stress might induce oxidative stress on the animals as an external inducer (Bernabucci, 2002). The establishment of mitigation strategies of GHGs is an urgent issue to alleviate the effect of climate change induced by GHGs emission on stress corrosion.

The present paper deals with global perspective on bilateral impact between livestock and GHGs emission and their mitigation options with biotechnological and physical approaches.

### **Possible control of indirect action of prebiotics, probiotics and secondary products on rumen methanogenesis**

The stoichiometric balance of VFA, CO<sub>2</sub> and CH<sub>4</sub> indicates that acetate and butyrate promote CH<sub>4</sub> production whereas propionate formation conserves H<sub>2</sub>, thereby reducing CH<sub>4</sub> production. Therefore, a strategy to mitigate ruminal CH<sub>4</sub> emission in indirect manner is to promote alternative metabolic pathway to dispose of the reducing power, competing with methanogenesis for H<sub>2</sub> uptake such as nitrate reduction (Takahashi et al., 1983; Takahashi and Young, 1991). Rumen manipulation with ionophores such as monensin has been reported to abate rumen methanogenesis (Mwenya et al., 2005). However, there is an increasing interest in exploiting secondary metabolites such as saponins and tannin (Pen et al., 2006; 2008, Jayanegara et al., 2012; 2014), prebiotics and probiotics as natural feed additives to solve problems in animal nutrition and livestock production as alternatives of the antibiotics (Takahashi, 2014). In general, tropical grasses for ruminants have lower digestibility compared to temperate grasses due to rich in lignin. However, tropical plants normally contain a high to medium content of secondary metabolites. It has been reported that many kinds of tropical feeds for ruminants have been suggested to mitigate rumen methane emission (Wanapat, et al., 2013).

Particular interest concerning bacteriocins which produced by lactic acid bacteria has increased recently. Bacteriocins, antimicrobial proteinaceous polymeric material substances, are ubiquitous in nature being produced by a variety of Gram-negative and Gram-positive bacteria, and typically narrow spectrum antibacterial substances under the control of plasmid. Nisin is produced by *Lactococcus lactis* subsp. *lactis* which is an amphiphilic peptide composed by 34 amino acids with two structural domains that are connected by a flexible hinge, and is classified into the group of lantibiotics. Nisin has a mode of action similar to ionophores, which show antimicrobial activity against a broad spectrum of Gram-positive bacteria and is widely used in the food industry as a safe and natural preservative. It is generally recognized as safe (GRAS) and given international acceptance in 1969 by the joint Food and Agriculture Organization/World Health Organization (FAO/WHO) Expert Committee on Food Additives.



**Table 3.** Effects of different levels of nisin on parameters of model for cumulative CH<sub>4</sub> or CO<sub>2</sub> production in mixed rumen cultures: CH<sub>4</sub> (ml) or CO<sub>2</sub> (ml) =  $a+b(1-c^{-et})^3$ , where  $a$  is the first CH<sub>4</sub> or CO<sub>2</sub> production (ml),  $b$  is the second CH<sub>4</sub> or CO<sub>2</sub> production (ml),  $c$  is CH<sub>4</sub> or CO<sub>2</sub> production rate (ml/min), and  $t$  is time (min).

	Nisin ( $\mu\text{mol } \ell^{-1}$ )						SEM	C vs. Nisin
	0	5	10	15	20	30		
<i>Cumulative CH<sub>4</sub> production</i>								
$a$	13.1	12.7	11.7	11.6	9.2	7.8	1.9	n.s.
$b$	203.67 <sub>a</sub>	180.84 <sup>a</sup> <sub>b</sub>	180.36 <sup>ab</sup>	155.88 <sup>b</sup>	159.55 <sup>ab</sup>	152.3	15.7	0.04
$a+b$	216.81 <sub>a</sub>	193.55 <sup>a</sup> <sub>b</sub>	192.04 <sup>ab</sup>	168.79 <sup>ab</sup>	167.52 <sup>ab</sup>	159.74 <sup>b</sup>	16.6	0.04
$c$	0.005	0.005	0.004	0.005	0.005	0.005	0.000	n.s.
$r$	0.991	0.991	0.994	0.992	0.995	0.995	0.002	n.s.
<i>Cumulative CO<sub>2</sub> production</i>								
$a$	62.1	63.3	60.8	67.0	54.1	57.2	5.5	n.s.
$b$	902.2	894.5	938.2	861.2	883.5	951.7	48.0	n.s.
$a+b$	964.3	957.8	999.1	928.2	937.6	1008.9	51.38	n.s.
$c$	0.005	0.005	0.005	0.005	0.005	0.005	0.000	n.s.
$r$	0.995	0.995	0.997	0.995	0.997	0.997	0.001	n.s.

n.s., Not significant

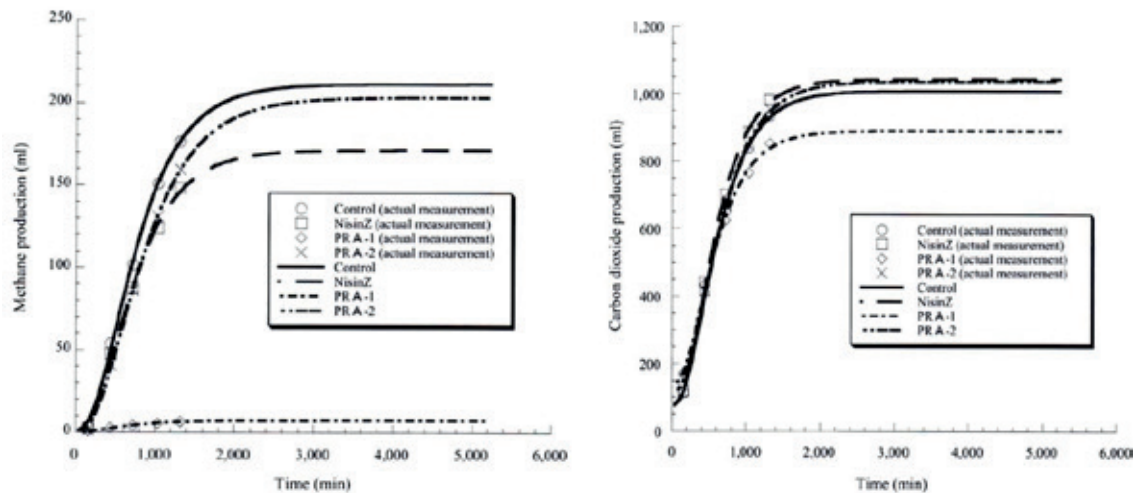
Orthogonal contrast, C vs. Nisin : comparison between control and nisin

$r$ , correlation coefficient. Values followed by the different superscript letters are significant different ( $p < 0.05$ ).

Recent works have indicated that *Lactococcus lactis* subsp. *lactis* produce nisin Z, which has been identified from Korean traditional fermented food “Kimchi” besides nisin A. They have similar antibacterial ability to mitigate methane emission (Sar, et al., 2005) (Table 3), to inhibit growth both of *Clostridium amoniphilum*, which is obligate amino-acid fermenting bacteria and lactic acid-producing ruminal Staphylococci and Enterococci. *Leuconostoc mesenteroides* ssp. *mesenteroides*, *Leuconostoc lactis* and *Lactococcus lactis* ssp. *lactis* were isolated from “Laban” which was a traditional fermented milk product in Yemen and determined the mitigating effect on in vitro rumen methane production. These strains isolated from Laban enhanced propionate production and decreased acetate/propionate ratio. In consequence, they reduced methane production remarkably. For *Leuconostoc mesenteroides* ssp. *mesenteroides*, in particular, the mitigating effect was amplified with GOS, which was degradable about 80% within 1 hour incubation in the artificial rumen fluid due to the stimulation of reduction reactions consuming metabolic hydrogen. However, direct involvement of bacteriocin or lower molecular substances produced by the strain on rumen methanogenesis remains to be elucidated.



Abatement of rumen methanogenesis by direct action of lactic acid bacteria as prebiotics producer



**Fig 2.** Effect of *Lactobacillus plantarum* TUA1490L culture on cumulative CH<sub>4</sub> and CO<sub>2</sub> production. PRA-1: *Lactobacillus plantarum* TUA1490L, PRA-2: *Leuconostoc citreum* JCM9698.

**Table 4.** Effect of a 24 h-old *Lactobacillus plantarum* TUA1490L culture and its cell-free supernatant on *in vitro* rumen methane and other fermentation variables after a 24 h fermentation period.

	Treatments			p-value
	Control (GY medium)	Culture	Cell-free supernatant	
Cumulative CH <sub>4</sub> (ml 24 h <sup>-1</sup> )	53.9 <sup>a</sup>	17.4 <sup>b</sup>	13.0 <sup>b</sup>	<0.001
Cumulative CO <sub>2</sub> (ml 24 h <sup>-1</sup> )	899	735	714	0.2
VFA(mM)				
Acetic	22.2 <sup>a</sup>	17.8 <sup>b</sup>	18.9 <sup>b</sup>	0.011
Propionic	12.1	12.0	12.6	0.512
Butyric	3.0 <sup>a</sup>	1.5 <sup>b</sup>	1.5 <sup>b</sup>	<0.001
Valeric	0.7 <sup>a</sup>	0.0 <sup>b</sup>	0.0 <sup>b</sup>	<0.001
Total VFA	38.0 <sup>a</sup>	31.3 <sup>b</sup>	33.0 <sup>b</sup>	0.02
A:P ratio	1.84 <sup>a</sup>	1.48 <sup>b</sup>	1.50 <sup>b</sup>	<0.001
pH	6.83	6.74	6.67	0.2
ORP (mV)	-356 <sup>a</sup>	-300 <sup>b</sup>	-283 <sup>b</sup>	0.004

SEM, standard error of the mean; ORP, oxidative reduction potential

<sup>a-c</sup> Means within a row with common superscripts do not differ ( $p < 0.05$ ).

Fig 2. shows suppressing effect of *Lactobacillus plantarum* TUA1490L culture on hydrogenotrophic rumen methanogenesis, whereas CO<sub>2</sub> production has not been affected by this culture. The protein resistant antimicrobials (PRA) maintained their antimicrobial effects after incubation with proteases, while nisin lost its activity. Therefore, the PRA was hypothesized to be a more sustained agent than nisin for the mitigation of rumen methane emission and a more direct effect of the agent on rumen methanogens (Table 2). *Lactobacillus plantarum* produces



bacteriocin from many foods. PRA was the antibacterial substance produced from a strain of *Lactobacillus plantarum* TUA1490L that was isolated from tomato in Japan. However, methane suppressing activity of PRA was not inactivated by protease treatment. Moreover, aeration cultivation is an essential process for activation of PRA to abate methanogenesis. Therefore, possible mechanism of PRA produced by *Lactobacillus plantarum* TUA1490L on rumen methane production might be assumed as resulting from the direct involvement of low molecule substance such as hydrogen peroxide due to the requirement of aeration for the preparation (Takahashi, 2013a).

Creation of renewable energy from anaerobic fermentation (biogas plant) of animal manures and the innovative reuse of the digested slurry to mitigate  $N_2O$

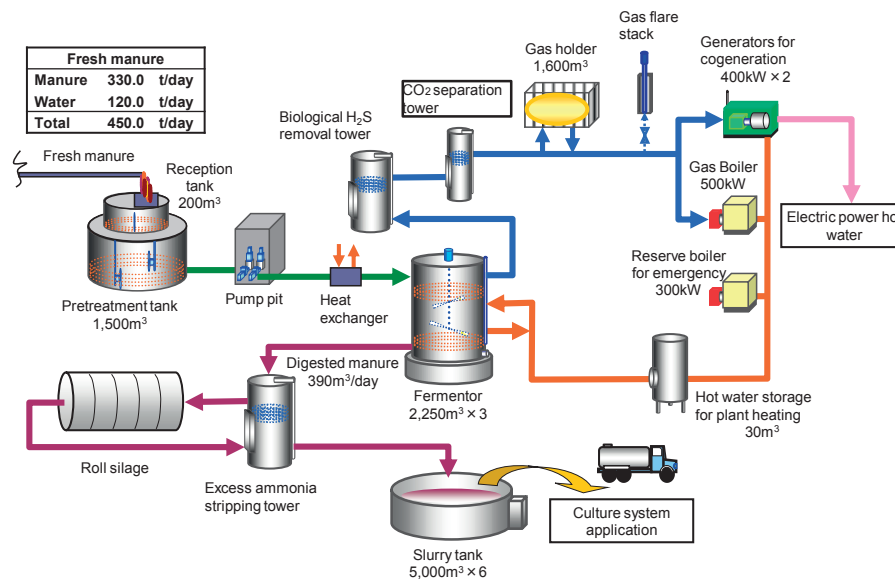


Fig. 3 Biogas recycling new biogas,  $CO_2$ ,  $H_2$ ,  $H_2O$ ,  $NO_x$  emission

The increased emissions of  $CH_4$  and  $N_2O$  from decomposing unmanaged and bio-based industrial wastes along with the expansion of human activities contribute to climate change as GHG. The biogas plant produce biogas including combustible  $CH_4$  as renewable energy using unused resources like animal manures, can provide fuel, heat and electricity (Umetsu et al., 2005), and minimize the impact on the environment thus reducing the amount of pollutants discharged. An advanced biogas plant system facilitated the ammonia stripping device from digested slurry to mitigate  $N_2O$  (Fig. 3) has been proposed and apply to nitrogen recycling-options such as liquid fertilizer, ammonolysis of cellulose biomass and  $NH_3$  fuel cell (Takahashi, 2013b). Additionally, hydroponic culture system using  $CO_2$  exhausted from the biogas system and the digested slurry as a culture solution will contribute to not only GHG mitigation, but saving water and nutrient resources for hydroponic culture system in semi-arid and arid areas (Fig. 4).

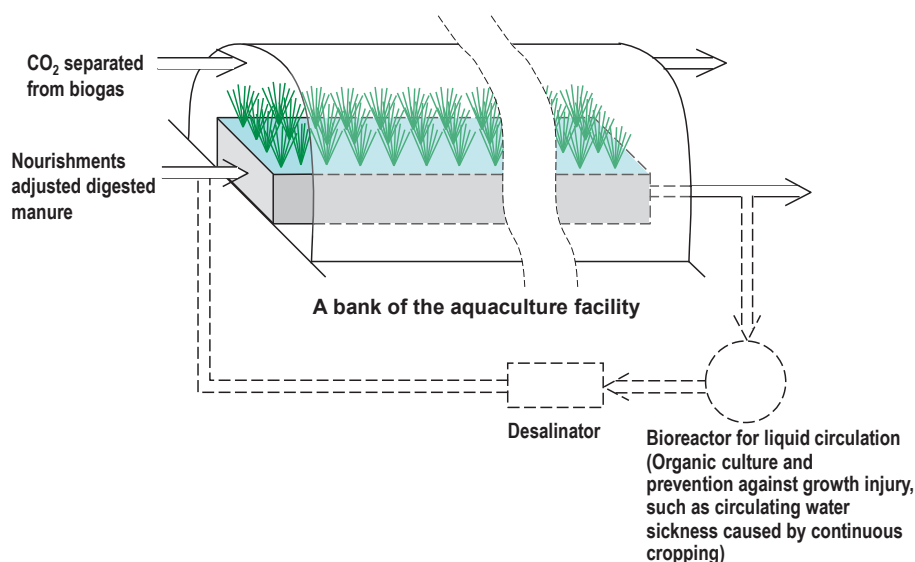


Fig. 4 A conceptual example flow of the nuticulture technology using the separated CO<sub>2</sub> from biogas and digested slurry

### Ammonia removal with heterotrophic denitrification to mitigate N<sub>2</sub>O emission from livestock wastewater

Improper management of livestock wastewater will cause eutrophication in the hydrosphere due to nitrate (NO<sub>3</sub><sup>-</sup>) and N<sub>2</sub>O emission in the atmosphere attributed to excess ammonia nitrogen (NH<sub>4</sub><sup>+</sup>-N). It is a common issue in Asian developing countries where abrupt expansions of population and urbanization have been progressing along with economic development. So far, most biotechnological approaches on ammonia removal from livestock wastewater have been implemented by aerobic nitrification and anaerobic denitrification using autotrophs (Carrera et al., 2003). However, the autotrophic bacteria are presumably unsuitable for livestock wastewater treatment because of high strength of ammonium and organic matters (Ruiz et al., 2003). Furthermore, the long retention for autotrophic nitrification has been designated due to slow proliferation rate of the bacteria (Richardson and Watmouth, 1999). In an attempt to seek ammonia removal ability of heterotrophic bacteria *Alcaligenes faecalis* strain No.4 was isolated from sewage sludge (Joo, et al. 2006). As an alternative way to mitigate N<sub>2</sub>O and NO<sub>3</sub><sup>-</sup> emission derived from animal agriculture, heterotrophic nitrification and aerobically denitrifying effect of *A. faecalis* strain No.4 on excess ammonia nitrogen was evaluated in wastewater collected from the piggery in suburb of Shanghai, China according to the procedure reported by Joo, et al., (2006). Fig. 5 shows progressive removal of ammonia from piggery wastewater using *A. faecalis* strain No. 4 was confirmed under proper controlling the C/N ratio and pH.

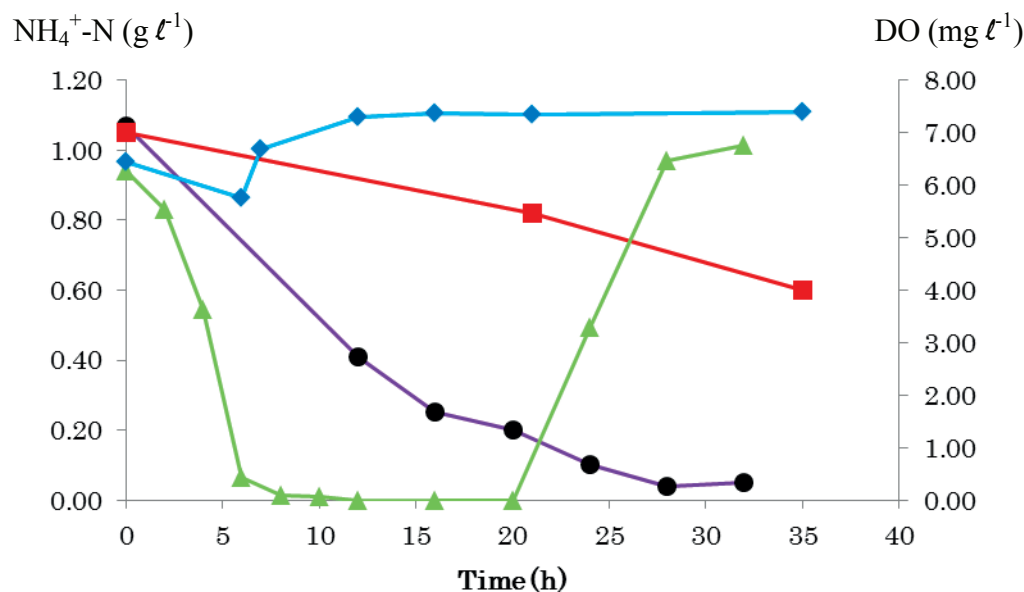


Fig 5. Ammonia removal using *Alcaligenes faecalis* No.4 with heterotrophic nitrification and aerobic denitrification, NH<sub>4</sub><sup>+</sup>-N: Ammonia nitrogen, DO: dissolved oxygen; Circle: NH<sub>4</sub><sup>+</sup>-N (*A. faecalis*), square: NH<sub>4</sub><sup>+</sup>-N (control), triangle: DO (*A. faecalis*), rhombus: DO (control)

### Heat stress and oxidative stress induced by hot environment and the mitigating effects of mechanical stimulating brushes (Rumenfibe)

Global warming, especially, critical or subcritical hot summer attributed to the abrupt increase in GHG emission has adversely affected the performances of livestock productivities due to the susceptibility to heat stress. Relationship between heat stress and oxidative stress has been reported in the various livestock species including poultry. Heat stress suffered hen has induced oxidative stress (Lin et al., 2008). Swine exposed hot environment has accelerated oxidative stress (Katsumata et al., 2004). Moreover, for primiparous cow exposed hot environment, oxidative stress markers, ascorbic acid and sulfhydryl (SH) residue concentration in plasma have declined due to oxidative stress (Tanaka et al., 2007). Thus, oxidative stress can be assumed part of the heat stress. Therefore, the mitigation of heat stress might be achieved by the abatement of oxidative stress.

**Table 5.** Effect of Rumenfibe on the oxidative markers in the plasma of pasture-based lactating cows in the Australian (Tasmania) spring and summer

Plasma	Control	Rumenfibe	<i>p</i> -value
dROMs <sup>1</sup> (Carr. U)	107	118	0.183
BAP <sup>2</sup> (μM)	4382 <sup>a</sup>	4686 <sup>b</sup>	0.036
OSI <sup>3</sup> (arbitrary units)	2.5	2.5	0.551
Ceruloplasmin (g/L)	0.17	0.19	0.145
SHp <sup>4</sup> (μM)	326	347	0.368
GSH <sup>5</sup> (μM)	3.4	3.6	0.572
AOPP <sup>6</sup> (μM Chloramine. T equi.)	36	37	0.705





<sup>ab</sup>Means within a row with common superscripts do not differ ( $p < 0.05$ ).

<sup>1</sup>dROMs: reactive oxygen metabolites; <sup>2</sup>BAP: biological antioxidant potential;

<sup>3</sup>OSI: oxidative stress index (dROMs/BAP x 100); <sup>4</sup>SHp: thiol groups;

<sup>5</sup>GSH: reduced glutathione; <sup>6</sup>AOPP: Advanced oxidation products.

Accordingly, mitigating effect of rumen mechanical stimulating brush (Rumenfibe, Meiwa Sangyo, Kyoto) on oxidative stress in pasture-based lactating dairy cow has been investigated in the Australian spring and summer. Table 3 shows plasma contents of oxidative stress markers (Golder, et al., 2016). Only BAP concentration in Rumenfibe treated cows was significantly ( $p < 0.05$ ) higher than that of control. Furthermore, the relatively higher values in plasma BAP concentrations were determined even in control cows than those reported in dairy cows (Celi and Raadsma, 2010; Golder et al., 2013; Talukder et al., 2014). However, no any significant improvements of productive performance and rumen parameters were observed by Rumenfibe administration, because the meteorological measurement suggested inadequate hot-environmental temperature and temperature-humidity index to induce heat stress due to the cool summer.

In consequence, the increased plasma BAP concentration in Rumenfibe treated cows may support the hypothesis that oxidative balance will be improved by Rumenfibe administration.

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## Feeding Strategies on Farms to Improve Livestock Productivity and Reduce Methane Production

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### Abstract

Nutrition and feeding methods are highly accounted for the successful ruminant productivity and the quality of the animal products produced. Roughage and concentrate sources both locally available and introduced are complementarily essential attributing to the efficient production systems. Furthermore, “Feeding the microorganisms, feeding the ruminants” is of great concern since the rumen is the fermentation vat in producing the fermentation end-products such as volatile fatty acid (VFA),  $\text{NH}_3\text{-N}$  and microbial protein useful for ruminant hosts. Whilst, the enteric green-house gas including  $\text{CH}_4$  and  $\text{CO}_2$  are being produced and eructed. The anaerobic fermentation process (Embden-Meyerhof-Parnas Pathway or glycolysis) is closely associated with rumen microbiome and fermentation end-products for productive functions. Feed resources, feed processing and feeding technology are essentially key factors to the efficient and successful ruminant production especially in the tropics. Diversity and distribution of roughage resources both quantity and quality will impact on the performance of livestock. Numerous agricultural crop-residues such as rice straw can be treated with urea (U) and lime (L) (1.5+1.5% U-lime) to enrich its nutritive value. Furthermore, fodder trees and shrubs including *Leucaena leucocephala* and *Flemingia macrophylla*, as well as whole cassava crop can be ensilaged (cassava top silage) to produce high quality protein roughages for ruminant feeding. Feeding of these roughage can result in efficient rumen fermentation and improve meat, milk yield, and milk quality, whilst rumen methane was reduced. These feeding interventions can be employed on farms for establishment (E), development (D), utilization (U), and sustainability (S) (EDU-S) of livestock production. Agricultural production system including animal production has been shown to impact on global warming especially from methane enteric fermentation of ruminants. Many approaches have been reported to mitigate rumen methane production; however, dietary plants containing plant secondary compounds (condensed tannins, saponins) have impacted on rumen microorganisms, hence can reduce rumen methane production. Nevertheless practical feeding implementations on-farms need to be employed and expanded among farmers/producers, not only to reduce global warming but for the economical advantage of the animal production and improvement of livelihoods, as well as conserving the environmentally-friendly atmosphere.

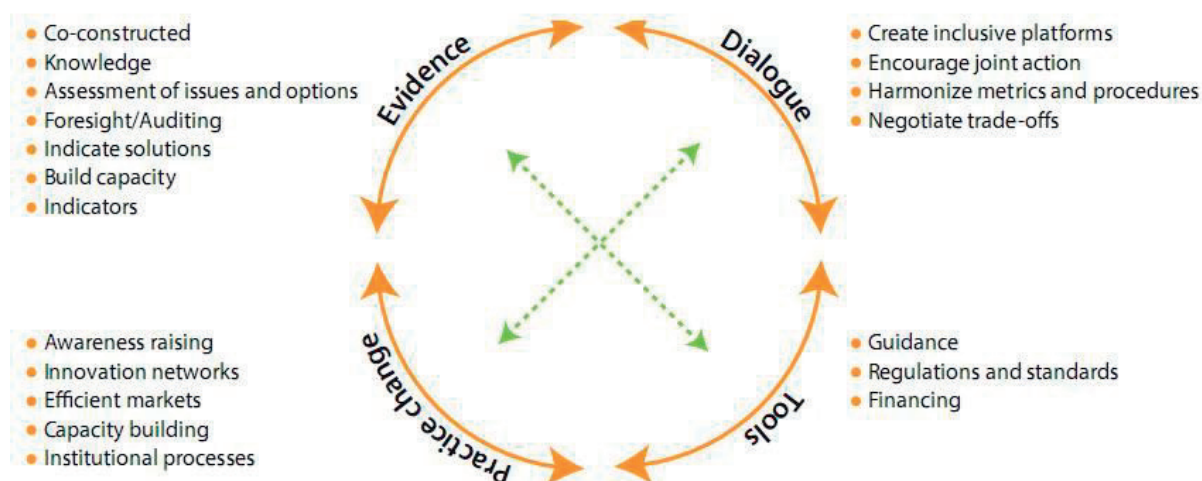
**Keywords:** livestock, sustainable production, feed resources, on-farm interventions, methane



## Introduction

Thornton (2010) stated the needs to develop breeds, nutrition and animal health to increase potential animal production efficiency especially in the developing countries. Rapid population growth will continue to be an important impediment to achieving improvements in food security. While urbanization and income growth rate (2.1% per capita) will further impact on the need of livestock products consumption. As Steinfeld et al. (2006) indicated that in the year 2050 the annual consumption per capita of meat and milk will be 44, 78 kg with total consumption of meat and milk 326, 585 Mt., for developing countries, while 94, 216 kg with total consumption of 126, 295 Mt., for developed countries, remarkable increase in developing countries. Smith et al. (2013) have reiterated the importance the role livestock production beyond the supply of milk, meat and eggs. Livestock can enhance food and nutrition security and providing income to support the livelihood and well-being of the household formers. The challenges are those how to manage the trade-offs to enable livestock's positive impacts to be achievable while minimizing the negative issues and to maintain environmentally-friendly. Godber and Wall (2014) additionally reiterated the importance of livestock production as an important contributor to sustainable food security, as the animal products account for one-third of global human protein consumption. Livestock-based food security will be more vulnerable to impacts of climate change in addition to prevailing lacks of technical support and economic, as well as other supporting infra-structure and available markets.

FAO (2014) has illustrated action plans to ensure livestock sustainability into practices encompassing the broad areas (Figure 1).



**Figure 1.** Operating Sustainability: Four broad areas of action (FAO, 2014)

Wanapat et al. (2015) reported that animal agriculture has been an important component in the integrated farming systems in developing countries. It serves in a paramount diversified role in producing animal protein food, draft power, farm manure as well as ensuring social status-quo and enriching livelihood. Ruminants are importantly contributable to the well-being and the livelihood of the global population. Ruminant production systems can vary from subsistence to intensive type of farming depending on locality, resource availability,

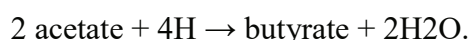
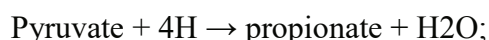
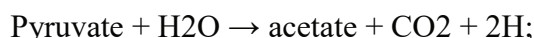
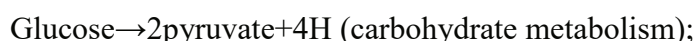


infrastructure accessibility, food demand and market potentials. The growing demand for sustainable animal production is compelling to researchers exploring the potential approaches to reduce greenhouse gases (GHG) emissions from livestock. Global warming has been an issue of concern and importance for all especially those engaged in animal agriculture. Methane (CH<sub>4</sub>) is one of the major GHG accounted for at least 14% of the total GHG with a global warming potential 25-fold of carbon dioxide and a 12-year atmospheric lifetime. Agricultural sector has a contribution of 50 to 60% methane emission and ruminants are the major source of methane contribution (15 to 33%). Methane emission by enteric fermentation of ruminants represents a loss of energy intake (5 to 15% of total) and is produced by methanogens (archae) as a result of fermentation end-products. Ruminants' digestive fermentation results in fermentation end-products of volatile fatty acids (VFA), microbial protein and methane production in the rumen. While, Wanapat et al. (2013) reported that the availability of local feed resources in various seasons can contribute as essential sources of carbohydrate and protein which significantly impact rumen fermentation and the subsequent productivity of the ruminant.

### **The rumen as a fermentation vat and factors affecting its function**

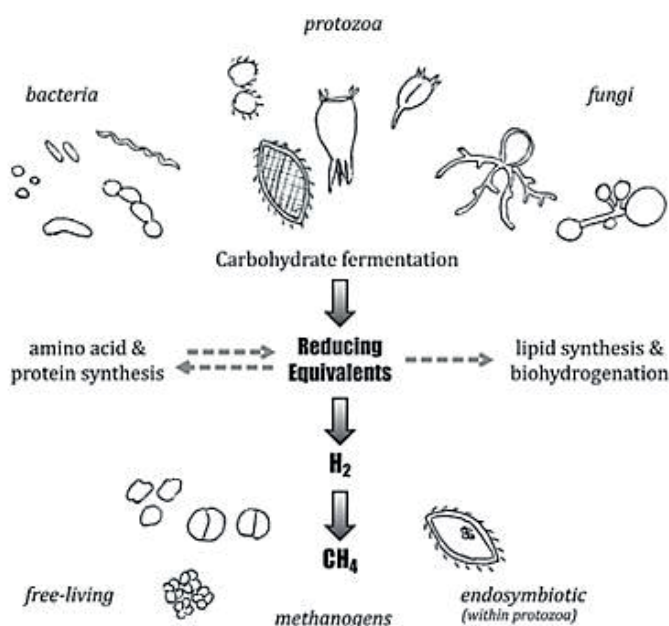
The rumen has an important role and function in preparing fermentation end-products for biosynthetic processes of ruminants. It is therefore essential that the rumen is healthy and is able to establish an optimum ecology in order to perform well with regard to rumen microorganisms (bacteria, protozoa and fungi), pH, substrates (e.g. roughage, energy, effective fibre etc), fermentation end-products (NH<sub>3</sub>-N, VFAs), and microbial synthesis VFAs, particularly propionate (C<sub>3</sub>), acetate (C<sub>2</sub>) and butyrate (C<sub>4</sub>), are major sources of energy, and allow the synthesis of glucogenic and lipogenic compounds, while NH<sub>3</sub>-N is an essential source of nitrogen for microbial protein synthesis.

Knapp et al. (2014) (Figure 2) stated that ruminant animals and microbes have evolved together, filling a niche based on the conversion of complex plant carbohydrates to energy that is beneficial to both the host animal and the microbial symbionts. The microbes include bacteria, protozoa, fungi, and archaea (Figure 2). The rumen ecosystem is an anaerobic environment, in which the degradation of plant material occurs in a very short time frame compared with other anaerobic ecosystems such as wetlands and estuaries, and the fermentation products are different. Some of the microbial species have coevolved with ruminants and hindgut-fermenting mammals and do not exist in any other environment (e.g., rumen protozoa). Also, the methanogens of ruminants and other mammalian herbivores are distinct from methanogens in other environments.





The metabolic hydrogen is converted to H<sub>2</sub> by hydrogenase-expressing bacterial species, and the H<sub>2</sub> converted to CH<sub>4</sub> by Archaea in the combined reaction:



**Figure 2.** Rumen microorganisms, including bacteria, protozoa, and fungi, ferment carbohydrates to obtain energy and generate significant amounts of reducing equivalents (FADH<sub>2</sub>, NADH, and others) in the process and VFA (not shown) and H<sub>2</sub> as end products. Methanogens, both free living and endosymbionts inside protozoa, convert H<sub>2</sub> to CH<sub>4</sub>. A small amount of reducing equivalents are utilized in lipid synthesis and FA biohydrogenation. Synthesis of amino acids can result in production or utilization of reducing equivalents, but the net amount is small. Protein synthesis utilizes reducing equivalents. Elevated concentrations of H<sub>2</sub> inhibit carbohydrate fermentation, providing a negative feedback mechanism. Organisms are not drawn to scale [after Czerkawski (1986)].(Cited by Knapp et al., 2014)

### Methane mitigation strategies

An abundance of CH<sub>4</sub> mitigation strategies have been studied and they can be classified into 3 broad categories: (Knapp et al., 2014)

1. Feeds, feeding management, and nutrition: feeding good-quality feeds can increase animal productivity and feed efficiency. Certain feeds can enhance propionate or decrease acetate production, decreasing H<sub>2</sub> that would be converted to CH<sub>4</sub>.

2. Rumen modifiers: feeding specific substances that directly or indirectly inhibit methanogenesis or using biological control (defaunation, bacteriocins, bacteriophages, and immunization) directed at reducing methanogens.

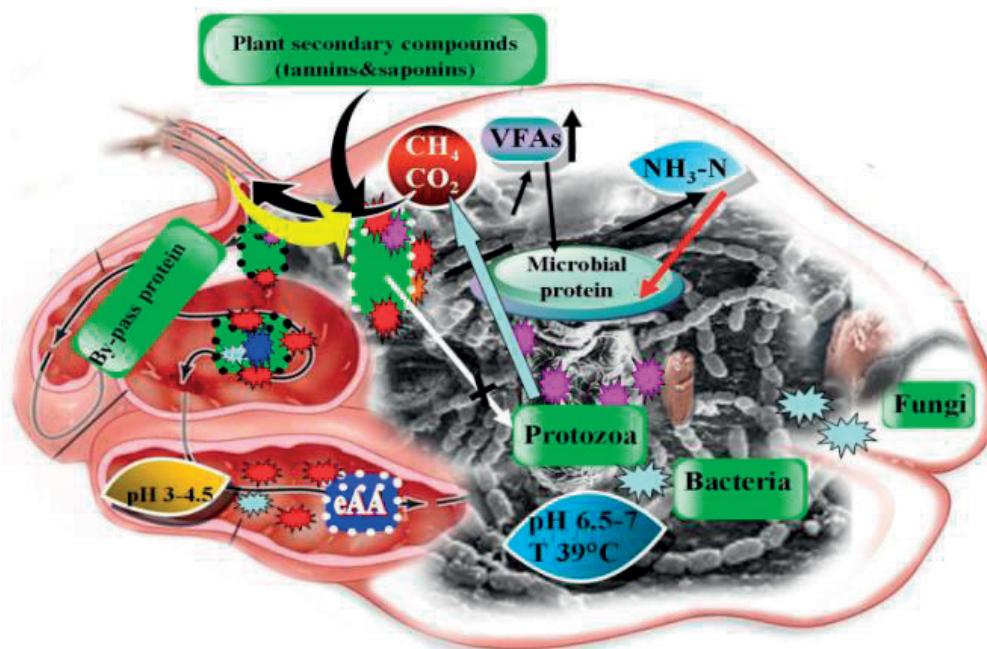
3. Increasing animal production through genetics and other management approaches: improving nutrient utilization for productive purposes to dilute out maintenance on an individual animal or a herd basis, increasing feed efficiency and decreasing CH<sub>4</sub> per unit of product (meat or milk). More details of rumen manipulation approaches can be found in Hook et al. (2010) (Table 1) and Wanapat (2000)



Methanogenesis is an important part of the energy metabolism in ruminants and measuring its production is critical in understanding ruminant livestock productivity. CH<sub>4</sub> emissions data can be combined with information relating to the rumen microbiome, metabolic processes, and digestion to provide invaluable insights into the efficiency of livestock systems. However, to date, improvements in livestock efficiency have mostly been made through advances in gut microbiology, nutrition, genetics, and health of the host animal and not through the inclusion of CH<sub>4</sub> emission information. Hill et al. (2016) has outlined and presented details relating to measuring rumen methane methods with advantages and disadvantages, as well as future research.

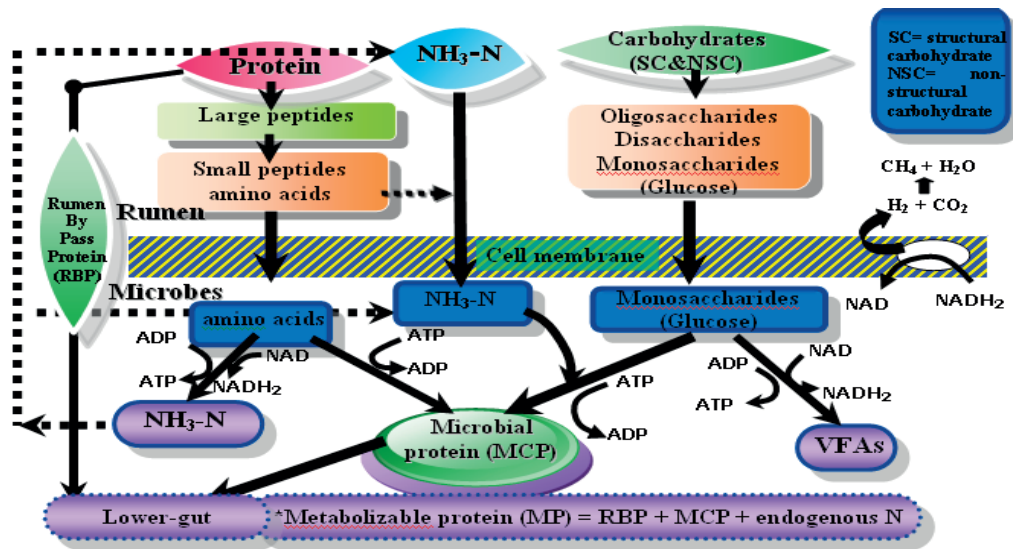
### Dietary manipulation in reducing rumen methane

There are several factors which can have great impacts on rumen methane production namely level of intake, frequency of feeding, type of roughages, ratio of roughage to concentrate, type and concentration of non-structural carbohydrates etc. All of these factors can play important roles on rumen pH, volatile fatty acids production, ammonia nitrogen and microbial protein synthesis and the consequences on rumen methanogens and methane production, protozoa and cellulolytic bacteria. Boadi et al (2004) and Hook et al. (2010) have proposed numerous potential ways as how to mitigate the rumen methane production. The main approaches are as follows: improving animal productivity, nutritional and management strategies (type of carbohydrates, level of intake, forages type and quality, feeding frequency, roughage treatment/processing, grazing management,), management of rumen fermentation (propionate enhancers, use of fats and essential oils, use of plant secondary compounds: condensed tannins and saponins etc (Table 1).



**Figure 3.** Role of plant secondary compounds (condensed tannins & saponins) on rumen fermentation process (Wanapat et al., 2012 )





**Figure 4.** Energy and protein metabolism in the rumen microbial protein synthesis and methane production. (Wanapat, 2012; modified from Nocek & Russell, 1988 ).

The profound example of local feed resources is cassava (*Manihot exculenta*, Crantz). As reported by Wanapat (2003), Wanapat and Kang (2015) that cassava chip, cassava hay and be used remarkably well for ruminant feeding providing as energy and protein sources, respectively. Furthermore, fermenting cassava root or chip with yeast could produce yeast-fermented cassava chip protein (YEFECAP) (Table 2) which can be used to replace soybean meal in ruminants (Boonnop et al., 2009; Boonnop et al., 2010; Polyorach et al., 2013; Wanapat and Kang, 2013b). Simple procedure of yeast fermentation with cassava (root, chip, pulp) has been demonstrated. Remarkable findings have been reported regarding the use of YEFECAP in ruminants (Table 2).

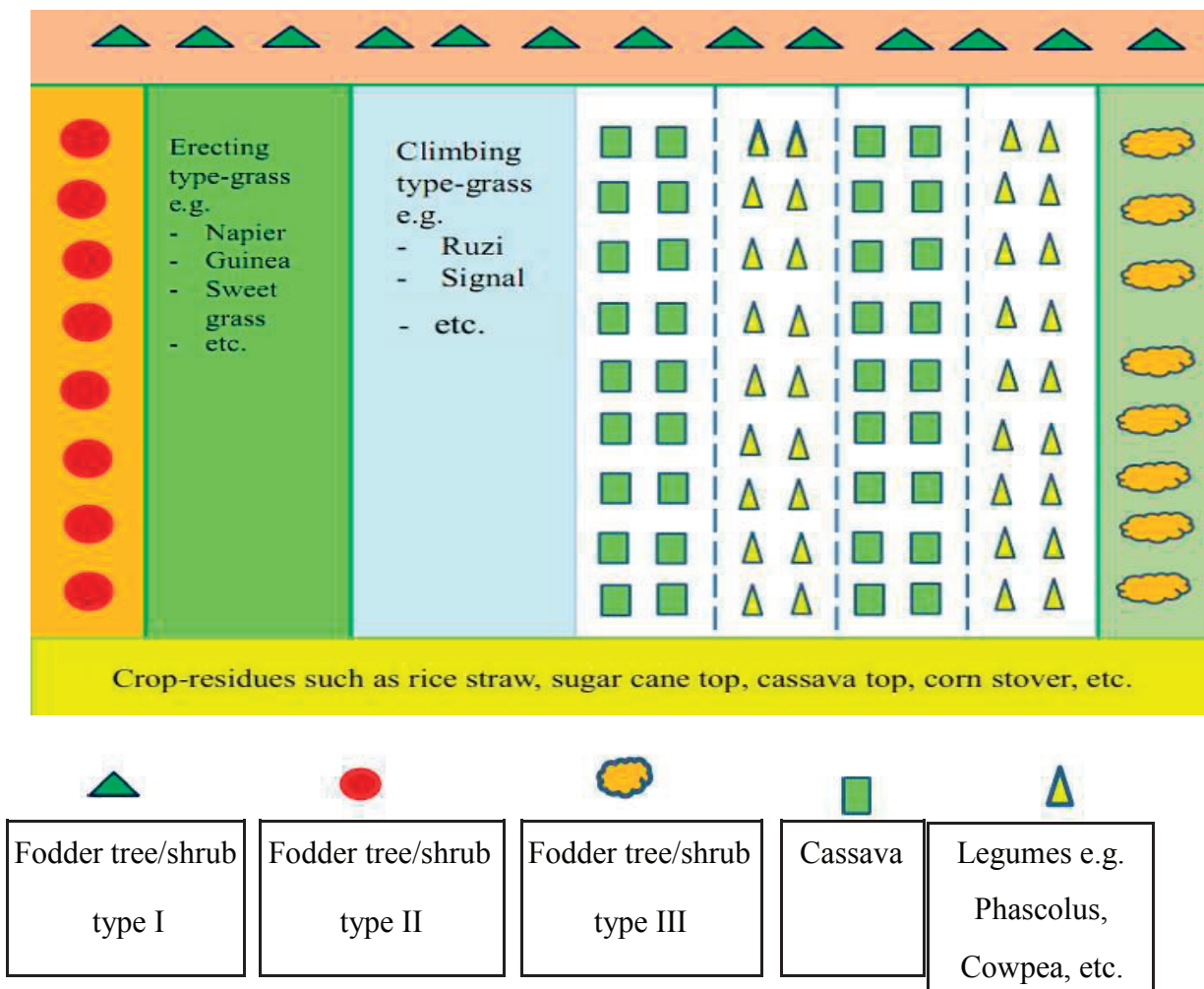
The use of fodder trees and shrubs has been developed through the process of pelleting; *Leucaena leucocephala* leaf pellets (LLP), mulberry leaf pellets (MUP) and mangosteen peel and/or garlic pellets, can be used as good sources of protein to supplement ruminant feeding (Table 10). Apart from producing volatile fatty acids and microbial proteins, greenhouse gases such as methane are also produced in the rumen. Several methods have been used to reduce rumen methane. The use banana flower powder (BAFLOP) has been used effectively in rumen manipulation (Kang et al. 2015) (Table 9). However, among many approaches, nutritional manipulation using feed formulation and feeding management, especially the use of plant extracts or plants containing secondary compounds (condensed tannins and saponins) and plant oils, has been reported. This approach could help to decrease rumen protozoa and methanogens and thus mitigate the production of methane. At present, more research concerning this burning issue the role of livestock in global warming - warrants undertaking further research with regard to economic viability and practical feasibility.



To achieve productive, profitable, sustainable and environmentally friendly in the tropics the following recommendations are highly recommended;

- E = Establishment of feed resources
- D = Development of feeding system
- U = Utilization of feeds including feeding method and processing
- S = Sustainability of the livestock production system

The proposed feeding system for ruminants, food-feed-system (FFS) to produce a year round feeding calendar, as well as to enrich the environment on-farm is illustrated in Figure 5 Under this system, both grass types (climbing and erecting) can be used to maximize the biomass by grazing and/or cut-and-carry. Root from cassava can be used as carbohydrate source while feed and whole top can be dried as hay as protein (Wanapat et al. 2013). Additionally, fodder trees/shrubs can be harvested intervally to use freshly as supplements and/or hay/silage making (Tables 5,6).



**Figure 5.** The proposed feeding system for ruminants, food-feed system (FFS) for the sustainable ruminant feeding system for smallholder farmers in the tropics.

**Table 1.** Methane abatement strategies, mechanism of abatement, and considerations for use.

Methane abatement strategy	Mechanism of abatement activity	Considerations when selecting abatement strategy
Dietary composition		
Increase hemicellulose /starch	Increased passage rate; greater proportion propionate versus acetate; reduced ruminal pH	Shift methanogenesis to hind gut or manure, risk of subacute ruminal acidosis (SARA)
Decrease cell wall components		
Grinding		
Lipids		
Fatty acids	Inhibition of methanogens and protozoa; greater proportion propionate versus acetate; biohydrogenation	Effect on palatability, intake, performance, and milk components; varies with diet and ruminant species; long-term studies needed
Oils		
Seeds		
Tallow		
Defaunation		
Chemical	Removes associated methanogens; less hydrogen for methanogenesis	Adaptation of microbiota may occur; varies with diet; maintenance of defaunated animals
Feed additives		
Methanogen Vaccine	Host immune response to methanogens	Vaccine targets; diet and host geographical location differences
Monensin	Inhibits protozoa and gram positive bacteria; lack of substrate for methanogenesis	Adaptation of microbiota may occur; varies with diet and animal; banned in the EU
Plant Compounds		
Condensed tannins	Antimicrobial activity; reduced hydrogen availability	Optimum dosage unknown; more <i>in vivo</i> research needed; long-term studies needed; may affect digestibility; residues unknown
Saponins		
Essential oils		
Organic Acids		
Fumarate	Hydrogen sink, greater proportion propionate versus acetate	Varies with diet; more <i>in vivo</i> research needed; long-term studies needed; may affect digestibility
Malate		

Source: Hook et al. (2010)



**Table 2.** Effect of YEFECAP as a protein source in concentrate mixtures on milk production, milk composition and economic return

Items	Treatments				SEM	Contrasts		
	T1	T2	T3	T4		L	Q	C
<b>Production</b>								
Milk yield, kg/d	13.5	14.0	14.5	15.0	0.27	**	Ns	ns
3.5% FCM <sup>1</sup> , kg/d	13.7	14.7	15.9	17.1	0.49	**	Ns	ns
<b>Milk composition, %</b>								
Protein	4.0	4.1	4.5	4.7	0.17	**	Ns	ns
Fat	3.2	3.3	3.4	3.5	0.06	**	Ns	ns
Lactose	4.5	4.6	4.6	4.7	0.07	ns	Ns	ns
Total solids	12.3	12.7	12.8	13.0	0.78	ns	Ns	ns
Milk urea N, mg/dl	14.8	12.5	12.3	12.0	0.58	*	Ns	ns
<b>Economic return, \$US/hd/d</b>								
Feed cost	2.5	2.6	2.6	2.7	0.14	ns	Ns	ns
Milk sale	9.5	9.8	10.2	10.5	0.19	**	Ns	ns
Profit	7.0	7.2	7.6	7.8	0.16	**	Ns	Ns

T1, T2, T3 and T4 are YEFECAP replacement of SBM at 0, 33, 67 and 100%, respectively

Source: Wanapat et al. (2013b)

Current research findings on using local feed resources such as Ipil ipil (*Leucaena leucocephala*), cassava hay and cassava top silage have revealed promising results in ruminant feeding improvements on rumen fermentation end products, nutrient digestibilities, microbial protein synthesis and methane reduction have been obtained. (Ampapon and Wanapat, 2016; Nyuyen et al., 2017; Wanapat et al., 2013) (Table 3,4,5,6,7,8).

**Table 3.** Effect of various treated rice straw on milk production and milk composition of lactating dairy cows.

Items	Rice straw			SEM
	Untreated	5.5% U	2%U+2%Ca(OH) <sub>2</sub>	
Milk yield, kg/day	10.8	11.1	12.1	2.83
3.5% FCM, kg/day	11.3	12.3	12.9	3.64
<b>Milk composition, %</b>				
Protein	2.8 <sup>a</sup>	3.3 <sup>b</sup>	3.4 <sup>b</sup>	0.34
Fat	3.8 <sup>a</sup>	4.1 <sup>b</sup>	4.3 <sup>c</sup>	0.02
Lactose	4.8	5.0	5.1	0.88
Solids-not-fat	8.3	8.7	8.5	0.98
Total solids	12.1	12.5	12.4	0.86

<sup>a,b,c</sup> Means in the same row with different superscripts differ ( $P < 0.05$ ).

Source: Wanapat et al. (2009)



**Table 4.** Effects of *Leucaena* silage levels feeding on rumen ecology and fermentation end products in dairy steers

Items	RST	RLS30	RLS60	LS	SEM	Significant level
Temperature (°C)	38.9	39.3	39.1	38.6	0.93	ns
NH <sub>3</sub> -N (mg/dl)	7.4 <sup>a</sup>	16.2 <sup>b</sup>	22.4 <sup>c</sup>	27.9 <sup>d</sup>	0.27	**
BUN (mg/dl)	5.75 <sup>a</sup>	15.02 <sup>b</sup>	17.9 <sup>c</sup>	21.75 <sup>d</sup>	0.86	**
Ruminal pH	6.7	6.7	6.7	6.6	0.3	ns
Total VFA (mmol/l)	79.5 <sup>a</sup>	97.1 <sup>b</sup>	101.3 <sup>c</sup>	88.2 <sup>d</sup>	1.2	*
VFA (mol/100 mol)						
Acetate (C <sub>2</sub> )	74.6 <sup>a</sup>	70.6 <sup>b</sup>	66.5 <sup>c</sup>	66.2 <sup>c</sup>	0.76	*
Propionate (C <sub>3</sub> )	15.9 <sup>a</sup>	19.6 <sup>b</sup>	24.9 <sup>c</sup>	25.2 <sup>c</sup>	0.5	*
Butyrate (C <sub>4</sub> )	9.8	9.7	8.6	8.5	0.92	ns
C <sub>2</sub> /C <sub>3</sub>	4.8 <sup>a</sup>	3.6 <sup>b</sup>	2.7 <sup>c</sup>	2.6 <sup>c</sup>	0.28	*
CH <sub>4</sub> production (mol/ 100 mol)	33.2 <sup>a</sup>	30.3 <sup>b</sup>	26.5 <sup>c</sup>	26.3 <sup>c</sup>	0.39	*

Means in the same row with different letters differ (\* $P < 0.05$ , \*\* $P < 0.01$ )

ns nonsignificantly different, SEM standard error of the means, NH<sub>3</sub>-N ammonia nitrogen, BUN blood urea nitrogen, VFA volatile fatty acid, CH<sub>4</sub> methane, RST rice straw, RLS30 70 % rice straw + 30 % *Leucaena* silage, RLS60 40 % rice straw + 60 % *Leucaena* silage, LS *Leucaena* silage

Source: Giang et al. (2016)

**Table 5.** Effect of cassava top silage (CTS) on rumen ecology and fermentation in dairy steers

Item	% CTS				SEM	<i>P</i> -value
	0	30	60	100		
Temperature, °C	38.5	38.7	38.5	38.6	0.10	0.44
pH	6.6	6.6	6.7	6.6	0.07	0.69
Roll-tube technique, CFU/ml						
Total viable bacteria, ×10 <sup>11</sup>	12.5	16.2	18.9	17.1	1.9	0.22
Cellulolytic, ×10 <sup>9</sup>	3.8	4.2	6.3	6.4	2.1	0.75
Amylolytic, ×10 <sup>7</sup>	1.1	2.1	2.9	2.4	0.5	0.22
Proteolytic, ×10 <sup>7</sup>	3.3	3.5	5.9	6.5	1.2	0.21
Total direct count, cell/ml						
Protozoa, ×10 <sup>5</sup>	5.0 <sup>c</sup>	4.2 <sup>b</sup>	3.8 <sup>bc</sup>	3.6 <sup>a</sup>	0.07	0.01
Total VFA, mmol/l	116.8	117.6	115.6	116.9	0.59	0.35
VFA, mol/100mol						
Acetate, %	68.1 <sup>c</sup>	63.7 <sup>b</sup>	62.4 <sup>a</sup>	62.3 <sup>a</sup>	0.36	0.01
Propionate, %	21.3 <sup>a</sup>	26.8 <sup>b</sup>	28.6 <sup>c</sup>	28.8 <sup>c</sup>	0.39	0.01
Butyrate, %	10.7 <sup>b</sup>	9.5 <sup>a</sup>	8.9 <sup>a</sup>	8.8 <sup>a</sup>	0.31	0.01
C <sub>2</sub> /C <sub>3</sub>	3.1 <sup>b</sup>	3.2 <sup>a</sup>	2.2 <sup>a</sup>	2.1 <sup>a</sup>	0.14	0.01
CH <sub>4</sub> production <sup>A</sup> , mol/100mol	29.1 <sup>c</sup>	25.2 <sup>b</sup>	23.8 <sup>a</sup>	23.7 <sup>a</sup>	0.67	0.01
NH <sub>3</sub> -N, mg%	9.2 <sup>a</sup>	19.6 <sup>ab</sup>	24.1 <sup>c</sup>	29.8 <sup>c</sup>	3.00	0.01
BUN, mg%	5.0 <sup>a</sup>	12.8 <sup>ab</sup>	19.3 <sup>bc</sup>	26.0 <sup>c</sup>	3.14	0.01

SEM = standard error of the mean, CFU = colony-forming unit.

<sup>a, b, c</sup>. Means in the same row with different superscripts differ ( $P < 0.05$ ) and ( $P < 0.01$ ) <sup>A</sup> Calculated according to Moss et al. (2000); CH<sub>4</sub> production = 0.45(C<sub>2</sub>) - 0.27(C<sub>3</sub>) + 0.4(C<sub>4</sub>).

Source: Viennasay et al. (2017)

**Table 6.** Effect of cassava top silage on milk yield and composition in lactating dairy cows

Items	Cassava top silage (kg/day of DM)				SEM	P-value
	0	0.75	1.50	2.25		
<b>Production</b>						
Milk yield, kg/day	12.7 <sup>a</sup>	13.2 <sup>a</sup>	13.3 <sup>a</sup>	14.0 <sup>b</sup>	0.93	0.04
3.5% FCM, kg/day <sup>1</sup>	14.6 <sup>a</sup>	14.9 <sup>a</sup>	16.1 <sup>b</sup>	17.2 <sup>c</sup>	1.01	0.03
<b>Milk composition, %</b>						
Fat	4.4	4.3	4.8	5.0	0.35	0.58
Protein	3.2	3.5	3.6	3.8	0.39	0.34
Lactose	4.4	4.4	4.3	4.5	0.14	0.75
Solids-not-fat	9.3	9.3	9.2	9.0	0.55	0.97
Total solids	14.1	14.0	14.2	13.9	0.58	0.45

<sup>a,b,c</sup> Means in the same row with different superscripts differ ( $P < 0.05$ ). <sup>1</sup>3.5% FCM (fat collected milk) =  $0.432 \text{ (kg of milk/d)} + 16.23 \text{ (kg of fat)}$ . Source: Wanapat et al. (2017)

**Table 7.** Effect of legume foliage supplementation on fermentation characteristics, blood urea nitrogen, and methane production in dairy steers

Items	Control	CH	FMH	CH+FMH	SEM	P-value
Temperature (°C)	38.8	38.9	38.6	38.9	0.05	0.26
NH <sub>3</sub> -N, mg%	8.51 <sup>a</sup>	9.06 <sup>b</sup>	9.28 <sup>b</sup>	9.12 <sup>b</sup>	0.20	0.04
BUN, mg%	10.2	9.5	10.8	10.4	0.42	0.86
Ruminal pH	6.8	6.8	6.5	6.6	0.16	0.10
Total VFA, mmol/l	121.4	124.3	120.5	126.7	2.35	0.49
Acetate, %	68.7 <sup>a</sup>	65.1 <sup>b</sup>	64.8 <sup>ab</sup>	62.2 <sup>b</sup>	1.35	0.04
Propionate, %	20.1 <sup>a</sup>	25.4 <sup>b</sup>	24.3 <sup>b</sup>	27.6 <sup>b</sup>	1.03	0.03
Butyrate, %	8.7	8.1	8.9	8.4	0.79	0.87
C <sub>2</sub> /C <sub>3</sub>	3.4 <sup>a</sup>	2.6 <sup>b</sup>	2.7 <sup>b</sup>	2.3 <sup>b</sup>	0.12	0.05
CH <sub>4</sub> production <sup>d</sup> , mol/ 100 mol	22.0 <sup>a</sup>	19.1 <sup>b</sup>	18.8 <sup>b</sup>	17.0 <sup>b</sup>	0.29	0.02

<sup>a, b, c</sup> Means in the same row with different superscripts differed ( $P < 0.05$ ) <sup>d</sup>Calculated according to Moss et al. (2000): Methane =  $0.45 \text{ (C2)} - 0.275 \text{ (C3)} + 0.4 \text{ (C4)}$  CH cassava hay meal, FMH Flemingia hay meal, SEM standard error of the means. Source: Phesatcha et al. (2016)



**Table 8.** Effect of legume foliage supplementation on urinary purine derivatives and microbial nitrogen supply in dairy steers

Items	Control	CH	FMH	CH+FMH	SEM	P-value
Purine derivatives, mmol/day						
Allantoin excretion	24.5	27.4	26.5	31.3	0.63	0.24
Allantoin absorption	75.6	88.1	87.4	98.7	0.16	0.91
MCP, g/day	325 <sup>a</sup>	387 <sup>a</sup>	452 <sup>b</sup>	482 <sup>b</sup>	1.14	0.02
EMNS, g/kg OMDR	23.1 <sup>a</sup>	25.4 <sup>a</sup>	28.7 <sup>b</sup>	30.2 <sup>b</sup>	2.56	0.04

<sup>a, b</sup> Means in the same row with different superscripts differ ( $P < 0.05$ ) CH cassava hay meal, FMH Flemingia hay meal, SEM standard error of the means, MCP microbial crude protein, EMNS efficiency of microbial N supply, OMRD organic matter digested in the rumen. Source: Phesatcha et al. (2016)

**Table 9.** Effect of banana flower powder supplementation on milk production and chemical composition

Item	Treatments				s.e.m.	Interaction B		
	BAFLOP		NaHCO <sub>3</sub>			R	B	R*B
R : C <sup>A</sup>	60 : 40	40 : 60	60 : 40	40 : 60				
Production, kg/day								
Milk yield	11.5	13.5	11.5	13.5	0.22	***	ns	ns
3.5% FCMC	12.9	12.9	12.5	12.1	0.59	ns	ns	ns
Milk composition, %								
Protein	35.4	25.6	24.6	28.1	4.56	ns	ns	ns
Fat	43.1	31.7	39.9	29.9	1.92	**	ns	ns
Lactose	48.5	47.6	47.8	49.3	2.04	ns	ns	ns
Solids-not-fat	91.0	80.1	79.4	84.4	9.80	ns	ns	ns
Total solid	137.1	111.8	109.9	114.3	2.04	**	**	**
Milk urea N, mg%	9.1	9.8	8.8	10.5	0.84	ns	ns	ns

BAFLOP, banana flower powder; NaHCO<sub>3</sub>, sodium bicarbonate. \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ . ns, not significant. <sup>A</sup>R : C, roughage:concentrate ratio at 60 : 40 and 40 : 60. <sup>B</sup>Interaction effect of R, R : C ratio and B, buffering agent. <sup>C</sup>3.5% FCM (fat-corrected milk) = 0.432 (kg of milk/day) + 16.23 (kg of fat). Source: Kang et al. (2015)



**Table 10.** Effects of plant secondary compounds and plant oil on methane gas production in various studies.

Substrates	Level	Methane,%	Animal	References
Garlic powder	16 mg	(-)22.0*	Buffalo (rumen fluid)	Kongmun et al. (2010)
Coconut oil	16 mg	(+)6.4*	Buffalo (rumen fluid)	Kongmun et al. (2010)
Soap berry fruit and mangosteen peel pellet	4%	10.0		Poungchompu et al. (2009)
Mangosteen peel poeder	100g/h/d	(-)10.5	beef cattle	Kongmun et al. (2009)
Tea saponins	0.01 0.02 0.03 0.04mg/mg diet	1.4 9.7 10.0 2.6		Wongnen and Wachirapakorn (2011)
Coconut oil	7%	(+)39.5*	beef cattle	Kongmun et al. (2009)
Coconut oil	7%	(-)10.2*	Buffalo	Kongmun et al. (2010)
Coconut oil and Sunflower oil	50:50 ratio at 5% in concentrate	10	Buffalo	Pilajun et al. (2010)
Coconut oil Garlic powder	8:4 (mg)	(-)18.9*	Buffalo	Kongmun et al. (2010)
Coconut oil + Garlic powder	7%+100g	(-)9.1*	Buffalo	Kongmun et al. (2010)
Eucalyptus oil	0.33-2 ml L-1	30.3-78.6%	Sheep	Sallam et al. (2009)
Eucalyptus oil	0.33-1.66 ml L-1	4.47-61.0%	Buffalo	Kumar et al. (2009)
Eucalyptus meal leaf	100 g/d	reduce	Cow	Manh et al. (2011, unpublished data); Khodyhotha et al. (2011, unpublished data);

Source: Wanapat et al. (2012)

## Conclusions

Feed resources and feeding systems are essentially important to the ruminant production efficiency in the tropics. EDU-S is highly recommended to be developed on farms to ensure the sustainable ruminant production. Improving the rumen fermentation efficiency using dietary strategy especially those with plant secondary compounds is potentially promising. Furthermore, the FFS using local feed resources as well as conserving them for long dry season is





recommended. Finally, processing such as pelleting of fruit or plant herbs require future research attention. Most importantly, increasing rumen fermentation efficiency, mitigating methane and hence enhancing ruminal productivity using dietary means would be highly appreciated by farmers of their practical implementations.

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## Strategies to Increase the Efficiency of Nutrient Utilization

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### **Abstract**

A key issue for the future will be the numbers of animals which we have. Whilst lower producing animals may have lower environmental impact (gaseous, faecal, urinary) per animal, the requirement for more animals to achieve a certain production of animal products may actually result in a higher environmental impact than a smaller number of high producing animals. This is likely to be a central part of our overall strategy – fewer high producing animals have a lower overall maintenance requirement and therefore a higher efficiency of nutrient utilisation. However there will continue to be a place for relatively low yielding animals when there are consuming diets based almost entirely on non human edible feed. The challenge is therefore to combine appropriate high genetic value animals with good nutrition and management strategies. In this context “good nutrition” should be nutrition which minimises the impact of the animal on its environment. Thus it might involve increased localisation – increased reliance on locally available feedstuffs including by- or co-products – and reduced usage of cereals and oilseed products (where there is competition with man) which often have high environmental costs associated with their production and transport. In particular, diets should be low in human edible feed. In ruminants continued attention should be focused on dietary interventions which mitigate greenhouse gas emissions – hopefully without incurring a penalty in production whilst increasing the efficiency of nutrient utilization. It beholds us an animal scientist to ensure our production systems use available feed resources as efficiently as possible – especially as resources will become increasingly scarce and expensive as competition with humans for food/feed increases. If we fail to address this challenge we will come under increasing attention and pressure from lobby groups, the press and politicians.

**Keywords:** animal, nutrient utilization, feeding, environment



## Introduction

At its simplest, efficiency is just an interrelationship between inputs and outputs. This encompasses the spectrum of scenarios from low input/ high output (“efficient”) to high input/ low output (“inefficient”). However this is simplistic – for a number of reasons. Is the comparison conducted a Biological Efficiency ? Or is it an Economic Efficiency. Considering biological efficiency, the inputs could be intake ( fresh matter or dry matter), energy (digestible, metabolizable or net), or protein (including the concept of HEP – human edible protein). The outputs could consider just a single product such as meat or milk or should consider the full range of uses to which the animal partitions the nutrients – thus a dairy cow does not just use nutrients for milk production but she partitions nutrients towards maintenance, body condition and reproduction as well as lactation. Even this consideration of biological efficiency fails to consider the effects which this input / output relationship has on the environment. The inputs and outputs both have effects on the environment in terms of the provision of the food which is consumed and the fact that the useful outputs are accompanied by other excretive and eliminative losses in the form of faeces, urine and gases which often have a major influence on the environment. This becomes particularly important when we consider economic efficiency which should not be just a simple consideration of feed costs versus meat value but should take account of the environmental impact of the production of feed and any environmental impact of waste outputs. Associated with this is the wider consideration of whether efficiency is best considered at the level of the individual animal (when the balance can be more accurately considered) or the population whereby the issue of the number of animals and their maintenance requirement is taken into account.

This paper deals with the range of definitions of efficiency and discusses how these must be clearly understood before one can address strategies to improve nutrient utilization.

### **Preliminary consideration: Animals or no animals**

Globally there is increasing debate as to whether we could feed the world more effectively, without animals, by solely consuming plant products. This would increase both biological and economic efficiency of food production and remove the negative impacts of livestock on the environment. There is an increasing research in this area and a recent review by Mottet et.al. (2017), *Livestock: on our plate or eating at our table*, addresses many of the key arguments. In 2015, almost 800 million people were still undernourished (FAO, 2015a). This includes insufficient access to balanced supply of macronutrients (carbohydrates, proteins and fats) but also “hidden hunger”, i.e. lack of, or inadequate intake of micronutrients, resulting in various forms of malnutrition, such as anaemia or vitamin A deficiency (FAO 2015). Food from animal sources contributes 18% of global calories (kcal) consumption and 25% of global protein consumption (FAOSTAT, 2016). But it also makes an important contribution to food security through the provision of high-quality protein and a variety of micronutrients – e.g. vitamin A, vitamin B-12, riboflavin, calcium, iron and zinc – that can be locally difficult to obtain in adequate quantities from plant-source foods alone (Randolph et al., 2007; Murphy and Allen, 2003). Livestock's contribution goes beyond the production of meat, milk and eggs, however, and a number of factors determine their overall impact on food security (Gerber et al., 2015). Positive contributions include: (1) the direct supply of essential macro- and micro-nutrients; (2) the contribution of domesticated animals to agricultural productivity through manure and draught power; and (3) the income generated by livestock production at household and national level. Potentially negative contributions to food security include: (1) animal feed rations containing products that can also serve as human food; (2) the fact that animal feed may be produced on



land suitable for human food production; and (3) the relatively low efficiency of animals in converting feed into human-edible products.

The paper by Mottet et al. (2017) aims to inform one important dimension of the debate on the contribution of animal production to food security. Beef production, in particular, is often criticized for its very high consumption of grain, with cited figures varying between 6 kg and 20 kg of grain per kg of beef produced (Eshel et al., 2014, Godfray et al., 2010; Garnett, 2009). The upper bound of this range is, however, based on feedlot beef production, which accounts for 7% of global beef output according to Gerber et al. (2015) and FAO (2009), and 13% according to this analysis. It does not apply to the other forms of beef production that produce the remaining 87–93% of beef. Indeed, debate on the subject often lacks recognition of the wide diversity in production systems and in the goods and services delivered by livestock (Smith, 2015). And while some of the global discussion on food security may address the question of the feed/food competition, it often fails to mention the diversity of animal diets around the world and the various levels of efficiency in production systems (Godfray et al., 2010; Flachowsky, 2010). Some well-documented studies (e.g. Eshel et al., 2014) covering the US livestock sector, are often quoted without clear reference to the geographic context they apply to (e.g. Carrington, 2014), and are therefore wrongly used to inform decision makers, and the public at large. For example, the literature often highlights the supposed efficiency of pigs and poultry in converting feed into meat. But these studies do not take account of the higher share of feed consumed in the form of grains edible by humans and of land suitable for food production used by monogastrics.

The livestock sector is expected to continue to grow. Demand for animal products is increasing in many parts of the world as a result of rising incomes, growing population and urbanization. Global demand for meat and milk is expected to increase by 57% and 48% respectively between 2005 and 2050 (Alexandratos and Bruinsma, 2012). Most of the past decades sector's growth took place in large-scale, specialized monogastric farms (FAO, 2009), and this trend can be expected to continue. This was achieved through an increased reliance by the sector on cultivated forages, grains and oilseed meals, but also on agricultural by-products such as brans, dried distillers' grains, pulps and molasses.

As this increase in demand for animal source food will have a major impact on global food systems and land use, there is a need to better inform policy makers and consumers about feed use and feed use efficiency in the livestock sector (Capper et al., 2013). To this end, the analysis by Mottet et al. (2017) addresses the food/feed competition looking at two main drivers: the feeding of human-edible materials to animals and the use of arable land to produce animal feed (instead of producing food directly). It relies on a new and unique database and provides broad quantitative estimates of livestock feed rations, feed demand, and related land use. It analyses the composition of feed rations and the efficiency with which human-edible and non-human-edible feed materials are converted into animal-source food and discusses land use implications. Their paper is meant to inform policy makers and the wider global community with a quantitative assessment of the role of livestock in current and future food security

### **Livestock and livelihoods**

Whilst ruminant livestock undoubtedly are major contributors to greenhouse gas emissions, can we really do without them? Many of the poorest people in the world live in rural areas of tropical developing countries. Most keep farm animals. Cattle, buffalo, sheep, goats, pigs and poultry are among the most important assets of the poor and are the mainstays of their farming. Livestock bring the poor income, food and fertiliser. These sustain their livelihoods,



assets, health and environments. Demand for livestock foods in developing countries is expected to more than double over the next twenty years. This ‘livestock revolution’ offers several hundred million people opportunity to raise themselves out of absolute poverty. The increasing demand for milk and meat makes livestock development an imperative for a more equitable and sustainable future. Conditions in the tropical developing world are often harsh for animals and people alike and these challenges may be further exacerbated by climate change.

Livestock production efficiency in the developing countries is just one-quarter that of developed regions. Limiting factors for the small-scale farmers and pastoralists in developing countries are a dearth of livestock feeds, a devastating disease burden – both human and animal, rapidly eroding livestock and forage biodiversity, poor access to markets, unresponsive policy environments, and degradation of natural resources.

So why are livestock so integral to this situation? The fight against poverty starts with rational use of available natural resources. Among those most readily available to the world’s poor are farm animals. One-third of the world’s 6 billion people depend on animals. Of the 1.3 billion people living in absolute poverty, 80% live in rural areas and of these, two-thirds—some 678 million poor—keep livestock. Livestock matter because their products matter. Their high-quality food nourishes families that eat too little and too poorly. Their milk, eggs and meat bring cash into households with no other means of earning money. Their manure fertilises soils exhausted by continuous cropping. Their importance as sources of power is critical for cultivation and transport of farm produce to markets where mechanised traction does not yet exist.

Cattle and other ruminants in developing countries are efficient food producers. They eat grass and shrubs instead of grain. They forage for food along roadsides and consume the stalks of harvested maize and the wastes of the vegetable garden. Ruminant animals transform biomass unavailable to humans into high-quality protein, fat and micronutrients. These are essential for human health and development. Half the world’s population is malnourished in ‘micro-nutrients’; three billion people are deficient in iron, 400 million in vitamin A. The most vulnerable are women of reproductive age and children. Women become anaemic, children stunted. A considerable number go blind, fall sick and die. This ‘hidden hunger’ is successfully combated by supplementing small quantities of milk or meat to the starchy staple diets of the poor.

In conclusion, - we can not do without ruminant animals!

Playing “The Devil’s Advocate”, perhaps we could do without non-ruminant animals !? For all their apparent feed conversion efficiency if one considers alternative efficiency measures – such as “human edible feed” or “human edible protein” to animal product, then they become less efficient than ruminants. As stated earlier, there is undoubtedly a high, and increasing, demand for animal products with the highest year on year increases being seen in eggs, poultry meat and pig meat – particularly in the emerging markets in developing countries. To ensure that we deflect unwanted critical attention from politicians and policy makers it will be vital that as animal scientists we can demonstrate that we have systems of animal production which do not harm the environment. Can we produce sufficient energy and protein crops locally to reduce transportation costs – costs of both a monetary and an environmental nature ? Or, if they are being produced elsewhere, can they be grown in an environmentally friendly manner – without destroying rainforest for the production of soya or oil palm !?, and can they be transported, nationally or internationally in an energy efficient manner.



### **Feed/food competition and the role of livestock in the bio-economy**

The annual feed intake of livestock (6 billion tonnes DM) represents 60% of the total food and feed combined biomass, including residues and by-products, or about 20% of the global human appropriation of biomass (Pelletier and Tyedmers, 2010; Imhoff et al., 2004; Krausmann et al., 2013). Today, crop production, processing and the agrifood chains produce large amounts of residues as well as co- and by-products, which constitute nearly 30% of global livestock feed intake. These products will be produced in larger amounts as the human population grows and consumes always more processed food. Livestock play, and will continue to play a critical role in adding value to these residual products, a large share of which could otherwise be an environmental burden.

But livestock also make an indirect contribution to the bio-economy and overall food output by increasing crop productivity through manure and draught power. For example, Gebresenbet and Kaumbutho (1997) estimate that cattle, together with camels, horses and donkeys, provide transport and draught power for ploughing fields on about 15% of farms in Southern Africa and 81% of farms in Northern Africa. In Europe, the share of manure in total nitrogen inputs was estimated at 38% and as much as 61% in the Netherlands (European Commission, 2012). These results are generally in line with the discussion put forward by van Zanten et al. (2015), arguing that livestock can make substantial contribution to protein supply, without triggering feed/food competition.

Given the rising demand for livestock products, the area of land needed for livestock is expected to increase if feed conversion ratios do not improve significantly. Efficiency should not only be considered at animal level but also at production system level. The main factors driving FCR within a given species are feed quality, followed by animal genetics and health conditions (Gerber et al., 2015). The scenarios used in their study provide some insight on a range of feasible FCR improvements which can be considered rather conservative compared to past improvements. Through improved feed formulation, genetic selection and better veterinary services, feed conversion ratios in poultry and pigs have been halved over the last three decades in Brazil and Thailand (de Haan et al., 2001), and Europe (Albers, 2013) and globally for broilers (FAO, 2009). Genetic improvements have led to increases in average milk production per lactation and the number of days required to achieve slaughter weights in poultry in the UK (Wilkinson, 2011). Marginal additional gains are expected in these systems and can be achieved through precision livestock farming and the development of feed additives. In other regions, especially in sub-Saharan Africa and South Asia, progress can also be made in feed quality through improved grasslands and better use of crop residues, including treatments to increase their feeding value. Herd management, and in particular the proportion of breeding stock that needs to be fed but does not contribute directly to human-edible output (e.g. replacement heifers), is also a major driver of FCR and can be improved through interventions in reproduction and animal health. Finally, the proportion of dual-purposes animals in herds/flocks, such as dairy cows or laying hens, for which maintenance energy is distributed over two products, also influences FCR significantly (Wilkinson, 2011).

Despite its crucial importance in animal systems and food security, there is no census-based global database on feed. All global analyses thus rely, at least partially, on modelling. Our estimates draw on an improved version of GLEAM's feed module (Gerber et al., 2013). Results were compared against literature and existing global statistics and appear in line with both the results of an extensive examination of 121 peer-reviewed papers, which are presented in the Supplementary Information section, as well as with FAOSTAT (2016) food balance sheets. They were also compared and found in line with other model-based assessments in terms of feed rations composition and feed use efficiencies for different species (Bouwman et al., 2005;





Steinfeld et al., 2006; Wirsenius et al., 2010; Herrero et al., 2013; Dijkstra et al., 2013) and impact of improvements in feed conversion ratios on land-use (Wirsenius et al., 2010). Although simplifications and generalizations were necessary given the paucity of data, our assessment includes more categories and rations than past work, including the distinction of cattle feedlots and a particular focus on human-edible feed materials and soybean cakes, and protein feed intake per kg of protein produced. This provides a more realistic estimate of FCR, as argued by Wilkinson (2011). The analysis of land-use implications also revealed that factoring in the quality of land (grassland convertibility to cropland) led to findings that defied simple conclusions of resource use by livestock, as also demonstrated by Peters et al. (2014).

The results presented by Mottet et al. (2017) regarding feed rations, animal productivity and land use are expected to improve economic modelling and projections, often based on crude statistics and livestock production data. For example, the fact that livestock consumes about one third of global cereal production and about 100 million tonnes of agricultural co- and by-products is a major element in modelling the aggregated demand for crop products, but so is the almost 50% of the feed intake coming from grasslands. Animal intake of cereals affects the price elasticity of demand, given that elasticity is typically greater for animal consumption (able to reduce the short cycle subsectors (feedlots, pigs) when prices are high, and expand when they are low, absorbing surpluses).

Livestock consume about 6 billion tonnes DM as feed per year, of which 86% is made of materials that are currently not eaten by humans. In addition, soybean cakes, which production can be considered as main driver of land-use, represent 4% of the global livestock feed intake. Livestock play a key role in the bio-economy by converting forages, crop residues and agricultural by-products into high-value products and services. The production of global feed requires 2.5 billion ha of land, which is about half of the global agricultural area. Most of this area, 2 billion ha, is grassland, of which about 1.3 billion ha cannot be converted to cropland (rangeland). This means that 57% of the land used for feed production is not suitable for food production.

Contrary to commonly cited figures, the estimates of Mottet et al. (2017) show that to produce 1 kg of boneless meat requires 2.8 kg human-edible feed in ruminant systems and 3.2 in monogastric systems (layers excluded). These global figures, however, conceal a vast range of feed conversion ratios and feed qualities, between and within species and production systems. Very low efficiencies in terms of overall feed input can be found in extensive grazing ruminant systems due not only to low productivity but also to low nutritional density of feed. But when expressed in terms of human-edible protein, those systems are efficient converters of vegetal protein into animal protein, better than industrial monogastric systems that consume less feed but larger amounts of human-edible feed and soybean cakes per unit of product. These results allow to nuance the severity of the feed/food competition that is often put forward.

The paper by Mottet et al. (2017) demonstrates that modest yield improvements can significantly reduce projected further land expansion for feed production. It also illustrates the complementarity between improving yields at animal level and crop level, and thus the need to evaluate options that improve the efficiency of the entire food system, i.e. the efficiency of the complex web of processes and flows that link natural resources to consumers that require more animal-derived foods. Animal production, in its many forms, plays an integral role in the food system, making use of marginal lands, turning co-products into edible goods, contributing to crop productivity and turning edible crops into highly nutritious, protein-rich food. Quantifying the land and biomass resources engaged in livestock production and the food output they generate, but also improving our modelling capacity by including trends in consumer preferences, climate change impacts, and industrial processes to improve the human edibility of



certain feed materials is arguably basic information needed as part of further research into the challenge of sustainably feeding 9.6 billion people by 2050

### **A UK example of re-defining efficiency of feed use by livestock (Wilkinson 2011)**

Livestock, particularly ruminants, can eat a wider range of biomass than humans. In the drive for greater efficiency, intensive systems of livestock production have evolved to compete with humans for high-energy crops such as cereals. Feeds consumed by livestock were analysed in terms of the quantities used and efficiency of conversion of grassland, human-edible ('edible') crops and crop by-products into milk, meat and eggs, using the United Kingdom as an example of a developed livestock industry. Some 42 million tonnes of forage dry matter were consumed from 2008 to 2009 by the UK ruminant livestock population of which 0.7 was grazed pasture and 0.3 million tonnes was conserved forage. In addition, almost 13 million tonnes of raw material concentrate feeds were used in the UK animal feed industry from 2008 to 2009 of which cereal grains comprised 5.3 and soyabean meal 1.9 million tonnes. The proportion of edible feed in typical UK concentrate formulations ranged from 0.36 for milk production to 0.75 for poultry meat production. Example systems of livestock production were used to calculate feed conversion ratios (FCR – feed input per unit of fresh product). FCR for concentrate feeds was lowest for milk at 0.27 and for the meat systems ranged from 2.3 for poultry meat to 8.8 for cereal beef. Differences in FCR between systems of meat production were smaller when efficiency was calculated on an edible input/output basis, where spring-calving/grass finishing upland suckler beef and lowland lamb production were more efficient than pig and poultry meat production. With the exception of milk and upland suckler beef, FCR for edible feed protein into edible animal protein were >1.0. Edible protein/animal protein FCR of 1.0 may be possible by replacing cereal grain and soyabean meal with cereal by-products in concentrate formulations. It is concluded that by accounting for the proportions of human-edible and inedible feeds used in typical livestock production systems, a more realistic estimate of efficiency can be made for comparisons between systems

### **An example illustrating the importance of fertility and replacement rate influencing efficiency through animal numbers (after Garnsworthy 2012)**

Dairy systems contribute 20 to 30% of livestock emissions and excretions in Europe. Cows convert food unsuitable for human consumption (grass, forages, byproducts) into high quality food (milk), but nutrient conversion is 20 to 30%, so 70 to 80% of nutrients are excreted. With indoor systems, wastes can be contained and spread on the land when plant uptake is optimal. The main driver of environmental impact is production efficiency, i.e. milk or wastes per unit input. Efficiency is a function of animal numbers (milking cows and replacements), milk yield and culling rate. Higher-yielding fertile cows produce more milk per lactation and lifetime, so 'unproductive' emissions and excretions for maintenance and rearing are spread over more litres of milk. Poor fertility in modern dairy cows increases culling, which offsets efficiency gains from high milk yield. Our recent work has studied nutrition, insulin and fertility. Insulin stimulates oestrous cycles, but reduces oocyte quality. Dietary manipulation of insulin at strategic phases of the reproductive cycle doubled the proportion of cows pregnant at 120 days in milk. Diets for high-yielding cows contain more concentrates, starch and fat, and less fibre than grass-based systems, which reduce methane emissions. Breeding for low methane emissions may be possible in future; we find variation among cows with the same milk yield and diet. Nitrogen and phosphorus excretions are reduced by increasing production efficiency and by reducing excess inputs. Scope for reducing excesses is greater in higher-yielding cows, but trade-off in



nitrous oxide emissions must be avoided. A whole-system approach is needed which considers environmental cost of diet formulation as well as economics.

### **Influences of the animal on the environment**

All species of farm livestock, intensive and extensive, may be seen to influence the various components of the environment – air, land and water. This may be either at a global or local level. Globally, one of the key influences is the area of land required to provide feed, either in the form of forage or non-forage materials. Additionally, the transportation of feedstuffs is a major contributor to greenhouse gas emissions. Locally, again the provision of feed is a major influencing factor whilst there will also be direct effects of the animal. Through the actions of grazing and treading ruminant animals can influence the appearance of the countryside, biodiversity, and soil properties. The inefficiencies of the digestive physiology and metabolism of the ruminant animal mean that there are major losses of energy and nutrients between what the animal consumes as feed and what is retained in its body. These losses occurring through eructated gases, faeces and urine have major influences on the environment. Historically, ration formulation and feeding systems have concentrated on achieving high levels of animal performance. This was often at the expense of other importance issues such as animal welfare, animal and human safety, product quality and the environment. It is critical that greater importance is attributed to minimising losses from ruminants when formulating diets and feeding systems. These losses are considerably smaller in the case of non-ruminants – the nature of their digestive systems results in smaller losses although of course their digestive tracts are greatly limited in the nature of the feedstuffs which they consume bringing them into direct competition with humans. Thus whilst the environmental influences associated with direct losses from non-ruminants are considerably smaller, there is a potentially greater indirect risk to the environment associated with the production and transportation of appropriate feedstuffs.

### **Air, land and water**

Activities associated with livestock (especially ruminants) have a major influence on air, land and water. Agriculture, especially livestock agriculture has been shown to be a major contributor towards global greenhouse gas emissions and therefore global warming. These emissions, primarily in the form of carbon dioxide, methane and nitrous oxide, arise through agronomic practices (associated with the production of feedstuffs), transportation, enteric losses from the animal associated with digestion and losses from manures and the soil. Additional to these gaseous pollutants, ruminants may also be major contributors to land and water pollution. It is therefore inevitable that attention focuses on ruminants as major contributors to global warming and the posing of the question – Do we need farm livestock (and their products of meat and milk) ?...or would the world be environmentally better off without them ?! Some key aspects relating to climate change are rehearsed along with a redressing of the balance by a consideration of the positive influences of livestock on livelihoods globally

### **Climate change**

The exact nature of any change in climate remains unclear but has been well reviewed. The likeliest scenario is one of increased variability, particularly at the extremes with overall increases in mean temperature and overall decreases in mean rainfall being likely. Climate change is now, somewhat belatedly, being taken seriously at a global, political level. By way of example, FAO Headquarters in Rome has hosted the High-Level Conference on World Food Security and the Challenges of Climate Change and Bioenergy in 2008. The Conference built



upon the work initiated at the earlier Expert meeting. All FAO member countries, relevant inter-governmental and non-governmental organizations and other institutions attended. The overall purpose of the Conference was to address food security and poverty reduction in the face of climate change and energy security. More specifically, the objective was to assess the challenges faced by the food and agriculture sectors from climate change and bioenergy in order to identify the steps required to safeguard food security within the broader context of action being recommended to address climate change and bioenergy at the global, regional and national levels. It thus contributed to the UN system efforts in the field of climate change which were and still are much needed. Whilst the discussions, and the hoped for action coming out of the discussions, have global relevance they are likely to have a particular impact on many climatically marginal areas within the tropics and our livestock production systems. Already we are seeing that many governments have legislated on greenhouse gas emissions from many sectors of industry including agriculture.

### **Livestock effects on Carbon and Nitrogen cycles**

Livestock's role in the carbon and nitrogen cycles underlying global climate change are closely connected to livestock's impact on land use and the change in land-use. Livestock's land use includes both grazing land and land dedicated to the production of feed crops and fodder. The methodology for estimating emissions is contentious. However, considering estimates of emissions along the entire commodity chain, livestock contribute about 18% to the global warming effect. Livestock contribute about 95 of total carbon dioxide but 37% of methane and 65% of nitrous oxide. It is expected that the later will substantially increase over the coming decades as pasture land, which is currently at its maximum expanse, is converted to arable production. Future expansion of the livestock sector will increasingly be based on crops and crop by-products. There are a variety of options which can be applied to reduce the impact of the animal on the environment by reducing emissions:

- Carbon sequestration on extensive grazing land
- Reduction in methane emissions from low input : low output ruminant production systems
- Reduction of methane and nitrous oxide emissions from animal waste, through energy recovery and improved waste management

### **Air**

The odour associated with livestock, particularly those kept at a high stocking density in a restrained space, is an example of an effect of the animal on its environment and is one which the general public, particularly those unconnected with livestock farming, might find offensive. This is particularly an issue with intensive pig and poultry enterprises..although it can also apply to intensive dairy units and cattle feedlots.

However the main effect of ruminant animals on their gaseous environment is through gaseous emissions associated with their digestion of feed in particular the enteric fermentation. Whilst some may be associated with the faeces, the majority is methane and carbon dioxide resulting from the activities of micro-organisms in the reticulo-rumen which is eructated out. This is an inevitable outcome of fermentation and may typically account for about 8% of the Gross Energy of the diet. This figure is only indicative and will vary depending on level of feeding and diet composition. There is therefore scope to reduce the output of these greenhouse gases by dietary manipulation including the addition of manufactured additives or naturally occurring plant compounds which have been shown to reduce proportions of methane produced



per kg feed ingested. This topic merits continued research as in a number of situations those compounds which reduce methane production do so by reducing the overall efficiency of rumen fermentation which results in fewer end products of fermentation available to the host animal and reduced performance. Thus whilst these compounds might seem attractive it is critical to look at them on the basis of not just the amount of methane produced per animal but by considering the amount of methane produced per unit of product produced.

Considering total levels of gaseous emissions from ruminants, one of the key influencing factors is the number of animals. This is critically important with the increasing global demand for meat and milk. As discussed, mitigations which reduce methane per animal tend to also reduce animal performance. This leads to a requirement for more animals to achieve a given level of animal production with the likelihood of greater total gaseous production. One of the greatest influences on reducing emissions would be to have fewer animals ! Instead of a large number of moderate / low producing animals, where the Animal Production Level (APL) is low with Maintenance (M) being a high proportion on  $M + \text{production}$  and the gaseous emissions are high relative to product production, move towards a lower number of high producing animals. Whilst these high producing high APL animals may be associated with higher individual emissions levels this is more than compensated for by the reduction in animal numbers. This strategy, as well as reducing total greenhouse gas emissions from the enteric fermentation, also impacts on the total feed required.

Mention was made earlier of the major contribution from agronomic and transport variables to the global emissions. More efficient utilisation of feeds by individual animals would reduce the overall requirements for feed production and transportation per animal although increasing demand for animal products might still result in high levels of output associated with these factors

### **Land and Water**

In addition to the influences of ruminant animals on their environment by grazing and treading, their major influence is through the impact of their faeces and urine on the land, and through run-off, on water.

Of course the addition either directly from the grazing animal, or indirectly as manure from housed animals, of these materials to the land has great positive benefits. It serves as a return of organic matter improving soil structure and inorganic material particularly nitrogen, phosphorous and potassium as well as other minerals. The return of these materials is vital for sustainability and can bring about monetary savings by reducing the reliance on artificial fertilizers. However there may be issues associated with overall quantities as well as geographic or spatial distribution.

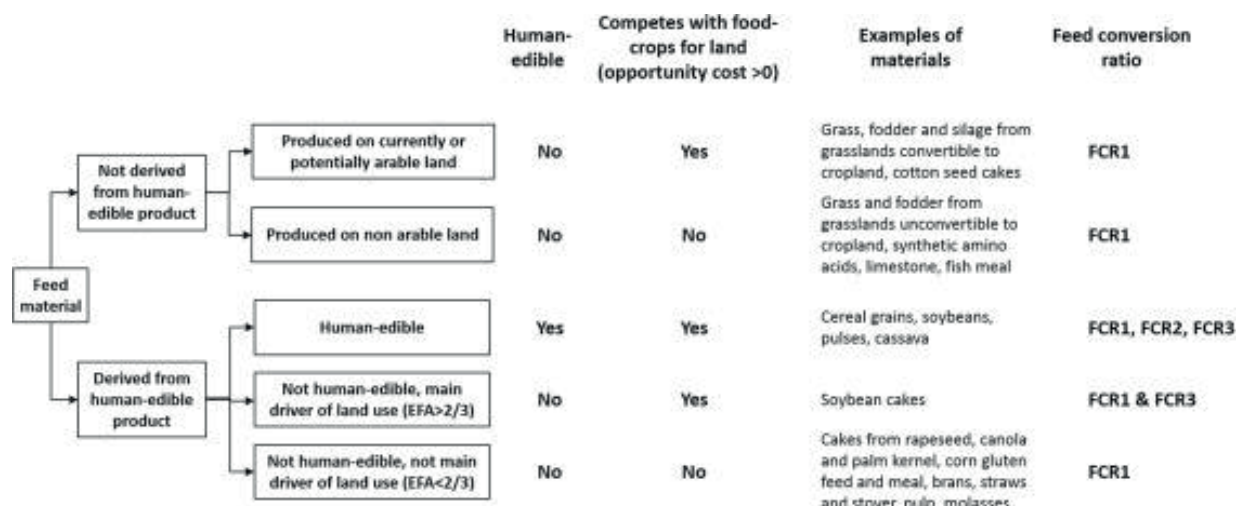
In intensive livestock housing the number of animals per unit of land may be extremely high relative to the available land area. In such situations the danger of run-off and leaching of nutrients endangers water courses and, due to the high biological oxygen demand of these products, fauna. Risks of loss of nutrients through volatilisation, run-off or leaching can also be exasperated through inappropriate storage of waste material.

Whereas production of gaseous emissions is primarily a problem specific to ruminants, production of faeces and urine is inevitable from all species. No diet is going to be digested with 100% efficiency – there will always be faeces produced – and as mentioned it is often a very valuable product. Similarly the metabolism of the animal and its nitrogen turnover is such that there will always be urine production – again a valuable product. However, should we be concerned about the amount and the composition of these materials and their effect on the environment?



Just as we can manipulate the ruminant animal’s diet to influence the gaseous emissions, so to we can manipulate faecal and urine output. A good example would be altering the degradability of the nitrogen component of a ruminant’s diet. For high producing animals a diet high in supply of undegradable protein will be required in order to meet the animal’s total tissue protein requirement. This may be met by feeding a supplement with a moderate degradability at a high inclusion rate. This meets the requirement for undegradable protein to contribute to the total tissue protein requirements but does so by markedly oversupplying the rumen degradable protein (RDP). This RDP is degraded in the rumen but contributes to an ammonia pool which exceeds the capabilities of the rumen micro-organisms to utilise it. Thus it ends up being taken up across the rumen wall into the blood and whilst some may be subsequently recycled through the rumen wall or via saliva, much of it passes to the liver and hence to the kidney and the urea is excreted. Much of this surplus RDP could be true protein which thus represents the waste of a feed resource and contributes to a high urinary nitrogen output in the faeces – which may or may not be useful but is certainly an expensive fertilizer nitrogen source! A more efficient way of meeting the animal’s requirement for undegradable protein would be by feeding a supplement with a low degradability (and is thus a good source of “by-pass protein”). Such supplements have the disadvantage that they may be more expensive but have the advantages that they are used more efficiently by the animal and result in less impact on the environment

**Highlights (after Mottet et al 2017)**



**Feed classification methodology**

- 86% of the global livestock feed intake in dry matter consists of feed materials that are not currently edible for humans
- Contrary to commonly cited figures, 1 kg of meat requires 2.8 kg of human-edible feed for ruminants and 3.2 for monogastrics
- Livestock consume one third of global cereal production and use about 40% of global arable land
- Livestock use 2 billion ha of grasslands, of which about 700 million could be used as cropland
- Modest improvements in feed conversion ratios can prevent further expansion of arable land dedicated to feed production.



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## Sustainable Intensification of Animal Systems in Emerging Economies

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### Abstract

Increasing global population and the concomitant rise in demand for livestock products is a challenge that the agriculture industry should address. There are two ways to increase production to meet demand: increase land area for agriculture or produce more within the area that is currently used for agriculture. There is little room for expansion and will not be enough for the expected 70% increase in demand. Therefore, the solution lies in increasing production (and reduce waste at the same time). However, higher production may negatively affect the environment; therefore, increases in production must be made in a more sustainable way. Livestock emit greenhouse gases that impacts environmental sustainability directly by belching (methane) and during manure storage and land application (mostly nitrous oxide). While agriculture in developed countries has served well in providing abundant, safe food, even more effort should be done to reduce the carbon footprint. The highest opportunity in increasing productivity lies in developing countries, particularly reducing emissions per unit of product produced. The ultimate goal is *sustainable intensification*: keeping the production gains made in intensification while continuing to improve the sustainability of our agricultural production systems. But it is also worth recognizing that the success producers have had in intensifying agricultural production has helped to reduce climate change.

**Keywords:** greenhouse gas, methane, livestock, manure

### Introduction

It has been generally accepted that the world population is expected to exceed 9 billion people by 2050. Supplying this population with safe, secure, and healthy food will challenge the world's agricultural resources and production systems. This is expected to increase the demand for livestock products, which will be challenging to meet because very little room for land expansion exists. It is expected that the largest growth will be in developing countries and will overtake developed countries in their consumption of livestock products (FAO, 2011). Due to this increasing global demand for livestock products, there are concerns over sustainable animal agriculture practices and particularly environmental impacts of livestock production (Kebreab et al., 2012). The environment can be impacted in several ways such as degradation of water quantity and quality, air emissions and other emerging issues such as hormones, antibiotics and other chemical pollutants. Godfray et al. (2014) summarized the challenges for the food system in the coming decades as (1) population growth including changes in demography, (2) increases in disposable income and expectations and consequent diet change, (3) resource scarcity,



particularly water, (4) global change, particularly climate change and its impact on food production, and (5) mitigation of greenhouse gas emission and at the same time adaptation to consequences to climate change.

Livestock industries are a significant source of greenhouse gas (GHG) emissions globally, however, in developing countries with less intensive agricultural practices, livestock contribute proportionally higher compared to developed countries which comprise less than 10% (Figure 1). Different countries and regions contribute different amounts both in relative to other industries and in absolute terms, which is primarily a function of the number of animals kept and their efficiency of feed utilization (Kebreab et al., 2012). Using a life cycle analysis, which is a tool used by many industries to quantify the environmental impact of products, FAO (2010) compared the total average life cycle emissions across different world regions. The report showed that the highest emissions per unit of product were found in developing regions with sub-Saharan Africa, South Asia, North Africa and the Near East emitting an average of 7.5, 4.6 and 3.7 kg CO<sub>2</sub>-eq. per kg of fat and protein corrected (FPC) milk, respectively. Industrialized regions such as North America and Europe, on the other hand, were found to exhibit the lowest emissions per kg of product.

Tilman et al. (2011) estimate that if current trends of greater agricultural intensification in richer nations and greater land clearing (extensification) in poorer nations were to continue, ~1 billion ha of land would be cleared globally by 2050, with CO<sub>2</sub>-C equivalent GHG emissions reaching ~3 Gt/y and N use ~250 Mt/ y by then. In contrast, if 2050 crop demand was met by moderate intensification focused on existing croplands of under-yielding nations, adaptation and transfer of high-yielding technologies to these croplands, and global technological improvements, their analyses forecast the need for land clearing to be reduced by ~0.8 billion ha, GHG emissions cut by a third, and global N use reduced by ~25 Mt/y.

The objective of the paper is to investigate the environmental sustainability of intensive agriculture and lessons learned to achieve similar level of efficiency in animal production in less intensive systems.

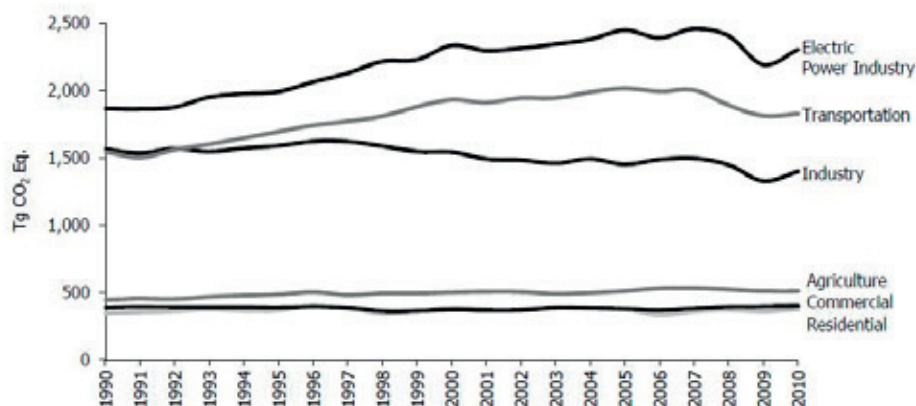


Figure 1. Emissions of greenhouse gases in the USA allocated to economic sectors. (From: Inventory of US Greenhouse Gas Emissions and Sinks 1990-2010, US Environmental Protection Agency).



## Environmental Sustainability

Although sustainability is usually seen as the ‘sweet spot’ which lies at the confluence between the environment, economics and social issues, this paper is mostly devoted to environmental sustainability without completely disregarding its implications on the other aspects. Due to limitation of space, the paper also focuses on cattle, particularly, dairy.

The main processes contributing directly to GHG emissions from livestock and thus affecting environmental sustainability are (1) enteric fermentation and (2) manure decomposition – both during storage or after application to soil (Kebreab et al., 2006). These processes are the largest sources of methane and nitrous oxide emissions in any animal production system (Figure 2). Methane is the main GHG gas eructated from cattle, which is 25 times more effective in trapping heat in the atmosphere than carbon dioxide over 100 year period (IPCC, 2006). Beauchemin et al. (2010) indicated that enteric methane was the largest contributing GHG in beef production accounting for 63% of total emissions, with about 84% of the enteric methane from the cow-calf herd, mostly from mature cows. In the dairy sector, methane contributes most to the global warming impact of milk - about 52% of the GHG emissions – from both developing and developed countries (FAO, 2010). Most of the lifecycle analyses conducted on emissions from livestock suggest that research into mitigation practices to reduce GHG emissions from cattle should focus on reducing enteric methane production from cows.

Methane production is directly related to level of intake and diet composition. Increased production levels associated with increased feed intake levels reduce methane per unit product in two principal ways (1) dilution of maintenance effect and (2) decreased proportion of feed converted to methane. Increased intake levels are associated with proportionally less methane production, because higher intake levels are associated with reduced rumen retention times, reduced rumen pH and a consequent shift towards propionic acid formation which reduces methane emissions (Ellis et al., 2008). Considering the energy transformations of a feed, Dijkstra et al. (2013) pointed out that the highest gains in efficiency can come from converting gross energy into digestible energy.

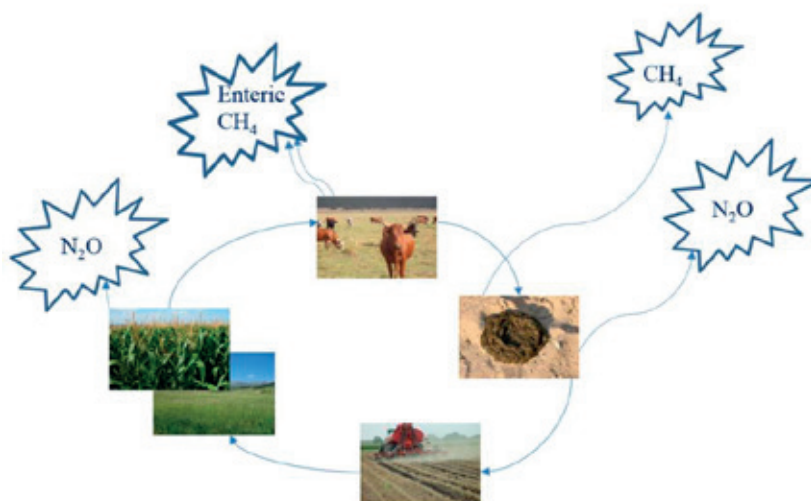


Figure 2. Main environmental stressors from an agricultural system.



## Feed Efficiency

Feed efficiency can simply be defined as a measure of how well cows utilize the ration. An analysis of methane emissions based on milk production in California showed that methane emissions have declined 52% compared to 1950 (Figure 3). This reflects a 200% increase in milk production due to genetic and nutritional improvements while cow numbers have increased just over 100% since 1950. This means that fewer and fewer numbers of animals are required to meet the growing demand, which is a more sustainable system. The maintenance requirements of animals are usually considered to be fixed. High production levels will increase feed conversion efficiency simply because the amount of metabolized feed needed for maintenance processes is diluted out (Figure 4). An increase in productivity (amount of product per animal) not only offers a pathway to satisfy increasing demands for milk and beef but also a possible mitigation approach to reduce the emission of various pollutants. Moraes et al. (2014a) showed the efficiency of energy utilization in beef cattle may be higher in modern beef cattle compared to those from decades ago. Because maintenance energy requirement is assumed not to change as a function of production, whereas the daily energy requirement increases as milk yield increases, the proportion of total energy used for maintenance is reduced (Dijkstra et al., 2013). For example, upon an increase in annual FPC milk production from 6,000 to 10,000 kg/cow, the energy requirements per kg milk (MJ/kg FPC milk) are reduced by 16% and 19% in the Dutch and UK systems, respectively. Similarly, Capper et al. (2009) and Capper (2011) showed that modern, high-production level dairy and beef cattle practices require considerably fewer feed resources than low-production level systems several decades ago. Average annual milk production in the USA increased from 2,074 kg/cow in 1944 to 9,193 kg/cow in 2007 and feed input per kg milk was reduced by 77% at the 2007 level compared with the 1944 level. Average daily growth rate of beef cattle in the USA increased from 0.75 (1977) to 1.08 (2007) kg/head, and feed input per kg gain was reduced by 19% at the 2007 level compared with the 1977 level.

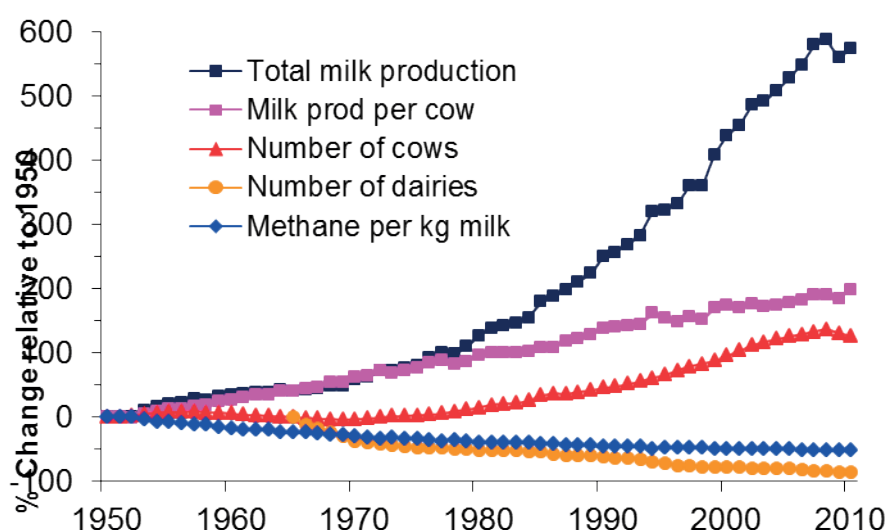


Figure 3. Comparative changes in the California dairy industry from 1950 to 2010. Adapted from Von Kyserlingk et al. 2013).



### Effect of Diet on Enteric Methane Emissions

Due to cost and time required to measure enteric methane production, prediction equations are widely used to calculate methane emissions. Mathematical equations range from simple fixed conversion values (IPCC 2006; Tier 1), to highly complex process-based models (e.g., Bannink et al., 2011). Various studies have shown that diet has a significant impact on methane emissions mainly due to fiber and fat contents. For example, Moraes et al. (2014b) developed a mathematical model that showed a strong positive relationship between dry matter intake and methane emissions. Fiber fractions were also positively related to methane emissions while increased levels of lipids in the diet were associated with reduced enteric methane emissions. Although the IPCC methodology recommends the use of Tier 2 method for estimating emissions, it does not differentiate between diets high in structural carbohydrates (e.g. cows on native pastures) and those based on digestible starch (grain) sources. Therefore applying the recommended methane conversion factor ( $Y_m$ ) of 6.5% GE across the world would not give a true assessment of efficiency. For example, Kebreab et al. (2008) found that the average  $Y_m$  in dairy cows in the US was 5.63% (range 3.78 to 7.43%) and in feedlot cattle the average  $Y_m$  was 3.88% (range 3.36 to 4.56%) for Holstein breeds. In tropical breeds, Kennedy and Charmley (2012) measured methane emissions from Brahman cattle using open-circuit gas exchange. They reported that methane emissions ranged from 5.0 to 7.2% of GE and were linearly related to dry matter intake (DMI). Arias et al. (2013) reported that in Chilean conditions the  $Y_m$  ranged from 6.2 to 7.9% GE with the lower value being associated with beef cows fed in feedlot and the higher value with pasture finished cattle. The variation was attributed to differences in digestibility and contents of fiber. Therefore, intensive systems can reduce methane emissions when considered per product basis.

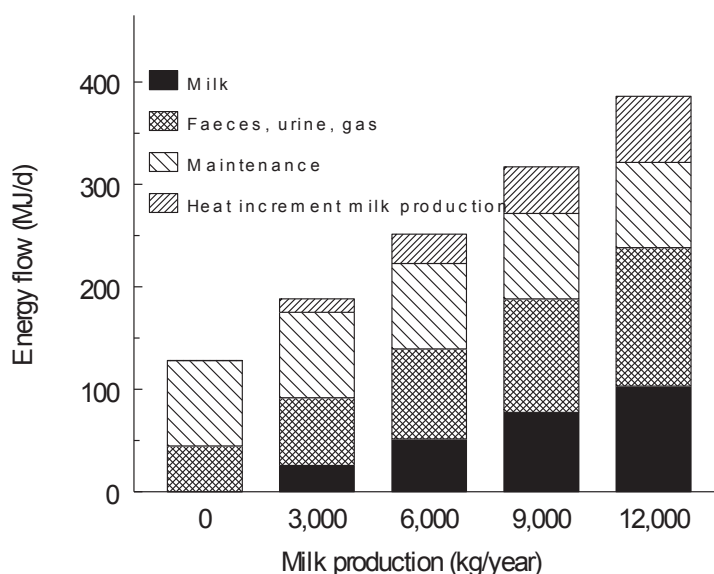


Figure 4. Milk production level (kg fat and protein corrected milk (FPCM) per cow per year) and partitioning of feed towards losses in feces, urine and gases, maintenance needs, heat increment for milk production, and milk production in dairy cattle. Adapted from Dijkstra et al., 2013).



Although the amount of feed required per unit of milk or meat is reduced when milk production levels increase, the associated higher feed intake levels generally coincide with a reduction in digestibility. The level of reduction in digestibility is usually more pronounced for structural carbohydrates compared with non-structural carbohydrates. For example, Robinson et al. (1987) evaluated the effect of feed intake level of a 1/3 hay, 2/3 concentrate diet on apparent digestibility of dairy cattle. Based on the intake and digestibility results reported, the decline in digestion of organic matter (OM), neutral detergent fiber (NDF) and starch was 2.9%, 6.9% and 0.1% per multiple of energy maintenance requirement, respectively. The digestibility of cellulose and hemicellulose are strongly related to methane production more so than soluble carbohydrate. Therefore, improved digestibility of feed in intensive systems increases the feed energy available to the animal, thereby reducing emissions of methane per unit of animal product. Forage preservation, forage composition and an increase in the concentrate:forage ratio may all reduce the proportion of dietary intake energy lost as methane by up to 30% (Kebreab et al., 2006). Appuhamy et al. (2006) evaluated over 40 extant models to predict methane emissions and they reported that those that include intake, digestible fraction of NDF and lipid were the most accurate. Supplementation of traditional diets with lipids is one of the most promising mitigation strategies due to its effectiveness in reducing methane, environmental safety, and animal health (Hristov et al. 2013). Decreasing fiber (neutral detergent fiber, NDF) proportion, while increasing the amount of lipids in dairy diet reduces enteric methane emissions. Caro et al. (2016) evaluated the global potential reduction of enteric methane emissions released from dairy cattle using mathematical models. The authors simulated the amendment of traditional diets in 183 countries aggregated to 11 regions. Amending dairy cattle diets involved increasing the concentration of lipid (up to 6 %) and decreasing the concentration of fiber, without affecting the total gross energy intake. The greatest potential reduction per unit of milk produced occurred in Africa followed by South America and Asia (55, 46 and 34 %, respectively). Lipid supplementation has also an indirect effect on methane and nitrous oxide emissions from manure management. Methane emissions from manure management was expected to decrease by 13%, while nitrous oxide emissions would increase by 21% due to diet amendment. On balance, the total potential reduction of GHG emissions through diet amendment was 104 MtCO<sub>2</sub>eq annually. Moreover, amending diets was simulated to increase global milk production by 13%.

## Conclusion

Sustainable intensification of agricultural systems, particularly animal systems in emerging economies have the potential in meeting the increasing global demand for meat and milk. When calculated per unit of product produced, these systems reduce greenhouse gas emissions and environmental impact of increasing productivity.

## Acknowledgements

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## Climate Smart Livestock Production Systems (CSLPS)- A Novel Approach to Balance the Changing Climate

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### **Abstract**

The livestock sector is contributing up to 18% of the global greenhouse gas (GHG) emissions increased from 1769 x 10<sup>9</sup> kg CO<sub>2</sub> equivalent in 1961 to 277 x 10<sup>9</sup> kg CO<sub>2</sub> in 2010. It was observed that tropics and sub-tropics of which the Himalayas is the largest range of up to 3-5 times faster warming than in the rest of the world. Environmental temperature is the most critical climatic having a direct impact on livestock production. Livestock can express full genetic potential at thermo-neutral zone where the heat production in animals is less and the energy can be utilized for production. The upper critical temperature is at lower level in high producing animals than low producing animals of same species or breed because of greater heat load of higher metabolic rate. Global warming strongly affects the performance of livestock due to which 2% loss of milk production in India have been observed. Exposure to elevated ambient temperature decreases fertility and semen quality in poultry, rabbits and horses. Heat stress in livestock prone to several diseases. Climate Smart Livestock Production (CSLP) aims at sustainable production, reducing climate change vulnerability, reduction of methane emission and improved food security and livelihood security. The present paper focused on Identification and classification of Livestock adoptable Bio-Climatic Health Zones, Climate Change Adaptation and disease resistance traits using SNP Techniques, Standard Operative Practices (SOP) in Livestock Management to be adopted in vulnerable areas of climate change, Institutional support from the technologies, strategies evaluation and capacity building of community, special focus on animal health, value addition and quality control of livestock produce and value addition through processing of livestock products to reduce the effluents under climate smart livestock production system. It is concluded that by adopting climate-resilient livestock production principles like using more appropriate genotypes, adapted and efficient indigenous animals, better management of available feed resources, conserving grasslands and improving water resource efficiency, food security and livelihood security can be assured.

**Keywords:** Climate Smart Livestock Production, Climate resilient livestock production

### **Introduction**

Climate Change is a global phenomenon. Climate change is one of the principal environmental problems of present times (IPCC, 2014). However, developing countries are more exposed to the hazards of climate change and are less resilient to them. (Morton, 2007). It is also important to note that the climate change is of great concern at local level, especially within developing countries like India (Gerlitz, 2015).

According to Intergovernmental Panel on Climate Change (IPCC, 2007), "It is a change in the state of the climate that can be identified by changes in the mean temperature and/or the variability of its properties and that persists over an extended period of time, typically decades



or longer". The global temperature have already increased by  $0.7^{\circ}\text{C}$  over the past century and are projected to further increase by a minimum of  $1.8^{\circ}\text{C}$  to a maximum  $4^{\circ}\text{C}$  before the end of this century, depending on our ability to act quickly to combat climate change as stated by Anantha padmanabhan et al. (2007). The temperatures by 2050 will be at least  $2^{\circ}\text{C}$  and perhaps as much as  $5^{\circ}\text{C}$ , above those of pre industrial times (IPCC, 2007, The World Bank, 2010), threatening sustainable food production worldwide. In India, there is an overall decrease in the seasonal mean rainfall. Over the period from 1901 to 2010, global mean sea level rose by 0.19m.

### **Contribution of Livestock to GHG emissions and climate change**

The livestock sector is one of the major contributors in agriculture, by some estimates contributing up to 18% of the global greenhouse gas (GHG) emissions (Thornton and Herrero, 2010). Of this, about one third is reported to be due to land use change associated with livestock production, another one third is nitrous oxide from manure and slurry management, and roughly 25% is attributed to methane emissions from ruminant digestion (Thornton and Herrero, 2010). Dourmad et al. (2008) in their report classified the contribution of livestock production system to global climate change directly through three main sources of the GHG emissions: the enteric fermentation of the animal, manure and production of feed and forage. Livestock are known to contribute to greenhouse gas (GHG) emissions. Dairy farming contributes to and is affected by climate change. Dairy production plays a role in GHGs emissions, particularly methane ( $\text{CH}_4$ ), which contributes to climate change. The study by Aydinalp and Cresser (2008) showed that most of the methane releases come from paddy fields (91%) and less significantly from animal husbandry (7%) and the burning of agricultural wastes (25%). According to Andin (2010), carbon footprints of agriculture and forestry contribute 30% of emission to atmosphere; 5% by all ruminants and 2% by dairy farming. Furthermore, about 50 to 70% of the GHG emission from dairies are originated by  $\text{CH}_4$  from digestive processes of the cow and manure storage.

Total GHG emission from livestock in world increased from  $1769 \times 10^9$  kg  $\text{CO}_2\text{eq}$ . in 1961 to  $277 \times 10^9$  kg  $\text{CO}_2$  in 2010. This increase in India was from  $225 \times 10^9$  kg  $\text{CO}_2\text{eq}$  in 1961 to  $392 \times 10^9$  kg  $\text{CO}_2\text{eq}$  in 2010 (Patra, 2014). The contribution of enteric methane emission from livestock in world was estimated to be  $1537.5 \times 10^9$  kg  $\text{CO}_2\text{eq}$  in 1961 and  $2372.5 \times 10^9$  kg  $\text{CO}_2\text{eq}$  in 2010. In India it has been estimated as  $209.5 \times 10^9$  kg  $\text{CO}_2\text{eq}$  in 1961 which rose to  $357.5 \times 10^9$  kg  $\text{CO}_2\text{eq}$ . Estimates (1961-2010) of methane emissions from manure of world livestock and in India rose from  $170.25 \times 10^9$  kg  $\text{CO}_2\text{eq}$  and  $13.0 \times 10^9$  kg  $\text{CO}_2\text{eq}$  to  $285.35 \times 10^9$  kg  $\text{CO}_2\text{eq}$  and  $27.4 \times 10^9$  kg  $\text{CO}_2\text{eq}$ , respectively.

According to the United Nations Environment Programme, when considering the entire food chain (including deforestation for grazing, forage production, and so on), meat production accounts for 18-25 per cent of the world GHG emissions. Left unchecked, animal production is predicted to account for 70 per cent of the sustainable level of all global GHG emissions by 2050. This level of global consumption poses severe sustainability challenges. (UNEP, 2011 and Pelletier and Tyedmers, 2010). Animals respond to an unfavourable ambient temperature in a very complex manner. Figure 2 and 3 show the most important influences of the climatic environment on the physiological processes of the animals, together with the consequences of these changes in their production potential and product quality.

### **Climate Change in Himalayan region and Jammu & Kashmir**

The United Nations Intergovernmental Panel on Climate Change clearly mentioned that Himalayan region will witness an increase of 5-10 rainy days by 2030s and the intensity of the rainfall is also expected to rise. The Intergovernmental Panel on Climate Change (IPCC, 2007) in its assessment report on global climate scenarios also has indicated that the pattern of global



warming will be more pronounced at high altitude zones, especially those in the tropics and sub-tropics of which the Himalayas is the largest range of up to 3-5 times faster warming than in the rest of the world. Alpine glaciers, such as the ones in the Himalayas, are particularly sensitive indicators of climate change. There is credible evidence that the glaciers in the Kashmir Himalayas are responding to the climate change. The rising temperatures and scanty snowfall in the winters have reduced the mass of the glaciers. Several dozens of smaller glaciers have completely vanished during the last 50 years in the state. (Itrat Bukhari, 2016).

The Third assessment report (TAR, 2001) of the Inter-Governmental Panel on Climate Change-analysis of the temperature trend in the Himalayas and its vicinity shows that, temperature variations are greater in the uplands than lowlands. Shrinkage of glaciers, thawing of permafrost, late freezing and earlier break up of ice on rivers and lakes, pole-wards and altitudinal shifts of plant and animal species, declines of some plant and animal populations, and earlier emergence of insects have been observed (IPCC, 2007). The Indian Network for Climate Change Assessment (INCCA, 2010) in 2010 reported an extreme shift in precipitation pattern with an average increase of 5-10 rainy days in Jammu and Kashmir region by 2030. The intensity of rainfall is likely to increase by 1-2mm/day particularly in the eastern part of Jammu and Kashmir (Das et al., 2014).

### **Significance of Climate for Livestock Production**

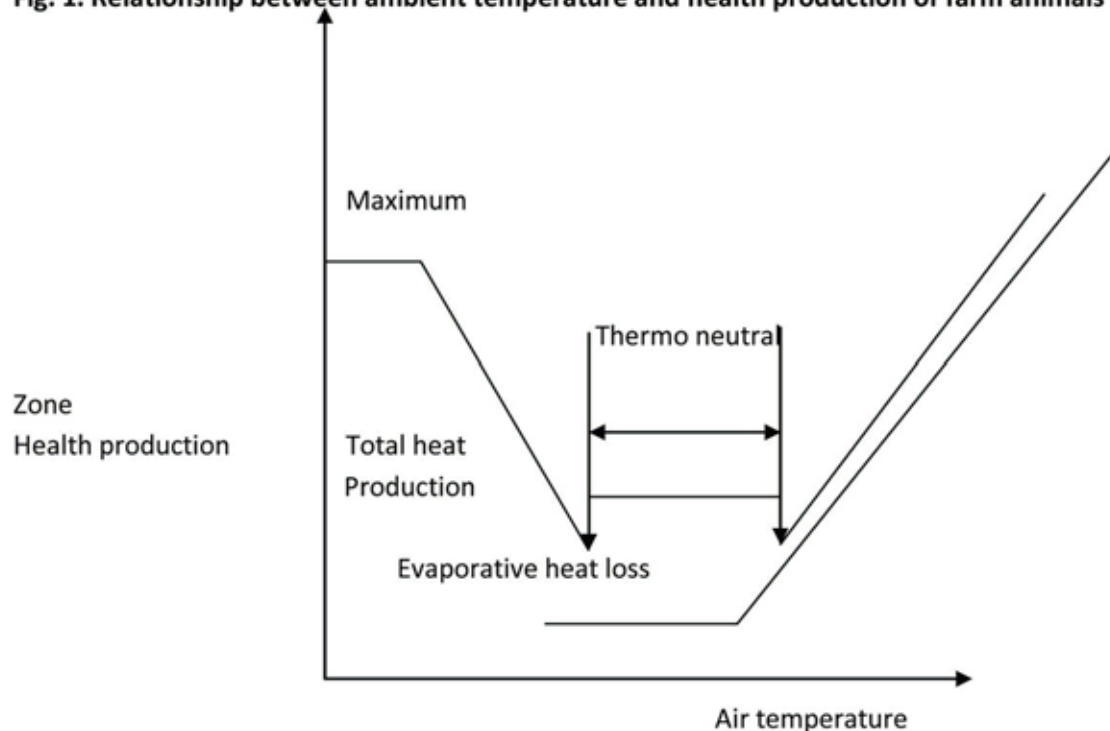
Environmental temperature is the most critical climatic factor followed by humidity, radiation and wind velocity having a direct impact on livestock production. The livestock can express their genetic potential only at their zone of thermo-neutrality, within which the metabolic rate is independent of environmental temperature. The upper and lower critical temperatures are of more significance in tropical, sub tropical and temperate regions.

The total of heat produced in the course of digestion, excretion and metabolism of nutrients is called heat increment. Within a certain range of ambient temperature and besides unvarying feed and nutrient intake the total heat production of the animal remains constant (Figure 1). This temperature range is called the thermo neutral environment zone. In a thermo neutral environment the heat production of the animal is at the minimum, and thus the dietary energy can be used for production (growth, egg and milk production) efficiently. Unfavourable temperatures (too cold or too hot environments) lead to an increased heat production by the animal, i.e. there is more loss of energy, and in consequence less energy remains for production at the same level of energy intake, and the efficiency of energy utilization deteriorates.

The upper and lower critical temperatures for different animal species and age groups are shown in Table 1. The species, age and body condition of the animals all have a significant influence on the critical temperature, but other environmental factors affecting their thermal sensation and heat dissipation, such as air velocity and air humidity, are also crucial. Increasing the airflow improves the efficiency of evaporative cooling, but higher humidity has the opposite effect. In cold, humid conditions the heat conductivity of wet hair increases, thus the animal becomes more sensitive to the lower ambient temperature. Based on these examples it can be seen that in case of high humidity levels the comfort zone of the animals becomes narrower, the lower critical temperature increases while the upper critical temperature decreases. Thermoregulation is the ability of the animals to maintain their body temperature in cold or hot environments, consisting of behavioural, physiological and anatomical responses that affect energy metabolism.



**Fig. 1. Relationship between ambient temperature and health production of farm animals**



**Table 1. Lower and upper critical temperature of farm animals at different age or body weight (FASS, 2010)**

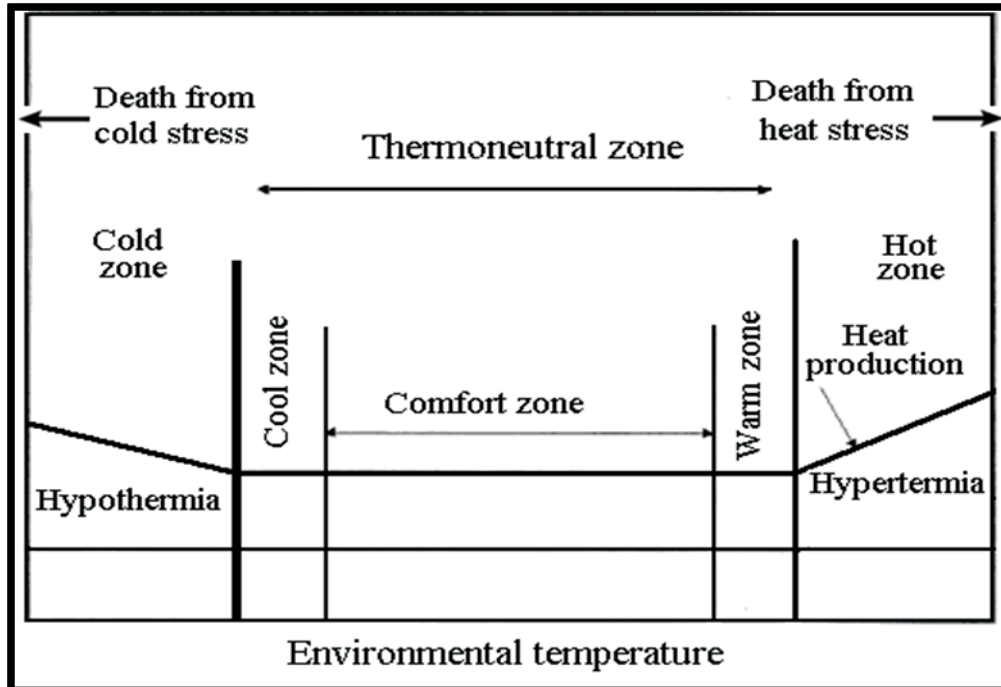
	Lower	Upper
	Critical temperature ( $^{\circ}\text{C}$ )	
Lactating sow with piglets	15 $^{\circ}\text{C}$ for sow 32 $^{\circ}\text{C}$ for piglets	26 $^{\circ}\text{C}$ for sow No practical upper limit for piglets
Prenursery , 3-15 kg	26	32
Nursery, 15-35 kg	18	26
Growing pigs , 35-75	15	25
Finishing pigs , 70-100 kg	10	25
Sow , boar > 100 kg	10	25
Dairy cow	-12/-1*	24
Newborn dairy calf	8-10	35
1 – day –old chicken	32	35
Finishing broiler	16	26
1-day-old turkey	35	38
Finishing turkey	16	26
Laying hen	16	27-29

The upper critical temperature is lower in exotic breeds and their crosses with indigenous breeds in comparison to pure indigenous breeds. The upper critical temperature for Haryana bulls is 32.0  $^{\circ}\text{C}$  but for its crosses having 50 per cent exotic blood of Holstein Friesian, Brown



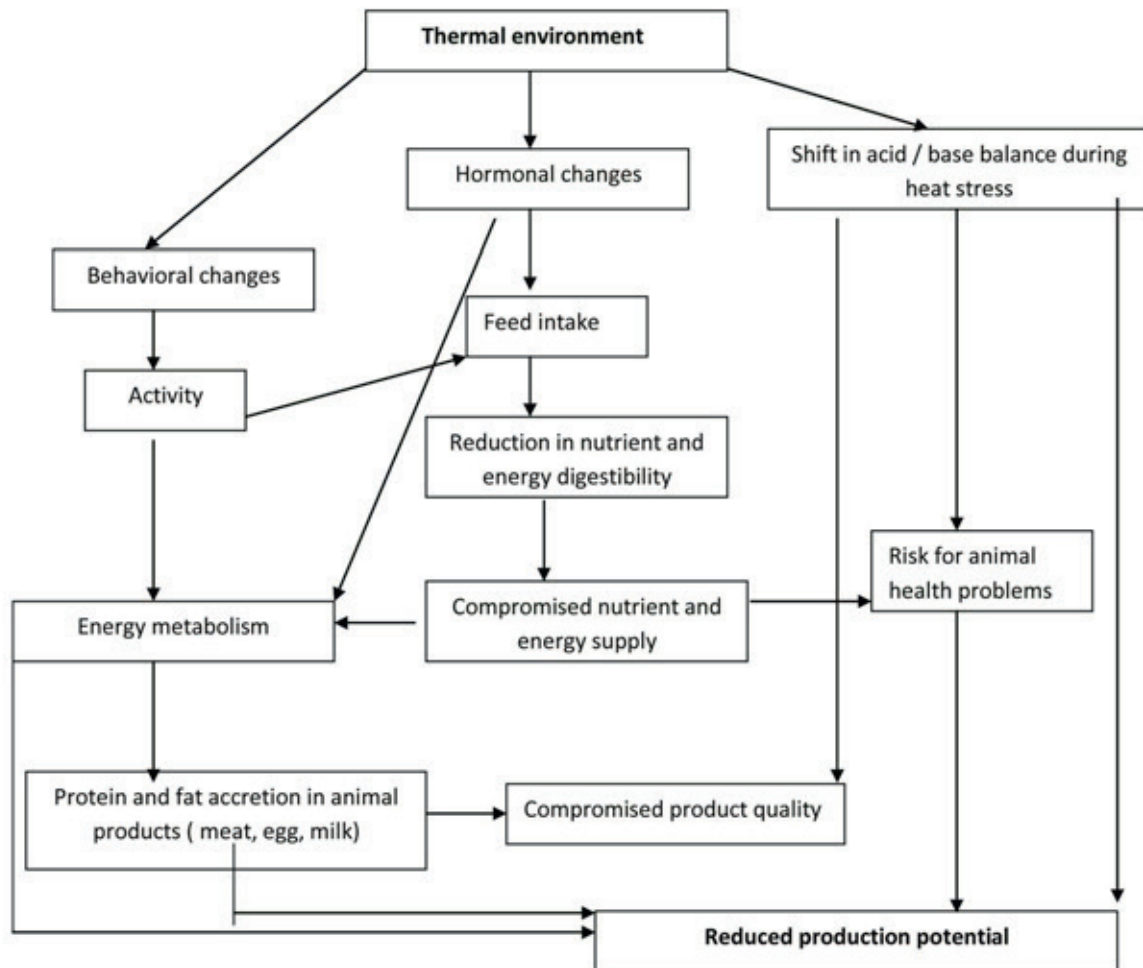
Swiss and Jersey is  $26.5^{\circ}\text{C}$ ,  $27.5^{\circ}\text{C}$  and  $29.0^{\circ}\text{C}$ , respectively. (Singh and Bhattacharyya, 1991). The upper critical temperature is at lower level in high producing animals in comparison to that in low producing animals of same species/breed because of greater heat load of higher metabolic rate.

**Fig. 2 Thermo Humid Comfort Zones:**





**Fig. 3 Schematic representation of the potential mode of action of inconvenient thermal environment on the production potential and risk of animal health problems**



### **Climate change on the metabolism and metabolic diseases of livestock**

At thermal equilibrium the difference between heat production and heat loss of the animal is zero. If heat production exceeds heat loss from radiation, convection, evaporation, and conduction, heat is stored and hyperthermia results in an increased body temperature. In farm animals with only a few sweat glands or none at all (poultry, swine), evaporation through rapid air exchange (panting) is one of the most important mechanisms for cooling the body. It is well known, that rectal temperature is a good indicator of internal body temperature. For this reason rectal temperature and respiratory rate are the usual indicators of heat stress even in cattle (Brown-Brandl et al., 2001; Kadzere et al., 2002).

Climate change and related variations in climatic conditions could have significant influence on the economic viability of livestock production systems in India. Any shifts in climatic conditions could affect animal agriculture in four primary ways; (1) feed –grain, production availability and price; (2) pastures and forage crop production and quality; (3) animal health growth and reproduction; and 4) disease and pest distribution.

The estimated annual loss in milk production due to heat stress is nearly 2% of the total milk production in India. The negative impact of temperature rise on total milk production for India has been estimated about 1.6 million tonnes by 2020 and more than 15 million tonnes by 2050 (Srivastava AK, 2010).



Climate change, particularly global warming, may strongly affect production performances of farm animals and the impact is shown worldwide on livestock production (Nienaber et al., 1999). During the heat wave in California in the year 2006, dairy producers lost more than 1 billion dollar in milk and animals. While vulnerability of animal production to climate change has hardly been documented in the context of India, experimental studies have been conducted on effects of season and climate on production, performance and other physiological parameters of dairy animals. These studies have shown that milk yield of crossbred cows in India (e.g., Karan Fries, Karan Swiss and Other Holstein and jersey cross) has negatively correlated with temperature humidity index (Shinde et al., 1990). On Macro level, research shows that the productivity of cross bred animals is lower in areas where the annual mean temperature is higher. Animals in production are more vulnerable to heat stress and further rise in temperature due to climate change will have additive impact.

### Climate change on animal reproduction and disorders

High environmental temperature may compromise reproductive efficiency of farm animals in both sexes affecting milk, meat and egg production and the results of animal selection. Wolfensen et al. (2000) reported that over 50 per cent of the bovine population is located in the tropics and it has been estimated that heat stress may cause economic losses in about 60 per cent of the dairy farms around the world. Pigs are very sensitive to hot conditions mainly due to low sweating capacity. Exposure to elevated ambient temperature decreases fertility even in poultry, rabbits and horses. Male birds appear to contribute more than females due to heat stress related infertility and high temperatures have a greater impact on semen quality (Karaca et al., 2002).

Stressful environmental temperature reduces the flow of blood to the uterine tract, damaging or killing the developing embryos, zygotes are most vulnerable to heat stress in initial stages of cleavage, silent heat and low conception rates are serious problems in buffaloes, low libido and poor quality semen are the main issues mostly affected by the climate change.

**Table 2. Temperature Humidity Index levels and effects on health disorders**

THI	Stress level	Effects
<72	None	Comfortable with high feed conversion efficiency
72-79	Mild	Dairy cows will adjust by seeking shade, increasing respiration rate and dilation of the blood vessels. The effect on milk production will be minimal
80-89	Moderate	Both saliva production and respiration rate will increase. Feed intake may be depressed and water consumption will increase. There will be an increase in body temperature. Milk production and reproduction will be decreased
90-98	Severe	Cows will become very uncomfortable due to high body temperature, rapid respiration (panting) and excessive saliva production. Milk production and reproduction will be markedly decreased.
> 98	Danger	Potential cow deaths can occur





### Climate change on animal health and spread of diseases

Climate Change drastically influences the reproductive and metabolic functions causing the ill effects on the health mainly resulted in

- (i) Temperature related illness and death
- (ii) The acclimation of the animals to meet the thermal challenges results in the lowered feed intake and alteration of many physiological functions that are linked with impaired health and the alteration of production and reproductive efficiency. The decrease in energy intake due to reduced feed intake results in a negative energy balance and partially explains why cows lose significant amounts of body weight when subjected to heat stress ( Beede and Collier, 1986).
- (iii) The increased respiration rate results in enhanced carbon dioxide being exhaled.
- (iv) Results of epidemiology study carried out in dairy cows indicated a higher incidence of Mastitis during periods of hot weather (Chirico et al., 1997)
- (v) Heat stress may be responsible for impairment of the protective value of colostrums in animals and for alteration of passive transfer of immunoglobulin in neonatal calves. (Donovan et al., 1986).
- (vi) Global warming will also affect the biology and distribution of vector borne infections.
- (vii) Several studies have assessed the relationships between heat stress and immune responses in cattle, chickens or pigs.

National Initiative on Climate Resilient Agriculture (NICRA at IVRI Izatnagar) demonstrated that expression of Cytokine and TLR-2 under heat stress was higher in crossbred cattle than indigenous Tharparkar breed that former were under higher immune stress.

Current health events driven by weather include African Rift Valley fever outbreaks, which can be predicted based on Indian Ocean weather some weeks to months before the outbreaks (Martin et al., 2008). Recent bluetongue and hantavirus movements into and across Europe as a result of warmer habitats for vectors (King et al., 2006 and Clement et al., 2009). The expanding range of fungus-induced destruction of amphibians (Pounds et al., 2006) and altered human food and water accessibility (Anderson et al., 2004). Water-borne disease incidence increases as extreme rainfall events become more frequent. Data demonstrate that more than two thirds of such outbreaks follow rain events in the upper quintile of intensity (Curriero et al., 2001)

### Modern concepts in the spread of diseases due to Climate Change:

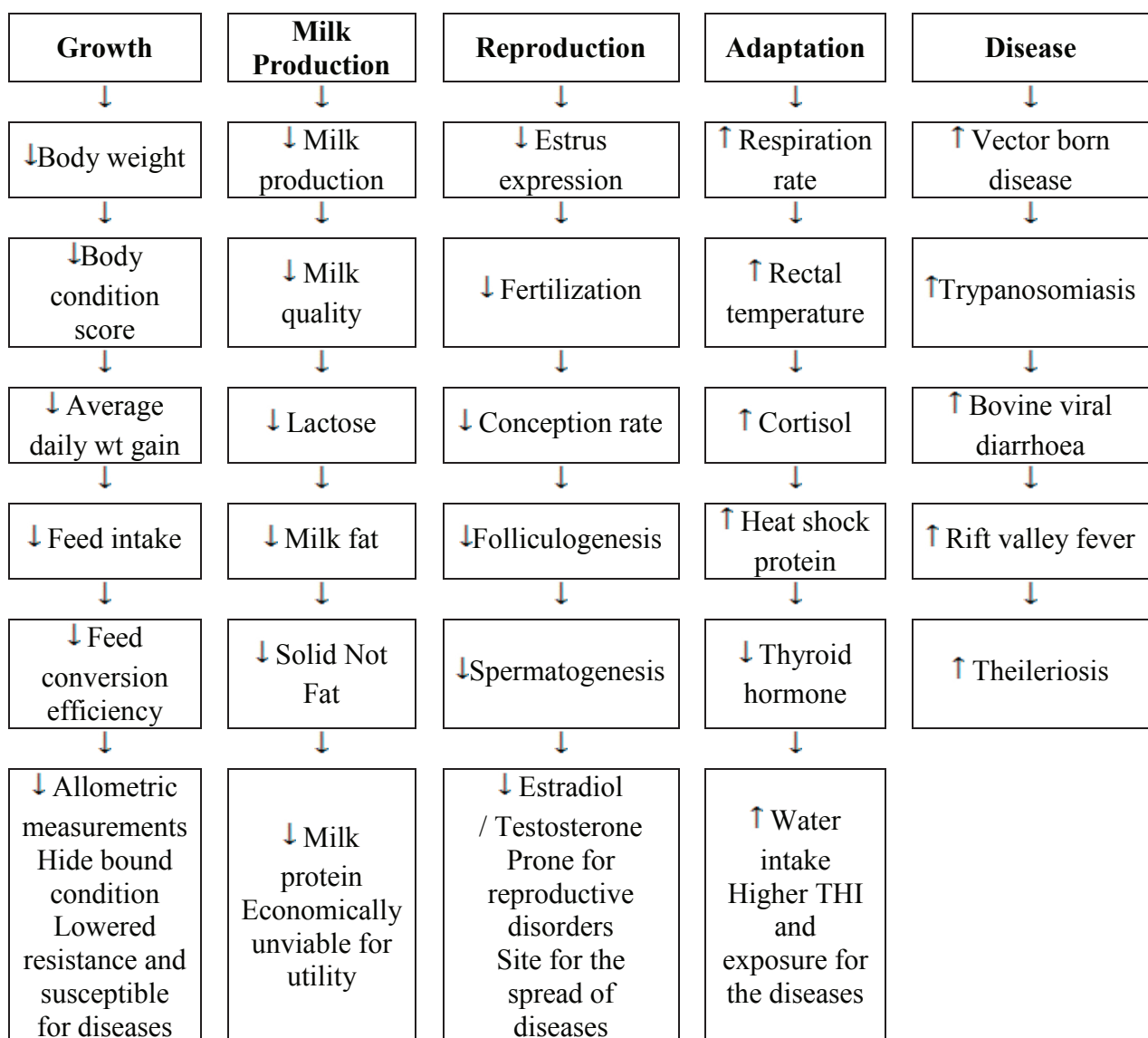
- Temperature- and precipitation-induced changes in higher elevation ecosystems may produce less obvious differential effects. Habitats of beneficial or foundation species may shrink, removing long time **physical barriers** to disease migration and allowing pathogens and vectors access to new populations or areas.(Lovejoy, 2008)
- Disease agents with external (eg, non host) portions to their life cycles are most apt to experience direct **biological impacts** from global climate change—in general, parasites and food-, water-, and vector borne diseases. *Argulus correghoni*, an ectoparasite of salmonids, for example, undergoes annual generational cycles under modest temperature rises, yielding a stair step increase in disease incidence(Marcogliese, 2008)
- Global climate change will indirectly affect disease agents whose transmission depends primarily on close **host-to-host contact**. Diseases predisposed by host stress, debilitation, malnutrition, or post disaster injury (which includes nearly all diseases) will see periodic spikes as populations respond to larger climatic effects and localized weather-based disasters
- The **inadequate food availability** during nesting is a great stressor that increases disease prevalence. At the same time, climate change may narrow available habitats, forcing birds of



several species to crowd into ever smaller areas of remaining resources and increasing the chance of within-species and cross-species disease transmission. This scenario is a likely explanation for the recent dispersion of highly pathogenic H5N1 avian influenza (Alto et al., 2009)

- **Favourable effects due to climate change:**
- **Exceeding species’:** upper or lower temperature ranges may cause species to shift habitats or die but a shift does not guarantee an increase in disease spread. Improved habitat suitability for agents or vectors does not guarantee they will enter, because other limiting factors may exist, such as competition, physical barriers, or predation.
- Habitat degradation may overwhelm “improvements” from temperatures and precipitation.
- GCC will decrease biodiversity, and pathogens, vectors, or hosts could be early victims.(Casis, 2009 and Lafferty, 2009).

**Fig. 4. Intrinsic and Extrinsic mechanisms of disease spread due to climate change**





Evidential Incidences of diseases in relation to Climate Change in India:

- The Ebola hemorrhagic fever, Rift valley fever, avian influenza H5N1, plague and Nipah virus are examples of newly emerging zoonotic diseases. Temperature and humidity are considered as a key feature of weather for dynamics of vector population and disease transmission.
- Clean water is known to promote breeding of vectors-mosquitoes like anophelies (Malaria), culex (Japanese encephalitis) and aedes (Dengue and chikungunya) while polluted water promotes breeding of *Culex quiquefasciatus* (Filariasis vector) (Joon, and jaiswal, 2012).
- The damage caused by vector-borne zoonoses is enormous. In India, seven genera of hard ticks and three genera of soft ticks have been reported (Ahmed et al., 2007). The most important genera are Hyalomma, Haemaphysalis, Rhipicephalus and Argus. Soil in several villages was found to be infested with two species of sandflies, *Phlebotomus argentipes* Annandale and Brunetti and *P. papatasi*. (Singh et al., 2008) The flea species *Xenopsylla cheopis* is prevalent in many parts of the country (Renapurkar D.M, 2008). The presence of *Leptotrombidium deliense*, the scrub typhus vector, in the Eastern Himalayas (Varma, 1969) has also been reported.
- In India, Japanese encephalitis virus has been isolated from 16 species of mosquitoes (ICMR, 2000). Avian influenza (H5N1) outbreaks were first reported in the SEA region in 2003. Sporadic outbreaks are continuing in many countries including in Bangladesh (2007), India (2006-2007), Indonesia (2004-2007), Myanmar (2006-2007) and Thailand (2003-2006). All these countries with the exception of Indonesia adopted a stamping-out policy for the control and eradication of avian influenza outbreak in poultry.
- The swine flu (H1N1) is a viral infection that originated from pig and was first isolated from pig in 1930s. The World Health Organization elevated the worldwide pandemic alert level to phase 5 (called when there is a strong signal that a pandemic is imminent and that the time to finalize the organization, communication and implantation of the planned mitigation measures is short), on 29<sup>th</sup> April, 2009. On 11<sup>th</sup> June, 2010 WHO raised the level to phase 6 (highest level) indicating that the flu has spread worldwide.

### **Climate change on livestock production systems and Health**

The first category of LPS includes grazing or pastoral system. These systems utilize more than 3 billion hectares of arid pasture, where agriculture is not feasible. Ruminants represent the most common domestic animals reared in these systems. The second includes the crops livestock system. The third category includes industrial or landless or commercial or Intensive livestock system. Small holder production systems existing in India are highly sensitive for the vagaries in climate. The possible impacts of climate change on various components of livestock production systems and remedial measures to control/counter are shown in Table 2.



**Table 3. Impacts of Climate Change on Livestock Production Health Systems and remedial measures**

Impact on	Resulting in	How livestock production Health system get altered	Remedial measure
Water Resources	Climate change will have a substantial effect on global water availability	<p>Water shrinkage results in shortage of drinking water resources, feed production and pasture yields including grasslands.</p> <p>Water intake for indigenous cattle increases from about 3 L per Kg dry matter intake at 10 ° C ambient temperature, to 5 L at 30 ° C and to about 10 L at 35 ° C. Water intake for <i>Bos taurus</i> at the same three temperatures is about 3, 8 and 14 L per Kg dry matter intake.</p> <p>ISRO has estimated that most of the glaciers in the Kashmir valley have disappeared and rest have decreased in size. Talib 2007 estimated a reduction of height of areas like Budgam to one fourth of the original size.</p>	<p>Water management is vital for increasing efficiency in the use of resources, adapting to mitigating climate change and sustaining productivity. Increase in organic content of the soil through conservation tillage, the soils water holding capacity increases, minimum zero tillage, erosion control, the use of crop residues to conserve soil moisture, improved soil cover through coverage through cover crops, increasing water infiltration, reducing evaporation and increasing storage of rainwater in soils are some possible solutions for the water management. Descheemaeker et al. ( 2010) cite feed management (improving feed quality, increasing feed water productivity, enhancing feed selection, strengthening grazing management), water management and animal management as three broad strategies for improving livestock-water productivity.</p>
Feeds	By altering the productivity of crops, forage and grasslands	<p>A rise in temperature to 30-35 ° C may increase the productivity of C4 species of crops which are less than 1 per cent of earths plant species, whereas C3 plant species accounted for 95% on earth which normally flourishes in cool, wet and cloudy climates, where light levels may be low, results in reduced harvest index due to high temp + CO<sub>2</sub> levels</p>	<p>Balance feeding and location specific feeding strategies, use of tested economically feasible technologies, supplementation of feed additives, plant derived liquid and yeast derived surfactant, bypass fat, protein in feed of high milk producing cows and buffaloes, densification and fortification of poor quality roughages are some of the scientific technical interventions to improve the feed efficiency which reduces the further contribution of livestock towards the GHG emissions.</p> <p>Carbon sequestration process using the traditional semi intensive systems of grazing methods have proved the beneficial effects in term long application. Feeding of over matured crops should be avoided. Treatment methods for delignification and breakage of lingo-cellulose complex have to be propagated.</p>
	Changes in composition of forages	<p>Temp + CO<sub>2</sub> increase results in decline in browse plants although marginal benefits in the legumes species.</p>	
	Quality in composition	<p>Higher temperature results in lignifications and reduces the digestibility and rate of degradation in the rumen.</p>	



<p>Livestock and Human health</p>	<p>Vector borne diseases</p>	<p>The expansion of vector populations into cooler areas (high altitudes) results in malaria and livestock tick-borne diseases or into more temperate zones such as blue tongue diseases.</p> <p>Temperature and humidity variations have a significant effect on helminth infections. The World Wild Life enlisted 12 germs that can spread into new regions due to climate change affecting human and wildlife health. These include Avian Influenza, Babesia, Cholera, Ebola, Intestinal parasites, Lyme disease, Red Tide (Algal Bloom), Plague, Rift Valley Fever, Tuberculosis, Sleeping Sickness and Yellow fever.</p> <p>Heat related mortality and morbidity could increase Increased rainfall variability is likely to increase food borne and water borne diseases.</p>	<p>Breeding of more disease resilient animals is the best approach to avoid the problem of transmitting the diseases. Emergence of gastro-intestinal parasites due to climate change should be minimised by adopting SOP for health management at both intensive and extensive livestock production systems. Production of vaccines against methanogens in the rumen is a potentially useful mitigation option for ruminants in land based grazing systems. (Wright and Klieve, 2011). Possibilities of using satellite images to predict outbreak of infectious diseases which reflect the reliable and up to date information on impacts of climate change on disease surveillance.</p>
<p>Biodiversity</p>	<p>Extinct of vulnerable species</p>	<p>FAO (2007) on animal genetic resources indicates that 20% reported breeds are now classified as at risk and that almost one breed per month is becoming extinct due to change in climate (CGRFA, 2007)</p> <p>A 2.5°C rise in global temperature would determine major losses between 20-30 percent of all plant and animal species.</p>	<p>Establishment of “Genetic Enhancement Centres” “Centres of Conservation of Indigenous Breeds” for a warming planet and rising oceans for the identified genotypes and their characterization and validation is required. Identification of genes resistant to biotic and abiotic stresses and genetic potential alleles for the adaptation to different bio climatic zones is required.</p>
<p>Pastoralists livelihood and socio-economic aspects</p>	<p>Resource based conflicts</p>	<p>The competition for the common property resources (CPR) and forest grazing resources leads conflict and competition resulting in excessive grazing and exploitation. This results in extinct of plant species, prolonged growing seasons, declined livestock yields,</p>	<p>Involvement of local communities and pastoralists in adaptation and refinement of traditional livestock production systems needs to be promoted. This is especially required for adopting good practices in respect of climate change adaptation and mitigation. Migration cycles are changing through time, due to changes in climate variability. It includes</p>



		pest and disease incidence and livelihood risks. Climate change management implications of livestock involve the financial implications which influence the socio economic conditions of the communities.	moving their livestock and families from one place to another, keeping different animal species making reciprocal arrangements with other pastoralist groups for access to pasture and water, developing water conservation techniques, observing early warning signs of impending drought and practising complementary livelihood activities. (UNDP Report, 2006; McKee, 2008; Trujano, 2008; Ehlers and Schetter, 2001)
Livestock systems	Intensive management systems are less sensitive than pastoral systems	Due to high sensitivity to climate change, small holder based pastoral systems will suffer complex, localised impacts of climate change in India.	Agro forestry is an integrated approach to the production of trees and non tree crops or animals on the same piece of land with the provision for carbon sequestration. Silvi pastoral systems in Central and South America in a Global Environmental Facility (GEF) funded project, resulted in 71 percent increase in carbon sequestration, increase in milk production by 10 per cent and farm income rose by 115 percent and reduced herbicide use by 60 percent is the successful model in livestock production system.

Understanding the complexity of the impacts of climate change on livestock production systems, the following concept of “**Climate Smart Livestock Production Systems**” has been discussed at various national and International Livestock forums with different nomenclatures. However, a sorted concept proposal with few successful models addressed on the issues is presented below.

### **Climate Smart Livestock Production Systems:**

Livestock Production systems are considered to be “Climate Smart” by contributing to increasing food security, adaptation and mitigation in a sustainable way. Any livestock management practice that improves productivity or the efficient use of scarce resources can be considered as “Climate Smart Livestock Production Systems” because of the potential benefits with regard to food security, even if no direct measures are taken to counter detrimental climate effects (Ayantunde et al., 2015) It works with the similar concept of Climate Smart Agricultural production systems (CSA) which aims at—Sustainability; increasing livestock productivity and income; reducing the climate change vulnerability; reducing emissions that cause climate change, protecting the environment against degradation and enhancing food security and improved livelihood of a given society. The concept of Climate Smart Livestock Production systems are in concurrence with the vision of enhanced productivity by enhanced resource efficiency (Leng, 1993 and Blummel et al., 2010).

The Climate Smart Livestock Production Systems focus on the following areas:

#### **1. Identification and classification of Livestock adoptable Bio-Climatic Health Zones:**

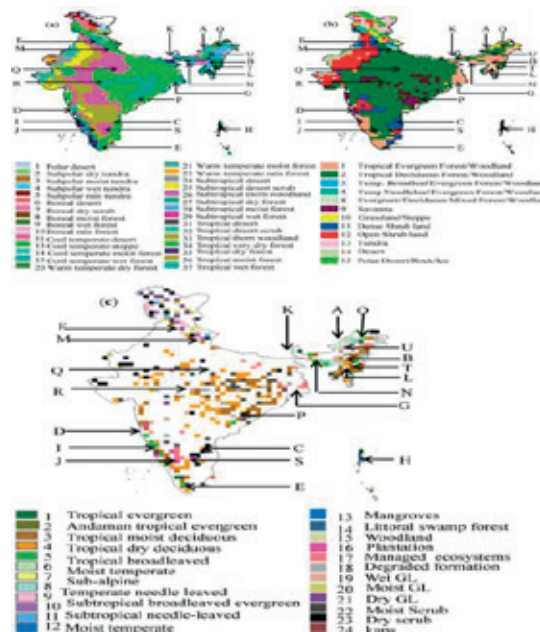
Establishment of long term observational and monitoring network of meteorological and ecological data for critical habitats, species and eco systems in the eco-regions of the country and



in Indian Himalayan region is needed. The suitability and ability of the breeds and species for their adoptability, survivability, production, fertility and productivity abilities in the changing climatic scenario need to be identified and a blue print on the bio climatic zones for the country in relation to the livestock adaptability need to be prepared. The scientific data on the rate of adaptability and comfortable zones and Temperature Humidity Indices for different breeds and species need to be restudied and compiled.

The variation in the species and breed population in the present breeding tracts over the decades is an indication of the farmer’s preferences, suitability of the breed/species, vector population and evidences of outbreaks and mapping of diseases to the changing climatic conditions. The national breeding policies and state priorities on the induction of breed/species should be based on meteorological data base and the rate of adaptability to the bio climatic zones.

Fig 5. Bio climatic Health zones of India



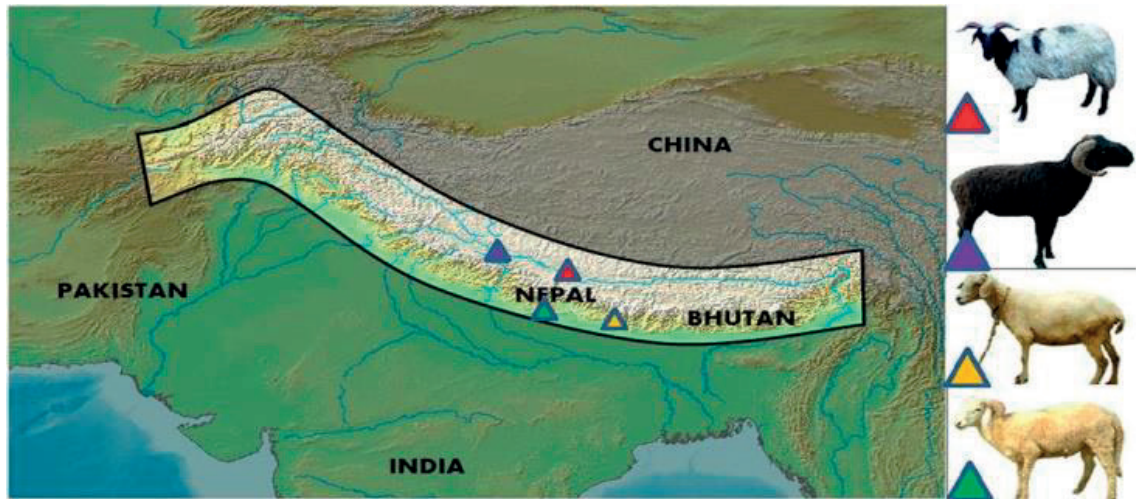
**Climate Change Adaptation and disease resistance traits using SNP Techniques:**

To identify genes underlying adaptation process, Gorkhale et al., (2016) genotyped genome-wide single nucleotide polymorphisms (SNPs) of four major sheep breeds living at different altitudes in Nepal and downloaded SNP array data from additional Asian and Middle East breeds. Using a  $d_f$  value-based genomic comparison between four high-altitude and eight lowland Asian breeds, discovered the most differentiated variants at the locus of *FGF-7* (*Keratinocyte growth factor-7*), which was previously reported as a good protective candidate for pulmonary injuries. A SNP upstream of *FGF-7* appears to contribute to the divergence signature. First, the SNP occurred at an extremely conserved site. Second, the SNP showed an increasing allele frequency with the elevated altitude in Nepalese sheep. Third, the electrophoretic mobility shift assays (EMSA) analysis using human lung cancer cells revealed the allele-specific DNA-protein interactions. Thus, it was hypothesized that *FGF-7* gene potentially enhances lung function by regulating its expression level in high-altitude sheep through altering its binding of specific transcription factors. Especially, *FGF-7* gene was not

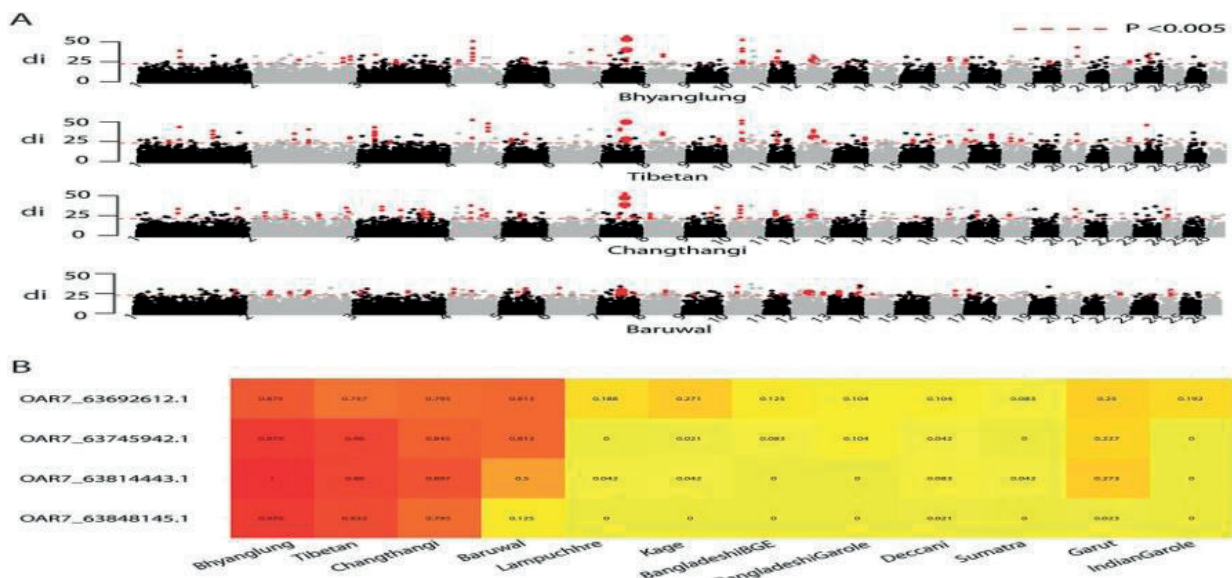


implicated in previous studies of other high-altitude species, suggesting a potential novel adaptive mechanism to high altitude in sheep at the Himalayas.

Fig 6. Indigenous sheep of Nepal (a) Bhyanglung ▲, (b) Baruwal ▲, (c) Kage ▲ (d) Lampuchhre ▲.



(A) Fig 7. Identification of directional selection for high-altitude adaptation.



(B) Manhattan plot of genome-wide distribution of di values for each of the four high-altitude sheep breeds. Red dots represent significant SNPs within merged regions. The larger red dots indicate common significant SNPs shared by the four breeds, and the threshold indicating signature of selection is denoted with a dashed red line. (B) A heat map of frequencies of major allele in high-altitude sheep of the top SNP loci for each tested populations.





Table 5. Genomic analysis identified a potential novel molecular mechanism for high-altitude adaptation in sheep at the Himalayas

S.No	Population	Number	Origin
	High Altitude Sheep		
1.	Bhyanglung	24	Nepal, Asia
2.	Baruwal	24	Nepal, Asia
3.	Tibetan	37	China, Asia
4.	Changthangi	29	India, Asia
	Low Altitude Sheep		
1.	Deccani	24	India, Asia
2.	Indian Garole	26	India, Asia
3.	Awassi	24	Middle east
4.	Lampuchhre	24	Nepal, Asia
5.	Kage	24	Nepal, Asia

## 2. Standard Operative Practices (SOP) in Livestock Management to be adopted in vulnerable areas of climate change

### a) Feeding Management:

The emission of 0.4 giga tonnes of CO<sub>2</sub> eq has been reported from feed production including the chemical fertilizer application. (FAO, 2013). The United Nations Food and Agriculture Organization has developed a concept of sustainable animal diets, which integrates the importance of efficient use of natural resources, protection of the environment, socio-cultural benefits and ethical integrity which recommends the supplementation of tree leaves, oil seed cakes, brans, urea-N and mineral mixtures to overcome the deficiency and to enhance the productivity. This approach also decreases emission of environment pollutants per unit of animal product formation. (Broadening Horizons, Feedipedia, 2016). 2.5 giga tonnes of CO<sub>2</sub> eq including forest and other natural vegetation and fossil fuels are emitted annually (FAO, 2013). Straws and crop residues are burnt every year causing environmental problem and soil degradation in addition to losing a valuable feed resource. Baling and silage making to compact and enrich the poor quality roughages will add to the feeding resources and improve the quality of feed which helps in reduction of emission of CO<sub>2</sub>.

Densified total mixed ration blocks or densified TMR pellets due to the addition of straw, oil seed meals and other nutrients into a complete diet without any deficiency of nutrient helps in improving the animal productivity and decreases the environmental pollutants. Use of unconventional feed ingredients with higher quality protein and energy source can reduce the shortage of feeds and environmental pollution. Several other simple and economically viable feeding technologies like wetting of straws, supplementation of good quality greens, increasing the nutrient density, supplementation of feed additives like antioxidants, minerals and plant products are of significance in the climate smart feeding strategies.

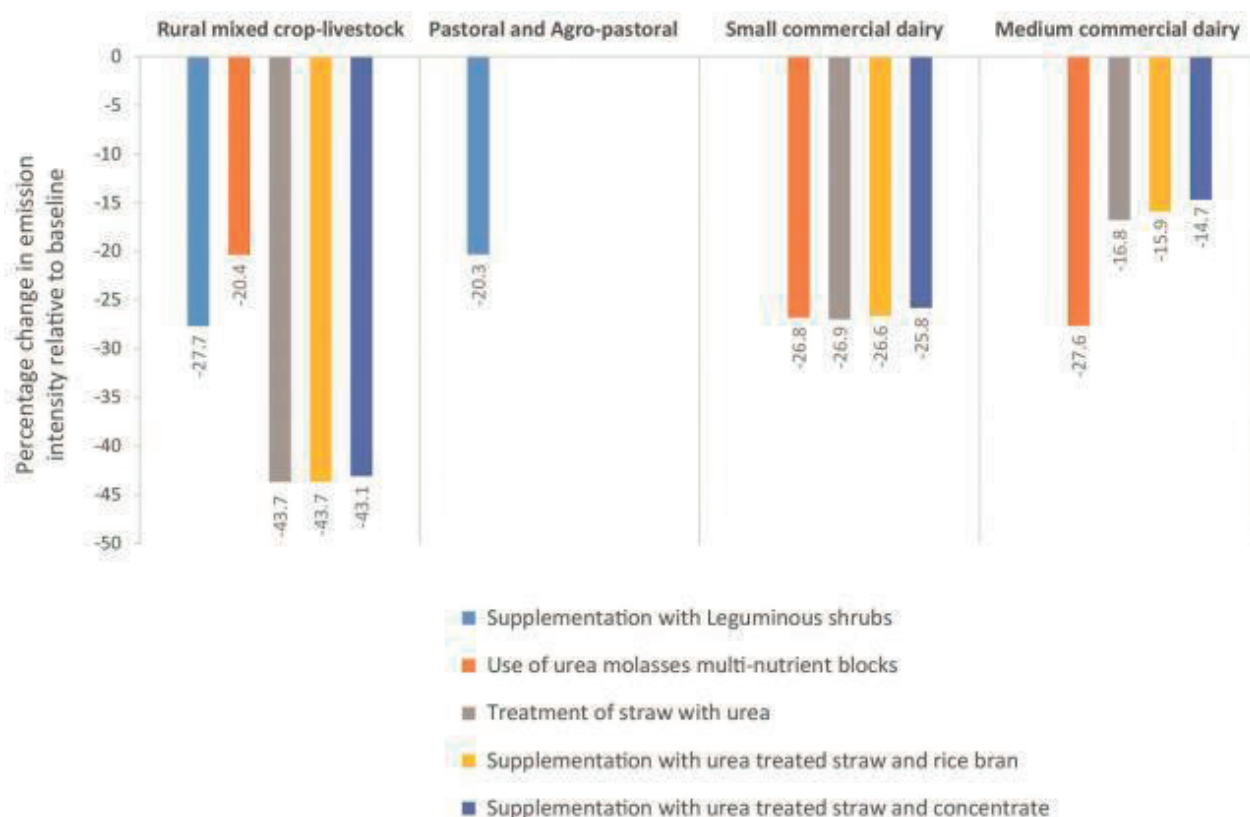
Grazing Management: Rotational grazing, which can be adjusted to the frequency and timing of the livestock grazing needs enhances the quality and digestibility of the forage, improves the productivity of the system and reduces the CH<sub>4</sub> emissions per unit of LWG (Eagle et al., 2012). Carbon sequestration results when grazing pressure is reduced as a means of stopping land degradation. Sanjiv Kumar et al., (2015) recorded effective carbon sequestration process through open grazing system in Deccani sheep flocks.

Use of IT solutions for Precision livestock feeding: *Herdman* is one of the most potential MIS application software packages for managing farm level data. Various application of DSS in dairy industry are cow culling decision support system, *MCLONE3*, -A decision support system



for management of liquid dairy and swine manure, *Dairypert*, *NutMan*, Dairypro-An individual Feed Allocation Decision Support System for the dairy farm , economics for opening a dairy farm for a given number of animals. In addition to MIS and DSS, data mining tools like Decision tree, Artificial neural network (ANN) and Fuzzy logic techniques are being used to predict the milk yield, estrus, lameness, mastitis et based on production, reproduction, health and behavioral parameters ultimately help in improving the efficiency of dairy farm. Data recording and analysis at farm level helps in precision feeding. The Food and Agriculture Organization (FAO) and the Global Research Alliance on Agricultural Greenhouse Gases (GRA) are collaborating on a project focusing on enteric methane emissions showed that the treatment of crop residues with urea results in an emission intensity reduction of 44% relative to the baseline. Supplementation of lactating cows with UMMB results in a reduction of emission intensity between 20-27%. These reductions are a consequence of the improved feed digestibility, increased animal feed intake and associated increases in milk production. All interventions returned a positive productivity outcome with increases in milk production ranging between 8-70%. Highest productivity impacts were found where urea treatment was used which provides both energy and nitrogen to the microorganisms in the rumen and thus improves the digestion and utilization of fibrous feed such as straw. It also provides readily available source of energy, protein and minerals for the dairy animal (Carolyn Opio, 2017)

**Fig 8:** Impact of feeding and nutritional approaches on emission intensity (kg CH<sub>4</sub>/kg milk)



**b) Housing Management:** Reducing the overcrowding and maximizing provision of shade, use of sprinklers for cooling animal shelters and improving the ventilation, animal houses installed with air cooling systems and Controlled environmental conditions for high profit and environmental friendly houses are the optional measures to combat the challenges of housing



management. Provision of community animal shelters, provision of and shelter and water bodies on the migratory tracts are the provisions to be made as policy initiatives.

Cyclone shelters and flood proof housing models need to be developed for the low lying areas of coastal areas of the country by the coordinated efforts of Veterinary institutions and National Institute of Designs and Civil Engineering domains of the reputed organisations.

### **c) Manure Management**

2.2 giga tonnes of CO<sub>2</sub> eq are emitted through manure storage, application and deposition (FAO, 2013). Hence, the practice of anaerobic digesters for bio gas and fertilizers, composting, improved manure handling and storage and application techniques like rapid incorporation should be adopted. Infrastructure adaptation measures like eco friendly housing and shade should be provided. Anaerobic digestion technology has been shown to be highly profitable in warm climates (Gerber et al 2008). Manure application practices can also reduce N<sub>2</sub>O emissions. Improved livestock diets, as well as additives, can substantially reduce CH<sub>4</sub> emissions from enteric fermentation and manure storage (FAO, 2006).

### **3. Institutional support for the technologies, strategies evaluation and capacity building of community**

NICRA, ICAR initiative works on understanding the unique traits in indigenous livestock responsible for higher heat tolerance, develop data base on genetic adaptation in cattle and buffalo, identify molecular markers under different stresses, to develop adaptation and mitigation strategies to thermal stress through nutritional and environmental manipulation, develop models for disease forecasting, identify markers for disease resistance, carryout epidemiological studies and technology demonstration and dissemination and farmers awareness programmes. The research findings from NICRA form a strong data base to develop “Climate Smart Livestock Production Systems” in the country.

Livestock Insurance schemes that are weather indexed may also be effective where preventative measures fail (Skees and Enkh-Amglala, 2002). Index based livestock insurance, based on satellite imagery are being piloted in several areas of drought prone northern Kenya (Barett et al., 2008; Mude, 2009). NABARD project initiated by Punjab State Council for Science and Technology, Government of Punjab illustrates sustainable interventions to achieve sustainable milk production through climate resilient bovine stock management. The project would benefit 3,000 small and marginalized farmers whose main occupation is livestock rearing in 3 vulnerable districts of Punjab namely Tarn Taran, Bhatinda and Ludhiana. The project includes maintenance of milch cross breed cattle and stray cattle in temperature regulated environment, improved feed management, and weather based livestock insurance. Cattle ponds for stray cattle would be developed with integration of climate smart elements. Weather based insurance tool would be developed which correlates variation in temperature and milk yield to evaluate monetary compensation. Weather forecasting and early warning systems are very important to enable the farmers to take preventive measures to reduce the morbidity and mortality from the extreme weather conditions should be institutionalized.

### **Assessment of Pastoral Resources**

Space technologies of ISRO have potential role to play in detecting and predicting the adverse effects of climate change, weather forecasting and warning systems, identification of potential grazing resources, vegetative mass, water bodies, ground water potentiality (Sarjan Reddy et al, 2007). Geographic information systems (GIS) are computerized information systems that allow for the capture, storage, manipulation, analysis, display and reporting of geographically referenced data (Marble, 1984, Parker, 1987, and Walsh, 1988) Essentially, the



technique is a combination of computerized mapping technology and database management systems (DBMS), in which spatial data sets from diverse sources are managed and analyzed.

Livestock herders are using GIS based maps, global positioning system (GPS) devices, mobile phones and the web to help them manage their flocks and herds. The IDRC-sponsored project 'sustainable management of pastoral resources in the Sahel', also referred to as the Cyber Shepherd initiative, set up in 2001 aims to enable Sahelian pastoralists to access accurate information on grazing lands in order to help them coordinate their movements and protect land and water resources during the dry season.

### **Mapping of ecologically sensitive pastures and development of rehabilitation centres**

The ecologically sensitive pastures like the alpine/ subalpine, Shola, Eastern Ghats, arid zones (e.g. *Sewan* grasslands of Rajasthan; semi-arid grasslands of Deccan; *Rollapadu* grasslands in the semi-arid tracts of Andhra Pradesh; *Banni* grasslands of Gujarat, etc.) are facing the highest threat due to unsustainable biotic interference. These pastures, with unique floristic compositions, have evolved to climax/ sub-climax stages over hundreds of years of ecological succession and it may not be possible to bring these back once these are destroyed. These ecologically sensitive pastures can be comprehensively mapped using GIS/ remote sensing and their extent worked out for each phyto-geographical zone.

#### **4. Special focus on animal health, value addition and quality control of livestock produce:**

A disease forecasting system providing a correlation between disease occurrence and changes in climate, vegetation cover etc. Need to be developed in line with National Animal Diseases Referral Expert System (NADRES).

National Animal Disease Reporting System (NADRS) in India is a comprehensive web based system with the objective to provide instant alerting system (SMS based) for outbreak of diseases, spread of diseases and remedial measures. This system will enable veterinary authorities to closely monitor, control and eradicate animal diseases, particularly those of a trans-boundary nature. NADRS is being implemented by the Department of Animal Husbandry, Dairying and Fisheries (DADF) through the National Informatics Centres (NIC). About 143 animal diseases scheduled in the prevention and control of infectious and contagious diseases in animals act, 2009 are included in this reporting system. The NADRS involves a computerized network, integrating both MIS and GIS, linking 7032 blocks spreads in five states of India including Rajasthan, Delhi, Gujarat, Kerala and Andhra Pradesh to the Central Disease Reporting and Monitoring Unit (CDRMU) in the DADF at New Delhi.

GIS has been included in decision support systems for control of infectious diseases in animals (Sanson et al 1994, Laube et al., 1997). GIS can be used to produce maps of disease incidence, prevalence, mortality, morbidity on farm, region, or national levels. The information is more easily understood when visualized on a map. There are a number of possible applications in the veterinary field. One of the most attractive uses is for the recording and reporting of disease information on a geographical basis. This allows for the spatial distribution of disease to be monitored over time. Other important veterinary applications relate to the epidemiological study of specific diseases, cluster analysis, modelling disease spread, and planning control strategies, mapping of ecologically sensitive pastures and development of rehabilitation packages. RS and GIS demonstrated potential applications in a number of public health problems including mosquito borne infections in relation to rainfall, soil salinity and resting areas, ticks and the variance, moisture and temperature of habitats, foxes and rabies through GIS and computer population models.



## 5. Value addition through processing of livestock products to reduce the effluents

Most of the commercial milk and meat processing units will be using several ICT tools to maintain quality standards and to improve efficiency in the processing plants which include dairy plants, feed mixing plants, slaughter houses, meat processing units, drug manufacture and vaccine production units etc.

Automation is a set of technologies that results in operation of machines and systems without significant human intervention and achieves performance superior to manual operation. The functions of many automatic milk and food processing equipments are based on ICT embedded technologies. Application of ICTs in processing of feed products reduces the manpower handling which helps to meet international hygienic and safety standards of the products e.g. Mother dairy. ERP software packages like SAP is useful in all commercial automation systems increase visibility into food-manufacturing operations by improving the transparency and traceability of vital business information. Enterprise Resource Planning (ERP) software database system of Suguna Poultry, India's pioneer in integrated poultry contract farming uses Oracle's Enterprise Resource Planning (ERP) software database system to enable its field agents to input data via Web sites on its contract growers' operations. Information tracked and consolidated includes number of chicks delivered, feed delivered, mortality rates and prices paid etc. This information is vital for the industry in scheduling of production, processing, and distribution of inputs including chicks, feeds, vaccines etc., and outputs including chicken and eggs.

The Indian Institute of Management, Ahmadabad (IIMA) has developed software-Dairy Information System Kiosk (DISK) which provides more functions at the milk collection centers, DISK database includes a complete history of all milch cattle owned by the farmers. Basic details of breed, history of disease, vaccinations, artificial insemination and pregnancy are maintained in the system. It also has decision support systems to forecast milk collection, and provide feedback to the farmers. Farmer's will also have access to a multimedia data base on large number of innovations captured by SRISHTI (an NGO working in co-operation with IIMA). DISK application is being pilot tested in two DCSs of Amul Dairy in Kheda district of Gujarat

M S Swaminathan Research Foundation offers mobile information service model for fisherman in partnership, with Qualcomm and TATA Tele services. It provides fisherman friendly mobile phones which help the in getting the details like weather, sea wave height and information regarding rich fishing zone.

Use of Touch screen Information Kiosk as part of the DFID-Animal Health Project, the Rajiv Gandhi College of Veterinary and Animal Sciences at Puducherry and Sri Venkateswara Veterinary University, Tirupati under RKVY Project, has designed a touch screen information kiosk to provide access to the farmers on dairy cattle management. The demand driven information in the form of text, pictures and animations with audio back up and could be accessed with the touch of a screen Even illiterate farmers can access the required information. The poultry Expert System (PES) was developed in Andhra Pradesh Agricultural University, (SVVU) using Visual Basic 6.0 and MS Access on selected dimensions including diseases, bio-security, summer management and drugs used in poultry farming. PES is an IT enabled tool for faster dissemination of expert advice on poultry farming in multiple locations at the same time

## Conclusion

There has been a significant shift in thinking around the way livestock is produced under conditions of changing climate. By using more appropriate genotypes, adapted and efficient indigenous animals, managing feed resources better, conserving grasslands and improving water



resource efficiency, significant gains can be made in securing more climate-resilient livestock production following the principles of “Climate Smart Livestock Production Systems”. These ideas need to feed into broader climate adaptation strategies, and gain greater support through collaboration of government and NGOs, research institutions and farmers. A particular focus, in this regard, should be practical and meaningful interventions to support small-scale livestock farmers, who play a vital role in supporting livelihoods and food security in India.

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## The Role of Ruminants on Environmental Pollution

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### **Abstract**

Ruminants are the least efficient subjects in producing animal protein, one of the main causes of deforestation, produce methane and thus contribute to global warming. With regard to the low efficiency, the literature reports information relating to industrialized countries such as the USA, without considering that more than 70% of cattle and almost all other ruminants live in areas of the planet, usually the poorest, where it is not possible to produce foods suitable for nourishing man. As far as deforestation is concerned, many of the areas currently used as pasture have been obtained by burning forests, but it is nowhere mentioned that all areas used today by man come from forest areas and that every year millions of hectares of forests are destroyed by fire.

As for the production of CH<sub>4</sub>, although ruminants physiologically produce methane, it is also true that the increase of methane in the atmosphere is preceded by the increase in heat, the main cause of which is the increase in CO<sub>2</sub>. The presence of this gas in the atmosphere is due to the use of fossil fuels, namely oil, coal and natural gas, the emission of which is due to multinationals that manage energy production.

**Keywords:** ruminants, deforestation, methane, global warming.

### **Introduction**

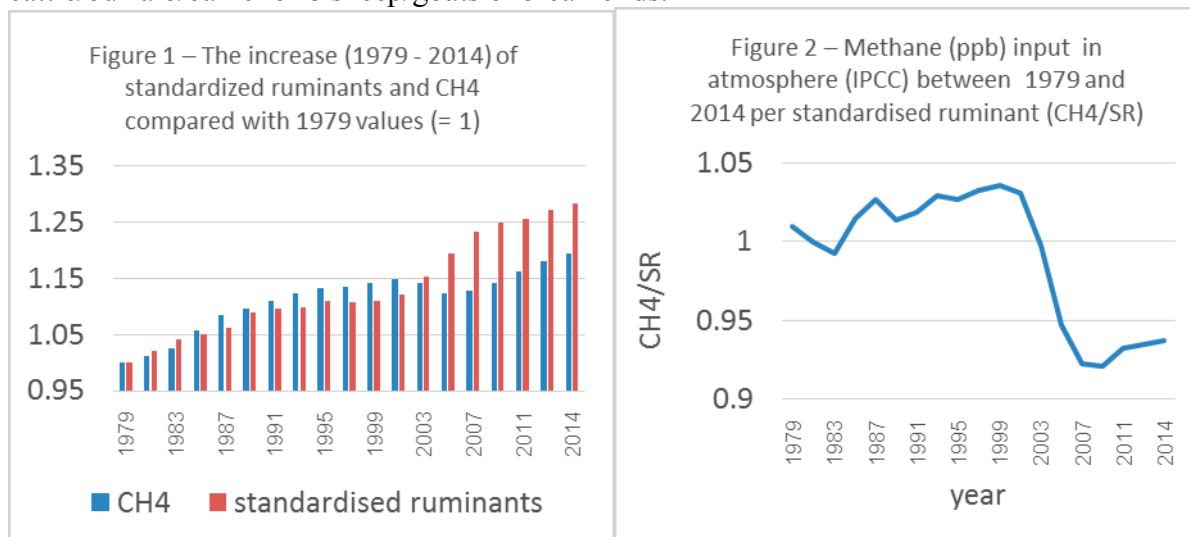
The increasing population will require new cultivated lands and/or further increase of the yields, also because new countries have been adopting the western alimentary style: as it has happened in China between 1980 and 2012 where the meat consumption has increased from 20 to 59 Kg pro capite. The developing countries increase the consumption of animal-derived products pursuing a status symbol. Animalists and scientists emphasize that consumption of animal-derived products is a non-secondary cause of GW. The main responsible would be ruminants that produce the methane that can be found in the atmosphere.

Currently, about 11-12% of the population (especially Asia and Sub-Saharan Africa) is undernourished; however, this value was 18% in 1990. One third of the food produced (1.3 billions of tons) is spoiled, due to inefficient logistics of distribution. It is likely that without this waste undernourished population would not exist. The food crisis of the last times has been determined by the speculation on foodstuffs and by grabbing of lands (land grabbing for 20 million hectares) in the South of the world, Africa but also Asia, for both the production of agrofuels and foodstuffs by developed countries and large state-owned companies, joint venture between public and private (De Castro, 2011). The lands are reclaimed from nomadic or sedentary populations who have owned them for generations but cannot demonstrate the ownership because in developing countries there is no guarantee of land rights but informal and traditional rules, recognized locally but not by international agreements. History teaches that less than 100 years ago the colonialists rather than renting those surfaces decimated the populations that owned them.



### Methane in the atmosphere and role of ruminants

Looking at the IPCC report, the value data of methane in the atmosphere (p.p.b) it is easily verified that the increase of methane in the atmosphere from 1979 up to 2014 (Figure 1) has followed that of the number of standardized ruminants (SR) that corresponds to 1 cattle/buffalo/camel or 8 sheep/goats or 5 camelids.



It is also easily verified that the increase of methane in the atmosphere from 1979 up to 2002 is higher than the number of standardized ruminants. After 2002, the opposite is true. A further proof of the spurious association between CH<sub>4</sub> in the atmosphere and the number of SR raised on the planet is given by the following observation. If the CH<sub>4</sub> value into the atmosphere is divided by the SR number, it is easy to see that the methane produced by the ruminants after 2002 decreases from 1.031 to 0.937 (Figure 2) ppb/SR while the digestive physiology has not been modified. Indirectly, this evidence shows that there are other sources that emit methane into the atmosphere that after 2002 were more active.

To be recalled here is that CH<sub>4</sub>, responsible for 18% of the greenhouse effect, is derived from the decomposition of solid urban waste in landfills and heating / anaerobic biomass digestion (both of these causes are difficult to quantify). Also derives from marshes (23%), fossil fuel extraction (20%), digestive processes of ruminants (17%), rice cultivation (12% - 18%). To these sources, it is necessary to add the emissions of oil activities and the considerable losses stemming from gas pipelines. The concentration in the atmosphere of 700 ppb estimated between 1000 and 1750 has increased to 1750 ppb in 2000 (+ 150%).

Between 1979 and 2014, according to FAO, standardized ruminants increased from 1 billion and 539 million heads (cattle = 1,217 billion) to 1 billion and 974 million heads (cattle = 1,494 billion), so they grew by 28%, while rice production rose from 216 to 741 million tons and the increase was 244%. If CH<sub>4</sub> sources (ruminant digestive processes = 17%, rice fields = 12%) are considered why scientists and press predominantly demonize the ruminants? It can not be excluded that they protect the interests of multinationals who produce seeds. The increase of methane in the atmosphere, as well as that of CO<sub>2</sub>, has been recorded especially since the advent of industrial era (end of XVIII century-beginning of XIX century) when the number of domestic ruminants in the planet was certainly lower than at present but the number of wild ruminants was absolutely higher. In the USA, for example, 89,3 million of bovine are currently bred but it is



known that in 1870, 60 million of bison together with a high number of wild ruminants were present (the estimates of the number of the Eurasian bison are unavailable). Since that time the CH<sub>4</sub> in the atmosphere has increased 2.5 times. CO<sub>2</sub> and methane increase after the temperature has increased, and not vice versa (Caillon et al., 2003). The atmospheric concentration of CO<sub>2</sub> has increased by 18% compared to the pre-industrial period (from 270 ppb to 319 ppb in 2005) since the carbon, stored in the subsoil for millions of years, has been used for energy purposes releasing CO<sub>2</sub> into the atmosphere, right in the period in which the number of forests on the planet decreased. The GW accelerates the melting of permafrost (20% of the world's land surface) from which large quantities of CH<sub>4</sub> are released thanks to the action of methanogenic bacteria. Methane has a half-life of 10 years and its release is aggravated by the CO<sub>2</sub> that has a half-life of 100 years. Despite this evidence, 483 coal-burning power plants have been planned between 2009 and 2019 and 710 more will be built between 2020 and 2030 (about a third in China).

It has been estimated that the human population present in urban areas would increase from 28% to 70 % from 1950 to 2050 (P. De Castro, 2015), and that 57 % of cattle population which is bred in Africa and Asia mainly for traction, will be replaced by mechanical tools. To sustain the current production instead of 1.5 billion cattle about 900 million heads will be sufficient, that being more efficient, will be fed diets with less forage and produce less CH<sub>4</sub>. In addition to the digestive processes of ruminants, the manure stocked for 3-4 months before its agronomic utilization also contributes to the release of CH<sub>4</sub> in the atmosphere. The daily introduction of manure and slurry in the biogas plants (in Italy there are 1300 active plants) results in the production of clean CH<sub>4</sub>. Doing so ruminants do not cause pollution, while providing clean energy. The use of this technique in a buffalo farm of 2500 equivalent adult animals, provides electric energy for 300 families. The heat produced by the plants can also be used to dry forages in an early vegetative stage (with protein content > 20%) also when it rains, saving 1 Kg of soy/head/day that can be used for human nutrition. In contrast to what is generally stated, soy is used to provide oil to humans (olive oil only satisfies 4% of lipid consumption) and extraction flour is given to animals! If we consider the activity of forage that is not used for pasture (hard parts and roots) in Brazil (1 head/ha) the organic CO<sub>2</sub> of the plants compensate that produced by bovines.

### **The Role of Ruminants on deforestation**

Although deforestation has slowed down, it still exists aggravating the GW. Industrialized countries have deforested both Europe and other countries in the past and today they invite developing countries to restore the forests. In Italy entire forests provided the wood for the construction of the roman fleet and for warming up the thermal baths, in the UK the forest that in the past concerned the whole Sherwood County (the Robin Hood's Sherwood forest) today is reduced to 423 hectares, the pau brazil (*Caesalpinia echinata*) of Atlantic mata was reduced in powder by the colonialists to produce a precious staining. It is also reminded that the deforestation of *Sequoia sempervirens* in the mountains at the western borders of Sierra Nevada was interrupted by the government of the United States only in 1890.



From my point of view, today the developed countries should increase the forested area and the Latin American countries should plant trees in the pasture. Between 2000 and 2007 in Brazilian Amazon 154,312 km<sup>2</sup> of forest has disappeared, an area equal to the surface of Greece and represents 0.134% of the total surface of the planet net of glaciers and deserts. If the deforestation drama is really in the interest of humanity, it is sufficient that each country allocates 0.134% of its forest area. Such countries should also devote more resources to preventing fires.

It is strange that only ruminant farming is held responsible for deforestation while other activities such as mining, soybean cultivation (almost 86 million hectares), cocoa (almost 7 million hectares), jatropha and other biofuel plants (for the production of 'Palm oil in Malaysia has been used 4.5 million hectares of forest), prey of precious woods are ignored. In Tanzania an emiro encircled 400,000 hectares for exclusive hunting rights by guarding them with armed troops in order to prevent Masai from using them as pasture. Grazing is considered unused land and then leased by the rulers to the best bidder. Above all, Africa is a wonderful reserve and land grabbing object. The African rainforests destroyed by humans have adversely affected biodiversity (the gorilla is now considered endangered by extinction).

### **Breeding of ruminants with intensive system and extensive system**

Despite the unique nutritional qualities of milk and meat supplied by ruminants, especially when raised to pasture, some scientists continue to recommend chicken meat, which we remember grows 50 times in 45 days and that of pork, animal that doubled the daily weight increment over the last 40 years, claiming that man is a species that physiologically should eat fruit.

Between 1979 and 2014 consumption of meat of ruminant increased by 140%. On the total, however, consumption of pork remained unchanged, that of poultry and ruminants increased significantly (+ 17%) and decreased (- 13%) respectively. Breeds of meat cattle, especially in Europe, provide hypertrophic muscle without flavor or are treated with hormones like in the US, maybe that push the consumer to prefer the less expensive meat of chicken and pork.

In my opinion, if bovine products continue to be less tasty and their nutritional characteristics will be increasingly dissimilar to those of freely grazed ruminants (optimum ratio  $\omega 3 / \omega 6$ , higher presence of CLA and unsaturated fatty acids) will be replaced, especially in Countries that do not have culinary traditions, by worms and crickets. The demonization regards red meat but not chicken meat although broilers between hatching and slaughter grow almost 50 times in 45 days and hence more quickly than a neoplastic mass.

With the intensive breeding to get one kg of meat of beef, 14 to 20 kg of dry matter are needed, which are made up of 9 to 13 kg of corn + soy. Intensive bovine animals account for less than 28% of the bovine present in the world. The others cattle and the two billion and 200 million of the small ruminants almost all utilize pasture which is practiced on areas that are not suitable for intensive agricultural process.



### **Why some scientists want to convince man to eat only fruit**

It is known that homo habilis evolved beyond its vegetarian roots and became omnivorous at least 1.8 to 2.5 million years ago; it dates back to that time the discovery of cuts on animal bones older than 2.5 million years. Between 1.8-2.5 million years ago and 600-350,000 years ago the volume (cm<sup>3</sup>) of human brain increased from 510-600 to over 1500 cm<sup>3</sup>. During this time it is assumed that hunting and meat consumption. These activities took place in groups and generated the first social relationships and the evolution of the use of the speech.

Despite this, animal activists and some scientists tend to convince the public opinion that man is a species that physiologically should eat fruit, like primates, closest relatives. It is well known that chimpanzees hunt other small monkeys (*Ptilocolobus temminckii* and *Colobus guereza*) and completes its feeding with small mammals. When they have been caught and killed, the meal is distributed to all members of the hunting group. Similar to that of the chimpanzee is the behavior of the Orange of Bonobo. Another example is *Macaca irus* (*macaca irus*) living in Indonesian coastal regions. It feeds on crabs and all kinds of crustaceans, molluscs, amphibians, worms and possibly fish trapped in some ponds in the low tide period. It also feeds on fruits, leaves and sprouts. Our ancestors, like the last indigenous peoples, have chosen, in my opinion, food driven by instinct. Our ancestors instinctively ate meat because they needed vitamin B12 which, unlike herbivores, are unable to synthesize and is present only in products of animal origin. Solutions to problems arising from population growth must be the result of its intelligence rather than the response to economic interests that do not always provide correct information. To sustain the current production instead of 1.5 billion cattle about 900 million heads will be sufficient, that being more efficient, will be fed diets with less forage and produce less CH<sub>4</sub>, but will give foodstuffs with lower nutraceutical characteristics.

### **Conclusions**

Solutions to problems arising from population growth must be the result of its intelligence rather than the response to economic interests that do not always provide correct information. To sustain the current production instead of 1.5 billion cattle about 900 million heads will be sufficient, that being more efficient, will be fed diets with less forage and produce less CH<sub>4</sub>, but will give foodstuffs with lower nutraceutical characteristics.

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## Milk Fatty Acid Profile: Influence of Feeding Model

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### Abstract

Nowadays, n6:n3 ratio and CLA content are two within the main important parameters to assess the nutritional value of milk for human health. This is due to their antioxidant, immunomodulating, anticarcinogenic and antiarteriosclerosis properties. By comparing the quality of milk from ruminant species in different experimental conditions, our research group showed that the feeding model is critical in determining milk fatty acid profile. In particular, we showed that, by increasing the forage content of the diet (F/C 70:30), it is possible to improve milk quality (high CLA content and n6:n3<4) without significantly affecting milk yield. The relation between milk fatty acids profile and pasture was also widely investigated in goats, whose milk is considered an important alternative in humans in case of allergy. In a number of studies carried out in goats, we showed that milk CLA content and n6:n3 ratio are also affected by pasture, stabling conditions, and SCD expression. All results suggest that feeding model is critical in determining milk quality, mainly in terms of human health.

**Keywords:** milk, fatty acid profile, CLA, feeding model

### Introduction

The interest of consumers for healthy foods is increasing year per year and, concerning milk, the fatty acid profile is considered one of the most important parameter to determine its nutritional value. Particularly, the n3 and n6 fatty acids, being essential for humans, must be provided through the diet. These fatty acids have double bonds in positions greater than C9, and humans cannot synthesize them due to the lack of the enzymes  $\Delta$ -12 and  $\Delta$ -16 desaturase (Hu et al., 2001). The main n6 fatty acid in cow milk fat,  $\alpha$ -Linoleic acid (C18:2), is required for the synthesis of arachidonic acid (C20:4, n6) and eicosanoids (Calder, 2012). The  $\alpha$ -Linolenic acid (C18:3, n3) can be converted to more biologically active polyunsaturated fatty acids with very long-chain: EPA and DHA. The recommended n6:n3 ratio for human health is between 2:1 and 4:1 (Simopoulos, 2002).

Similarly, high importance has been recently given to the CLAs that are reported to have immunomodulating, anticarcinogenic and antiarteriosclerosis properties (Pastushenko et al., 2000; Whigham et al., 2000). They form a group of positional and geometric fatty acid isomers derived from  $\alpha$ -Linoleic acid of which milk fat is the richest dietary source (Parodi, 1999). The major isomer of CLA is cis-9, trans-11, also called rumenic acid, it represents up to 80% of total CLA in milk. Ruminant CLA comes from two sources: (1) rumen biohydrogenation, (2) endogenous synthesis in the mammary gland by the activity of Stearoyl-CoA desaturase (SCD) on trans11 C18:1 (TVA, transvaccenic acid), the intermediate product of several PUFA (i.e.



linolenic acid) biohydrogenation (Griinari et al., 2000). Stearoyl-CoA desaturase (SCD) is also the rate-limiting enzyme in the biosynthesis of mono-unsaturated fatty acids (MUFAs) by the introduction of a cis double bond between carbons 9 and 10 in a spectrum of saturated fatty acids, with preference for C16:0 and C18:0. The expression of SCD is known to change according to animal species, tissue, dietary conditions and environmental factors such as age (Martin et al., 1999), insulin (Daniel et al., 2003) and CLA (Choi et al., 2000).

In recent years, our research group is working on the influence of feeding models on the fatty acid profile and CLA levels in milk from different ruminant species. In a very recent trial whose results are not yet published, forty dairy cows (around 100 days in milk) were divided into two groups and fed, along three months, diets with 60:40 or 70:30 forage:concentrate (F:C) ratio. Milk yield was slightly influenced by the feeding model, even if it has to take in account that the cows were in middle lactation. The total of saturated fatty acids was similar between groups while the group fed higher F:C ratio showed significantly ( $P<0.05$ ) higher values of  $\alpha$ -Linolenic acid (% of total FA: 0.117 vs 0.023) as well as total n3 fatty acids (% of total FA: 0.220 vs 0.124). By contrast,  $\alpha$ -Linoleic acid as well as total n6 FA were higher in group fed lower F:C, even if the differences were not significant. The n6:n3 ratio was significantly ( $P<0.01$ ) lower in group fed higher F:C ratio. Finally, this last group showed also significantly ( $P<0.05$ ) higher total CLA levels (% of total FA: 0.82 vs 0.48) mainly due to c9 t11 CLA (% of total FA: 0.82 vs 0.48).

Higher  $\alpha$ -Linolenic acid, total n3 FA and CLA were found in milk from cows fed significant portion of daily dry matter forage-based diets also by other authors (Butler et al., 2008; Slots et al., 2009; Stergiadis et al., 2012).  $\alpha$ -Linolenic acid was higher in milk from group fed higher F:C ratio, probably due to the higher content of this acid in the diet; according to Couvreur et al. (2006) forage contains a high percentage of unsaturated fatty acids, with  $\alpha$ -linolenic acid being the predominant n3 FA. The diet affected milk content of c9 t11 CLA and total CLA according to Bergamo et al. (2003) in buffalo, Sanz Sampelayo et al. (2007) in goats and Tsiplakou et al. (2010) in sheep. As suggested by Kelly et al. (1998) the higher concentration of milk CLA could be also due to the type and source of dietary carbohydrates, which may influence microbial fermentation altering the rate of CLA production or their utilization by rumen microbes and, therefore, the concentration of CLA in milk fat. In addition, the higher levels of  $\alpha$ -Linoleic and  $\alpha$ -Linolenic acid – the main precursors of c9 t11 CLA – in the forage can explain the higher CLA content in milk of group fed higher F:C ratio (Kemp and Lander, 1984; Kim et al., 2000).

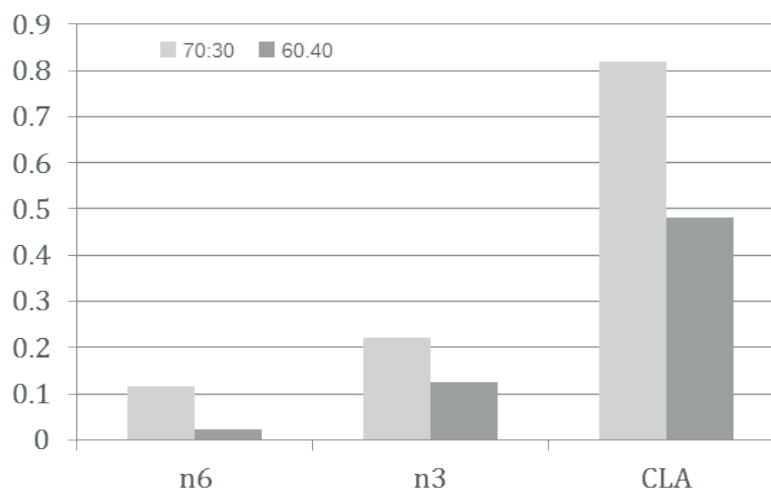






Figure 1. n6, n3 and CLA content (% of total FA) in milk from cows fed diet with 60:40 or 70:30 FC ratio

Goat milk, considered an alternative for consumers who are allergic to cow's milk, shows higher levels of butyric (C4:0), caproic (C6:0), caprylic (C8:0), capric (C10:0), lauric (C12:0), myristic (C14:0), palmitic (C16:0), linoleic (C18:2), but lower in stearic (C18:0), and oleic acid (C18:1). Thus, almost 20% of the fatty acids in goat's milk are short chain fatty acids, which are readily digested (Jennes, 1980) while the level of medium chain fatty acids (55%) is relatively high (Boza and Sanz Sampelayo, 1997). Increasing milk PUFA content through animal diet improves milk nutritional value; in an autochthonous goat population, the Cilentana one, bred in south of Italy, we have studied for several years the influence of pasture on milk fatty acids profile and CLA contents (D'Urso et al., 2008; Tudisco et al., 2010) as well as on the Stearoyl-CoA desaturase (SCD) gene expression (Tudisco et al., 2012; Tudisco et al., 2014). Our researches were carried out comparing results from groups housed in a stable and fed alfalfa hay and concentrate and groups which had free access to pasture (9.00 a.m - 4.00 p.m.), constituted by 60% leguminosae (*Trifolium alexandrinum*, *Vicia* spp.) and 40% graminee (*Bromus catharticus*, *Festuca arundinacea*, *Lolium perenne*). Also, these groups received the same levels of concentrate once back in stable. In all the trials milk yield was unaffected by feeding model.

D'Urso et al. (2008) found, in milk from grazing goats, significantly ( $P < 0.01$ ) higher fat content (53.7 vs. 61.5 g/day) as well as total PUFA (4.00 vs 3.67 g/100 g of fat;  $P < 0.05$ ) c9 t11 CLA (0.778 and 0.513 g/100 g of fat;  $P < 0.01$ ), t10 c12 CLA (0.046 vs. 0.029 g/100 g of fat;  $P < 0.01$ ) and total CLA (0.84 vs. 0.56 g/100 g of fat;  $P < 0.01$ ). The  $\alpha$ -Linolenic and  $\alpha$ -Linoleic acids were slightly higher for grazing groups thus n6:n3 ratio was not affected by the feeding model. Accordingly, Tudisco et al. (2010) reported significantly ( $P < 0.01$ ) higher fat (65.9 vs. 54.3 g/day), c9 t11 CLA (0.810 vs. 0.542 g/100g of fat), t10 c12 CLA (0.041 vs. 0.024 g/100g of fat), total CLA (0.87 vs. 0.58 g/100g of fat) and total PUFA (4.52 vs. 3.60 g/100 g of fat;  $P < 0.05$ ) in milk from grazing group. In addition, in such group they found significantly higher levels of  $\alpha$ -Linolenic acid (0.81 vs. 0.53 g/of fat;  $P < 0.01$ ) positively affecting n6:n3 ratio which was lower than in milk from stable group. In contrast with the results of Valvo et al. (2007) which found lower values of saturated fatty acids in milk of grazing sheep than in milk of sheep housed in stall, in both our trials this parameter was not different among the groups.

The higher levels of  $\alpha$ -Linolenic acid in milk from grazing group could be due to the higher content of linoleic acid in the pasture compared to the alfalfa hay (Tudisco et al., 2010) and this result agrees with Cabiddu et al. (2004) who compared milk fatty acid profile from grazing and stall-housed sheep. Griinari and Baumann (1999) pointed out that grazing in pasture is a good way for increasing the level of milk PUFA, as also confirmed by Banni et al. (1996) in sheep and by Dhiman et al. (1999) in dairy cows. Our results agree also with researches carried out on dairy buffalo (Bergamo et al., 2003), and dairy goats (Tsiplakou et al., 2010). In addition, Tudisco et al. (2010) found the highest PUFA values in milk of grazing group in June and September, according to the fatty acid profile of pasture along the trial as reported also by Tsiplakou et al. (2006) on grazing sheep.

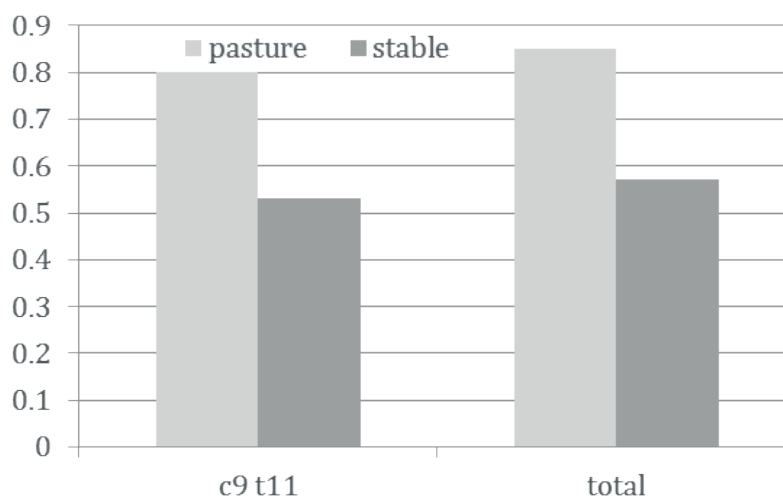


Figure 2. c9 t11 CLA and total CLA content (g/100g of fat) in goat milk (average data from our trials).

The higher value of c9 t11 CLA and t10 c12 CLA found in the milk of grazing groups could be also attributed to the higher level found in the pasture of  $\alpha$ -Linoleic and  $\alpha$ -Linolenic acids, which are recognized as their main precursors (Kemp and Lander, 1984; Kim et al., 2000). In addition, the lower content of c9 t11 CLA in milk of goats housed in stall, may also be related to the loss of precursor fatty acids during the hay making process (Aii et al., 1988). Also, the different levels of CLAs by sampling month in milk of grazing group, agree with that of their precursors in the pasture. However, particularly the c9 t11 CLA originates also by SCD in the mammary gland (Grinari and Bauman, 1999). According to Lock and Garnsworthy (2003), a part of increase of this isomer maybe due to the apparent increase of SCD activity in grazing animals.

Ruminant mammary SCD gene expression has been studied by examining mRNA levels in samples of mammary tissue (Beswick and Kennelly, 2000; Baumgard et al., 2002; Peterson et al., 2003) or in milk somatic cells (Boutinaud et al., 2002; Murrieta et al., 2006; Feng et al., 2007).

Tudisco et al. (2012) compared the expression levels of SCD gene in grazing vs stable-fed goats, by extraction of total RNA from milk somatic cells. The authors also investigated the correlation of this parameter with milk CLA content. The feeding model significantly affected CLA levels and SCD expression (arbitrary units:  $1.5 \pm 0.5$  vs  $0.39 \pm 0.11$ , for grazing and stable groups, respectively;  $P < 0.01$ ). According to Lock and Garnsworthy (2003), about 80% of c9 t 11 CLA is produced by SCD. The authors therefore focused their attention on the SCD activity which can be measured by comparing the product:substrate ratios of certain fatty acids. Indeed, there are four main products of SCD activity in the mammary gland of ruminants: C14:1, C16:1, cis 9 C18:1 and CLA, produced from C14:0, C16:0, C18:0 and trans11 C18:1, respectively. According to Lock and Garnsworthy (2003), the best indicator of SCD activity is the C14:1/C14:0 ratio because all of the C14:0 in milk fat is produced via de novo synthesis in the mammary gland; consequently, desaturation is the only source of C14. Increasing C14:1/C14:0 ratio values would indicate increase SCD activity. Tudisco et al. (2012) found higher values of this ratio in milk from grazing group, suggesting a higher SCD activity.



Successively (Tudisco et al., 2014), we investigated on the influence of season of grazing on goat milk FA profile, CLA content and SCD gene expression. Values of milk c9 t11 CLA and total CLA significantly increased from April (mg/100 g of fat: 0.51 and 0.54) to June (mg/100 g of fat: 0.90 and 0.98), decreased in July (mg/100 g of fat: 0.75 and 0.81) and again increased in August (mg/100 g of fat: 1.12 and 1.21), showing the same trend of both  $\alpha$ -Linoleic and  $\alpha$ -Linolenic acids in the pasture. By contrast, the SCD expression had an opposite behavior.

The relations between fatty acids profile of pasture forage plants and milk CLA content in sheep grazing from April to September were investigated by Meluckova et al (2008).  $\alpha$ -Linolenic acid in the pasture decreased from May to August, and subsequently it slightly increased in September. Milk CLA showed the same trend.

## Conclusions

The feeding model highly affects milk nutritional value. Increasing diet forage:concentrate ratio and/or breeding ruminants at grazing results in significantly lower milk n6:n3ratio and higher levels of CLAs, widely recognised as having benefits for human health. Finally, it has to be underlined that, in our experimental conditions, the improvements in milk fatty acid profile has been achieved without detrimental effects on milk yield.

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## Development of MBY in Yunnan and Diverse of Grass Resources

In P.R. China

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### Abstract

Cattle (1/4 Murray Grey, 1/2 Brahman, and 1/4 Yunnan Yellow cattle crossbred, MBY) has been inter se breeding since 1980s. The results provided the genetic information to match the standards of new beef breed in South China. Adult bulls weigh from 600 to 900 kg and cows from 450 to 600 kg in grazing conditions, which is intermediate in size among beef breeds. They readily convert grass to beef and finish economically with high quality of beef. Bulls are characterized by a distinct hump over the shoulder and neck. Cows calve easily, have high mothering ability, and milk well to rear quick growing calves. The calves weigh about 30.0 kg at birth and wean at 6 months under 24 hour grazing system. MBY cattle are natural polls or horns, and vary in coat color from red, yellow, silver, spotted to pure black. MBY heifers mature early at the age of 12 months and show puberty. All the performances inherit stably. MBY cattle have high parasite resistance and heat tolerant ability. They can thrive not only in hot weather, but also in snowing weather. MBY cattle are now extensively being used as paternal resource to cross Yunnan Yellow cattle and its hybrids. Yunling cattle and its hybrids had better adaptability in South Yunnan, of which the body weights in different growth stages were prior to those of local yellow cattle, displaying its superior breeding potential for extension in Yunnan province and South China. MBY cattle not only gained sound profits through developing corn silage and sugarcane tip's silage as local roughage resource for finishing F1 weaned calves of Yunling cattle × Dehong Yellow cattle, but it reduced resource waste and pollution, and decreased feeding costs in beef industry to promote more economic, social and ecological benefits. Better effects of hybridization was considered a reference as an example for Yunling cattle extension in South China through the crossbreeding combination of (Yunling cattle × Dianzhong Yellow cattle), of which F1 generation were showing the characterizations of tolerance to crude fodders, resistance to heat and tick, good adaptability and easily care.

**Keywords:** Yunling cattle, crossbred, grass, China



## **Advances in Meat Processing Technology, Slaughtering House Facility and Development: JARVIS**

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### **Abstract**

JARVIS was founded in 1902 in Connecticut, US. always committed to the research, development and manufacture of meat processing machinery, especially for slaughtering tools. All of the tools are approved by the United States Department of Agriculture (USDA) and European Community (EC) to meet stringent quality standards. In order to reach better service for Chinese customers and for continue developing business in China, in February 2006, Jarvis US invested and established a finance company in Beijing which is Jarvis Machinery Manufacturing (Beijing) Corporation. Since year 2006, Jarvis has provided advanced slaughtering tools and meat processing equipment for many domestic enterprises. Besides slaughtering tools, Jarvis also provides customers with high quality consumables and accessories. At present 90% of domestic slaughtering or meat processing enterprises have strategic cooperation with Jarvis China. In addition, has been repeatedly invited to the oversea exhibitions, such as in Thailand, Jarvis China participated in every conference of VIV Asian International Exhibition of Intensive Animal Husbandry. VIV Asian International Exhibition of Intensive Animal Husbandry is an international, professionalized, commercial and modern high-end animal husbandry brand exhibition, is one of the most high profile and charisma conference in Asia, Jarvis company staff actively broadcast products in exhibition, get acquaintance of customer needs, provide customers with professional slaughtering and meat processing machinery scheme.

**Keywords:** meat processing machinery, slaughtering tools, animal husbandry



## Thai Dairy Productivity: Milk Quality and Days Open

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### **Abstract**

Dairy farming plays an important role in agricultural sector in Thailand at which Thai dairy products have been growing well in ASEAN markets. Dairy products have increased due to expansion of domestic markets and exportation to ASIAN countries. However, numerous difficulties are affecting dairy productivity particularly farm and feeding management. The effects of environment on milk production, milk quality and fertility have been studied and several recommendations and suggestions were reported. The current knowledge and experiences of dairy farmers and improvement in genetics and milk processing are acceptable considering the small and medium scale dairy farms are operated under tropical climate conditions. During the last three years, the governmental policy (Thai Milk Board) of dairy farming, school milk program and dairy industry put down a great impact on productivity and milk quality. In 2016, an average somatic cells in bulk tank milk was dramatically decreased to be at 464,000 cells/ml with 12.23% of total solids. Meanwhile, days open (calving to conception) was 199.75days and milk production was 4,310 kg/lactation. Furthermore, the cost to produce milk of Thailand was 14.66 baht/kg. In addition, average milk consumption of Thai people was only 18 liters per head per year. Therefore, Thai dairy productivity, milk quality, days open and also milk consumption remain to be improved. Several studies showed that dairy herd health and production management program (DHH&PM) is a key success to improve dairy productivities but how to apply DHH&PM to every dairy farm in Thailand is still the question.

**Keywords:** dairy productivity, milk quality, days open, DHH&PM, Thailand

### **Introduction**

Dairy farming in Thailand began in 1926 by Indian immigrants and after World War II (1945) consumption of milk and other dairy products began to be promoted by the Thai government. During 1950s, Thai and European governments had cooperatively established commercial dairy production. Artificial insemination service centers and dairy cattle farming project were also established by government organization. Dairy farming has been extensively introduced to Thai farmers during 1960s. At that time, only 1-5 cows were raised in each farm (Pichet, 2011). Department of Livestock Development (DLD), Dairy Farming Promotion Organization of Thailand (DPO), and Nongpo Dairy Cooperative have been responsible for intensively promoting dairy farming and dairy industry ever since. It was operated as a smallholder dairy farming system, however, more recently it has been developed into an intensive production system with mostly 30-60 cows per farm. The annual report from Office of Agricultural Economics (2016) showed that the current number of dairy cows and dairy cattle in Thailand were 241,824 and 626,216, respectively with the total milk production of 1,126,513





tons/year. The average milk production per cow per day was 12.89 kg/cow/day. Furthermore, the cost to produce milk was 14.66 baht/kg with price of milk at 18.01 baht/kg (Table 1). A total of 212 milk collecting centers and 121 dairy factories are running in Thailand. Despite that, powdered milk is being regularly imported into Thailand. Exportation of Thai dairy products has increased 12.52 % and importation of powdered milk and dairy products has increased 2.99% (Office of Agriculture Economics, 2017). The average milk consumption in Thailand was only 18 liters per head per year. Approximately 40% (1,323/3,328 Ton/day) of milk was provided to the school milk program. Milk quality and reproductive performance of dairy cattle are concerned as important information to evaluate Thai dairy efficiency. Recently, the average milk production of each cow was  $4,310 \pm 1,078$  kg/lactation with % milk fat, % milk protein, and somatic cell counts (cells/ml) were  $3.57 \pm 0.65$ ,  $3.10 \pm 0.31$  and  $410,000 \pm 67,000$ , respectively (DLD, 2016; DPO, 2017). Recently, the 20 years plan of Thailand 4.0, a ten years plan of national strategic plan for dairy and dairy products (2017-2026) and a five years plan of national food and mount disease (2017-2021) are paying attention to support dairy farmers and dairy industry with targets of international standard of milk quality for consumer, increase both domestic consumption and exportation of Thai dairy products. Large scale dairy farms and area wide integration approach as well as pipe line milking system and TMR feed center are introduced.

Typically, crossbred Holstein Friesians cattle are raised under tropical conditions. Genetic improvement program of dairy cattle in Thailand has been intensively running and annual dairy sire summary has been continuously reported by DLD and DPO for over 10 years. Crossbreeding was used to combine the advantages of Holstein for milk yield with native of local cattle or crossbreds for tropical adaptability and fertility. This system has helped to increase the milk production in the following generations. However, when Holstein fraction is higher than 90%, cows require highly intensive management and health care. The percentages of Holstein breed within the cows and the quality of roughage sources showed a certain effect on calving interval and services per conception but not really on milk performances (König et al., 2005). We accepted that Thai-Tropical Holstein breed was selected for genetic potential on optimal production, longevity under tropical environment, trouble free and maximum profit. However, most Thai dairy farmers lacked sufficient knowledge and understanding of genetic selection and mating strategies. Most of farmer did not know how to used EBV and among farmers who knew the benefit of EBV for selection, they do not frequently used it (Borisutsawat, 2016). Lately, Jersey and Brown Swiss breed were introduced to increase totals solids in milk according to goal of milk quality of Thailand.

### **Milk quality**

Food chain on milk production in Thailand was set up under DLD regulation using GAP for dairy farm (Dairy Farm Standard/Good Agricultural Practice; GAP) and GMP for milk collecting centre (Good Manufacturing Practice; GMP) and then FDA (Food and Drug Administration, Ministry of Public Health) is taking care dairy products to consumer using GMP to certify all dairy factories (Aiumlamai et al., 2013). Recently, all GMP at milk collecting centers and dairy factories are regularly certified and numbers of certified GAP dairy farms are increasing. Bulk tank milk from dairy cooperatives has been regularly sampled by DLD and found that a large number of dairy farms have problems with high levels of somatic cell counts (SCC) and low percentages of total solids (TS), fat, and protein in their raw milk. Mastitis remains the most important problem for dairy farming in Thailand. Factors affecting milk quality



and quantity in Thailand were studied and found that milking hygiene, mastitis control program, and proper milking machine were the major factors causing mastitis in Thailand. It is clear that feeding has a impact on milk composition (Aiumlamai et al., 2012). The pricing of milk quality at dairy factory (bulk tank milk) is determined by %Fat, % Protein, % SNF (solids not fat), % TS, Freezing point, MB, SPC, LPC, Coliform, SCC and antibiotics while the pricing at dairy cooperatives (bulk milk) is determined by antibiotics, MB, %Fat, SNF, SCC and farm standard. In 2015, Milk Board of Thailand began to set a strong policy on quality of raw milk using in the school milk program of Thailand. The policy aimed to lower somatic cells to be below 700,000 cells/ml and increase total solids to be above 12%. In each semester, regulation of milk quality for school milk was stronger. The improvement of milk quality was clearly showed in 2016, DLD reported the milk quality in bulk tank milk with the average SCC at 464,000 cells/ml and 70% of milk collecting centers had their SCC lower than 500,000 cells/ml. In addition, the average TS was 12.23 of which only 60% of milk collecting centers had TS higher than 12.15%. Furthermore, % of fat, protein, and SNF were 3.7, 3.07 and 8.53, respectively. In 2017, regulation of milk quality for school milk is set at level of SCC to be below 550,000 cells/ml and % of TS to be above 12.25. The goal of milk quality of Thailand is set for the level of somatic cells to be below 400,000 cells/ml and % of total solids in milk must be higher than 12.50 (a ten years plan of national strategic plan for dairy and dairy products 2017-2026). Therefore, all producers must try harder to get better quality of raw milk in the process. This policy will definitely has a great impact on improvement of milk quality of Thailand. However, there are a lot of discussions on 12.5% of TS concerning genetic of Thai-Tropical Holstein breed and feeding in dairy farms in Thailand.

### **Reproductive performance: days open**

The reproductive performance of dairy cattle in Thailand remains lower than that of the general target for dairy cattle, as the days open and conception rates are 140-200 days and 20-60%, respectively. In 2016, DLD reported the average calving to conception, average calving interval, average age at first calving of dairy heifers were 199.75days, 466.7 days, and 33.01 months, respectively. Factors negatively affecting fertility in dairy cattle in Thailand are nutrition deficiency, improper feeding management, reproductive diseases, heat stress, inappropriate artificial insemination and inadequate herd health services. Studies have shown that during the hot and humid season, in which the temperature and humidity index (THI) was higher than 75, the conception rate was significantly decreased. Furthermore, heat and humidity stress may lower milk yield during summer and rainy seasons (Aiumlamai et al., 2012). Poor body condition scores (BCS) are usually found in postpartum dairy cows which negatively influence reproductive performance. Ovulation and estrus after calving are delayed, hormonal treatments could improve pregnancy rate and shorten days open. Responses to any treatment are variable and related to those factors that influence duration of post partum first ovulation such as BCS and parity (Aiumlamai et al., 2009; Kaewlanum et al., 2008; Ninnasopha et al., 2014; Rhodes et al., 2003). Therefore, reduction of days open need to be seriously concerned since it is an important key to improve productivity of dairy cattle in Thailand.

### **Nutrition: production, health and fertility**

Crop residues and rice straw are widely used in feeding practices, which adversely affect milk production, milk quality, fertility, and health. Rice straw treatment, tropical grasses, total mixed ration (TMR) and silage were studied and introduced to improve milk production and quality (Wanapat, 2005). Studies on metabolic profiles in dairy cows showed subclinical ketosis or negative energy balance or acidosis and laminitis. Negative energy balance (NEB) in



periparturient dairy cows is commonly found in Thai small holder farms. These NEB cows showed suboptimal milk yield and had a delayed first estrus after calving and lower conception rate (Rukkhwamsuk, 2010). Ruminant acidosis causing laminitis was found in Thai small holder farms and related to reduction in fertility (Seesupa et al., 2016). This problem is likely to be caused by improper feed and feeding management. Dairy cows raised in small holder farms are confronted to the problem of improper nutritional management and its consequences, which may lead to impairment of their production, health, and fertility. Farmers should pay attention on feed and feeding management during transition period and optimized BCS at calving, to properly prepare the rumen function and to offer a good quality and balanced diet to the cows. However, limited of proper feed and cost of feed in each region lead to improper feeding in dairy farms reflected to milk quality and production. Therefore, nutrition and feeding play role in health, milk production and compositions as well as fertility. Dairy farmers and dairy extensions have to understand and be able to apply proper feed for dairy cattle.

### **Health related problems**

Several studies reported that mastitis, repeated breeder, metritis, metabolic problems, blood parasitic infestations and lameness were the most common clinical problems in dairy cows. For dairy heifers, major problems were respiratory infections, parasitic infestations, and lameness. The presence of IBR, BVD, neosporosis, ureaplasmosis, brucellosis, leptospirosis, blood parasites as well as foot and mouth disease (FMD) in dairy cattle have been reported in several studies in Thailand, which affects health, fertility, and production (Aiumlamai, 1999; Aiumlamai et al., 2010). *Trypanosoma evansi* infection in cattle could cause abortion, 20-40% of a subclinical trypanosomosis was reported and resulted in a decrease in milk yield (Pholparka et al., 1999). Recently, bovine respiratory disease complex syndrome seems to highly infect dairy cattle in many areas causing low milk production and poor fertility and death was found in severe cases. Lately, contamination by mycotoxins in dairy feed is a problem in dairy cattle in Thailand which causing low production and fertility and death can be found in some cases. Health care services are provided by veterinarians and para-veterinarians under DLD, DPO, various dairy co-operatives, Veterinary Teaching Hospitals, and private sectors. Veterinary bovine practitioners are working on individual sick animal and only a few veterinarians using herd health management to improve productivity of the farm (Aiumlamai et al., 2012).

### **Herd health and production management (HH & PM): a key success for productivity**

Although herd health and production management (HH & PM) has been practiced for over 20 years in Thailand, the official support from Ministry of Agriculture was started in 2010. HH&PM is aimed to improve performance of dairy cows in milk production, milk quality, reproduction, and growth. Recently, it had been intensively practice under government policy from DLD and Dairy Cooperatives, Ministry of Agriculture during this decade. Mostly HH&PM pay attention on milk quality and fertility while the traditional services focus on clinically diseased animal. Several studies showed that HH & PM could improve productivity in Thailand (Rojanasthien et al., 2009; Tanchaen et al., 2016). Therefore, HH & PM services should be applied more in this region because these practices are ultimately meant to improve income, and/or reduce costs, control diseases, and enhance animal health, production should be regarded in an economic perspective. The records kept by farmer, practitioner and farm advisor are the basis of all information about herd and farm performance. Poor record keeping is a major problem for herd management in Thai dairy farmers. Without good records, no meaningful evaluation and analyses can be undertaken, and therefore, the process of assessment and decision making cannot be properly conducted. Veterinarian, dairy extension and farm advisor need to use



HH & PM as a tool to improve productivity of dairy farm in area. Training of veterinarian and dairy extensions should be adequate, they should familiarize themselves with the approach at the level of population instead of individual sick animal. Then, they must try to convince farmers to understand and participate in HH & PM. Based on priorities set, the veterinarian can start regular farm visits for animal monitoring, farm condition and data collection (Table 2). All data collected during farm visit will be converted into herd and farm performance index. These indicators are then compared to the goals set and reference figures for the region (Table 3). The implementation of HH & PM in the region will cost money. However, the question is not how much money will be invested in problem solving and prevention but rather what the ration of cost to profit would be. Once, the HH & PM is running on more farms, they can be operated on the basis of membership fees (Aiumlamai and Noordhuizen. 2002). Veterinarians and dairy extension staffs should apply the HH & PM services and must have skills in information handling, decision analysis, and epidemiological techniques. They must access a complex information system and be able to monitor, evaluate, analyze and interpret the collected data in order to optimize health and production performance, and foresee impending problems (Brand et al., 1997). HH & PM is a great tool to combine different disciplines together in order to improve overall dairy production in the region, to optimize individual farm performance, to increase social standing of farmer's community and to improve consumer safety and meet quality demands related to dairy products.

## Conclusions

Dairy productivity of Thai dairy cattle remains to be improved both in milk quality and fertility. National regulation (DLD and FDA) and Milk Board of Thailand play an important role in improving the quality of milk and marketing both by reducing mastitis and bacteria count as well as increasing total solid in milk. In addition, fertility of cows is needed to be seriously concerned, particularly on how to reduce days open.

Table 1 Number of dairy cattle, milk production, consumption, cost and price of milk in Thailand.

Year	Dairy cows/cattle (heads)	Total milk production (tons)	Consumption (tons)	Milk production of cow (kg/head/day)	Cost of milk production (baht/kg)	Price of milk at collecting center (baht/kg)
2012	229,041/573,048	1,022,190	990,836	12.23	14.47	16.61
2013	237,730/589,779	1,095,314	1,047,550	12.62	15.34	16.92
2014	230,064/605,017	1,067,338	1,025,181	12.71	15.62	16.91
2015	232,115/608,367	1,084,162	1,046,216	12.80	14.17	17.74
2016	236,200/616,420	1,111,247	1,077,910	12.89	14.66	18.01
2017*	241,824/626,216	1,126,513	1,087,085	?	?	?
%Change	0.38/1.79	1.58	1.67	?	?	?

Source: The office of Agriculture Economics, 2017 \*Expected data



Table 2. Dairy herd performance monitoring.

Milk quality and mastitis	Bulk tank SCC % Milk fat, %Milk protein, %Total solids in milk, Fat to Protein ratio C.M.T of each cow Milk production of each cow Milking system
Reproduction	Calving to first AI, Calving to conceive, Calving interval, Services/pregnancy, Days open, DIM Age at first breeding, Age at first calving Reproductive disorders Sire information
Cow health& Nutrition (no. cow < 60 DIM)	BCS, Hoof score, Feces score Milk fever, Ketosis, Acidosis, Lameness, LDA, Off feed
Culling	Mastitis, Reproductive, Feet/Legs, Disease/Injury, Low production
Feed and feeding management	%CP, ME, Concentrate: Roughage ratio, mineral, vitamin
Farm Environment	Stable, water, shade, GAP

Applied from Aiumlamai, S. and J. Noordhuizen. 2002; Brand et al., 1996

Table 3. Example of performance reference values for Thai dairy farms.

**Milk quality**

bulk tank somatic cells	< 500,000cells/ml
%fat	> 3.35 (3.60)
%protein	> 3.0 (3.0-3.20)
%SNF	> 8.25 (8.50)
%total solids	> 12.15 (12.25)
Fat to Protein ratio	1.00-1.30

**(Goal of Thai milk quality: SCC < 400,000 cells/ml, TBC < 300,000 cfu/ml and %TS > 12.5)**

**If average days in milk in all lactating cows 150 days, cows proportion are:**

cows pregnant, lactating	42 %
cows non-pregnant, lactating	41 %
cows pregnant, non-lactating	17 %
cows non-pregnant, non-lactating	0 %



### Reproductive Indices of dairy herd:

average of heifers at conception	15 months
average of first calving	27 months
average lactation length	305 days
average dry period length	60 days
average interval calving to first service	60 days
average interval calving to conception	85 days
average number of days open	100 days
average calving interval	365 days
average services per conception	1.5 times
average annual abortion rate	3%
average annual culling rate	25%

### Replacement in herd

< 40% of herd

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Applied from Aiumlamai, 2010; Brand et al., 1996; DLD, 2016

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## Current Non-ruminant Production and Future Prospects in Vietnam

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### Abstract

Non-ruminant production plays very important role in livestock production in Vietnam. Non ruminant meat products represent about 90% of total meat consumed. In which, pork meat represent more than 70% and poultry meat represents about 20% and it has increasing tendency in the future. However, the non-ruminant production is raised mainly in small scale (in small households), and there are some species animals are raised in a farm household. Non-ruminant production is facing to many difficulties in term of sustainable development such as out -broken epidemics (bird flu, PRRS, FMD,...), strong fluctuation of animal products, problem of environmental pollution, problem of food safety... Vietnamese government is applying the policy of restructure of livestock production in order to develop animal production in term of sustainable development.

**Keywords:** non-ruminant production, small scale, sustainable development

### Introduction

Livestock production sector plays an important role in the socio-economic development of Vietnam (it represents about 30% of agricultural GDP). In the period of 2011 -2015, the livestock sector has a high growth rate, about 4,5-5% annually. The animal-origin products meet basically the domestic consumption demand, some products even were exported to other countries (Hoang Thanh Van, 2016). In 2013, Vietnam had exported about 40,000 tones de pork (Statica, 2016). Livestock production not only provides enough food for domestic consumers demand but also generates employment and income for a high number of farmers in rural areas (about 6.5 million households or 42% total households in rural areas engaged in livestock production, and shares about 14% in total household income) (Lucila Lapar, Ma., 2015). In the coming years, livestock production sector in Vietnam will be projected to rise due to the rapid increase of consumption demand in the domestic market.

#### **Non - Ruminant Production in Vietnam in the last decade**

The non-ruminant production sector in Vietnam plays very important role in livestock production development. In recent years, due to the rapid growth of the economy, the livestock production has been developed significantly to meet the increasing demand of domestic consumption. The changes have seen in not only population of livestock herds, but also the production scale of animal farms. Figure 1 and 2 show the variation of population of pig and poultry over some last years. Figure 3 and 4 indicate the variation in meat production and consumption in the last decade.



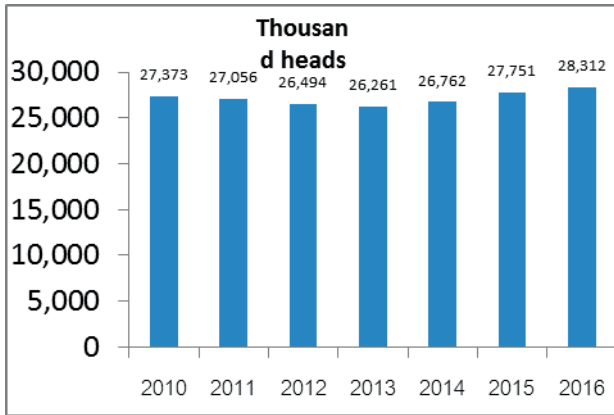


Figure 1. Changes in Swine Population from 2010 to 2016

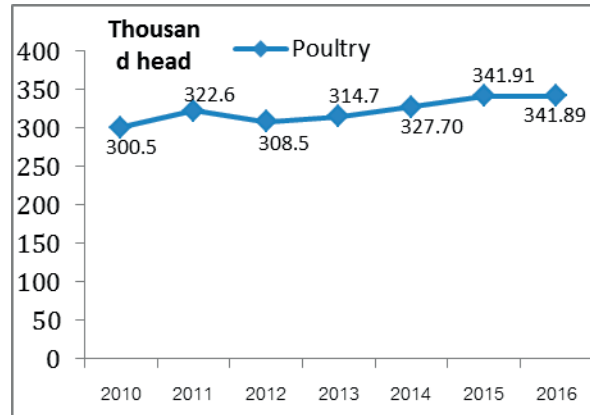


Figure 2. Changes in population of poultry herds from 2010 to 2016

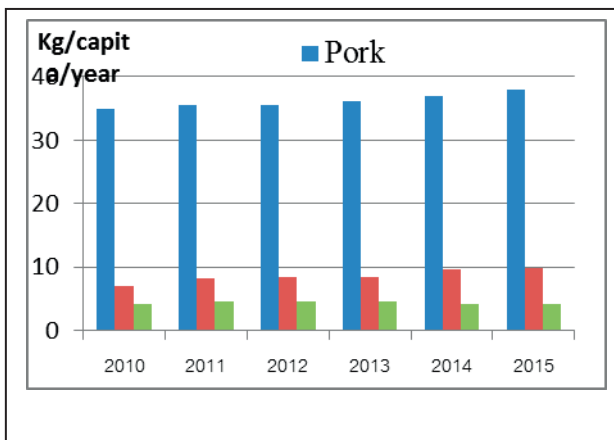


Figure 3. Changes in average meat consumption per capita per year

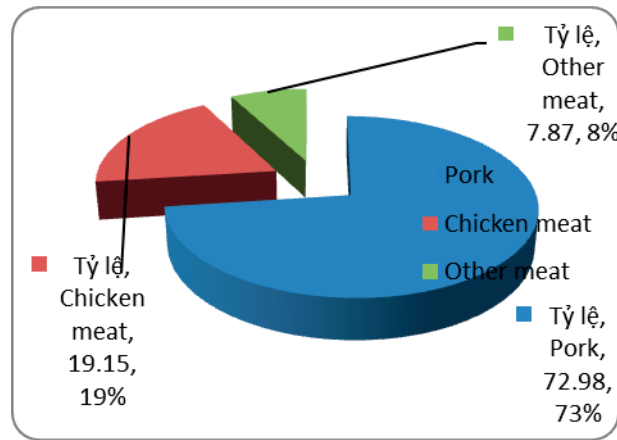


Figure 4. Proportion of different kinds of consumed meat

According to figure 1 and 2, the population of livestock herds (pig and poultry) has varied slightly over some last years. The pig population varies about 27,000 thousand heads, and poultry populations have been increased considerably: from 300,000 thousand to more than 340,000 thousand heads in 2010 in 2015 for poultry population, and from 128.5 thousand heads to 275.3 thousand heads in the same period (more than 2 times in 5 years) (Figure 3).



The pork production has risen from 2.012 thousand tons per year in 2010 to 3.491,6 thousand tons per year in 2015 (Figure 4). The statistical data indicates that pork is one of the most important types of meat in Vietnam with the average meat consumption per capita per year risen from 35.1kg live weigh in 2010 to 37.9kg in 2015 (Figure 3), sharing 72.98% of total consumed meat in the market.

### Characteristics of livestock Production systems

#### a) Small scale of animal production

Almost of households in countryside practice animal production (48% of farm household raising pig and 91% households raising chickens) (GSO, 2016). Animal production is mainly practiced in farm household at small scale (Table 1 and 2).

**Table 1.** Structure of pig farm households' size by ecological regions

	Total		% of HH			
	Pig population (1000 heads)	%	1-5 pigs/HH	6-9 pigs/HH	10-49 pigs/HH	≥50 pigs/HH
Whole country	4,131,513	100	77.54	8.89	12.79	0.78
Red river delta	870,504	21.07	72.00	7.59	19.46	1.21
Northern midlands and mountain areas	1,204,391	29.15	80.24	10.02	9.46	0.28
North Central and Central coastal areas	1,238,887	29.99	85.02	7.71	7.04	0.23
Central Highlands	210,796	5.10	74.50	9.73	14.93	0.84
South East	110,075	2.66	43.16	10.63	38.87	7.34
Mekong River Delta	496,860	12.03	71.44	10.63	16.81	1.12

Source: GSO, 2011

The proportion of pig farm households' size having less than 10 heads represents about 87%, and for households raising more than 50 pigs represent only 0.78% of total pig farm households. The small swine farms are mainly in the North and Centre of Vietnam. In the Southern provinces (South East and Mekong Delta) the scale of farm is larger.

**Table 2.** Structure of chicken farm households' size by ecological regions

	Total		% of HH			
		%	1-49 chickens/H H	50-99 chickens/H H	100-999 chickens/H H	≥1000 Chickens/H H
Whole country	7 864 730	100	89.60	7.16	3.03	0.21
Red river delta	1 785 463	22.70	86.89	8.41	4.32	0.38
Northern midlands and mountain areas	1 726 313	21.95	86.68	9.18	3.93	0.21
North Central and Central coastal areas	2 243 199	28.52	91.47	6.38	2.10	0.05
Central Highlands	527 392	6.71	91.58	5.95	2.32	0.14
South East	398 841	5.07	88.30	8.33	3.07	0.31
Mekong River Delta	1 183 522	15.05	93.96	3.93	1.84	0.27

Source: GSO, 2011

For chicken production, it's the same situation as swine production. The number of chicken farm households at large scale is very small. That means the households raising chicken aim to be auto-consumption more than for the market.

However, one of the most remarkable changes in livestock production in Vietnam over some last years is the intensification and rapid development of large-scale farms. The statistical data indicated that over the last years, the number of pig farms with less than 10 heads of pigs has reduced 2.2 million farms (equal to a reduction of 38.5% in the total pig farms) from 2006 to 2011. On the other hand, the amount of medium-scale farms (with a pig herd size of 10 to 49 heads) and large-scale farms (with more than 50 heads of pigs per farm) has an increase of 3.4% and 80%, respectively (GSO 2012).

The trend in poultry production is also similar to that of pig production. The number of large-scale farm (with more than 1000 heads of chicken) in 2011 was 16.6 thousand farm, equal to 4.32 times higher than that in 2006 (GSO 2012).

*b) Developing integrated farm households and role of animal production in households' economy*

The Vietnamese peasants are mainly practicing agriculture at very small scale (about 0.25 ha/HH in Red River Delta and 0.5 ha in the Mekong delta), that's why, they generally having many economical activities (crop production, animal production, aquaculture and off-farm activities).

Almost of farm households have crop production and animal production which are the



main activities in farm households. In animal production, the pigs and chickens are the most popular but cattle production is not important as pasture limit.

### **Challenges of Livestock Production Development in Term of Sustainable Development**

#### **- Low productivity**

Low productivity in animal production (such as the number of weaned pigs/sow/year in Vietnam is equal to 40-50% compare with developed countries) is one of constraints in livestock production. In the southern east area northern one sow produce annually 1212 kg live weight of fattening pigs, but in the mountainous area, one sow produce only 530 kg live weight. This is about 2800 to 3100 kg/sow/year in Canada and United States (Nguyen Thanh Son et al.,..2016). This is caused by the important population of unimproved sows herd. The locale breed of sow represent about 12%, crossbred sows represent about 70%, the rest are exotic sows (Nguyen Thanh Son et al.,..2016).

#### **- Disease**

The epidemiology is the foremost challenge in animal production. Since 2003, many animal farms in nearly all provinces have been facing with the outbreak and repetition of several infectious diseases such as avian influenza, PRRS (Porcine Reproductive & Respiratory Syndrome). In 2007, the PRRS had occurred at 13.355 farm households in 14 provinces, causing a loss of approximate 30.000 heads of pigs. In 2008, the disease had been broken in 28 provinces and the number of culling pigs was 10 times higher than that in 2007 (Binh, Dao et al. 2010). In 2010, the PRRS has broken in 49 provinces and 812,947 heads of pigs were contaminated and 442,961 pigs have been destroyed (Department of Veterinary, 2010).

FMD (Feet and Mouth Disease) is one of important diseases in Vietnam. In 2010, this disease has been broken in 13 provinces with about 3000 contaminated animal heads. Besides, there are some other diseases that have been occurred such as diarrhea symptoms, Pasteurellosis,...which caused an important loss in animal production.

#### **Strong variation of input and output price**

The third challenge is the big fluctuation of animal feed and product price in the market. On average, the price of animal feed in Vietnam is 10% to 20% higher than that in surrounding countries. One of the reasons is the excessive dependence on the imported raw materials for feed formulation. In which, about 45% of energy feed, more than 70% of protein and more than 85% of additives are imported. Thus, farmers have many difficulties in expanding their production scale. On the other side, the price of output products has decreased remarkably (figure 7). In 2017, the extreme falling of both pigs and poultry products caused a big loss for producers. The average price of live fattening pigs in southern market downed from 41,100 dong/kg in November 2016 to 26,000 dong/kg in July 2017, equivalent to a decrease of 63.26% (figure 7). Farmers are now facing extreme challenges from the price fluctuation of both input and output market.

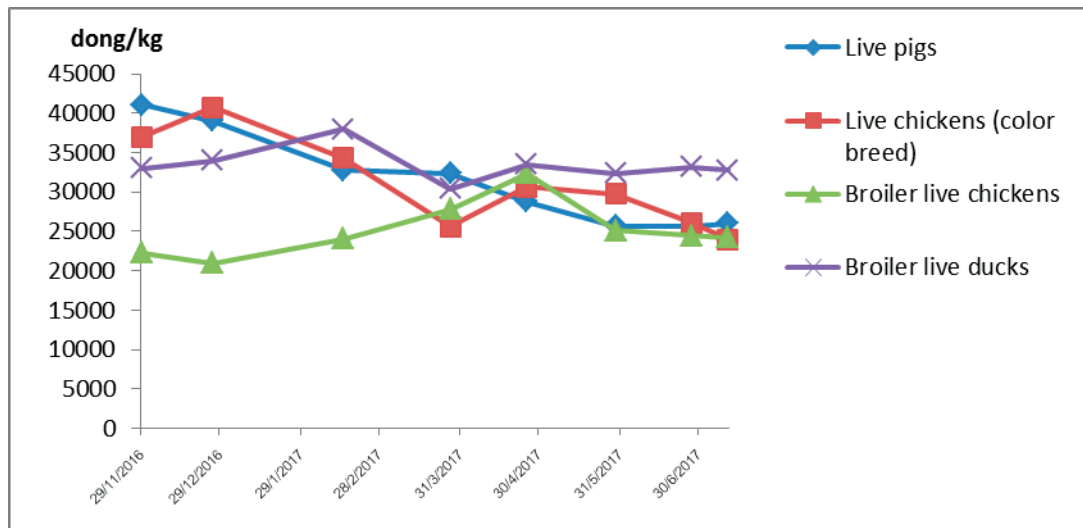


Figure 7. Variation of several livestock products in the Southern market of Vietnam

- Pollution of Environment

The fourth challenge is the threats of environmental pollution from intensive animal production. The expansion of animal herd size produces an extreme amount of wastes (including liquid and solid wastes) that need a comprehensive and effective management and treatment system. However, a high proportion of animal wastes have not been managed and treated well. In estimation, around 40 to 50% of total produced animal waste have been treated before discharging into the surrounding environment.

- Safe products

The food safety is now also an increasing concern of domestic consumers. The overuse of antibiotic and other chemical substances for both purposes of disease prevention and treatment and growth stimulation causes an alarm of food safety. By MARD (2015), there were 7 feed meals that used forbidden substances (Auramine O, Salbutamon, Clenbuterol,...) in order to increasing the lean meat, growth rate, creating better color of the products and preventing the diseases. According to Department of Veterinary of Ho Chi Minh city (2015), the inspection results at eight slaughterhouse showed that 62.5% of the inspected farms using the forbidden substances with 13.9% of total pork samples detected. Another inspection result stated by Ministry of Agriculture and Rural Development also pointed out that there were 16% of meat samples detected with forbidden substances and 7.6% of meat samples with antibiotic residue.

## Future Prospects of non-ruminant production in term of sustainable development in Vietnam

### Development of concentrated intensive farming system with a high biosecurity, food safety, and environmental protection

According to the development strategy of livestock production sector towards 2020 issued by the Ministry of Agriculture and Rural Development, livestock production will be fostered to achieve a rapid growth rate (5.5-6% per year in 2015-2020) and share a high proportion in total agricultural output value (about 42% in 2020). In order to gain that goal, the



livestock production will be oriented toward a concentrated intensive farming system with a high biosecurity, food safety, and environmental protection. In 2020, the share of livestock products provided by intensive large-scale farms will be 68-70% in total animal-original products. The environmental issues will be well-controlled by developing the waste treatment system at the livestock farms with about 78-80% of total animal farms having the waste treatment system in 2020 (Ministry of Agriculture and Rural Development 2008). Furthermore, the disease prevention and control program should be implemented effectively to limit its affects on livestock production of farm households.

### **Restructure of livestock production sector throughout supporting for small-medium farms**

Beside, the government also set up a program of restructuring the livestock production sector. The restructure program of livestock production sector will be focused on the small and medium-scale farms to enhance its value-added and sustainability. The main contents of this program is to support small and medium farms by providing partly the expenses of breeding (support half of expense of semen for artificial insemination) and expenses of waste treatment system construction (support by 50% of total expenses of waste treatment system construction, but not more than 5 million dongs per farm) (Prime Minister 2014).

### **Redistribution of livestock production by geographical regions**

According to Decission N. 984-QD-BNN, program of restructuring the livestock production sector will change the distribution of animal population. In period of 2013 to 2020, the pig population in Red River Delta will decrease from 25.74% to 15% of total swine population of the country. By contrast, the share of pig population in the Northern mountainous area will be increased from 24,1% in 2013 to 30% in 2020 ((Ministry of Agriculture and Rural Development 2014). The redistribution of animal population will create more jobs for the remote and backward areas, decrease the environmental pollution threats from livestock waste. In order to achieve this goal, the government should support for the movement of the animal farms from the delta to surrounding mountainous and hilly regions throughout the land and loan access as well as tax policy.

### **Improvement of animal breeds and breeding supply systems**

The improvement of animal breeds is of great importance in increasing the productivity, performance and product quality of the livestock production. Firstly, the strategy should be focussed on the importation and selection of high productive potential breeds as the basic for crossbreeding programs. According the restructuring program of livestock sector, the share of exotic sows will projected to increase from 19.8% in 2013 to 30-33% in 2020 in order to produces more than 75% of hybrid fattening pigs (Ministry of Agriculture and Rural Development 2014). The breeding supply system needs to be reorganized and improved to provide producers with good quality of animal breeds.

Secondly, it is important to conserve the native breeds to keep the biodiversity, especially some breeds that are in danger of disappearing. To do so, the list of breeds under the priority of conservation should be identified. Moreover, the conservation of native breeds will be effective if there is a good plan for not only conservation, but also exploitation and development of the native breed. Farmers need to be guided and supported by the government to conserve the native breed within their original regions.



### **Promoting the commodity chains of animal products**

The setting of commodity chain enables farmers to plan their production appropriately and meet and adapt better to the change of demand in the market. The development of commodity chain also helps to manage the quality and safety of animal origin products. In order to improve the commodity chains, the long-term benefits of all actors should be taken into consideration. For producers, especially small and medium farms often have less power in the market than other intermediate actors (middlemen, wholesaler, and retailers). Therefore, the government should have appropriate institutional policy to protect the small producers as well as to force them to obey some the general regulations and responsibilities.

The cooperation among farmers and between farmers with other agents in the commodity chains should be encouraged to limit the negative impacts of the market. Small and medium farms should be cooperated with each other to share the technical knowledge and experience as well as to help each other in dealing with some problems. For large-scale farms or intensive production system, the development of contract farming system will be a good way to sustain the production under the context of extreme fluctuation in the market.

### **Facilitating the adoption of improved production technologies and organic livestock production**

Recently, Vietnamese government has set a priority to encourage the adoption of advanced and modern techniques in livestock production sector in order to increase the productivity and product quality of livestock production systems. In 2012, the government launched a program on the development of advanced and modern techniques in livestock production sector according to the Decision 1895/QĐ-TTg (Prime Minister 2012). The goals of this program is to foster the development of several agricultural production centers that adopted modern techniques in genetic and animal breeding selection, disease prevention, housing system, animal feed formulation, etc. In order to achieve this goal, the state has implemented some supportive policies for both producers and business firms who adopt the modern and advanced techniques in animal production.

Another strategy for sustainable livestock production is the incentive for the adoption of Good Animal Husbandry Practices and organic livestock production. Since 2008, the has been approved by the Decision number 1504/QĐ-BNN-KHCN for poultry (Ministry of Agriculture and Rural Development 2008) and Decision number 1506/QĐ-BNN-KHCN for pigs (Ministry of Agriculture and Rural Development 2008). These guidelines encourage farm households to raise the animals properly to ensure the biosecurity, food safety and alleviation of environmental contamination. A number of supportive policies has been implemented to promote the application of VietGAHP of livestock keepers in recently. The adoption of VietGAHP in recent years has proven effective in improving livestock productivity, production, and household income.

## **Conclusions**

Over the last decade, the livestock production in general and non-ruminant production sector in particularly has changed significantly in to more and more intensive production system. There is a decrease in the number of small-scale farms, while an increase trend in number of medium and large-scale farm holdings. Pig and poultry production systems have been all



developed at a high rate in order to meet the increasingly requirement of animal origin products of consumers. Animal production always plays an important role in farm households' economy. It is not only an activity in order to poverty decrease but also improve households' economy in countryside.

In the coming years, the non-ruminant production sector in Vietnam has several opportunities and challenges for its development. The increasing demand for food of animal origin products both in domestic and international markets brings an opportunity for the continuous growth of livestock production.

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## Ruminant production in Lao People's Democratic Republic

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### Abstract

Livestock is one of the fastest growing agricultural subsectors in Lao People's Democratic Republic. This growth is driven by the quickly increasing demand for livestock products approximately 3.5-4.0 percent annually, lead to expanded livestock production in Laos. Pork meat still represents the largest proportion of meat consumed in the Laos followed by bovine and poultry meat consumption was estimated at 7.29 and 4.21 Kg/capita/year. Ruminant production in Laos is still dominated by small-scale or backyard producers. Ruminant graze around the communal areas along roads and fallow cropland around the village during the day and return home later in the day to the nearby villages. Smallholder farmers still use industrial feed during early age or reproduction period of livestock development to save some money. However, there are many feed resources in Laos that can be used as alternatives to use for feeding to livestock animals, most of these are rich in either fiber (e.g., taro leaves and cassava leaf) or carbohydrate (e.g., cassava pulp).

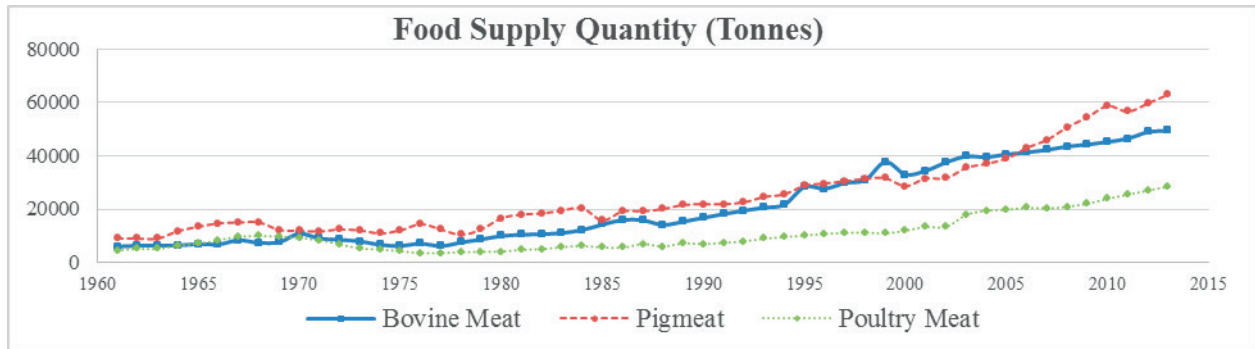
**Keywords:** ruminant production, feed resource, agro-industrial by products

### Introduction

Currently, livestock is one of the fastest growing agricultural subsectors in Lao People's Democratic Republic (Laos). This growth is driven by the quickly increasing demand for livestock products approximately 3.5-4.0 percent annually, lead to expanded livestock production in Laos (Ministry of Agriculture and Forestry, 2016), this demand being driven by increased incomes and urbanization. However, meat consumption appears somewhat unstable, peaking and falling in recent years. Despite a shift toward higher bovine meat consumption, pork meat still represents the largest proportion of meat consumed in the Laos (40%). FAOSTAT (2017) reports that pork consumption was estimated at 9.31 kg/capita/year during 2013, followed



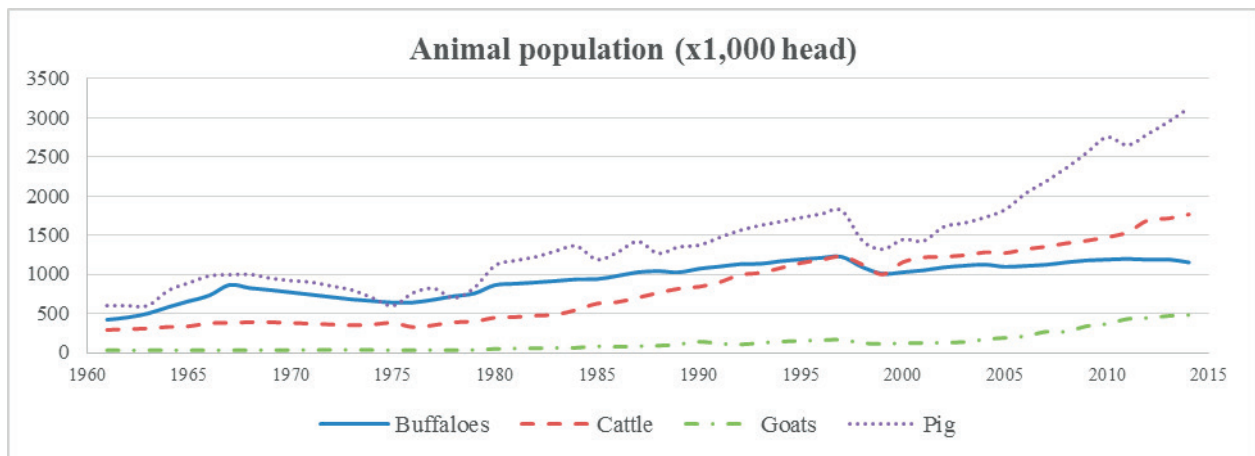
by bovine and poultry meat consumption was estimated at 7.29 and 4.21 Kg/capita/year. The resultant trends in meat consumption in Laos is shown in Figure 1.



**Figure 1** Food supply quantity in Laos.

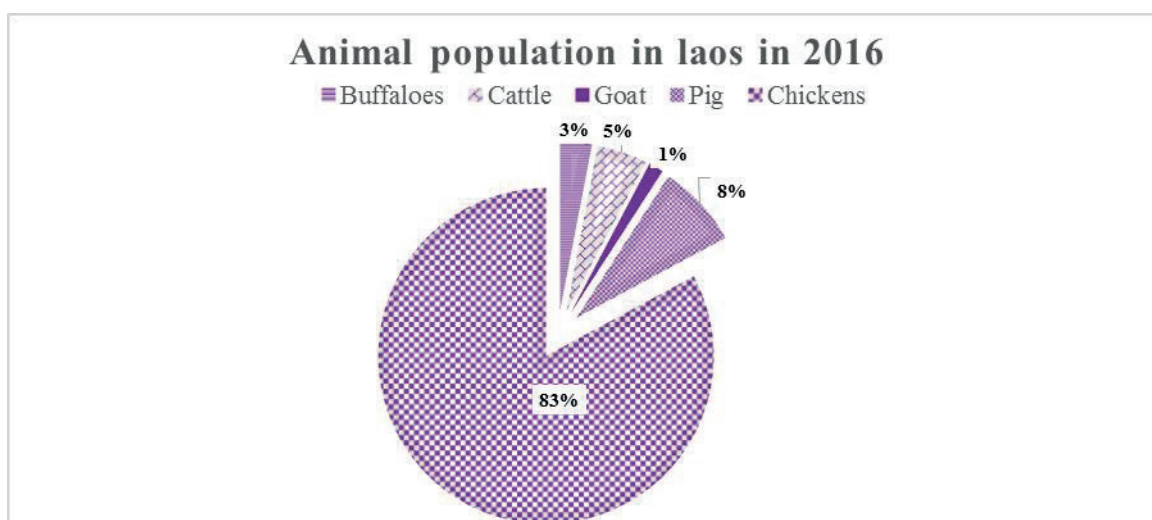
**Source:** FAOSTAT (2017)

Livestock numbers have increased steadily over the last 15 years, with total meat production increasing from 19,479 tonnes in 1961 to 140,863 tonnes in 2013 (FAO, 2017). However, over the last 10 years, buffalo, cattle, goat, pig and poultry numbers have been reported as remaining relatively static indicating populations may have plateaued at 1.15, 1.88, 0.55, 3.15 and 32.63 million head respectively in 2016 (Figure 2 and Figure 3) (Ministry of agriculture and forestry, 2016; FAOSTAT, 2017).



**Figure 2** Animal population in Laos.

**Source:** FAOSTAT (2017)



**Figure 3** The proportion of the animal population in Laos.

**Source:** Ministry of agriculture and forestry (2016)

However, livestock production in Laos is mostly small scale and follows traditional production systems. The extent livestock production in Laos can reduce poverty, meet growing domestic meat demand and lift livestock exports is problematic. However, main limitations to livestock production in Lao PDR is lack of available feed, particularly in the dry season, therefore, researcher have been looking for several alternative feed resources to conduct research on livestock feed, feed crops, and the use of agricultural and industrial wastes as livestock feed for encouraging animal production in Laos.

### **Non ruminant production in Laos**

In 2016, swine and poultry population in Laos were 3.15 and 32.63 million head and accounted for 8% of the total livestock production for that year. Commercial intensive of swine or poultry production systems accounted for approximately 10% of this total, with smallholder farmers contributing the remaining 90%. In Laos's poultry industry, its poultry consumption has been self-sufficient; thus no import is needed. However, unlike pork, most swine production is produced by small-scale farmers. In 2016, about 70% of the swine farms had capacity of only 100-200 swine, the import of swine for consumption and breeding approximately 21,570 and 15,244 head respectively (Ministry of agriculture and forestry (2016)).

In Laos, medium- to large-scale farmers often use industrial feed to raise swine and poultry. While smallholder farmers use self-made feed to raise their animals. Normally, feed costs represent at least 60-70% of the total production costs. Corn meal, rice bran, broken rice and soybean meal are the major feed ingredients for non-ruminant production. However, the prices of feed ingredients have varied over the years based on supply and demand conditions. Therefore, have a substantial impact on the prices of non-ruminant animal. However, smallholder farmers still use industrial feed during early age or reproduction period of livestock development to save some money but growth rate in these pigs is related to issues associated with their feeding.

**Table 1.** Non ruminant production in Laos in 2016 (Unit: Head)

	Swine		Poultry		Swine		
	Total	Commercial	Total	Commercial	Import for consumption	Import for breeding	Export
Northern	1,177,602	26,715	9,552,393	314,506	-	1,091	0
Central	968,122	236,729	11,002,580	1,906,412	21,570	8,342	725
Southern	1,006,461	61,521	12,078,698	1,267,588	-	5,811	24,970
Total	3,152,185	324,965	32,633,671	3,488,506	21,570	15,244	25,695

**Source:** Ministry of agriculture and forestry (2016)

There are many feed resources in Laos that can be used as alternatives to use for feeding to monogastric animals. Most of these are rich in either fiber (e.g., taro leaves and cassava leaf) or carbohydrate (e.g., cassava root). However, almost all are low in protein (Table 2).

**Table 2.** Feed resources for non-ruminant production in Laos

Feed resources	Animal	Used in diet (%)	CP in Diet (%)	FI (Kg/d)	ADG (Kg)	FCR	References
Taro leaves silage	Native pigs	25	12.00	0.65	0.07	3.78	Kaensombath et al. (2016)
Cassava leaf meal	Native pigs	10	13.00	0.79	0.20	4.14	Sivilai et al. (2016)
		20	14.00	0.79	0.21	3.94	
Taro leaves silage	Native pigs	21	12.90	0.85	0.29	3.00	Kaensombath (2017)
Cassava leaves silage		19	11.40	0.78	0.26	3.00	
Taro leaves silage	Native pigs	40	9.00	0.57	0.14	4.04	Sivilai et al. (2017)

CP= Crude protein, FI = Feed intake, ADG = average daily gain, FCR = Feed conversion ratio

## Ruminant production in Laos

Ruminant production in Laos is still dominated by small-scale or backyard producers. In 2016 these smallholder farmers represented approximately 99% in Laos despite efforts of the Laos government to develop commercial-scale farms. In 2016, there were 6 and 170 buffalo and cattle commercial farms, buffalo and cattle farm were found most frequently in Central region (Table 3) (Ministry of agriculture and forestry, 2016). At present, small-scale production predominates.

There are more than 1.15 and 1.88 million buffalo and cattle-raising smallholders in the country. Laos obviously is the larger cattle producer which produced around 1.22 million of cattle in 1997 with annual growth rate of 2.16% in the period 2000-2014 (FAOSTAT, 2017). The growth in cattle production is mainly to fulfill increasing domestic demand. An average Laos in 2013 consumed 7.29 Kg/Capita/Year of bovine meat, almost tripled compared to a Thai consumer (2.56 kg) (FAOSTAT, 2017). Goats are generally left to graze freely all year in small groups in forest and fallow cropland. Farmers tend to restrict the number of goats they raise to avoid excessive damage to crops for which the owner is held responsible. There usually is good local market demand for goat meat which is one of the reasons for the relatively high rate of increase in the goat population (4.66% per annum) (FAOSTAT, 2017). However, export of livestock products from Laos is very limited. The export value of meat is quite low and has fluctuated over the years.

**Table 3.** Ruminant production in Laos in 2016 (Unit: Head (Number of commercial farm))

	Buffalo		Cattle		Cattle		
	Total	Commercial	Total	Commercial	Import for consumption	Import for breeding	Export
Northern	280,502	200 (1)	490,256	4,198 (50)	-	5	-
Central	565,457	523 (5)	1,053,867	8,196 (64)	6,515	606	992
Southern	310,034	0 (0)	337,783	16,447 (56)	-	112	-
Total	1,155,993	723 (6)	1,881,906	28,841 (170)	6,515	723	992

**Source:** Ministry of agriculture and forestry (2016)

In Laos, the cattle graze around the communal areas along roads or wetlands around the village during the day and return home later in the day to the nearby villages, nutritional requirements of ruminant animals are mainly met through fodder crops, shrubs, grasses and agro industrial wastes (Napasirth et al., 2010) (Table 4). However, main limitations to ruminant production in Laos is lack of available feed, particularly in the dry season, therefore, researcher have been looking for several alternative feed resources to conduct research on livestock feed, feed crops, and the use of agricultural and industrial wastes as livestock feed for encouraging animal production in Laos. However, improving Lao cattle and buffalo productivity will be difficult to achieve without provision of a more seasonally balanced supply of nutrients and improved farm management (Nampanya et al., 2016)

**Table 4.** Feed resources for ruminant production in Laos

Feed resources	Animal	Used in diet (%)	CP in Diet (%)	FI (%BW)	ADG (Kg)	References
Fresh cassava leaves	Native cattle	13.55	-	2.15	0.16	Napasirth et al. (2010)
Dried cassava leaves		11.50	-	2.33	0.24	
Rice straw	Native cattle	Ad lib	-	2.26	0.20	Napasirth and Maniseng (2012)
5%Urea treated rice straw		Ad lib	-	2.23	0.24	
<b>Cassava pulp</b>	Native cattle	41	13.3	3.03	0.78	Napasirth et al. (2016)
<b>Cassava pulp</b>	Native cattle	56	14.0	2.75	0.67	Napasirth et al. (2017)
<b>Cassava pulp</b>	Native buffalo	28.5	13.9	5.45	0.98	Lorvhanseuy et al. (2017)

CP= Crude protein, FI = Feed intake, ADG = average daily gain, FCR = Feed conversion ratio

## Conclusions

Increasing demand for livestock products, lead to expanded livestock production in Laos. Pork meat still represents the largest proportion of meat consumed in the Laos followed by bovine and poultry meat consumption was estimated at 7.29 and 4.21 Kg/capita/year. Livestock production in Laos is dominated by small-scale or backyard producers. Ruminant graze around the communal areas along roads and fallow cropland around the village. Smallholder farmers use industrial feed during early age or reproduction period to save some money. However, there are many feed resources in Laos that can be used as alternatives to use for feeding to livestock animals, most of these are rich in either fiber (e.g., taro leaves and cassava leaf) or carbohydrate (e.g., cassava pulp).



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## **Tropical Animal Genetic and Environment Impact**

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### **Abstract**



## Potential Use of Alternative Protein Source in Ruminant Diet

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### Abstract

Feeding livestock with animal wastes results in reducing feed cost and lower prices of animal products. Rumen digesta, by-product from slaughterhouse, is consisted of fermented and non-fermented of dietary feeds at various stages of digestion in the rumen. Furthermore, it is consisted of the end products of microbes metabolic activities such as microbial protein, amino acids, vitamins, volatile fatty acids (VFA) and contains no anti-physiological factors, which beneficially affects in the animal production. Recent studies have shown that transfer of rumen digesta from animals fed an appropriate diet to target animals could enhance fiber digestion in ruminant. Ensiling of rumen digesta with agro-industrial by products helps to improve the nutritional value agro-industrial by products like improving protein contents and increases their digestibility. In addition, utilization of dried rumen digesta as protein source in the diet could improve *feed utilization and reducing feed cost in fish, poultry, and rabbit*. In addition, dried rumen digesta could enhance rumen fermentation, feed utilization and rumen microorganisms in *in vitro*, beef cattle and buffalo which had no adversary affect to the animals. Thus, feeding of rumen digesta is recommended since it enhanced feed utilization, has a positive economic impact, controlled environmental pollution and hazards that accrue from inadequate waste disposal.

**Keywords:** rumen digesta, protein source, environmental pollution, ruminant

### Introduction

Livestock production in Thailand plays an important role both in supplying meat, milk, eggs for domestic consumption and for export. Animal feeds generally account for up to 70 percent of the cost of production and within these costs, protein sources are likely to have a significant impact. Under the prevailing conditions and level of livestock production in Thailand, an increase in production can be anticipated. A number of local protein sources have been used in animal rations (Wanapat, 2014). There has been much recent focus on protein in ruminant feeding because of restrictions to the use of animal proteins, efforts to reduce costs of ruminant production and concerns arising from the low recovery of N in production systems and loss of N compounds to the environment (Cherdthong and Wanapat, 2010). Soybean meal (SBM) has long been considered the best source of supplemental protein in concentrate diets because of its high protein content and great amino acid profile. However, the high price of transporting from regions of soybean production, the increase in the world population, and the growing demand for protein-rich foods that do not compete with human food sources have encouraged the search for alternative feeds to replace soybean meal in animal diets (Cherdthong et al., 2014; 2015).

The growing livestock industry is producing enormous volume of wastes, which makes the urban and rural areas increasingly becoming burdened with improper disposal, that pose problems to both humans and environment (Cherdthong and Wanapat, 2013). The attitude of





humans towards overcoming these problems is usually quite negative either because nothing is done to remedy the damages or, it is motivated only by a sheer necessity that is undertaken with reluctance. However, researchers have recently made some farmers to realize that livestock wastes can be converted into animal feed. This necessitates the recycling of various wastes as livestock feed ingredients to arrest the competition between man and his animal for food. Feeding livestock with animal wastes results in reducing feed cost and lower prices of animal products (Agbabiaka et al., 2012). It contributes to self-sufficiency in protein and makes possible the integration of animal production, which can in turn solve some problems arising from improper waste disposal (Esonu et al., 2010). Therefore, this review paper is aimed at presenting and discussing the effects of rumen digesta as alternative protein source on feed utilization, rumen fermentation and blood metabolites in ruminants.

### **Rumen digesta**

Digested digesta in the rumen is not uniform, but rather stratified into gas, liquid, and particles of different sizes, densities, and other physical characteristics. Most material in the mat has been recently ingested, and as such, has considerable fermentable substrate remaining. Microbial fermentation proceeds rapidly in the mat, releasing many gases. As fermentation proceeds, fermentable substrate is exhausted, gas production decreases, and particles lose buoyancy due to loss of entrapped gas. Digesta in the mat hence goes through a phase of increasing buoyancy followed by decreasing buoyancy. Simultaneously, the size of digesta particles—relatively large when ingested—is reduced by microbial fermentation and, later, rumination. Incomplete digestion of plant material here will result in the formation of a type of bezoar.

One of such is from slaughterhouse namely rumen digesta, which consisted of fermented and non-fermented of dietary feeds at various stages of digestion in the rumen. Rumen digesta causes water pollution by entering into the rivers, streams, and local water sources. Rumen digesta also causes environmental pollution by its conversion into methane and carbon dioxide. FAOSTAT (2012) reported that the slaughterhouse in Thailand was generated rumen digesta at 41,000 tones of DM/year from 1.2 million of ruminant animals. Therefore, conversion of these wastes into animal feed will increase the flexibility of ration formulation and reduce environmental pollution.

The dried rumen digesta (DRD) contains 19.4% crude protein (CP) and 42.2% neutral detergent fiber (NDF) (Cherdthong and Wanapat, 2013). However, chemical composition probably different among countries influenced by the 1) pre-slaughtered feeding regimen, 2) length of the holding period between feeding and slaughter, 3) season of the year, 4) the type of feed resources diversity, 5) selectivity of pasture by different ruminants in different locations on the nutritive value of DRD (Agbabiaka et al., 2012). Furthermore, it is consisted of the end products of microbes metabolic activities such as microbial protein, amino acids, vitamins, volatile fatty acids (VFA) and contains no anti-physiological factors (Okpanachi et al., 2010), which beneficially affects in the rumen and also enhancing the potential for ruminal microbial activity. Recent studies have shown that replacing SBM by DRD to animal diet improve *feed utilization and reducing feed cost in fish* (Agbabiaka et al., 2012), *poultry* (Esonu et al., 2010), *and rabbit* (Mohammed et al., 2013). *Our previous works* has demonstrated that utilization of DRD could improve rumen fermentation, feed utilization and rumen microorganisms in *in vitro* (Cherdthong and Wanapat, 2013), beef cattle (Cherdthong et al., 2014; 2015), and buffalo (Seankamsorn et al., 2017). Thus, results on rumen digesta study in ruminant animals will be addressed below.

### **Utilization of rumen digesta to small animals**



It has been reported that rumen digesta contain no anti-nutritional factor (Agbabiaka et al., 2012). This has prompted some researchers to use the material to replace one protein source or the other. Odunsi (2003) combined rumen digesta with blood meal to replace groundnut cake (GNC) or fish meal in layers diet and reported low values for parameters monitored namely; feed intake, weight gain, hen day egg production, egg weight and shell thickness in the laying birds that were fed the test diet. He attributed this to the un-palatable nature of the diet due to the influence of the blood meal which agreed with the findings of Adeniji (2002) in a study carried out on pullets.

Mohammed et al., (2013) used rumen digesta as replacement for soy bean in rabbit diet, and reported that there was a linear increase in feed intake and weight gain of rabbits as the level of rumen digesta increased in the diet. Feed intake was reported to increase up to 40% level of inclusion of rumen digesta. Their findings were similar to those of Whyte and Wadak (2002) and Mohammed et al., (2013) when a similar diet was fed to rabbits. Rabbits fed diets containing rumen digesta compare well in terms of performance with those on conversational feeds (Esonu et al., 2002). Esonu et al., (2002) suggested that the improved performance could be attributed to the higher protein of the undigested starchy and fibrous carbohydrates, long chain fatty acids and partially digested diet protein material due to the influence of the microbial protein in the digesta. On carcass characteristics evaluation, Esonu et al. (2006) reported that the values of the dressed weight, gizzard, and heart were similar to those of the control group, and the values indicated that the material was well utilized by the birds. The reasons advanced was that the improved performance could probably be due to adequate dietary crude fiber level, which may have activated the intestine and more occurrence of peristaltic movement, more enzyme production resulting in efficient digestion and utilization of the nutrients.

### **Rumen digesta transfer study**

The technique involves transfer of rumen digesta from animals fed an appropriate diet to target animals in order to enhance fiber digestion by stimulation of rumen microbes has been developed by Winugroho et al. (1993). Rumen transfer was used in order to improve utilization of dried Calliandra (*Calliandra calothyrsus*) in sheep which has been previously reported to be of low nutritive value compared to the fresh Calliandra (Widiawati and Winugroho, 1996). The rumen digesta from the cattle was then cultured in buffer solution and incubated in a 39°C water bath for 8 weeks. One group was given rumen digesta 3 kg and 1.5 kg on days 1 and 2, respectively at the beginning of the preliminary period. Rumen digesta was administered by using a stomach tube. The data revealed that cross bred Ongole cattle were the best animal donors for sheep. Widiawati and Winugroho (1996) found that rumen fill transfer could improve the utilization of a diet supplemented with sun-dried Calliandra.

In addition, the comparative study between swamp buffalo and Thai native cattle in potential transfer of buffalo rumen digesta into cattle have been elucidated by Wanapat et al. (2003). The rumen digesta (about 50% by weight of total digesta) from each rumen fistulated buffalo were transferred to rumen fistulated cattle. These transfer were done as quickly as possible to avoid extended exposure of digesta to the air. After completed transfer, all lids of fistulae were closed. Based on these studies, diurnal fermentation patterns in both cattle and buffaloes were revealed. It was found that rumen NH<sub>3</sub>-N was a major limiting factor. Rumen digesta transfer from buffalo to cattle from buffalo to cattle was achievable. Monitoring rumen digesta for 14d after transfer showed an improved rumen ecology in cattle as compared to that of original cattle and buffalo. It is probable that buffalo rumen digesta could be transferred (Wanapat et al., 2003). However, further research should be undertaken in these regards in order to improve rumen ecology especially for buffalo-based rumen.



### **Ensiled of rumen digesta with roughages**

Silage can be made from plant materials with suitable moisture content depending on the methods of storage, degree of compression and water content of feed materials. Ensiling of rumen digesta with agro-industrial by products not only helps to improve the nutritional value agro-industrial by products like improving protein contents and reducing pH, fiber contents and lignin. Furthermore, the ensilation of rumen digesta with agro-industrial by products increases their digestibility. Thus, by ensilation of rumen with crop residue can save environment and at same time it can fulfill the nutritional requirement of the ruminants.

The acceptability and feeding value of rumen digesta could be enhanced by ensiling, a controlled microbial fermentation used to improve the feeding value of feed ingredients as reported by Gerald and Thomas (2006). Ensiling aids in masking odour of feed materials including rumen digesta, as odour is one of the factors affecting its efficient utilization (Maigandi et al., 2002). Ensiling of rumen digesta was found to improve the nutrient in the rumen digesta (Maigandi et al., 2002) although they further stated that if the process exceeds four weeks, the protein in the material may be converted to non-protein nitrogen and could hinder the supply of the needed protein to the animal for normal growth. Muhammad et al. (2016) concluded that ensiling rumen digesta with cowpea hay at 60% moisture improve feed intake and digestibility as well as average daily gain increases from 71.43g/day to 90.77g/day when compared with control group.

### **Feeding dried rumen digesta (DRD) in ruminant animals**

Ruminants are herbivores that utilize a symbiotic relationship with the rumen microorganisms to exploit fiber feeds as a source of nutrients. Rumen content contains high number of microorganisms, including bacteria, protozoa and fungi. Bacteria are the most numerous of these microorganisms and play a major role in the biological degradation of dietary fiber. *Fibrobacter succinogenes*, *Ruminococcus albus* and *Ruminococcus flavefaciens* are the predominant cellulolytic bacterial species found in the rumen (Wanapat and Cherdthong, 2009). In recent years, there has been increasing research directed towards rumen ecology and rumen manipulation. Replacing high quality protein sources by DRD could improve rumen fermentation and digestibility of nutrient in ruminants. Therefore, conversion of these wastes into ruminant feed will increase the flexibility of ration formulation and reduce environmental pollution (Cherdthong et al., 2015).

### **Nutrient digestibility as affect by DRD**

Inclusion of rumen digesta could improve digestibility of nutrients in ruminants (Table 1). This could be related to the considerable amounts of semidigested material and/or to unknown factors that enhanced rumen microorganisms. For example, the biological value of microbial protein in the rumen digesta was found to be high, high minerals and B-vitamin and no anti-physiological factors (Okpanachi et al., 2010) as well as end-products of rumen fermentation were contained in DRD, thus may result in efficient digestion of nutrients. Cherdthong et al. (2014) demonstrated that the aNDF digestibility increased with an increase of DRD in the diet and was highest when inclusion of 100% DRD and led to increased in rice straw intake. The results are in agreement with previous studies in *in vitro*, supplementation of 8% DRD showed higher *in vitro* true digestibility (11.5%) than no DRD supplemented group (Cherdthong and Wanapat, 2013). Furthermore, Seankamsorn et al. (2017) reported that buffalo fed with DRD pellets at 150 g/d had the highest CP and NDF digestibility. Increase of digestibility coefficients of CP and aNDF in buffalo fed with DRDP at 150 g/d indicated the availability of more potential for the proliferation of rumen microbes and fro improvement of feed intake and digestibility from the current study. It is known that aNDF exerts a significant



effect on rice straw intake regulation and is responsible for the physical limitation of dietary intake. Van Soest (1994) explained that the digestibility of aNDF is directly influenced by factors such as ingestion and feed composition, feed processing, and inherent animal factors. Similarly, Mohammed et al. (2013) found the rabbits fed with 20-40% of bovine blood-rumen content mixture (BBRCM) utilized fiber better than those fed 0% BBRCM diet and could be due to the presence of bacteria in caecum that act on the fiber material effectively.

In addition, the technique involves transfer of fresh rumen digesta from sheep to target animals could improve the utilization of a diet in order to enhance fiber digestion by stimulation of rumen microbes (Widiawati and Winugroho, 1996). Wanapat et al. (2003) revealed that monitoring fresh rumen digesta from buffalo for 14 days after transfer showed an improved rumen ecology and bacterial activities in cattle as compared to that of original cattle and buffalo; thus, buffalo rumen digesta could be efficiently transferred.

**Table 1** Effect of rumen digesta on nutrient digestibility in ruminant

Product	Results	Animal	Source
DRD powder (8%)	<i>In vitro</i> true digestibility (11.5%) higher than no DRD supplemented group	<i>In vitro</i>	Cherdthong and Wanapat (2013)
DRD replacing SBM (100%DM in concentrate diet)	Digestibility of NDF increased from 55.6 to 59.9%	Beef cattle	Cherdthong et al. (2014)
DRD pellets (150 g/d)	CP and NDF digestibility were highest	Swamp buffalo	Seankamsorn et al. (2017)
Fresh rumen digesta	Fresh buffalo rumen digesta could be efficiently transferred to cattle in order to improve digestibility.	Beef cattle Swamp buffalo	Wanapat et al. (2003)
Ensiled rumen digesta	Ensiling rumen digesta with cowpea hay at 60% moisture improve feed intake and digestibility	Sheep	Muhammad et al. (2016)

### Effect of DRD on rumen fermentation

Fermentation of substrate by rumen microorganisms results in production of microbial protein, gases and VFAs. Feeding of DRD did not adversely affect on rumen pH, temperature and ruminal NH<sub>3</sub>-N. Furthermore, VFAs, produced by fermentation of organic matter in the rumen, can have a major effect on production and product composition in ruminants. The relative proportions in which VFA are produced, are influenced by a number of factors, including substrate composition, substrate availability and rate of depolymerization, and microbial species present. Seankamsorn et al. (2017) found that concentration of ruminal total VFA was not altered by DRD pellets supplementation and the average values ranged from 115 to 120 mmol/l (Table 2), and these were slightly higher than those previously reported by Cherdthong et al. (2014), who demonstrated that total VFA concentrations in the rumen of buffalo fed with DRD ranged from 112 to 114 mmol/l. These results have clearly shown that the utilization of DRD in concentrate mixture could be substituted for SBM as high as 100% in Thai cattle ration without statistically adverse effect on VFA concentration. Supplementation of DRDP levels did not significantly affect VFA profiles except propionic acid which was highest when DRDP was supplemented at 150 g/d (26.4 mol/100 mol). The improvement of propionic acid concentration



could be related to the enhancement of nutrient intakes, digestibility, and microbial activity. Thus, a higher propionic acid production rate indicated better energy yield while a shift in acetic to propionic acid ratio better explained efficiency of energy use in 150 g DRDP supplementation. Agreement with findings reported by Cherdthong and Wanapat (2013) revealed that the *in vitro* molar proportion of propionate was significantly higher when compared with the group that received no DRD supplement. Furthermore, Wanapat et al. (2003) demonstrated that rumen digesta transfer from buffalo to cattle was fluctuated in acetic acid concentration while propionic acid and butyric acid were similar indicating an active role of rumen microbes and on-going fiber fermentation by cellulolytic bacteria. Therefore, replacing imported commercial feedstuffs with DRD could also save the energy for transporting imports and possibly reduce the environmental impact of burning the waste or using it for landfill.

**Table 2** Concentrations of total volatile fatty acid (TVFA) and VFA profiles of swamp buffalo fed with various levels of dried rumen digesta pellets (DRDP)

	Supplementation of DRDP, g/day				SEM	P value
	0	50	100	150		
Total VFA, mmol/l	119	117	115	120	4.21	0.84
VFA profiles, mol/100 mol						
Acetic acid	61.8	63.0	64.5	56.9	3.28	0.45
Propionic acid	21.9 <sup>b</sup>	21.9 <sup>b</sup>	22.6 <sup>b</sup>	26.4 <sup>a</sup>	1.13	0.03
Butyric acid	16.3	15.0	12.8	16.7	2.16	0.61

**Source:** Seankamsorn et al. (2017)

### Effect of DRD rumen microorganisms

The rumen is a dynamic system, in which resident microbes must adapt continuously to changes in diet composition, form, quantity and frequency of consumption. Cherdthong et al. (2015) uses of real-time PCR to quantify predominant cellulolytic bacteria revealed that population of total bacteria and *R. flavefaciens* were also increased with increasing levels of DRD in the diets (Table 3). Population of total bacteria and *R. flavefaciens* at 4 h post feeding were highest when inclusion 100% of DRD in the ration ( $17.6 \times 10^{11}$  and  $5.2 \times 10^8$  copies/ml rumen fluid, respectively). This study could be related to the considerable amounts of semi-digested material and/or to unknown factors in DRD that enhanced rumen bacteria (Liu et al., 2002). Okpanachi et al. (2010) reported that the biological value of microbial protein, minerals, vitamin and end products of rumen fermentation in the DRD were found to be high, while no anti-physiological, thus could result in efficient bacterial cell synthesis and related to feed digestion in ruminants. Seankamsorn et al. (2017) demonstrated that the average population of fungal zoospores increased as the level of DRDP supplementation increased and was significantly higher at 150 g of DRDP supplementation (Table 4). Furthermore, feeding of pellets could be provided a continuous  $\text{NH}_3\text{-N}$  and energy supply for microbial protein synthesis and increased microbial activities in the rumen. Thus, increasing fungal zoospores concentration could improve fiber digestibility and rice straw intake. Agreed with Cherdthong and Wanapat (2013) indicated that supplementation of DRD at 8 mg could increase *in vitro* microbial biomass at 23.4 mg. In addition, Wanapat et al. (2003) revealed that monitoring fresh rumen digesta from buffalo for 14 days after transfer showed an improved rumen ecology and bacterial activities in cattle as compared to that of original cattle and buffalo, thus buffalo rumen digesta could be efficient transferred.



**Table 3.** Replacing dried rumen digesta (DRD) for soybean meal (SBM) in Thai cattle feed on predominant cellulolytic bacterial species

Item	DRD replacing SBM, %DM				SEM	P-value
	0	33	67	100		
Cellulolytic bacteria, copies/ml						
Total bacteria, x 10 <sup>11</sup>	9.8	10.3	11.3	12.2	1.01	0.78
<i>F. succinogenes</i> , x 10 <sup>9</sup>	5.2	5.4	5.5	5.6	0.53	0.45
<i>R. flavefaciens</i> , x 10 <sup>8</sup>	1.8 <sup>a</sup>	2.4 <sup>a</sup>	3.6 <sup>ab</sup>	5.2 <sup>b</sup>	0.49	0.02
<i>R. albus</i> , x 10 <sup>8</sup>	0.9	0.9	0.9	0.9	0.16	0.17

**Source:** Cherdthong et al. (2015)

**Table 4.** Effect of various levels of dried rumen digesta pellets (DRDP) on microbial population

	Supplementation of DRDP, g/day				SEM	P value
	0	50	100	150		
Ruminal microbes, cell/mL						
Protozoa, x 10 <sup>6</sup>						
0 h post feeding	1.50	1.74	1.73	1.75	0.36	0.95
4 h post feeding	2.00	2.38	1.75	1.63	0.27	0.31
Mean	1.75	2.06	1.74	1.69	0.27	0.76
Fungal zoospore, x 10 <sup>4</sup>						
0 h post feeding	1.38	1.38	1.33	1.34	0.27	0.83
4 h post feeding	2.21	2.38	2.63	3.25	0.55	0.79
Mean	1.80 <sup>b</sup>	1.88 <sup>b</sup>	1.98 <sup>b</sup>	2.30 <sup>a</sup>	0.11	0.04

**Source:** Seankamsorn et al. (2017)

### Effect of DRD on microbial protein synthesis and nitrogen balances

According to the National Research Council (NRC, 2001), microbial protein synthesis in rumen is important for the demand of the protein in ruminants. The absorbable protein in small intestine is the key for the demand. Cherdthong et al. (2014) elucidated that microbial crude protein (MCP) and EMNS were significantly increased when DRD inclusion in the diet and highest with 100% DRD replacement [425.9 g/d and 34.2 g /kg of organic matter digested in the rumen (OMDR), respectively], which is reflected in increased bacterial population in the rumen. These results were in agreement with Wanapat et al. (2003) who reported that the EMNS varied between 18.2-45.0 g /kg of OMDR. From the results above, MCP and EMNS may be differ from substances contained in DRD such as microbial protein, amino acids, vitamins and VFA rather than the protein quantity from SBM (Cherdthong and Wanapat, 2013). Therefore, conversion of DRD wastes into ruminant diet not only enhances microbial N synthesis and increase the flexibility of ration formulation but also reduces environmental pollution (Ørskov 2007).

N consumed by cattle ultimately appears in either the urine, feces or is interchanged with the body's N reserves (Südekum et al., 2005). Cherdthong et al. (2014) reviewed that amount of N intake and total urinary N excretion (%) were not significantly different with level of DRD in the diet and ranged from 18.1 to 19.9 g/d and 62.9-70.8%, respectively (Table 5). This effect could be considered to be a positive environmental effect because urinary N is rapidly converted to NH<sub>3</sub>, which is recognized as a notable air pollutant. Fecal N excretion was also not changed (29.2 to 37.1% of total N excretion), suggests feeding DRD may allow for great without negatively affecting N excretion. N absorption, N retention and N retention to N intake ratios were unaffected by 100% DRD inclusion in concentrate diet indicated that the nutrient value of DRD is comparable with that of SBM as a protein source for cattle.

**Table 5.** Effect of replacing soybean meal (SBM) with dried rumen digesta (DRD) on N utilization of cattle

Item	DRD replacing SBM, %DM				SEM	P-value
	0	33	67	100		
N intake, g/d	18.1	19.0	19.9	18.8	1.08	0.17
Total N excretion, g/d	6.2	6.8	7.2	6.8	0.98	0.14
Fecal excretion, g/d						
Output, kg/d	0.9	1.0	1.0	0.9	0.54	0.10
Total N, g/d	2.3	2.5	2.1	2.4	0.94	0.12
% N excretion	37.1	36.8	29.2	35.3	4.50	0.43
Urinary excretion						
Output, l/d	3.5	4.4	3.7	4.2	2.39	0.23
Total N, g/d	3.9	4.3	5.1	4.4	2.44	0.21
% N excretion	62.9	63.2	70.8	64.7	7.44	0.67
N absorption, g/d	15.8	16.5	17.8	16.4	3.21	0.31
N retention, g/d	11.8	12.2	12.7	12.0	3.45	0.32
% of N retention to N intake	65.2	64.2	63.8	63.8	6.32	0.65

**Source:** Cherdthong et al. (2014)

### Limitation of rumen digesta utilize as protein source

The production of animal feed increasingly relies on the global acquisition of feed material, increasing the risk of chemical and microbiological contaminants being transferred into food-producing animals (Wanapat, 2004). Animal feed contamination provides a comprehensive overview of recent research into animal feed contaminants and their negative effects on both animal and human health. There are many concerns when rumen digesta was introduced as protein sources in animal feeds. Firstly, the contamination of pathogen from animal host may influence the quality of rumen digesta. Thus, sources of rumen digesta should be considered and confirmed that animal host was not infected by pathogen. In addition, preparation process of rumen digesta before feed to the animals such as treated-heat or sun-dry, and pellets could be reduced the pathogen contamination in rumen digesta. Secondly, the quality of rumen digesta may not be stable which depends on many factors such as feeding regimen, the type of feed resources diversity, and selectivity of pasture by different locations (Agbabiaka et al., 2011). Thirdly, it has required the time when dry form of rumen digesta was used in animal ration. Two to three days are required for sun-dry rumen digesta until 95% DM. Lastly, the high fiber content in rumen digesta might affect digestibility in animals, thus optimum levels of supplementation should also be considered.

### Conclusions

Based on this review it could be concluded that inclusion of rumen digesta in animal diets might be enhanced feed utilization and reducing feed cost without adversely affecting the animals. Therefore, conversion of these wastes into animal feed will increase the flexibility of ration formulation and reduce environmental pollution.

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## The Influence of Climate Change and the Strategy for Producing Tropical Animals

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### Abstract

Although there are many issues that animal producers face, environmental problems, economic pressure and animal feed. It is predicted that animal production in developing countries, especially in tropical region under climate change condition will continue to sustain world growth in the future. Meat production, in these areas, the efficiency of livestock is generally lower than that obtained in Europe and North America. Although many factors may be involved, climatic factors are one of the key limiting factors and the first of animal development. In addition, under climate change condition, livestock stress from heat stress. Heat stress influences direct and indirect to livestock. Direct effects influence themselves. Indirect effects influence quantity and quality of feed and forage crop, and also influence pathogen and parasite. In heat stress condition, livestock can be decreased growth, milk, meat, wool, egg production, health and welfare of livestock. The paper is a review of the influence of climate change and the strategy for producing tropical animals to reduce heat stress in the context of these tropical livestock production systems. Strategies can be suggested for 6 methods to decrease heat stress. Under the heat stress, better production should be resolved. It is possibly to modify food components that can promote higher consumption and high efficiency. How to increase heat exchange between the environment and animals and those who change their environment to prevent or limit heat stress can use it to improve performance under hot weather. The new tools, It will allow scientists to improve the accuracy and efficiency of screening to withstand heat stress.

**Keywords:** livestock, heat stress, cooling, tropical

### Introduction

Livestock play an important role in the agricultural sector in developing countries. The livestock sector contributes 40% to the GDP of the agricultural sector. Increasing of population and food consumption, it means increasing of consumption from animal protein. It is necessary to expand livestock production (FAO, 2009). Animal production is affected by climate change. Severity and seasonal fluctuations of climate change affect growth, milk yield, egg production, reproduction, health and disease epidemics. In addition, seasonal violence and fluctuations also have a direct effect on crop production, which affects the production of forage crops, raw materials, animal feeds. The quantity and quality of the crop will affect the livestock and will result in reduced productivity and reproduction (Sejian, 2013). Climate change is a major threat to sustainability of livestock around the world. Therefore, the adaptation and mitigation of severe climate play an important role in combating the effects of climate change in the livestock sector (Sejian *et al.*, 2015a). Impact on livestock performance in many regions, including its harmful effects, climate change may show that climate change in the short term (a couple of years) or the

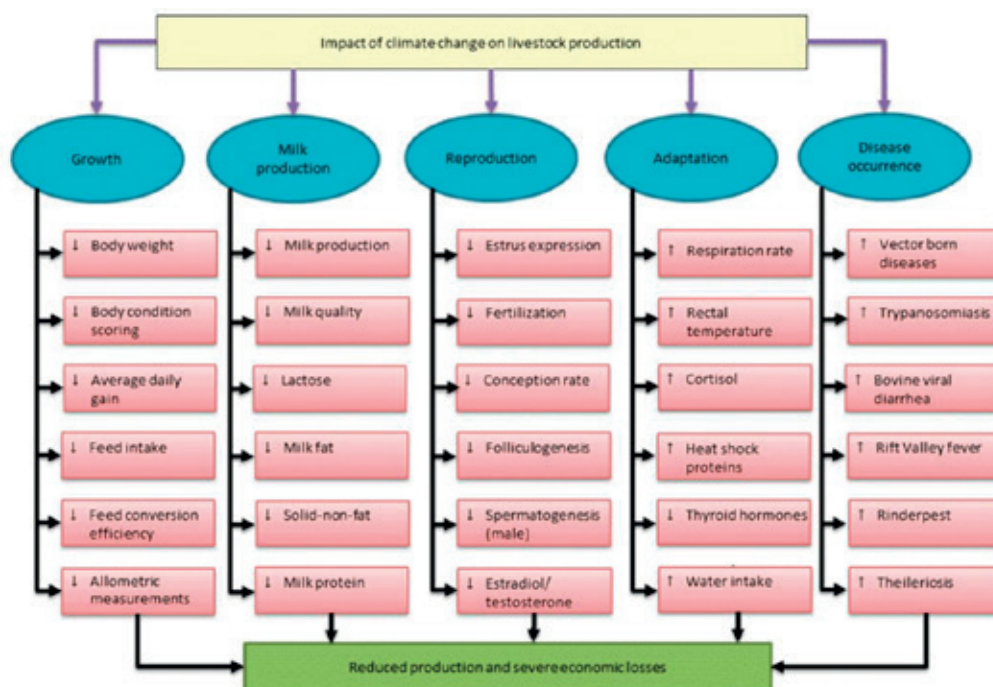


change of over a decade ago. Climate change is generally associated with rising global temperatures. Predictions for various climate models suggest that in the coming year, in 2100, the global average temperature may be raising 1.1-6.4 degrees celsius in 2100. Livestock production is exposed to extreme weather conditions such as extreme heat waves, floods, and droughts. Severe events also result in the death of livestock (Gaughan and Cawsell-Smith, 2015). Livestock can adapt to hot weather. However, the survival mechanisms of the response may negatively impact productivity. Therefore, it is important to anticipate the impact of climate change on livestock production.

### Direct effects of climate change on livestock

The most significant direct effects of climate change on livestock production are heat stress. Heat stress affects productivity of livestock. Growth, meat, milk, egg, and fertility decline. Poor health, morbidity, and mortality rates increase. Therefore, the increasing of climate temperature directly affects the production performance of livestock (Figure 1).

The direct impact of climate change, as a result of temperature increasing and the simultaneous climate change, causes heat stress, which affects growth, reproduction, milk production, wool production, healthy, and animal welfare (Walter *et al.*, 2010) Heat stress causes reducing of feed intake and low growth. The native cattle are tolerance to high temperatures (Walter *et al.*, 2010). Extreme temperatures are caused by extreme weather conditions that affect *Bos taurus* more than *Bos indicus*, resulting in reduced milk yield, meat production, and feeding time. Animals like to be in the shade, shorter grazing time. (Robertshaw and Finch, 1976). In addition, the mortality rate and illness from heat stress will increase. Climate change will increase the risk of drought and floods such as El Niño in the future and may result in higher mortality rates. Seriously due to drought, this will result in food security and water security. It will lead to increased conflicts with natural resources, and food insecurity in the region. Similarly, El Niño may result in flood-related epidemics diseases (Van den Bossche, 2008).



**Figure 1.** describes the various impacts of climate change on livestock production (Sejian *et al.*, 2016).



### **Indirect effects of climate change on livestock**

The loss of livestock production is largely due to the indirect effects of climate change resulting from the reduction of water resources that affect the production of forage crop, feed grains, and the quality of feed. In the past decade, crops and forage crops continued to higher, increased carbon dioxide, and fluctuating water availability due to changing precipitation patterns. Climate change may adversely affect yield, breed characteristics and quality, which may affect animal feed production. It has a negative impact on other ecological roles (Giridhar and Samireddypalle, 2015). Due to the fluctuations of rainfall during crop seasons in many regions of the world, with the emergency outlook evident from the effects of climate change, livestock production systems are often more vulnerable than positive ones. Climate change also influences water availability and quality. Changes in temperature and weather can affect the quality, quantity and distribution of rainfall, river flow, and groundwater. Climate change may result in faster flow of water, lower groundwater levels, and longer drought that affect the water use of agriculture and drinking water. Water loss affects animal physiology that results in weight loss, low reproductive rate (Naqvi *et al.*, 2015). By these reasons, it is required further research on the risks of climate change in water resources to support the development of adaptation strategies for agriculture, in addition, emerging diseases, including those caused by climate change-induced vectors, can cause serious economic losses.

Climate change has an indirect effect on the quantity and quality of feed and forage crops. Due to temperature, humidity and water shortage, there is spread of livestock and plant diseases. By these reasons, they affect to low quality and quantity pasture and water shortages in arid areas, and also caused by severe weather and significant human water related factors (IPCC, 2001).

Frequency and distribution of disease are reported due to climatic variability. However, estimating the actual effects of climate change on animal health in the long term is still challenging (Van den Bossche, 2008), which is difficult to separate non-climate factors from climate factors. The best way to assess the future impact of climate change is through the relationship between climate and its effects on the biological processes that affect the transmission of disease (Rogers and Randolph, 2006).

### **Concept of multiple stressor impacts on livestock**

Livestock raised in a tropical environment may generally have more than one stress factor. Stress from various causes affects animal production, reproduction, and health. Most researches have researched the effects of environmental stress on livestock. This is generally research of one factor stress in a covering time experiment. Many of the equilibrium factors are difficult to manage, analyze and interpret (Sejian *et al.*, 2010). When animals are manipulated to heat stress and nutritional stress by segregation of these effects, it does not attack growth and reproductive performance as if animals are stressed at the same time from 2 factors (Sejian *et al.*, 2011). Combination Stress from combination factors is important for growth, reproduction, and health. Each stressed livestock has different stress levels (Heat and nutrition) (Sejian *et al.*, 2010). Stress occurs simultaneously from both causes. The effect on the biological functions required for adaptation and maintenance during stressful periods may be severe (Sejian *et al.*, 2013). Therefore, any research on the effects of climate change on livestock can solve many types of stress. Because climate change affects many factors that cause stress at the same time.



Table 1. Effect of thermal, nutritional, combined and multiple stresses on growth and reproductive performance of Malpura ewes

Parameters	Control	Thermal stress	Nutritional stress	Combined stresses	Multiple stresses
Body weight (kg)	39.67 ± 2.65 <sup>a</sup>	35.19 ± 1.46 <sup>ab</sup>	30.39 ± 1.50 <sup>b</sup>	30.04 ± 1.35 <sup>b</sup>	29.55 ± 1.22 <sup>b</sup>
Average daily gain (g)	169.14 ± 0.01 <sup>a</sup>	47.71 ± 0.07 <sup>b</sup>	-122.57 ± 0.06 <sup>c</sup>	-138.00 ± 0.07 <sup>c</sup>	-88.00 ± 0.05
Ewes in heat (%)	85.71 <sup>a</sup>	57.14 <sup>b</sup>	85.71 <sup>a</sup>	71.43 <sup>ab</sup>	41.7 <sup>c</sup>
Estrus duration (hour)	38.00 ± 2.41 <sup>a</sup>	23.40 ± 3.34 <sup>b</sup>	28.50 ± 5.68 <sup>bc</sup>	18.75 ± 3.75 <sup>bd</sup>	14.4 ± 2.78 <sup>c</sup>
Estrus cycle length (day)	18.17 ± 0.31 <sup>b</sup>	20.28 ± 0.74 <sup>ab</sup>	18.00 ± 0.27 <sup>b</sup>	22.25 ± 1.67 <sup>a</sup>	23.56 ± 1.45 <sup>a</sup>
Conception rate (%)	71.43 <sup>a</sup>	42.86 <sup>ab</sup>	57.14 <sup>ab</sup>	28.57 <sup>b</sup>	-
Lambing rate (%)	71.43 <sup>a</sup>	42.86 <sup>ab</sup>	57.14 <sup>ab</sup>	28.57 <sup>b</sup>	-
Estradiol (pg/mL)	14.58 ± 0.96 <sup>a</sup>	12.06 ± 0.73 <sup>b</sup>	12.80 ± 0.91 <sup>b</sup>	10.04 ± 0.74 <sup>c</sup>	7.19 ± 0.23 <sup>d</sup>
Progesterone (ng/mL)	3.31 ± 0.56 <sup>c</sup>	4.48 ± 0.32 <sup>ab</sup>	3.98 ± 0.26 <sup>bc</sup>	5.19 ± 0.27 <sup>a</sup>	7.34 ± 0.28 <sup>d</sup>

Combined stresses-thermal and nutritional stress, Multiple stresses-thermal, nutritional and walking stress. Means and SEM within a row having different superscripts differ significantly ( $P < 0.05$ ) (Sejian *et al.*, 2016).

Livestock raised under climate change condition are generally subjected to more than one stress at a time. This greatly influences the livestock production and reproduction under such environmental conditions. Nearly all studies on the effect of environmental stress on farm livestock productivity have generally implicated one stress at a time since comprehensive, balanced multifactorial experiments are technically difficult to manage, analyze, and interpret. Hence, few reports evaluating effects of multiple stresses on farm animals are available in the literature. However, researchers have described several hypothetical schemes highlighting how two stressors can synergistically influence normal physiologic functions in mammalian species. Thermal stress in livestock is aggravated when feed restriction is involved. These effects are often manifested in changes in the blood biochemical parameters, enzymes, and thyroid hormone levels in livestock. Generally, when nutrition is not compromised, livestock species cope with heat stress better. Further, several findings have shown that livestock species tolerate and adapt to nutritional stress more than thermal stress. However, when both stresses are present, severe impacts on all the biologic functions in the livestock have been observed. Hence, it may be pertinent to conclude that the combined effects of two stressors may have severe impact on biologic functions in livestock species (Sejian *et al.*, 2016).



### **Impact of climate change on livestock production**

Animal productivity is optimized within narrow environmental conditions. When the temperature is either below or above the threshold values for peak animal production, efficiency is compromised because nutrients are diverted to maintain euthermic as a means of maintaining body temperature which invariably takes priority over product synthesis such as milk, meat, fetus, etc. Heat stress negatively impacts a variety of productive parameters including milk yield, growth, reproduction, and carcass traits. In addition, heat load increases healthcare costs and animals can succumb to severe thermal stress (especially lactating cows and sows). Therefore, environmental heat stress is a significant financial burden to the industry. Advances in management (i.e., cooling systems, barn construction) have alleviated some negative impacts of thermal stress on animal agriculture (Sejian *et al.*, 2016).

Animals with heat stress eat less feed and more water. There is a change of endocrine function by heat stress. The energy requirement for maintainant is higher. The production efficiency is reduced (Gaughan and Cawsell-Smith, 2015). Environmental stress causes weight loss, low growth rate, poor body score condition, low milk yield, low milk quality, low milk fat, low essential fatty acids, low solid not fat, low lactose, but higher stearic acid. Highly productive animals will be most affected. Adaptation to prolonged stress may come with productivity losses. It is needed to solve management problems to increase or maintain the level of production and more friendly environment, which is not sustainable. It may look better when using local livestock with smaller size and low production. They are indigenous species that have adaptation traits. They are less impacted, less cost of production which should be important. It is better than trying to breed heat tolerance and high yielding breeds with no adaptability traits (Gaughan, 2015).

Basal metabolic heat production increases with enhanced production (Sejian *et al.*, 2016). Therefore, genetic selection base upon traditional production traits may increase susceptibility of animals to thermal stress. Consequently, an animal or a breed's annual productivity needs consideration before introducing novel genetics into a particular geography. Understanding the biology and mechanisms of how heat stress jeopardizes animal performance, therefore, is critical in developing compatible approaches to ameliorate current challenges facing animal production and future mitigating strategies to improve animal well-being, performance, and economics.

Heat stress adversely affects milk production and its composition in dairy cow, especially dairy cows of high genetic merit (Renaudeau *et al.*, 2012). The effective environmental heat loads above 35°C activate the stress response systems in lactating dairy cows. In response dairy cows reduce feed intake which is directly associated with negative energy balance, which largely responsible for the decreasing in milk synthesis (Renaudeau *et al.*, 2012). Moreover, maintenance requirements of energy also increase by 30% in heat stress dairy cow. Therefore, energy intake will not be enough to cover the daily requirements for milk production. West (2003) reported a decreasing in dry matter intake by 0.85 kg with every 1°C rise in air temperature above a cow's the thermo neutral zone, this decrease in feed intake approximately 36% of the decrease in milk production (Rhoads *et al.*, 2009). Drop in milk production up to 50% in dairy cows might be due to reduced feed intake for metabolic adaptations to heat stress as heat stress response to alters post-absorptive carbohydrate, lipid, and protein metabolism a part of reduced feed intake. Increasing in basal insulin levels improves insulin response in heat-stressed cows. Heat stress during the dry period (last 2 months of gestation) reduces mammary cell proliferation and so, decreases milk yield in the following lactation. Moreover, stress during the dry period negatively affects the function of the immune cell in dairy cows facing calving and also extend to the following lactation. The negative impacts of stress on lactation length, dry period, calving interval, milk constituents and milk yield in Murrah buffaloes. Hot and humid



environment not only affects milk yield but also effects milk quality. Kadzere *et al.*(2002) reported that milk fat, solids-not-fat (SNF) and milk protein percentage decreased by 39.7, 18.9 and 16.9%, respectively.

### **Impact of climate change on livestock reproduction**

Climate change is a long-term shift in the statistics of the weather such as temperature, radiation, and wind and rainfall characteristics of a particular region. Sustainability in livestock production system is largely affected by climate change. An imbalance between metabolic heat production inside the animal body and its dissipation to the surroundings results to heat stress under high air temperature and humid climates. The foremost reaction of animals under thermal weather is increase in respiration rate, rectal temperature and heart rate. The anticipated rise in temperature due to climate change is likely to aggravate the heat stress in livestock, adversely affecting their productive and reproductive performance and even death in extreme cases. The predicted negative impact of climate change on agriculture would also adversely affect livestock production by aggravating the feed and fodder shortages.

Fertility characteristics are affected by heat stress. Conception rates in dairy cows may be reduced by 20-27% in summer and cattle are subjected to heat stress. In particular, dairy cows exhibit silent symptoms due to decreased luteinizing hormone and follicle-stimulating hormone secretion. They are lack of reproductive performance due to heat stress. The oocyte and embryonic development are decreased (Naqvi *et al.*, 2012). Heat stress reduces follicle growth in cows by progesterone secretion. The luteinizing hormones stimulate follicle and ovarian changes in the ovulation cycle. Heat stress also involves the development of abnormal embryo and increasing of embryonic mortality in cows. Heat stress during pregnancy reduces fetal growth and can increase fetal loss. The secretion of hormones and enzymes that regulate the reproductive system may be altered by heat stress in the bull. It affects the spermatogenesis by inhibiting the spread of spermatocytes.

High temperature and humidity affects cellular functions by direct alteration and impairment of various tissues or organs of the reproductive system in both the sexes of the animal. Reproductive functions of livestock are vulnerable to climate changes and both female and males are affected adversely. Heat stress also negatively affects reproductive function (Amundson *et al.*, 2006). The climate change scenario due to rise in temperature and higher intensity of radiant heat load will affect reproductive rhythm via hypothalamo- hypophyseal-ovarian axis. The main factor regulating ovarian activity is GnRH from hypothalamus and the gonadotropins i.e. FSH and LH from anterior pituitary gland. Heat mitigation measures and strategies need to be adopted not only to reduce thermal stress but also to curtail fertility losses and other health consequences on animals. The expression of estrus and conception rate is recorded low during summer in crossbred cattle and buffaloes. Low estradiol level on the day of estrus during summer period in buffaloes may be the likely factor for poor expression of estrus in this species.

Bull is recognizing as more than half of the herd and hence, bull's fertility is equally or more important for fertilization of oocyte to produce a good, viable and genetically potential concepts. It is well known that bull testes must be 2-6°C cooler than core body temperature for fertile sperm to be produced. Therefore, increased testicular temperature results from thermal stress can changes in seminal and biochemical parameters leads to infertility problems in bulls. The significant seasonal difference in semen characteristics was reported by several studies. The seasonal effects on changes in testicular volume, hormonal profiles, sexual behavior and semen quality that affect the reproductive performance of males.



### **Impact of climate change on livestock adaptation**

By physiological conditions, when livestock are in heat stress, they will have a mechanism to maintain body temperature within physiological limits. Livestock begin to compensate and adapt to the immune system and stress, which are important factors in survival. However, this may affect the decrease in production potential.

Physiological changes related to physiological responses such as respiratory rate, heart rate, and rectal temperature stress to the livestock is related. Heat stress, which affects the hypothalamic-pituitary-adrenal axis, causes corticotropin secretion, which stimulates somatostatin. It is an important mechanism by which stressful animals reduce hormone stress, reduce growth hormone and thyroid levels make animals live in hot weather. It helps protecting cells from increased environmental temperatures. To improve production and reproductive traits using functional genomics to identify genes that express during heat stress, it can lead to identification of animals with good genetic characteristics that can tolerate heat stress. Including the knowledge management of functional genome to create products, medicine, and supplement using for heat tolerance livestock. Research evaluating genes that have been implicated in responding to adaptation to heat stress by microarray analysis or global genomic studies. It has been suggested that heat chock proteins play an important role in adaptation to heat stress.

### **Impact of climate change on livestock diseases**

Changes in temperature and rainfall are the most significant climatic variables affecting the outbreak of livestock diseases. In temperate region, in particular, the longer of winter affects to the greater risk of emerging diseases. Especially, the disease has birds and insect as carrier. The longer winter causes longer time for migration birds. The migration birds still remain in the migration area that the temperature and humidity are fluctuation. The migration birds are disease carriers, such as new influenza and other infectious diseases (eg babesiosis, theileriosis, anaplasmosis), rheumatic fever, and some types of bluetongue disease may become very prevalent. The geographic range may be widespread if rainfall is increased. This may result in increased disease spread in livestock, such as rubella, osteoporosis (CAE), equine anemia (EIA), horse flu, marek's disease (MD), and diarrhea from the bovine viral diarrhea. There are several fast-paced emergencies that rapidly that still spread throughout the large area. Outbreaks of diseases such as foot and mouth disease or avian influenza affect many animals and cause deterioration of the environment and the health of the surrounding communities (Sejian et al., 2016).

The impact of climate change on the spread and geographic spread of animal diseases has been shown to be associated with changes in the spread of disease carriers and animals that are susceptible to temperature changes and rainfall.

Increased temperatures affect certain pathogens and parasites that lead to the development of pathogens. This may shorten the cycle, and more infection. However, some pathogen and parasite is more sensitive to temperature and affect survival. Other effects that should be considered are humid and dry conditions. Pathogens and parasites that are susceptible to humid and dry conditions may be affected by precipitation changes, soil moisture, and frequency of flooding. Wind changes may also affect the spread of certain pathogens.

Temperature and humidity are the limits of vector distribution at low temperature. The temperature will vary, as will occur when the humidity is too high. So much colder and higher areas, which were previously too cold for some carriers, may begin to grow when climate change. Temperate regions may warmer and carriers can be present if rainfall or humidity is increased. Conversely, these regions may not have much influence on the carrier if the moisture





level remains unchanged or decreases. Changes in temperature and humidity will result in an increase or decrease in the prevalence of disease carriers (Anyamba *et al.*, 2002).

The ability of certain insects to carry the virus varies with temperature. Increasing temperature will change the balance between infective period and incubation period, increases or decreases the proportion of infected carriers that live long enough to pass on. Another effect on the vectors considered is the movement of the wind and may have a significant impact on climate change in vector distribution, especially when the pattern of climate change is changed. The report shows that there is a connection between the movement of the wind and the epidemic of culicoides and mosquito-borne diseases (Seller and Maarouf, 1993). A good example of a british outbreak of blue tongue in 1998 (Renaudeau *et al.*, 2012).

### **Strategies to alleviate stress from climate change in tropical animals**

It can apply a variety of environmental and technical solutions to reduce the effects of hot climate. However, environmental applications to alleviate heat stress in farm animals are unsuitable if nutrition, disease control, or breeding factors restrict the performance of the animal. For example, in laying hens, stocking rate density should be reduced under higher temperatures to prevent heat from radiant heat between animals and excessive heat stress. (Burmeister *et al.*, 1986). In addition, during the hottest period of the day, animal stress must be avoided. Consequently, animals should not be manipulated during hot spells to avoid stress-related mortality (Amand *et al.*, 2004). Finally, heat can be minimized by adopting simple and basic rules for designing animal facilities (shape, orientation, thermo-physical properties of construction materials, ventilation, opening facilities, etc.). In cattle, Collier and Beede (1985) suggested that physical modification of the environment or genetic selection for more heat tolerant cattle would be the primary means of reducing adverse effects of the environment on animal production and dairy profitability. This assumption is valid for pigs and poultry. Classically, these methods can be divided into two groups: those who modify the environment to prevent or limit heat stress at the animal's exposure, or to heat up the exchange between animals and the environment.

1. Methods for reducing environmental temperature. These environmental modifications try to reduce heat stress by reducing radiation or surrounding animal temperatures. For outdoor animals, shade (natural or artificial) is the easiest and most cost-effective way to reduce solar heat.
  - 1.1. Trees are effective and natural shading materials that shade the animals together with beneficial cooling due to the moisture evaporating from the leaves. Artificial shades have been used with success for heat-stressed animals in confinement or in intensive situations. Shade is effective in protecting cows from solar radiation, but does not alter the temperature or humidity around the cows to maximize sensible routes of heat loss (West, 2003).
  - 1.2. Various cooling systems have also been evaluated. Air temperature can be lowered by air conditioning, but the expense of such types of mechanical air cooling make it impractical for cooling livestock animals (West, 2003).
2. Methods for enhancing animal heat losses. Increased heat exchange generally means increased heat loss from the surface of the body by increasing the heat loss mechanism. Air movement is an important factor in relieving heat stress due to both convective and evaporative heat losses. Natural ventilation rate can be maximized using a well-oriented semi-opened building with high and well isolated roof (Holik, 2009).



3. Changes in macronutrients composition. Feed intake is depressed in hot weather, it is an adaptation to reduce metabolic heat production. The metabolic utilization of crude proteins (CPs) or fiber is higher than for starch or fat. The higher heat increment of CP is partly related to the deamination of excess of amino acids for urea synthesis and a higher protein turnover. The energy losses associated with the metabolic utilization of digestible fiber are mainly related to the losses of combustible gases and heat arising from fermentation and during the production of ATP from the oxidation of short-chain fatty acids, which is less efficient compared with ATP gains from the oxidation of glucose. From that, it has been suggested that low-CP or fiber diets should attenuate the depressed intake associated with heat stress.
  - 3.1. using energy or protein concentrate diets to overcome the low DM intake
  - 3.2. using low increment diets to improve DM intake.
4. Water management. Water is an essential nutrient for livestock animals, especially during a thermal stress. Water intake during heat stress is a limiting factor for survival and performance, as water has a fundamental role in the heat exchange system for temperature regulation and maintenance of hydration balance. Whatever the species, water restriction enhances the negative effect of thermal stress on animal performance. In hot conditions, water losses increase (evaporation by panting and sweating) and water ingested in feed and generated by metabolism is reduced. Consequently, drinking water consumption has to increase to cover the requirements of a heat-stressed animal. In warm climate, a key husbandry practice is to provide an abundant and clean source of drinking water close to the feeding area. Moreover, in many high-temperature regions, drinking water provided to livestock animals is often warm. Whatever the species, some studies demonstrated that a provision of chilled water would improve animal performance reducing Tb through absorbed heat energy (Jeon *et al.*, 2006).
5. Early heat conditioning in poultry. Apart from management considerations to limit heat-stress, strategies for early acclimation to high Ta are currently under study in chickens. The physiological basis of these techniques is the fact that the neuroendocrine response to heat can be modified during the period when the thyroid and adrenal axes are set up (Piestun *et al.*, 2009a).
6. Genetic selection. Whatever the species, selection for improved performance has led to increased metabolic heat production, which increases their susceptibility to heat stress. The rapid development of livestock production throughout the world has resulted in high-performance stocks, coming from international breeding companies located in North American and Western Europe, and being imported into developing countries with hot climate conditions. Because of the sporadic and short-term nature of acute heat stress in selection conditions, animal survival should rely on managements practices such as cooling methods, feeding strategies and animal management in the case of a heat wave (Cahaner, 1996).

## Conclusions

Heat stress affects the performance of animals in tropical countries, which are contributing to significant economic losses in the livestock industry. The severity of heat stress will become a problem in the future as global warming continues, especially if the genetic selection for growth and milk production continues. Significant advances in environmental management, including improved cooling and housing systems and changing feeding strategies, can reduce the effect of heat stress on performance. The effectiveness of these solutions depends



on many factors related to livestock and production systems. In practical terms, the level of decision making to modify the animal environment depends on the cost of improving the environment, with the value of improving the environment. Increasing the level of production and efficiency of livestock enterprises is essential. However, most economic considerations will determine the level of environmental management used for the livestock system. Due to the high genetic variation between and within the species, there is no doubt that it is possible to choose to with stand heat stress, although all practical problems remain unresolved. In terms of research requirements, additional work is needed to accurately predict the effect of heat stress on animal production in practical conditions. In fact, the reason of laboratory and field equations to predict the effect of temperature on animal performance is very questionable.

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## ***Session 1-Orchid ballroom I***

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### **Metabolizable Protein Requirements of Lactating Buffaloes (*Bubalus bubalis*) Fed on Silage Based Diet**

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#### **Abstract**

Fifteen lactating Murrah buffaloes (*Bubalus bubalis*) were allocated into three treatments ( $n=5$ ) on the basis of milk production ( $7.10 \pm 0.40$ ) and days in milk (DIM;  $118 \pm 5.0$ ). The nutrient requirements of buffaloes were met by feeding maize silage: concentrate mixture in proportion of 60:40 respectively. The MP level in second treatment (Control; 11.1% on DM basis) was designed to meet the current Indian Council of Agricultural Research (ICAR, 2013) guidelines, whereas in treatment one and three, MP levels were 10% lower (10% LMP) and 10% higher (10% HMP), respectively than ICAR (2013) requirements. Results revealed that feeding of diets containing varying levels of MP did not affect ( $P > 0.05$ ) body weight, milk production and milk composition. The estimated MP requirement for maintenance and 6% fat corrected milk yield (6% FCM) in experimental buffaloes were  $2.56 \text{ g/kg } W^{0.75}$  and  $66.78 \text{ g/kg } 6\% \text{ FCM}$ , respectively. The estimated MP requirements for maintenance and 6% FCM were 3.11% lower and 1.13% higher than recommendation of ICAR (2013), respectively. In conclusion, performance of lactating buffaloes were not affected by feeding diets containing 10% higher or 10% lesser MP than ICAR (2013) recommendation.

**Keywords:** murrah buffaloes, metabolizable protein, maize silage, milk production

#### **Introduction**

The domestic water buffalo (*Bubalus bubalis*) contributes a significant share of global milk production and is the major milk producing animal in several countries. In spite of the immense potential of milk production, we seem to know inappreciable about its nutrient requirements, productive capabilities and limitations. Buffaloes must receive sufficient nutrients to supply the nutrient secreted in their milk, and for maintenance. Most of the time, information concerned to feeding of cows are utilize for feeding of buffalo. But there are differences in feed utilization efficiency; therefore, the nutrient requirements of both species may not be same.

The protein requirements of dairy animals were expressed in terms of crude protein (CP), digestible crude protein (DCP) or rumen undegradable protein (RUP) in existing literature (Paul et al. 2002) but these information no longer be used alone to estimate the accurate protein delivered to animals. Simultaneously, with increasing environmental concern, nowadays



research is being focused on improving the efficiency of N utilization and optimum production. Studies have shown that altering the supply of RUP alone cannot explain variations in milk production responses (NRC, 2001). The new metabolizable protein (MP) system (NRC, 2001) opens the possibility for fine-tuning on protein nutrition of dairy cows as compared to the old crude protein or absorbed protein model. Reviewing available information clearly indicated that changes in milk production in dairy animals were related to the MP rather than to either CP or RUP. Therefore, replacing conventional CP, DCP, and RUP system with MP system provides better idea to define protein utilization and diet formulation as this system best fits with the biology of ruminants. The equations used for MP requirements in the CPM Dairy software were not applicable in buffaloes as greater differences observed in the estimated and recorded MP requirements of buffaloes (Bovera et al. 2007). The objective of this study was to determine the MP requirements and effects of varying dietary MP levels on feed utilization and milk production in Murrah buffaloes.

## Materials and Methods

### Experimental setup and dietary treatments

The experiment was conducted over 96 days at the experimental unit of the cattle yard of ICAR-National Dairy Research Institute, Karnal (India), and was approved by the Institutional Animal Ethics Committee, Government of India, approval number, IAEC/21/14 dated 04.01.2014. In a completely randomised block, 15 lactating Murrah buffaloes (*Bubalus bubalis*) were randomly assigned into three dietary treatments (five buffaloes per treatment) based on parity, milk yield ( $7.10 \pm 0.40$  kg/d), and days in milk ( $118 \pm 5.0$  d). Feeding regimen was similar in all the treatments except the buffaloes in 10% HMP and 10% LMP treatments were fed on concentrate mixture containing 10% lower (10.0% of DM) and 10% higher (12.2% of DM) MP content, respectively than control group (11.1% DM). The dietary MP level of control group was designed to meet requirements of buffaloes as per ICAR (2013). Nutrient requirements of buffaloes were met by feeding a diet consisting of 60 parts maize silage and 40 parts concentrate mixture in two equal daily portions at 05:30 and 17:30 h. The compound feed was provided separately as a meal to ensure its complete intake. Water was offered ad libitum during the entire experiment. Buffaloes were individually housed in tie-stalls and milk was collected twice daily at 06:00 and 18:00h. Daily feeds offered and residue left was recorded to determine the net feed intake. Milk, faeces, and urine excreted during 24 h were recorded for 8 days, and daily representative samples were taken for further analyses.

### Laboratory analyses

Samples were analyzed for DM, total nitrogen and ether extract (AOAC, 1995). Fibre content of samples was analyzed by determining NDF (McQueen and Nicholson, 1979), ADF and ADL (AOAC, 2005), NDICP and ADICP (Licitra et al., 1996). TDN, DE, and ME content were determined as per NRC (2001). Pooled milk samples were analyzed for fat, protein, lactose, total solids, and solid not fat (SNF) using a precalibrated milk analyzer (Lacto Star, FUNKE GERBER, Article No 3510, Berlin). The 6% FCM was calculated by using the equation given by Rice et al. (1970).

### Calculation and statistical analyses

The requirements of CP, DCP and MP for maintenance and milk production (6% FCM, kg/d) were determined by using following regression analysis model (as used by Solis et al., 1991):  $Y = a + b1X1$

Where  $Y$  is the MP, CP and DCP intake (g/kg  $W^{0.75}$  kg/day) and  $X_1$  is the 6% FCM (kg/kg  $W^{0.75}$  kg/day). The regression constant and regression coefficients give estimates of nutrient



requirements for maintenance and milk production. The data were analyzed using the general linear model procedure (SAS Inst. Inc. 2003 Cary, NC, Version 9.2) as a completely randomized design with animal as experimental unit and group as a fixed effect. Data of entire experiment were subjected to Two Way ANOVA.

**Table 1.** Ingredients and chemical composition of experimental concentrate mixture dry matter basis

Item	Maize silage	Concentrate mixture <sup>a</sup>		
		10% LMP	Control	10% HMP
Ingredient, % of DM				
Maize		30	25	22
Bajra		10	10	10
Mustard cake (deoiled)		10	15	18
Cotton seed cake		17	20	25
Soybean meal		--	3	6
De-oiled rice bran		30	24	16
Mineral mix		2	2	2
Salt		1	1	1
Chemical composition, g/kg of DM				
DM	324	893	911	916
CP	107	185	193	208
ME, MJ/kg DM	9.1	117	117	116
MP	65	100	111	122

<sup>a</sup>MP level of control group was designed to meet requirements of buffaloes as per ICAR (2013). 10% HMP and 10% LMP group contains 10% lower (10.0% of DM) and 10% higher (12.2% of DM) MP content, respectively than ICAR (2013) recommendation.

## Results and discussion

### Feed intake and nutrient utilization

The CP, DCP, and MP intakes were significantly higher ( $P < 0.05$ ) in 10% HMP and lower in 10% LMP than buffaloes fed on basal diet (Table 2). There were no significant ( $P > 0.05$ ) effects of varying dietary MP levels on daily DM intake, body weight change, and digestibility of DM, EE, and NDF. Apparent CP digestibility showed significant ( $P > 0.05$ ) effect of dietary MP contents and digestibility was reported higher in buffaloes fed on 10% HMP and control diets. In contrary to the findings of present study, Faverdin et al. (2003) and Wang et al. (2007) found increased DM intake in Holstein dairy cows fed on diets containing different levels of MP. Faverdin et al. (2003) infused different levels of soya protein and found no effect on apparent digestibility of DM, OM, NDF, or ADF and very small effects on ruminant fermentation variables. Increasing MP supply beyond NRC (2001) recommendations for mid lactating dairy cows had no effect on BW gain and body condition score (BCS) of animals (Voltolini et al., 2008).

### Milk yield and composition

Data for milk yield, 6% FCM and composition indicated that increasing or decreasing the MP by 10% than ICAR (2001) recommendations did not affect milk production and milk composition in experimental buffaloes (Table 2). Bovera et al. (2007) observed non significant changes in milk production when buffaloes were fed higher amount of energy and protein as calculated by CPM dairy software. Increasing MP supply beyond NRC (2001) recommendations





in mid lactating dairy cows grazing elephant grass pasture did not affect 3.5% FCM, milk fat, protein, lactose, and total solids contents (Voltolini et al., 2008). In contrast, Wang et al. (2007) observed increased milk yield, 4% FCM and milk protein (%) in Chinese Holstein cows producing average 30.2 kg/d milk. Increased milk yield from 33.90 to 36.20 kg/d with increasing MP from 8.1 to 10.2% was also observed by Raggio et al. (2004) in dairy cattle. Blouin et al. (2002) observed higher daily milk yield in lactating cows fed on high MP diet, whereas; percentage of fat and protein tended to increased with low MP diet. Lee et al. (2012) found decreased DM intake and milk yield in high producing dairy cows fed the metabolizable protein deficient diets.

**Table 2.** Effects of varying dietary MP levels on feed utilization, milk yield and milk composition in lactating buffaloes

Attributes	Group			SEM
	10% LMP	Control	10% HMP	
Intake, kg/d				
DM	13.2	13.3	13.3	0.03
CP	1.8 <sup>c</sup>	1.9 <sup>b</sup>	2.0 <sup>a</sup>	0.02
DCP	1.1 <sup>c</sup>	1.4 <sup>b</sup>	1.3 <sup>a</sup>	0.03
MP	0.89 <sup>c</sup>	0.94 <sup>b</sup>	1.0 <sup>a</sup>	0.06
Apparent digestibility, %				
DM	62.4	62.6	63.6	0.52
CP	63.6 <sup>b</sup>	66.2 <sup>a</sup>	66.6 <sup>a</sup>	0.77
Milk production, kg/d				
Milk yield	7.3	7.3	7.3	0.02
6% FCM	9.0	9.0	9.0	0.04
Milk composition, %				
Fat	7.2	7.2	7.3	0.05
Protein	4.1	4.2	4.2	0.03

<sup>a-c</sup> Means within same row with different superscripts differ significantly ( $P < 0.05$ ).

No effect of feeding diets having varying MP levels on milk yield and composition in experimental lactating buffaloes might be due to more efficient utilization of N in lactating buffaloes fed on diet containing lower MP level.

#### **Protein requirements of lactating buffaloes**

The comparison of predicted daily protein requirements with existing feeding literature and recommended protein requirements of Murrah buffaloes based on prediction equation are presented in Table 3. The estimated MP requirement for maintenance and milk production was 2.56 g/kg  $W^{0.75}$  and 66.78 g/kg 6% FCM, respectively. Estimated MP requirement for maintenance was 3.11% lower whereas; MP requirement for 1 kg 6% FCM milk was marginally higher (1.13%) than the ICAR (2013) recommendations.



**Table 3.** Comparison of predicted daily protein requirements in buffaloes with existing feeding literature

Protein	Feeding recommendation						
	Present Study	ICAR (2013)	Paul et al. (2001)	ICAR (1985)	Pathak and Verma (1993)	Kearl (1982)	Sen et al. (1978)
Maintenance requirement, g/kg W <sup>0.75</sup>							
MP	2.56	2.65	NA	NA	NA	NA	NA
CP	5.02	4.87	5.42	NA	2.81	3.43	NA
DCP	3.19	NA	3.14	2.63	1.68	2.86	2.84
Requirement/kg 6% FCM, g/kg W <sup>0.75</sup>							
MP	66.78	66.00	NA	NA	NA	NA	NA
CP	116.05	124.00	90.30	NA	110.00	108.00	NA
DCP	71.77	NA	55.20	68.00	66.00	76.00	57.00

NA, not available.

CP requirements were in the range of previous studies reported by Kearl (1982), Paul et al. (2002), and ICAR (2013). The CP requirement estimated by Pathak and Verma (1993) are very low because they were derived from non-producing animals. DCP requirement for maintenance reported by Mudgal and Kumar (1978), Tiwari and Patle (1983) and Paul et al. (2002) was 3.20 g/d, 3.00g/d, and 3.14 g/d, respectively and these were comparable with the findings of present study.

## Conclusions

Although, the intake of CP and MP and apparent digestibility of CP increased as the dietary MP increased but varying dietary MP level did not alters performance of lactating buffaloes. The MP requirement determined by the ICAR (2013) is 3.11% higher for maintenance and 1.13% lower for 6% FCM in lactating buffaloes in present study. Therefore, balancing the protein content of diet in dairy animals in terms of MP is the best possible way for efficient utilization of dietary N.

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## Supplementation of Mangosteen Peel and Banana Flower Pellet (MABAP) to Improve Ruminal Fermentation and Milk Production in Dairy Cows

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### Abstract

The objective of this experiment was to evaluate the influence of mangosteen peel and banana flower pellet (MABAP) on ruminal fermentation and milk production in dairy cows fed on rice straw based diet. Mangosteen peel powder (MSP) is a fruit peel that contains plant secondary compounds (condensed tannins and saponins) and banana flower powder (BAFLOP) is a buffering agent in the rumen. Fourteen crossbred dairy cows (75 % Holstein-Friesian × 25 % Brahman), 404 ± 50.0 kg of body weight (4 years old) and 90 ± 5 day-in-milk with daily milk production of 11 ± 2.0 kg/day, were randomly assigned to receive two dietary treatments (T1 = non-supplementation of MABAP and T2 = supplementation of MABAP at 150 g/head/day) according to a T-test design. All animals were fed concentrate mixtures at 1.0% of body weight and rice straw was fed ad libitum for 35 days with the first 30 days of feed adaptation and the last 5 days of samples collection. The results revealed that there was no difference between treatments on ruminal pH, temperature, ammonia nitrogen and blood urea nitrogen. Total volatile fatty acids, acetic acid, propionic acid and butyric acid were similar among treatments. Moreover, protozoa population was reduced by MABAP supplementation. In addition, estimated rumen methane production was not different by MABAP supplementation ( $P < 0.05$ ). Milk yield and milk composition were the greatest in cows fed with MABAP. In conclusion, supplementation of MABAP as a source of plant secondary compounds exhibited and buffering agent had no negative effect on feed intake, nutrient digestibility while improve ruminal fermentation characteristics and milk production in lactating dairy cows.

**Keywords:** lactating dairy cows, buffering agent, plant secondary compound, rumen enhancer

### Introduction

Russell and Rychlick (2001) stated the important of rumen ecology on its fermentation efficiency and the subsequent production of livestock. There were many factors which could alter rumen ecology for example using high grain with low fiber diet can reduce rumen pH. The use of feed additives such as antibiotics and buffers can improve rumen fermentation efficiency. Research and development regarding methane (CH<sub>4</sub>) production in ruminants have been receiving considerable attention in which mitigation of the rumen CH<sub>4</sub> has been the main issue (Boadi and Wittenberg 2002; Wanapat et al., 2009; Hook et al., 2010; Bodas et al., 2012). This methane is not only related to environmental problems but also is associated with energy losses 8–12 % of ingested gross energy (Bhatta 2015). Plants rich in secondary metabolites (saponins, tannins, essential oils, etc.) have antimicrobial activity which can be exploited for selective inhibition of a particular group of microbes in the rumen and its fermentation (Wallace et al., 2002; Kamra et al., 2015). Mangosteen (*Garcinia mangostana*) peel is a fruit by-product containing a high level of



condensed tannins (CT) and saponins (SP) which exert a specific effect against rumen protozoa, while the rest of the rumen biomass remains unaltered (Poungchompu et al. 2009). Furthermore, Wanapat et al. (2014) reported that supplementation of MSP (100 g DM/day) in buffaloes can increase concentration of C<sub>3</sub> and microbial protein synthesis. In addition, Oskoueian et al. (2013) confirmed that the use of flavonoids especially naringin and quercetin could significantly suppress protozoal and methanogen population in the *in vitro* gas production experiment. High carbohydrate feeds are critical component of ruminant diets in supporting product efficiency while the value 5.5 is the critical point since there is an increase rumen disorder. Banana flower powder has been reported to be used as a rumen buffering agent due to its high mineral elements (Kang and Wanapat 2013; Kang et al., 2014; 2015). The previous findings showed that using banana flower powder (BAFLOP) as buffering agent could enhance ruminal pH and fermentation efficiency in *in vitro* using buffalo and dairy steer rumen fluid (Kang and Wanapat, 2013), and *in vivo* of dairy steers (Kang et al., 2014) and lactating dairy cattle (Kang et al., 2015). Therefore, the present study aimed at investigating the effect of mangosteen peel and banana flower pellet (MABAP) on feed intake, digestibility, rumen fermentation and milk production in lactating dairy cows.

## Materials and methods

Fourteen crossbred dairy cows (75 % Holstein-Friesian × 25 % Brahman), 404 ± 50.0 kg of body weight (4 years old) and 90 ± 5 day in milk with daily milk production of 11 ± 2.0 kg/day, were randomly assigned to receive two dietary treatments (T1 = non-supplementation of MABAP and T2 = supplementation of MABAP at 150 g/head/day) according to a t-test design. Untreated rice straw was offered *ad libitum*. The experiment was conducted for 35 days with the first 30 days of feed adaptation and the last 5 days of samples collection.

Rumen fluid was collected by stomach tube connected with vacuum pump at 0 and 4 h post feeding on the last day of each period. The pH and temperature of the rumen fluid was immediately measured using a portable pH and temperature meter (HANNA instrument HI 8424 microcomputer, Singapore). Rumen fluid samples were then filtered through four layers of cheesecloth. The next part was immediately fixed with 10% formalin solution (1:9 v/v, rumen fluid: 10% formalin) to measure microbial populations by total direct counts of bacteria, protozoa and fungal zoospores (Galyean, 1989). Samples were used for volatile fatty acids (VFA) and NH<sub>3</sub>-N analysis where 5 ml of H<sub>2</sub>SO<sub>4</sub> solution (1 M) was added to 45 ml of rumen fluid. The mixture was centrifuged at 1,600 × g for 15 min, and the supernatant was stored at -20°C prior to VFA analyses using high-performance liquid chromatography (HPLC). Cows were milked twice daily using a bucket-type milking system and milk was weighed at each milking period. Milk samples were composited daily, for both the morning and evening milking, and preserved with 2-bromo-2-nitropropane-1, 3-dial and stored at 4°C until analysed for milk composition (fat, protein, lactose, total solids, and solid-not-fat) by infrared method using Milko-Scan 33 (Foss Electric, Hillerod, Denmark). Milk urea nitrogen (MUN) was determined using Sigma kits #640 (Sigma Diagnostics, St. Louis, MO, USA). All data obtained from the experiment were subjected to T-test design using PROC of SAS (Statistical Analysis System (SAS Institute Inc., 2013). The results are presented as mean values and differences among means with P<0.05 were accepted as representing statistical differences while 0.05<P<0.10 were accepted as tendency.

## Results and discussion

Ruminal fermentation characteristics and blood metabolites affected by MABAP supplementation are presented in Table 2. There were no effects of MABAP supplementation on ruminal pH (6.7–6.8) and temperature (39–39.5°C) in the present study and these were in optimal



ranges for fiber and protein digestion (Fabio et al., 2008; Wanapat and Pimpa, 1999; Zicarelli et al., 2011). Moreover,  $\text{NH}_3\text{-N}$  and BUN were not significantly different between treatments ( $P>0.05$ ) which was similar to the findings of Kongmun et al. (2011), Manasri et al. (2012) and Wanapat et al. (2008). Total VFA, C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub> concentrations and methane production were not affected by MABAP supplementation. Moreover, Ngamsaeng et al. (2006) reported that supplementation of MSP at 100 g/(head x day) in cattle did not show any effects on total VFA or individual VFA concentration which was in contrast to the findings of this paper. Protozoal population were remarkably reduced with supplementation of the MABAP ( $P<0.05$ ). Shokryzadan et al. (2016) reported that mangosteen peel (MSP), containing condensed tannins and saponins, which can affect rumen microbes to reduce enteric methane emission. Condensed tannins and saponins have been shown to influence of rumen ecology by significantly lowering the concentration of C<sub>2</sub>, reduce ruminal protozoa, methanogen population and reducing CH<sub>4</sub> production (Guo et al., 2008; Pongclompu et al., 2009). However, milk yield and 3.5% FCM were not significantly different by MABAP supplementation but milk composition were greatest in cows supplementation of MABAP, especially milk fat was significantly increased in cows supplementation of MABAP. Milk yield (kg/day) to rumen methane production (mol %) were significantly increased by MABAP supplementation.

## Conclusions and recommendations

Supplementation of MABAP had no effect on ruminal pH, temperature,  $\text{NH}_3\text{-N}$  and BUN, while the population of protozoa was decreased by MABAP supplementation. Milk compositions were greatly increase in cows fed MABAP. Therefore, this study suggested that MABAP could be used as a plant source to modify rumen fermentation and microbial population without negatively affecting in lactating dairy cows.

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**Table 1.** Feed ingredient of mangosteen peel and banana flower pellet (MABAP)

Item	MABAP, %
Mangosteen peel powder	70.0
Banana flower powder	20.0
Cassava chip meal	5.0
Urea	4.0
Sulfur	1.0

**Table 2.** Effect of mangosteen peel and banana flower pellet (MABAP) on rumen fermentation and microbial population in dairy cows

Items	Supplementation of MABAP,g/(head day)		P-value
	T1(0)	T2(150)	
pH	6.7	6.8	0.07
Temperature, °C	39.5	39.0	0.32
Ammonia nitrogen, %	12.5	12.7	0.24
Blood urea nitrogen, %	9.6	9.7	0.20
Total volatile fatty acid (mmol/liter)	107.3	112.5	0.53
Acetic acid (C <sub>2</sub> ), %	67.5	66.5	0.26
Propionic acid (C <sub>3</sub> ), %	21.5	22.1	0.09
Butyric acid (C <sub>4</sub> ), %	11.0	11.4	0.15
C <sub>2</sub> :C <sub>3</sub>	3.1	3.0	0.08
Methane (mol/100 mol)*	28.9	28.4	0.09
Total direct counts, cell/ ml			
Protozoa, x10 <sup>5</sup>	4.7	3.3	0.01
Fungi zoospores, 10 <sup>5</sup>	2.8	2.7	0.25

\*Methane=0.45(C<sub>2</sub>)-0.275(C<sub>3</sub>) + 0.4(C<sub>4</sub>) (Moss et al., 2000).

**Table 3.** Effect of mangosteen peel and banana flower pellet (MABAP) on milk production

Items	Supplementation of MABAP,g/(head day)		P-value
	T1(0)	T2(150)	
Milk yield (kg/d)	9.8	10.2	0.07
3.5% FCM (kg/d)	9.3	11.6	0.35
Milk composition			
Protein	3.1	3.6	0.05
Fat	3.8	4.2	0.03
Lactose	4.5	4.3	0.47
Solids-not-fat	8.2	8.6	0.25
Total solids	12.3	13.4	0.20
Milk yield (kg/day)/ rumen methane, mol %	0.32	0.40	0.02



## Energy Utilization, VFA, and A/P Ratio of Kacang Goat Fed Total Mixed Ration Containing Different Treatments of Soybean Meal

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### Abstract

This study was focused on the energy utilization, volatile fatty acids (VFA), and A/P ratio of Kacang goat fed TMR containing different treatments of soybean meal. Fourteen yearling Kacang bucks, 17.6±1.2 kg were arranged in completely randomized design consisted of 3 different treatments those were SBM control (n=5): untreated SBM; SBM50 (n=5): 50% untreated SBM + 50% formaldehyde-protected SBM; SBM100 (n=4): 100% formaldehyde-protected SBM. The TMR consisted of 30% *Pennisetum purpureum*, 30% *gliricidia* leaves, 19.2% cassava waste product, 13.8% wheat bran, 7% SBM, 1% mineral mix were mixed containing 14-15% crude protein and 56-60% TDN. The average daily gain (ADG) during 70 days weighed weekly was calculated using linear regression. Data were analyzed by analysis of variance using the SPSS statistics software version 19. The energy intake and digestible energy (DE) of SBM control (13.0 MJ and 7.7 MJ) were higher than those of SBM50 (10.2 MJ and 5.8 MJ), but they were relatively the same as SBM100 (11.3 MJ and 6.7 MJ). Energy conversion ratio (energy intake, DE, and metabolizable energy/ME) also had the same pattern. Digestible energy (% intake energy), fecal, urine, methane energy loss (MJ), and ME were similar between the treatments. Intake, digested, and metabolizable energy (MJ/kg BW<sup>0.75</sup>) were also the same between the treatments. Total VFA and A/P ratio before feeding was also similar between the treatments. In fact, the A/P ratio at 3 hours and 6 hours of SBM control were higher than those of SBM50, but they were relatively the same as SBM100. It can be concluded that energy utilization of untreated SBM was better than those of 50% formaldehyde-protected SBM, but it was similar to those of SBM100. The control group had A/P ratio that was higher than SBM50 group. In fact, total VFA was similar between the treatments.

**Keywords:** *gliricidia* leaf, Kacang goat, metabolizable energy, methane energy, A/P ratio

### Introduction

Many efforts have been done to improve the low productivity of Kacang goat. Total mixed ration (TMR) containing sources of energy and protein could improve the performance of the



goat. Adiwiniarti *et al.* (2016) reported that soybean meal (SBM) was better than fish meal to improve the productivity of Kacang goat. Soybean meal is one of protein sources that were palatable but highly degradable in the rumen. Darlis *et al.* (2000<sup>a</sup>) reported that crude protein digestibility of goat fed chopped rice straw+SBM (71.1%) was higher than that of goat fed chopped rice straw+SBM+sago meal (66.7%). Many efforts have been done to increase rumen undegradable protein (RUP) in ruminant using formaldehyde, especially in cattle (Widyobroto *et al.*, 2010; Suhartanto *et al.*, 2014). This study observed the use of SBM treated by 1% of formaldehyde for Kacang goat compared to untreated SBM. It focused on the energy utilization, volatile fatty acids (VFA), and A/P ratio.

## Materials and Methods

Fourteen yearling Kacang bucks, 17.6±1.2 kg were arranged in completely randomized design consisted of 3 different treatments those were control (n=5): untreated SBM; SBM50 (n=5): 50% untreated SBM + 50% formaldehyde-protected SBM; SBM100 (n=4): 100% formaldehyde-protected SBM. The TMR consisted of 30% *Pennisetum purpureum*, 30% *gliricidia* leaves, 19.2% cassava waste product, 13.8% wheat bran, 7% SBM, 1% mineral mix were mixed containing 14-15% crude protein and 56-60% TDN. Soybean meal was protected using 1% of formaldehyde calculated based on dry matter SBM.

Feces and urine of each goat were collected, weight, and sampled during 14 days of collection period. Daily feces were sun dried for 3 days, and oven dried at 55°C for 24 h (Solaiman and Shoemaker, 2009). Urine was collected daily into flasks containing 100 ml of 20% H<sub>2</sub>SO<sub>4</sub> (Singh and Kundu, 2013). At the end of collection period, collected dried feces sample and urine were homogenized individually, frozen until being analyzed. Energy of feed, feces, urine was determined using bomb calorimeter. Digestible energy (DE) was calculated from dietary gross energy intake minus fecal energy. The difference between DE and energy loss from urine and methane was determined as metabolizable energy (ME). Methane loss was measured using methane analyzer for 10 min, repeated every 3 hours during 2 days (Kawashima *et al.* 2001 *cit* Purnomoadi, 2014). Conversion factor of 9.45 kcal/L was used to convert methane gas volume to energy (Islam *et al.*, 2000). Volatile fatty acids (acetic, propionic, and butyric acids) analyzed from rumen fluid at 0, 3, and 6 h after feeding were determined by Gas Chromatography (Shimadzu GC-8). The average daily gain (ADG) during 70 days weighed weekly was calculated using linear regression. Data were analyzed by analysis of variance using the SPSS statistics software version 19.

## Results and Discussion

### Energy intake, digestible energy, and metabolizable energy

The energy intake and digestible energy (DE) of control (13.0 MJ and 7.7 MJ) were higher ( $P<0.05$ ) than those of SBM50 (10.2 MJ and 5.8 MJ), but they were relatively the same ( $P>0.05$ ) as SBM100 (11.3 MJ and 6.7 MJ) (Table 1). In fact, the energy intake and DE (MJ/kg BB<sup>0.75</sup>) were not significantly different ( $P>0.05$ ) between the treatments. This indicated that high energy intake and digestible energy was caused by the differences of the body weight.

Energy conversion ratio (energy intake, DE, and metabolizable energy/ME) of control group was better ( $P<0.05$ ) than those of SBM50 group, but it was similar to SBM100 group (Table 2). It showed that goats fed untreated SBM more efficiently converted energy to body weight gain than goats fed 50% formaldehyde-protected SBM. However, digestible energy (% intake energy), ME (MJ/day), energy intake (MJ/kg BB<sup>0.75</sup>), digestible energy (MJ/kg BB<sup>0.75</sup>), and metabolizable energy (MJ/kg BW<sup>0.75</sup>) were similar between the treatments. Metabolizable



energy in this study (4.6 to 6.1 MJ/day) was lower than those reported by Islam *et al.* (2000): 4.4 to 8.9 MJ/day and Wang and Xue (2016): 8.3 to 8.5 MJ/day.

**Table 1.** Energy intake, digestible energy, and metabolizable energy of Kacang goats fed total mixed ration containing different treatments of soybean meal

Parameter	Treatments			P value
	SBM control	SBM50	SBM100	
Energy intake (MJ/day)	13.0 <sup>a</sup>	10.2 <sup>b</sup>	11.3 <sup>ab</sup>	0.013
Energy intake (MJ/kg BB <sup>0.75</sup> )	1.3	1.1	1.1	0.094
Energy loss from:				
Fecal energy (MJ/day)	5.2	4.4	4.57	0.106
Urinary energy (MJ/day)	0.4	0.4	0.3	0.880
Methane energy (MJ/day)	1.3	0.9	1.0	0.418
Digestible energy (MJ/day)	7.7 <sup>a</sup>	5.8 <sup>b</sup>	6.7 <sup>ab</sup>	0.016
Digestible energy (MJ/kg BB <sup>0.75</sup> )	0.8	0.6	0.7	0.093
Digestible energy (%)	59.2	56.3	59.8	0.300
Metabolizable energy (MJ/day)	6.1	4.6	5.4	0.087
Metabolizable energy (MJ/kg BB <sup>0.75</sup> )	0.6	0.5	0.5	0.281
Metabolizable energy (%)	47.0	45.4	48.3	0.830

Note: SBM control: untreated SBM; SBM50: 50% untreated SBM + 50% formaldehyde-protected SBM; SBM100: 100% formaldehyde-protected SBM

<sup>a,b</sup>Means with different superscripts within a row are significantly different (P<0.05)

**Table 2.** Energy conversion ratio

Parameter	Treatments			P value
	SBM control	SBM50	SBM100	
GE conversion ratio (MJ/g)	0.2 <sup>a</sup>	0.4 <sup>b</sup>	0.2 <sup>ab</sup>	0.036
DE conversion ratio (MJ/g)	0.1 <sup>a</sup>	0.2 <sup>b</sup>	0.1 <sup>ab</sup>	0.035
ME conversion ratio (MJ/g)	0.1 <sup>a</sup>	0.2 <sup>b</sup>	0.1 <sup>ab</sup>	0.030

Note: SBM control: untreated SBM; SBM50: 50% untreated SBM + 50% formaldehyde-protected SBM; SBM100: 100% formaldehyde-protected SBM

<sup>a,b</sup>Means with different superscripts within a row are significantly different (P<0.05)

### Volatile fatty acids and A/P ratio

Total of VFA produced was not significantly different (P>0.05) between the treatments at 0 h, 3 h, and 6 h after feeding (Table 3). Acetate-propionate ratio (A/P ratio) before feeding was also similar (P>0.05) between the treatments. In fact, the A/P ratio at 3 hours and 6 hours of control were higher (P<0.05) than those of SBM50, but they were relatively the same as SBM100. This might be cause of the digested dry matter of control (452.0 g) tended a little bit higher (P=0.08) than those of SBM50 group (324.1 g). The digested crude fiber of control was 86.5 g and those of SBM50 goats was 65.6 g. Acetate production was influenced by the roughage fermentation, and goats have potentially degraded dry matter (*in situ*) and acid detergent fiber (*in vivo*) of rice straw+SBM or SBM+sago meal better than sheep (Darlis *et al.*, 2000<sup>b</sup>). Darlis *et al.* (2000<sup>b</sup>) reported A/P ratio in goat (5.1) that was higher than A/P ratio in this



study. Jelantik *et al.* (2012) reported total VFA in Kacang goat fed urea-ammoniated grass hay and sun-dried fish or fish meal was 44.1 to 61.1 mM and A/P ratio was 3.65 to 4.28.

**Table 3.** Volatile fatty acids and A/P ratio

Parameter	Treatments			P value
	SBM control	SBM50	SBM100	
Total VFA (mM/L):				
0 h	134.0	91.4	90.2	0.075
3 h after feeding	143.1	111.1	116.3	0.512
6 h after feeding	141.4	113.4	120.7	0.383
Acetate (mM/L):				
0 h	98.7	66.8	66.9	0.091
3 h after feeding	107.2	78.8	85.6	0.426
6 h after feeding	105.3	81.0	88.2	0.28
Propionate (mM/L):				
0 h	23.4	16.4	15.8	0.079
3 h after feeding	25.8	22.6	22.7	0.416
6 h after feeding	26.2	22.8	23.3	0.653
Butyrate (mM/L):				
0 h	11.9 <sup>a</sup>	8.3 <sup>ab</sup>	7.5 <sup>b</sup>	0.021
3 h after feeding	10.0	9.7	8.1	0.528
6 h after feeding	9.8	9.6	9.2	0.948
A/P ratio:				
0 h	4.2	4.1	4.2	0.94
3 h after feeding	4.2 <sup>a</sup>	3.5 <sup>b</sup>	3.7 <sup>ab</sup>	0.02
6 h after feeding	4.1 <sup>a</sup>	3.6 <sup>b</sup>	3.8 <sup>ab</sup>	0.048

Note: SBM control: untreated SBM; SBM50: 50% untreated SBM + 50% formaldehyde-protected SBM; SBM100: 100% formaldehyde-protected SBM

<sup>a,b</sup>Means with different superscripts within a row are significantly different ( $P < 0.05$ )

## Conclusion

It can be concluded that energy utilization of untreated SBM was better than those of 50% formaldehyde-protected SBM, but it was similar to those of 100% formaldehyde-protected SBM. The control group had A/P ratio that was higher than SBM50 group, but similar to SBM100 group. In fact, total VFA was similar between the treatments.

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## Feed Intake and Digestibility of Dairy Cows Affected by Mao (*Antidesma thwaitesianum* Muell. Arg.) Pomace Meal Supplementation

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### Abstract

The current study was designed to determine the effect of mao (*Antidesma thwaitesianum* Muell. Arg.) pomace meal (MPM) supplementation on feed intake and nutrient digestibility in dairy cows. Four crossbred dairy cows (75% Holstein-Friesian with 25% Thai native breed) in early-lactation, 442±30 kg of body weight (BW) and 45±5 days in milk were randomly assigned according to a 4×4 Latin square design to receive 4 dietary treatments. The treatments were different levels of MPM supplementation at 0, 100, 200, and 300 g/head/day, respectively. Cows were fed with concentrate diets at a ratio of concentrate to milk yield of 1:1.5, and urea-treated (3%) rice straw was fed *ad libitum*. It was found that supplementation of MPM had no effect on DM intake and digestibility of DM, OM, CP, EE, NDF and ADF ( $P>0.05$ ). From the current study, it can be concluded that supplementation of MPM did not show any negative effect on DM intake and nutrient digestibility in dairy cows.

**Keywords:** Mao pomace meal, intake, digestibility, dairy cows

### Introduction

Mao or mamao or makmao (*Antidesma thwaitesianum* Muell. Arg.) is favored by consumers in the local market in Thailand because of its good color and taste. Currently, mao juice and mao wine industry produce very large amounts of pomace (seed and skin pulp), which is considered as an environmental problem. Mao pomace contains a large amount of plant secondary compounds, especially condensed tannins and saponins which have potential use for manipulating rumen fermentation. Our previous study (Gunun et al., 2016) reported that supplementation of mao seed meal to diets of goats improve rumen fermentation, especially propionate and N utilization, without affecting the feed intake and nutrient digestibility. Gunun et al. (2014) found that potential extent of gas production (a+b) and *in vitro* true digestibility were decreased when supplemented with mao pomace meal (MPM) at 12-20 mg/0.5 g of diet.



Supplementation of high levels of tannins and saponins to ruminant diets usually reduces feed intake because of reduced palatability, decreased rate of digestion and development of conditioned aversion (Mueller-Harvey, 2006; Li and Powers, 2015). However, utilization of condensed tannins and saponins in MPM for dairy cows has not been studied. Therefore, the objective of this study was to investigate the effect of MPM supplementation on feed intake and nutrient digestibility in dairy cows.

### **Animals, treatments and experimental design**

Four crossbred early-lactation dairy cows (75 % Holstein-Friesian × 25 % Thai native breed) with  $442 \pm 30$  kg of body weight (BW) and  $45 \pm 5$  days in milk with daily milk production of  $11 \pm 1.0$  kg/day were randomly assigned according to a  $4 \times 4$  Latin square design to receive 4 dietary treatments. The treatments were as follows: supplementation with MPM at 0, 100, 200 and 300 g/head/day. Fresh mao pomace was provided from Wanawong fruit wine Ltd., Phuphan, Sakon Nakhon, Thailand; and sundried for 2 to 3 days, then ground to pass a 1 mm sieve. Cows were fed the concentrate diets at a concentrate to milk yield ratio of 1:1.5. The urea-treated (3%) rice straw was fed *ad libitum*. The ingredients and chemical composition of diets are shown in the Table 1. Cows were housed in individual pens. Clean fresh water and mineral blocks were available at all times. The experiment was conducted for four periods, each lasting for 21 days. The first 14 days were for adaptation period, whereas the last 7 days were for sample collection.

### **Data collection and sampling procedures**

Feed intakes and refusals were measured and recorded. The BW was measured daily during the sampling period prior to feeding. Feeds were sampled and fecal samples were collected of each individual cows on each treatment during the last 7 days of each period. Feeds were sampled daily during the collection period and were composited by period prior to analyses. Fresh fecal samples (about 500 g) were collected twice daily by rectal sampling (morning and afternoon) after milking, two successive samples were combined and used as one sample and composited for storage in the freezer. Composite samples were dried at 60 °C, ground (1-mm screen using Cyclotech Mill; Tecator, Hoganas, Sweden) and then analysed for dry matter (DM), ash, ether extract (EE) and crude protein (CP) content (AOAC, 2016), neutral detergent fiber (NDF), acid detergent fiber (ADF) (Van Soest et al. (1991) and acid-insoluble ash (AIA). AIA was used to estimate digestibility of nutrients (Van Keulen and Young, 1977). The condensed tannins and saponins were analyzed by using the vanillin-HCl method as modified by Wanapat and Pongchompu (2001). All data were subjected to analysis of variance according to a  $4 \times 4$  Latin square design using the general linear models procedures (SAS, 1996). Orthogonal polynomial contrasts were used to estimate the effect of MPM supplementation. Significant effects were identified at  $P < 0.05$ .



**Table 1.** Ingredients and chemical composition of the diet used in the experiment

Item	Concentrate	UTRS <sup>a</sup>	MPM <sup>b</sup>
Ingredient, % dry matter			
Cassava chip	65.0	-	-
Soybean meal	19.3	-	-
Rice bran	8.1	-	-
Urea	2.6	-	-
Molasses	3.0	-	-
Mineral and vitamin mixture	1.0	-	-
Salt	0.5	-	-
Sulfur	0.5	-	-
Chemical composition			
Dry matter, %	85.9	55.2	93.8
Percentage of dry matter			
Organic matter	92.2	89.8	95.8
Crude protein	18.1	5.8	8.6
Ether extract	0.5	0.8	3.6
Neutral detergent fiber	24.9	78.4	72.1
Acid detergent fiber	15.2	56.9	63.6
Ash	7.8	10.2	4.1
Condensed tannins	-	-	9.2
Saponins	-	-	9.8

<sup>a</sup>UTRS, Urea-treated (3%) rice straw.

<sup>b</sup>MPM, Mao pomace meal.

## Results and discussion

Results of feed intakes and nutrient digestibilities as affected by MPM supplementation are presented in Table 2. The results showed that feed intake and nutrient digestibility were not influenced by MPM supplementation ( $P>0.05$ ). Similarly, Gunun et al. (2016) found that supplementation with mao seed meal did not change feed intake and nutrient intakes of goats. Kumar et al. (2017) reported that supplementation of tea seed and tea seed saponin extract did not affect DM intake of Gaddi kids. It has been reported that feed intake was reduced by high doses ( $>50$  g/kg DM) of condensed tannin uptake (Beauchemin et al., 2008). Saponins are bitter in taste, highly soluble in water and at higher dose level may depress intake due to low palatability (Li and Powers, 2015). However, in the present study, dietary tannins and saponins sources had no effect on total DM intake when used at a suitable level ( $<50$  g/kg DM) as a supplement. In addition, tannins and saponins have been implicated for their inhibitory effect on feed digestion (Santoso et al., 2007; Patra and Saxena, 2011). Supamong et al. (2017) reported that supplementing of *Delonix regia* seed meal containing CT and SP at 270 g/head/day reduced



DM and organic matter (OM) digestibility in beef cattle. However, Gunun et al. (2016) who elucidated that mao seed meal supplementation did not affect on nutrient digestibility in goats.

**Table 2** Effect of mao pomace meal (MPM) supplementation on feed intake and nutrient digestibility in dairy cows

Item	Level of MPM <sup>b</sup> (g/head/day)				SEM	Contrast		
	0	100	200	300		L	Q	C
DM intake								
UTRS <sup>a</sup>								
kg/d	5.1	5.2	5.5	5.5	0.52	ns	ns	ns
%BW	1.1	1.1	1.1	1.2	0.06	ns	ns	ns
Concentrate								
kg/d	7.9	8.2	8.1	8.2	0.16	ns	ns	ns
%BW	1.7	1.8	1.7	1.8	0.06	ns	ns	ns
Total intake								
kg/d	13.0	13.6	13.8	14.0	0.62	ns	ns	ns
%BW	2.8	2.9	2.9	3.0	0.06	ns	ns	ns
g/kg BW <sup>0.75</sup>	130.2	134.5	133.9	139.3	2.58	ns	ns	ns
Apparent digestibility, %								
Dry matter	55.7	54.3	54.3	58.1	1.17	ns	ns	ns
Organic matter	59.7	58.7	58.8	62.1	1.12	ns	ns	ns
Crude protein	57.6	49.7	50.1	53.3	2.36	ns	ns	ns
Ether extract	51.5	47.8	53.6	53.9	3.16	ns	ns	ns
Neutral detergent fiber	49.2	50.6	48.2	53.8	1.84	ns	ns	ns
Acid detergent fiber	37.9	43.7	41.4	46.1	1.83	ns	ns	ns

<sup>a</sup>UTRS, urea-treated (3%) rice straw.

<sup>b</sup>MPM, mao pomace meal.

SEM, standard error of the means; L, linear; Q, quadratic; C, cubic; ns, not significant.

## Conclusions

MPM supplementation did not showed negative effects on intake and nutrient digestibility in dairy cows fed on urea-treated rice straw based diet.

## Acknowledgements

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## Performance and Physiological Status of Kids Milking by Milk Replacer Containing Cricket Meal

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### Abstract

Goat kids mortality in Indonesia is still high, more than 20%. This condition is due to the low nutrient intake of kids, high market of goat milk so that decreasing of immune status and the poor nutrition status of the ewes, especially for duplet and triplet litter size. Nutrient improvement through the utilization milk replacer is important to reduce the mortality. Good quality protein from insect is one alternative solution for making milk replacer. The aim of this research was to evaluate the performance and physiological or healthy status of kids milking by milk replacer containing cricket meal. The randomised completely design was used in this experiment by using twelve pre-weaning kids and divided into three treatments. The treatments were control with Goat milk (GM) where the kids milking by their mother, the Cow Milk (CM) group was the kids milking by cow milk and the Milk Replacer (MR) group was the kids milking by milk replacer containing cricket meal. Result showed that there was no significance difference of physiological status parameter in kids milking by different milk (milk replacer). Meanwhile the performance of kids was significance difference ( $P < 0.05$ ) affected by the treatments. The average daily gain of control was around 120 g/h/d, and higher than cow milk and cricket milk replacer treatments (98 and 109 g/h/d). The palatability of cricket meal milk replacer was higher than cow milk. It is concluded that cricket meal can be used as a part of milk replacer, substitute of casein without any negative effect to the healthy status, although the performance still a little bit lower than control.

**Keywords:** cow milk, cricket meal, milk replacer, physiological status and pre weaning

### Introduction

Statistic data of Indonesian goat population in 2017 (18.6 million heads) shows that there is increasing population during last five years. Milk and meat now is getting more popular and goes to the big market, especially for sate and yoghurt. High milk yield with high price and high litter size give an opportunity to the farmers to increase their income. On the other hand, the performance of kids are decreasing and mortality increase due to the lack of nutrient requirements. The kids get their milk only a week after parturition and after that all the milk goes to the market. Another problem is if ewes with high litter size, more than three kids, will cause the kids get worst condition. Milk replacer is one of the alternative solution to solve the kids performance before weaning. Utilization of local feedstuff for milk replacer can help to solve the problem. Insect such cricket is one of protein sources which can be used for substitute casein or soy bean meal. Insect is one of potential commodity as alternative a new protein source for animal feed stuff (Sánchez-Muros et al., 2014). Crickets meal contains 57.07%



of crude protein, 10.28% of crude fiber, and 13.13% of fat (Astuti et al., 2016). Cricket also has contain various of amino acid 6.75% valine, 5.1% lysine, 4.46 isoleucine, 11.62% alanine, 7.22% arginine, and 6,77% glycine and chitin 8.7% (Wang, 2005). Meanwhile the fatty acids contain are 50.32% palmitic acid (16:0), 32.06% stearic acid (18:0), 9.77% oleic acid and 2.34% linoleic acid (Chakravorty et al., 2014). Commonly crickets used as feed for fish, birds, and poultry, while for ruminant feed is very rare (Makkar, 2014). Moreki et al. (2012) reported the utilization of cricket meal as protein source to substitute of soybean meal in chicken ration could increase feed conversion ratio. The result from the previous study by using raw material cricket meal for creep feed ration in sheep showed that there were no significance difference performance with the control ration, it means that cricket meal could be used savely (Astuti, et al., 2016). Based on the situation, this study was aimed to evaluate the utilization of cricket meal in milk replacer on performance and physiological status of goat kids.

## Material and Methods

Twelve crossbred Sapera male (saanen x etawah) local pre-weaning goat kids, one week old were used in this experiment and divided into three treatments by using completely randomised block design. The initial average body weight were 3.78 kg. The treatments were control, where the kids were given goat milk (GM), kids were given cow milk (CM) and kids were given milk replacer (MR), four times a day (morning, at noon, afternoon and night). Parameters measured were nutrients intake, ADG, respiration rates, heart rates and rectal temperatur, and evaluated during eight weeks.

Table 1. Nutrient composition of goat milk and milk replacer

Nutrients (%)	Treatments			Commercial MR
	GM	CM	MR	%
Protein	31.54	25.72	21.97	18-22
Fat	50.00	15.59	25.72	10-20
Lactosa	32.31	35.07	-	-
Calcium	0.92	1.25	1.62	1.00
Phospor	1.85	1.64	0.87	0.70

GM = goat milk, CM = cow milk, MR = milk replacer.

The ingredients for making milk replacer were egg yolk powder, skim powder, full cream, wheat meal, fish oil, premix, and CaCO<sub>3</sub>. Total dry matter offered of milk replacer was 3.5% of BW and then diluted in warm water (37°C) with ratio 1:4 .

## Result and Discussion

The average environment temperature during morning and afternoon were lower than at noon, meanwhile for the humidity in the morning and afternoon higher than at noon (Table 2). Yousef (1985) reported that the thermoneutral zone for tropical goat is around 18-30°C with humidity is around 55% relative. The ideal temperature for dairy goat is around 21 °C (Ensminger, 2002). The temperature during this research was around 26.50 - 29.40 °C with the humidity from 58 – 64% relative, so that the condition was very confinient for the animals. It was the comfort zone for the animals. On the other hand, high temperature and humidity can caused animal heat stress so that the consumption will decrease.



Table 2 . Environment condition during research

Week	Morning		Noon		Afternoon	
	Temp. (°C)	Humid. (%)	Temp. (°C)	Humid. (%)	Temp. (°C)	Humid. (%)
1	26.4	64	29.1	56	28.0	70
2	26.9	56	30.8	59	28.4	60
3	26.7	71	29.3	68	25.5	58
4	27.8	65	28.5	60	26.0	50
5	26.0	57	30.2	46	28.5	70
6	26.5	61	28.8	59	27.0	68
7	25.5	69	30.4	53	28.0	71
8	26.3	65	28.2	67	26.7	68
Average	26.5±0.67	63.5±5.29	29.4±0.95	58.5±7.15	27.3±1.13	64.4±7.56

Data table 3. showed that DM, protein, fat and calcium intake of cricket meal MR treatment was significance higher than another two treatments ( $P<0.05$ ), meanwhile, phosphor intake of all treatments showed the same. Main factor which affect to the consumption are palatability, milk quality, temperature of environment, age, physiological status and body weight ( Mc. Donald et al., 2011).

Table 3. Nutrient intakes of goat kids during 8 weeks evaluation

Nutrients (g/h/d)	Treatments		
	GM	CM	MR
Dry matter	98.20±7.79 <sup>c</sup>	120.05±5.24 <sup>b</sup>	203.87±3.07 <sup>a</sup>
Protein	30.97±2.46 <sup>b</sup>	30.88±1.35 <sup>b</sup>	43.97±0.66 <sup>a</sup>
Fat	31.73±2.52 <sup>b</sup>	18.72±0.82 <sup>c</sup>	51.40±0.77 <sup>a</sup>
Calcium	0.90±0.07 <sup>c</sup>	1.50±0.07 <sup>b</sup>	3.30±0.05 <sup>a</sup>
Phospor	1.82±0.14	1.97±0.09	1.77±0.03

GM = goat milk, CM = cow milk, MR = milk replacer. Different font at the same colom is significance difference ( $P<0.05$ )

The average daily gain (ADG) and final body weight of kids with goat milk and milk replacer treatments were same and higher than cow milk treatment (Table 4), although the dry matter intake in milk replacer treatment was higher than in goat milk treatment ( $P<0.05$ ). This condition was suggested by the differences of availability (digestibility and absorption) of nutrients of both treatments, where the goat milk is better than milk replacer. The enzyme activities and mothering immunity compound in goat milk is significantly affected to the growth rates of the kids. This condition was not happen in cow milk treatment, where the performance profil was the lowest compared to others.

Table 4. Performance pre-weaning goat kids during 8 weeks

Performance	Treatments		
	GM	CM	MR
Initial BW (kg)	3.75 ± 0.34	3.70± 0.32	3.88 ± 0.33
Final BW (kg)	9.65±0.54a	8.53 ± 0.51b	9.23 ± 0.53a
ADG (g/h/d)	120.41 ± 10a	98.47±20.96b	109.18±12.37a



GM = goat milk, CM = cow milk, MR = milk replacer. Different font in the same colom is significance difference P<0.05.

The singlet kid usually has higher gain than duplet or triplet litter size. In this study all kids were produced from singlet and duplet litter size. The total milk from the ewes for the duplet kids should be divided for both kids, so that it affected to the performance. The goat milk has mothering immunity for the kids compare to the artificial milk replacer without mothering immunity compound (Fig. 1). The high final body weight of goat milk treatment was supported by efficiency of nutrient uptakes and its mothering immunity, meanwhile the performance of kids treated by milk replacer containing cricket meal was due to the high nutrients (protein, fat and calcium) intake only.

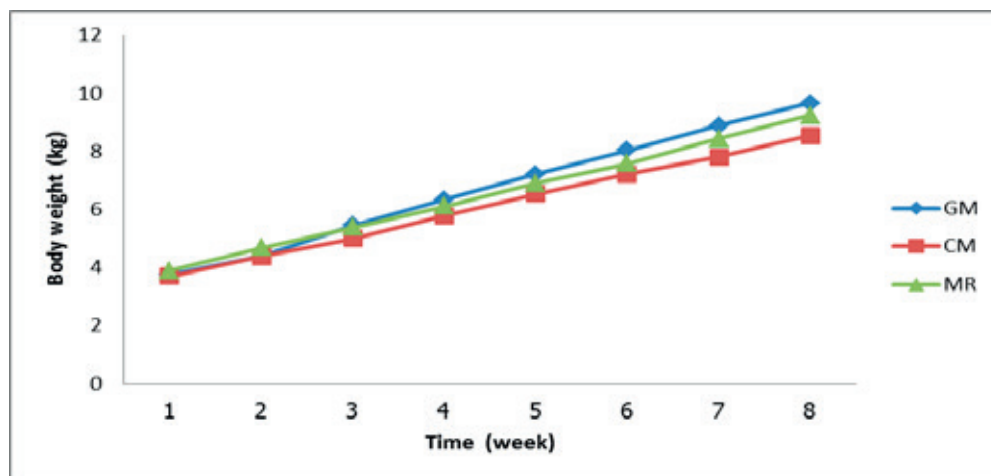


Figure 1. The growth rate of the kids until weaning weight.

Table 5. Physiological status of goat kids during 8 weeks pre-weaning

Parameters	Treatments	Times		
		Morning	Noon	Afternoon
Rectal temp. (°C)	GM	38.87±0.26	39.4±0.24	39.36±0.33
	CM	38.90±0.28	39.45±0.16	39.19±0.28
	MR	38.84±0.26	39.55±0.14	39.13±0.33
	Average	38.87±0.27	39.47±0.18	39.23±0.31
Heart rates (x/min)	GM	82.53±4.66	111.21±7.37	87.82±5.98
	CM	84.86±6.63	92.09±9.27	92.09±9.27
	MR	95.88±14.41	112.96±9.42	87.64±11.39
	Average	87.76±8.57	105.42±8.64	89.18±8.88
Respiration (x/min)	GM	32.50±4.44	42.25±5.00	34.61±4.62
	CM	37.43±5.59	40.36±5.81	42.81±4.45
	MR	40.41±6.62	41.36±5.90	40.14±6.54
	Average	36.78±5.55	41.32±5.57	39.19±5.20

GM = goat milk, CM = cow milk, MR = milk replacer

The physiological status of animal such as rectal temperature, hearth rates and respiration rates were non significance among the treatments and in normal condition (Table 5). Frandson,



et al. (2009) reported that rectal temperature, respiration rates and heart rates were 38.5 - 40 °C, 26 - 54 x/min and 70 - 135 x/min, respectively. The respiration rates are affected by body temperature and environment condition, body condition and intakes. This research showed that milk replacer containing cricket meal resulted good response to the physiological status of the kids, without any negative effect.

## Conclusion

It was concluded that cricket meal could be used in goat kids milk replacer with good palatability and resulted ADG around 109 g/h/d, without any negative effect to the health status of the animal.

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ANN-01-0080

## Variations in Milk Composition between Morning and Afternoon Milking in Dairy Cow

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### **Abstract**

The objectives of this study were to compare the milk composition between milk samples from morning and afternoon milking in dairy cow. Thirty mid-lactation Holstein dairy cows were used in this study. Cows were milked two times a day (morning milking at 07.00 h and afternoon milking at 15.00 h). Milking interval was 16 h and 8 h between afternoon milking to morning milking and morning milking to afternoon milking, respectively. Separate morning and afternoon milk samples were collected every day for one week and analyzed for milk composition. Milk production (kg/h/day) of morning milk was significantly higher ( $P < 0.001$ ) than afternoon milk at 9.3 and 4.6, respectively. Fat content (%) from morning milk was significantly lower ( $P < 0.001$ ) than afternoon milk at 2.6 and 3.9, respectively. There were no significant differences in other milk composition parameters. Based on these results, a conclusion can be made that milk from morning and afternoon milking varies in fat content. Therefore, milk samples for milk composition analysis should be concerned with milking time.

**Keywords:** milk composition, milk sampling, milking time

### **Introduction**

Understanding the variability in milk yield, fat and milk composition percentage is important when milk samples were taken for analysis in research work or for making management decisions. Usually, milk samples were taken from morning milk plus afternoon milk from individual cows for milk composition analysis. Milk fat content in milk varies between morning and afternoon milking. Gilbert et al. (1973) reported that milk fat percentage of milk from afternoon milking was higher than morning milking. Moreover, Erb et al. (1952) found that milk fat was 9.6% lower in the milk from morning milking as compared to afternoon milking. However, milking interval may affect milk yield and result in milk fat content of morning milk and afternoon milk. Williams et al. (2010) found that milk fat content was not different between morning milk and afternoon milk when the milking interval was exactly 12:12 h (12 h for morning milking to afternoon milking and 12 h for afternoon milking to morning milking of next day). While milk fat was higher in afternoon milk than morning milk when milking intervals were 14:10 h and 16:8. Traditionally, farmer practices in Thailand, milking interval is 14:10 to 16:8. Therefore, the objectives of this study were to compare the milk composition between milk samples from morning and afternoon milking in dairy cow.



## Materials and Methods

The study was conducted using 30 mid-lactating dairy cows research farm of faculty of Natural resource, Rajamangala University of Technology Isan, Sakon Nakhon Campus, Thailand. Cows were milked two times a day at 07.00 h (morning milking) and 15.00 h (afternoon milking). Milk production was recorded daily. Separate morning and afternoon milks sampling were collected every day for one week and analysis for milk compositions by using milkoscan. The milk production and milk composition (fat, protein, lactose, solid not fat and relative density) were analyzed using a paired-T test using SPSS software to compare between morning and afternoon milk.

## Results and discussions

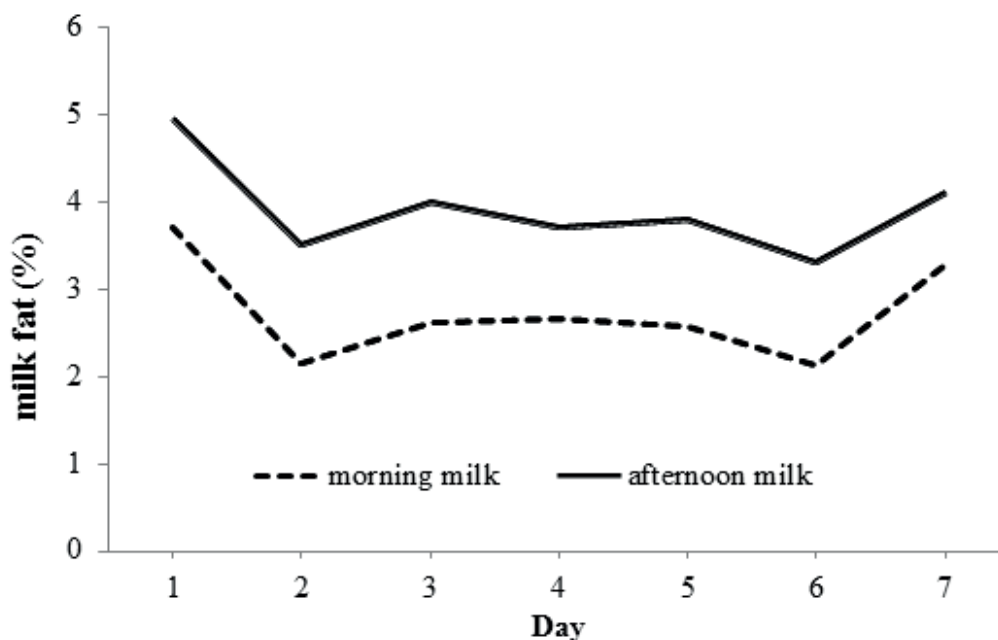
The influence of milking time on milk production and milk composition are shown in Table 1. The milking time (morning or afternoon milking) had a highly significant effect ( $P < 0.001$ ) on the means of milk production and fat concentration. There were no significant differences between morning milk and afternoon milk for protein, lactose, solid not fat concentration and relative density.

**Table 1.** Influence of milking time on milk production and milk composition in mid-lactating dairy cow.

Characteristic	Morning milk	Afternoon milk	P value
Milk production (Kg/h/day)	9.3	4.6	***
Concentrations (%)			
Fat	2.6	3.9	***
Protein	3.1	3.2	ns
Lactose	4.8	4.8	ns
Solid not fat	8.5	8.7	ns
Relative density	1.026	1.026	ns

Milk production (kg/h/day) of morning milk was significantly higher ( $P < 0.001$ ) than afternoon milk. Fat content (%) from morning milk was significantly lower ( $P < 0.001$ ) than afternoon milk (Figure 1).

Milk production showed a higher production in the morning than in the afternoon of this study agrees with reported from various studies (Everett and Wadell, 1970; Gilbert et al., 1973; Quist et al., 2008; Williams et al., 2010). Milk production is related to the inter-milking period preceding that milking (O'Brien et al., 1998; Remond et al., 2009) and directly proportional to inter-milking interval (Williams et al., 2010). Longer milking interval from afternoon milking to next day morning milking could result in higher milk production of morning milking.



**Figure 1.** Mean of 30 cows for milk fat concentration (%) for each milking (morning milking and afternoon milking) over 7-d milking period

Our finding of higher fat concentration in the afternoon milking than morning milking was consistent with finding of Gilbert et al., (1973), who found that fat was higher in the afternoon milking. Wheelock (1980) reported that when milking intervals are not equal, milk fat percentage will be highest after the shortest interval. The shorter milking interval from morning milking to afternoon milking of the same day in this study could be the reason why higher fat percentage of afternoon milking than morning milking.

## Conclusion

Milk samples from morning and afternoon milking were varies in fat content. Therefore, milk sample for milk composition analysis should be concern about milking time.

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## Session 2-Arawan I

ANN-01-0052

### Feeding POAB at Different Level of Amino Acids in The Diet of Broiler Chickens

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#### Abstract

Antibiotics are commonly used in poultry diets in order to prevent diseases and to improve the performance. However, there has been growing public concern about the risk of bacterial resistance associated with the routine use of Antibiotic Growth Promoter (AGP) in livestock feeds. The organic acid mixtures might be more efficient than some antibiotic growth promoter in improving broiler performance. The diet supplemented with organic acid and palm fat (POAB) in the broiler diet showed an increase in body weight gain (2963.00 g) compared to the control group (2694.55 g) at the end of 42 day of experiment. A similar trend was found in the result of crude fat, methionine and lysine digestibility. It showed that the POAB able to improve the nutrients digestion of the chickens. From the results, the POAB able to improve the growth performance by increase the digestibility of the chickens. Moreover, the treatment groups supplemented with POAB promote the growth of beneficial bacteria which was indicated by higher lactic acid bacteria (LAB) count compared to the treatment without organic acids.

**Keywords:** organic acids, palm fat, inhibitory activity, growth performance, broiler

#### Introduction

Organic acids are natural constituents of plant and animal tissues and their use as feed additives is now being studied worldwide to replace antibiotics. A wide range of organic acids with variable physical and chemical properties are available for poultry, of which many are used in the drinking water or mixed with the feed (Huyghebaert et al., 2011; Menconi et al., 2014). Besides that, organic acids also contributed greatly to the profit in poultry production as it provides consumer with health and nutritious poultry products (Adil et al., 2010). The recommended rates of commercially produced organic acid usually between 0.2% and 1.0%. (Lückstädt et al., 2004). The mode of action of organic acids in animal diets has not been clearly clarified; this inadequate understanding has limited the application of organic acids in broiler diets. However, several possible mechanisms have been proposed and most of them have been associated with: (1) reduced pH in diets and subsequent reduction of the pH in the GIT, (2) better nutrient utilization in diets by increasing nutrient retention, and (3) inhibition of pathogenic bacterial growth (Afsharmanesh et al., 2005; Mroz, 2005). The purpose of this study was to investigate the effect of dietary supplementation with organic acids (OA) with different level of amino acids on the growth performance, digestibility and microflora count in broiler chickens.



## Material and Methods

A total of 384 day-old chickens were used in this study. This experiment will be conducted in a poultry research unit, UPM. The chickens were randomly assigned into 8 treatment groups with 6 replicates per treatment. Each experimental unit was consisted of 8 chickens. Starter diet was feed from day 0 to day 21, while grower diet was feed from day 21 to day 42. The POAB used in this study was supplied by Sunzen Lifescience Sdn Bhd. The chickens were allocated to the following treatments:

For starter diet, T1: 21% Crude protein (CP) (Lys 1.18%; Met 0.45%), T2: 21% CP (Lys 1.0%; Met 0.45%), T3: 21% CP (Lys 1.18%; Met 0.4%), T4: 21% CP (Lys 1.0%; Met 0.4%), T5: 21% CP (Lys 1.18%; Met 0.45%) + POAB, T6: 21% CP (Lys 1.0%; Met 0.45%) + POAB, T7: 21% CP (Lys 1.18%; Met 0.4%) + POAB, T8: 21% CP (Lys 1.0%; Met 0.4%) + POAB.

For grower diet, T1: 19% CP (Lys 0.95%; Met 0.39%), T2: 19% CP (Lys 0.75%; Met 0.39%), T3: 19% CP (Lys 0.95%; Met 0.25%), T4: 19% CP (Lys 0.75%; Met 0.25%), T5: 21% CP (Lys 0.95%; Met 0.39%) + POAB, T6: 21% CP (Lys 0.75%; Met 0.39%) + POAB, T7: 21% CP (Lys 0.95%; Met 0.25%) + POAB, T8: 21% CP (Lys 0.75%; Met 0.25%) + POAB.

The experiment was conducted for 42 days. The individual body weight (BW) and cage feed intake (FI) were recorded weekly and live weight gain (WG), average daily weight gain (ADG) and feed conversion ratio (FCR) were calculated. At weeks 6, 12 chickens, respectively from each treatment were slaughtered for sampling of jejunum, duodenum, ileum and ileal digesta for further analysis. Ileal digesta samples were taken for nutrient analysis and apparent digestibility measurement.

## Results and Discussions

### Growth performance of the broiler chickens

Table 1 showed the growth performance for the starter stage. there was no significant difference among the treatment groups during the initial body weight. While for the final body weight, T5 showed a significant highest body weight among all the treatment groups. This might due to the higher amino acids level supplemented together with the POAB in the diet. Moreover, the treatment groups which supplemented with POAB also showed a significant better body weight to the treatment groups although had the same level of amino acids provided in the diet. For the feed intake, T1 showed a significant different compared to T4. While for the FCR, T4 showed the highest FCR value among the treatment groups. Besides that, the treatment groups supplemented with POAB showed a significant lower FCR compared with the treatment group without POAB.

Table 2 showed the growth performance for the grower stage. For the final body weight, T5 showed the highest body weight among the treatment groups follow by T6 and T7. The higher body weight of the chickens can be found in the treatment groups supplemented with POAB although the amino acids supplementation in the diet is the same compared with the diets without POAB. While for the T8 (Lys 0.75%; Met 0.25%) showed a similar or comparable results with T1 (Lys 0.95%; Met 0.39%) which had a higher amino acids level in the diet. This can be concluding that POAB able to help to improve the growth performance of the chickens.

Table 3 represented the overall growth performance of the chickens in 42 days. Similar trends are found in the starter and grower stage. The treatment group supplemented with POAB had a significant higher performance. Most of the treatment groups with POAB also showed a significant lower FCR compared to the treatment group without supplementation of POAB. The improvement in the FCR could be possibly due to better utilization of nutrients resulting in increased body weight gain (Adil et al. 2010). Moreover, the dietary supplementation of organic



acids increased the body weight and FCR in broiler chicken (Fascina et al. 2012). Brzóska et al. (2013) reported that the organic acids (0.3–0.9%) had a growth-enhancing and mortality-reducing effect in broiler chickens, with no significant effect on carcass yield or proportion of individual carcass parts.



**Table 1.** Growth Performance For 0-21 days with different dietary treatments

Treatment**	Growth Performance (0-21 days)*					
	Initial BW	Final BW	Cumulative WG	Cumulative FI	FCR	Average Daily Gain
T1	45.19	818.43 <sup>bc</sup>	773.25 <sup>bc</sup>	1091.04 <sup>a</sup>	1.41 <sup>bc</sup>	36.82 <sup>cb</sup>
T2	45.23	814.02 <sup>d</sup>	768.66 <sup>e</sup>	1083.33 <sup>ab</sup>	1.41 <sup>bc</sup>	36.60 <sup>e</sup>
T3	45.19	807.35 <sup>f</sup>	762.16 <sup>f</sup>	1081.46 <sup>b</sup>	1.42 <sup>ab</sup>	36.29 <sup>f</sup>
T4	45.29	804.12 <sup>g</sup>	758.75 <sup>g</sup>	1083.33 <sup>ab</sup>	1.43 <sup>a</sup>	36.13 <sup>g</sup>
T5	45.27	826.39 <sup>a</sup>	781.08 <sup>a</sup>	1089.82 <sup>ab</sup>	1.39 <sup>e</sup>	37.19 <sup>a</sup>
T6	45.25	820.83 <sup>b</sup>	775.58 <sup>b</sup>	1083.33 <sup>ab</sup>	1.39 <sup>de</sup>	36.93 <sup>b</sup>
T7	45.19	817.45 <sup>c</sup>	772.27 <sup>cd</sup>	1086.57 <sup>ab</sup>	1.40 <sup>cd</sup>	36.77 <sup>cd</sup>
T8	45.25	815.47 <sup>cd</sup>	770.23 <sup>de</sup>	1086.35 <sup>ab</sup>	1.41 <sup>bc</sup>	36.67 <sup>de</sup>
SEM	0.02	1.03	1.03	1.02	0.01	0.05

\*The results are presented as mean value. Value with different subscripts within column differ significantly at  $p < 0.05$ ; SEM, Standard error of the means

\*\*T1: 21% Crude protein (CP) (Lys 1.18%; Met 0.45%), T2: 21% CP (Lys 1.0%; Met 0.45%), T3: 21% CP (Lys 1.18%; Met 0.4%), T4: 21% CP (Lys 1.0%; Met 0.4%), T5: 21% CP (Lys 1.18%; Met 0.45%) + POAB, T6: 21% CP (Lys 1.0%; Met 0.45%) + POAB, T7: 21% CP (Lys 1.18%; Met 0.4%) + POAB, T8: 21% CP (Lys 1.0%; Met 0.4%) + POAB.





**Table 2.** Growth Performance for 21-42 days with different dietary treatments

Treatment	Growth Performance (21-42 days)					
	Initial BW	Final BW	Cumulative WG	Cumulative FI	FCR	Average Daily Gain
T1	818.43 <sup>bc</sup>	2739.74 <sup>cd</sup>	1921.30 <sup>cd</sup>	3338.90 <sup>c</sup>	1.73 <sup>cd</sup>	91.49 <sup>cd</sup>
T2	814.02 <sup>d</sup>	2654.47 <sup>e</sup>	1840.45 <sup>e</sup>	3245.92 <sup>d</sup>	1.76 <sup>c</sup>	87.64 <sup>e</sup>
T3	807.35 <sup>f</sup>	2584.77 <sup>f</sup>	1777.42 <sup>f</sup>	3267.71 <sup>de</sup>	1.83 <sup>b</sup>	84.63 <sup>f</sup>
T4	804.12 <sup>g</sup>	2435.96 <sup>g</sup>	1631.84 <sup>g</sup>	3146.32 <sup>e</sup>	1.92 <sup>a</sup>	77.70 <sup>g</sup>
T5	826.39 <sup>a</sup>	3008.27 <sup>a</sup>	2181.87 <sup>a</sup>	3695.96 <sup>a</sup>	1.69 <sup>d</sup>	103.89 <sup>a</sup>
T6	820.83 <sup>b</sup>	2862.00 <sup>b</sup>	2041.17 <sup>b</sup>	3496.79 <sup>b</sup>	1.70 <sup>d</sup>	97.19 <sup>b</sup>
T7	817.45 <sup>c</sup>	2766.05 <sup>c</sup>	1948.59 <sup>c</sup>	3369.38 <sup>c</sup>	1.70 <sup>d</sup>	92.78 <sup>c</sup>
T8	815.47 <sup>cd</sup>	2717.45 <sup>d</sup>	1901.97 <sup>d</sup>	3351.16 <sup>c</sup>	1.71 <sup>cd</sup>	90.57 <sup>d</sup>
SEM	1.03	23.85	22.89	25.66	0.01	1.09

\*The results are presented as mean value. Value with different subscripts within column differ significantly at  $p < 0.05$ ; SEM, Standard error of the means

\*\***T1:** 19% CP (Lys 0.95%; Met 0.39%), **T2:** 19% CP (Lys 0.75%; Met 0.39%), **T3:** 19% CP (Lys 0.95%; Met 0.25%), **T4:** 19% CP (Lys 0.75%; Met 0.25%), **T5:** 21% CP (Lys 0.95%; Met 0.39%) + POAB, **T6:** 21% CP (Lys 0.75%; Met 0.39%) + POAB, **T7:** 21% CP (Lys 0.95%; Met 0.25%) + POAB, **T8:** 21% CP (Lys 0.75%; Met 0.25%) + POAB.



**Table 3.** Growth Performance for 0-42 days with different dietary treatments

Treatment	Growth Performance (0-42 days)					
	Initial BW	Final BW	Cumulative WG	Cumulative FI	FCR	Average Daily Gain
T1	45.19	2739.74 <sup>cd</sup>	2694.55 <sup>cd</sup>	4429.94 <sup>cde</sup>	1.64 <sup>cd</sup>	64.16 <sup>cd</sup>
T2	45.23	2654.47 <sup>c</sup>	2609.24 <sup>c</sup>	4329.26 <sup>c</sup>	1.66 <sup>c</sup>	62.12 <sup>c</sup>
T3	45.19	2584.77 <sup>f</sup>	2539.58 <sup>f</sup>	4349.17 <sup>de</sup>	1.71 <sup>b</sup>	60.47 <sup>f</sup>
T4	45.29	2435.96 <sup>s</sup>	2390.67 <sup>s</sup>	4229.65 <sup>f</sup>	1.77 <sup>a</sup>	56.92 <sup>s</sup>
T5	45.27	3008.27 <sup>a</sup>	2963.00 <sup>a</sup>	4782.63 <sup>a</sup>	1.61 <sup>d</sup>	70.55 <sup>a</sup>
T6	45.25	2862.00 <sup>b</sup>	2816.75 <sup>b</sup>	4586.79 <sup>b</sup>	1.63 <sup>cd</sup>	67.07 <sup>b</sup>
T7	45.19	2766.05 <sup>c</sup>	2720.86 <sup>c</sup>	4465.95 <sup>c</sup>	1.64 <sup>cd</sup>	64.78 <sup>c</sup>
T8	45.25	2717.45 <sup>d</sup>	2672.20 <sup>d</sup>	4437.51 <sup>cd</sup>	1.66 <sup>c</sup>	63.62 <sup>d</sup>
SEM	0.02	23.85	23.85	16.33	0.01	0.57

\*The results are presented as mean value. Value with different subscripts within column differ significantly at  $p < 0.05$ ; SEM, standard error mean

**\*\*Starter:** T1: 21% Crude protein (CP) (Lys 1.18%; Met 0.45%), T2: 21% CP (Lys 1.0%; Met 0.45%), T3: 21% CP (Lys 1.18%; Met 0.4%), T4: 21% CP (Lys 1.0%; Met 0.4%), T5: 21% CP (Lys 1.18%; Met 0.45%) + POAB, T6: 21% CP (Lys 1.0%; Met 0.45%) + POAB, T7: 21% CP (Lys 1.18%; Met 0.4%) + POAB, T8: 21% CP (Lys 1.0%; Met 0.4%) + POAB.

**Grower:** T1: 19% CP (Lys 0.95%; Met 0.39%), T2: 19% CP (Lys 0.75%; Met 0.39%), T3: 19% CP (Lys 0.95%; Met 0.25%), T4: 19% CP (Lys 0.75%; Met 0.25%), T5: 21% CP (Lys 0.95%; Met 0.39%) + POAB, T6: 21% CP (Lys 0.75%; Met 0.39%) + POAB, T7: 21% CP (Lys 0.95%; Met 0.25%) + POAB, T8: 21% CP (Lys 0.75%; Met 0.25%) + POAB.

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### **Digestibility of the broiler chickens**

Table 4 showed the result of digestibility during the starter stage and grower stage. For the digestibility of crude protein, the digestibility of the treatment groups supplemented with POAB showed a higher significant result compared with the treatment group without POAB. But there is no significant different among the treatment supplemented with POAB and without POAB. A similar trend was found in the result of crude fat, methionine and lysine digestibility. It showed that the POAB able to improve the nutrients digestion of the chickens. From the results, it showed that the POAB able to improve the growth performance by increase the digestion of the nutrients. Diets supplemented with POAB (T7 and T8) able to show a similar or better results compared with the diet with higher level of amino acids.

Treatment groups supplemented with POAB clearly showed a better digestibility to the treatment without POAB. Besides that, Hernández et al. (2006) and Garcíá et al. (2007) reported that supplementation of formic acid (0.5% or 1.0%) in broiler finisher diet was found to improve apparent ileal digestibility (AID) of CP (72.5% or 73.5%, respectively) as compared with control (60.7% CP). Similar finding also reported by Samanta et al. (2010) that organic acids raised gastric proteolysis and improved the digestibility of protein and amino acids.



**Table 4.** Digestibility result for starter and grower stage with different dietary treatments

Treatment**	Digestibility Coefficient (%) - Starter Diet*					Digestibility Coefficient (%) - Grower Diet*				
	Dry Matter	Crude Protein	Crude Fat	Methionine	Lysine	Dry Matter	Crude Protein	Crude Fat	Methionine	Lysine
T1	90.72	67.67 <sup>b</sup>	68.73 <sup>b</sup>	82.46 <sup>b</sup>	75.58 <sup>b</sup>	88.47 <sup>bcd</sup>	67.39 <sup>b</sup>	69.32 <sup>b</sup>	82.62 <sup>b</sup>	77.74 <sup>bc</sup>
T2	90.51	67.15 <sup>b</sup>	69.07 <sup>b</sup>	82.41 <sup>b</sup>	75.45 <sup>b</sup>	88.19 <sup>cd</sup>	67.59 <sup>b</sup>	68.91 <sup>b</sup>	82.52 <sup>b</sup>	77.39 <sup>c</sup>
T3	90.75	67.39 <sup>b</sup>	69.21 <sup>b</sup>	82.83 <sup>b</sup>	75.82 <sup>ab</sup>	88.34 <sup>d</sup>	66.79 <sup>b</sup>	68.82 <sup>b</sup>	82.67 <sup>b</sup>	77.53 <sup>c</sup>
T4	90.55	67.61 <sup>b</sup>	69.38 <sup>b</sup>	82.67 <sup>b</sup>	75.51 <sup>b</sup>	88.21 <sup>d</sup>	66.54 <sup>b</sup>	68.35 <sup>b</sup>	82.26 <sup>b</sup>	77.50 <sup>c</sup>
T5	91.25	72.33 <sup>a</sup>	74.57 <sup>a</sup>	85.78 <sup>a</sup>	77.52 <sup>a</sup>	88.96 <sup>abc</sup>	71.84 <sup>a</sup>	74.32 <sup>a</sup>	85.54 <sup>a</sup>	79.82 <sup>a</sup>
T6	91.21	72.29 <sup>a</sup>	74.26 <sup>a</sup>	85.48 <sup>a</sup>	77.50 <sup>a</sup>	88.97 <sup>abc</sup>	71.24 <sup>a</sup>	74.33 <sup>b</sup>	85.43 <sup>a</sup>	79.11 <sup>ab</sup>
T7	91.19	72.14 <sup>a</sup>	74.19 <sup>a</sup>	85.51 <sup>a</sup>	77.38 <sup>a</sup>	89.56 <sup>a</sup>	72.29 <sup>a</sup>	74.37 <sup>b</sup>	85.75 <sup>a</sup>	79.61 <sup>a</sup>
T8	91.18	72.13 <sup>a</sup>	73.34 <sup>a</sup>	85.44 <sup>a</sup>	77.34 <sup>a</sup>	89.14 <sup>ab</sup>	71.73 <sup>a</sup>	74.06 <sup>b</sup>	85.67 <sup>a</sup>	79.19 <sup>ab</sup>
SEM	0.12	0.51	0.58	0.33	0.25	0.12	0.53	0.66	0.39	0.25

\*The results are presented as mean value. Value with different subscripts within column differ significantly at  $p < 0.05$ ; SEM, Standard error of the means

\*\***Starter:** T1: 21% Crude protein (CP) (Lys 1.18%; Met 0.45%), T2: 21% CP (Lys 1.0%; Met 0.45%), T3: 21% CP (Lys 1.18%; Met 0.4%), T4: 21% CP (Lys 1.0%; Met 0.4%), T5: 21% CP (Lys 1.18%; Met 0.45%) + POAB, T6: 21% CP (Lys 1.0%; Met 0.45%) + POAB, T7: 21% CP (Lys 1.18%; Met 0.4%) + POAB, T8: 21% CP (Lys 1.0%; Met 0.4%) + POAB. **Grower:** T1: 19% CP (Lys 0.95%; Met 0.39%), T2: 19% CP (Lys 0.75%; Met 0.39%), T3: 19% CP (Lys 0.95%; Met 0.25%), T4: 19% CP (Lys 0.75%; Met 0.25%), T5: 21% CP (Lys 0.95%; Met 0.39%) + POAB, T6: 21% CP (Lys 0.75%; Met 0.39%) + POAB, T7: 21% CP (Lys 0.95%; Met 0.25%) + POAB, T8: 21% CP (Lys 0.75%; Met 0.25%) + POAB.



### **Microflora counts with different dietary treatments**

Table 5 showed the microflora counts with different dietary treatments in week 3 and week 6. A similar trend was observed in week 3 and week 6. In terms of bacterial count, the lactic acid bacteria count (LAB) in week 6 showed slightly higher than week 3. Besides that, the lactic acid bacteria count also showed a significant higher result compared with *Enterobacteriaceae* (ENT) in each treatment group.

Moreover, the treatment groups supplemented with POAB promote the growth of beneficial bacteria which was indicated by higher LAB count compared to the treatment without organic acid. As for the *Enterobacteriaceae*, the treatments group with POAB also showed a lower growth compared with treatment without POAB.

From the result, it showed that the supplementation of POAB able to increase the LAB and reduced ENT cell population. Decreased faecal pH values may also affected the decreased of pathogenic bacteria in broiler excreta. However, reduction of amino acids of the feed had no effect on the microbial population in the faecal samples.



**Table 5:** Microflora count with different dietary treatments in week 3 and week 6.

Parameter*	Dietary Treatment**							
	T1	T2	T3	T4	T5	T6	T7	T8
Faecal pH	7.32 ± 0.02 <sup>b</sup>	7.47 ± 0.03 <sup>a</sup>	7.43 ± 0.01 <sup>a</sup>	7.40 ± 0.03 <sup>a</sup>	5.49 ± 0.03 <sup>c</sup>	5.39 ± 0.01 <sup>d</sup>	5.50 ± 0.02 <sup>c</sup>	5.37 ± 0.01 <sup>d</sup>
<b>Microbial Count Week 3, Log CFU/g</b>								
Lactic acid bacteria	6.92 ± 0.04 <sup>b</sup>	6.88 ± 0.04 <sup>b</sup>	6.87 ± 0.04 <sup>b</sup>	6.92 ± 0.02 <sup>b</sup>	7.07 ± 0.03 <sup>a</sup>	7.07 ± 0.03 <sup>a</sup>	7.04 ± 0.02 <sup>a</sup>	7.12 ± 0.01 <sup>a</sup>
<i>Enterobacteriaceae</i>	5.78 ± 0.07 <sup>a</sup>	5.88 ± 0.05 <sup>a</sup>	5.79 ± 0.06 <sup>a</sup>	5.92 ± 0.03 <sup>a</sup>	4.71 ± 0.08 <sup>b</sup>	4.71 ± 0.07 <sup>b</sup>	4.68 ± 0.09 <sup>b</sup>	4.74 ± 0.09 <sup>b</sup>
<b>Microbial Count Week 6, Log CFU/g</b>								
Lactic acid bacteria	7.66 ± 0.01 <sup>b</sup>	7.78 ± 0.12 <sup>b</sup>	7.87 ± 0.07 <sup>b</sup>	7.86 ± 0.07 <sup>b</sup>	8.12 ± 0.09 <sup>a</sup>	8.13 ± 0.06 <sup>a</sup>	8.15 ± 0.10 <sup>a</sup>	8.15 ± 0.09 <sup>a</sup>
<i>Enterobacteriaceae</i>	5.82 ± 0.05 <sup>ab</sup>	5.96 ± 0.02 <sup>a</sup>	5.51 ± 0.34 <sup>bc</sup>	5.15 ± 0.03 <sup>cd</sup>	4.82 ± 0.09 <sup>de</sup>	4.77 ± 0.11 <sup>de</sup>	4.63 ± 0.03 <sup>e</sup>	3.26 ± 0.04 <sup>f</sup>

\*The results are presented as mean value + SEM. Value with different subscripts within column differ significantly at  $p < 0.05$

\*\***Starter:** T1: 21% Crude protein (CP) (Lys 1.18%; Met 0.45%), T2: 21% CP (Lys 1.0%; Met 0.45%), T3: 21% CP (Lys 1.18%; Met 0.4%), T4: 21% CP (Lys 1.0%; Met 0.4%), T5: 21% CP (Lys 1.18%; Met 0.45%) + POAB, T6: 21% CP (Lys 1.0%; Met 0.45%) + POAB, T7: 21% CP (Lys 1.18%; Met 0.4%) + POAB, T8: 21% CP (Lys 1.0%; Met 0.4%) + POAB.

**Grower:** T1: 19% CP (Lys 0.95%; Met 0.39%), T2: 19% CP (Lys 0.75%; Met 0.39%), T3: 19% CP (Lys 0.95%; Met 0.25%), T4: 19% CP (Lys 0.75%; Met 0.25%), T5: 21% CP (Lys 0.95%; Met 0.39%) + POAB, T6: 21% CP (Lys 0.75%; Met 0.39%) + POAB, T7: 21% CP (Lys 0.95%; Met 0.25%) + POAB, T8: 21% CP (Lys 0.75%; Met 0.25%) + POAB.

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## Conclusion

From the result, it showed that organic acid supplementation had a beneficial effect on the performance of broiler chicken. Organic acids improved nutrient digestibility by reducing microbial competition with the chickens for nutrients and endogenous nitrogen losses, by lowering the growth-depressing microbial metabolites. Furthermore, the organic acids in poultry might have a direct effect on the gastrointestinal tract (GIT) bacteria population, reducing the level of some pathogenic bacteria by lowering the pH and improve the population of beneficial bacteria which help in nutrients digestion.

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## Effects of Purple Glutinous Rice Residue Meal in Concentrate Diets on Growth Performance in Growing Pigs

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### Abstract

The objective of this experiment was to investigate the effect of supplementation of purple glutinous rice residue meal in diets on average daily gain (ADG), feed conversion ratio (FCR), feed intake, and back-fat thickness, cholesterol, HDL, LDL and blood triglycerides in growing pigs. Sixteen pigs cross bred with an average  $43.5 \pm 2$  kg body weight were fed four experimental diets in a randomized completely block design. The animals were randomly assigned to each sequence of feeding and the dietary treatments were: T<sub>1</sub> = control diet, T<sub>2</sub> = control diet + 5% purple glutinous rice residue meal, T<sub>3</sub> = control diet+10% purple glutinous rice residue meal and T<sub>4</sub> = control diet+15% purple glutinous rice residue meal. The results found that ADG, FCR, body weight gain, blood cholesterol, HDL, LDL and triglycerides were not significantly difference among treatments ( $P>0.05$ ). In conclusion, results demonstrated that treatment of purple glutinous rice residue meal could be supplemented at 5-10% without adverse effect on performance of growing pigs.

**Keywords:** purple glutinous rice residue meal, growing pigs, supplemented, cholesterol

### Introduction

In Thailand, pig production intensified significantly during the last decade, with many economic, epidemiological and environmental implications. Strategies toward more sustainable future developments are currently investigated, and these could be informed by a detailed assessment of the main trends in the pig sector, and on how different production systems are geographically distributed (Thanapongtharm et al., 2016). These production systems are referred to as 'intensive' in the sense that a high amount of infrastructure, technology, health care and feeds are used to increase the productivity of high-yielding animals on the farm, resulting in increased outputs (kg meat per animal space per year) (Svendsen and Svendsen, 1997). Many of these plants are used as foods and medicines by both humans and animal species (Cowan, 1999; Mahady, 2002). Purple rice (*Oryza sativa* L. var. *glutinosa*) or black rice, where it is known as "Khao Kam" is an indigenous Thai glutinous rice strain characterized by purple pigments in the husk and the pericarp. The color is determined by a number of distinct anthocyanins (El-Sayed et al., 2006; Rerkasem et al., 2015). Anthocyanins, a member of classes of flavonoids, are responsible for the color of purple rice bran. Major components of anthocyanins in black rice with purple bran are cyaniding-3-glucoside and peonidin-3-glucoside and are mainly located in



the aleurone layer of the rice bran (Hu and others 2003). Rice bran, a byproduct of rice milling, is a constituent (approximately 10%) of the whole rice grain and consists of the bran layers (pericarp, seed coat, nucellus, and aleurone) and the germ (Rohrer and Siebenmorgen, 2004). Feeding purple rice instead of white rice and corn lowered total plasma cholesterol in fattening, though only at certain time points during fattening (Peawong et al., 2011). Overall, these properties of purple rice indicate that there might be also direct or indirect effects on the quality of the meat from pigs. The objective of the present study was to test whether feeding purple glutinous rice residue meal to pigs improves average daily gain (ADG), feed conversion ratio (FCR), feed intake, and back-fat thickness, cholesterol, HDL, LDL and blood triglycerides in growing pigs.

## Materials and Methods

### Animals and management

The experiment was performed using 16 growing pig, divided into 4 equal groups. The animals were kept in groups in pens (4 animals each). The pigs' weight was at approximately  $43.5 \pm 2$  kg BW. Each pen was equipped with a self-feeder and nipple drinker to allow *ad libitum* access to feed and water throughout the experiment.

### Experimental design and treatments

The animals were randomly divided into four treatment groups comprising 2 replications according to Randomized Completely Block Design (RCBD). Sixteen Duroc×(Large White×Landrace) crossbred piglets (barrows:gilts, 1:1) were randomly assigned to four isocaloric and isonitrogenous diets at  $43.5 \pm 2$  kg body weight in a randomized complete block design. They were housed in individual pens of a size of 1.5×2.0 m for on average of 16 weeks. The experimental treatments included: T1 = control diet, T2 = control diet + 5% purple glutinous rice residue meal, T3 = control diet+10% purple glutinous rice residue meal and T4 = control diet+15% purple glutinous rice residue meal. Drinking water was provided by nipples available at all time. All diets were provided in mash form and formulated to meet or exceed the NRC (1998) recommendations for all nutrients, regardless of treatment.

### Sampling and measurement

Individual body weight and feed consumption per pen were measured at the end of each week to 16th week to determine the average daily gain (ADG), mean total feed Intake, kg/pig, feed conversion ratio (FCR), and back-fat thickness, cholesterol, HDL, LDL and blood triglycerides. At the end of the trial, all pigs were weighed and scanned with ultrasound to determine back-fat thickness and loin muscle area.

### Statistical analyses

All data were subjected to the GLM procedures of SAS (1996) as a completely randomized design, with each pen serving as the experimental unit. Differences among all treatments were separated by Duncan's multiple range tests. Mean values and standard error (SE) were reported. Probability values less than 0.05 were considered as significant (Steel & Torrie, 1980).

## Results and Discussion

Initial and final weight of pig did not differ among all the treatments ( $P>0.05$ ). Body weight gain were increased by 5%, 10%, 0% and 15% of purple glutinous rice residue meal supplemented, respectively ( $P>0.05$ ). In addition, ADG and FCR were not significantly difference ( $P>0.05$ ) when used 5-10% purple glutinous rice residue meal supplemented as show in Table 1.



**Table 1** Main effects of purple glutinous rice residue meal supplemented on growth performance.

Items	Purple glutinous rice residue meal supplemented (%)				SEM	P-value
	0	5	10	15		
Initial weight (kg)	41.75	43.25	42.50	43.00	2.45	NS
Final weight (kg)	58.00	63.50	60.75	54.50	4.51	NS
Body weight gain (kg)	16.25	20.25	18.25	11.50	1.38	NS
ADG (kg/day)	0.15	0.18	0.17	0.11	0.72	NS
FCR	4.58	3.91	4.42	4.33	0.19	NS
Mean total feed Intake, (kg/pig)	73.04	78.75	76.86	71.45	1.37	NS

NS = not significantly different ( $P>0.05$ ), SEM=standard error of mean, ADG=average daily gain, FCR=feed conversion ratio

The effects of purple glutinous rice residue meal supplemented on pig performance have been published and the current study not found effect on growth. However, the mean total intake of the pigs on the four diets did not show any significant ( $P>0.05$ ) differences. This could have rendered the diets more palatable and possibly enticed the pigs to eat more of the diets. This assertion agrees with that of Anyika et al. (2009) who had earlier stated that feed intake can be influenced by level of palatability, source of nitrogen and the level of essential amino acids. Meat color was among the few physicochemical traits which were not significantly affected (Table 2).

**Table 2** Effects of purple glutinous rice residue meal supplemented on back-fat thickness, meat color and blood chemical in growing pig.

Items	Purple glutinous rice residue meal supplemented (%)				SEM	P-value
	0	5	10	15		
Backfat thickness (mm)	1.13	1.12	1.12	1.14	0.03	NS
Loin, meat color						
L*	38.23	50.34	44.35	53.67	2.28	NS
a*	8.35	6.24	5.76	5.30	0.50	NS
b*	11.70	10.72	10.79	13.58	0.58	NS
Blood chemical						
0 hr post feeding						
Cholesterol (mg/dL)	61.25	71.50	61.25	76.00	3.04	NS
Triglyceride (mg/dL)	32.25	30.00	23.25	21.50	2.20	NS
HDL (mg/dL)	25.23	24.75	24.25	29.50	0.80	NS
LDL (mg/dL)	30.30	40.50	32.75	41.25	2.36	NS
4 hr post feeding						
Cholesterol (mg/dL)	60.25	78.00	60.00	74.75	4.20	NS
Triglyceride (mg/dL)	36.25	48.00	31.75	39.00	5.25	NS
HDL (mg/dL)	26.75	27.00	24.50	31.50	1.31	NS
LDL (mg/dL)	26.25	40.75	28.25	34.75	2.82	NS

NS = not significantly different ( $P>0.05$ ), SEM=standard error of mean, <sup>a, b</sup> means in the same row with different superscripts differ significantly ( $P<0.05$ ), HDL= High Density Lipoprotein, LDL=Low Density Lipoprotein



The results for the serum biochemical assay for cholesterol, and high and low lipoproteins are shown in Table 2. The blood cholesterol, HDL and LDL levels did not indicate significant ( $P>0.05$ ) differences between treatment.

### Conclusions

Considering the data obtained from this study, the dietary 5-10% of purple glutinous rice residue meal supplementation had effect on growing pig while partially exerted beneficial effects on growth performance.

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## Effect of Diet Containing Dragon Fruit Peel Meal Fermentation for Productivity of Kampung Chickens

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### **Abstract**

The objective of this research was to determine the effect of diet containing different dragon fruit meal fermentation for productivity of Kampung chickens as been implemented for 3 month . The study design used is completely randomized design where used with 4 treatments and 5 replications of each has 10 birds . A number of 200 age 2 weeks of Kampung chickens were used in this experiment . The treatments were RD0= control diet without dragon fruit meal fermentation, RD1= diet with dragon fruit meal fermentation 5% ; RD2= diet with dragon fruit meal fermentation 7% and RD3 = diet with dragon fruit meal fermentation 9%.The variable studied were : feed consumption, feed conversion ,final body weight , body weight gain, carcass weight . Data obtained was analyzed with of covariance and followed by Ducant's multiple range test ( Steal and Torrie, 1993), when significant differences ( $P < 0,05$ ) among treatments were found . Results of this experiment showed that feed consumption , feed conversion , final body weight , body weight gain, carcass weight , carcass percentage was no significant ( $P > 0,05$ ) among the treatments RD0, RD1, RD2, but FCR, final body weight, non carcass RD3 treatment gave significant ( $P < 0,05$ ). It can be concluded that dragon fruit meal fermentation until 7% on the diet gave no significant effect for Kampung chicken productivity but treatment usage dragon fruit 9% have significant different on FCR final body weight ,non carcass compared with others treatments.

**Keywords:** carcass, dragon fruit, fermentation, Kampung chickens, productivity

### **Introduction**

Poultry feed in Indonesia is still problems , because of most widely used feed components are imported. Feed is very influential on the production and productivity of livestock, because it has contribute 70-80% of the cost of production of a farm. Kampung chicken is one of native chicken breeds in Indonesia production egg and meat to improved nutrition for human (Henuk et al., 2015). The high feed costs due to raw materials derived from imported commodities and its use compete with humans. The high price of feed is indirectly require that farmers are looking for alternative feed ingredients so it can lower the feed costs and maximize revenues.

Dragon fruit is a key raw material in the manufacture of juices, jams, syrups, chips or other food ingredients. Dragon fruit peel is agricultural waste which has not been widely used by the community, especially in Indonesia (Mustika, 2014). According Citramukti (2008) part of the dragon fruit between 30-35% is the fruit component and 40-70% are dragon fruit skin the still rarely or even not been fully utilized, although some studies have reported peel dragon fruit



contains high antioxidant and contents phenolics in the dragon fruit peel amounted 28.16 mg/100 g, in addition to having antioxidant also contain anthocyanins (Nurliyana et al., 2010). The low protein and high crude fiber in fruit peels is a constraint in the utilization as animal feed especially Kampung chickens.

An increase in the value of dragon fruit skin can be done by applying biofermentation by utilizing microbial services, ie utilizing the ability of the yeast *Sacharomyces cerevisiae* contained in tape. *Sacharomyces cerevisiae* can increase fibrous fiber digestibility and can act as a probiotic in poultry (Ahmad, 2005). At the time of fermentation by yeast, the crude fiber content of ration can be degraded, so it can be utilized by poultry. Another benefit of fermentation products is to suppress the enzyme activity of 3-hydroxy-3-methylglutaryl Co-A reductase that serves to synthesize cholesterol in the liver (Tanaka et al., 1992).

Research on dragon fruit peel for livestock feed is still rarely done according Mustika et al. (2014) dragon fruit peel can be given up to the level of 1% for quail. While for the fermentation product has been done, according to Astuti et al. (2016) dragon fruit feed meal fermented until 6% on the diet gave no significant effect for broiler productivity. The purposes of this research were to evaluate the effect of diet containing different dragon fruit meal fermentation for productivity of Kampung chickens.

## Materials and methods

This research was carried out for 3 months at Teaching Farm, Campus Bukit Animal Science, University of Udayana. Bukit Jimbaran, Denpasar – Bali. Indonesia. The study used 200 native chicks with 2 week age.

**Table 1.** Composition Ingredients Ration and Nutrient Content of Diets (age 2-3 months)

Ingrediens (%)	Composition (%)				
	RD0	RD1	RD2	RD3 1)	
Corn	43.57	41.39	40.86	40.34	
Fish Meal	8.00	8.00	8.00	8.00	
Soybean Meal	18.44	18.49	18.51	18.53	
Race Brand	25.00	21.93	20.43	18.53	
Dragon Fruit Skin	0.00	5.00	7.00	9.00	
Flour Fermented					
Coconut Oil	4.79	5.00	5.00	4.00	
Premix	0.10	0.10	0.10	0.10	
CaCo3	0.10	0.10	0.10	0.10	
Nutrient of Diets					*Standard
Energy KCal/kg	2900	2900	2900	2900	2900
Crude Protein (%)	20.00	20.00	20.00	20.00	20.00
Crude Fat (%)	10.35	10.14	9.95	9.76	8.00
Crude Fiber (%)	3.08	3.73	3.90	4.10	5.00
calcium/Ca (%)	0.65	0.73	0.77	0.80	0.90
Phosphor/P (%)	0.67	0.64	0.62	0.60	0.60

- 1) RD0= Ration without used dragon fruit skin flour (control); RD1 = Ration with 5% fermented dragon fruit skin flour ;
- 2) RD02 = Ration with 7% fermented dragon fruit skin flour and RD03 = Ration with 9% fermented dragon fruit skin flour
- 3) \* Standard (Scott et al., 1982)



Dietary treatments were formulated by recommendation Scott et al. (1982) (Table 1). This research uses a completely randomized design (CRD) with 4 treatments and 5 replications, every unit is filled with 10 chickens. Treatments were as follows: RD0= Ration without used dragon fruit skin flour (control); RD1 = Ration with 5% fermented dragon fruit skin flour; RD02 = Ration with 7% fermented dragon fruit skin flour and RD03 = Ration with 9% fermented dragon fruit skin flour. All feed and water were provided *ad libitum*. The variable studied were: feed consumption, feed conversion, final body weight, body weight gain, carcass weight. Feed intake and Body Weight for individual chicks were recorded weekly. Slaughter weight is obtained by weighing a live chicken at the end of the study after were fasted for 12 hours. Kampung chickens of carcasses are obtained by cutting by dividing body parts such as feathers, neck, viscera, head and two legs. Percentage of carcass obtained by dividing carcass weight with slaughter weight multiplied 100% (National Standardization Agency, 1995 and Dewi et al, 2014).

Data were analyzed statistic by ANOVA and when there are significant differences continued test Duncan (Steel and Torrie, 1993). The data were analyzed using statistic application program SPSS 17.

## Results and discussion

### Performance

The effects of ration for performance of Kampung chickens aged 2 – 8 weeks is summarized in Table 2. The results of the study showed that the Kampung chickens fed RD3 consumed feed (879.85 g /6 weeks) and produced better performance as their body weight gain (273.45g/6 weeks) with their feed conversion ratio (3.22) ( $P < 0.05$ ) compared with their fed RD0, RD1, RD2. The body weight gain of Kampung chickens were higher with better feed utilization indicated that they were given the rations with used 9% dragon fruit skin flour fermentation improved the process of digestion of feed in their digestive tract. This is likely due to dragon fruit skin flour fermentation containing various microbes that degrade fiber and probiotic microbes so it will be able to increase the ration digestibility and metabolism. According Weiss and Hogan (2007) that material having the antioxidant content of livestock can reduce the effects of free radicals such as increasing feed consumption. According Mustika *et.al.* (2014) it is because free radicals can cause oxidative stress in livestock resulting in lower feed consumption. Oxidative stress is a state of imbalance between the amount of free radicals and antioxidants in the body, that can trigger the occurrence cell damage and lowered immune system (Nurliyana et al., 2010).

### The effect of ration for Carcass

In this research the average value of chicken slaughter weight ranging between 351.00-392,00 grams (Table 2). Results of analysis variance no significant effect ( $P > 0.05$ ) among the treatment RD0, RD1, RD2, and RD3. Result of percentage carcass at this research approximately 56.41 to 58.13%. Research done by Mulyono et al. (2009) and Dewi et al. (2015) also found that utilization of waste from the rumen of Bali cows as bio-inoculant and supplement products in the ration of broiler chickens proven to improve the quality and digestibility of the ration based nonconventional waste thus produced higher carcass quality and can reduce non-carcass weight.



**Table 2.** Effect of Treatment for Performance and Slaughter Weight, percentage Carcass and Percentage Carcass Portion Kampung Chickens.

Variables	Treatment 1				SEM
	RD0	RD1	RD2	RRD3	
Body weight (8 week/g)	347.00b	361.45ab	376.88ab	392.27a	12.13
Body weight gaining (g)	229.08b	243.27b	258.28ab	273.45a	10.21
Feed consumption	862.15ab	837.44b	839.69b	879.85a	18.26
Feed conversion	3.77a	3.45ab	3.26b	3.22b	0.02
Slaughter weight (g)	351.00	359.80	389.60	392.00	2.05
Carcass Weight(g)	197.02	200.82	226.46	221.44	0.2
Carcass (%)	56.13	55.81	58.13	56.41	0.15
Breast (g)	47.64a	49.74b	56.92a	54.66ab	1.26
Wing (g)	32.66	30.56	35.22	34.94	0.9
Thigh (g)	63.14	65.78	74.68	74.04	0.56
Backs (g)	53.58	54.74	59.64	57.5	1.47

Explanation :

- 1) RD0= Ration without used dragon fruit skin flour (control);
- 2) RD1 = Ration with 5% fermented dragon fruit skin flour ; RD02 = Ration with 7% fermented dragon fruit skin flour and RD03 = Ration with 9% fermented dragon fruit skin flour.2) Values with the same superscript in the same row shows the difference was not significant ( $P>0,05$ ). ;
- 3) SEM : *Standard Error of The Treatment Means*

This difference is due to the age , chicken strains and the type of feed given. The factors that affect the percentage chicken carcass such as age and body weight (Brake et al., 1993). Analysis of variance showed no significant differences ( $P>0,05$ ) on carcass percentage. According to Haroen (2003) that the carcass weight is closely associated with slaughter weight and body weight gain. It is also likely caused by the composition of the feed used in this research has a balance of protein and energy are the same. Energy is required for all activities of life and the production of meat, so that the energy shortage can cause stunted growth (Fadilah et al., 2007). Based on the analysis of variance showed that the results were significantly different ( $P<0,05$ ) on the weight of breast it shows that RD0, RD2 treatments are relatively the low with RD1 and RD3. It is also suspected chickens consumes the feed with the same nutrients, so it may cause breast percentage was different. Breast value in this study ranged from 47.64 to 56.92g .This value which average difference was likely caused by differences in the strains Kampung chickens used. The use of dragon fruit peel flour was no significant ( $P>0,05$ ) on the wing and back weight. This is because the weight of wings and back carcass weight showed no significantly different results. As stated Achmanu et al. (1997) that will affect the percentage of carcass weight of carcass and its parts. The breast and thighs grow more dominant during growth than on the wings (Abubakar and Nataamijaya, 1999).

Results of analysis variance showed of feeding treatment on the percentage thigh does not occur significant differences ( $P>0,05$ ). The results showed that all treatments are relatively the same effect on the percentage thigh. The weight of carcass parts is directly determined by carcass weight (Widhiarti, 1987).

## Conclusions

It can be concluded that dragon fruit meal fermentation until 7% on the diet gave no significant effect for Kampung chicken productivity but treatment usage dragon fruit 9% have significant different on FCR final body weight ,non carcass compared with others treatments.





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## Effect of Rice Wine By-Product as Alternative Protein Source on Growth Performance of Broiler Chickens

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### Abstract

The study was conducted on the influence of rice wine residue on live performance, digestibility, processing yields, and meat quality of broiler chickens was assessed at 42 day of age. A total of 250 (1-day old) mixed-sex broilers were assigned to 5 dietary treatments: control diet (T1); 25% rice wine residue in the diet (T2); 50% rice wine residue in the diet (T3); 75% rice wine residue in the diet (T4), and 100% rice wine residue in the diet (T5). The birds were arranged in a Completely Randomized Design. Each treatment was provided in a 3-stage feeding program. Supplementation of inclusion level of rice wine residue in the diet had no effect on production performance at 42 day of age ( $P>0.05$ ). However, replacement the source of protein up to 50% of rice wine residue in the diet had reduced body weight and improved average daily gain ( $P<0.05$ ) when compared with the control at 15-28 day of age. Utilization of rice wine residue in broiler diets may have potential to use as an alternative protein source when supplemented lower than 50% inclusion level.

**Keywords:** rice wine, by product, protein source, growth performance, broiler

### Introduction

By-products may be defined as left over feedstuffs from agricultural industries. In recent years, large amounts of industrial waste was released to the environment. Those waste had been considered and required for special treatment to dispose. By-products used as animal feedstuffs would reduce environmental pollution and economic loss. The use of by-products can be fulfilled nutritional requirement for animals in which many by-products have contained high level of crude protein and those may have high potential as animal feed (Xu et al., 2006). However, protein is one of the most expensive ingredients in poultry diets. One of agricultural by-products that has been interested for alternative source of protein is rice wine residue. Rice wine is a traditional alcoholic beverage in Asian countries. Fermented rice starch is the process of making rice wine by converted to sugar by amylolytic process of fungi. Then, sugar is converted to alcohol by the fermentation of yeast cells. At the end of the process, rice wine residue is separated from the liquid and can be used as feedstuffs. Those residue is rich in nutritive values which is derived from both rice and microorganisms, which can be utilized as a protein source. Vechklang et al. (2011) reported that rice wine residue contained high level of protein (38% CP) and several essential amino acids. The aim of this study was to evaluate the effect of inclusion levels of rice wine residue as alternative protein source on body weight, feed intake, and feed conversion ratio of broiler chickens.



## Materials and Methods

### Feed preparation

The rice wine residue was separated from the liquid after the end of the fermentation process. The closed containers were used to obtain the residue from the factory in Nakhon Ratchasima, Thailand. The residue was dried before being used as broiler diets. The proximate analysis of fresh and dried rice wine residue were measured dry matter, protein, total lipid, fiber and ash according to the standard methods of AOAC (1995). Feed were formulated according to NRC (1994) recommendation.

### Animal and experimental design

A total of 250 mixed sex broilers were raised in 20 floor pens to 42 day of age in floor pens bedded with new rice husk (5 pens/diet; 10 birds/pen; 1x1.15 m<sup>2</sup>/pen) using a three stage feeding program. The starter feed was fed on days 1-14, the grower feed on days 15-28, and the finisher on days 29-42. Birds were provided five dietary treatments (T1) control diet, (T2) added 25% dried rice wine residue in the diet, (T3) added 50% dried rice wine residue in the diet, (T4) added 75% dried rice wine residue in the diet, and (T5) added 100% dried rice wine residue in the diet. The ingredients composition of the diets are shown in Table 1 and 2. All birds were weighed on a per pen basis at 14, 28, and 42 day of age and body weights (BW), average daily weight gain (ADG), average daily feed intake (ADFI), adjusted feed conversion (FCR), and mortality were determined. The data were analyzed by the GLM procedure of SAS (Statistical Analysis System, SAS Institute Inc., Cary, N.C.). All percentage data was transformed to arcsine values prior to analysis. The Tukey's test was used to compare and separate means when main effects were significant ( $P < 0.05$ ).

## Results and Discussion

Before formulated broiler feed diets, rice wine residue was dried. Crude protein, moisture and dry matter of fresh and dried rice wine residue were determined as shown in Table 1. Dried rice wine residue contained high crude protein (37% CP) and may use as alternative source of protein in diets.

**Table 1** Crude protein, moisture and dry matter of fresh and dried rice wine residue.

Items	Crude protein (%)	Moisture (%)	Dry matter (%)
Fresh rice wine residue	37.1	88.2	11.8
Dried rice wine residue	7.5	4.4	95.6

In poultry feed, most concerned is given to protein products, due to the importance of protein as a major constituent of the biologically active compounds in the body (Beski et al., 2015). Replacement of rice wine residue as a protein source in the diet had no effect on productive performance at 14 and 42 day of age ( $P > 0.05$ ). No differences ( $P > 0.05$ ) were detected in mortality between the treatments (data not shown). However, the dietary treatments had a significant effect ( $P < 0.05$ ) on body weight and average daily weight gain at 15-28 day of age.

Birds raised on up to 50% inclusion level of rice wine residue had lowered body weights and average daily weight gain than other treatments (Table 2). This experimental feed containing high level of dried rice wine residue can be described by the less palatability of the diets when the feed contains high level of rice wine residue. The result was in agreement with Vechklang et al. (2011), who reported that adding higher amount of rice wine residue in fish diets (up to 22.5%) had no effects on growth performance of Nile tilapia. However, adjusted feed conversion were significantly ( $P < 0.05$ ) lower with the 100% rice wine residue diet at 29-42 day of age



compared to other treatments. Rice wine is composed primarily of the extracted residues of rice during rice wine production and is a high protein and energy source.

**Table 2** Effect of inclusion level of rice wine residue as an alternative protein source on growth performance of broiler chickens.

Items <sup>1</sup>	T1 <sup>2</sup>	T2	T3	T4	T5	SEM <sup>3</sup>	P-value
1-14 d							
BW (g)	395	391	370	375	370	8.04	NS <sup>4</sup>
ADG (g/d)	69	69	66	66	67	1.06	NS
ADFI (g/bird/d)	80	80	81	81	82	0.70	NS
FCR	1.02	1.03	1.08	1.08	1.07	0.02	NS
15-28 d							
BW (g)	760 <sup>ab</sup>	788 <sup>a</sup>	700 <sup>ab</sup>	695 <sup>ab</sup>	667 <sup>b</sup>	26.7	0.033
ADG (g/d)	55 <sup>ab</sup>	56 <sup>a</sup>	50 <sup>ab</sup>	50 <sup>ab</sup>	48 <sup>b</sup>	1.87	0.027
ADFI (g/bird/d)	103	102	103	103	103	0.31	NS
FCR	1.90	1.82	2.07	2.07	2.16	0.08	NS
29-42 d							
BW (g)	738	720	685	868	933	71.24	NS
ADG (g/d)	150	147	140	152	155	5.39	NS
ADFI (g/bird/d)	148	148	149	149	148	0.70	NS
FCR	2.68 <sup>ab</sup>	2.93 <sup>ab</sup>	3.13 <sup>a</sup>	2.32 <sup>ab</sup>	2.15 <sup>b</sup>	0.19	0.013
1-42 d							
BW (g)	2093	2053	1910	2093	2133	84.61	NS
ADG (g/d)	43	42	38	44	45	2.30	NS
ADFI (g/bird/d)	121	120	121	121	120	0.37	NS
FCR	2.07	2.10	2.28	2.07	2.03	0.09	NS

<sup>1</sup>BW = Body weight (g), ADG = Average daily gain (g/d), ADFI = Average daily feed intake (g/bird/d), FCR = Feed conversion ratio (adjusted for mortality)

<sup>2</sup>T1 = Control; T2 = 25% Rice wine residuals in the diet, T3 = 50% Rice wine residuals in the diet, T4 = 75% Rice wine residuals in the diet, T5 = 100% Rice wine residuals in the diet.

<sup>3</sup>SEM = standard error of the mean,

<sup>4</sup>NS = Not significant

<sup>ab</sup>Means within a row with difference superscripts differ significantly.

## Conclusion

Based on its high protein composition required for broiler growth, dried rice wine residue would have potential to use as a protein source and might be able to partially replace in broiler diets. Dried rice wine residue contains high nutritional contents, especially, it is high in protein level (37% CP).

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## A Comparison of Fat-Soluble Antioxidants in Wild and Farm-Reared Chukar Partridges (*Alectoris Chukar*)

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### Abstract

This study assessed differences in antioxidant (carotenoid, retinol, retinol-ester, vitamin E and coenzyme Q10) composition of egg yolk and tissue in chukar partridges (*Alectoris chukar*) newly hatched from eggs of birds maintained in captivity on commercial maize-soybean based diets and birds from the wild whose diet was obtained from the natural environment. All eggs were incubated in a commercial hatchery. Day-old chicks from both groups were sacrificed and dissected for antioxidant analysis. Fat soluble antioxidant concentrations of egg yolk and tissues were determined by HPLC. Total carotenoids, retinol, alpha-tocopherol, and total vitamin E concentration of wild egg yolks were significantly higher compared to yolks from farm-reared birds ( $p < 0.05$ ). However, gamma tocopherol, and coenzyme Q10 were not significantly different in the yolks of either wild or farmed birds ( $p > 0.05$ ). The concentration of total carotenoids in all tissues of wild chukar one-day old partridges was significantly higher than in farmed one-day old chukar partridge tissues ( $p < 0.05$ ). Alpha tocopherol, free-retinol, retinol-esters and total vitamin A were significantly higher in most tissues of wild chukar when compared to farmed chicks ( $p < 0.05$ ). Coenzyme Q10 concentrations of heart, kidney and brain tissues of farm-reared chukar day old chicks were significantly higher than tissues from wild birds, although leg and breast tissues of wild chicks were significantly higher than in farmed birds ( $p < 0.05$ ). These findings suggest that maternal access to antioxidants in the diet of farmed chukar partridges could positively influence fat soluble antioxidant concentrations in the egg yolk and tissues of day old chicks.

**Keywords:** antioxidant, carotenoid, chukar partridges, coenzyme q10, newly hatched, retinol-esters, vitamin a, e reared, wild



## Used Dried Cassava Leaves with Enzymes from Fermented Tomato Pomace with *Aspergillus niger* in Laying Duck Diet on Nutrient Digestibility

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### Abstract

This study was aimed to determine effects of cassava leaves with enzymes from fermented tomato pomace with *Aspergillus niger* replacing laying duck diets on nutrient digestibility. Twelve laying ducks (aged 30 weeks, 4 ducks per treatments) were used in complete randomized design (CRD). There were three dietary treatments (T1 = basal diet; T2 = basal diet substituted cassava leaves 20%; and T3 = basal diet substituted cassava leaves 20% + enzymes 0.2%). The laying ducks were randomly taken to individual cages for 17 days period for total collection method. The results showed that nutrient digestibility were significantly difference ( $P < 0.05$ ), not including EE and NDF digestibility. Cassava leaves replaced laying duck diet were reduce nutrient digestibility when compare with control diet. However, laying duck fed the diet with cassava leaves and enzymes was improved ADF digestibility ( $P < 0.05$ ) and tend to higher most of nutrient digestibility than ducks fed the diet with cassava leaves ( $P > 0.05$ ).

**Keywords:** laying duck, fibrolytic enzymes, *Aspergillus niger*, nutrient digestibility

### Introduction

Consumption rate of egg in Thailand increased by 4.77% annually during 2010-2014, mainly due to the fact that eggs are available at lower costs than other protein sources (Agricultural Statistics of Thailand, 2015). Khaki Campbell ducks in Thailand has the important animal for produced egg. It is one of good protein sources for human. The price of duck feeds were raising and more expensive. Farmers must find ways to cut the cost of feed.

Cassava Leaves contain an average of 25% crude protein, depending on the cultivar and climatic conditions. It was recommended that farmer blending of cassava leaves as a source of protein and vitamins. Although the potential for the use of cassava leaves in the feeding of laying animals is thinkable, there were high fiber content. Could be reduced nutrient bioavailability, nutrient uptake and digestibility (Latif and Muller, 2015).

Fibrolytic enzymes have the ability the break down these structural polysaccharides, making the nutrients available to the animal (Bedford, 2000). There are various benefits to be gained from the use of enzymes in poultry diets. Some of the benefits influencing the performance of poultry are increased feed value of the dietary raw materials and increased nutrient utilization (Sarmiento-Franco et al., 2003, Botha, 2011).





Thailand produces fresh tomato around 145,600 ton per year (Agricultural Statistic of Thailand, 2009). Tomato was one of the most popular vegetables used as ingredients in many kind of food and also commercially in form of juice, paste and sauce. Tomato waste products from cannery factories, which producing a considerable large amount of wet tomato pomace as a byproduct. The utilization of tomato pomace constitutes an efficient and inexpensive agro-industrial substrate for fibrolytic enzymes via fungal solid state fermentation approach and suitable mean for its valorization.

The main goal of this research is improve utilization of dried cassava leaves with enzymes from fermented tomato pomace with *Aspergillus niger* on nutrient digestibility of laying duck.

## Materials and Methods

A total of 12 ducks excluded from receiving feed, 1.64 kg BW and 30 weeks old, were randomly allocated to 12 pens in an environmentally open room conditions throughout the experimental period (7 days of preliminary period and 7 days of data collection). Each ducks were put in individual cage for excreta and endogenous collection by using total collection method. Excreta was collected in seven days, then sample was analyzed in laboratory to measure nutrient content according to standard methods and calculate digestibility. The experiment using a completely randomized design with three treatments and four replications. Dietary treatments were based on laying diet contained 18% crude protein and substituted by dried cassava leaves and enzymes. Laying ducks were received dietary treatments as followed: Treatment 1 (T1) = Basal diet, Treatment 2 (T2) = Basal diet substituted 20% dried cassava leave, Treatment 3 (T3) = Basal diet substituted 20% dried cassava leave plus enzymes 0.2%. Diets were presented in mash form and given ad-libitum of feed per duck per day; unconsumed feed was measured each morning and free access to water. The treatment means were compared using analysis of variance (ANOVA) according the method of Steel and Torrie (1984).

## Results and Discussion

The data results of the feeding treatment of laying duck diet substitution by cassava leaves with or without enzymes on nutrient digestibility were presented on Table 1. Results showed that the substituted of cassava leaves in duck diets have significant decreased nutrient digestibility ( $P < 0.05$ ). However, addition enzymes in diet containing cassava leaves (T3) tend to increase nutrient digestibility ( $P > 0.05$ ) and significant increased for ADF digestibility ( $P < 0.05$ ) when compared with dietary diets containing cassava leaves (T2).

The enzymes from tomato pomace fermented with *Aspergillus niger* was contain xylanase (Saykhammy et al., 2017). Previous research report that exogenous xylanase can partially hydrolyze the arabinoxylans and release the enclosed nutrients for the birds to use (Williams et al., 1997). Consequently, birds can digest the nutrients more easily and achieve better growth performance. This experiment shown that additional enzymes in the diets tend to improved nutrient digestibility similarly with Adeola et al. (2007) reported that supplementation of wheat-based diets with cocktail enzymes resulted in improvements in DM and nitrogen digestibility.

**Table 1.** Dry matter intake and nutrient digestibility of laying duck

	T1	T2	T3	SEM	Sig
DMI (g/d)	104.85	109.88	130.05	9.551	0.198
DM (%)	75.09 <sup>b</sup>	64.20 <sup>a</sup>	67.98 <sup>ab</sup>	2.302	0.024
CP (% DM)	73.75 <sup>b</sup>	57.86 <sup>a</sup>	62.20 <sup>a</sup>	3.425	0.025
EE (% DM)	73.93	72.03	77.04	1.515	0.114
NDF (% DM)	53.17	39.22	42.68	4.108	0.074
ADF (% DM)	45.75 <sup>c</sup>	16.76 <sup>a</sup>	34.84 <sup>b</sup>	3.107	0.000
Ash (% DM)	60.64 <sup>b</sup>	44.02 <sup>a</sup>	50.36 <sup>a</sup>	3.723	0.033
Ca (% DM)	66.79 <sup>b</sup>	55.78 <sup>a</sup>	55.36 <sup>a</sup>	2.925	0.036
P (% DM)	71.31 <sup>b</sup>	54.67 <sup>a</sup>	59.28 <sup>a</sup>	2.908	0.008

<sup>a,b</sup> Means within column with difference superscripts differ significantly (P<0.05)

## Conclusion

In conclusion, effective enzymes from fermented tomato pomace with *Aspergillus niger* onto diet tended to increase utilization of cassava leaves in diet and improved nutrient digestibility.

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***Session 3-Ochid ballroom I***

ANN-01-0001

**Effect of Increasing Energy and Protein Ration on Nutrient Digestibility and Performance of Bali Heifer Calves****Ni Nyoman Suryani\*, I Wayan Suarna, I Gede Mahardika, and Ni Putu Sarini***Faculty of Animal Husbandry, Udayana University Denpasar,  
Bali, 80232, Indonesia**\*Corresponding email: mansuryanifapet@unud.ac.id***Abstract**

The purpose of this study was to determine the effect of energy and protein levels on performance of Bali heifer calves. The study was conducted in Bali, Province of Indonesia on 12 Bali heifer calves with initial body weight  $102,5 \pm 4,6$  kg/head in a randomized block design. The treatment given is four types of ration consists of Metabolizable energy (ME) protein levels: ME 2000 kcal : 12% CP; ME 2100 kcal : 13% CP; ME 2200 kcal : 14% CP and ME 2300 kcal : 15% CP, respectively as treatment A, B, C and D. Variables in this research were average daily weight gain (ADG), feed conversion ratio (FCR), nutrient intake and digestible nutrient. The results showed that increase ME ration up to 2300 kcal/kg significantly ( $P < 0.05$ ) increase energy intake. Crude protein consumption is also highest in treatment D (is 423.53 g/d). Increased consumption of energy and nutrients due to increased nutrient digestibility. Average daily weight gain of heifer in treatment D (0.33 kg/d) is the highest as compared to other treatments. Based on the results of this study, can be concluded that increase in energy and protein ration will improve nutrient digestibility thereby increasing consumption and ending on improving weight gain of Bali heifer calves.

**Keywords:** energy, protein, heifer bali cattle, growth performance**Introduction**

Livestock performance is influenced by environmental factors (70%) and genetic (30%). One environmental factor that is very influential is the nutrient content of the ration. Nutrition for heifers of prospective parent important to consider to produce quality cattle that will be born later, because nutrition plays an important role in the growth performance, production and reproduction. In addition, maintaining calf prospective parent is an integral part of the productivity of cows after becoming a mother to produce milk. Bali cattle, if it is maintained nutritional status according to their needs, then appropriate genetic potential growth is very likely to be achieved. Suryani and Mariani (1996) reported, a weight gain of Bali cattle can reach 760 g/day/head when rations supplemented with concentrates. Even, it can reach 900 g/day when given complete ration based on urea-ammoniation treated rice straw supplemented with S and Zn mineral (Partama et al., 2003). Another research of Suryani (2012) found that provision of various kinds of forage such as 15% elephant grass + 20% rice straw + 25% gliricidia + 10% calliandra and a 30% concentrate able to produce weight gain 880 g/d.



In Indonesia, 99.81% of cattle has traditionally maintained with scale of ownership 2-3 tails. Only 0.0041% of cattle reared in a professional manner with of ownership thousands to tens of thousands of tail (Badan Pusat Statistik, 2011). Because traditionally maintained, then nutrition is not a major focus so Bali cattle genetic potential is not reached optimally. Until now to fulfillment the nutritional needs especially the energy and protein of Bali cattle heifer still refers to the standard of temperate countries. Therefore, this study was conducted to evaluate the effect of energy and protein ration on heifer calves performance.

## Materials and Methods

### Cattle

The study used 12 of Bali heifer calves of 9 months. Each calves reared in individual cages. Given feed consists of forages and concentrates. Concentrate feed was given in the morning. While forage given fresh after concentrate feed. The composition of the ration is presented in Table 1 and the nutrient content of the ration are in Table 2.

Table 1. The composition of the ration treatment of Bali cattle heifer

Composition	Treatments			
	A	B	C	D
Concentrate	36.5	40.6	44.0	47.25
Urea	0.6	0.65	0.5	0.75
Molasses	2.4	3.25	5.0	5.0
King grass	60.0	55.00	50.0	45.0
Coconut oil	0.0	0.0	0.0	1.5
Vitamin/Mineral	0.5	0.5	0.5	0.5
Total	100.0	100.0	100.0	100.0

Table 2. The nutrient content of ration

Nutrient of ration	Treatments			
	A	B	C	D
Crude Protein (%)	12.06	13.11	13.97	15.05
ME (kcal/kg)	2045.38	2103.57	2201.85	2297.60
Crude Fiber (%)	27.21	26.24	25.02	23.92
Calcium (%)	0.20	0.60	1.29	1.47
Phosphor (%)	0.57	1.02	1.81	1.97

### Design of Experiments

These experiments used a randomized block design with 4 dietary treatments with 3 animals per treatment as replicates. The dietary treatment given is four types of ME : CP levels of ration: ME 2000 kcal/kg : 12% C; ME 2100 kcal/kg : 13% CP; ME 2200 kcal/kg : 14% CP and ME 2300 kcal/kg : 15% CP respectively, as treatment A, B, C and D.

### Variables Observed

#### Dry Matter, Organic Matter and Nutrient Intake

DM Intake was calculated by multiplying fresh feed weight with DM content of the ration, subtracted with DM of the unconsumed feed. Organic Matter Intake obtained by reducing the DM Intake with ash contained in the ration. Nutrient intake obtained by subtracting nutrient content of consumed feed with nutrient content of the unconsumed feed.



### **Digestible Dry Matter, Digestible Organic Matter and Digestible Nutrient**

Dry matter and nutrient digestible measured by total collection period for 7 days. Observations over the total collection are done from 08:00 am until 8:00 am the next day. Rations and the rest of the rations are taken each 200 g each day and at the end the total collection was mixed and decomposed according with their treatment. After that, taken each 200 g for analysis nutrient contain. The same was done to determine content of nutrients in the feces. Digestible nutrient is calculated by the following formula:

Digestible Dry Matter (g/d) = Dry Matter intake (g) – Dry Matter in feces (g)

Digestible Organic Matter (g/d) = Organic Matter intake (g) – Organic Matter in feces (g)

Digestible nutrient (g/d) = nutrient intake (g) – nutrient in the feces (g)

### **Weight gain**

Calves were done every two weeks. Cattle's weight gain was obtained by subtracting the initial body weight to the final body weight. Daily live weight gain is a weight gain during the study divided by the length of the study.

### **Data Analysis**

The data obtained in this study were analyzed by analysis of variance. If the results are significantly different ( $P < 0.05$ ) between treatments, the analysis followed by orthogonal contrast test at 5% level according to Steel and Torrie (1995).

## **Results and discussions**

### **Digestible Nutrient**

The quality of ruminant livestock feed is determined by its digestibility. The digestibility of the feed is closely related to its chemical composition and the crude fiber has the greatest influence on digestibility. Crude fiber in ruminant livestock ration is essential to keep the rumen condition healthy and support the synthesis of microbial proteins by maintaining stable rumen conditions (Xu et al., 2014). Ration digestibility is defined as the part of the ration that is not excreted in the feces so it is assumed that the part is absorbed by the livestock body. The digestibility is expressed on a dry matter basis (McDonald et al., 2002).

Digestible energy of Bali heifer calves increased significantly ( $P < 0.05$ ) by increasing protein and energy ration. The highest digestible energy yields in heifer treated D ration which is 7814.34 kcal GE/d, same as 70.92% compare with digestible energy. A treatment is 59% consistently heifer fed D diet resulted highest digestibility in DM, OM, CP, CF and EE (Table 3). Increased protein and energy rations from 12% CP and 2000 kcal ME/kg to 15% CP and 2300 kcal ME/kg will significantly increase ( $P < 0.05$ ) the digestible DM, OM, CP and EE by 28.86%; 29.15%; 51.93% and 89.71%. However, the digestibility of crude fiber, although there was an increase with increasing protein and energy ration, but statistically did not show the difference ( $P > 0.05$ ).

The results of this study are not much different from some other researchers. Digestible energy growing Hereford heifers fed ration contain 17.6% CP and ME 2.64 Mcal/kg is 74.0% and digestible DM is 74.9% (Joan et al., 1986). While feeding 14.63% CP and GE 16.66 MJ/kg to 311 kg body weight Chinese Holstein heifers resulted in DM, CP and GE digestibility 70.2%, 71.6% and 71.5% respectively (Dong et al., 2017).



Table 3. Effect of level energy protein ration on digestible nutrient of Bali heifer calves.

Variables	Treatments				SEM
	A	B	C	D	
Digestible energy, kcal GE/d	5634.71a	6123.88ab	7001.80bc	7814.34c	381.25
Digestible dry matter g/d	1638.20a	1705.51a	1949.30ab	2111.03b	170.04
Digestible organic matter g/d	1427.12a	1483.06a	1693.73ab	1843.08b	143.02
Digestible crude protein g/d	205.13a	228.98a	275.50b	311.66b	21.64
Digestible crude fiber g/d	463.02a	439.57a	479.31a	491.36a	44.93
Digestible ether extract g/d	49.57a	52.50a	68.78b	94.04c	6.28

Superscripts with different small letters in the same row indicate significant difference ( $P < 0.05$ )

SEM = "Standard Error of the Treatment Means"

Energy intake of heifers treated with treatment C and D respectively 12.68% and 15.56% significantly higher ( $P < 0.05$ ) than the treatment A. Increased energy ration will significantly improved nutrient digestible. High energy intakes of heifer have no negative effect on the development of mammas and may even have improved growth. Although increased growth rates during the post-puberty period have no effect on milk production (Lohakare et al., 2012).

The nutrient digestibility had a positive effect of increasing the nutrient intake as shown in Table 4. Significant improvement ( $P < 0.05$ ) occurred in heifers treated with ration containing ME 2200 kcal/kg: 14% CP and ME 2300 kcal/kg: 15% CP (Table 4). Feeding rations contained 14% crude protein: ME 2200 kcal/kg and 15% Crude protein: ME 2300 kcal/kg also significantly ( $P < 0.05$ ) increased protein intake. The results of this study on Bali heifer calves are much lower than that of other cattle. Research conducted by Devant et al. (2000) in Friesian crossbred heifers given rations containing 14.4% crude protein result DOM 3.2 kd/d, protein intake 602.5 g/d, DMI 4.49 kg/d and generate Average Daily Weight Gain 1.21 kg/d.

Table 4. Effect of energy protein level in ration on nutrient intake of Bali heifer calves.

Variables	Treatments				SEM
	A	B	C	D	
Energy intake, kcal/d	9531.64a	9509.71a	10740.45b	11015.06b	232.39
Dry matter intake, g/d	2729.72a	2661.24a	2980.20b	3001.48b	112.34
Organic matter intake, g/d	2342.00a	2276.98a	2533.89b	2556.54b	95.74
Crude Protein intake, g/d	321.41a	335.42a	392.80b	423.53c	14.85
Crude fiber intake, g/d	706.17a	710.53a	744.66a	727.46a	28.25
Ether extract intake, g/d	85.47a	88.01a	105.40b	124.97c	4.12

Superscripts with different small letters in the same row indicate significant difference ( $P < 0.05$ )

SEM = "Standard Error of the Treatment Means"

## Performance

Table 5. Effect of energy protein level in ration on performances of Bali heifer calves.

Variables	Treatments				SEM
	A	B	C	D	
Initial body weight, kg	102.67a	104.33a	101.33a	101.67a	1.53
Final body weight, kg	124.67a	126.67a	127.33a	129.00a	1.69
Daily weight gain, kg/d	0.27a	0.27a	0.32b	0.33b	0.82
Feed Conversion Ratio	10.31a	10.00ab	9.48ab	9.14b	0.49

Superscripts with different small letters in the same row indicate significant difference ( $P < 0.05$ )

SEM = "Standard Error of the Treatment Means"

Increased energy intake in the treatment of C and D is not only caused by increased energy digestibility, but also due to higher energy ration content. Heifers more efficiently utilizes nutrients when fed with 2200-2300 kcal /kg and 14-15% protein seen from daily weight gain and



FCR. The acceleration of growth before puberty is useful in decreasing age at first calving. However, it must be accompanied by increased intake of protein. Few studies reported that insufficient concentrations of dietary CP in rapidly growing pre-pubertal heifers may impair mammary development and decrease first lactation yield (Lohakare et al., 2012). That's why in this study, increasing energy ration followed by increasing CP on diet. According to Brown et al. (2005), heifers can be fed for accelerated growth rates prior to puberty in addition to decrease age at first calving also to decrease the cost of raising replacement heifers.

Feeding dairy heifers a balanced diet is always important. Heinrichs (2017) recommended ration contain 14 to 15% CP for pre-pubertal heifers based on 2.15% BW DMI/d and energy to allow 1.75 to 2.00 pounds of average daily gain or approximately 130 kcal of metabolizable energy per pound of metabolic body weight. Brown et al. (2005) fed moderate diet contain 21.3% CP (M = moderate protein and energy intake) and high diet contain 30.3% CP (H= high protein and energy intake) to female Holstein calves. The result that ADG and DMI heifer calves fed M diet respectively 0.379 kg/d and 0.847 kg/d compared with H diet respectively 0.668 kg/d and 1.213 kg/d. This result indicates that fed the calves with high diet at the beginning period will reduce growth rate after weaning. The recommended rate of gain after weaning is 800 – 1000 g/d.

Growth rate of F1 Angus x Chinese Xiangxi yellow cattle were unaffected by dietary energy (TDN : 80% vs 70%) and protein levels (14.3% vs 11.9%). The cattle fed an high energy (TDN 80%) diet had a significantly lower DMI (6.76 vs. 7.48 kg DM/d,  $P < 0.01$ ) and FCR (9.38 vs. 11.13,  $P < 0.01$ ) than those fed an low energy (TDN 70%) diet. The ADG and final BW were not affected by the energy or protein levels. Compared with the low energy (TDN 70%: CP 11.9) diet, the high energy (TDN 80% : CP 14.3%) group had ADG 0.77 vs 0.71 kg/d and final body weight 410.44 vs 399.17 kg (Li et al., 2014).

This study found the relationship between metabolizable energy with average daily gain following the equation  $Y = 0.0145 X + 199.55$  where  $Y =$  Average Daily Gain (g/d) and  $X =$  metabolizable energy (k.cal/d) with  $R^2 = 0.88335$  as presented in Figure 1.

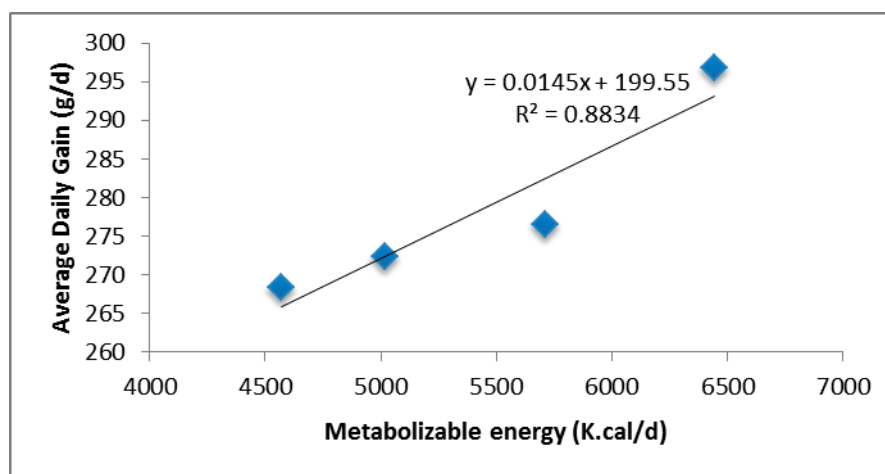


Figure 1. Correlation of ME (k.cal/d) and Average Daily Gain (g/d)

The relationship between protein intake with average daily gain following the equation  $Y = 0.2363 X + 191.44$  where  $Y =$  Average Daily Gain (g/d) and  $X =$  Protein intake (g/d) with  $R^2 = 0.80739$  as presented in Figure 2.

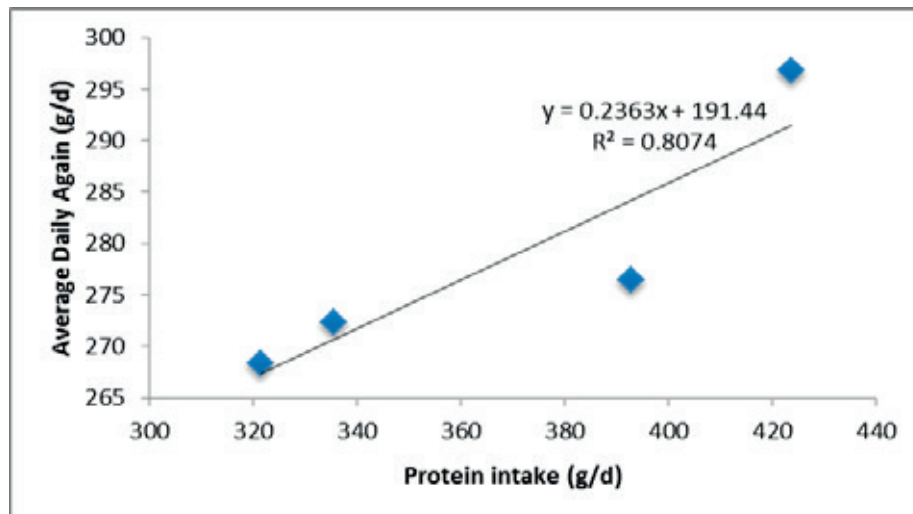


Figure 2. Correlation of protein intake (g/d) and Average Daily Gain (g/d)

## Conclusion

Based on these results, it can be concluded that the increase in energy and protein ration will improve nutrient digestibility thereby increasing intake and ending on improving weight gain of Bali cattle heifer.

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## Metabolizable Energy of Cassava Pulp for Thai Native Beef Cattle

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### Abstract

This study evaluated the nutritive values of cassava pulp for Thai native beef cattle. The experiment was carried out at Khon Kaen Animal Nutrition Research and Development Center, Department of Livestock Development, Thailand during May to October, 2016. The experiment was conducted as a crossover design with 4 Thai native beef cattle maintained in 2 groups and fed 2 dietary treatments in two 20-d periods by using respiration animal calorimetry methodology, a 14-d for preliminary and a 6-d for collecting data in each period. The dietary treatments were: 1) basal feed; control, CTL and 2) basal feed 70.20% + cassava pulp 29.80%, CVP (DM basis). The results showed that addition of 30% cassava pulp to the diet did not affect energy balance, methane production, fecal N, nutrient digestibility except for CP, TDN, DE content and ME content. The TDN, DE content and ME content of cassava pulp were 74.39 %, 12.88 MJ/kg and 11.30 MJ/kg, respectively.

**Keywords:** cassava pulp, metabolizable energy, Thai native beef cattle



## Application of Pressurized Heating in Production Process of Bali Cattle Fur Meal to Its Nutrient

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### Abstract

Pressurized heating is one of technology that widely applied in the process of making fur meal. The pressurized heating process affects the nutrient composition in the meal product so that it needs to be further study. The purpose of this study was to evaluate the effect of applying pressurized heating to the nutrient composition of Bali Cattle fur meal. A number of 5 kg fur waste from Bali cattle obtained from the processing industry of skin cracker at the slaughterhouse, Tamangapa, Makassar, Indonesia. The pressurized heating process uses autoclave by applying four pressure levels, namely: P0 = 0 Psi (no pressurized heating/control); (P1 = 15 Psi); (P2 = 18 Psi) and (P3 = 21 Psi). The heating process was doing for 10 hours. The study started from the stage of supply of raw materials, washing, drying, weighing, and pressurized heating, drying, grinding and testing. The results showed that the application of pressure heating process at different levels did affect the nutrient content of Bali cattle fur meal. The application of the pressurized heating process to fur wastes up to 21 Psi did not reduce the nutrient component in Bali cattle fur meal.

**Keywords:** pressurized heating, nutrient, fur waste, Bali cattle, fur meal

### Introduction

The fur of Bali cattle is one kind of waste produced by skin cracker processing industry in Indonesia. The potential production fur waste of Bali cattle is huge, but not utilized. This is because Bali Cattle is the largest population of livestock in Indonesia (Anonymous, 2017). The result of the preliminary study showed that the waste potential of the unhairing process of skin cracker industry was significant. Percentage of fur waste from slaughterhouse can reach 3% of body weight. If in every day, this industry processed 10 sheets of skin with an average weight of 250 kg, then the potential of fur waste produced is  $10 \times 250 \text{ kg} \times 3\% = 75 \text{ kg/day}$  or  $2.250 \text{ kg/month} \approx 2.3 \text{ tons/month}$  (Said, 2014). The potential of this waste is very large, but not utilized. Fur meal from Bali cattle has a very high nutrient composition, but its digestibility is very low. Fur waste has a protein structure of keratin, which is a non-dissolved fibrous protein (Yamamura et al., 2002). Method processing of fur meal has been done in various, including physical, chemical and microbiologically. The use of enzymes from bacteria is one of the methods that environmentally friendly (Deivasigamani and Alagappan, 2008). To improve the digestibility, appropriate process technology has needed. One of the technologies was used pressurized heating. Pressurized heating is known to break the disulfide bond on the keratin protein component that constitutes the fur waste structure (Kim and Patterson, 2000). Fur meal is a potential alternative source of protein. It has high protein content, low carbohydrates, optimal amino acid content, and little anti-nutritional properties (Zhang et al., 2000). Fur meal contains



keratin proteins that can combine with other ingredients of glycerol to made into composite film sheets (Barone and Arikan, 2007). The purpose of this study was to evaluate the effect of the application of pressurized heating to the nutrient composition of Bali cattle fur meal.

## Materials and Methods

Fur waste obtained from processing industry of skin cracker Bali cattle at the slaughterhouse, Tamangapa, Makassar, Indonesia. A total of 5 kg fur waste sample was used as research material. Electric autoclave (75X-240V), oven (Memmert), beaker glass 250 ml (Pyrex), Erlenmeyer (Pyrex), porcelain cup (laboratory standard), analytical scales (Sartorius TE 214S) were used as main equipment.

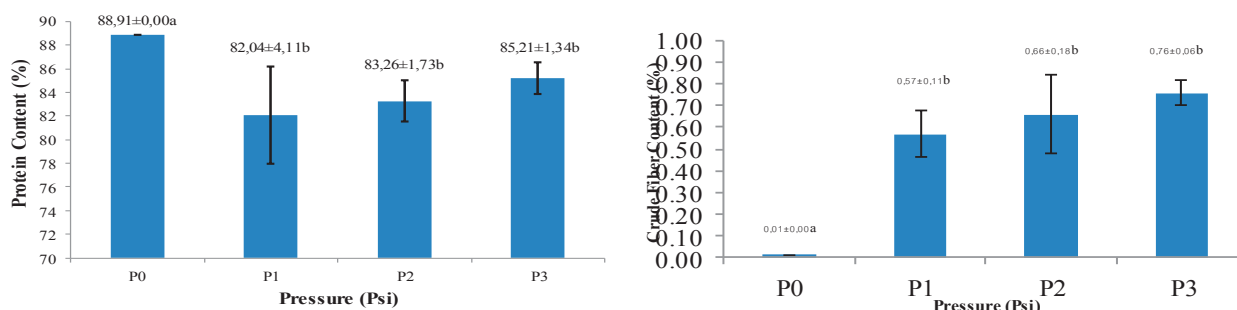
Research designed experimentally. There are four levels of pressurized heating applied, namely P0 = 0 Psi (no pressurized heating/control); (P1 = 15 Psi); (P2 = 18 Psi) and (P3 = 21 Psi). The heating process was done for 10 h. Data analyzed by using statistical program SPSS Version 15.0. The treatment showed a significant effect, then tested the significant difference with Duncan's Multiple Range Test (DMRT) at 5% (Steel and Torrie, 1991).

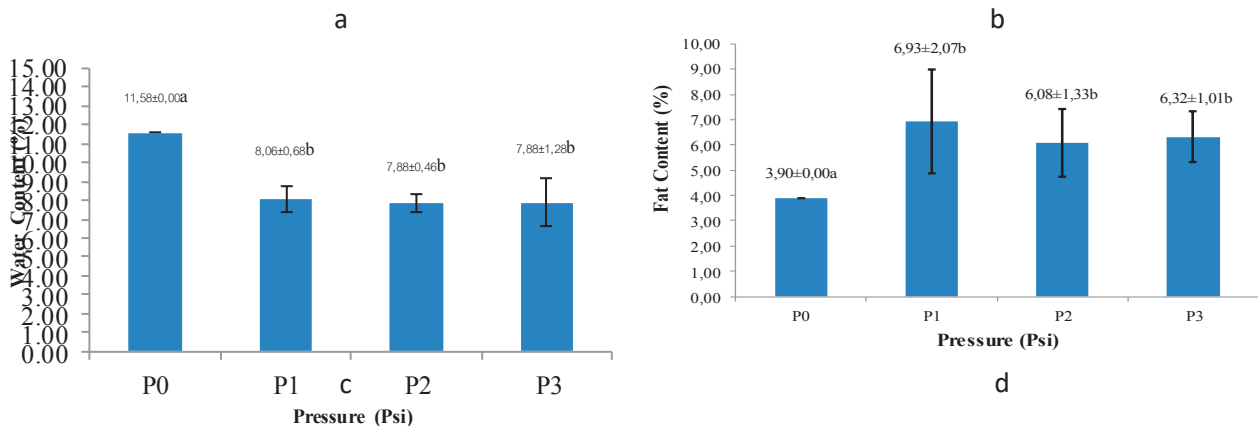
Fur waste of Bali cattle from skin cracker industry processed according to the standard of the fur processing industry. The samples of fur waste were washed with running water. The fur waste dried in an oven at 60°C for 10 h. The fur weighed and inserted into the glass beaker, then placed in the autoclave. The pressure on the autoclave was set according to the treatment. The pressurized heating process carried out for 10 hours. The heated of fur wastes then dried at an oven temperature of 100°C for 8 h. A sample of fur waste in milled with a blender for 1 minute until it becomes fur meal. The fur meal then fed into the vacuum plastic to further testing the nutrient composition. Nutrient compositions for fur meal of Bali cattle observed included: protein content, fat content, moisture content, and crude fiber content.

## Results and Discussion

### Protein content

Protein is one of the nutrients needed for livestock growth. The availability of protein in livestock feed affected by the manufacturing process. Application of pressurized heating process in the process of feed ingredients will affect the chemical composition of the feed material. The ratio of the protein content of the fur meal product to the different pressure heating treatments is presented in Figure 1a. The result of variance analysis showed that the application of pressurized heating treatment had a significant effect ( $P < 0,05$ ) on the protein content of Bali cattle fur meal than control (Hamri, 2016). Chicken feather meal (80-90%) has the protein content similar to Bali cattle fur meal (82.04-85.21%) (Taskin et al., 2012)(Kim and Patterson, 2000). Increased levels of pressure during the heating process does not affect the nutrient composition of Bali cattle fur meal. Fur waste is a by-product of livestock that has potential as the source of protein in livestock (Van-Heughten and Van-Kempen, 2002). In the heating process accompanied by pressure will cause the process of dissolving protein components and protein denaturation. The heating process will break the disulfide bond (S-S) of the amino acid cystine on the fur structure (Kim and Patterson, 2000). The amino acid profile of the fur meal has a similarity to the amino acid profile in fishmeal and soybean meal (Sarmwatanakul and Bamrongtum, 2000).





**Figure 1** Nutrient composition of Bali cattle fur meal in different pressurized heating.

1a, protein content of Bali cattle fur meal; 1b, crude fiber content of Bali cattle fur meal; 1c, water content of Bali cattle fur meal, and 1d, fat content of Bali cattle fur meal. P0 = 0 Psi (no pressurized heating/control); (P1 = 15 Psi); (P2 = 18 Psi); (P3 = 21 Psi). Different superscripts (a,b,c,d) in each treatment showed significant differences ( $P < 0.05$ ).

### Fiber content

Based on the results of statistical analysis of the data in Figure 1b shows that there was a significant difference between the samples using pressurized heating with no pressurized heating (control) ( $P < 0.05$ ). The value of crude fiber in Bali cattle fur waste heated higher than control (0.01%). In the pressurized heating process, there was due to the presence of skin tissue. The result of this process has higher fiber content values (0.57-0.76%). The skin of the Bali cattle contains fibers unsolved, which can affect the fiber content of Bali cattle fur meal. The ratio of the fiber composition to Bali cattle fur meal was similar to the fiber content of chicken feather hydrolysate (0.5%) (Puastuti, 2007). The fiber content in the form of NDF and ADF related to the feed digestion process (Liu et al., 2014).

### Water content

The water content in a feed material determined by the method of the manufacturing process and the way of storage. The amount of nutrient in the form of a water component in a feed material indicates the amount of water attached to the particles of the material. The difference in nutrient composition in Bali cattle fur meal in different processes presented in Figure 1c. The result data of analysis of variance shows that the application of pressurizing heating on the process production of Bali cattle fur meal significantly ( $P < 0.05$ ) to the composition of water content. The use of pressurizing heating in the production process decreases the water content of Bali cattle fur meal products during the process compared to control (Hamri, 2016). Feed ingredients that have high water content have the potential to grow microorganisms (Richana and Sunarti, 2004).

### Fat content

The fat has an important role in the growth and development of livestock because fat serves as a source of energy reserves. These vitamins were the type of fat-soluble in the vitamin. The fats derived from feed ingredients can be stored in the tissues of body cells in the form of fat reserves. Figure 1d shows that the application of pressurized heating process on Bali cattle fur



meal has a significant effect ( $P < 0.05$ ) on fat content compared to control. The heating process increases the fat content of the product. It may be influenced by the presence of more dominant fat glands in Bali cattle (Hamri, 2016). The fat particles present in the flour products derived from the hydrolysis of the fat glands present in the skin of Bali cattle. The facts contained in the feed required by livestock as a source of energy (Liu et al., 2014). The fat content of chicken feathers (1.2%) is lower than the fat content of Bali cattle fur meal (6.08-6.93 %) (Zerdani et al., 2004).

## Conclusion

The pressurized heating process has significantly affected for the nutrient composition of Bali cattle fur meal during the process on the protein content, fat content, moisture content and crude fiber content. Pressurized heating process (P1, P2, and P3) have different nutrient composition than control (P0). The application of pressurized heating treatment (P3) (21 Psi) have the nutrient composition of Bali cattle fur meal with better properties.

## Acknowledgements

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## Effect of $\beta$ -Glucan Supplementation on Feed Intake, Digestibility and Rumen Fermentation in Thai Native Beef Cattle

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### Abstract

Four 1.5 year old male Thai native beef cattle with an initial body weight (BW) of  $100 \pm 20$  kg were randomly assigned according to a  $4 \times 4$  Latin square design to study the effect of Beta-Glucans ( $\beta$ -glucan) on feed intake, digestibility and fermentation. The dietary treatments were different supplemented levels of  $\beta$ -glucan at 0, 1.6, 3.1, and 4.7 gram (g), respectively. Rice straw intake and total feed intake were significantly different among treatments ( $p < 0.05$ ) and was the highest in the 4.7 g  $\beta$ -glucan supplement. Intakes of organic matter (OM), crude protein (CP), neutral detergent fiber (aNDF) and acid detergent fiber (ADF), digestible OM intake, digestible OM fermented in the rumen and metabolizable energy showed no difference among treatments ( $P > 0.05$ ). The experimental diets had no effect ( $P > 0.05$ ) on the apparent digestibilities of DM, OM, aNDF and ADF while CP digestibility were increased with the increase of  $\beta$ -glucan in the diet; especially at 4.7g  $\beta$ -glucan. No differences ( $P > 0.05$ ) were found in ammonia nitrogen concentration ( $\text{NH}_3\text{-N}$ ) and blood urea N among treatments. The population of protozoa was significantly different among treatments and was highest in supplemented group with  $\beta$ -glucan at 4.7 g. Based on this study, it could be concluded that supplementation of  $\beta$ -glucan at 4.7 g could improve rice straw intake, CP digestibility and protozoa population in Thai native beef cattle

**Keywords:** beta-glucans, feed intake, digestibility, fermentation, Thai native beef cattle

### Introduction

In animal husbandry, the control and prevention of infectious diseases is of great economic importance. Antibiotics have been used in animal production for many years both as prophylactic agents and growth promotor.  $\beta$ -glucan are naturally occurring forms of carbohydrate found in yeast, fungi, oats, and barley that have been shown to stimulate the mammalian immune system (Taylor et al., 2002). Recently, many studies have shown that supplementation  $\beta$ -glucan to animal diet improve feed utilization in chicken (Redmond et al., 2010), pigs (Xiao et al., 2004), sheep (Khalkhane et al., 2013), and Holstein calves (Tao et al., 2015). However, studies on the supplementation of  $\beta$ -glucan in Thai native beef cattle have no data. Therefore, this study was conducted to evaluate the effect of  $\beta$ -glucan on feed intake, digestibility and rumen fermentation in Thai native beef cattle fed on rice straw.





## Materials and methods

### Dietary, animals, experimental design, and feeding

Four Thai native beef cattle with initial body weight (BW) of  $100 \pm 20$  kg were randomly assigned according to a  $4 \times 4$  Latin square design to receive four treatment by  $\beta$ -glucan at 0, 1.6, 3.1, and 4.7 g/d, respectively.  $\beta$ -glucan (FUBON<sup>®</sup>) was obtained from The Siamese Intercorp Co., Ltd, Thailand which contained  $\beta$ -glucan more than 20%. All animals were fed rice straw ad libitum as roughage source while additional concentrate was fed at 0.5% BW daily and offered in two equal meals per day at 07.00 and 16.00 h. The proportions of concentrate ingredients and the chemical composition of the concentrates,  $\beta$ -glucan, and rice straw are shown in Table 1. All cattle were kept in individual pens while clean fresh water and feed blocks were available at all times. Individual intakes of rice straw and concentrate were recorded daily by weighing the offered and refused feeds during the morning feeding. The experiment was conducted for four periods of 21 days each.

### Sample collection and sampling procedures

Concentrates, rice straw and  $\beta$ -glucan were sampled daily during the collection period and were composited by a period prior to chemical analyses. Feed offered, refusal and fecal samples were collected for chemical analysis namely DM, N, ash, acid detergent fiber (ADF) ND Neutral detergent fiber (NDF). Approximately, 45 mL of rumen fluid was taken from the rumen by a stomach tube connected to a vacuum pump at 0 and 4 h after feeding on the last day of each period. Ruminal pH and temperature were determined using a portable pH and temperature meter (Hanna Instruments HI 8424 microcomputer, Singapore). Ruminal  $\text{NH}_3\text{-N}$  concentration was analyzed using a Kjeltach Auto 1030 Analyzer. Rumen fluid was used for direct counts of protozoa (Boeco, Singapore). A blood sample was collected from the jugular vein at the same time as rumen fluid sampling into tubes containing 12 mg of EDTA, and the plasma was separated by centrifugation at  $500 \times g$  for 10 min at  $4^\circ\text{C}$  and stored at  $-20^\circ\text{C}$  until analysis of plasma urea N. Statistical analysis accounted for the  $4 \times 4$  Latin square design using the GLM procedure of SAS (1996).

## Results and discussions

### Feed intake, nutrient intake.

The effect of  $\beta$ -glucan supplementation on feed intake, nutrient intake in Thai native beef cattle is presented in Table 2. Supplementation of  $\beta$ -glucan at 0 to 4.7 g in cattle were not affected on intake of DM, OM, CP, NDF, and ADF. However, supplementation with  $\beta$ -glucan was significantly improved their rice straw intake ( $\text{g/kg BW}^{0.75}$ ) when compared to the no-supplemented group ( $p < 0.05$ ). The rice straw intake was higher than no  $\beta$ -glucan supplementation (65.1- 71.4  $\text{g/kg BW}^{0.75}$ ). In addition, total fed intake were also increased from 84.1 to 90.3  $\text{g/kg BW}^{0.75}$  when  $\beta$ -glucan supplementation at the highest level. The greater feed intakes by  $\beta$ -glucan supplementation could be attributed to enhanced palatability of  $\beta$ -glucan and increasing in nutrient digestibility. Palatability can be improved by using ingredients preferred by animal or by using feed additives, such as flavors, that make the diet more acceptable and encourage greater feed intake. Flavors are feed additives that attempt to enhance the taste and smell of feed to stimulate feed intake. Similar results were reported by Tao et al. (2015) who found that  $\beta$ -glucan supplementation at 75 mg/kg feed in pre-ruminant Holstein calves improved intake of feed.

### Digestibility of nutrients

Table 3 presents the data for apparent digestibility of nutrients. The experimental diets has no effect ( $P > 0.05$ ) on the apparent digestibilities of DM, OM, NDF and ADF. Digestibility of DM ranged from 63.5-64.9%. However, the digestibility of CP was significantly different among treatment and supplementation at 4.7 g/d  $\beta$ -glucan was the highest (63.2%) than those of the



other diets. It could be therefore inferred that increased nutrient digestibility by supplementary yeast  $\beta$ -glucan is associated with improved rumen microorganisms especially cellulolytic bacteria. These results indicated the availability of more potential for the proliferation of rumen microbes and for improvement of feed intake and digestibility from the current study. The supplementation of yeast  $\beta$ -glucan at 75 mg/kg feed also increased the apparent digestibility of CP (Tao et al., 2015).

### **Rumen fermentation and protozoal population**

The effect of various levels of  $\beta$ -glucan on ruminal pH, rumen temperature, concentrations of rumen  $\text{NH}_3\text{-N}$ , and protozoal population is presented in Table 4.  $\beta$ -glucan supplementation did not affect rumen pH and temperature and were ranged from 6.7-6.8 and 38.9-39.2, °C, respectively ( $P > 0.05$ ) which was reported as a suitable range for feed digestion activity of rumen microorganisms. Concentration of  $\text{NH}_3\text{-N}$  in the rumen fluid is the net result to  $\text{NH}_3\text{-N}$  production from the feed, fermentation of protein or hydrolysis of urea, absorption through the rumen wall and passage out of the rumen and utilization by microbes. The current experiment found that concentration of ruminal  $\text{NH}_3\text{-N}$  were not changed among  $\beta$ -glucan level supplementation and ranged from 16.2-17.4 mg/dl. However, these values were above the optimum level for rumen micro-organism growth (15–30 mg %), as stated by Wanapat and Pimpa (1999).

The population of protozoa at 4 h post feeding was increased with the increasing of  $\beta$ -glucan supplementation and was significantly highest at 4.7 g of  $\beta$ -glucan. This may be related to the factors in  $\beta$ -glucan that enhanced rumen protozoa. One of the possible explanations of this finding seems to be an enrichment of  $\beta$ -glucan with constituents which are digested and utilized by the examined protozoa as a source of energy. Similarly, Belzecki et al. (2012) demonstrated that enrichment of culture medium with the examined  $\beta$ -glucan results in an increase in the number of ciliates protozoa over 36 and 15% when the growth medium was supplemented with pachyman (1,3- $\beta$ -glucan) and pustulan (1,6- $\beta$ -glucan), respectively.

### **Conclusion**

Supplementation of  $\beta$ -glucan at 4.7 g improved rice straw intake, total intake and digestibility of CP in Thai native beef cattle. Furthermore, these findings should be further suggested in rumen cellulolytic bacteria study by using real-time PCR to elucidate the actual effect of  $\beta$ -glucan on nutrient digestibility.

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**Table 1** Ingredient and chemical composition of concentrate,  $\beta$ -glucan and rice straw used in the experiment

Item	Concentrate	$\beta$ -glucan	Rice straw
Ingredients, kg DM			
Cassava chips	55.0		
Rice bran	11.0		
Coconut meal	12.9		
Palm kernel meal	13.5		
Urea	2.6		
Pure sulfur	1.0		
Mineral premix <sup>a</sup>	1.0		
Molasses, liquid	2.0		
Salt	1.0		
Chemical composition			
Dry matter, %	93.2	94.0	96.5
		-----%DM-----	
Organic matter	90.7	92.0	90.2
Ash	9.3	8.0	9.8
Crude protein	13.3	35.0	2.8
Neutral detergent fiber	13.4	-	78.2
Acid detergent fiber, %DM	7.7	-	58.3
$\beta$ -glucan <sup>b</sup>	-	>20	-

<sup>a</sup>Minerals and vitamins (each kg contains): Vitamin A: 10,000,000 IU; Vitamin E: 70,000 IU; Vitamin D: 1,600,000 IU; Fe: 50 g; Zn: 40 g; Mn: 40 g; Co: 0.1 g; Cu: 10 g; Se: 0.1 g; I: 0.5g.

<sup>b</sup> $\beta$ -glucan (FUBON<sup>®</sup>) was obtained from The Siamese Intercorp Co., Ltd, Thailand.



**Table 2** Influence of different levels of  $\beta$ -glucan on feed intake and nutrient intake in Thai native beef cattle

	Supplementation of $\beta$ -glucan, g DM				SEM	P value
	0	1.6	3.1	4.7		
Rice straw intake						
kg/day	1.9	2.0	2.0	2.1	0.45	0.55
g/kg BW <sup>0.75</sup>	65.1 <sup>a</sup>	67.9 <sup>a</sup>	68.2 <sup>b</sup>	71.4 <sup>c</sup>	0.54	0.04
Concentrate intake						
kg/day	0.6	0.5	0.6	0.6	0.11	0.32
g/kg BW <sup>0.75</sup>	19.1	18.6	18.8	18.7	0.63	0.92
$\beta$ -glucan intake						
g/day	0.0	1.6	3.1	4.7	-	-
kg/day	0.0000	0.0016	0.0031	0.0047	-	-
g/kg BW <sup>0.75</sup>	0.000	0.054	0.106	0.160	-	-
Total intake						
kg/day	2.5	2.6	2.6	2.7	0.69	0.55
g/kg BW <sup>0.75</sup>	84.1 <sup>a</sup>	86.6 <sup>b</sup>	87.1 <sup>b</sup>	90.3 <sup>c</sup>	1.12	0.02
% BW	2.2	2.3	2.3	2.4	0.12	0.36
Nutrient intake, kg/d						
Dry matter	2.5	2.6	2.6	2.7	2.66	0.35
Organic matter	2.2	2.3	2.3	2.4	2.54	0.45
Crude protein	0.1	0.1	0.1	0.1	1.03	0.16
Neutral detergent fiber	1.6	1.6	1.6	1.7	2.01	0.28
Acid detergent fiber	1.2	1.1	1.1	1.2	1.98	0.19

<sup>a,b,c</sup>Means in the same row with different superscripts differ ( $P < 0.05$ ).

**Table 3** Apparent nutrient digestibility in Thai native beef cattle fed different levels of  $\beta$ -glucan

	Supplementation of $\beta$ -glucan, g DM				SEM	P value
	0	1.6	3.1	4.7		
Digestibility coefficients, %						
Dry matter	64.0	63.5	63.8	64.9	1.09	0.25
Organic matter	67.1	66.5	66.4	68.8	1.25	0.99
Crude protein	61.6 <sup>a</sup>	60.2 <sup>a</sup>	60.3 <sup>a</sup>	63.2 <sup>b</sup>	0.12	0.03
aNeutral detergent fiber	59.5	59.4	59.2	60.6	0.19	0.68
Acid detergent fiber	58.7	58.3	58.2	59.2	0.21	0.38

<sup>a,b</sup>Means in the same row with different superscripts differ ( $P < 0.05$ ).



**Table 4** Effect of different levels of  $\beta$ -glucan on rumen fermentation and blood urea-nitrogen in Thai native beef cattle

	Supplementation of $\beta$ -glucan, g DM				SEM	P value
	0	1.6	3.1	4.7		
Ruminal pH						
0 h post feeding	6.8	6.7	6.8	6.9	1.02	0.22
4 h post feeding	6.5	6.6	6.6	6.7	1.69	0.35
Mean	6.7	6.7	6.7	6.8	1.58	0.28
Ruminal temperature, °C						
0 h post feeding	38.2	38.6	38.5	38.4	1.99	0.96
4 h post feeding	39.6	39.5	39.7	39.9	2.36	0.12
Mean	38.9	39.1	39.1	39.2	2.03	0.54
NH <sub>3</sub> -N, mg/dl						
0 h post feeding	14.5	13.9	15.5	15.9	4.89	0.66
4 h post feeding	17.8	18.5	17.9	18.8	5.36	0.47
Mean	16.2	16.2	16.7	17.4	5.01	0.78
Protozoa, x 10 <sup>6</sup>						
0 h post feeding	1.1	1.5	1.2	1.4	0.23	0.12
4 h post feeding	2.1 <sup>a</sup>	2.9 <sup>a</sup>	3.8 <sup>b</sup>	4.8 <sup>c</sup>	0.30	0.02
Mean	1.6	2.2	2.5	3.1	0.29	0.05

<sup>a,b,c</sup>Means in the same row with different superscripts differ (P<0.05).



## Ruminal Nitrogen Release from Limestone-Urea Mixture

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### Abstract

The *in sacco* technique was used to determine the ruminal nitrogen (N) disappearance from the limestone-urea mixture. The mixtures of limestone-urea were created with 4 levels of urea based on the Ca content of limestone: 100, 75, 50 and 25%. Two rumen fistulated Ettawah local cross bred goats were used in the *in sacco* technique. The ruminal incubation times were 0.75; 1.5; 3, 6, 12, 24, and 48 h. The ruminal DM and N disappearances (expressed as % of the initial amount) were calculated based on the their content of sample residues. The data were then used to estimate ruminal degradability kinetic parameters of DM and N. The rapidly soluble fraction and potentially degradable fraction of DM and N were biggest and smallest, respectively, in the mixture of 25% limestone and 75% urea. The mixture of 75% limestone and 25% urea had smallest rapidly soluble fraction and biggest potentially degradable fraction for ruminal DM and N degradabilities, respectively. The limestone could reduce the percentage of ruminal N disappearance from the mixture of limestone-urea.

**Keywords:** limestone, urea, N release, rumen, *In sacco*

### Introduction

It is well known that ruminant relies on rumen microbial protein synthesis. Ruminal ammonia, volatile fatty acids and keto acids are the main components utilized in the microbial protein synthesis. Urea is commonly used partly as an ammonia source in ruminant feed. The use of urea should be synchronized with the rapidly available carbohydrate to avoid the effect of excessive ruminal ammonia. In addition, the ruminal ammonia release from urea containing feed should be delayed because urea is hydrolyzed rapidly in the rumen.

Some efforts attempt to slow the ruminal ammonia release from urea containing feed. Cherdthong et al. (2011a) created urea-Ca mixture as a promising supplement for the ruminant. The limestone is commonly utilized as a good Ca source for ruminant (Crawford et al., 2008; Dias et al., 2008; Nunez et al., 2014). There are some limestone mountains in Java island especially in the province of Central Java. The limestone from limestone mountains contain high Ca and other elements which may be utilized to bind urea. Cherdthong et al. (2011b) postulated that the hydrogen bonding plays an important role in the mixture of urea-CaSO<sub>4</sub> causing the slower rate of NH<sub>3</sub>-N formation in rumen. The objective of this study was to determine the ruminal nitrogen (N) disappearance from limestone-urea mixtures with different portions of limestone and urea. This was accomplished using the *in sacco* technique.



## Materials and Methods

### Creation of limestone-urea mixture and the *in sacco* technique

The limestone samples were collected from three locations of limestone mountain in Subdistrict of Pamotan, Rembang Regency, Province of Central Java. The limestone was dissolved with water (1:1; w/w) and stirred at 50°C for 10 min. The limestone pulp then was mixed with urea to find 4 levels of urea including 100, 75, 50 and 25% of Ca concentration of limestone. Table 1 shows the portions of limestone and urea those were tested for their ruminal nitrogen releases.

Two female local Ettawah cross bred goats with body weight average of 30 kg and aged at 36 months were used in this *in sacco* technique. Animals were fitted with ruminal cannulas with inside diameter of 3 cm. Goats were housed in individual metabolic cages and fed on a diet containing 70% elephant grass, 10.7% rice bran, 17% coconut meal, 1.1% cassava waste, 1.0% sugarcane molasses, 0.2% vitamin-mineral premix. The diet was offered at daily maintenance level and drinking water was available throughout experimental period.

The *in sacco* technique used nylon bags with dimension of 2 X 6 cm and mean pore size of 46 µm. Each sample of 3 g was placed in the bag which was attached with 0.25 m weighted chain. The bags were suspended in rumen of sheep via cannula 30 min after morning feeding. Incubation times were 0.75, 1.5, 3, 6, 12, 24, and 48 h. Three replicates of each sample were tested for each tested limestone-urea mixture and incubation time. There were intervals of 24 h between each series of incubation time to minimize the effect of previous test.

After the time of incubation bags were removed and immersed in ice water for 15 min, the bags were washed with water using a washing machine for 3 min. The bags and sample residues were then dried at 70°C for 48 h before weighing, and sample residues were prepared for chemical analyses.

### Chemical and statistical analysis.

Sample residues were then determined their nitrogen (N) contents based on kjeldahl method. The ruminal DM, and N disappearances (expressed as % of the initial amount) were calculated based on the their content of sample residues. The degradability data were then calculated to obtain the kinetic of ruminal DM and N degradabilities according to the equation described by (Ørskov and McDonald, 1979):

$$P = a + b(1 - e^{-ct})$$

where P is the amount degraded at time  $t$ ,  $a$  is the rapidly soluble fraction,  $b$  is the potentially degradable fraction,  $c$  is the rate of degradation of fraction  $b$ . The parameters of ruminal degradability kinetic were focused on rapidly soluble fraction (fraction  $a$ ), potentially degradable fraction (fraction  $b$ ), and the degradation rate of fraction  $b$  ( $c$ ) for DM and N, respectively. The data were tested using analyse of variance based on a completely randomized design.

## Results and Discussion

The mixture of limestone-urea is known as a supplement for ruminant, mainly as the sources of mineral (CaCO<sub>3</sub>) and nitrogen, respectively. The limestone from some mountains in the Province of Central Java is commonly utilized for cement material and animal feed supplement. However, there is a little information concerning with nutritive value of the limestone. Cherdthong et al. (2011a,b) studied the urea-calcium mixture proofing as the supplement for ruminant.

Figure 1 shows the time course of ruminal incubation for releasing the nitrogen from limestone-urea mixtures. The amount of DM and N disappearances elevated at the early times of ruminal incubation in L0U100, L25U75, and L50U50. The disappearance amount of N in L0U100 or urea was near to be 100% at the first hour of ruminal incubation. Suhada et al. (2016) assumed that





95% of urea was rapidly hydrolyzed in rumen within first hour after feeding. The mixture of L75U25 indicated a delay in ruminal N release from limestone-urea mixture. The ruminal DM and N degradability kinetics of limestone-urea mixtures are presented in Table 2.

Decreasing levels of urea in the mixture decreased ( $P < 0.05$ ) the rapidly soluble fraction and the degradation rate of potentially degradable fraction, and increased ( $P < 0.05$ ) potentially degradable fraction of DM and N (Table 2). While the rapidly soluble fraction is composed of some readily available feed materials, the potentially degradable fraction is composed of slowly available feed materials in rumen.

Islam et al. (2004) reported that the increase in maturity of whole crop rice silage reduces the rapidly soluble fraction and increases potentially degradable fraction of DM, indicating a lower quality of the feed. The higher rapidly soluble fraction in *Leucaena* leaf than that of *Acacia* leaf indicates that *Acacia* is provided with a higher portion of by pass protein, because protein of *Acacia* is degraded more slowly (Abdulrazak et al., 2001). Likewise, the cotton seed meal may be a good source of by pass protein because the rapidly soluble fraction of crude protein is degraded more slower than that other tropical protein resources (Promkot et al., 2007). By referring these facts, the mixture of L75U25 may be provided with a ruminal slow release of nitrogen because the rapidly soluble fraction and the degradation rate of potentially degradable fraction were smallest, whilst potentially degradable fraction of DM and N was biggest (Table 2).

The capacity binding of limestone for urea could be assumed not so big, the ratio of limestone-Ca to urea in the L75U25 mixture was 1:0.3 (Table 1). In addition, limestone is commonly utilized as a good source of Ca for the ruminant diet (Crawford et al., 2008; Dias et al., 2008; Nunez et al., 2014), but the ruminal solubility of urea may be much greater than that of limestone. Therefore, urea was almost hydrolyzed completely and there was a residual limestone after 48 h ruminal incubation (Table 2).

## Conclusion

The limestone could reduce ruminal nitrogen disappearance from limestone-urea mixture but its implication for ruminant supplement remains to be studied.

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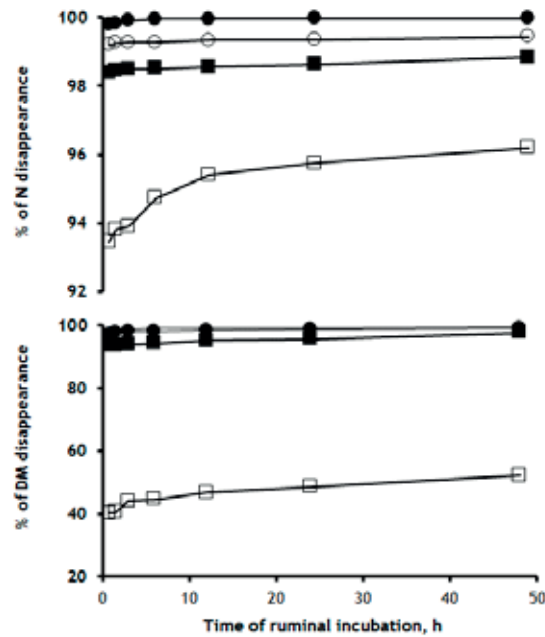
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**Figure 1.** Percent of DM and N disappearances of L0U100 (●), L25U75 (○), L50U50 (■), and L75U25 (□) throughout the ruminal incubation times.

**Table 1.** Compositions of Limestone-urea mixture

Lime stone – urea mixture	Urea	Limestone	N	Ca
L0U100	100	0	46.16	0
L25U75	75	8	41.77	4.95
L50U50	50	33	24.02	20.44
L75U25	25	58	10.66	35.92

**Table 2.** Parameters of ruminal degradability kinetics of limestone-urea mixture

Items	Limestone-urea mixture				SEM
	L0U100	L25U75	L50U50	L75U25	
Dry Matter					
Fraction <i>a</i> , %	98.86 <sup>a</sup>	97.64 <sup>a</sup>	93.72 <sup>b</sup>	42.47 <sup>c</sup>	7.11
Fraction <i>b</i> , %	0.59	1.59	3.89	10.69	1.78
<i>c</i> , %/h	5.56	6.54	3.40	2.24	0.89
Nitrogen					
Fraction <i>a</i> , %	99.86 <sup>a</sup>	99.24 <sup>ab</sup>	98.43 <sup>b</sup>	93.70 <sup>c</sup>	0.75
Fraction <i>b</i> , %	0.11 <sup>b</sup>	0.20 <sup>b</sup>	0.40 <sup>b</sup>	2.58 <sup>a</sup>	0.36
<i>c</i> , %/h	17.70 <sup>a</sup>	3.73 <sup>b</sup>	2.80 <sup>b</sup>	6.50 <sup>b</sup>	2.14

<sup>a,b,c</sup>*P* < 0.05; SEM: standard error of means.



## Effect of Sulfur Levels Supplementation in Fermented Total Mixed Ration Containing Fresh Cassava Root Using *In Vitro* Gas Production Technique

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### Abstract

The objective of the present study was to evaluate the effect of sulfur levels supplementation in fermented total mixed ration (TMR) containing fresh cassava root as energy source on kinetic of gas, ruminal fermentation, and nutrient digestibility using *in vitro* gas production technique. The experimental design was a 3×4 factorial in Completely randomized design. Dietary treatments were levels of sulfur supplementation at 0, 1 and 2% in TMR (Factor A) and time of fermentation at 0, 7, 14 and 21 days (Factor B). It was found that gas production from soluble fractions (a), gas production from the insoluble fraction (b), gas production rate constants for the insoluble fraction (c), potential extent of gas production (a+b) were not altered when increasing concentration of sulfur ( $P>0.05$ ). Cumulative gas production (at 96 h of incubation) was significantly different when increase sulfur 2% ( $P>0.05$ ) whereas there was no influence by different time of ensiling. Ruminal pH was similar in all treatments while  $\text{NH}_3\text{-N}$  concentration tended to be higher for 2% sulfur than those in other groups. Fermented TMR ensiled for 21 day had the highest in *in vitro* dry matter digestibility, *in vitro* neutral detergent fiber digestibility (IVNDFD) and *in vitro* acid detergent fiber digestibility (IVADFD) while protozoa population was not altered by the type of TMR. It could be concluded that 2% sulfur levels supplementation in TMR containing fresh cassava root fermented for 21 days could improve kinetic of gas and nutrient digestibility while maintain ruminal fermentation parameters.

**Keywords:** fresh cassava root, sulfur, fermented total mixed ration (FTMR), ruminant

### Introduction

Cassava (*Manihotesculenta*, Crantz) is a crop of major importance in the tropical. It is systems primarily for the starchy root which is used for human industry food or as an energy source for non-ruminant or ruminant livestock feed (Wanapat and Khampa, 2007). Fresh cassava root as an energy supplement is interesting in ruminant diets, because of its low cost and reduce process compared with cassava chip, and convenient use for the farmer (Cherdthong et al., 2017). Moreover, during the rainy season, it is difficult to sun-dry cassava to generate cassava chip. However, feeding of fresh cassava root in ruminants is limited since it contains a high level of hydrocyanic acid (HCN) which is responsible for poisoning. Fresh cassava root contains HCN at about 90-114 mg/kg fresh basis (Nguyen et al., 1997; Cherdthong et al., 2017). In ruminants, Cherdthong et al. (2017) reported that supplemented 1.5% of fresh cassava root with feed block containing 4% sulfur did not adverse effect on roughage intake, rumen fermentation and blood



urea nitrogen whereas hydrocyanic acid was reduced. Furthermore, the fermented total mixed ration (FTMR) is a basic method to potentially improve nutrient utilization, extend the long preservation and could reduce some anti-nutritive substance in feeds (Wongnen et al., 2009). FTMR is roughage with concentrate mixing and then fermenting under anaerobic conditions (*ie* ensiling) in a sealed container. Thus, the objective of the present study was to evaluate the effect of sulfur levels supplementation in FTMR containing fresh cassava root as energy source on kinetic of gas, ruminal fermentation, and nutrient digestibility using *in vitro* gas production technique.

## Materials and Methods

The experimental design was a 3×4 factorial in Completely randomized design, and dietary treatments were based on two factors; A: level of sulfur supplementation at 0, 1 and 2% in TMR and B: time of fermentation at 0, 7, 14 and 21 days. Fresh cassava root was collected from Khon Kaen province areas, Thailand. The FTMR was roughage with concentrate mixing and then fermenting under anaerobic conditions (*ie* ensiling) in a sealed container. Sample of FTMR, rice straw for roughage source was dried at 60°C for 48 h, then ground to pass through a 1-mm sieve used for chemical analysis. Ingredient and chemical composition of FTMR in the experiment shown in Table 1. Two castrated male, rumen-fistulated dairy cattle with body weight of 450 ± 30 kg were used as rumen fluid donors. Rumen fluid was collected from animals fed with concentrate (14% CP and 75% TDN) at 0.5% of BW. Samples of 0.5 g of FTMRs were weighed into 50 ml serum bottles. For each treatment, bottles were sealed with rubber stoppers and aluminium caps and incubated at 39°C (96 h) for *in vitro* gas test according the method of (Menke and Steingass, 1988). During the incubation, data of gas production was measured immediately after incubation at 0, 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 48, 72 and 96 h by using a pressure transducer and a calibrated syringe. Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979) for determined kinetics of gas. Fermentation liquor was sampled for measured pH, NH<sub>3</sub>-N. *In vitro* digestibility for dry matter (IVDM), neutral detergent fiber (IVNDFD) and acid detergent fiber digestibility (IVADFD) were also measured. All data from the experiment were analyzed as a completely randomized design using the GLM procedure of SAS.

## Results and discussions

### Chemical composition of the diets

The chemical compositions of DM, OM, Ash, CP, NDF, and ADF in FTMR with various levels of sulfur after ensiling and fresh cassava root are shown in Table 1. FTMR consisted of CP, NDF and ADF at 9.5- 9.8%, 56.5-60.2 and 22.8-23.4% DM, respectively. The composition of the fresh cassava root consisted of CP, NDF and ADF at 2.1%, 53.1 and 31.2 % DM, respectively and were similar to that reported by Cherdthong et al. (2017). However, chemical composition of fresh cassava root might be variable depend on many factors such as variety, soil fertility or state of growth etc.

### Gas kinetics and cumulative gas production

The values for the estimated parameters obtained from the kinetics of gas production models for substrates studied are given in Table 2. It was found that gas production from soluble fractions (a) ranged from -2.4 to 5.7 and was not significantly different among treatments



( $P > 0.05$ ). Furthermore, gas production from the insoluble fraction (b), gas production rate constants for the insoluble fraction (c), potential extent of gas production (a+b) were also not altered when increasing concentration of sulfur ( $P > 0.05$ ). Cumulative gas production (at 96 h of incubation) was significantly different when increase sulfur 2% ( $P > 0.05$ ) whereas was not influenced by different time of ensiling. This enhanced performance of cumulative gas production could be due attributed to higher nutrients and digestibility to precursor protein production, resulting in effective ruminal fermentation.

### **Ruminal parameters, *in vitro* digestibility**

As shown in Table 3, ruminal pH was similar in all treatments and ranged from 6.0 to 6.7. The ruminal pH were in the optimum range for microbe activity, as reported by Van Soest (1994). Similarly, Vasupen et al. (2006) show that ruminal pH was not affected by FTMR. Type of FTMR supplementation did not effect ruminal  $\text{NH}_3\text{-N}$  concentration among dietary treatments. However,  $\text{NH}_3\text{-N}$  trend to higher for 2% sulfur than those other groups, which is the optimal range of  $\text{NH}_3\text{-N}$  for microbial protein synthesis (Satter and Slyter, 1974). FTMR ensiled for 21 day had highest in *in vitro* dry matter digestibility, *in vitro* neutral detergent fiber digestibility (IVNDFD) and *in vitro* acid detergent fiber digestibility (IVADFD) this increase was likely to the fermentation of fibre during the preparation of the FTMR to reflects higher microbial biomass, fibrolytic enzyme degraded some cellulose into sugars (Blümmel et al., 1997). In addition, adding sulfur in the ration might be improving microbial synthesis and enhanced digestibility. Sulfur has long been recognized as an essential element for ruminant microorganism, growth and normal cellular metabolism as metabolism is closely related to nitrogen metabolism (Cherdthong et al. 2011). The animal relies on microorganisms in the rumen to convert sulfate to hydrogen sulfite which is used to synthesize methionine and cysteine for microbial growth. Moreover, Vasupen et al. (2006) reported that the fermented fiber fraction in FTMR is increase digested by microbes in the rumen key to increased fiber digestion after feeding FTMR in dairy cows. Protozoa population was not altered by the type of FTMR.

### **Conclusion**

Based on this experiment, it could be concluded that 2% sulfur levels supplementation in TMR containing fresh cassava root fermented for 21 days could improve kinetic of gas and nutrient digestibility while maintain ruminal fermentation parameters. However, these finding should be applied further *in vivo* experiment in order to increase animal performance.

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**Table 1.** Ingredient and chemical composition of fermented total mixed ration used in the experiment (g/kg dry matter (DM)).

Item	Factor 1	Factor2	Factor3	Fresh cassava root
Ingredients, % DM				
Rice straw	400	400	400	
Fresh cassava root*	400	400	400	
Soybean meal	50	50	50	
Palm kernel meal	50	40	30	
Rice bran	30	30	30	
Urea	20	20	20	
Pure sulfur	0	10	20	
Mineral premix	10	10	10	
Molasses, liquid	30	30	30	
Salt	10	10	10	
Chemical composition, %DM				
Dry matter	469.5	466.8	456.0	32.00
Organic matter	954.1	955.0	957.2	92.60
Ash	42.0	44.5	46.9	3.70
Crude protein	95.6	97.7	94.9	2.08
Neutral detergent fiber	565.3	601.7	583.7	53.13
Acid detergent fiber	227.8	234.0	229.5	31.19

\* Fresh cassava root contained 32%DM





**Table 2.** Effect of fermented total mixed ration on gas kinetics and cumulative gas at 96 h after incubation.

Item/Trt	0% S			1% S			2% S			P-value						
	21D	14D	7D	0D	21D	14D	7D	0D	21D	14D	7D	0D	A	B	A*B	SEM
Gas production kinetic																
a	2.4	1.4	-1.2	-2.4	2.8	2.1	5.7	5.1	0.3	4.1	-1.1	-1.2	0.29	0.58	0.87	1.53
b	66.0	53.0	44.1	49.6	43.5	54.1	57.8	44.5	54.2	88.6	61.8	72.3	0.13	0.64	0.59	2.98
c	0.1	0.1	0.2	0.2	0.1	0.2	0.0	0.0	0.1	0.1	0.1	0.2	0.43	0.71	0.44	0.20
a+b	68.5	54.4	42.9	46.9	46.3	56.2	63.5	49.6	54.5	92.6	60.7	71.1	0.11	0.43	0.34	2.85
Cumulative gas, ml	73.3 <sup>b</sup>	57.7 <sup>b</sup>	45.9 <sup>b</sup>	71.3 <sup>b</sup>	50.2 <sup>b</sup>	57.8 <sup>b</sup>	62.1 <sup>b</sup>	49.0 <sup>b</sup>	115.6 <sup>a</sup>	97.2 <sup>a</sup>	132.6 <sup>a</sup>	77.6 <sup>a</sup>	0.03	0.99	0.69	3.76

\* S: level of sulfur in diet. D: day of fermentation in diet. A: P-value level of sulfur in diet. B: P-valueday of fermentation in diet. a, the gas production from the immediately soluble fraction; b, the gas production from the insoluble fraction; c, the gas production rate constant for the insoluble fraction ratio ; a+b, the gas potential extent of gas production. SEM, standard error of mean; ns, not significant.

**Table 3.** Effect of fermented total mixed ration on pH, ammonia-nitrogen and *in vitro* DM digestibility using *in vitro* gas production.

Item/Trt	0% S			1% S			2% S			P-value						
	21D	14D	7D	0D	21D	14D	7D	0D	21D	14D	7D	0D	A	B	A*B	SEM
pH	6.05 <sup>a</sup>	6.15 <sup>b</sup>	6.65 <sup>b</sup>	6.46 <sup>b</sup>	6.00 <sup>a</sup>	6.70 <sup>b</sup>	6.60 <sup>b</sup>	6.66 <sup>b</sup>	6.10 <sup>a</sup>	6.08 <sup>b</sup>	6.25 <sup>b</sup>	6.66 <sup>b</sup>	0.85	0.02	0.05	0.27
NH <sub>3</sub> -N, mg%	22.5 <sup>a</sup>	22.3 <sup>a</sup>	22.2 <sup>a</sup>	21.4 <sup>a</sup>	21.6 <sup>a</sup>	21.5 <sup>a</sup>	22.1 <sup>a</sup>	20.8 <sup>a</sup>	27.0 <sup>b</sup>	26.0 <sup>b</sup>	25.8 <sup>b</sup>	28.8 <sup>b</sup>	0.95	0.01	0.89	0.98
IVDMD, %	61.0 <sup>a</sup>	59.0 <sup>a</sup>	57.0 <sup>a</sup>	59.0 <sup>a</sup>	62.0 <sup>a</sup>	61.0 <sup>a</sup>	56.5 <sup>a</sup>	58.0 <sup>a</sup>	62.5 <sup>b</sup>	61.0 <sup>b</sup>	60.5 <sup>b</sup>	59.8 <sup>b</sup>	0.01	0.16	0.01	0.68
IVNDFD, %	35.7	32.5	24.1	29.1	35.1	32.0	34.0	25.5	43.1	39.4	34.7	29.0	0.16	0.33	0.51	1.77
IVADFD, %	22.3	21.7	27.3	21.7	17.0	22.0	20.3	22.5	15.9	18.0	23.7	18.7	0.16	0.41	0.65	1.41
Protozoa x 10 <sup>6</sup> cell/mL	6.5	4.3	5.3	9.5	6.5	8.0	4.0	8.0	8.0	7.8	4.0	4.0	0.96	0.08	0.20	1.21

\* S: level of sulfur in diet. D: day of fermentation in diet. A: P-value level of sulfur in diet. B: P-valueday of fermentation in diet. IVDMD, %: *in vitro* dry matter digestibility. IVNDFD, %: *in vitro* neutral detergent fiber digestibility. IVADFD, %: *in vitro* acid detergent fiber digestibility. SEM, standard error of mean; ns, not significant.

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## The Performance of Extension Agent in Improving Adoption The Technology Beef Cattle Feed

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### Abstract

This study aims to determine the performance of extension agent in improving the adoption of beef cattle feed technology. The research was conducted in Bulukumba District, South Sulawesi Province. The sample of extension agent as respondents from the extension population was determined randomly in each village, with 64 respondents. Primary data were obtained through interviews using questionnaires and focus group discussions. Indicators to measure extension performance refers based on responsiveness, responsiveness, and service quality. The results showed that the extension materials provided by extension workers, most of the extension workers provided material related to artificial reproduction or insemination (75.5%), followed by materials of feed technology (53.4%), and others. Thus, it can be concluded that the performance of extension workers in general has been running well, but need to improve the performance of extension workers in the adoption of beef cattle feed technology, which has an impact on increasing production, income and welfare of farmers.

**Keywords:** performance extension, adoption, feed technology, beef cattle

### Introduction

Extension agent is a learning process for the main actors and business actors so they are willing and able to help and organize themselves in accessing market information, technology, capital and other resources, in an effort to increase productivity, business efficiency, income, and welfare, and increase awareness in preservation of environmental functions. The extension system is the whole set of skills development, knowledge, skills, and attitude of the main actors and business actors through counseling. Law of the Republic of Indonesia Number 18 Year 2009 states Extension of animal husbandry and animal health is one of the efforts of farmers empowerment which aims to increase knowledge, skills, and change their attitude and behavior, implemented through non formal education). The extension of revitalization policy is considered important because the extension agents is the spearhead of agricultural development. Harianto, et al., (2014) states that extension agents play an important role in the development of livestock in a region, because it is an agent of change and as a technical implementer in the community. Extension agent are required to always



access new information as best as possible in order to develop cattle, be it information of a farming technology, capital and marketing access. The most important measure of livestock development is the new information that farmers get, the knowledge of farmers in applying a technology and the number of visits given by extension agents to the farmers. Performance is an implementation of the plan that has been prepared. Implementation of performance is done by human resources who have the ability, competence, motivation and interests. How organizations value and treat their human resources will influence their attitudes and behaviors in performance. Meanwhile, according to Bernardin & Russel in (Mardikanto, 2008) defines performance as an outcome record generated from the function of certain extension workers.

The contribution of the organization's members to its organization can be measured by performance appraisal. The lack of achievement of the target of agricultural human resource improvement through counseling is caused by the method / media of counseling less appropriate with the socio-economic condition of farmers and the material submitted is not in accordance with the needs of farmers (Subarna, et al., 2006). Such discrepancies are caused by problems: (a) the interaction between extension workers and farmers is less intensive; (b) lack of mastery of material from extension workers; and (c) low extension sensitivity to problems that occur in farmers (responsivness). Extension work is greatly influenced by various factors. Generally the performance of agricultural extension workers is influenced by the individual variables of extension workers, psychologists and organizations that extension workers perform extension tasks. Individual variables can be classified in capabilities and skills, personal and demographic backgrounds. Furthermore, psychological variables can be formulated in perceptual, attitude, personality, learning and motivation, while organizational variables can be divided into resource variables, leadership, rewards, structure and job design (Sapar, et al., 2012). Therefore, it is necessary to increase the adoption of beef cattle feed technology with improvement and improvement in extension performance in conducting extension program in farmers. The problem of lack of technology adoption of beef cattle feed on farmers is caused by the extension method is less appropriate with the socio-economic condition of farmers and the material submitted is not in accordance with the needs of farmers (Subarna, et al., 2006). Such discrepancies are caused by problems: (a) the interaction between extension workers and farmers is less intensive; (b) lack of mastery of material from extension workers; and (c) low extension sensitivity to problems that occur in farmers (responsivness). Therefore, this study aims to determine the performance of extension workers in increasing adoption of cattle feed technology of beef cattle.

## Materials and Methods

The research was conducted in Bulukumba Regency South Sulawesi Province. The sample of extension workers as respondents from the extension population was determined randomly in each village. The number of samples which after calculated using the Slovin formula (Umar, 1997), obtained by extension respondents as respondents as much as 64 counselors. Data collected from this research are primary data obtained by using questionnaire and through focus group discussion. The indicators used to measure extension performance refers to the indicators of public organization performance proposed by Bestina et al. (2005), namely responsiveness, responsiveness, and service quality. Responsiveness indicator is identification of infrastructure requirement in feed processing technology; guidance, coaching, mentoring feed processing technology. Responsibility includes encouraging, motivating, inviting breeders to carry out feed processing; extension activities benefit



the farmers. Quality of service is the satisfaction of extension services, and the satisfaction of communication (talking, hanging out, discussion) conducted by extension workers. Measurement of each item of the question is scored with the lowest level gets a score of one and the highest level gets a score of four. Data analysis is done through descriptive approach including frequency and percentage.

## Results and Discussion

Bulukumba Regency is one of the regencies located in the South of Sulawesi and is approximately 153 km from the capital of South Sulawesi Province, famous for its phinisi boat industry which provides economic added value for people geographically located at coordinates 05°20' - 05° 40' South Latitude and 119° 58' to 120° 28' East Longitude. Boundaries with the North is Sinjai Regency, East with Bone regency, South by Flores Sea and West side with Bantaeng Regency. Bulukumba Regency is high in livestock density of 66.33 e / km<sup>2</sup> and livestock ownership is 0.82 head per household. The description of the profile of respondent's extension based on the survey results can be seen the general condition of the respondent's extension, that is age, education level and experience as presented in Table 1. Based on the research result, showed the characteristics of extension workers in Bulukumba Regency as shown in Table 1. Age of respondent's extension indicates that around more 70% extension workers with age between 31-50 years, 20.3% with age more 50 years, rest less than 30 years old. Age extension is very urgent in carrying out duties as extension workers. Thus, the age of extension counselor during the assessment is classified as a productive age. According to Palebangan, et al., (2006) states that based on the age of productive and non-productive age, the productive age ranges from 15 to 55 years, and at this age the extension's physical ability is very influential to work optimally.

The educational level of respondents is 93.7% who completed education in college. Extension educator level is an indicator of population quality and is a key variable in human resource development. Adequate education of counselors will make it easier to transfer farming innovations and technologies (Murwanto, 2008).

**Tabel 1.** Karakteristik Extension Agent Responden

No	Characteristik Extension Agent	The Number of (responden)	Persentase (%)
1	Age Extension Agent		
	<30 year	8	12,5
	31-40 year	21	32,8
	41-50 year	22	34,4
	>50 year	13	20,3
2	Education		
	Graduate High School	4	6,25
	Graduate College	60	93,7
7	Experience		
	<5 year	24	37,5



>5-10 year	18	28,1
>10-15 year	7	10,9
>15 year	15	23,4

Extension methods and techniques are a collection of various ways in which the extension process can be applied so that the counseling becomes more effective and efficient. The choice of method is not always the same according to time and place, but depends on the problem, the situation and the conditions. A particular method will be more effective when it suits the existing problem, but on the contrary even using sophisticated methods does not mean anything if it is less relevant to the existing context. In the implementation of counseling provide material related to beef cattle feed is still very less that is only 53.4% compared to artificial insemination technology (75.5%) and others. Artificial insemination technology is a lot of materials provided by extension workers, whereas the problem that is often much needed farmers is a feed problem. This is supported by the statement of Budiman (2001) states that the development of food has problems, among others: a) the feed raw material is not wholly fulfilled from local so that still rely on import, b) local feed raw material not yet optimally utilized, c) availability local food is not continuous and lack of quality, d) the use of legume crops as a source of feed is not optimal, e) the utilization of sleep and land integration is still low, f) the application of feed technology is still low, g) national feed production is uncertain due to less data accuracy exact, and h) the research and its application are not aligned. Performance of agricultural extension (performance) is a response or individual behavior towards the success of work achieved by the individual in an organization in accordance with the duties and responsibilities given to him that carried out effectively and efficiently based on a certain period of time in order to achieve organizational goals (Bahua, et al., 2010).

Table 2 shows that extension performance is related to responsiveness, ie extension workers rarely carry out identification of infrastructure needs in feed processing technology. In terms of responsibility, the results showed that the extension has been able to encourage, motivate, invite the farmers to carry out the processing of feed for the development of beef cattle. In addition, 51.6% of farmers expressed satisfaction with counseling guidance services, coaching, mentoring conducted by extension workers, and satisfaction of communication (talking, socializing, discussing) conducted by extension workers.

Hartati, et al (2011) stated that the performance of extension workers can be seen from their efforts to develop themselves, that is capable of mastering, material, technique, and extension methods that will be submitted to farmers based on philosophy, principles and ethics extension.

**Tabel 2.** The performance of the counselors in improving technology adoption beef cattle feed

No	Description	Criteria	Number of Responden	Persentase (%)	
1	Responsivitas Counselors undertook the identification of needs of infrastructures in technology feed	Never	9	14,1	
		Rarely	29	45,3	
		Often	26	40,6	
		always	0	0,0	
	Counselors undertook the identification of needs of infrastructures in technology of the an agricultural waste to feed	Never	26	40,6	
		Rarely	16	25,0	
		Often	19	29,7	
	Guidance counselors do / guidance and assistance processing technologies feed	always	3	4,7	
		Never	11	17,2	
		Rarely	16	25,0	
	Counselors do guidance / guidance and assistance process technology an agricultural waste to feed	Often	31	48,4	
		always	5	7,8	
		Never	16	25,0	
		Rarely	22	34,4	
	2	Responsibilitas Counselors unable to give spirit , motivation , call farmers to implement processing feed	Often	26	40,6
always			0	0,0	
Never			16	25,0	
Rarely			22	34,4	
Counselors unable to give spirit , motivation , call farmers to implement processing an agricultural waste to feed		Often	26	40,6	
		always	0	0,0	
		Never	16	25,0	
		Rarely	22	34,4	
3		Quality service Our satisfaction towards the services outreach guidance, coaching, mentoring done by extension officers	Dissatisfied	13	20,3
			not satisfied	8	12,5
			satisfied	33	51,6
			very satisfied	10	15,6
		Dissatisfied not satisfied satisfied very satisfied	very satisfied	10	15,6
			Dissatisfied	12	18,8
			not satisfied	7	10,9
	satisfied		33	51,6	
	Our satisfaction against communication (talking, hang out, discuss) conducted by the extension officers to breeders	very satisfied	12	18,8	
		Dissatisfied	12	18,8	
		not satisfied	7	10,9	



## Conclusion and recommendations

In providing extension materials, most extension agent provided less than 53.4% of feeding cattle feed material, compared to material related to artificial insemination of 75.5%, and other materials. In general the performance of extension agent in identifying the needs of infrastructure facilities in beef cattle feed technology to improve the adoption of feed technology on beef cattle ranchers is still less than optimal.

It is suggested that in order to increase the adoption of beef cattle feed technology, it is necessary to improve and improve the extension worker performance in increasing adoption of beef cattle feed technology so that the productivity of beef cattle business can increase.

## Acknowledgements

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## Session 4-Arawan I

ANN-01-0012

### **Use of *Bacillus subtilis* to Produce Feather Meal for Animal Feeds and Organic Fertilizers**

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#### **Abstract**

Steam pressure cooked methods have been used to produce feather meals, however, the meals produced under these hard conditions showed low agricultural values, especially pepsin digestibility. The quality of fermented feather meal (FFM) produced in the laboratory by *Bacillus subtilis* 531-7 (BCRC 910793) was better than the quality of commercial hydrolyzed feather meals (HFMs). FFM was produced from HFM by fermentation with *Bacillus subtilis* 531-7 for 24 h, 48 h or 72 h. The pepsin digestibility of FFMs were in the range from 62.8 to 86.4 %, whereas the pepsin digestibility of HFMs were in the range from 23.5 to 80.7 %. Crude protein of hydrolyzed feather meal (HFM) was also improved when fermented with *Bacillus subtilis* 531-7. The crude protein of FFMs were in the range from 84.3 to 97.5 %, whereas the crude protein of HFMs were in the range from 84.5 to 93.3 %. From our survey, it indicated that fermentation was a good method to improve the quality of HFM, especially when hydrolyzed FM of the low pepsin digestibility was used.

**Keywords:** *Bacillus subtilis* 531-7 (BCRC 910793), hydrolyzed feather meals (HFMs), fermented feather meal (FFM), animal feed, organic fertilizer

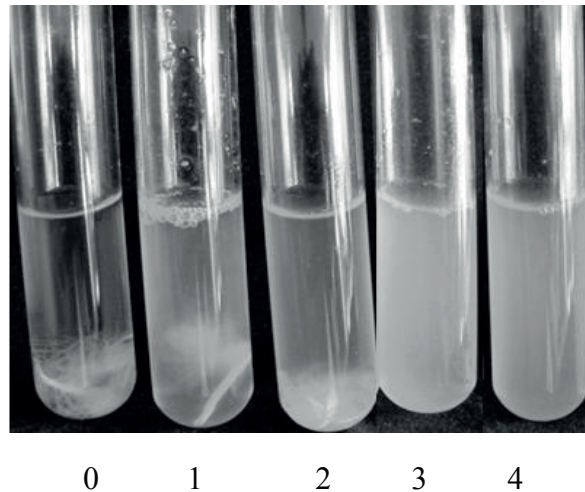
#### **Introduction**

Feather is a waste from the poultry processing industry, and it contains over 90 % crude protein, and about 15 % N. Therefore, degradation of feather to feather meal is important for agricultural applications, such as additives for animal feed and organic fertilizer (Choi, et al., 1991; Hadas and Kautsky, 1994).

Steam pressure cooked methods have been used to produce feather meals, however, the meals produced under these hard conditions showed low agricultural values, especially pepsin digestibility. The pepsin digestibility was highly correlated with the degree of reduction of disulfide bonds (Zhang et al., 2014), which in turns correlated with steam pressure and temperature (Blasi et al., 1991). Fermentation of chicken feather waste by microorganisms is a good alternative (Grazziotin et al., 2006). Several factors contribute to improve the quality of feather meal. One is the composition of the raw material used to make hydrolyzed FM (HFM), the second factor is the selection of the processing conditions for HFM, and the third factor is the selection of appropriate hydrolyzed FM for making fermented FM.

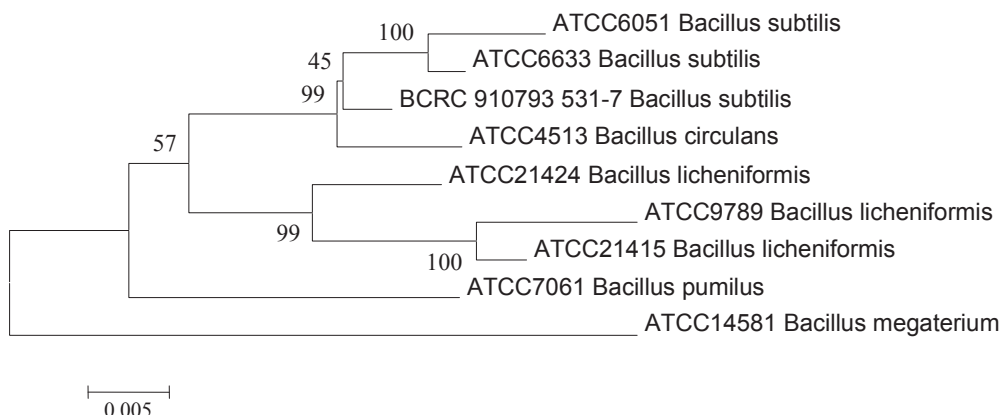


Thus, screening program to isolate micro-organism exhibiting feather degradation ability has been conducted in this laboratory since 2013, and one isolate collected from compost degraded whole chicken feather completely within 4-6 days was obtained (Figure 1).

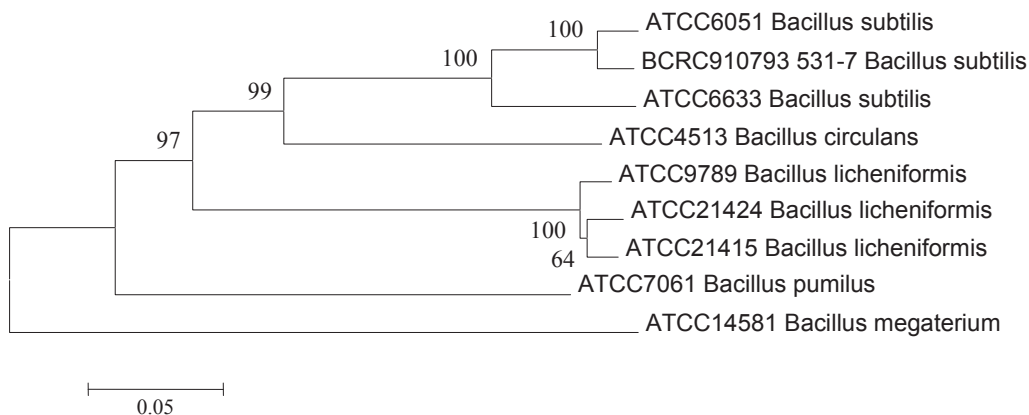


**Figure 1.** Whole chicken feather degradation. 0: Control (no degradation); 1: Partially degraded, except rachis; 2: Partially degraded and rachis started to degrade; 3: Rachis degraded further; 4: Rachis degraded completely.

The DNA data and the phylogenetic tree of this bacterium indicated it is *Bacillus subtilis*, and named as *Bacillus subtilis* 531-7 (BCRC 910793) (Figures 2 and 3).



**Figure 2.** Neighbor-joining (NJ) phylogenetic tree of *Bacillus subtilis* 531-7 with other *Bacillus* strains based on 16S rDNA partial gene sequence



**Figure 3** Neighbor-joining (NJ) phylogenetic tree of *Bacillus subtilis* 531-7 with other *Bacillus* strains based on *gyrB* gene partial gene sequence.

## Materials and Methods

### Production of hydrolyzed feather meal (HFM) :

Wet chicken feathers were collected for screw-extrusion-dehydration, followed by pyrohydrolysis, drying, cooling, filtering, then grinding to powder (HFM).

### Production of fermented feather meal (FFM)

Hydrolyzed feather meal (HFM) was fermented with bacteria for 24 h, 48 h or 72 h, then filtering, drying, grinding to powder (FFM).

Qualities of hydrolyzed feather meals (HFMs) and fermented feather meals (FFMs) were compared with two factors: pepsin digestibility, and crude protein. The percentage of crude protein, and the percentage of pepsin digestibility using a 0.2 % pepsin solution were determined by AOAC method (AOAC International, 1997). Bacteria which have feather digesting enzyme will convert the protein in feather into a digestible form.

## Results and discussion

Pepsin digestibility of hydrolyzed feather meal (HFM) were improved when hydrolyzed feather meal (HFM) was fermented with *Bacillus subtilis* 531-7 (Table 1). Normally a minimum pepsin digestibility of 70 % or 75 % is considered to be an acceptable value to assure that the feather meal will not result in animal illnesses. For examples, the pepsin digestibility of sample 1 (HFM) and 2 (HFM) were increased from 23.5 % to 62.8 %, and from 29.2 % to 67.4 %, respectively, when product of HFM was fermented with *Bacillus subtilis* 531-7 for 24 h (D1, Table 1). The pepsin digestibility of sample 1 product (HFM) could be further increased to 83.4 % (FFM) when fermented time was set at 72 h (D3, Table 1). For sample 4 HFM product, the pepsin digestibility was increased from 78.9 % to 84.9 % when fermented time was 72 h (D3, Table 1). The data indicated that the pepsin digestibility could be increased significantly when the low quality of HFM products were fermented.

**Table 1.** Pepsin digestibility of hydrolyzed feather meal (HFM) and fermented feather meal (FFM)

HFM Commercial product	HFM	FFM	FFM/HFM	FFM-HFM	(FFM-HFM)/HFM
1	23.5	62.8 (D1)	2.7	39.3	167
		83.4 (D3)			
2	29.2	67.4(D1)	2.3	38.2	131
3	92.8	96.0(D1)	1.0	3.2	3.4
4	78.9	70.9 (D1)	0.9	-8	-10.1
		84.9 (D3)			

D1:24 h fermentation; D3:72 h fermentation

Crude protein of hydrolyzed feather meal (HFM) was also improved when fermented hydrolyzed feather meal (HFM) with *Bacillus subtilis* 531-7 (Table 2). Normally a minimum 80 % is considered to be an acceptable value for the feather meal. For example, the crude protein of sample 1 (HFM) was increased from 82.3 % (HFM) to 96.1 % (FFM) when fermented time was 24 h (D1, Table 2). The crude protein of sample 1 (HFM) could be further increased from to 96.1 % (FFM) when fermented time was 72 h (D3, Table 2).

**Table 2.** Crude protein of hydrolyzed feather meal (HFM) and fermented feather meal (FFM)

HFM Commercial product	HFM	FFM	FFM/HFM	FFM-HFM	(FFM-HFM)/HFM
1	82.3	96.1 (D1)	1.2	13.8	16.8
		97.5 (D3)			
2	86.8	96.0(D1)	1.1	9.2	10.6
3	93.3	90.0(D1)	0.96	-3.3	-3.5
4	84.5	84.3 (D1)	0.99	-0.2	-0.002
		93.5 (D3)			

Particle size of HFM might have some effect on the pepsin digestibility. For example, the pepsin digestibility of sample 3 (HFM) was 92.8 % (Table 1), and the major distribution of particle size was less than 0.15 mm in a ratio of 50.7 % (Table 3). The pepsin digestibility of sample 1 (HFM) and 2 (HFM) were 23.5 % and 29.2 %, respectively (Table 1), and the major distribution particle size for these two samples were larger than 0.42 mm in a ratio of 76.3 % and 80.7 %, respectively (Table 3). These data suggested that particle size might be important for pepsin digestibility, therefore, the major particle size of FFMs were controlled less than 0.45 mm (Table 3).

**Table 3.** Particle size of hydrolyzed feather meal (HFM) and fermented feather meal (FFM)

Commercial product	HFM			FFM		
	>0.42 mm	0.42~0.15 mm	<0.15 mm	>0.42 mm	0.42~0.15 mm	<0.15 mm
1	76.3	23.7	0	33.2	40.5	26.3
2	80.7	19.3	0	41.6	40.8	17.6
3	16	33.3	50.7	33.8	52.7	13.5
4	16.0	39.9	44.1	42.9	36.5	20.6

## Conclusion

The effect of increasing pepsin digestibility was significant when *Bacillus subtilis* 531-7(BCRC 910793) was fermented with HFM sample of low pepsin digestibility. However, the effect of increasing was limited when *Bacillus subtilis* 531-7(BCRC 910793) was used to ferment with sample of high pepsin digestibility. From this study, it indicated that fermentation of HFM with feather degrading bacteria was a good method for improving or hydrolyzed FM of low pepsin digestibility.

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## Assessment of Metabolizable Energy, Nutrients Digestibility and Fatty Acids Composition of Fat Crystals Derived From Crude Palm Oil in Chickens

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### Abstract

Present study was planned and carried out to assess the metabolizable energy, nutrients digestibility and fatty acids composition of fat crystals derived from crude palm oil in chickens. A total of twenty adult male chickens having 10 weeks age and good health condition were focused for current study in order to determine the metabolizable energy, nutrient digestibility and fatty acids composition fat crystals were derived from palm oil. Our results revealed that the percentage of dry matter, crude protein, ether extract and crude fiber was found at 95.3, 5.4, 94.0 and 0.4% respectively; while, protein and fat digestibility of fat crystals derived from palm oil (FCPO) was 82.14 and 71.04%. Gross energy (GE), true metabolizable energy (TME) and apparent metabolizable energy (AME) were found at 9207, 6591 and 6401 kcal/kg respectively. Gas chromatography–mass spectrometry (GC-MS) results indicated that, in FCPO the major saturated fatty acids (total 49.36%) was palmitic acid (43.43%), whereas; among total unsaturated fatty acids (50.65%); 40.66% were monounsaturated fatty acids and 9.99% were polyunsaturated fatty acids (PUFA). On the basis of our findings it is concluded that FCPO bears an excellent capability for used as an alternative and admirable fat source for the chickens.

**Keywords:** chicken, fat crystals derived from crude palm oil, metabolizable energy, nutrients digestibility, fatty acids

### Introduction

Statistic data of Indonesian goat population in 2017 (18.6 million heads) shows that there is increasing population during last five years. Milk and meat now is getting more popular and goes to the big market, especially for sate and yoghurt. High milk yield with high price and high litter size give an opportunity to the farmers to increase their income. On the other hand, the performance of kids are decreasing and mortality increase due to the lack of nutrient requirements. The kids get their milk only a week after parturition and after that all the milk goes to the market. Another problem is if ewes with high litter size, more than three kids, will cause the kids get worst condition. Milk replacer is one of the alternative solution to solve the kids performance before weaning. Utilization



of local feedstuff for milk replacer can help to solve the problem. Insect such cricket is one of protein sources which can be used for substitute casein or soy bean meal. Insect is one of potential commodity as alternative a new protein source for animal feed stuff (Sánchez-Muros et al., 2014). Crickets meal contains 57.07% of crude protein, 10.28% of crude fiber, and 13.13% of fat (Astuti et al., 2016). Cricket also has contain various of amino acid 6.75% valine, 5.1% lysine, 4.46 isoleucine, 11.62% alanine, 7.22% arginine, and 6.77% glycine and chitin 8.7% (Wang, 2005). Meanwhile the fatty acids contain are 50.32% palmitic acid (16:0), 32.06% stearic acid (18:0), 9.77% oleic acid and 2.34% linoleic acid (Chakravorty et al., 2014). Commonly crickets used as feed for fish, birds, and poultry, while for ruminant feed is very rare (Makkar, 2014). Moreki et al. (2012) reported the utilization of cricket meal as protein source to substitute of soybean meal in chicken ration could increase feed conversion ratio. The result from the previous study by using raw material cricket meal for creep feed ration in sheep showed that there were no significance difference performance with the control ration, it means that cricket meal could be used safely (Astuti, et al., 2016). Based on the situation, this study was aimed to evaluate the utilization of cricket meal in milk replacer on performance and physiological status of goat kids.

## Material and Methods

Twelve crossbred Sapera male (saanen x etawah) local pre-weaning goat kids, one week old were used in this experiment and divided into three treatments by using completely randomised block design. The initial average body weight were 3.78 kg. The treatments were control, where the kids were given goat milk (GM), kids were given cow milk (CM) and kids were given milk replacer (MR), four times a day (morning, at noon, afternoon and night). Parameters measured were nutrients intake, ADG, respiration rates, heart rates and rectal temperature, and evaluated during eight weeks.

Table 1. Nutrient composition of goat milk and milk replacer

Nutrients (%)	Treatments			Commercial MR	GM = goat mil k, CM = cow
	GM	CM	MR	%	
Protein	31.54	25.72	21.97	18-22	
Fat	50.00	15.59	25.72	10-20	
Lactosa	32.31	35.07	-	-	
Calcium	0.92	1.25	1.62	1.00	
Phospor	1.85	1.64	0.87	0.70	

milk, MR = milk replacer.

The ingredients for making milk replacer were egg yolk powder, skim powder, full cream, wheat meal, fish oil, premix, and CaCO<sub>3</sub>. Total dry matter offered of milk replacer was 3.5% of BW and then diluted in warm water (37°C) with ratio 1:4.

## Result and Discussion

The average environment temperature during morning and afternoon were lower than at noon, meanwhile for the humidity in the morning and afternoon higher than at noon (Table 2). Yousef (1985) reported that the thermoneutral zone for tropical goat is around 18-30°C with





humidity is around 55% relative. The ideal temperature for dairy goat is around 21 °C (Ensminger, 2002). The temperature during this research was around 26.50 - 29.40 °C with the humidity from 58 – 64% relative, so that the condition was very confinient for the animals. It was the comfort zone for the animals. On the other hand, high temperature and humidity can caused animal heat stress so that the consumption will decrease.

Table 2 . Environment condition during research

Week	Morning		Noon		Afternoon	
	Temp. (°C)	Humid. (%)	Temp. (°C)	Humid. (%)	Temp. (°C)	Humid. (%)
1	26.4	64	29.1	56	28.0	70
2	26.9	56	30.8	59	28.4	60
3	26.7	71	29.3	68	25.5	58
4	27.8	65	28.5	60	26.0	50
5	26.0	57	30.2	46	28.5	70
6	26.5	61	28.8	59	27.0	68
7	25.5	69	30.4	53	28.0	71
8	26.3	65	28.2	67	26.7	68
Average	26.5±0.67	63.5±5.29	29.4±0.95	58.5±7.15	27.3±1.13	64.4±7.56

Data table 3. showed that DM, protein, fat and calsium intake of cricket meal MR treatment was significance higher than another two treatments (P<0.05), meanwhile, phospor intake of all treatments showed the same. Main factor which affect to the consumption are palatabilty, milk quality, temperature of environment, age, physiological status and body weight ( Mc. Donald et al., 2011).

Table 3. Nutrient intakes of goat kids during 8 weeks evaluation

Nutrients (g/h/d)	Treatments		
	GM	CM	MR
Dry matter	98.20±7.79 <sup>c</sup>	120.05±5.24 <sup>b</sup>	203.87±3.07 <sup>a</sup>
Protein	30.97±2.46 <sup>b</sup>	30.88±1.35 <sup>b</sup>	43.97±0.66 <sup>a</sup>
Fat	31.73±2.52 <sup>b</sup>	18.72±0.82 <sup>c</sup>	51.40±0.77 <sup>a</sup>
Calcium	0.90±0.07 <sup>c</sup>	1.50±0.07 <sup>b</sup>	3.30±0.05 <sup>a</sup>
Phospor	1.82±0.14	1.97±0.09	1.77±0.03

GM = goat milk, CM = cow milk, MR = milk replacer. Different font at the same colom is significance difference (P<0.05)

The average daily gain (ADG) and final body weight of kids with goat milk and milk replacer treatments were same and higher than cow milk treatment (Table 4), although the dry matter intake in milk replacer treatment was higher than in goat milk treatment (P<0.05). This condition was suggested by the differences of availability (digestibility and absorption) of nutrients of both treatments, where the goat milk is better than milk replacer. The enzyme activities and mothering immunity compound in goat milk is significantly affected to the growth rates of the kids. This condition was not happen in cow milk treatment, where the performance profil was the lowest compared to others.

Table 4. Performance pre-weaning goat kids during 8 weeks



Performance	Treatments		
	GM	CM	MR
Initial BW (kg)	3.75 ± 0.34	3.70 ± 0.32	3.88 ± 0.33
Final BW (kg)	9.65 ± 0.54a	8.53 ± 0.51b	9.23 ± 0.53a
ADG (g/h/d)	120.41 ± 10a	98.47 ± 20.96b	109.18 ± 12.37a

GM = goat milk, CM = cow milk, MR = milk replacer. Different font in the same colom is significance difference P<0.05.

The singlet kid usually has higher gain than duplet or triplet litter size. In this study all kids were produced from singlet and duplet litter size. The total milk from the ewes for the duplet kids should be divided for both kids, so that it affected to the performance. The goat milk has mothering immunity for the kids compare to the artificial milk replacer without mothering immunity compound (Fig. 1). The high final body weight of goat milk treatment was supported by efficacy of nutrient uptakes and its mothering immunity, meanwhile the performance of kids treated by milk replacer containing cricket meal was due to the high nutrients (protein, fat and calcium) intake only.

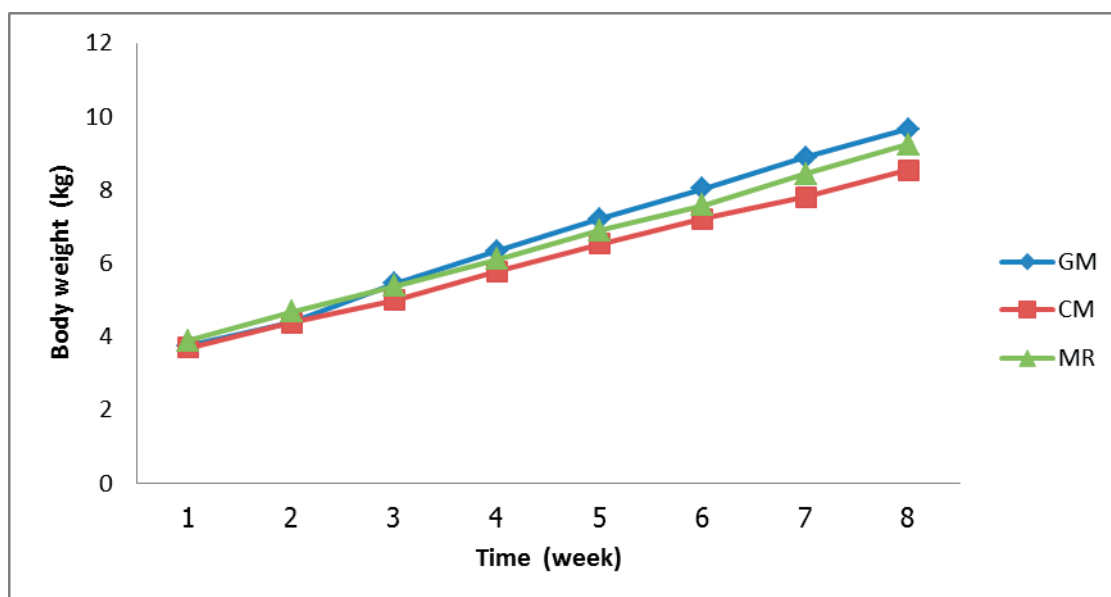


Figure 1. The growth rate of the kids until weaning weight.

Table 5. Physiological status of goat kids during 8 weeks pre-weaning

Parameters	Treatments	Times		
		Morning	Noon	Afternoon
Rectal temp. (°C)	GM	38.87 ± 0.26	39.4 ± 0.24	39.36 ± 0.33
	CM	38.90 ± 0.28	39.45 ± 0.16	39.19 ± 0.28
	MR	38.84 ± 0.26	39.55 ± 0.14	39.13 ± 0.33
	Average	38.87 ± 0.27	39.47 ± 0.18	39.23 ± 0.31



Heart rates (x/min)	GM	82.53±4.66	111.21±7.37	87.82±5.98
	CM	84.86±6.63	92.09±9.27	92.09±9.27
	MR	95.88±14.41	112.96±9.42	87.64±11.39
	Average	87.76±8.57	105.42±8.64	89.18±8.88
Respiration (x/min)	GM	32.50±4.44	42.25±5.00	34.61±4.62
	CM	37.43±5.59	40.36±5.81	42.81±4.45
	MR	40.41±6.62	41.36±5.90	40.14±6.54
	Average	36.78±5.55	41.32±5.57	39.19±5.20

GM = goat milk, CM = cow milk, MR = milk replacer

The physiological status of animal such as rectal temperature, hearth rates and respiration rates were non significance among the treatments and in normal condition (Table 5). Frandson, et al. (2009) reported that rectal temperature, respiration rates and heart rates were 38.5 - 40 °C, 26 - 54 x/min and 70 - 135 x/min, respectively. The respiration rates are affected by body temperature and environment condition, body condition and intakes. This research showed that milk replacer containing cricket meal resulted good response to the physiological status of the kids, without any negative effect.

## Conclusion

It was concluded that cricket meal could be used in goat kids milk replacer with good palatability and resulted ADG around 109 g/h/d, without any negative effect to the health status of the animal.

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## Effect of Dietary Supplementation of Cinnamon and Curcumin on Performance, Humoral Immune Responses, and Blood Lipid Profile in Rabbits

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### Abstract

A total of seventy-two V-line rabbits of both sexes, 5 weeks old, with initial weights of  $639.4 \pm 25.97$  g were used for the study. Rabbits were randomly allocated to 6 treatment groups, each of which included 4 replicates of 3 rabbits. The experiment lasted for 6 weeks to be finished at 11 weeks of age and dietary treatments were as follows: 1) Control (basal diet); 2) basal diet+ 100 mg/kg antibiotic tylosin; 3) basal diet+ 0.5 g /kg turmeric (as a source of curcumin); 4) basal diet+ 1.0 g/kg turmeric; 5) basal diet+ 0.5 g/kg cinnamon; 6) basal diet+ 1.0 g /kg cinnamon. The results showed that the highest body weight and weight gain values and the best feed conversion ratio were recorded in the groups given 1.0 g /kg diet turmeric or cinnamon. The highest feed intake values were observed in the control and Tyrosin fed groups, while the lowest feed intake value was recorded in cinnamon fed groups. Tylosin, cinnamon and turmeric supplementation significantly ( $P \leq 0.05$ ) improved digestibility of DM, OM, CP, CF, EE and NFE and also, improved the nutritive values of nutrients as TDN and DCP in comparison with control. Serum lipid profile improved by phytogetic feed additives compared with tylosin and control groups. Phytogetic feed additives reduced Malondialdehyde ( $P \leq 0.05$ ), while, increased total antioxidant capacity concentrations and glutathione peroxidase activities. Immunostimulatory effects of phytogetic feed additives were recorded. In conclusion, we state that cinnamon and turmeric could have beneficial effects on performance, digestibility, and immunomodulatory and play an important role as an exogenous antioxidant.

**Keywords:** rabbits, cinnamon, turmeric, antibiotic, performance, immunity, blood lipid profile

### Introduction

Turmeric is a yellow powder driven from the rhizome of “*Curcuma longa*” with extensive use as spices particularly in south and south-east Asia and Middle East countries. Curcumin, the yellow pigment of turmeric, the main active component is turmeric (Jagetia and Aggarwal, 2007). Antioxidative properties have been reported for turmeric and curcumin in several studies (Wei and Shibamoto, 2007). Also, it has been shown that turmeric and curcumin possess hypolipidemic effects (Babu and Srinivasan, 1997). Anti-inflammatory and immune system modulating effects of turmeric (South et al., 1997). Cinnam-aldehyde is the major component of cinnamon, creating about 65 percent of the extracted essential oil (Mountzouris et al., 2009). Immune system stimulating



effects has been reported for cinnamaldehyde (Nofrarias et al., 2006). Also, considerable antibacterial (Chang et al., 2001) and antifungal (Soliman and Badeaa, 2002) properties have been found for cinnamon essential oil. The aim of the present study is to determine the effects of cinnamon and turmeric powder as phyto-genic feed additives instead of antibiotic on growth performance, digestibility, immune response, blood serum lipid profiles and antioxidant status in growing rabbits through Egyptian summer season

## Materials and methods

A total of seventy-two V-line rabbits of mix male and female, 5 weeks old, with initial weights of  $639.4 \pm 7.15$  g were used for the study. Rabbits were randomly allocated to 6 treatment groups, each of which included 4 replicates of 3 rabbits. The experiment lasted for 6 weeks to be finished at 11 weeks of age and dietary treatments were as follows: 1) Control (basal diet); 2) basal diet+ antibiotic (Tylosin 100 mg/kg); 3) basal diet+ 0.5 g /kg cinnamon; 4) basal diet+ 1.0 g/kg cinnamon; 5) basal diet+ 0.5 g/kg turmeric (as a source of curcumin); 6) basal diet+ 1.0 g /kg turmeric. The experimental diets were offered to rabbits *ad libitum*. Rabbits fed diet containing 17 % crude protein, 12.5 % crude fiber, 3.2 % fat and 2750 digestible energy. 6 ml of blood sample was taken from the ear vein with a sterile syringe. The blood sample was put into a sterile vacutainer tube without an anticoagulant for serum biochemical analysis. Blood serum metabolites were estimated using commercial kits (Bio Merieux, France) according to the procedure outlined by the manufacturer. Serum immunoglobulin IgG was determined using ELISA technique. Three rabbits of each treatment were immunized with 0.1 ml of a 2.5% Sheep Red Blood Cells (SRBCs) via the marginal ear vein at 15 days after starting the dietary treatment supplementation, to measure antibody titer against sheep red blood cells. The dosage of SRBC for inoculation was pre-determined by a separate trial. Antiserum to SRBCs was collected 14 days post challenge according to Wegmann and Smithies, (1966). The agglutination titer was expressed as the log<sub>2</sub> of the reciprocal of the highest serum dilution giving complete agglutination (Nelson et al., 1995). All data were analyzed using one way analysis of variance (ANOVA) using SPSS 11.0 statistical software (SPSS, Inc., Chicago, IL, 2001). Significant differences between means were detected using new Duncan multiple range test (Duncan, 1955).

## Results and discussion

Results of the present study are illustrated in Table 1. The highest body weight and the best feed conversion ratio were recorded in the groups given 1.0 g /kg turmeric or cinnamon. Besides, the highest weight gain value was found in the group given 1.0 g cinnamon/ kg diet. Moreover, the highest feed intake values were observed in the control and tyrosin groups, while the lowest feed intake values was recorded in cinnamon fed group. In fact, mortality percent was not different among the groups that may be due to good hygienic condition of the farm and good sanitary status of the digestive system. The most efficient feed conversion ratio in rabbits fed diets supplemented with antibiotic, turmeric and cinnamon powder reveals that the impact of growth promoter substances may be related to a more efficient use of nutrients. Cinnamon and turmeric improved the performance that may be due to reduced levels of peroxidation (blood serum MDA), and or due to the active components that enhance digestion and absorption of dietary nutrients. The results presented by Hussein (2012) reported that *curcuminoids* and *curcumin* of turmeric increased utilization of feed, resulting in enhanced growth. Cinnamon has strong antibacterial properties, anticandidial, antiulcer, analgesic, antioxidant and hypocholesterolaemic activities (Mastura et al.,



1999). Thus, alike antibiotics, turmeric and cinnamon may control and limit the growth and colonization of numerous pathogenic and non-pathogenic species of bacteria in chickens' gut resulting in balanced gut microbial ecosystems that leads to better feed utilization reflected by improved feed conversion ratio. In general, antibiotic tylosin, cinnamon and turmeric supplementation significantly ( $P \leq 0.05$ ) improved digestibility of DM, OM, CP, CF, EE and NFE as compared with control group. There is evidence to suggest that herbs, spices, and various plant extracts have appetite- and digestion-stimulating properties and antimicrobial effects (Kamel, 2001). Total digestible nutrients (TDN) and digestible crude protein (DCP) were significantly ( $P \leq 0.05$ ) improved by the different feed additives in comparison with the control group. The different phytogetic feed additives significantly ( $P \leq 0.05$ ) increased antibody titers against SRBCs compared with control and tylosin fed groups after 14 days of vaccination. This may be attributed to that, the use of different phytogetic feed additives eliminate immune response against some diseases. Serum IgG was enhanced ( $P \leq 0.05$ ) in rabbits fed diets containing phytogetic additives and tylosin as compared with control. However, the effect of phytogetic additives was more pronounced than tylosin. It was observed that serum total lipids, triglycerides, total cholesterol and low density lipoprotein (LDL) significantly ( $P \leq 0.05$ ) decreased with different levels of cinnamon and turmeric as compared with the group received tylosin in their diet and control group. On the other hand, high density lipoprotein (HDL) concentration was significantly ( $P \leq 0.05$ ) increased in the groups fed cinnamon and turmeric as compared with tylosin and control fed groups.

The present results were in agreement with those of Ciftci et al. (2010) reported that total cholesterol of the serum ( $P \leq 0.01$ ), thigh and breast meat ( $P \leq 0.05$ ) were found to be lower in both cinnamon groups (500 and 1000 ppm of cinnamon oil). Also, Alagawany *et al.* (2016) found that triglycerides, total cholesterol and low density lipoprotein concentrations were linearly and quadratically decreased with increasing the dietary proportion of turmeric. Rabbits exposed to Egyptian high temperature conditions during summer season resulted in significant decrease ( $P \leq 0.05$ ) in serum total antioxidant capacity (TAC) and glutathione peroxidase (GP) and significant increase in malondialdehyde (MDA), however, feeding rabbit's phytogetic cinnamon and turmeric supplemented diet improved the TAC and antioxidant enzyme (GP) and reduced the MDA concentration in comparison with the control group. Antibiotic tylosin also significantly ( $P \leq 0.05$ ) improved TAC and GP in comparison with control, however, the phytogetic feed additives were more effective than antibiotic. Quiles et al. (2002) reported that supplementation with *Curcuma longa* reduces oxidative stress and attenuates the development of fatty streaks in rabbits fed a high cholesterol diet. Abdel-Daim and Abdou (2015) reported that pretreatment of Thallium acetate intoxicated rats with curcumin induced a significant decrease ( $P \leq 0.05$ ) in serum MDA level along with a significant increase ( $P \leq 0.05$ ) in TAC levels compared with the Thallium acetate group (induced liver injury). Ciftci et al. (2010) reported that cinnamon oil (1000 ppm) reduced MDA level ( $P \leq 0.05$ ) and increased GSH-Px and catalase (CAT) activities ( $P \leq 0.01$ ) in broiler chickens. These effects are due to the antioxidant property of cinnamon oil (Lin et al. 2003). The protective role of essential oils may result from its antioxidative defense mechanism through the induction of antioxidant enzyme activities (Hsu and Liu 2004). Choiem Hwang (2005) reported that the intake of medicinal plants in rats results in an increase in antioxidant enzyme activity and a decrease in MDA.

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**Table 1.** Effect of Tylosin, turmeric and cinnamon on growing V-line rabbits

istics	Control		Tylosin 100 mg/kg		Turmeric (g/kg)		Cinnamon (g/kg)	
			0.5	1.0	0.5	1.0	0.5	1.0
<b>performance:</b>								
Initial body weight, g	630.2±29.5	633.4±27.9	636.8±27.3	645.7±15.4	638.9±29.3	645.7±15.4	638.9±29.3	651.2±26.4
Final body weight, g	1887.9 <sup>d</sup> ±54.5	1969.7 <sup>c</sup> ±44.8	1999.9 <sup>b</sup> ±42.1	2135.2 <sup>a</sup> ±32.7	1993.9 <sup>b</sup> ±52.2	2135.2 <sup>a</sup> ±32.7	1993.9 <sup>b</sup> ±52.2	2154.6 <sup>a</sup> ±58.2
Total weight gain, g	1257.6 <sup>c</sup> ±21.4	1336.3 <sup>d</sup> ±22.7	1363.1 <sup>c</sup> ±19.7	1489.5 <sup>b</sup> ±20.7	1355.0 <sup>c</sup> ±22.4	1489.5 <sup>b</sup> ±20.7	1355.0 <sup>c</sup> ±22.4	1503.4 <sup>a</sup> ±24.7
Total feed intake, g	3559.5 <sup>a</sup> ±79.2	3521.7 <sup>a</sup> ±101.2	3474.6 <sup>b</sup> ±98.1	3447.8 <sup>c</sup> ±98.2	3381.4 <sup>d</sup> ±96.0	3447.8 <sup>c</sup> ±98.2	3381.4 <sup>d</sup> ±96.0	3388.0 <sup>d</sup> ±97.1
Feed conversion ratio	2.83 <sup>a</sup> ±0.03	2.63 <sup>bc</sup> ±0.10	2.54 <sup>c</sup> ±0.08	2.31 <sup>d</sup> ±0.09	2.49 <sup>c</sup> ±0.02	2.31 <sup>d</sup> ±0.09	2.49 <sup>c</sup> ±0.02	2.25 <sup>d</sup> ±0.04
No. of dead rabbits	2/12 (16.7%)	0/12 (0%)	1/12 (8.33%)	1/12 (8.33%)	0/12 (0%)	1/12 (8.33%)	0/12 (0%)	1/12 (8.33%)
<b>Digestibility of:</b>								
Dry matter, %	66.07 <sup>c</sup> ±1.09	71.66 <sup>b</sup> ±1.18	69.13 <sup>b</sup> ±1.23	73.05 <sup>a</sup> ±1.15	73.89 <sup>a</sup> ±1.12	73.05 <sup>a</sup> ±1.15	73.89 <sup>a</sup> ±1.12	74.27 <sup>a</sup> ±1.06
Organic matter, %	89.10 <sup>c</sup> ±1.67	91.52 <sup>a</sup> ±1.52	90.50 <sup>b</sup> ±1.54	92.75 <sup>a</sup> ±1.56	92.05 <sup>a</sup> ±1.59	92.75 <sup>a</sup> ±1.56	92.05 <sup>a</sup> ±1.59	92.90 <sup>a</sup> ±1.32
Crude protein, %	62.17 <sup>c</sup> ±1.16	65.94 <sup>b</sup> ±1.15	64.99 <sup>b</sup> ±1.18	70.97 <sup>a</sup> ±1.20	70.04 <sup>a</sup> ±1.19	70.97 <sup>a</sup> ±1.20	70.04 <sup>a</sup> ±1.19	72.54 <sup>a</sup> ±1.23
Crude fiber, %	42.84 <sup>c</sup> ±1.09	48.00 <sup>b</sup> ±1.04	45.42 <sup>b</sup> ±1.02	52.52 <sup>a</sup> ±1.13	53.03 <sup>a</sup> ±1.10	52.52 <sup>a</sup> ±1.13	53.03 <sup>a</sup> ±1.10	53.15 <sup>a</sup> ±1.07
Ether extract, %	46.19 <sup>c</sup> ±2.09	51.61 <sup>a</sup> ±2.20	48.83 <sup>b</sup> ±2.12	51.14 <sup>a</sup> ±2.21	50.56 <sup>a</sup> ±2.19	51.14 <sup>a</sup> ±2.21	50.56 <sup>a</sup> ±2.19	52.48 <sup>a</sup> ±2.23
Nitrogen free extract,%	55.60 <sup>b</sup> ±1.89	59.62 <sup>a</sup> ±1.73	58.40 <sup>a</sup> ±1.85	59.75 <sup>a</sup> ±1.46	59.15 <sup>a</sup> ±1.52	59.75 <sup>a</sup> ±1.46	59.15 <sup>a</sup> ±1.52	59.80 <sup>a</sup> ±1.48
<b>Nutritive values:</b>								
TDN, %								
DCP, %	55.71 <sup>c</sup> ±0.23	62.80 <sup>b</sup> ±0.21	59.24 <sup>b</sup> ±0.24	60.08 <sup>b</sup> ±0.28	61.39 <sup>b</sup> ±0.30	60.08 <sup>b</sup> ±0.28	61.39 <sup>b</sup> ±0.30	64.38 <sup>a</sup> ±0.19
DE (kcal/kg)	10.84 <sup>c</sup> ±0.07	11.49 <sup>b</sup> ±0.06	11.37 <sup>b</sup> ±0.12	12.41 <sup>a</sup> ±0.08	12.20 <sup>a</sup> ±0.13	12.41 <sup>a</sup> ±0.08	12.20 <sup>a</sup> ±0.13	12.67 <sup>a</sup> ±0.17
<b>Antibody titers and serum</b>	2467.95 <sup>d</sup> ±5.83	2782.04 <sup>b</sup> ±6.77	2624.33 <sup>c</sup> ±6.23	2661.54 <sup>c</sup> ±6.27	2719.57 <sup>b</sup> ±4.75	2661.54 <sup>c</sup> ±6.27	2719.57 <sup>b</sup> ±4.75	2852.03 <sup>c</sup> ±6.17
<b>IgG:</b>								
SRBCs								
Serum IgG	0.694 <sup>c</sup> ±0.01	0.724 <sup>c</sup> ±0.03	0.908 <sup>b</sup> ±0.02	0.953 <sup>a</sup> ±0.04	0.963 <sup>a</sup> ±0.02	0.953 <sup>a</sup> ±0.04	0.963 <sup>a</sup> ±0.02	0.968 <sup>a</sup> ±0.03
	209.6 <sup>c</sup> ±5.781	246.7 <sup>bc</sup> ±27.28	285.0 <sup>b</sup> ±10.41	298.3 <sup>ab</sup> ±12.02	340.0 <sup>a</sup> ±17.32	298.3 <sup>ab</sup> ±12.02	340.0 <sup>a</sup> ±17.32	341.3 <sup>a</sup> ±18.89
<b>Serum lipid profile:</b>								
Total lipids (mg/dL)	247.00 <sup>a</sup> ±10.11	229.00 <sup>b</sup> ±4.41	212.00 <sup>c</sup> ±7.62	204.00 <sup>c</sup> ±6.43	205.00 <sup>c</sup> ±6.84	204.00 <sup>c</sup> ±6.43	205.00 <sup>c</sup> ±6.84	201.00 <sup>c</sup> ±6.56
Triglycerides (mg/dL)	55.66 <sup>a</sup> ±1.15	54.02 <sup>a</sup> ±2.51	53.00 <sup>b</sup> ±1.00	52.33 <sup>b</sup> ±0.33	52.00 <sup>b</sup> ±0.58	52.33 <sup>b</sup> ±0.33	52.00 <sup>b</sup> ±0.58	52.00 <sup>b</sup> ±1.20
Cholesterol (mg/dL)	93.66 <sup>a</sup> ±0.66	94.12 <sup>a</sup> ±4.84	72.13 <sup>b</sup> ±1.15	71.00 <sup>b</sup> ±4.70	73.15 <sup>b</sup> ±4.81	71.00 <sup>b</sup> ±4.70	73.15 <sup>b</sup> ±4.81	76.00 <sup>b</sup> ±2.00
HDL (mg/dL)	33.66 <sup>b</sup> ±0.88	34.00 <sup>b</sup> ±0.58	45.33 <sup>a</sup> ±1.20	44.73 <sup>a</sup> ±0.33	42.23 <sup>a</sup> ±1.20	44.73 <sup>a</sup> ±0.33	42.23 <sup>a</sup> ±1.20	43.20 <sup>a</sup> ±0.33
LDL (mg/dL)	20.31 <sup>a</sup> ±7.35	20.87 <sup>a</sup> ±2.33	17.67 <sup>b</sup> ±7.62	18.98 <sup>b</sup> ±0.58	15.93 <sup>b</sup> ±2.67	18.98 <sup>b</sup> ±0.58	15.93 <sup>b</sup> ±2.67	14.16 <sup>b</sup> ±1.73
<b>Antioxidant status:</b>								
TAC (mM/L)								
MDA (nmol/mL)	1.60 <sup>c</sup> ±1.23	1.72 <sup>b</sup> ±1.57	2.21 <sup>a</sup> ±1.85	2.24 <sup>a</sup> ±2.56	2.24 <sup>a</sup> ±3.25	2.24 <sup>a</sup> ±2.56	2.24 <sup>a</sup> ±3.25	2.27 <sup>a</sup> ±2.28
GSP (nmol/mL)	20.11 <sup>a</sup> ±3.25	19.19 <sup>a</sup> ±6.18	17.34 <sup>b</sup> ±3.04	17.11 <sup>b</sup> ±5.47	16.84 <sup>b</sup> ±6.77	17.11 <sup>b</sup> ±5.47	16.84 <sup>b</sup> ±6.77	16.20 <sup>b</sup> ±8.22
	10.51 <sup>c</sup> ±2.58	11.92 <sup>b</sup> ±3.57	12.37 <sup>a</sup> ±4.12	12.44 <sup>a</sup> ±5.42	12.50 <sup>a</sup> ±4.36	12.44 <sup>a</sup> ±5.42	12.50 <sup>a</sup> ±4.36	12.91 <sup>a</sup> ±1.95

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## Study on The Growth Performance, Meat Quality and Bone Breaking Strength of Broilers fed Dietary Rice Hull Silicon

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### Abstract

Bone problems have been accepted as a main issue perilously affecting the broilers' health and welfare that result severe economic loss. Current study was planned to investigate the influence of dietary rice hull silicon (RHS) on the performance, meat quality and bone breaking strength of broilers. A total of one hundred 10-days old Arbor Acre chicks were used for the study. Birds were divided into five groups. One group was kept as control, while other groups were provided 2.5, 5.0, 7.5 and 10.0 ppm dietary RHS along with their basal diets. Results showed that diet containing various levels of dietary RHS did not adversely affect ( $P>0.05$ ) the body weight, feed intake, and feed conversion ratio. Significant difference was found in group supplemented with 7.5 ppm dietary RHS against drip loss of thigh meat compared to other groups ( $P<0.05$ ), while against thawing loss although lowest value was observed in the same group but statistically no significant difference was found among all groups. Similarly, against shear force and thawing loss of breast meat; 7.5 ppm dietary RHS group revealed significant difference ( $P<0.05$ ) compared to all other groups and tibia breaking strength was also increased significantly ( $P<0.05$ ), whereas; against breaking strength of femur no significant effect was observed among all groups ( $P>0.05$ ). Keeping in view findings of current study it was conclude that the dietary RHS can be used as a natural mineral in broilers' diet to enhance meat and bones quality. At the level of 7.5 ppm RHS is more beneficial for broilers to improve their growth performance and product quality.

**Keywords:** broilers, bone breaking strength, meat quality, rice hull silicon

### Introduction

Modern commercial broilers are bred for having such strains though have maximum feed conversion ratio and fastest growth rate in minimum time period. With certain approaches of broiler breeders, no doubt, growth rate and weight gain of broilers have improved a lot and within 5 to 6 weeks birds gain about 2.5 to 3.0 kg weight, but this rapid weight gain has also been a single main cause to increase the rate of skeletal and leg deformities in broilers. It is well studied that minerals are important components of bones. Bone mineralization makes bones harder enough which resultantly empower the skeleton to withstand against gravity and additional loading (Shim et al., 2012). Traditionally, calcium and phosphorus are accepted as major minerals for animal diets, while other trace minerals have been ignored, especially silicon. Recently, silicon is classified as an essential trace mineral for cartilages growth, normal bones



development and improving the bones' quality (Pietak et al., 2007; Incharoen et al., 2016). Some testimonies indicate that silicon is associated with calcium metabolism, formation and stabilization of extracellular bone matrix (Reffitt et al., 2003; Jugdaohsingh, 2007). According to Incharoen et al. (2016) also suggested that dietary silicon can be used as a mineral additive to enhance the bones and meat quality of broilers.

As Thailand is an agricultural country and rice cultivation is the main occupation of the peoples. Several millions tons of rice is produced by the country each year. Rice hulls are obtained as major by-products when rice is processed at rice mills. Recent past studies have indicated that the rice hulls are rich source of silicon and that rice hulls extracted silicon may positively be associated with the bone breaking strength of broilers, especially at the dose rate of 0.75% (Incharoen et al., 2016). Thus, current study was planned in order to check the effects of dietary rice hull silicon (RHS) on the performance, meat quality and bone breaking strength of broilers on different dietary supplementation levels.

## Materials and Methods

### Experimental design and birds' management

RHS was produced according to the method of Incharoen et al. (2016). It was mainly containing SiO<sub>2</sub> (96.15%) followed by Na<sub>2</sub>O (0.91%) and K<sub>2</sub>O (0.60%). Animal related trials of the present study (No. 590506) were approved and regulated by the Naresuan University Animal Care and Use Committee (NUACUC). Arbor Acres broiler chicks were purchased from the commercial hatchery (Charoen Pokphand Foods PCL., Thailand). All chicks were placed in a brooding zone and hastily equipped to water and diet. At 10 day of age, a total of 100 chicks were divided into five groups, each with four replicates of five chicks. The control group was fed a basal diet (Table 1) and the other groups were fed the basal diet supplemented with RHS at 2.5, 5.0, 7.5, and 10.0 ppm, respectively. Experimental diets were provided on *ad libitum* and water on free access basis throughout the study period. Birds were maintained according to the hybrid strain guidelines till age of 45 days. The body weight and feed intake of each bird was recorded weekly.

### Data collection and measurement

Sampling protocols were followed according to the Naresuan University Animal Care and Use Committee (NUACUC) guidelines. At the end of the trial, four birds from each group were slaughtered to assess the meat quality and bone breaking strength. Broilers' carcass were scalded in warm water (60±5°C), de-feathered and eviscerated. Muscle samples were collected from both sides of the breast and thighs for evaluation of meat quality. Collected meat samples were dissected, weighed and then transferred into polyethylene bags. Samples were stored in refrigerator at temperature of 4°C for 24 hours. After refrigeration, each sample was individually wiped and weighed to check the drip loss, which was expressed as a percentage of the initial weight.

For thawing loss, each fresh meat samples were individually weighed, placed into polyethylene bags and then transferred to the de-freezer at -21°C for 48 hours. Subsequently, frozen samples were thawed for 24 hours in refrigerator at 4°C and finally weighed. Thawing loss was expressed as a percentage. To measure the shear force, skinless breast and thigh meat samples were firstly cooked and then checked on texture analyzer (model QTS20, Brookfield Instruments, UK). However, for measuring the bone breaking strength, samples of tibias and femurs were used. After separating bones from meat, samples were collected and then dried in hot oven at 95°C for overnight. Bones breaking strength were measured by using universal testing machine (model 441, Instron, Ltd., England), according to the method modified by Incharoen et al. (2016).

**Table 1.** Ingredients and chemical composition of the basal diet

Ingredients	Percentage (%)	
	Grower diet	Finisher diet
Corn	12.50	13.20
Broken rice	45.47	44.00
Soybean meal (45% CP)	30.38	29.86
Palm oil	4.10	5.10
Fish meal (57% CP)	7.00	7.00
Calcium Carbonate	0.50	0.50
Dicalcium Phosphate	0.10	0.10
Concentrate mixture <sup>1/</sup>	0.20	0.20
DL-methionine	0.20	0.20
Total	100.00	100.00
Calculated chemical composition		
Crude protein	22.00	20.00
Crude fat	6.16	7.14
Crude fiber	2.69	2.66
Calcium	0.63	0.76
Phosphorus	0.52	1.20
ME (kcal/kg)	3,150.00	3,200.00

<sup>1/</sup>Supplied per 100 kg of diet, vitamin A (15,000,000 IU); vitamin D3 (3,000,000 IU); vitamin E (2600 IU); vitamin K (35g); vitamin B1 (2.5g); vitamin B2 (6.5g); vitamin B6 (275.5g); vitamin B12 (26mg); pantothenic acid (11.04g); nicotinic acid (35g); biotin (15.1mg), choline chloride (250g); Copper (1.6g); Manganese (60.2g); Iron (1.6g); Zinc (45g); Iodine (400mg) and Selenium (160mg).

### Statistical analysis

Statistical analysis was performed by One-way Analysis of Variance (ANOVA) using the SPSS version 17.0 statistical software package (SPSS Inc., Chicago, USA). Differences among the groups were analysed by Duncan's multiple range test.  $P < 0.05$  were considered significant.

### Results and Discussion

Results regarding the effects of dietary RHS on broilers performance are presented in Table 2. The current data indicates that no negative impact on the body weight, feed intake and feed conversion ratio was observed among all groups during 10 to 45 days of age. In addition, no considerable difference was found among all groups against dressing percentage, weight of breast, fillet, wing and thigh plus drumstick ( $P > 0.05$ ) (Table 3). These encouraging results conclude that dietary RHS has no adverse influence on the broilers' performance during grower and finisher phase. Our results are in agreement Incharoen et al. (2016) who noted that no significant data on broiler performance were observed among the dietary silicon groups. Similarly, Bintas et al. (2014) also reported that dietary silicon-based supplement did not significantly affect the overall body weight gain, feed intake and feed conversion ratio of broiler chickens. Furthermore, the supplementation of silicon-based natural or modified clinoptilolite has also no significant impacts on total broiler productivity during age of 1 to 42 days (Wu et al., 2013). Conversely, some researchers have reported that dietary supplementation with silicon-



based clinoptilolite improves the health status, body weight gain and feed efficiency of the animals (Papaioannou et al., 2004). Tran et al. (2015) also reported comparable results when he was working on the dietary silicon and found positive influence on the ammonia reduction, weight gain and feed conversion in turkeys. Some other reports indicate that concentrated mixture with 70% silicon was found to enhance the nutrients digestibility in growing-finishing swine (Yan et al., 2010). These fickle effects of dietary silicon-based supplement may be induced by other factors such as pureness, origin, nature, chemical components, concentration and particle size etc.

**Table 2.** Effects of dietary RHS on the growth performance of broilers during 10-45 days of age

Parameters	Control	Dietary RHS (ppm)				<i>P</i> -value
		2.5	5.0	7.5	10.0	
Body weight (g/b)	2,519	2,508	2,477	2,580	2,528	0.598
Feed intake (g/b)	4,445	4,618	4,460	4,566	4,679	0.146
Feed conversion ratio	1.66	1.73	1.70	1.71	1.70	0.320

The results regarding the effects of dietary RHS on breast and thigh meat quality of broilers are shown in the Table 3. Drip loss of breast meat tended to be lower in dietary RHS groups, and lowest value was found in 7.5 ppm RHS group. Likewise, thawing loss also tended to decrease in dietary RHS groups and significantly decreased ( $P < 0.05$ ) in 7.5 ppm RHS group compared to all other groups. Meanwhile, shear force was higher in all RHS groups, with significantly highest in the 7.5 ppm RHS group ( $P < 0.05$ ). Regarding drip loss, thawing loss and shear force of thigh meat; no significant difference was found ( $P > 0.05$ ) among all groups excepting the 7.5 ppm RHS group which showed lowest drip loss and significant difference compared to all groups ( $P < 0.05$ ). Given results are comparable with Incharoen et al. (2016) who reported that dietary silicon is the essential mineral for improvement of meat quality in term of decreasing the cooking and thawing losses, especially at the level 0.75% in broiler diets. Although, 72% silicon-based mineral products also have beneficial impacts on the muscle firmness but this phenomenon might be affected by the interaction/metabolism of metal ions and subsequently alteration in the minerals content of tissues (Yan et al., 2010). Therefore, our current results seem to be more related with dietary RHS induced mineral component modification of muscular tissue that results improvement in the texture and meat quality in term of drip and thawing loss reduction.

About bone breaking strength results are presented in the Figure 1. Breaking strength of femur was higher in the group supplemented with 7.5 RHS followed by 5.0, control, 10.0 and 2.5. Although data showed variability among the means but statistically no significant difference was found within all groups against breaking strength of femur. Regarding breaking strength of tibia significant difference was found in 7.5 RHS group ( $P < 0.05$ ) followed by 5.0, 2.5, 10.0, while in control group no significant difference was found compare to 10.0 RHS group. These findings are related with Jugdaohsingh et al. (2004) who noted that higher silicon concentration is related with production, strength of bones and cartilages. Our results are also comparable with Short et al. (2011) who demonstrated that dietary silicon supplementation has ability to reduce the lameness in broiler chicken.

## Conclusion

Present study concludes that dietary RHS has no adverse influence on the broiler performance during the grower and finisher period. Moreover, RHS play a key role in improving the bone breaking strength and reducing the drip or thawing loss, that results enhancement of overall meat quality and better performance by the birds. On the basis of current results it seems

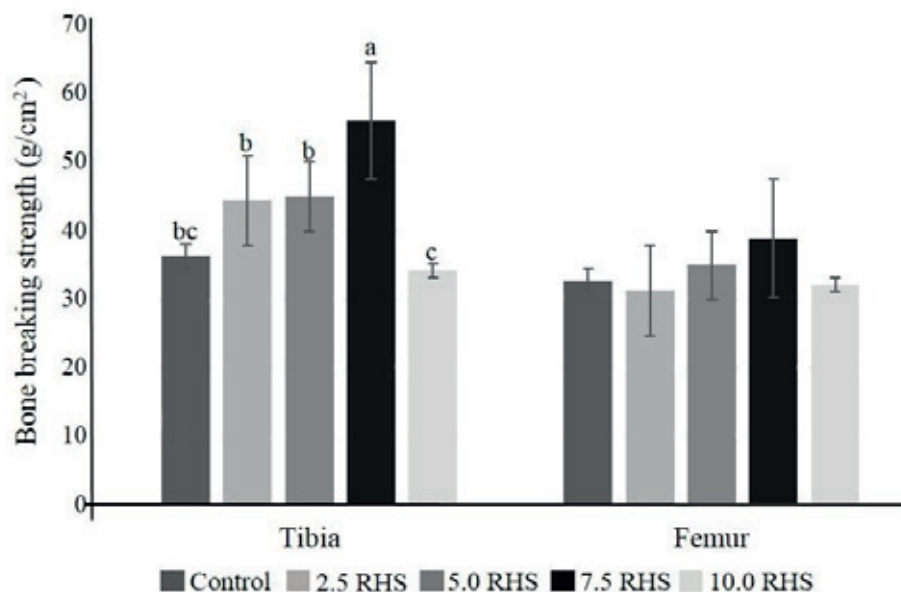


that dietary RHS can be used as an important trace mineral for broilers' feeding especially at the concentration of 7.5 ppm.

**Table 3** Effects of dietary RHS on meat quality of broilers at 45 days of age

Parameters	Dietary RHS (ppm)					<i>P</i> -value
	Control	2.5	5.0	7.5	10.0	
<b>Breast meat quality</b>						
Drip loss (%)	14.13	13.80	13.25	9.09	13.32	0.092
Thawing loss (%)	14.24 <sup>bc</sup>	11.09 <sup>ab</sup>	11.69 <sup>ab</sup>	8.08 <sup>a</sup>	17.81 <sup>c</sup>	0.050
Shear force (kg)	5.35 <sup>b</sup>	6.43 <sup>b</sup>	6.49 <sup>b</sup>	9.13 <sup>a</sup>	6.19 <sup>b</sup>	0.030
<b>Thigh meat quality</b>						
Drip loss (%)	12.38 <sup>ab</sup>	13.31 <sup>ab</sup>	13.51 <sup>ab</sup>	8.46 <sup>a</sup>	14.22 <sup>b</sup>	0.050
Thawing loss (%)	14.77	11.07	11.60	7.25	14.21	0.058
Shear force (kg)	8.86	7.04	8.88	9.26	8.56	0.328

<sup>a-c</sup>Means within rows with different superscripts are significantly different ( $P < 0.05$ ).



**Figure 1.** Effects of dietary RHS on bone breaking strength of broilers at 45 days of age

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## The Effects of Organic Corn Level Decreasing in Organic Laying Hen Diets on Egg Production and Egg Quality

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### Abstract

Two hundred and forty with 75 weeks old laying hen (CP brown) were randomly assigned to 5 experimental groups (Completely randomized design; CRD), 4 replicates of 48 birds. The laying hens were fed the non-organic control diet and organic diets those including organic corn at 0, 10, 20 and 30 % levels for 6 weeks. There were no significant differences in egg production, feed conversion ratio and feed intake by decreasing organic corn in the laying hen diets ( $P>0.05$ ). The organic corn levels had no effect on egg weight, yolk index, egg shell strength and shell color excepted egg weight and yolk index of 20% organic corn groups was lowest ( $P<0.05$ ) in the 6 and 4 weeks respectively. At the 2 weeks, the egg shell thickness of organic groups were lower than that of control ( $P<0.05$ ) excepted that of 10% organic corn group were not different from control. At the 4 weeks, the egg shell thickness of 20% organic corn group was lowest ( $P<0.05$ ). At the 6 weeks, the egg shell thickness of 10% organic corn group was lowest ( $P<0.05$ ). The haugh unit of 0% organic corn group was lowest ( $P<0.05$ ) during the experimental period. The yolk color was decreased with decreasing organic corn levels ( $P<0.05$ ). Therefore, it can be reduce the organic corn level in organic feed by using the organic broken rice without any effect on egg production performance but have to concern in egg yolk color.

**Keywords:** organic feed, egg production, egg quality, laying hen

### Introduction

The increasing of consumer interest in organic products lead to increasing of organic livestock production. The number of organic farms in Europe were increased in 1982s -2001s (Hermansen et al., 2004). The important factors for organic poultry production are organic feed and rearing system. Faculty of Animal Science and Technology, Maejo University, Chiang Mai, Thailand had started the organic feed project since 2015s. We found that organic corn production was very low while the organic soy bean was not difficult to buy in the organic market. Therefore, the alternative organic energy source should be provide instead of organic corn or combine with organic corn. In this study, the organic corn levels were decreased by using organic broken rice in laying hen diets. The effects of organic corn levels decreasing in laying hen diets on egg production and egg quality were observed.



## Materials and Methods

### Experimental Design

Two hundred and forty with 75 weeks old laying hen (CP brown) were randomly assigned to 5 experimental groups (Completely randomized design; CRD), 4 replicates of 48 birds. The laying hens were received feed and water *ad libitum* during the entire experimental period. Feed intake and egg productions were recorded weekly. Egg quality were determined every 2 weeks, 28 eggs per treatment were collected to determine egg weight, egg shell-breaking strength, egg shell thickness, egg yolk index, haugh unit, yolk color and egg shell color.

### Experimental Diets

The control diets represented a conventional corn-soybean based on layer feed, and the diets consist of organic corn at the level of 0, 10, 20 and 30% (Table 1).

### Statistical Analysis

All data were statistically analyzed using one-way ANOVA, and significant differences among the treatments were determined with Duncan's multiple range test using the Windows statistics software (SAS University Studio) at the level of  $P < 0.05$ .

## Results and Discussion

### Egg Production

There were no significant differences in egg production, feed conversion ratio and feed intake by decreasing organic corn in the laying hen diets ( $P > 0.05$ ) (Table 2). Similarly, Rama Rao et al. (2000) found that the using of broken rice instead of corn had no effect on hen-day egg production and feed conversion ratio in broiler breeders and Rama Rao et al. (2001) reported that broken rice can be used as principle energy sources in place of corn in broiler breeder diets without affecting egg production, fertility or hatchability. The similar egg production performance might be according to the utilization of energy from corn and broken rice based diets were similar in laying hen (Jadhao et al., 1999)

### Egg Quality

The organic corn levels had no effected on egg weight, yolk index, egg shell strength and shell color excepted egg weight and yolk index of 20% organic corn groups was lowest ( $P < 0.05$ ) in the 6 and 4 weeks respectively (Table 3). At the 2 weeks, the egg shell thickness of organic groups were lower than that of control ( $P < 0.05$ ) accepted that of 10% organic corn group were not different from control. At the 4 weeks, the egg shell thickness of 20% organic corn group was lowest ( $P < 0.05$ ). At the 6 weeks, the egg shell thickness of 10% organic corn group was lowest ( $P < 0.05$ ). The haugh unit of 0% organic corn group was lowest ( $P < 0.05$ ) during the experimental period. These findings are contrary to those of Jahao et al. (1999), who found that corn and broken rice based diets had no affected on egg quality characteristic in laying hen. The yolk color was decreased with decreasing organic corn levels ( $P < 0.05$ ). Similarly, Jahao et al. (1999) and Rama Rao et al. (2000) found that broken rice reduce egg yolk color in laying hens and broiler breeders respectively. The egg yolk color is highly affected by plant material type and intake of hen (Hammershoj and Johansen, 2016), where the different carotenoids in corn and broken rice are reflected in yolk color.

**Table 1.** Ingredients and calculated chemical composition of experimental diets

Ingredients	Control diet	Organic corn level (%)			
		0	10	20	30
Ground corn	61.36	-	10.00	20.00	30.00
Soybean meal (44% CP)	26.18	-	-	-	-
Full fat soybean meal	-	34.30	34.26	34.20	34.17
Broken rice	-	55.74	45.78	35.94	26.06
Rice bran oil	2.61	-	-	-	-
Fine limestone	7.60	5.80	5.80	5.90	6.11
Dicalcium phosphate	1.25	3.10	3.10	2.90	2.60
Salt	0.50	0.50	0.50	0.50	0.50
Premix <sup>1</sup>	0.50	0.50	0.50	0.50	0.50
Probiotic	-	0.05	0.05	0.05	0.05
Phytase <sup>2</sup>	-	0.01	0.01	0.01	0.01
Total	100	100	100	100	100
Calculated chemical composition (%)					
Crude Protein	17.00	17.00	17.00	17.00	17.00
Metabolizable energy (Kcal kg)	2.89	3.43	3.44	3.46	3.47
Crude fiber	3.30	1.72	1.83	1.94	2.05
Crude fat	2.74	9.11	9.32	9.54	9.75
Calcium	3.26	3.18	3.17	3.15	3.15
Available phosphorus	0.39	0.38	0.41	0.41	0.41
DL-methionine	0.45	0.00	0.02	0.04	0.06
L-lysine	0.87	0.00	0.03	0.05	0.08

<sup>1</sup> Premix supplies (per Kg premix) vitamin A 2,000,000 IU, vitamin D3 400,000 IU, vitamin E 3,500 IU, vitamin K3 0.18 g, vitamin B2 0.8 g, vitamin B6 0.56 g, vitamin B12 2 mg, Panthotinic acid 1.89 g, Nicotinic acid 4 g, Follic acid 60 mg, Blotin 18 mg, Coline 95g, Copper 2g, Manganese 16 g, Iron 12 g, Iodine 120 mg, Zinc 16 g, Cobalt 60 mg and Selenium 32 mg.

<sup>2</sup> Phytase, 5000 FTU/g



**Table 2.** The effects of organic corn level on egg production, feed conversion ratio and feed intake

Weeks		1	2	3	4	5	6	1-6
Egg production (%)								
Control diet		79.82	79.06	80.41	75.46	68.82	67.96	75.26
	0	81.43	78.98	78.75	72.79	66.13	68.27	74.39
Organic corn	10	84.18	81.47	72.56	77.68	72.14	75.34	77.23
levels (%)	20	82.14	78.57	80.06	77.08	72.92	68.15	76.49
	30	78.87	76.78	82.44	80.55	77.97	78.19	79.13
FCR								
Control diet		2.32	3.49	3.22	2.06	2.35	2.44	2.65
	0	2.44	3.39	3.04	2.07	2.48	2.19	2.52
Organic corn	10	2.36	3.32	3.51	2.12	2.37	2.17	2.60
levels (%)	20	2.48	3.26	2.93	1.96	2.06	2.25	2.64
	30	2.61	3.52	2.84	2.01	2.01	2.13	2.49
Feed in take (g/b/d)								
Control diet		125.35	180.06	169.48	101.42	103.15	107.51	131.16
	0	133.22	176.96	158.28	97.14	107.08	100.32	128.87
Organic corn	10	134.41	180.93	166.23	108.75	111.59	104.73	134.44
levels (%)	20	135.12	166.79	154.81	98.63	97.02	96.43	124.80
	30	138.39	176.98	155.35	112.39	104.11	110.68	132.95

**Table 3.** The effects of organic corn level on egg quality

Weeks	Diet	Egg weight (g)	Yolk index (%)	Egg shell strength (Kg force/cm <sup>2</sup> )	Egg shell thickness (mm)	Haugh unit	Yolk color	Shell color (% light)	
2	Control	66.24	4.59	3.34	0.3755 <sup>a</sup>	81.92 <sup>a</sup>	8.36 <sup>a</sup>	28.54	
	Organic corn levels (%)	0	68.24	4.59	3.32	0.3503 <sup>b</sup>	75.57 <sup>b</sup>	2.00 <sup>e</sup>	28.79
		10	66.82	4.56	3.33	0.3623 <sup>ab</sup>	78.78 <sup>ab</sup>	3.39 <sup>d</sup>	27.62
		20	65.89	4.51	3.16	0.3592 <sup>b</sup>	78.36 <sup>ab</sup>	5.54 <sup>c</sup>	29.91
		30	67.26	4.55	3.27	0.3506 <sup>b</sup>	81.06 <sup>ab</sup>	7.17 <sup>b</sup>	29.08
4	Control	65.47	4.78 <sup>a</sup>	3.35	0.3640 <sup>ab</sup>	84.92 <sup>a</sup>	7.54 <sup>a</sup>	26.49	
	Organic corn levels (%)	0	65.36	4.65 <sup>ab</sup>	2.89	0.3660 <sup>ab</sup>	79.39 <sup>b</sup>	1.18 <sup>e</sup>	27.57
		10	67.25	4.74 <sup>a</sup>	3.05	0.3508 <sup>ab</sup>	80.24 <sup>ab</sup>	2.29 <sup>d</sup>	25.74
		20	65.57	4.55 <sup>b</sup>	3.08	0.3495 <sup>b</sup>	81.27 <sup>ab</sup>	3.50 <sup>c</sup>	30.82
		30	65.63	4.65 <sup>ab</sup>	3.38	0.3710 <sup>a</sup>	82.25 <sup>ab</sup>	5.00 <sup>b</sup>	26.29
6	Control	64.55 <sup>a</sup>	5.05	3.21	0.3444 <sup>ab</sup>	85.54 <sup>ab</sup>	7.78 <sup>a</sup>	25.92	
	Organic corn levels (%)	0	66.74 <sup>a</sup>	5.11	3.14	0.3520 <sup>a</sup>	81.23 <sup>b</sup>	1.11 <sup>e</sup>	27.25
		10	66.16 <sup>a</sup>	5.07	3.06	0.3334 <sup>b</sup>	84.03 <sup>ab</sup>	2.75 <sup>d</sup>	26.39
		20	61.16 <sup>b</sup>	5.16	2.93	0.3394 <sup>ab</sup>	84.09 <sup>ab</sup>	3.75 <sup>c</sup>	27.25
		30	64.54 <sup>a</sup>	5.17	2.98	0.3407 <sup>ab</sup>	87.23 <sup>a</sup>	5.82 <sup>b</sup>	26.91

<sup>a-e</sup> Mean followed by different letters are significantly different (P < 0.05)



## Conclusion

It can be reduce the organic corn level in organic feed by using the organic broken rice without any effect on egg production performance but have to concern in egg yolk color. Therefore, natural pigments should be add to enhance egg yolk color if egg yolk color is an important factor for organic egg marketing.

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## Factors Effecting on Rabies Immunity Titer in Canine

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### Abstract

There are many factors effecting on rabies immune response in vaccinated dog. This study aimed to investigate the effects of sex, age, time of vaccination and vaccine brands on rabies immunity response in canine. A total of 233 canines (male = 115 and female = 118) were used from Pattaya city. Fluorescent Antibody Virus Neutralization Test (FAVN test) was found that the sex and vaccines companies had no effect on the immune response ( $P > 0.05$ ). Considering on age and vaccination cycle were found highest level in dogs were 4-6 and 7-9 years old (8.31 and 7.99 IU/mg, respectively) and lowest level in dogs were younger than 3 years old (4.41 IU/mg) ( $P < 0.05$ ). Dog which one time of vaccination was highest 7.28 IU/mg ( $P < 0.05$ ). However, the dogs have been five times vaccinated were lowest immune titer (4.84 IU/mg). Immune titer was significant increase ( $P < 0.01$ ) one month post vaccination. The results can be concluded that the rabies vaccine should be continued on an annual basis especially in dog aged 0-3 years old and more than 10 years old due to the immune response was not efficiency as the age of 4-9 years old.

**Keywords:** sex, age, time of vaccination, vaccine company, rabies titer

### Introduction

Pattaya City has set out measures to eliminate rabies from this area. In order to make Pattaya a rabies-free area within years 2020 by rabies prevention campaign will be continued for the dogs of both Pattaya residents and nearby areas. In order to make effective rabies vaccination in dog, the study to finding factors effecting to rabies immune response. There are many factors could effect to immune response such as sex, age or type of vaccine. In the Pattaya City was protected rabies by vaccination campaign to served community. The mobile veterinary clinics are free of charge to serve covering 41 communities.

Therefore, the objective of this study was to investigate the factors affecting on rabies immunity titer in dog to use as a prevention guideline of rabies disease.

### Materials and Methods

A total of 233 canines living in Pattaya were used in this study. The characteristic of dogs were showed in Table 1. The dogs were injected by 4 types of rabies vaccines (A, B, C and D). Blood sample were taken from brachial vein just before and one month after vaccine injection. One dog may be injected 1, 2, 3, 4 and 5 time. Effect of sex, age of the dog, type of vaccines and time of vaccine injection on immune response (titer) were analysis by using of



analysis of variance. Comparison among the factors (sex, age, type of vaccine and time of vaccine injection) were analyzed by using Duncan New's Multiple Range Test significant of  $P < 0.05$  was declare.

### **Samples Collection and Immune Response Analysis**

Before vaccination, blood samples were collected from canines who had received the first vaccination on 3-month-of age to determine immune levels. Blood samples were collected again after rabies vaccination for 1 month to determined immune levels by fluorescent antibody virus neutralization (FAVN) and real time RT-PCR respectively.

### **Results and Discussions**

This study investigated the influence of sex, age and types of rabies vaccines on immune response to use as a guideline to prevent rabies infection. It might be affected to human mortality in Pattaya city. The number and demographic characteristics percentage of canine in Pattaya city as shown in Table 1. It was found that males and females had almost similar numbers of 115 and 118 (49.36 and 50.64 %, respectively).

The number of dog ages from  $<1$  to 3 years, 4-6 years, 7-9 years, 10-12 years, and  $>13$  years old were 86, 48, 30, 39 and 33 dogs (36.91, 20.60, 12.88, 16.74, and 12.88 %, respectively). Time of vaccination found that one time vaccination are highest number compared to other groups. Considering types of vaccines companies (A, B, C and D) were found the dogs number are 38, 33, 125 and 33 (16.31, 15.88, 53.65 and 12.88 %, respectively) as shown in Table 1.

The various factors on immune responses are sex, age, time of vaccination and types of vaccine as shown in Table 2. Results shows that sex and types of vaccine did not significant difference ( $P > 0.05$ ). However, dogs age and time of vaccination were significantly different on immune response ( $P < 0.05$ ). The dogs age from  $>1$  year - 3 years, 4-6 years, 7-9 years, 10-12 years and 13 years old were various responses on immunological values of 4.41, 8.31, 7.99, 6.29, and 6.51 IU/mg, respectively

It was interesting that adult dogs (4-6 and 7-9 years old) had higher levels of immunity than young and old dogs. According to HohenEsch et al. (2004) reported that the immune response in adult dogs at 3.15 + 0.8 years old are better immune responses than old dogs. The observation of lymphocytes and CD4 + T cells were found high values in adult canines. In contrast, Lymphoproliferative activity and mitogens were reduced in old dogs (HogenEsch and Thompson, 2010).

Considering of the time of vaccination showed linear reduced on one time vaccination was highest value, followed by second, third, fourth and fifth time of vaccination (7.28, 6.12, 5.52, 5.38, and 4.84, respectively).

The interaction of sex, age, time of vaccinations and types of vaccine were no statistical different on immune response ( $P > 0.05$ ) (Table 2). The level of immunity of canines before and after vaccination were highly statistically significant ( $P < 0.01$ ) as shown in Figure 1. As the similar results with Teerapong (2013) showed that the non-vaccination dogs was found rabies diagnosed, because they don't have any immunity to disease prevention. In addition, the WHO (2010) reported that pets should be prevention by vaccinated to reduce the chance of infection. According to Ahmed et al. (1985) reported that sex hormones affected on thymus gland to induce immunity such as immunoglobulin M (IgM) and immunoglobulin G (IgG). Immunity levels and immune responses in female are better levels and responses than males. As the same results with HohenEsch et al. (2004) was found very old canines, the level of immunity was low there are greater chances of infection than younger canines.



## Conclusion

Results above can be concluded that sex and types of vaccine do not affect immunity levels. The age range from 4-6 and 7-9 years old, and one time vaccination were highest immune response. Immunity of the dogs after vaccination was higher than dogs before vaccination as well as rabies infection are also reduced when vaccination. Therefore, rabies vaccination can be prevented rabies disease infection, the pet owner must have a good understanding of vaccinations and cooperate with government agencies to reduce the number of rabies infected. This leads to the goal of rabies prevention in Pattaya city to be a rabies-free area in the future.

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**Table 1.** Number and demographic characteristics percentage of canine in Pattaya city

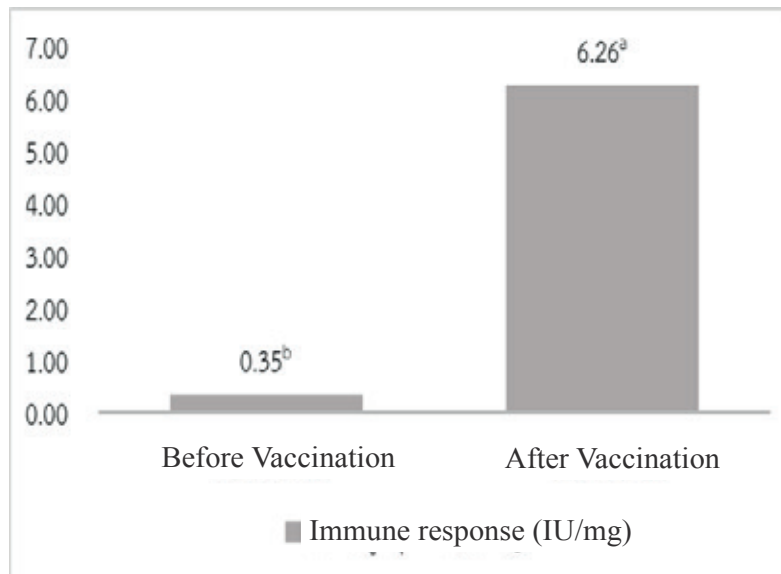
Data base	Number (head)	Percentage
1. Sex	115	49.36
Male	118	50.64
Female		
Total	233	100
2. Age		
0-3 Years	86	36.91
4-6 Years	48	20.60
7-9 Years	30	12.88
10-12 Years	39	16.74
>13 Years	30	12.88
Total	233	100
3. Time of vaccine		
1 Time	94	40.34
2 Time	32	13.73
3 Time	29	12.45
4 Time	49	21.03
5 Time	29	12.45
Total	233	100
4. Vaccines Company		
A	38	16.31
b	37	15.88
c	125	53.65
D	33	14.16
Total	233	100

**Table 2** Effect of sex, age and rabies vaccines on immune response in serum

Items Factors	Immune response	
	Before (IU/ml)	After (IU/ml)
A: Sex		
Male	0.23	5.95
Female	0.46	6.56
Sig.	ns	Ns
B: Age		
< 1-3 Year	0.21 <sup>b</sup>	4.41 <sup>c</sup>
4-6 Year	0.13 <sup>b</sup>	8.31 <sup>a</sup>
7-9 Years	0.06 <sup>b</sup>	7.99 <sup>a</sup>
10-12 Years	0.18 <sup>b</sup>	6.29 <sup>b</sup>
> 13 Years	1.61 <sup>a</sup>	6.51 <sup>b</sup>
Sig.	*	*
C: Time of Vaccine		
1 Time	0.65	7.28 <sup>a</sup>
2 Time	0.11	6.12 <sup>b</sup>
3 Time	0.18	5.52 <sup>bc</sup>
4 Time	0.20	5.38 <sup>bc</sup>
5 Time	0.11	4.84 <sup>c</sup>
Sig.	ns	*
D: Vaccines Company		
A	0.20	4.33
B	1.21	7.80
C	0.23	6.60
D	0.03	5.49
Sig.	ns	ns
AxB	ns	ns
AxC	ns	ns
AxD	ns	ns
BxC	ns	ns
BxD	ns	ns
AxBxC	ns	ns
AxBxD	ns	ns
AxCxD	ns	ns
BxCxD	ns	ns
AxBxCxD	ns	ns

**Notes:** ns = non significance different (P>0.05)

\* = significance different (P<0.05)



**Note:** <sup>a-b</sup> Highly significance different ( $P < 0.01$ )

**Figure 1** Immunity levels of before and after vaccination in serum



## **Antibacterial Activity of *Phaleria macrocarpa* Fruit Extracts: An *In Vitro* Study**

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### **Abstract**

*Phaleria macrocarpa* is one of the most important medicinal plants. This plant is native to Indonesia, which is originated from Papua. Its fruit is widely used as herbal medicine due to its bioactive compounds such as phenolic, benzophenone, terpenes, and alkaloid. They contribute to the antimicrobial activities of the fruit extract. The objective of this study was to investigate antibacterial activity of *Phaleria macrocarpa* fruit extract against pathogenic bacteria (*Escherichia coli* and *Salmonella* sp.) and nonpathogenic bacteria (*Lactobacillus* sp.). The antibacterial activity was determined by agar well diffusion method. Antibacterial activity was in completely randomized design with 5 treatments and 4 replicates, followed with orthogonal contrast for significant different effect. The treatments consist of control (-): water, control (+): tetracycline, and *Phaleria macrocarpa* fruits extract with different feed to solvent ratio (F:S) g/ml (P1 1:50, P2 1:100, P3 1:150). The result showed that tetracycline performed highest antibacterial activity. *Phaleria macrocarpa* fruits extract with different feed to solvent ratio could not inhibit of nonpathogenic bacteria, but could inhibit pathogenic bacteria without significant results. Our study clearly indicates that *Phaleria macrocarpa* fruits extract could inhibit colonization of pathogenic bacteria.

**Keyword:** antibacterial activity, pathogenic and nonpathogenic bacteria, *Phaleria macrocarpa*

### **Introduction**

Pathogenic bacteria have been considered as a crucial problem in poultry industry and can cause various diseases in human. Antibiotic is the one of manners to reduce the effect from pathogenic microbes in poultry. Most of poultry industry in Indonesia use antibiotics to maintain health and production efficiency. In veterinary, antibiotics have been used for therapeutic. While in poultry industry antibiotics have been used for growth promoter. The antibiotic serves as growth promoter by inhibiting the growth or destroying microorganisms either pathogenic or non-pathogenic, but can also give negative effect for consumer (Kirbis, 2007). The use of antibiotic for poultry diets was totally banned in Europa Community in January 2006 (Dono, 2013). One of alternatives that can be used to replace antibiotic in poultry industry is phytobiotics.

Phytobiotic is a component derived from plant and incorporated in feed to improve production qualities of livestock either on the way to improve the effect of feed or improve animal production traits, as well as, to improve characteristics of the products. Phytochemicals in phytobiotic are well known to have antimicrobial ability. One of phytobiotics that commonly used in Indonesia is *Phaleria macrocarpa* fruits. *Phaleria macrocarpa* is an important medical



plant. It grows in Papua Island, Indonesia as tropical areas. The four major parts of *Phaleria macrocarpa* that are mostly enriched in medicine are stems, leaves, egg shell of the seeds and fruits. *Phaleria macrocarpa* fruits have an eclipse shape; occur in various sizes with diameter ranging from 3 – 5 cm, have smooth surface and changing their color from green when young into red or maroon when ripening. The fruit is the most poisonous if directly eat (Ma ma lay et al., 2014; Othman et al., 2014). Its fruit is widely used as herbal medicine due to its bioactive compounds such as phenolic, benzophenone, terpenes, and alkaloid. They contribute to the antimicrobial activities of the fruit extract (Alara and Olera, 2016). Hendra et al. (2011) reported that *Phaleria macrocarpa* fruits was studied using the disc diffusion method against eight bacterial strain.

The objective of the present study is to investigate antibacterial activity from *Phaleria macrocarpa* fruits extracts: An in vitro study against gram-negative bacteria (*Escherichia coli* and *Salmonella* sp.) and gram-positive bacteria (*Lactobacillus* sp.).

## Material and Methods

*Phaleria macrocarpa* fruits were collected from Yogyakarta, Indonesia, in April 2016. The fruits were grinded, and then dried in an oven at approximately of 50°C. The dried powder was macerated with 96% aqueous ethanol and extracted at room temperature for 72 hours. The extract was filtered with Whatman No. 1 filter paper. The filtrate was evaporated to dry using water bath at 70°C.

The antibacterial assay of *Phaleria macrocarpa* fruit extracts was carried out with the disc diffusion method. All the microorganisms were incubated at 37°C for 24 h with inoculation into nutrient broth. One hundred microliters of the inoculate were spread over plates containing sterile nutrient agar (1 g agar powder, 1 g peptone, 0,5 g NaCl, 1 g meat extract and 100 ml aquadest). All of instrument were sterilized with autoclave 121°C for 15 m. Paper filter discs (6 mm) impregnated with 20 µL of each extract were placed on the surface of the media. The plates were left for 30 min at room temperature to allow the diffusion of the extracts and incubated at 30°C for 24 h. Tetracycline was also included in the test as a reference for positive control. Finally, the inhibition zone around the disc was measured and the experiments were run in four replicates. (Cavalieri et al., 2005)

## Results and Discussion

The *in vitro* antibacterial activity of *Phaleria Macrocarpa* fruits extract was evaluated by agar diffusion method. Results from the present study are shown in Table 1. Tetracycline as positive control had the largest inhibition zone of all bacterial tested. Water as negative control did not have activity to against of all bacterial tested, and *Phaleria Macrocarpa* fruits extract with different feed and solvent ratio had effective to against pathogenic bacteria, but cannot against non-pathogenic bacteria. Inhibition zone of tetracycline for 3 bacterial tested was 17.7, 9.0, and 17.7 respectively of *Eschericia coli*, *Salmonella* sp. and *Lactobacillus* sp. Tetracycline showed the highest results, but had negative effect. Tetracycline inhibits both of pathogenic and non-pathogenic bacteria. As feed additive, tetracycline affects not only animal health, but also public health. Sattar et al. (2014) ; Chopra and Roberts (2001) reported tetracycline is broad-spectrum microbial, exhibiting activity against a wide range of gram-positive and gram-negative bacteria and can cause resistant organisms in human body. Tetracycline will diffuses throughout the body and found in highest concentrations in kidney, liver, spleen, and lungs.

*Phaleria Macrocarpa* fruits extract showed inhibition zones of *Escherichia coli* and *Salmonella* sp., but not significant compare to other extraction. Ethanol is one of organic solvents that can be used for extraction of plant compounds which have antimicrobial activity,



and easier to penetrate the cellular membrane of membrane to extract the intracellular ingredient of plant material (Shodikin, 2010 and Wang, 2010). Maceration was applicable to obtain plant extract that have antimicrobial substances. So the combination between ethanol and the maceration can give the best results from *Phaleria macrocarpa* fruits extract, and can give maximum inhibition zones of bacteria tested. Soniya et al. (2013) argued that the inhibition zones from this result depending on the type of extract, plant species, and bacterial tested.

*Phaleria macrocarpa* fruit contains flavonoid, tannin, and polyphenol compound. These compounds were shown antimicrobial activity against gram positive and negative bacteria. Hendra et al. (2011); Alara et al. (2016), reported the flavonoid of *Phaleria macrocarpa* fruits content of kaempferol, myricetin, naringin, quercetin and rutin. Its components might contribute to antimicrobial activity, with some mechanisms of action such as inhibition of nucleic acid synthesis, cytoplasmic membrane function and energy metabolisms leading to pathogens. Kirbis (2007) also explain that the mechanism action of bioactive compounds as antimicrobial is divided into four categories: inhibition of cell wall synthesis, damage to cell membrane function, inhibition of nucleic acid synthesis or function, and inhibition of protein synthesis.

Non pathogenic bacteria, *Lactobacillus sp.*, was more resistant to *Phaleria macrocarpa* fruits extract. *Lactobacillus sp.* is one of lactic acid bacteria; it's the important component group of intestinal microflora. Lactic acid bacteria can produce antimicrobial agents that have antagonistic activity to against many microorganisms, including pathogenic bacteria by production of antimicrobial substance such as organic acids, hydrogen peroxide and bacteriocins (Lonkar et al., 2005; Arokiyarny and Sivakumar, 2011). Arias et al. (2013) reported that *Lactobacillus* strain reduced of both pathogen strains *Salmonella typhimurium* and *Escherichia coli*. The mechanism action was by reduced pH and produced organic acids.

## Conclusion

The results of this research suggest that *Phaleria microcarpa* fruits extract is effective to inhibit pathogenic bacteria (*Escherichia coli* and *Salmonella sp.*) without affecting nonpathogenic bacteria (*Lactobacillus sp.*).

## Acknowledgments

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**Table 1.** Antibacterial activity of *Phaleria macrocarpa* fruit extracts

Bacteria	Diameter of inhibition zone (mm)				
	Control (-)	Control (+)	P1	P2	P3
<i>Escherichia coli</i>	0.0	17.7	3.7	3.6	2.3
<i>Salmonella</i> sp.	0.0	9.0	3.6	3.7	2.9
<i>Lactobacillus</i> sp.	0.0	17.7	0.0	0.0	0.0

Notes: Control (-): water, Control (+), tetracycline, P1: *Phaleria macrocarpa* fruit extracts with F:S ratio was 1:50, P2: *Phaleria macrocarpa* fruit extracts with F:S ratio was 1:100, P3: *Phaleria macrocarpa* fruit extracts with F:S ratio was 1:150

**Table 2.** Orthogonal contrast test of antibacterial activity of *Phaleria macrocarpa* fruit extracts

Contras between treatment	Statistics	
	<i>Escherichia coli</i>	<i>Salmonella</i> sp.
Control (-) VS Control (+);P1;P2;P3	**	**
Control (+) VS P1;P2;P3	**	**
P1 VS P2;P3	Ns	Ns

Notes: \*\*:  $p < 0.01$ , Ns: no significant

Control (-): water, Control (+), tetracycline, P1: *Phaleria macrocarpa* fruit extracts with F:S ratio was 1:50, P2: *Phaleria macrocarpa* fruit extracts with F:S ratio was 1:100, P3: *Phaleria macrocarpa* fruit extracts with F:S ratio was 1:150



**Session 5-Orchid ballroom I**

ANN-01-0050

**Development Strategies for Dairy Cattle Production System and Milk Products in Northeast of Thailand: Policy Framework and Challenges****Theerachai Haitook<sup>a\*</sup>, Samruay Ninking<sup>b</sup>, Phruetthinun Chukasem<sup>b</sup>, Wuttikorn Srakaew<sup>a</sup>, and Naritsara Suayroop<sup>a</sup>**

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**Abstract**

Dairy cattle production in the northeast of Thailand has been promoting for over 40 years. The productivity of the dairy cattle and products of milk had its progressed consequently, currently ranged the second largest productivity in the country. Northeast Region Dairy Cooperative Federation Limited, (NEDF) establishment aimed to strengthen the dairy cooperatives and their members 'capacity in increasing of self-reliance, improving of production system and related business to achieve the goal to be leader in dairy production in the country and ASEAN region. The development strategies plan was developed as the guideline to mainstreaming the development direction. There were 5 main development strategy issues: 1) organization development and collaboration 2) production development, 3) milk processing and products marketing improvement 4) Development of knowledge, research and technology transfer and 5) business and services unit development. The article aims to reflect the development needed and to advocate the plan as the policy development. The detail of strategies and the project were defined as the activities to be conducted to accomplishment the development missions and goal of dairy production system in the period of 2018-2022.

**Keywords:** dairy cattle, development strategies, northeast of Thailand, policy

**Introduction**

Dairy cattle farming in northeast of Thailand has been growing up in productivity, be a main income sources of farms and plays the important role on contribution in providing high quality of nutrient supplies improving of living quality of the people in the region. The establishment of Northeast Dairy Federation Limited (NEDF) in 2014 by the dairy cooperatives in the region aimed at a representative body in facilitating of development, therefore, plays as the linkage to the higher level of relate dairy cattle development institution in area of policy forming and supports the development intervention of the dairy cooperation and dairy farming (NEDF, 2014). However, NEDF is a new establishment organization, required a lot of supporting in improving it institutional capacity, including strategic plan development planning needed. NEDF hold the meetings and workshops to develop their own development strategies plan as a tool and guideline for the development and the policy advocacy. The paper aims to reflect the development interventions for further advocating as policy framework and guideline to enhance the industry dairy cattle production enterprise in the region.



## Materials and Methods

The dairy cattle development strategies plan undertook the bottom up approach of participatory development and policy making. The activities were conducted during the month of June-August 2017. The aims was to develop the dairy cattle development strategies in northeast of Thailand. The 26 dairy cooperatives chairman and officers in the region had participation in the workshops. The activities were conducted in Khon Kaen Province. The SWOT analysis was conducted to mapping the status of problem and constraints issues, situation and development need of the dairy cooperatives of the region. The planning method adopted the logical framework approach as a tool for working out the development strategies. The activities were supported from Provincial Cooperative Office and Dairy Co-operative Federation Limited of Thailand.

## Results and discussions

### Current status of dairy cattle development in the northeast of Thailand

Northeastern is the important region for dairy cattle production and high development potential. The expanding and intensification on dairy cattle farming has been increasing since after the establishment of the Dairy Farming Promotion Organization of Thailand (DPO) in 1960, in Muaklek District, Sara bury Province. In 1978, the government had policy to increase milk yield in the region from 50 tons per day 892 tons per day in 1988 (DLD, 1984). Later on the dairy farming was expanded to cover 16 provinces in the northeast such as Surin, Sisaket, Sakon Nakhon, Khon Kaen and Udorn Thani Province (Vongpralup et. al, 1992) the productivities the keep increasing in intensification up to the present. In 2015, the total number of dairy cattle in the region was 128,030 heads, consisted of 125,266 female dairy cattle with 55,691 milking cows (44.46% of total female), whereas the rest of 2,764 heads were male dairy cattle. The total number of dairy farmers was 4,048 households or 24.9% of total household raised dairy cattle in the countries. Total milk yield in the northeast was 815 tons per day or 24.8% of total country milk yield per day, the dairy production ranged for second among the region of the (Dairy cattle and milk products development committee, 2017).

### Role and function of Northeast Region Dairy Cooperative Federation Limited (NEDF)

The Northeast region Dairy Cooperative Federation Limited (NEDF) was established in 2014 (NEDF, 2014). The aim was the strengthening on promoting capacity of dairy cattle cooperatives to be partnership in carrying on business, be unity and support each other based on humanity principle for better socio-economic and living quality of members. The 26 dairy cooperatives in the northeast were the member. Under this, the total milk produces at the amount of 470.2 tons per day from approximately 35,000 milking cows of the total number of 80,678 dairy cattle, in 3,680 households (unpublished data). The NEDF developed their own by-law and agreement among the cooperatives and also sought for the collaboration and supporting for development from government and other. NEDF served as the representative of dairy cooperative on products management, selling milk and other supply purchasing, supporting in milk product processing, saving and loan services, policy advocating and promoting dairy farming.



### Policy framework of conceptual and development strategies of NEDF

Figure 1 demonstrates the conceptual framework as a roles and responsibility for industrial dairy cattle development in northeast region by NEDF. The development goal will be the achievement in dairy cattle production industrial in the ASEAN region. The northeast region exhibited its potential as a center for development. Dairy cattle farming, the occupation operating based under the forming of group or cooperative organization establishment, and community-based development.

Table 1 shows the results based plan of development strategies of NEDF. The organization requires a strong supporting from 26 cooperatives and Dairy Cooperative Federation Limited of Thailand, Milk board, government and other collaborations agencies ether within the country or oversea development agencies in order to strengthen their development capacity.



Figure 1. Conceptual framework of northeast region dairy farming cooperative

Table 1. The result oriented of development strategies framework and achievement goal

Goal: Dairy production systems in northeast of Thailand become a leader of dairy production industry in the region and be resilient to the changing environment	
Strategies issue 1. Development of the organization and collaboration	
Objective 1. Strengthens the organization management system capacity on dairy production industry and milk processing and advocating the policy related dairy development in the northeastern region	Output 1.1. Management capacity of northeast dairy cooperative federation limited increased
	Output 1.2. Advocated of development policy to nation development plan
	Output 1.3. The management capacity of all dairy cooperative in the region increased
	Output 1.4. Dairy cooperative member understanding on cooperative approach, their role and responsibility, development participatory and beneficiary increased
	Output 1.5. Effectiveness of management capacity of the dairy cooperative network increased
	Output 1.6. Participated to the Dairy Co-operative Federation of Thailand Limited, milk board on developing of national dairy development plan
Strategies issue 2. Dairy production system improvement	
Objective 2. Improved the dairy production system and farming	Output 2.1. Established driving forces on dairy farming system standard improvement
	Output 2.2. Increased of the efficiency of the replacement cow



standard based on national regulation policy	in to the herd
	<i>Output 2.3.</i> Increased feeding resources, feed and feeding managed improving
	<i>Output 2.4.</i> Effectiveness herd health management
	<i>Output 2.5.</i> Effectiveness milking system improvement
Strategies issue 3. Milk processing and products marketing	
Objective 3. Effectiveness raw milk management system, processing, products logistic and expanded markets	<i>Output 3.1.</i> On site of milk collecting system and well logistic transportation of raw milk
	<i>Output 3.2.</i> Raw milk processing and milk products management system
	<i>Output 3.3.</i> Established marketing system and increased milk product consumer
	<i>Output 3.4.</i> Demand of milk products of the neighboring country increased
Strategic issues 4. Research and development, knowledge management and transferring of technology	
Objective 4. Strengthening the capacity on research and development, technology transfer for effective development on industrial dairy farming system	<i>Output 4.1.</i> Effectiveness on knowledge management system on dairy production and management aspect
	<i>Output 4.2.</i> Established research and development unit for improvement of dairy production system and products
	<i>Output 4.3.</i> Established the training and consultancy unit for effectiveness on technology transferring and consultancy services
Strategic issue 5. Development of business unit services	
Objective 5. Strengthening the capacity on services unit on dairy farming and milk processing supply for minimizing cheaper price of all supplied items	<i>Output 5.1.</i> Established production unit of cow replacement
	<i>Output 5.2.</i> Establishment of a high quality dairy beef production, processing and marketing unit
	<i>Output 5.3.</i> Established the services on low cost completed diet supply
	<i>Output 5.4.</i> Established of mechanic, equipment and tool supplied for dairy farming

### Development challenge and target of improvement

The changing of world's dairy cattle industry affected to Thailand dairy cattle industry and its development direction. The development of dairy cattle based on the country's dairy cattle and dairy milk products development strategies. The development strategy plans for 2017-2026, which is based on government policy of "Thailand 4.0" approach, consensus to the twelfth national economic and social development plan. The dairy cattle and milk products development committee (2017) defined the key performance indicators for the successfulness in dairy cattle development were 1) the benefit and services accessible dairy farmers from the dairy farmer organization not less than 80%, 2) dairy farmers' income increasing 5% per years, the annual average of daily milk yield per cow increase 4%, all dairy farms in the country receive the Good Agriculture Practice (GAP) standard, raw milk quality pass the quality standard increase not less than 10% annually (somatic cell not more than 400,000cell/ml, solid excluded fat not less than 8.75%, fat not less than 3.75% and also microorganism not more than 300,000 CFU/ml), consumption rate of processed milk increase 4% per year which is from 14 l/caput/year to 20 l/caput/year. Therefore, the increasing of milk products per year at rate of 5%, including the



completing in establishment of data and information for the management center on dairy cattle industry

### **Resilient dairy cattle farming and sustainability**

The dairy farming sustainability could be accomplishment via the enhancement of resilient farming, institutional capacity and technological adoption capacity. Resilient dairy farming covers the arena of good farm management skills, adapted appropriated technology suited to society, natural resources and environment management, appropriate land uses and good infrastructure, and risk management. Dairy farmers have some weakness on all aspects but the most is capacity to cope with risks aspect that could affect the lost in production system. Farm management capacity need to be improved in the direction to farming standard of Good Agriculture Practice (GAP) to produce high quality of milk and safety food product. Improving of milk processing based on Good Manufacturing Practice (GMP) with diversity of products. NEDF need to establish the well balance of demand and supply via well establishment of supply chain of raw milk and milk products, also covering to the expanding marketing channel of products, etc. These will be future scenario of sustainable industrial dairy farming of the region.

### **Conclusions**

Dairy cooperatives and NEDF need to build up their capacity to perform all tasks to serve for better quality of cooperative member's livelihood, improving networking of collaboration, resources exchanging, etc. It requires supporting and collaboration for enhancing their intuitional and technical capacity on sustainable industrial dairy cattle production development. The strategic plan developed as policy framework to advocate being a part of national dairy cattle development plan for future sustainable dairy productivity.

### **Acknowledgements**

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## Beef Cattle Feeding Management of Smallholder Farmers in Kon Tum City, Vietnam

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### Abstract

This study reports a survey which was conducted to describe the cattle feeding and management practices of smallholder farmers in Kon Tum City, Vietnam. Forty-five farmers raising cattle in two sub-districts (Kroong and Vinh Quang), located in the capital city of Kon Tum Province were randomly selected for an interview. Most of farmers in Kroong (40.0%) raised crossbreed beef cattle, but about 41.3% of cattle in Vinh Quang were native breed. On average the interviewed farmers raised 4 cattle per household and most of them had cows aged older than 3 years which were mainly kept for fattening. Cattle feeding were mainly based on grazing (Kroong) and cut and carry (Vinh Quang) with concentrate supplemented at 12 kg/h/d. Farmers in both sub-districts relied native grasses and crop residues as common roughage source. Concentrate utilization in Vinh Quang included commercial and homemade concentrate, homemade concentrate were the most common used by small-holder farmer with utilization of local feed ingredients such as cassava chip, rice bran and boiled rice, whereas commercial feed were mainly used as a concentrate supplemented in Kroong. In conclusion, cattle management by smallholder farmers was assessed as very low in terms of management and feeding. Farmers still raise their cattle in the traditional way with local feed resources based on natural grasses and rice straw; to improve cattle production, development strategies for improved feeding systems has been recommended.

**Keywords:** cattle feeding, management, smallholder farmers, Vietnam

### Introduction

Livestock productions in Vietnam are predominantly raised in small-scale household production units. At present, smallholder producers supply the majority of meat in the market, which most of households operating individually in the production and marketing of livestock and livestock products (Lapar et al., 2003). In Vietnam, most rural households raised several livestock species including pigs, poultry and cattle, and some households had small fish ponds. Traditionally, cattle had been used for draught power and asset accumulation, and many smallholders now raised one to three cattle as part of a diversified smallholder livelihood. Cattle were raised to preserve cash: farmers bought cattle whenever cash was available and sold animals when funds for major expenses were needed. Thus, cattle were a cash reserve rather than a way of generating regular income for the family. Farmers grazed cattle on grass, herbs and



shrubs growing along road sides, fields and waterways, and in nearby forests. In intensively cropped lowland area, farmers supplemented grazing with freshly cut native grasses and crop residues such as rice straw. Over the past decades, beef cattle production in Vietnam has achieved a positive development trend. There has been a dramatic increase in cattle meat production chiefly due to numerous support strategies under cattle development programme for the whole country (Hoang, 2011). Although this reports an attractive opportunity for smallholder farmers, there are a number of obstacles that need to be addressed in order for the opportunity to be realized, particularly in relation to feed quantity and quality. Cattle production is constrained by limited resources, low fertility sandy soils, and harsh climatic conditions, including high temperatures, a long dry season and flooding in the wet season. Expanding cattle production is restricted by the limited quantity and quality of feeds, and poor husbandry practices leading to long calving intervals, high calf mortality rates, low growth rates, and consequently low cattle productivity and efficiency. Appropriate solutions must be based on understanding the current situation of beef production system (Parsons et al., 2013). Thus, the objective of this study was to describe beef cattle production and management practices of smallholder farmers in Kon Thum city, Vietnam.

## Materials and Methods

The survey was carried out in two sub-districts (Kroong and Vinh Quang), located in the capital city of Kon Tum Province (Fig.3). The individual farms participating in this project were selected based on beef cattle are mainly kept by smallholder farmer more than 2 head per farm, with the help of the chief of village and provincial animal husbandry officer of Kon Tum Province. Forty-five farmers raising cattle from each research site (total 90 farms) were randomly selected for the interview using a semi-structured questionnaire. The questionnaire was designed to understand cattle herd size and structure, husbandry practices and feeding management. All data from the survey were checked and transferred into the same unit of measurement. The quality variables were coded into categorical values, both qualitative and quantitative data, were stored in Windows Excel and analyzed using (SPSS package version 19).

## Results and Discussions

### Cattle herd size structure

According to the result of the survey, the most farmers in Kroong (40.0%) had crossbreed beef cattle (Brahman x Vietnamese Yellow) in their farm while native beef cattle (Vietnamese Yellow) (Fig.1) were the most common for farmers in Vinh Quang (41.3%). The percentage of crossbred cattle is an indicator of the level of intensity of animal husbandry (Le and Koops 2003). Moreover, there were 22.2% of famers in both studies area raised both crossbred and native beef cattle in their farm (Table 1). Farmer in both studies area raised on average 4.28-4.89 cattle per household (Table 2). Most household in Kroong had cows older than 3 years, but bulls older than 3 years were more important for farmers in Vinh Quang. Most farmers in both study areas were raised cattle for fattening purpose (66.7 and 42.2%). The rural famers in Vinh Quang were primarily kept 2 bull cattle for used as draught power (12%), while no famers in Kroong used cattle for draught power (Table 2). According to Knips (2004), cattle are also relatively common in peri-urban areas, where they are kept for draught purposes.

### Feeding management

The methods of cattle feeding by smallholder famers are shown in Table 3. In Kroong, the main method of cattle feeding was grazing (66.7%), but cut and carry was common feeding (97.7%) in Vinh Quang. However, famers in both studies area has been used concentrate as supplemented, and utilization of crop residue was the most extensive form of production such as



rice straw and maize stover (Fig. 2). Rice straw was the major and very important feed component for ruminants. In Vietnam, over 20 million tons of rice straw are produced and normally piled up and stored for many months (Ly, 1992). However, natural grass was the most common feed resource for cattle in both studies area. In Kroong, some famers had grown elephant grass (*Pennisetum purpureum*) for used as a roughage source and commercial feed were used as a main concentrate supplemented, however some farmers used commercial concentrate mixed with rice bran or boiled rice. On the other hand, natural grass was the most common for cattle in Vinh Quang and concentrate utilization including commercial and homemade concentrate. Homemade concentrate were mixed by using local feed ingredients such as cassava chip, rice bran and some famer has been used boiled rice. More than 50% of surveyed households in Kroong had extension training on livestock and crop production such as cattle, goat, pig, cassava and rice. Thus in Kroong cattle production is more intensive, relying on good quality feed resource, whereas Vinh Quang is primarily semi-intensive and extensive.

## Conclusion

The cattle production of small-holder farmers in Kon Tum City, Vietnam was assessed as very low in terms of feeding and management practices. Farmers raised their cattle in the traditional way with local feed resources based on natural grasses and rice straw. Therefore, the development strategies for smallholder beef cattle production need to consider the interaction between livestock and availability of local feed resources in order to be effective and sustainable.

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A



B

Fig.1 Native beef cattle (Vietnamese Yellow, A) and crossbred beef cattle (Brahman x Vietnamese Yellow, B)



A



B

Fig. 2 Utilization of rice straw (A) and maize stover (B) as a roughage source



A



B

Fig. 3 Small-holder farmers in Kroong (A) and Vinh Quang (B)

**Table 1.** Cattle breed and production purpose of surveyed households

Items	Kroong (n=45)		Vinh Quang (n=45)	
	Frequency	Percent (%)	Frequency	Percent (%)
Beef cattle breed				
Crossbreed	18	40.0	16	35.6
Native	17	37.8	19	42.2
Crossbreed + Native	10	22.2	10	22.2
Purpose				
Breeding	10	22.2	4	8.9
Fattening	30	66.7	19	42.2
Caw calves for sale	5	11.1	10	22.2
Draught power	0	0	12	26.7

**Table 2.** Average number of difference type of cattle rising per household

Type of cattle	Kroong (n=45)	Vinh Quang (n=45)
	Mean $\pm$ SD	Mean $\pm$ SD
Bulls (> 3 years)	1.00 $\pm$ 1.96	1.82 $\pm$ 1.26
Young Bulls (0.5 – 3 years)	1.22 $\pm$ 1.42	0.60 $\pm$ 1.05
Male Calves (0 – 0.5 years)	0.40 $\pm$ 0.83	0.26 $\pm$ 0.61
Cows (> 3 years)	1.60 $\pm$ 2.91	0.68 $\pm$ 0.97
Heifer (0.5 – 3 years)	0.48 $\pm$ 0.99	0.57 $\pm$ 1.07
Female Calves (0 – 0.5 years)	0.24 $\pm$ 0.64	0.35 $\pm$ 0.74
Total	4.89	4.28

**Table 3.** Feeding management of surveyed households

Items	Kroong		Vinh Quang	
	Frequency	Percent (%)	Frequency	Percent (%)
Roughage utilization				
Grazing	30	66.7*	27	58.7*
Cut and carry	27	60.6*	44	97.7*
Crop residue	33	73.3*	41	91.1*
Combination of roughage utilization (n =45)				
Grazing	8	17.8	1	2.2
Cut and carry	1	2.2	2	4.4
Grazing, Cut and carry	3	6.7	1	2.2
Grazing, Crop residue	10	22.2	11	24.4
Cut and carry, Crop residue	14	31.1	16	35.6
Grazing, Cut and carry, Crop residue	9	20.0	14	31.1
Concentrate utilization (n =45)				
Commercial	32	71.1	13	28.9
Homemade	4	8.9	11	24.4
Not used	9	20.0	21	46.7

\* = % of combination of roughage utilization



## Formaldehyde Protected Soybean Meal in Total Mixed Ration for Kacang Goat to Increase the Production

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### Abstract

Kacang goats are one of indigenous goats in Indonesia raised traditionally in rural areas. The goats are grazed and fed natural grass, therefore the productivities are low. Kacang goats had improved their performance using soybean meal (SBM) and fish meal in the ration, but they preferred SBM to fish meal. This research investigated Kacang goat fed SBM protected with 1% of formaldehyde to increase undegradable protein in the rumen, so that the productivity of goat would increase. Fourteen heads of yearling Kacang buck, 15.8-19.8 kg (average: 17.6 kg) were arranged in completely randomized design. The treatments were control (n=5): untreated SBM; SBM50 (n=5): 50% untreated SBM + 50% formaldehyde-protected SBM; SBM100 (n=4): 100% formaldehyde-protected SBM. The rations consisted of 30% *Pennisetum purpureum*, 30% *gliricidia* leaves, 19.2% cassava waste product, 13.8% wheat bran, 7% SBM, 1% mineral mix were mixed containing 14-15% crude protein and 56-60% TDN. The average daily gain (ADG) was calculated using linear regression of 94 days body weights. Data were analyzed by one way ANOVA using SPSS statistics software version 19. Kacang goat did not like treated SBM, therefore differences of their intakes were reflected to their ADG. The ADG and slaughtered weight of control goats was the highest (78.9 g and 25.1 kg), while those of SBM100 goats (56.5 g and 23,0 kg) were relatively the same as SBM50 goats (41.5 g and 21.7 kg). Carcass weights of control goats (11.6 kg) were higher than those of SBM50 goats (9.5 kg), but both of them were similar to those of SBM100 goats (10.1 kg). Carcass percentages were similar between the treatments those were 46.2% (control) and 43.8% (SBM50 and SBM100). The meat+fat:bone ratio and meat:bone ratio was similar between the treatments (averages: 4.0 and 3.6). The physico-chemical quality of the chevon were similar, those were pH: 6.0, WHC: 39.8%, cooking loss: 37.2%, tenderness: 6.8 kg/cm<sup>2</sup>, while moisture, protein, fat, and collagen content were 72.8%, 21.6%, 2.6% and 1.9% respectively. The productivity of Kacang goat can be improved using untreated SBM that had better palatability.

**Keywords:** carcass, chevon quality, daily gain, Kacang goat, SBM



## Introduction

Kacang goats are one of indigenous goats in Indonesia raised traditionally in rural areas. The goats are grazed and fed natural grass, therefore the productivities are low. Kacang goats had improved their performance using soybean meal (SBM) and fish meal in the ration. In fact, the dry matter intake (DMI) of ration containing SBM was higher than those of ration containing fish meal (Adiwinarti *et al.*, 2016).

Dry matter digestibility of soybean meal supplemented in rice straw diet for goat was 59.5% (Darlis *et al.*, 2000). Formaldehyde has been used to decrease the digestibility of SBM in the rumen (Suhartanto *et al.*, 2014). Suhartanto *et al.* (2014) reported that *in vitro* dry matter degradability of SBM was 89.9%, while those of formaldehyde-protected SBM were 52.3% (0.5% formaldehyde) and 35.3% (1% formaldehyde). This research investigated Kacang goat fed SBM protected with 1% of formaldehyde to increase undegradable protein in the rumen, so that the productivity of goat hopefully would increase.

## Materials and Methods

Fourteen heads of yearling Kacang buck, 15.8-19.8 kg (average: 17.6 kg) were arranged in completely randomized design. The treatments were control (n=5): untreated SBM; SBM50 (n=5): 50% untreated SBM + 50% formaldehyde-protected SBM; SBM100 (n=4): 100% formaldehyde-protected SBM. The rations consisted of 30% *Pennisetum purpureum*, 30% *gliricidia* leaves, 19.2% cassava waste product, 13.8% wheat bran, 7% SBM, 1% mineral mix were mixed containing 14-15% crude protein and 56-60% TDN. Soybean meal was protected using 1% of formaldehyde that was calculated based on dry matter SBM. The average daily gain (ADG) was calculated using linear regression of 94 days body weights. Goats were weighed and slaughtered after being fasted for 12 h with free access of drinking water. The goats were slaughtered as Pratiwi *et al.* (2007), but carcasses in this research included kidney and fat around the kidney. *Biceps femoris* muscles were used for physical and chemical quality. Physical quality of chevon included pH, water-holding capacity, cooking loss, and tenderness using Warner-Bratzler shear force value were observed based on Shirima *et al.* (2013) and Moawad *et al.* (2013). Chemical quality of chevon included water, fat, protein content, and collagen were determined using near infrared spectroscopy (NIRS) (Prevolnik *et al.*, 2004). Data were analyzed by one way ANOVA using SPSS statistics software version 19.

## Results and Discussion

### The average daily gain, carcass, and meat production

The average daily gain of the goats was influenced by feed intake. The dry matter intake (DMI) of SBM50 goats (541.1 g) were lower ( $P < 0.01$ ) than those of control goats (701.3 g), however the DMI of SBM50 goats and control goats were not different significantly ( $P > 0.05$ ) from SBM100 goats (606.9 g). This indicated that Kacang goat did not like formaldehyde-treated SBM mixed with untreated SBM. Differences of their intakes were reflected to their ADG, slaughter weight, carcass weight, and meat weight. The ADG and slaughtered weight of control goats was the highest, while those of SBM100 goats were relatively the same as SBM50 goats (Table 1). The carcass and meat weight of SBM50 goats were lower than those of control goats. However, the carcass and meat weight of SBM50 goats and control goats were not different significantly ( $P > 0.05$ ) from SBM100 goats (Table 1 and Table 2). The ADG of goats fed untreated SBM (control) was higher than those of Kacang goats reported by Restitrisnani *et al.* (2013): 23.5 to 69.4 g. However, Rahman *et al.* (2014) reported that crossbred Boer goat fed soy waste product and *Pennisetum purpureum* had weight gain of 80.2 g/d. The carcasses weights of Kacang goats reported by Gafar *et al.* (2013) were 10.7 to 12.2 kg.



Carcass percentages were similar between the treatments those were 46.2% (control) and 43.8% (SBM50 and SBM100). This indicated that the high percentage of carcass was influenced by the high carcass weight and slaughter weight. Carcass percentages of Kacang goats in this study were lower than those of Kacang goats reported by Gafar *et al.* (2013): 53.3 to 56.8%, but relatively the same as those reported by Hutama (2014): 46.7%, and higher than those reported by Sumardianto *et al.* (2013): 40.9% or Adiwiniarti *et al.* (2015): 38.8%. Differences of carcass percent were caused by the different feeding management system.

**Table 1.** The average daily gain, slaughter weight, carcass weight, and carcass percentage

Parameters	SBM (control)	SBM50	SBM100
ADG (g)	78.9 <sup>Aa</sup>	41.5 <sup>B</sup>	56.5 <sup>Bb</sup>
Slaughter weight (kg)	25.1 <sup>Aa</sup>	21.7 <sup>B</sup>	23.0 <sup>Bb</sup>
Carcass weight (kg)	11.6 <sup>A</sup>	9.5 <sup>B</sup>	10.1 <sup>AB</sup>
Carcass percent (%)	46.2	43.8	43.8

<sup>A,B</sup> Row means with different superscripts differ significantly at  $P < 0.01$ , while <sup>a,b</sup> row means with different superscripts differ significantly at  $P < 0.05$ .

The meat+fat:bone ratio and meat:bone ratio was similar between the treatments (Table 2). The average of meat+fat:bone ratio was 4.0 and those of meat:bone ratio was 3.6. Meat:bone ratio of Kacang goat in this study was higher than those reported by Sumardianto *et al.* (2013): 2.6 or Adiwiniarti *et al.* (2015): 2.2.

**Table 2.** Carcass components and meat-bone ratio

Parameters	SBM (control)	SBM50	SBM100
Meat (kg)	8.1 <sup>a</sup>	6.5 <sup>b</sup>	7.2 <sup>ab</sup>
(%)	71.1	69.6	73.1
Fat (kg)	1.1	0.9	0.7
(%)	9.5	9.2	7.4
Bone (kg)	2.2	2.0	1.9
(%)	19.4	21.2	19.5
Meat+fat-bone ratio	4.2	3.7	4.2
Meat-bone ratio	3.7	3.3	3.8

<sup>A,B</sup> Row means with different superscripts differ significantly at  $P < 0.01$ , while <sup>a,b</sup> row means with different superscripts differ significantly at  $P < 0.05$ .

The physical and chemical quality of the chevon were similar, the averages were pH: 6.0, WHC: 39.8%, cooking loss: 37.2%, tenderness: 6.8 kg/cm<sup>2</sup>, while moisture, protein, fat, and collagen content were 72.8%, 21.6%, 2.6% and 1.9% respectively (Table 3). The pH of chevon in this study was lower than those reported by Adiwiniarti *et al.* (2015) in Kacang goats managed traditionally (pH: 6.3). The high pH caused the high WHC as Judge *et al.* (1989) stated that the higher pH (5.2 to 6.8), the more protein bound water, and the higher WHC. The tenderness was similar between the treatments because the collagen contents were also the same.

The water content was influenced by the fat content. High water content in this study might be caused by low fat content of the chevon as reported by Mirdhayati *et al.* (2014) and Hwangbo *et al.* (2009). Mirdhayati *et al.* (2014) reported that water content of Kacang goats was 73.8 to 74.5% and the fat content was 0.4 to 0.5%, while Hwangbo *et al.* (2009) reported that moisture content of Korean Black goat fed total mixed rations was 74.3 to 74.8% and the fat content was 1.4 to 1.7%. The protein content (21.0 to 22.2%) was lower than those reported by Mirdhayati *et*



*al.* (2014): 23.2 to 23.5%, but it were relatively the same as reported by Hwangbo *et al.* (2009): 21.7 to 22.5%, and higher than those reported by Adiwintarti *et al.* (2015): 19.6 to 19.7%.

**Table 3.** Physical and chemical quality of the chevon

Parameters	SBM (control)	SBM50	SBM100
Physical quality:			
pH	6.0	6.0	6.0
WHC (%)	39.1	40.2	40.4
Cooking loss (%)	37.1	38.0	36.3
Tenderness (kg/cm <sup>2</sup> )	6.9	6.8	6.8
Chemical quality:			
Moisture (%)	72.3	72.9	73.2
Protein (%)	22.2	21.5	21.0
Fat (%)	2.8	2.5	2.3
Collagen (%)	1.9	2.0	2.0

<sup>A,B</sup> Row means with different superscripts differ significantly at  $P < 0.01$ , while <sup>a,b</sup> row means with different superscripts differ significantly at  $P < 0.05$ .

## Conclusion

The productivity of Kacang goat can be improved using untreated SBM that had better palatability.

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## Effects of Different Tropical Grasses on Feed Intake and Blood Metabolite of Goats

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### Abstract

This experiment was designed to determine the effect of roughage sources of tropical grasses on feed intake and blood metabolite of goats. Four goats with average liveweight of 21±1 kg were randomly assigned according to a 4x4 Latin square design to receive four total mixed rations (TMR) containing native grass (NG), tropical grass (TCG), ruzi grass (RG), and whip grass (WG) as roughage source. TMR was offered on *ad libitum* basis. Based on this experiment, there were no significantly differences ( $P>0.05$ ) among treatments regarding DM intake. Likewise, mean temperature, ruminal pH, BUN, glucose, and PCV concentrations were not affected ( $P>0.05$ ) by dietary treatments.  $\text{NH}_3\text{-N}$ , however, appeared to be significantly lowered ( $P<0.05$ ) in NG treatment when compared to others. Based on this study, rumen fermentation and blood metabolite of goats did not affected by grass type in TMR.

**Keywords:** roughage sources, tropical grasses, feed intake, blood metabolite, goats

### Introduction

Roughages are the main feed source and therefore important for ruminant production. Feeding of such low quality forages adversely affects the intake and digestibility of animals adversely and thereby reduces performance of livestock production in the tropical areas (Wanapat, 1999). This is particularly important during the dry season where availability and quality of forage often become severely limited. Because of during the dry season, grasses and legumes stop growing so the farmers need to find alternative roughages for their animals. The most promising forage grasses in the tropical areas are pangola (*Digitaria eriantha* Steud., synonym *D. decumbens*), Napier (*Pennisetum purpureum*), Ruzi (*Brachiaria ruziziensis*), Para (*Brachiaria mutica*), Purple guinea (*Panicum maximum* TD 58), Atratum (*Paspalum atratum*), Mulato (*Brachiaria hybrido* cv. Mulato), Nile (*Acroceras macrum*), and native grasses such as tropical carpet grass (*Axonopus compressus*), whip grass (*Hemarthria compressa*) as a supplemental energy source. However, roughages produced in tropical areas for goat, sheep, beef and dairy cattle have great variability in chemical composition and nutritive value compared with roughages produced in temperate areas. Moreover, studies comparing intake and blood metabolite of animals feeding on these native roughages are limited and still need to be determined. Therefore, our objective was to study the effect of these roughage sources produced



in a tropical environment on intake, ruminal parameters, and blood metabolites of crossbred goats.

## Materials and Methods

### Animals, treatments, and experimental design

Four male crossbred (Thai native x Anglo Nubian) goats at ages about 12 months old with  $21.0 \pm 1.0$  kg body weight were randomly assigned according to a 4x4 Latin square design to investigate the effect of these roughage sources produced in a tropical environment. The four roughage sources were as follows: mixed native grass (MNG), tropical carpet grass (TCG, *Axonopus compressus*), ruzi grass (RG, *Brachiaria ruziziensis*), and whip grass (WG, *Hemarthria compressa*). These were harvested, chopped into small pieces (2-3.0 cm) and collected sample at  $45 \pm 2$  days of regrowth. All harvested material was weighted, sun-dried for 3-5 days, and stored for subsequent feeding. The four experimental diets were formulated in roughage: concentrate ratio of 40:60 as total mixed ration diet. The diets were formulated to provide the nutrient allowances to meet or exceed the NRC (1981) requirements of growing goats (Table 1).

All goats were kept individually in pens (0.50x1.20m) under well-ventilated sheds where water and mineral salt were available at all time. The experiment was conducted for 4 periods, and each period lasted for 21 days. During the first 14 d of each period, all animals were fed by respective diets for *ad libitum* intake, whereas during the last 7 d, the animals were moved to metabolism crates for total collection during the time goats with restriction to 90% of the previous voluntary feed intake to ensure total feed intake. Feeds were provided twice times in two equal portions daily at 0800 and 1600 h. For determination of daily DMI, refusals were collected and weighed daily before feeding. Feed samples obtained each time were oven dried at 60°C for 72 h, grounded to pass through a 1-mm sieve, and composited by period on an equal weight basis, and analyzed for DM, ether extract, ash, and CP content (AOAC, 1995). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed by the technique of Van Soest et al. (1991). Goats were individually weighed before the morning feeding at the beginning and ending of each experimental period. At the end of each period, rumen fluid was collected from all goats by using a stomach tube at 0 and 4 h-post feeding during the digestibility trial. Rumen fluid was strained through 4 layers of cheese cloth and immediately measured for pH using a pH meter (HANNA instruminals HI 98153 microcomputer pH meter, Singapore) fitted with a combined electrode. The ruminal fluid was then acidified with 3 mL of 1 M H<sub>2</sub>SO<sub>4</sub> added to 30 mL of ruminal fluid. The mixture was centrifuged at 16,000×g for 15 min, and the supernatant was stored at -20°C before NH<sub>3</sub>-N analysis by using the micro-Kjeldahl methods (AOAC, 1995). A total of approximately 10 mL of blood samples were collected from a jugular vein at the same time as ruminal fluid sampling and added into tubes containing 12 mg of EDTA. Plasma was separated by centrifugation at 2500×g for 15 min at 5°C and stored at -20°C until analysis. Plasma glucose and packed cell volume (PCV) were measured by using commercial kits (No. 640, Sigma Chemical Co., St. Louis, USA). All data were subjected to the analysis of variance by using Proc. GLM and treatment means were calculated and compared by using Duncan's New Multiple Range Test at significance level of  $\alpha = 0.05$ .

## Results and discussion

The results showed that (Table 2) overall mean of feed intakes of each goat in terms of total DMI (%BW, and g/kg BW<sup>0.75</sup>) were not significantly ( $P > 0.05$ ) affected by dietary treatments, ranging from 2.97 to 3.04% BW. Similarly, William et al. (2013) reported that crossbred East African x Norwegian goat had voluntary feed intake 2.6-3.2% and 2.9-3.2% BW in goats Hararghe Hihgland (Wallie et al., 2012). These data indicate that tropical roughages



have positive effects on feed intake. This could imply that DMI was potentially regulated by the energy density and nitrogen of the diet.

**Table 1.** Ingredients and chemical composition of experimental diets (% DM basis).

Item	Treatments <sup>1</sup>			
	MNG	TCG	RG	WG
Native grass	40.0	-	-	-
tropical carpet grass	-	40.0	-	-
Ruzi grass	-	-	40.0	-
Whip grass	-	-	-	40.0
Ground corn	39.2	39.2	39.2	39.2
Soybean meal	7.2	7.2	7.2	7.2
Fish meal	0.4	0.4	0.4	0.4
Leucaena leave meal	5.0	5.0	5.0	5.0
Palm kernel cake	5.0	5.0	5.0	5.0
Molasses	2.1	2.1	2.1	2.1
Dicalcium phosphate	0.4	0.4	0.4	0.4
Salt	0.2	0.2	0.2	0.2
Mineral and vitamin Mix <sup>2</sup>	0.5	0.5	0.5	0.5
Nutrient content, % DM				
DM <sup>3</sup>	85.49	85.18	85.63	85.49
Ash	6.98	6.42	6.45	6.19
OM	93.02	93.58	93.55	93.81
CP	14.05	14.52	14.35	14.87
EE	2.65	2.95	2.78	3.05
NDF	50.85	47.61	48.46	46.69
ADF	23.89	22.44	25.11	23.8

<sup>1</sup>Treatments: mixed native grass = MNG; tropical carpet grass = TCG; ruzi grass = RG; whip grass = WG; average CP = 7.21, 11.52, 7.97, and 12.52%; average NDF = 78.9, 75.4, 74.5, and 68.9%; average ADF = 37.2, 37.9, 38.5, and 36.5%, respectively, DM basis.

<sup>2</sup>Minerals and vitamins (each kg contains): Vitamin A: 10,000,000 IU; Vitamin E: 70,000 IU; Vitamin D: 1,600,000 IU; Fe: 50 g; Zn: 40 g; Mn: 40 g; Co: 0.1 g; Cu: 10 g; Se: 0.1 g; I: 0.5 g.

<sup>3</sup>DM: dry matter; OM: organic matter; CP: crude protein; EE: ether extract; NSC: non-structural carbohydrate; NDF: neutral detergent fiber; ADF: acid detergent fiber; ADL: acid detergent lignin.

**Table 2.** Intake data of goats fed on mixed native grass, tropical carpet grass, ruzi grass, and whip grass based diets.

Item	Treatment <sup>1</sup>				SEM <sup>2</sup>
	MNG	TCG	RG	WG	
Total DMI, kg/d	0.70	0.69	0.69	0.69	0.02
DMI, %BW	3.04	2.97	2.98	3.08	0.14
DMI, g/kg W <sup>0.75</sup>	66.50	65.26	65.37	66.96	3.02

<sup>1</sup>Treatments: mixed native grass = MNG; tropical carpet grass = TCG; ruzi grass = RG; whip grass = WG.

<sup>2</sup>SEM = Standard error of the mean (n=4).



In the present study, ruminal temperature, pH, and BUN were similar across treatments and the values were in the range of 39.20 to 39.31 °C, 6.35 to 6.46, and 11.69 to 15.24 mg/dL, respectively, which also are the optimal levels of those parameters for microbial activity and digestion of protein (6.0-7.0) (Russell and Wilson, 1996). Although not statistically different from TCG, RG, and WG, a reduction of BUN in NG compared with other treatments was observed. Whilst NH<sub>3</sub>-N was significantly ( $P < 0.05$ ) influenced by the treatments with goat fed with NG having significantly lower value of 6.96 mg/dL as compared with other treatments. Increased rumen NH<sub>3</sub>-N level resulted in increased BUN level across all treatments. It has been reported that NH<sub>3</sub>-N and BUN concentration were closely related and that when dietary crude protein percentage increased, concentration of BUN and solubility and degradability of protein in the rumen of cattle also increased (Cressman et al., 1980). Therefore, the differences in ruminal NH<sub>3</sub>-N and BUN concentration across treatments indicated in this study may have been related directly to crude protein level and the extent of crude protein degradation of each feed. No significant effect ( $P > 0.05$ ) of dietary treatments was detected for blood glucose and PCV, with the values were within the normal ranges of 50-75 mg/dL and 22-38 mg/dl, respectively (Lloyd, 1982).

**Table 3.** Blood metabolites of goats fed mixed native grass, tropical carpet grass, ruzi grass, and whip grass based diets.

Item	Treatment <sup>1</sup>				SEM <sup>2</sup>
	MNG	TCG	RG	WG	
Temperature, °C	39.31	39.30	39.20	39.25	0.17
Ruminal pH	6.41	6.41	6.35	6.46	0.03
NH <sub>3</sub> -N mg/dL	6.96 <sup>b</sup>	10.89 <sup>a</sup>	10.71 <sup>a</sup>	8.92 <sup>ab</sup>	1.07
BUN, mg/dL	11.69	15.24	12.87	14.01	0.89
Glucose, mg/dL	67.37	64.87	66.25	68.37	2.09
PCV, %	26.87	28.37	28.75	27.62	0.33

Means within rows followed with different superscript letters are statistically different ( $P < 0.05$ ).

<sup>1</sup>Treatments: mixed native grass = MNG; tropical carpet grass = TCG; ruzi grass = RG; whip grass = WG.

<sup>2</sup>SEM = Standard error of the mean (n=4).

NH<sub>3</sub>-N = Ammonia nitrogen; PCV = Packed cell volume; BUN = Blood-urea nitrogen.

## Conclusion

Based on the results of this experiment, it could be concluded that the use of NG, TCG, RG, and WG as roughage sources had no effects on feed intake and blood metabolites of goats. However TCG, RG, and WG improved rumen fermentation mainly by increasing the high level NH<sub>3</sub>-N and BUN. However, further researches on feeding trial of roughage sources are recommended to investigate its effects on animal performances and production such as meat and milk.

## Acknowledgements

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## Application of Tunnel-Ventilated Barn in Tropical Dairy Industry: A Review

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### Abstract

Dairy cow cannot dissipate excessive body heat efficiently under the tropical climate which leads to heat stress. Apart from that, this paper investigates the usefulness of tunnel-ventilated barn in tropical dairy industry. Tunnel ventilation technology is technique used to alleviate the impact of heat stress in dairy cattle significantly. Tunnel ventilation barns work to enhance convective heat loss by removing excess heat and humidity from the immediate surroundings of the animals. Sustainability of tunnel ventilation in the dairy industry is determined by using aspects (economic, social and ecological). In conclusion, the tunnel-ventilated barn is suitable for tropical dairy industry regard to mitigating heat stress as environmental effects of tropics.

**Keywords:** tunnel ventilation, climate, heat stress, dairy industry, sustainability

### Introduction

Dairy housing is an important component in the tropical dairy industry and plays a vital role in dairy cattle performances. West (2003) found that dairy cow cannot dissipate sufficient body heat in tropics that leads to heat stress. Temperature-humidity index (THI) is commonly used as an indicator of cow heat stress designed for dry environments (Armstrong, 1994). Chen et al., (2009) labelled heat stress when THI is less than 72. Hahn (1999) reported, the response to increased heat load in cows includes increased respiration rates. Dairy cows panted 54 times/minute under heat stress condition in subtropics (Shiao et al., 2011) and may be higher in tropics. However, they are not always conducive to production (Smith et al., 2006a).

Techniques used to alleviate the impact of heat stress in dairy cattle include shade, fans, sprinklers (Chan et al., 1997). This is greatly facilitated by the use of tunnel ventilation technology (Smith et al., 2006a). In a randomised controlled study of milk production, Shiao et al., (2011) reported that cows in the tunnel-ventilated barn produced 3% more milk than cows in the conventional free-stall barn. Smith et al., (2006b) also reported that tunnel ventilation increased milk yield over the 10-wk trial by  $2.8 \pm 0.19$  kg/cow per day in 2003. De Paepe et al., (2012) illustrated the standard model of tunnel-ventilated barn of the International Centre for Eremology (I.C.E.), Ghent University, Belgium in Fig. 1. A detailed description of the I.C.E. tunnel-ventilated barn is given by Cornelis et al., (2004). This paper will discuss the tunnel-ventilated barn in ecology, social, and economic perspective.



### Tunnel Ventilation System

Nienaber and Hahn (2007) divided ventilation system into two types: natural ventilation system and tunnel ventilation system. They labelled natural ventilation system as a barn without fans, while fans are set in tunnel ventilation system. The tunnel ventilation system is primarily set to maintain an appropriate and stable indoor environment for animals and also worker (Rong et al., 2017).

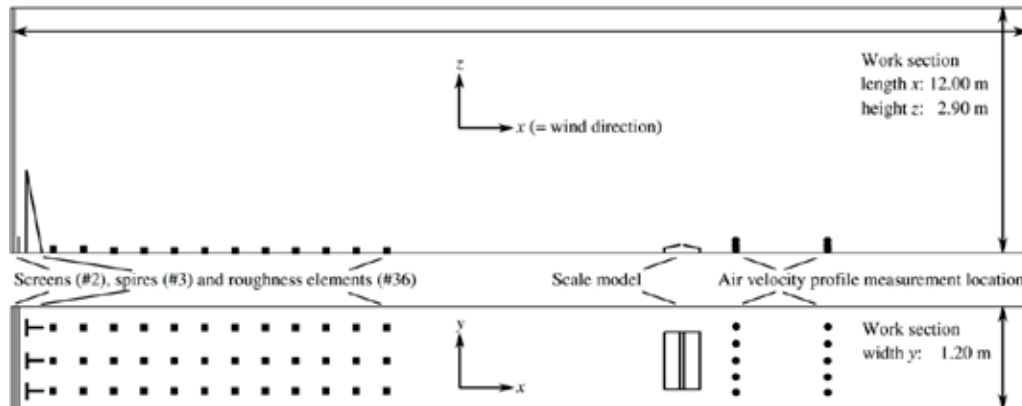


Fig 1. Longitudinal cross section and top view of the wind tunnel work section, with  $x = 0$  m at the far left and the scale model placed at  $x = 7.67$  m. The filled circles denote the positions where air velocity profiles were registered at  $x = 8.51$  m and  $x = 9.59$  m.

West (2003) investigated the model of tunnel ventilation using evaporative cooling, fans with injection of high pressure mist, and combinations of cooling over feed bunks and free stalls. Unlike West, Smith et al., (2006b) demonstrated the model of tunnel ventilation as containing feed bunks, water tanks, free-stall bedding areas, controlled lighting, and waste management flush. The  $27.5 \times 9.2$  m barn was outfitted with two 7.6-m evaporative cooling cells at one end and four 1.2m, 1-horsepower exhaust fans at the other. The pumps used to cycle water over the cooling cells which can set the temperature.

### Sustainability Assessment on Tunnel Ventilation in Tropical Dairy Industry

Sustainability in dairy industry is determined by using aspects (economic, social and ecological). Difficulty for determining the sustainability of farming systems is the combination of the different attribute measures into a sustainability function (Van Calker et al., 2006). Decomposition of overall sustainability into aspects and attributes is shown in Table 1.

Table 1. Decomposition of overall sustainability of dairy farms into aspects and attributes (based on Van Calker et al., 2005)

No.	Sustainability Aspects			
	Ecological	External Social	Internal Social	Economic
1	Eutrophication	Food safety	Working condition	Profitability
2	Groundwater quality	Animal welfare		
3	Dehydration of the soil	Animal health		
4	Acidification	Landscape quality		
5	Global warming			
6	Ecotoxicity			



### **Ecological sustainability**

Environmental effects always become the first attention when the dairy farm is built. Van Calker et al., (2008) demonstrated the indicators of each ecological attributes. Signs of eutrophication, groundwater quality, dehydration of the soil, acidification, global warming, and ecotoxicity are eutrophication potential ( $\text{NO}_3$  equiv./ha), nitrate concentration in groundwater ( $\text{NO}_3$  mg/l), water use (m<sup>3</sup>/ha), acidification potential ( $\text{SO}_2$  equiv./ha), global warming potential ( $\text{CO}_2$  equiv./1000 kg milk) and eco-toxicity (1,4 dichlorobenzene equiv./ha), respectively. In this paper, I argue that ecological effect of tunnel-ventilation is based on building materials of the barn. However, no single study exists which explains specific environmental effects of tunnel-ventilated barn related to building materials although extensive research has been carried out on tunnel ventilation system.

### **External social sustainability**

Curtis (2007) found that improvements in animal welfare are often used to justify to the public modern husbandry methods or new cattle housing. Manteca and Jones (2013) reported that, on the farm, some of the main welfare issues are related to neonatal mortality. Neonatal mortality was here defined as between 3 d and 1 mo of age; this definition seems consensual (Heinrichs and Radostits, 2001).

Some studies has reported that cows in the temperate climate have lower mortality rate than in the tropics. Among 4,839 births in Sweden, mortality was 2.6 and 0.7% for the 0 to 90-d period (excluding stillbirth) and the 1 to 7-d period, respectively (Olsson et al., 1993). Among 4,097 live-born Iranian calves, 6.5% died within 90 d (Azizzadeh et al., 2012). However, Ako (2013) labelled the standard of neonatal mortality is 3%.

Tunnel ventilation system only takes a part in cooling dairy cow. It is not yet clear whether the calves are reared in tunnel-ventilated barn. There is some evidence that the presence of other cows, the restriction of movement, and the impossibility to perform regular nesting behaviour may negatively affect the cow's welfare.

### **Internal social sustainability**

Humans are an important part of the cow's environment, both directly when working with them or being in close proximity and indirectly via management decisions on housing design (Velarde et al., 2015). Tunnel ventilation system as dairy housing ensures the optimum environment for cows and workers. However, the tunnel ventilation system is far apart from stockmanship. Waiblinger and Spoolder (2007) demonstrated that the term 'stockmanship' covers the way in which animals are handled, the quality of their daily management and the health care.

### **Economic sustainability**

Tunnel-ventilated barn as a mechanical ventilation is a relatively high energy consumption (Weeks, 2008) and can also bring about higher production cost leading to higher milk price for consumers. However, typical dairy industry in tropics gives a regular income to industry workers as milk is produced every day (Moran, 2005). Schelhaas (1999) found that its high cost





necessitates its use for making products with high value. In fact, tunnel-ventilated barn had no effect on milk composition (Smith et al., 2006b), but increased milk yield significantly.

Economically, an analysis of all associated cost for employing tunnel ventilation shows that payback, measured in sustained milk production, is achievable, especially for longer barns (Gooch and Stowell, 2003). Van Calker et al., (2008) labelled net farm income as the sign of profitability. However, the research to date has tended to focus on the physiological effects rather than the economic accounting of tunnel ventilation system.

## Conclusion

The tunnel-ventilated barn is suitable for tropical dairy industry regard to mitigating heat stress as environmental effects of tropics. The model of tunnel-ventilated barn of every research conducted is relatively similar. This study has highlighted the physiological implications of the barn and the efficiency in cow's productivity, land-use, and working activity.

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## Genetic Polymorphisms of Alpha<sub>S1</sub>-Casein (CSN1S1) Gene in Indonesian Local Goat Population Reared in South Sulawesi Province

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### Abstract

Alpha<sub>S1</sub>-Casein gene is a gene that controls the quality of the milk protein. Alleles of Alpha<sub>S1</sub>-Casein gene can be grouped into four levels of expression for the quality of the protein in milk, which is high (A, B1, B2, B3, B4, C, H, L and M), intermediate (E and I), weak (D, F and G) and null alleles (O1, O2, and N). The purpose of this study was to characterize Alpha<sub>S1</sub>-Casein gene in Indonesian Local goats by knowing allele frequencies and heterozygosity values by using PCR-RFLP techniques using XmnI restriction enzyme. This study found 2 alleles, ie. An allele with frequencies of 0.94, and B allele with 0.06. These two alleles were included in the group of high expression casein (high protein quality). In the local goat population, H<sub>o</sub> values obtained were 0.223 and H<sub>e</sub> was 0.376. The results showed that the genetic diversity of Alpha<sub>S1</sub>-Casein gene was low.

**Keywords:** alpha<sub>S1</sub>-casein gene, local goat, alleles, protein

### Introduction

The role of goat milk in human nutrition has so far been increasing, due to its nutritional superiority compared to cow's milk. Goat milk has a smaller size of the fat globula, which results in better digestibility, and has a higher proportion of short and medium chain fatty acids than cow's milk (Silanikove et al., 2010). This characteristic of goat's milk makes it suitable for babies and children (Lopez-Aliaga et al., 2010).

The quality of goat's milk is strongly influenced by protein content and is one of the main criteria in the sale of goat milk in many countries. Milk protein is divided into two types namely casein and whey. In goats the ratio of casein and whey is 80: 20. Casein is the largest compiler of milk protein consisting of four types of polypeptide ie.  $\alpha_{S1}$ -CN,  $\beta$ -CN,  $\alpha_{S2}$ -CN and  $\kappa$ -CN. Among the protein casein, alpha<sub>S1</sub> ( $\alpha_{S1}$ -CN) represents more than 40% in cow's milk (Farrell et al., 2004), whereas in goat's milk, it ranges from 0 - 25% (Boulanger et al., 1984). In goat's milk, casein alpha<sub>S1</sub> is an important variable that can be used as an identifier on goats (Maga et al., 2012).



The presence of casein alpha<sub>S1</sub> is related to the total amount of protein and casein in goat's milk (Ambrosoli et al., 1988; Clark and Sherbon, 2000). Casein alpha<sub>S1</sub> is encoded by the CSN1S1 gene, and has so far identified about 18 allele variants (Devold et al., 2010). Polymorphism of goat casein genes is closely related to the quality of milk proteins. Casein genes in goats are present on chromosome 6 about 250 kb in length (Martin et al., 2002).

The objective of this study was to characterize the alpha<sub>S1</sub>-casein (CSN1S1) gene in Indonesian Local goats by knowing allele frequencies and heterozygosity values by using PCR-RFLP techniques using *XmnI* restriction enzyme.

**Materials and Methods**

**a. Blood Samples and DNA Extraction**

DNA samples were taken from 180 heads goat from two regions ie. Enrekang and Jeneponto regency in South Sulawesi province. Blood samples were collected approximately 2 ml through the jugular vein using venojet in vacuttainer tubes with EDTA. DNA were isolated and purified with Genomic DNA extraction kit (Genejet Genomic DNA Extraction Thermo Scientific).

**b. CSN1S1 genotyping with PCR-RFLP**

The following primers were used to amplify the CSN1S1 gene :

Primers	DNA Sequens	Allele	Enzym Restriksi	Sources
Cn-F (F)	5'-TGGGTTGTTTCCTTCTAATG-3'	F	<i>XmnI</i>	Soares et al, 2009
Cn-F (R)	5'-CCTGAGCACTATTGGGAAC-3'			
Cn-O (F)	5'-GAAAGGGATGCCATGATAGATG-3'	O		
Cn-O (R)	5'-TTGGACTTGCCACAAGCTAGC-3'			
Cn-E (F)	5'-TCAAAACATGCAGCATAACTAAC-3'	E		
Cn-E (R)	5'-AGTCAGTGGCCTTTATACCAG-3'			

PCR reaction were performed in 25 µl aliquots contained 100 ng of DNA template, 0.25 mM of each primer, 150 µM dNTP mix, 2.5 mM Mg<sup>2+</sup>, and 0.5 µl Taq DNA polymerase supplied with 1x buffer. The following PCR protocol was used : 2 min early denaturation at 94 °C, then following 35 cycles : denaturation for 45 seconds at 94 °C, annealing temperature at 57 °C x 60 sec, followed by an extension : 72 °C x 60 sec, which then ends with a final extention at temperature 72 °C for 5 mins with PCR machine (SensoQuest, Germany). The yield and specificity of the PCR reactions were evaluated by electrophoresis of the product in 1.5 % agarose gel with ethidium bromide in 1x TBE buffer.

PCR products obtained from Cn-F primer for targeting F allele was analyzed using RFLP through cutting using the restriction enzyme cutting sites in the CSN1S1 gene. A total of 4 µl PCR product is added 0.5 µl of a restriction enzyme (5U); 0.5 µl and 5 µl of enzyme buffer milique water to a volume of 10 µl, then performed incubation for 3 hours at 37 °C.

**c. Statistical analysis**

The genotype and allele frequencies were calculated based on Nei & Kumar (2000)

formulation.  $X_{ii} = \frac{n_{ii}}{N} \times 100\%$  and  $X_i = \frac{(2n_{ii} + \sum_{j \neq i} n_{ij})}{2N}$  where  $X_{ii}$  = ii<sup>th</sup> genotype frequency,  $X_i$  = i<sup>th</sup> allele frequency,  $n_{ii}$  = number of sampel of ii genotype,  $n_{ij}$  = number of sample of ij genotype, and  $N$  = total sampel. Observed ( $H_o$ ) and Expected heterozygosity ( $H_e$ ) based on Nei's heterozygosities (1973) and computed using PopGene32 software version 1.31 (Yeh et al., 1999).  $H_o = \sum_k w_k \sum_{i \neq j}^q X_{kij}$  and  $H_e = 1 - \sum_k w_k \sum_i^q x_{ki}^2$ . Where  $H_o$  = observed within-population heterozygosity,  $H_e$  = expected within-population heterozygosity,  $w_k$  = relative population size,  $X_{kij}$  (i≠j) = the frequency of A<sub>i</sub>A<sub>j</sub> in the k<sup>th</sup> population.



## Results and discussions

### Alpha<sub>S1</sub>-Casein (CSN1S1) Allele Variations

Allele of Alpha<sub>S1</sub>-Casein gene obtained from PCR-RFLP results using three different primary types (Cn-O, Cn-E and Cn-F) ie. allele A and B that are classified into high allele levels. The results obtained as Aditama (2014) has demonstrated that Alpha<sub>S1</sub>-casein with high-expression alleles such as A and B are commonly found in PE goat in Central Java.

This finding supported by Grosclaude et al., (1987); Moiola et al., (1998); Rando et al., (2000); Bevilacqua et al., (2002); Ramunno et al., (2005); Sacchi et al., (2005); Sztankoova et al., (2007) that the alleles of the CSN1S1 loci in goat are grouped into 4 levels for protein quality in milk; high alleles (A, B1, B2, B3, B4, C, H, L and M), intermediate alleles (E and I), weak alleles (D, F and G) and the null alleles (O1, O2, and N).

### Allele and genotype frequencies

The results of the analysis of genotype and allele frequencies at alpha<sub>S1</sub>-casein genes in local goat can be seen in Table 1.

**Table 1.** The frequency of genotypes and alleles in the gene CSN1S1

Allele Locus CSN1S1					Genotype CSN1 S1 "High"		
High		E	F	O	AA	AB	BB
A	B						
0.94	0.06	-	-	-	0.88	0.06	0.06

Based on Table 1 it can be seen that the genotype frequencies are only high-expression "high" genotypes consisting of genotypes AA, AB and BB. AA frequency value is higher when compared with the AB and BB. This condition is unlike the research of Soares et al. (2009) which, in addition to obtaining high-expression "high" genotypes, also obtains the genotype of EE, FF and other alleles.

The allele frequencies obtained from this study were allele A (0.94) and allele B (0.06). An allele and the B allele obtained are classified into "high" allele levels. The number of alleles in this study was fewer than the alleles found in Soares et al. (2009) that identified some alleles ie. E, F, O, "high" alleles and other alleles.

Table 1 shows the frequencies of the A and B alleles in the Alpha<sub>S1</sub>-Casein gene are polymorphic according to Nei (1987) which says that an allele is said to be polymorphic if it has allele frequencies equal to or less than 0.99 and that Indrawan et al (2007) said that genetic diversity in the population is determined by the number of genes that have more than one allele (polymorphic genes), and the number of alleles in each of these genes.

### Heterozygosity Value

Observed heterozygosity (Ho) and expected heterozygosity value (He) of Alpha<sub>S1</sub>-casein gene can be seen in Table 2.

**Table 2.** Observed and expected heterozygosity of CSN1S1 gene in local goat population.

Observed Heterozygosity (Ho)	Expected Heterozygosity (He)
0.223	0.376

Based on the data in Table 2 it can be seen that the observed heterozygosity value (Ho) is lower than the expected heterozygosity value (He). This means that the genetic diversity in the local goat population under study is still low because of the possibility of inbreeding. This is as obtained by Mastrangelo et al., (2012), which derives a Ho value lower than the value of He.



According to Tambasco et al. (2003) the difference between the observed heterozygosity value ( $H_o$ ) and the expected heterozygosity ( $H_e$ ) can be an indicator of the genotype imbalance in the observed population. This imbalance indicates natural selection or artificial selection.

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## Induction of Follicular Growth and Atresia: Expression of Aromatase mRNA in the Ovary of *Bos Indicus*

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### Abstract

Aromatase activity and follicular estradiol (E2) concentration are significantly increased during the initiation of the bovine preovulatory LH surge and correlated with the developmental stages of follicular growth. However, in follicular atresia, the expression of aromatase mRNA is not well reported especially in bovine. The objective of this study was to determine effect of FSH or FSH-saline treatments on follicular growth and atresia, expression of aromatase mRNA, and peripheral E2 concentrations in beef cows. Twelve mature Brahman crossbred cows, (24-30 months of age; 250-300 kg of body weight) were randomly to received intramuscular FSH injections (Ovagen™, ICPbio Reproduction, Auckland, New Zealand) for 3 days to induce follicular growth (decreasing daily doses of 10, 8, 6 mg on days 17, 18 and 19) or FSH-saline to induce follicular atresia (10 mg FSH on day 17 and normal saline on days 18 and 19) or saline injections for 3 days as a control. Treatment of 3 days FSH increased ( $P < 0.05$ ) total numbers of follicles per cow compared to the others ( $21.3 \pm 0.4$ ,  $7.5 \pm 0.3$ , and  $4.0 \pm 0.4$  for 3 days FSH, 1 day FSH, and saline, respectively). Expression of aromatase mRNA in granulosa cells was greatest ( $P < 0.05$ ) in cows received 3 days FSH compared to cows received 1 day FSH and the control cows. Concentrations of E2, in pooled follicular fluids samples in small and medium follicles, were also greatest ( $P < 0.05$ ) in cows received FSH for 3 days, but were not different ( $P > 0.05$ ) in large follicles. This study indicates that induction of bovine follicular growth and atresia can be conducted using FSH and FSH-saline treatments. The expression of aromatase mRNA, follicular E2 concentrations are involved in the final growth or atresia of preovulatory follicle. This FSH induced model will provide the useful for studying the mechanisms regulating bovine follicular growth.

**Keywords:** follicular growth, aromatase, FSH, ovary, bovine

### Introduction

Bovine follicular growth and atresia are characterized by 2 or 3 follicular waves. At each follicular wave, a cohort of follicles is initiated to grow, but only the dominant follicle continues to grow and ovulate or the follicle will be atretic (Beg et al., 2006). Because the development and regression of follicles are associated with major structural and functional changes, it is



important to classify follicles accurately as healthy or atretic at all stages of development (Rodgers et al., 2010). As the follicular wave progresses, one follicle emerges as the dominant follicle and the remaining follicles of the cohort regress. Regression of these subordinate follicles occurs at the end of the FSH transient, a subsequent decrease of plasma FSH concentrations (at the end of the FSH transient) may cause a loss of aromatase activity and initiate regression of future subordinate follicles. As exogenous FSH is known to prevent atresia in subordinate follicles in ruminants. Towards the end of the FSH transient, the future dominant follicle and the largest subordinate follicle are similar in size and growth rate (Ginther et al., 2001), yet these two follicles diverge as FSH concentrations decline. The future dominant follicle becomes more sensitive to FSH, and can maintain P450arom expression in a low-FSH environment (Fortune et al., 2001). All these studies have been conducted in cattle breeds of European origin (*Bos taurus*); little information is available in *Bos indicus* about sizes of follicles, follicular E2 concentrations, and aromatase expression of follicles during FSH treatments. Thus, a full understanding of the regulation of aromatase expression and activity has important implications for the control of fertility. Therefore, the objective of this study was to determine effect of FSH or FSH-saline treatments on follicular growth and atresia, expression of aromatase mRNA, and peripheral E2 concentrations in *Bos indicus*.

## Materials and Methods

### Animals and treatments

All experimental procedures were approved by the animal ethic committee of Khon Kaen University. Twelve mature Brahman crossbred cows, (24-30 months of age; 250-300 kg of body weight) were randomly to received intramuscular FSH injections (Ovagen<sup>TM</sup>, ICPbio Reproduction, Auckland, New Zealand) for 3 days to induce follicular growth (decreasing daily doses of 10, 8, 6 mg on days 17, 18 and 19) or FSH-saline to induce follicular atresia (10 mg FSH on day 17 and normal saline on days 18 and 19) or saline injections for 3 days as a control. At slaughter (day 20), ovaries were collected and transported for analyses. Day of first detection of estrous behavior was designated as day 0 of the subsequent estrous cycle.

### Classification of follicles

Ovaries were collected and swiftly transported to the laboratory. All visible follicles were then classified by diameter into large (>10 mm), medium (7-10 mm) or small (3-6 mm) as described (Moonmanee et al., 2013).

### Follicular fluid and granulosa cells collections

Follicular fluids were aspirated for E2 evaluations and granulosa cells were collected from one ovary for estradiol assays. Granulosa cells were recovered for the extraction of nucleic acids, and the culture medium frozen at -20 °C for quantification of mRNA.

### Estradiol assays

Concentrations of E2 were determined with commercial ELISA kits (DRG Instruments, GmbH, Marburg, Germany; Moonmanee et al., 2013) in unextracted follicular fluid diluted (1:250 for E2) with PBS. Sensitivities of these assays were 10.0 pg/mL for E2. Intraassay CV was 5.91 % for E2.



## Quantification of aromatase mRNA

Total RNA were extracted from each individual granulosa cells using TRIzol reagent (Invitrogen), as previously described. The extracted RNA was directly dissolved in 10  $\mu$ l DNase I solution (Invitrogen) plus 1 U/ $\mu$ l RNase for DNA degradation, as suggested by the manufacturer. The concentration of RNA was not measured or normalized before reverse transcription. The total content of RNA from granulosa cells was immediately reverse transcribed into cDNA using a High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA) according to the manufacturer's protocol. The cDNA was then stored at -20°C until use. The extracted DNA was dissolved in 20  $\mu$ l of 8 mM sodium hydroxide as suggested by the manufacturer. To adjust the pH, 1.72  $\mu$ l of 0.1 M Hepes was added, and then 3.3  $\mu$ l of ultrapure H<sub>2</sub>O was added to a final volume of 25  $\mu$ l. The samples were immediately used for quantitative PCR to avoid DNA degradation (Ferreira et al., 2016).

## Statistical analysis

Data are expressed as the mean  $\pm$  SEM per cow, unless otherwise stated. For all parameters, differences between treatments means were contrasted by Duncan's New Multiple Range Test (Steel et al., 1997). The real-time RT-PCR data were tested for the normality of residuals and for the homogeneity of variance.

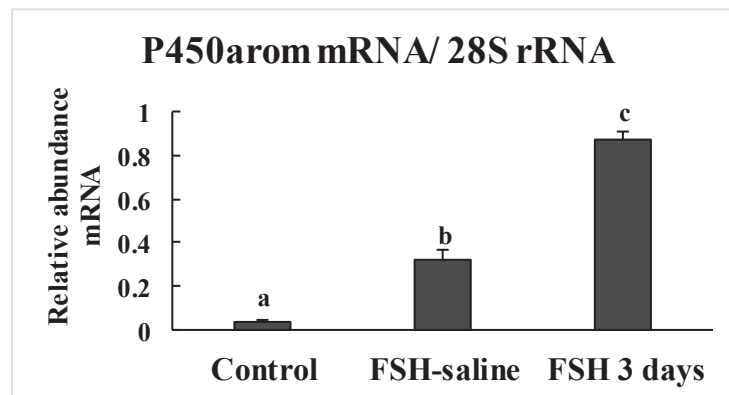
## Results and discussions

Treatment of 3 days FSH increased ( $P < 0.05$ ) total numbers of follicles per cow compared to the others ( $21.3 \pm 0.4$ ,  $7.5 \pm 0.3$ , and  $4.0 \pm 0.4$  for 3 days FSH, 1 day FSH, and saline, respectively). Concentrations of E<sub>2</sub> in pooled follicular fluids samples in small and medium follicles, were also greatest ( $P < 0.05$ ) in cows received FSH for 3 days, but were not different ( $P > 0.05$ ) in large follicles (Table 1). In order to test the hypothesis, a reduction in FSH concentrations decreases P450arom mRNA expression and enzyme activity. In this experiment, the treatments of FSH alone 3 days increased P450arom mRNA abundance ( $P < 0.05$ ; Figure 1) compared with FSH-saline and controls. The withdrawal of FSH or did not treatments FSH decreased P450arom mRNA abundance. This is consistent with reports showing that FSH decreased follicular atresia but increased mRNA of aromatase protein in bovine granulosa cells (Silva and Price, 2002). Thus, in this study indicates that induction of follicular growth can be conducted using FSH treatments. Treatment of FSH-saline could induce follicular atresia and reduce aromatase mRNA in ruminant (Moonmanee et al., 2013).

**Table 1.** Total number of follicles and follicular E<sub>2</sub> concentrations on the hormone treatments.

Measurement	Control	FSH-saline	FSH 3 days	P value
Number of cows (n)	4	4	4	-
Total number of follicles (n)	$4.0 \pm 0.4^c$	$7.5 \pm 0.3^b$	$21.3 \pm 0.4^a$	$< 0.05$
Follicular E <sub>2</sub> concentrations (ng/mL)				
Small follicle (3-6 mm)	$115.8 \pm 5.5^b$	$119.3 \pm 5.2^b$	$138.1 \pm 4.9^a$	$< 0.05$
Medium follicle (7-10 mm)	$280.9 \pm 4.2^c$	$294.5 \pm 4.5^b$	$306.8 \pm 3.7^a$	$< 0.05$
Large follicle (>10 mm)	$254.7 \pm 4.9$	$257.4 \pm 4.7$	$259.4 \pm 4.4$	0.21

Means  $\pm$  SEM are expressed per cow.



**Figure 1.** P450arom mRNA abundance in the bovine granulosa cells after hormone treated. Data for mRNA were corrected for loading (28S rRNA).

## Conclusion

This study indicates that induction of bovine follicular growth and atresia can be conducted using FSH and FSH-saline treatments. The expression of aromatase mRNA, follicular E2 concentrations are involved in the final growth or atresia of preovulatory follicle. This FSH induced model will provide the useful for studying the mechanisms regulating bovine follicular growth.

## Acknowledgements

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## The Effect of Bull (*Bos indicus*) and Extender Medium to Additional Antioxidant $\alpha$ -tocopherol of Cryopreservation Sperm Post-Thawing to Minimize of Repeat Breeding

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### Abstract

The aim of this research was to observe the effect of bull (*bos indicus*) and extender medium with the addition antioxidants  $\alpha$ -Tocopherol of cryopreservation sperm *post-thawing* to minimize of *repeat breeding*. The treatments were in completely randomized factorial design 2-way pattern, as factor A was bull Ongole Grade (OG) and Brahman and factor B was the extender medium egg yolk skim + standard and egg yolk skim +  $\alpha$ -Tocopherol each treatment was repeated 12 times. The observed variables were: motility, viability, abnormality and membrane integrity of spermatozoa. The effect between treatments was measured Independent Sampel T-Test. The results revealed the best result of Brahman bull with the motility average of  $49.11 \pm 2.02\%$ , the mean of viability was  $53.28 \pm 2.73\%$ , the average of abnormality was  $14.67 \pm 0.94\%$ , and the mean of spermatozoa membrane integrity was  $45.30 \pm 4.29\%$ . The result of the research on treatment extender medium of EYS+ $\alpha$ -Tocopherol showed the best result with the motility average of  $49.77 \pm 1.08\%$ , mean viability was  $54.77 \pm 0.62\%$ , average abnormality was  $14.45 \pm 1.07\%$ , the average of spermatozoa membrane integrity was  $47.40 \pm 1.32\%$ . The results revealed that there was a very significant difference ( $P < 0.01$ ) between extender medium EYS+STD with EYS+ $\alpha$ -Tocopherol on the mean of motility, viability and integrity membrane of spermatozoa and no significant difference ( $P < 0.05$ ) on spermatozoa abnormality. In conclusion, the use of Brahman bull with the addition of extender medium of EYS+ $\alpha$ -Tocopherol produced the best motility, viability, and membrane integrity of spermatozoa.

**Keywords:** sperm cryopreservation,  $\alpha$ -tocopherol, bull

### Introduction

Livestock sector has an important role in human resource development. Increasing people's welfare will be followed with increased consumption of livestock products, and contribute to the economy in the livestock sub-sector. Increased livestock productivity can be accelerated by means of reproductive biotechnology in order to maintain and support the increasing population and genetic quality of livestock. Artificial insemination (AI) is one of applied technologies in Indonesia which is easy and efficient (Feradis, 2010). If the cattle do not show pregnancy after insemination, it is called *repeat breeding*. *Repeat breeding* is female reproductive abnormalities that have normal estrous cycle and obvious estrous symptoms but



when mated to 2-3 times or more but no pregnancy (Susilawati et al., 2015). The success factor of AI is determined by the bulls and the extender medium. Ongole grade (OG) and Brahman bull are germplasm that need attention in the livestock sub-sector. AI success in maintaining sperm quality can be done with extender, the problem is mainly sperm storage, so it can be overcome with cryopreservation of sperm that requires the extender medium. Each of the most widely distributed extender medium has different advantages, such as skim egg yolk and andromed. However, the problem is that there is a low level of motility in the quality of the frozen sperm. This is due to the effect of *cold shocks* on frozen cells and the intracellular changes due to water discharges associated with ice crystalline formation. One way is to provide the addition of  $\alpha$ -*Tocopherol* antioxidants (Abdi et al., 2015).

## Materials and methods

A fresh sperm bull of OG and Brahman each with age of 3-4 years and body weight with the range of 600-750 kg. The feed consisted of 60% forage and 40% concentrate given twice daily ie morning and evening, while drinking water is given *ad libitum*.

### Extender medium preparation and addition of $\alpha$ -*Tocopherol*, glycerol

A total of 50 ml of skim; 20 ml of egg yolks; 1000 IU of penicillin; 1 mg / ml of streptomycin and 14% glycerol plus 1000 IU penicillin and streptomycin 1 mg / ml were homogenized. The extender medium was added with 0.4 gram/100 ml  $\alpha$ -*Tocopherol* antioxidants extender (Udin et al., 2013). Then put in erlenmeyer and stir thoroughly. Furthermore, the solution is stored in the refrigerator until it is used (Deichsela et al., 2016). The extender medium used were EYS + STD and EYS +  $\alpha$ -*Tocopherol*. The amount of  $\alpha$ -*Tocopherol* added was 0.4 grams/100 ml (Kutluyer et al., 2014). The addition of  $\alpha$ -*Tocopherol* in the sperm extender should be noted for its solubility. Because  $\alpha$ -*Tocopherol* is insoluble in water before adding the solution to the sperm extender,  $\alpha$ -*Tocopherol* was first dissolved in ethanol with an antioxidant ratio of ethanol of 1: 5 (Herdis et al., 2005). A total of 14% were removed from the extender medium then filled a vacuum with 14% glycerol and mixed with sperm (Akeel et al., 2012)

### The collection of sperm

The collection of sperm was performed using an artificial vaginal method filled with cold water and with 2/3 of artificial vaginal volume. Then given a lubricant, with the aim to facilitate the penis entry during collection so as not to injure the penis (Belala et al., 2016).

### Extender of sperm up to freezing

Extender sperm is divided into two parts, first extender with EYS + STD and second with EYS +  $\alpha$ -*Tocopherol* there are three extender: Primary A, extra A and B. Primary A extender contains skim milk and yolk, B extender contains skim milk, yolk, glycerol, and glucose. Then the *printing straw* preparation is done by printing the caption on the empty straw. Straw is distinguished by the color of the bull with each having a light blue and dark blue straw (OG and Brahman) in accordance with SNI standards. Then the equilibration process execution lasts for 2



hours in *cool top* with 5°C temperature. The *cooling process* is done by inserting in a closed measuring cup and placed on a beaker glass filled with water, then placed in a *cool top* (Belala et al., 2016). The next step is the process of *filling and sealing* which is a process of *filling* sperm that has been diluting and clamping straw by using automatic *filling and sealing* machine (Susilawati et al., 2015). *Pre-freezing* sperm evaluation is performed microscopically which includes motility, viability, and abnormality. Standard sperm motility of liquid before freezing is 60%. The last process is the freezing of sperm. Before being introduced into liquid N<sub>2</sub>, the sperm is placed above the liquid N<sub>2</sub> surface to evaporate at -120° C for 9 minutes. *The freezing* process is carried out by spraying liquid N<sub>2</sub> -196°C. Then stored in storage container containing liquid N<sub>2</sub> (Judycka et al., 2016). Evaluation of frozen sperm was performed after straw in thawing in waterbath with temperature of 37°C for 15 seconds. *Post-thawing* sperm evaluation was performed with a standard motility of 40% (Muino et al., 2008).

### Parameters Measured

The percentage of motility, viability and abnormality is done by shedding the sperm on an object glass and observed by a microscope. The surviving spermatozoa are colorless, while the dead absorb the color. Percentage of membrane integrity can be done by using HOST solution.

### Data analysis

Data were analyzed with SPSS version 20.0 for windows. Randomized Complete Random Design (RAL) with Factorial (2x2) ie 2 bulls (OG and Brahman) and 2 ekstender medium (EYS + STD and EYS + $\alpha$ -Tocopherol and followed with T-Test.

## Results and discussion

### Evaluation of Fresh Sperm

The average sperm volume obtained was  $7.06 \pm 1.17$  ml / ejaculate in accordance with Feradis (2010) that the sperm volume in bullis in the range of 5-10 ml. The sperm color was whitish cream. Ismaya (2014) reported that good bull sperm color (normal) is usually like milk or cream whitish and turbid. The mass movement of sperm +++, this result is in accordance with Ismaya (2014) that the assessment of spermatic movements with +++ is very good with marked big waves, looks dark, active and fast moving. The average sperm concentration in OG and Brahman bull was  $1.694 \pm 0.45$  per ml and  $2.214 \pm 0.27$  per ml. The results of this study in accordance with Hafez (2008) who reported that the sperm concentration in bull in the normal range with an average of 800-2000 per ml. The sperm motility in OG and Brahman bull were  $81.83 \pm 2.75\%$  and  $84.17 \pm 1.89\%$ . Ismaya (2014) reported, sperm motility in bull  $81,3 \pm 9,3\%$ . The viability of sperm obtained from each OG and Brahman bull sperm were  $80.67 \pm 1.89\%$  and  $83.67 \pm 1.29\%$ . These results are still within the range based on Hafez (2008). The average sperm abnormality rates in OG and Brahman bull were:  $15.03 \pm 0.49\%$  and  $14.07 \pm 0, 75\%$ , respectively. This result is in accordance with Hafez (2008) who states that abnormalities in bull





are below 20%. In general, the sperm assessment is macroscopically still in the normal category or eligible for further processing (Guimares et al., 2010)

## Evaluation sperm post thawing

### Percentage of post thawing spermatozoa motility

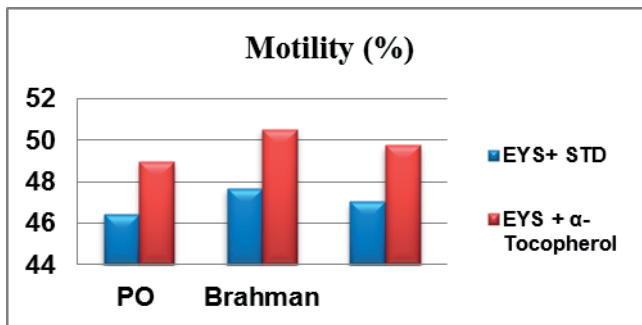


Fig. 1 Meaning of spermatozoa motility in bulls and extender medium to sperm quality frozen *post-thawing*

The results showed the difference in the number of frozen sperm motility with bulls and different extender medium. Brahman males get the highest frozen sperm motility value with a median of  $49.11 \pm 2.02$ . Not significantly different of ( $P < 0.05$ ) with bull OG which have average  $47.71 \pm 1.83$ . This is because bull OG and Brahman are local bull with the same breed (*Bos indicus*).

Mean of motility for factor B:  $47.05 \pm 0.90$  and  $49.77 \pm 1.08$ . In the treatment of medium extender using EYS with  $\alpha$ -Tocopherol obtained the highest motility value. This is because  $\alpha$ -Tocopherol is the largest antioxidant and can cause antioxidant effects, which can as a hydrogen ion donor capable of converting free radicals, ie peroxy radicals into less reactive Tocopherol radicals, so as not to damage the chain fat (Abdi et al., 2015). The content of  $\alpha$ -Tocopherol may act as an antioxidant to protect spermatozoa from free radical attack,  $\alpha$ -Tocopherol works by searching, reacting and destroying the chain of free radical reactions (Susilawati et al., 2015).

### Percentage of post thawing spermatozoa viability

The highest viability of spermatozoa was Brahman bull and extender medium with the addition of  $\alpha$ -Tocopherol antioxidants. If it is compared to EYS + STD that obtain lower spermatozoa viability. This is because the use of an extender medium with the addition of antioxidants is beneficial to the presence of free radicals during cryopreservation sperm process, thus as not to cause *cold shock*, so can increase the viability of the spermatozoa. This is probably because  $\alpha$ -Tocopherol is the main antioxidant contained in the cell membrane. Its role as a free radical inhibitor on the membrane surface can only be done by vitamin E (Akeel et al., 2012)

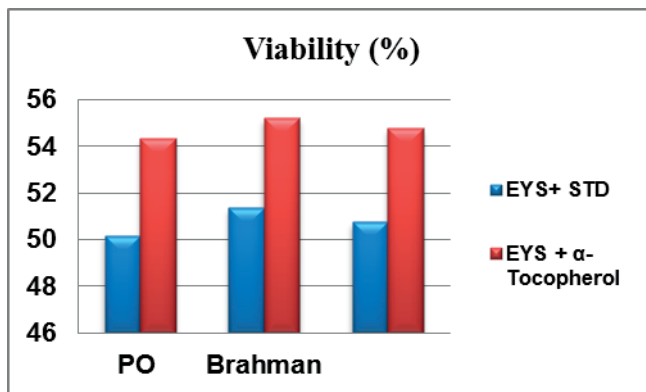


Fig. 2 Meaning of spermatozoa viability in bulls and extender medium to sperm quality frozen *post-thawing*

### Percentage of post thawing spermatozoa abnormality

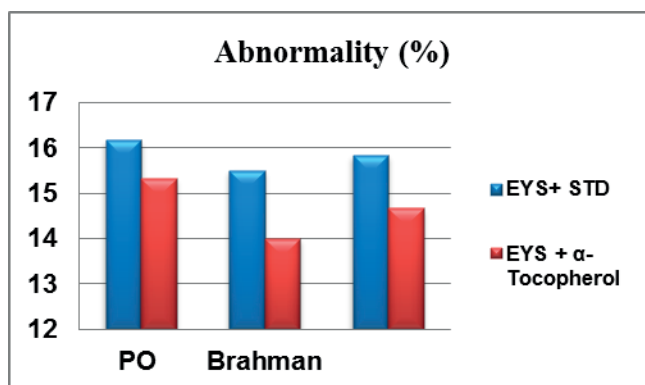


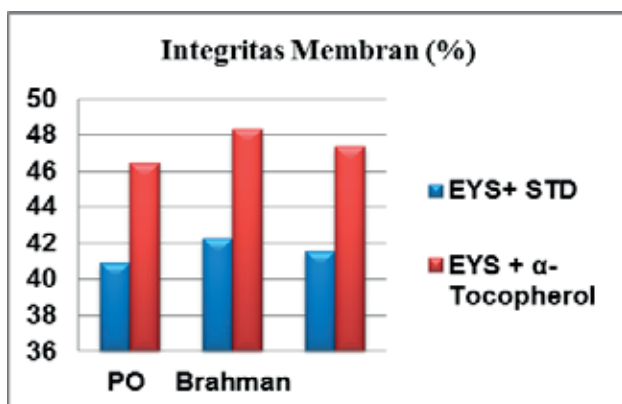
Fig. 3 Meaning of spermatozoa abnormality in bulls and extender medium to sperm quality frozen *post-thawing*

Standardization of frozen sperm distribution post thawing, the quality of sperm produced should provide viability or percentage of live spermatozoa at least 40% with progressive movements forward to the fore at least ++ (Ismaya, 2014). Based on the above table, bulls and extender treatment with mean spermatozoa viability for each A factor were  $52.25 \pm 2.94\%$  and  $53.28 \pm 2.73\%$  respectively and the B factor was  $50.76 \pm 0.84\%$  and  $54.77 \pm 0.62\%$ .

The table on the average of spermatozoa abnormality for each factor A is A1 and A2 are  $15.75 \pm 0.59\%$  and  $14.75 \pm 1.06\%$  respectively. While for the average of spermatozoa abnormality for each factor B that is B1 and B2 respectively are:  $15.84 \pm 0.47\%$  and  $14.67 \pm 0.94\%$ . Based on the results of this study, medium extender EYS without the addition of antioxidants  $\alpha$ -Tocopherol obtain higher abnormalities.

### Percentage integrity of post thawing spermatozoa membrane

Measurement of membrane integrity percentage with HOST method serves for functional evaluation of sperm membrane integrity. Membrane integrity is a condition that shows the mechanism of membrane physiological functions that are maintained as control of ion transport, so that the fluid outside the cell can not enter the cell (Jishage *et al.*, 2005). The success parameters for sperm quality can be measured using the percentage of integrity of this membrane. If the plasma membrane is damaged then the metabolic process will be disrupted, the synthesis of ATP (energy source) is not running normally so it will be fatal for the sperm that is the decrease of motility and sperm survival itself (Maria *et al.*, 2015; Susilawati *et al.* 2015).



**Fig. 4** Meaning of spermatozoa integritas membran in bulls and extender medium to sperm quality frozen *post- thawing*

The graphs on the average integrity of spermatozoa membranes for each A factor were  $43.69 \pm 3.94\%$  and  $45.30 \pm 4.29\%$ , respectively. While for factor B each was  $41.59 \pm 0.97\%$  and  $47.40 \pm 1.32\%$ . Brahman bull with an  $\alpha$ -Tocopherol extender produce high integrity values of sperm membranes.

## Conclusion

The use of Brahman bull with the addition of medium extender EYS+  $\alpha$ -Tocopherol produces the motility, viability, and integrity of the best spermatozoa membrane. The addition of the  $\alpha$ -Tocopherol antioxidant to the ekstender egg yolk skim (EYS) provides excellent motility, viability and integrity of the spermatozoa membrane. So, can minimize rate of *repeat breeding* in livestock.

## Acknowledgments

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## Early Embryonic Development, Corpus Luteum and Metabolite of PGF in Lactating Dairy Cows Supplemented with Palm or Sunflower Oil

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### Abstract

Supplementations of plant oils in diets have important effects on reproductive performance in postpartum dairy cows. The aim of this study was to examine effects of plant oil (sunflower oil [SO] and palm oil [PO]) supplements in diet on early embryonic development, corpus luteum (CL), and metabolite of prostaglandin F (PGFM) in lactating dairy cows. Cows (n= 42) were randomly assigned into one of two different plant oil supplements: 4% PO and 4% SO. All cows were fed ad libitum roughage and dietary concentrate with oil supplement beginning at 28 days prior to expected calving date and until day 111 postpartum. Blood samples were collected to analyze serum PGFM concentration on days 5 to 35 after parturition. CL volume and P4 concentration were determined on day 5, 9, and 13 of the estrous cycle. Oocyte characteristic and embryonic development were collected on day 83, 97 and 111. Serum PGFM concentrations were greater ( $P < 0.05$ ) in cows fed 4% SO than those fed 4% PO on days 15 to 30 after parturition. Volume of CL and P4 concentrations on day 13 in cows fed 4 % SO were greater ( $P < 0.05$ ) than those fed 4% PO diet ( $8,290 \pm 679$  vs.  $7,875 \pm 594$  mm<sup>3</sup> and  $5.0 \pm 0.6$  vs.  $3.9 \pm 0.3$  ng/mL, respectively) but oocyte characteristics and embryonic development were not affected ( $P > 0.05$ ) by PO or SO supplement. Thus, cows fed 4% SO may improve uterine health after calving via the metabolite of PGF and steroid production.

**Keywords:** corpus luteum, metabolite of PGF, early embryonic development, progesterone concentration, dairy cows

### Introduction

Inadequate dietary energy intake and poor body condition can negatively affect reproductive function in lactating dairy cows (Staples et al., 1998). Uterine diseases, lower percentage of pregnancy per AI, high culling and economic losses, these might decrease by fat supplementations (Dirandeh et al., 2013). Fat supplements (such as sunflower oil [SO], linseed oil, and palm oil [PO]) also have importantly the type of fatty acid and had beneficial effects on the follicle growth and ovulation (Juchem et al., 2010; Silvestre et al., 2011), estradiol and progesterone concentration (Staples et al., 1998), uterine health (Navanukraw et al., 2009), oocyte, and early embryonic development in dairy cattle (Bilby et al., 2006). For example, lactating dairy cows were fed with calcium salt of PO diet increased the number of blastocysts



produced in vitro following transvaginal ovum pick-up (OPU; Fouladi-Nashta et al., 2004). Supplemental fat in diet increased the average size of the dominant follicle (Beam and Butler, 1997). Larger ovulating dominant follicle resulted in larger CL (Sartori et al., 2002). Furthermore, previous study reported fish oil changed the fatty acid composition of the endometrium (i.e increased eicosapentaenoic and docosahexaenoic acid and reduced arachidonic acid) in manner that would reduce secretion of  $\text{PGF}_{2\alpha}$  (Bilby et al., 2006). We hypothesized that cow feeds with different plant oil will affect early embryonic development, oocyte characteristic, CL volume, serum concentrations of PGFM and P4. Therefore, the aim of this study was to examine effects of plant oil (PO and SO) supplements in diet on early embryonic development, CL and serum concentrations of PGFM in lactating dairy cows.

## Materials and Methods

### Animals, diet and experimental design

All experimental cows and procedures were approved by the Animal Ethic Committee of Khon Kaen University. The experiment was conducted at a semi-commercial dairy farm located in Khon Kaen, Thailand. Multiparous cows ( $n=42$ ) were blocked by parity, and randomly assigned to receive two dietary treatments as follow: 4% PO and 4% SO. All diets were formulated for 16.5% CP, 5.6% EE, 59.0% NDF, and 25.8% ADF (DM basis) as previously described (Navanukraw et al., 2009). Diets were formulated to meet NRC (2001) nutrient requirement and fed twice daily for the duration of experiment. Each cow was adjusted in accordance with individual milk yields as described (Navanukraw et al., 2009).

### Ultrasonographic observation

Transrectal ultrasonography of CL was performed using an ultrasonography equipped with a 7.5 MHz OPU probe (HS-2000, Honda Electronics Co., Ltd., Toyohashi, Japan). An image of the CL volume was frozen on screen at its maximal area and vertical and horizontal diameters were measured using the integral electronic calipers to determine the mean maximum cross-sectional diameter of the CL as previously described (Bilby et al., 2006).

### Synchronization for ovum pick-up (OPU) and OPU procedure

Cows were synchronized by two injections of  $\text{PGF}_{2\alpha}$  given 11 days apart at 42 and 53 days postpartum. All cows were evaluated twice daily for estrous detection after exhibiting at least one estrous cycle. Follicular aspiration was performed every two weeks for a total of 6 weeks of dietary treatment. For each cow, in each OPU session, the number of visibly aspirated follicles and size were recorded. Follicular contents from all visible ( $\geq 3$  mm) follicles were collected into a 50 mL conical tube containing of oocyte collection medium as described (Ponter et al., 2012).

### In vitro maturation and fertilization

Cumulus oocyte complexes (COCs) were isolated from the follicular aspirates and washed three times in collection medium without heparin (TCM-199). The quality of COCs, in vitro oocyte maturation and fertilization were followed by Ponter et al. (2012) and Thammasiri et al. (2016).

### Blood sampling, PGFM and P4 concentrations

On Days 5 to 35 after parturition, blood samples were collected from coccygeal veins on the following day. The blood was centrifuged at  $2,600 \times g$  for 30 minutes and stored at  $-20^\circ\text{C}$  until PGFM analysis. Serum concentrations of PGFM were assayed using an ELISA kit (Cayman Chemical, Ann Arbor, MI, USA) as described (Dirandeh et al., 2013). Intra-assay



coefficient of variation (CV) was 6.22%. For serum P4 analysis, blood samples were centrifuged (3,000 X g for 20 min at 4°C), and serum was harvested and stored at -20 °C as described (Navanukraw et al., 2004). Serum concentrations of P4 were determined by competitive ELISA (Crane et al., 2006). Intra- CV was 8.75% and assay sensitivity was 0.025 ng/mL.

### Statistical analyses

Data is presented as means  $\pm$  SEM. Repeated measures on concentration of PGFM and P4, the number of oocyte and CL volume were analyzed using the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC). Percentages of oocyte quality and embryonic development were analyzed using Chi-square test (SAS Inst. Inc., Cary, NC). For all analyses, statistical significance was declared at  $P < 0.05$ .

### Results and discussion

In previous study, Navanukraw et al. (2009) reported plant oil supplementation in dietary concentrate improved milk yield ( $P < 0.05$ ), follicle size ( $P < 0.05$ ), uterine health ( $P < 0.01$ ) and P4 concentration after parturition ( $P < 0.05$ ) of cows fed 4% PO and 4% SO supplements that were greater than that of control cows (no oil supplementation).

### Oocyte characteristic and early embryonic development

There were no significant ( $P > 0.05$ ) differences between diets for the total number of oocytes collected, healthy oocytes, oocyte quality and early embryonic development (Table 1).

**Table 1.** Oocyte characteristic and early embryonic development in cows fed dietary treatments

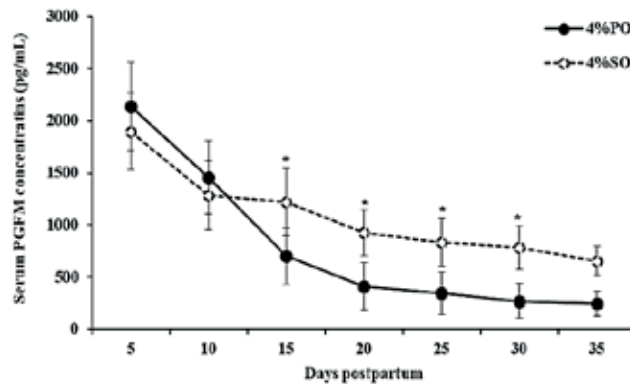
Response	Treatment		SEM
	4% PO	4% SO	
Number of cows	21	21	
Total oocytes, n	15.2	16.1	2.4
Healthy oocytes, n (%)	8.7 (57.2)	9.1 (56.5)	0.7
Oocyte quality 1, n (%)	3.4 (22.4)	4.5 (27.9)	0.8
Oocyte quality 2, n (%)	5.3 (34.8)	4.6 (28.6)	0.9
Cleavage rate, n (%)	6.4 (73.5)	6.6 (72.5)	0.4
Morula rate, n (%)	3.1 (35.6)	3.0 (33.0)	0.4
Blastocyst rate, n (%)	1.8 (20.7)	1.6 (17.6)	0.5

### Serum PGFM concentrations

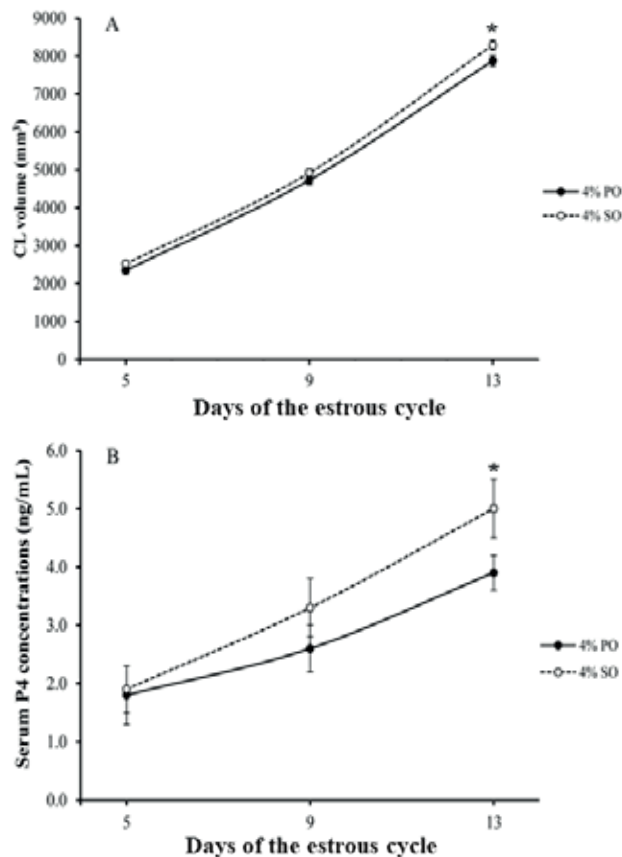
Concentrations of serum PGFM were similar at first 10 days postpartum, and were not affected ( $P > 0.05$ ) by diet treatment. On day 15, 20, 25 and 30 postpartum, serum PGFM concentrations were greater ( $P < 0.05$ ) for cow fed 4% SO than cow fed 4% PO.

### Volume of CL and P4 concentrations

The effect of dietary treatment on volume of CL and P4 concentrations is summarized in Fig. 2. No differences between treatments in mean volume of CL and P4 concentrations from the day of 5 to 9 after estrus (day 0). In additionally, volume of CL and P4 concentrations on day on day 13 of the estrous cycle in cows fed 4 % SO were greater ( $P < 0.05$ ) than those fed 4% PO diet ( $8,290 \pm 679$  vs.  $7,875 \pm 594$  mm<sup>3</sup> and  $5.0 \pm 0.6$  vs.  $3.9 \pm 0.3$  ng/mL, respectively; Fig. 2).



**Figure 1.** Mean serum concentrations of PGFM in cows fed 4% PO (filled bars), and 4% SO (hatched bars). \*  $P < 0.05$ .



**Figure 2.** Volume of CL (A) and P4 concentrations (B) in cows fed 4% PO (filled bars), and 4% SO (hatched bars). \*  $P < 0.05$ .

## Conclusion

In this study, supplementation of 4% PO and 4% SO did not have effects on oocyte characteristics and embryonic development. Increased PGFM concentrations after parturition, CL volume and P4 concentration on day 13 of the estrous cycle were observed in cows fed 4% SO. Therefore, supplement 4% SO may improve uterine health in lactating cows via the PGFM and steroid production.





## Acknowledgements

This study was financially supported by a grant from Thailand Research Fund (TRF) under Research and Researchers for Industries (RRI) and National Research Council of Thailand (NRCT).

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## Development of Corpus Luteum in Goats: Interaction Between Progesterone Concentration and Luteal Cell Proliferation

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### Abstract

Knowledge of the mechanisms of corpus luteum (CL) development is important for a better understanding of luteal function and its regulation. Significant remodeling in cell proliferation for luteal response of progesterone (P4) production occurs at the specific time during CL development. Thus, the aim of this study was to evaluate the relationship between P4 concentrations and luteal cell proliferation. Plasma and luteal tissue samples were collected from mature goats (n=24) on day 3, 6, 9, 12, 15 and 18 of the estrous cycle. Luteal growth and cell proliferation were evaluated using the indices of hyperplasia. The fresh weights of CL were followed the quadratic growth equation model ( $R^2=0.984$ ,  $P<0.0001$ ) increasing from days 3 to 12 and declined from days 15 to 18 of the estrous cycle. The P4 concentrations in goats were followed the quadratic growth regression model increasing from days 3 to 12 and declined from days 15 to 18 of the estrous cycle. Positive correlations were found for the hyperplasia as follows: (1) P4 concentrations and RNA concentration ( $R^2=0.951$ ,  $r=0.903$ ,  $P<0.001$ ), (2) P4 concentrations and protein concentration ( $R^2=0.876$ ,  $r=0.767$ ,  $P<0.001$ ), (3) P4 concentrations and DNA content ( $R^2=0.828$ ,  $r=0.686$ ,  $P<0.001$ ), (4) P4 concentrations and DNA concentration ( $R^2=0.669$ ,  $r=0.448$ ,  $P<0.001$ ). Thus, development of CL and P4 concentration were accordingly correlated with the specific growth equation by which proliferation of luteal cells during development plays a role in luteal function, i.e. progesterone production in goats.

**Keywords:** corpus luteum, progesterone, cell proliferation, goats

### Introduction

Corpus luteum (CL) plays an important role in reproductive function because it is the primary source of circulating progesterone (P4). After ovulation, the CL grows and vascularizes substantially and rapidly (Reynolds et al., 2000). Inadequate P4 production is a major cause of infertility and embryonic loss since P4 is a necessary requirement for both endometrium development and embryo survival (Webb et al., 2002). Increased total luteal volume and reduced circulating P4 levels, 7 days after ovulation, were observed in double compared to single ovulating cows (Lopez et al., 2005). Thus, luteal tissue provides an outstanding model for studying the critical factors necessary for normal tissue growth, regression, and function. Thus, the CL provides outstanding model for studying interactions between CL function and cell proliferation. Therefore, the objective of this study was to evaluate the CL development,



regression and functions during the estrus cycles and correlation between CL development and P4 concentration during proliferation of luteal cells.

## Materials and Methods

### Animal and experiment design

Native goats used in this study were characterized as brown or black in color with a black strip along the back and small in size with short upright ears. Twenty-four native nonpregnant goats were used with the average age and BW of 12 month and  $19.1 \pm 0.6$  kg, respectively. Plasma and luteal tissue samples were collected from mature goats (n=24) on day 3 (n=4), 6 (n=4), 9 (n=4), 12 (n=4), 15 (n=4) and 18 (n=4) of the estrous cycle. All mature female goats exhibited at least 2 normal consecutive estrous cycles ( $42 \pm 0.30$  d). Surgical laparotomy, ovariectomy and blood sample collection were performed on days 45, 48, 51, 54, 58 and 61 of the experiment (days 3, 6, 9, 12, 15 and 18 of the estrous cycle respectively). All experimental procedures were managed according to the guidelines approved by the Animal Ethics Committee of Khon Kaen University.

### Blood samples and P4 concentrations

Blood samples (7 ml) were taken by jugular venipuncture for progesterone (P4) determinations on days 3, 6, 9, 12, 15 and 18 of the 3<sup>rd</sup> estrous cycle. The serum was stored at -20 °C until assayed for P4. Serum P4 concentrations were determined by competitive ELISA (Navanukraw et al., 2014).

### DNA, RNA and protein concentrations

DNA, RNA and protein concentrations were determined in homogenates of luteal tissues using previously described procedures (Ricke et al., 1999). One hundred mg luteal tissues were briefly homogenized in PBS containing 3 mM NaN<sub>3</sub> and 1 mM EDTA (pH 7.2) using a Polytron (Brinkmann, Westbury, NY). Tissue homogenates were analyzed for concentrations of DNA using the diphenylamine procedure, for concentration of RNA using commercial RNA isolation kit (Invitrogen, CA, USA) and concentrations of protein using the Bradford protein assay (Bradford, 1976). Standards for DNA, RNA and protein were DNA type I from calf thymus and BSA (fraction V), respectively (Sigma-Aldrich, St. Louis, MO). Tissue DNA and protein contents were calculated by multiplying their concentrations by total luteal weight. The DNA content was used as an index of tissue hyperplasia, and the ratio of protein/DNA was used as an index of tissue hypertrophy (Ricke et al., 1999).

### Statistical Analyses

Data are presented as mean  $\pm$  SEM. Continuous data were analyzed using GLM and regression procedures of SAS (SAS, 2001).

## Results and discussion

Fresh weights of CL were similar on days 3 and 6 of the estrous cycle and were less ( $P < 0.0001$ ), on days 9, 12, and 15, which also were similar than those on day 15 and 18 were similar (Table 1). In contrast, fresh weights of CL were followed the cubic growth regression model ( $Y = 0.07570x^3 - 6.131197x^2 + 111.80214x - 143.3333$ ,  $R^2 = 0.8954$ ,  $P < 0.0001$ ). However, the fresh weight of CL was followed the quadratic growth equation model ( $R^2 = 0.984$ ,  $P < 0.0001$ ) increasing from days 3 to 12 and declined from days 15 to 18 of the estrous cycle.



The P4 concentration in goats were followed the quadratic growth regression mode ( $y = -0.3876x^2 + 3.1311x - 0.9884$ ,  $R^2 = 0.9847$ ,  $P > 0.0001$ ) increasing from days 3 to 12 and declined from days 15 to 18 of the estrous cycle.

The CL of goats grows extremely and exhibits substantially high rates of cellular proliferation from days 2-12 of the estrous cycle (Thammasiri et al., 2012). Based upon the linear increase in luteal weight and DNA content, the doubling time for ovine CL was approximately 58-83 h from days 2-12 after estrus. The maximum diameter of CL is reached 6-9 days after ovulation and regression starts between days 13 and 16 in ewes (Jablonka-Shariff et al., 1993). The CL has been known as one of the most angiogenic tissues, and angiogenic growth factors have been reported in all stages of luteal development especially during early- and midluteal stages (Grazul-Bilska et al., 2006). Among angiogenic growth factors, NO and eNOS have been shown as potent vasodilators that stimulate vascular endothelial growth factor and angiogenesis in goats (Navanukraw et al., 2014).

The P4 concentrations in goats were followed the quadratic growth regression model increasing from days 3 to 12 and declined from days 15 to 18 of the estrous cycle. Positive correlations were found for the hyperplasia as follows: (1) P4 concentrations and RNA concentration ( $R^2 = 0.951$ ,  $r = 0.903$ ,  $P < 0.001$ ), (2) P4 concentrations and protein concentration ( $R^2 = 0.876$ ,  $r = 0.767$ ,  $P < 0.001$ ), (3) P4 concentrations and DNA content ( $R^2 = 0.828$ ,  $r = 0.686$ ,  $P < 0.001$ ), (4) P4 concentrations and DNA concentration ( $R^2 = 0.669$ ,  $r = 0.448$ ,  $P < 0.001$ ).

## Conclusion

Development of CL and P4 concentration were accordingly correlated with the specific growth equation by which proliferation of luteal cells during development plays a role in luteal function, i.e. progesterone production in goats.

## Acknowledgments

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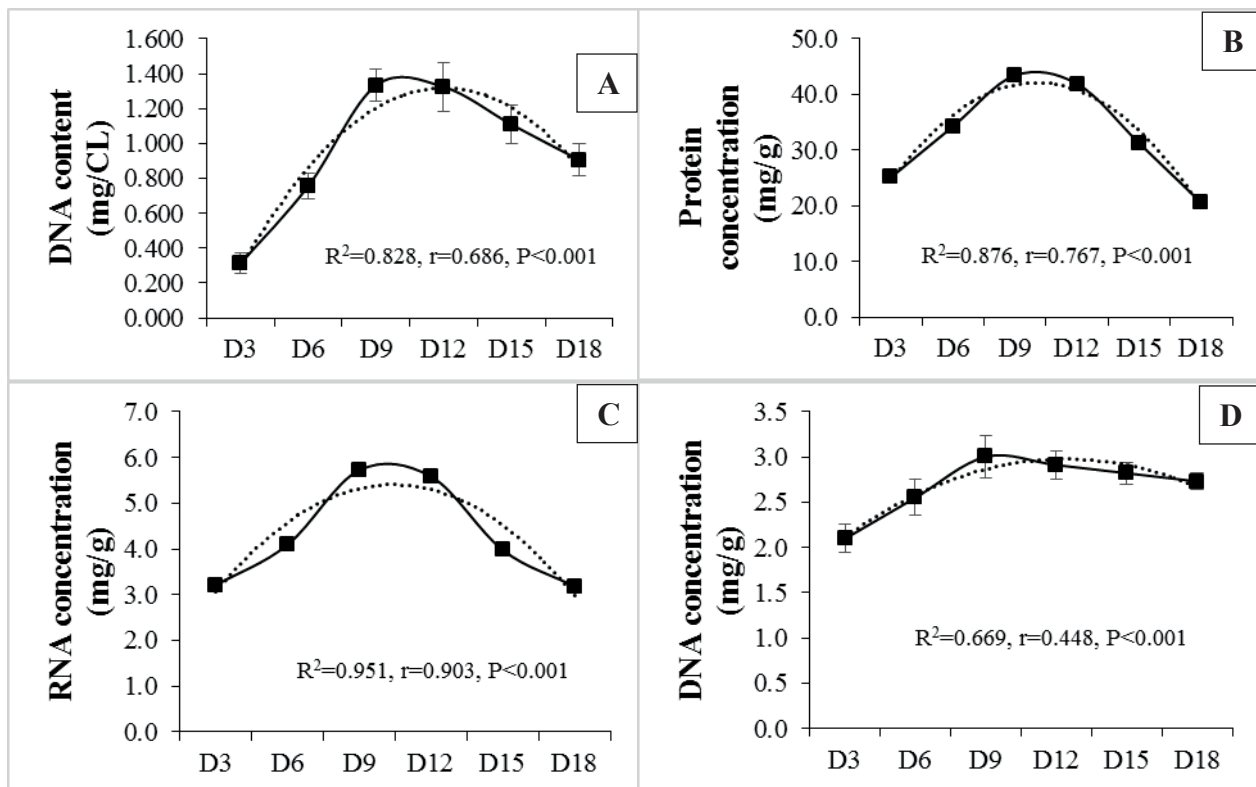
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**Table 1.** Fresh weight, P4 concentration, RNA concentration, Protein concentration, DNA content and DNA concentration on day 3, 6, 9, 12, 15 and 18 of the estrous cycle

Item	Day of the estrous cycle						P-value
	3	6	9	12	15	18	
P4 concentration (ng/ml)	1.8±0.2 <sup>c</sup>	3.5±0.1 <sup>c</sup>	5.1±0.2 <sup>a</sup>	5.3±0.2 <sup>a</sup>	3.8±0.1 <sup>b</sup>	2.3±0.2 <sup>c</sup>	<.0001
Fresh weight (mg)	147.3±20.6 <sup>c</sup>	296.2±17.5 <sup>c</sup>	445.1±18.7 <sup>a</sup>	452.3±27.7 <sup>a</sup>	391.4±22.7 <sup>ab</sup>	330.5±21.7 <sup>bc</sup>	<.0001
RNA concentration (mg/g)	3.2±0.1 <sup>c</sup>	4.1±0.1 <sup>b</sup>	5.7±0.03 <sup>a</sup>	5.6±0.1 <sup>a</sup>	4.0±0.1 <sup>b</sup>	3.2±0.1 <sup>c</sup>	<.0001
Protein concentration (mg/g)	25.1±1.5 <sup>c</sup>	34.3±0.8 <sup>b</sup>	43.4±1.5 <sup>a</sup>	41.9±2.6 <sup>a</sup>	31.2±2.0 <sup>b</sup>	20.5±1.5 <sup>c</sup>	<.0001
DNA content (mg/CL)	0.3±0.1 <sup>b</sup>	0.8±0.1 <sup>c</sup>	1.3±0.1 <sup>a</sup>	1.3±0.1 <sup>a</sup>	1.1±0.1 <sup>a,b</sup>	0.9±0.1 <sup>b,c</sup>	<.0001
DNA concentration (mg/g)	2.1±0.2 <sup>b</sup>	2.6±0.2 <sup>a,b</sup>	3.0±0.2 <sup>a</sup>	2.9±0.2 <sup>a</sup>	2.8±0.1 <sup>a</sup>	2.7±0.1 <sup>a</sup>	0.0264

<sup>a,b,c</sup>Means ± standard error



**Figure 1.** (A) DNA content (mg/CL), (B) Protein concentration (mg/g), (C) RNA concentration (mg/g) and (D) DNA concentration (mg/g) in goats CL compare with P4 concentration (ng/ml) in goats' plasma circulating from days 3-18 of estrous cycle





## **Optimization Total Digestible Nutrients - Protein Ratio to Achieve Good Feed Conversion Ratio in Indonesian Native Beef Cattle**

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### **Abstract**

A study was carried out to determine the ratio of total digestible nutrients (TDN) to Crude Protein (CP) of the diet for beef cattle with good efficiency through Acetate-Propionate (A/P) ratio. This study used 42 cattle from 4 experiments and 12 treatments conducted in the Laboratory of Meat and Dairy Animal Production, Faculty of Animal and Agricultural Sciences, Diponegoro University, Indonesia. The cattle used in the experiments had  $206.89 \pm 7.82$  kg body weight. The cattle were allowed to diets containing 8.59 – 13.20% CP and 42.80 – 75.43% TDN. Correlation analysis were used to describe the relationships among A/P ratio, feed conversion ratio (FCR) and TDN/CP ratio. The results showed that A/P ratio had negative relationship with FCR ( $r = 0.54$ ), while TDN/CP ratio had positive relationship with A/P ratio ( $r = 0.57$ ). According to the results, it can be concluded that TDN/CP ratio of diet for beef cattle should be 4.72 to obtain a good A/P ratio less than 3 and feed conversion ratio 7.19 or less.

**Keywords:** cattle, total digestible nutrients, crude protein, feed conversion ratio

### **Introduction**

The rapidly growing population drive the demand of livestock product that is one of the fastest growing agricultural subsectors in developing countries. As a consequence, the expansion of agricultural production needs to take place in a way that allows the less well-off to benefit from increased demand and that moderates its impact on the environment. (Wanapat et al., 2015) and might give an effect on the availability of feed resources. The efforts to increase cattle production in livestock industry is always accompanied by the increasing of feed cost as a consequence. Feed costs account for 50 – 70% of the total cost in animal bussiness operation (Verbeke et al., 2016). Therefore, a high body weight gain along with the efficiency of feed utilization could be used as indicator of the success of a livestock industry. Cattle with 250 – 450 kg of body weight have feed efficiency of 6.00 – 7.69 (Kearl, 1982) and 300 – 400 kg of body weight have feed efficiency of 8.4 – 10 for 1 kg of daily weight gain (NRC, 2000). One of the indicators of feed efficiency in cattle production is asetate: propionate ratio of rumen liquid. The increased proportion of propionic acid could partially explain the improvement in feed efficiency (Geay et al., 1992). A study by Da Silva et al. (2016) found that a diet with acetate to propionate ratio of 3.22 was associated with feed efficiency ratio (FCR) of 7.3 and average daily weight gain (ADG) of 1.3 kg. Another study showed that acetate to propionate ratio of 3.6 resulted in 6.05 FCR and 1.32 kg ADG (Geay et al., 1992). Propionic acid has a positive relationship with dietary energy utilization efficiency. The higher propionic acid indicates that the efficiency of



energy use in cattle is better (Cherdthong et al. 2014). An increasing dietary energy level could increase feed intake and digestibility (Zhou et al., 2015) but the increasing protein level without being balanced by energy level in the diet would not affect animal performance (Da Silva et al., 2016). Therefore, the efficiencies of dietary protein and energy are important to improve the efficiency of animal production. Based on the above explanation, it is necessary to determine the ideal TDN to CP ratio through feed efficiency and A/P ratio approach because TDN to CP ratio is one of the ways to get the most economical to purchase and good feed efficiency.

## Materials And Methods

This study used data from 4 studies and 12 treatments using Indonesian native cattle measured in Laboratory of Meat and Dairy Animal Production, Faculty of Animal and Agricultural Sciences, Diponegoro University, Indonesia. Cattle used in this study were 42 heads, weighed  $206.89 \pm 7.82$  body weight. They were fed diets containing 8.59 – 13.20% CP and 42.80 – 75.3% TDN. Feed given in this study consisted of 70% of concentrate and 30% Napiergrass. Studies being the sources of data were selected based on the following criteria: being run in a complete randomized design, the animals were at the same age (about 1.5 years old), and measuring acetate, propionate, FCR, TDN (%) and CP (%). The simple statistics of the data are shown in Table 1.

**Table 1** Statistics of variables in 4 studies and 12 treatments

	TDN	CP	A/P ratio	ADG	DMI	FCR
Number of animal	42	42	42	42	42	42
Average	56.2	11.3	3.3	771.0	5189.2	6.8
Standard deviation	6.7	1.8	0.6	129.1	762.0	1.2
Median	57.9	12.8	3.3	798.5	5295.4	6.8
Minimum Value	42.8	8.6	2.4	492.5	3840.0	4.9
Maximum Value	75.4	13.2	4.7	1014.9	6392.1	9.5

\*ADG: Average daily gain, DMI: Dry matter intake

## Parameters and Data Analysis

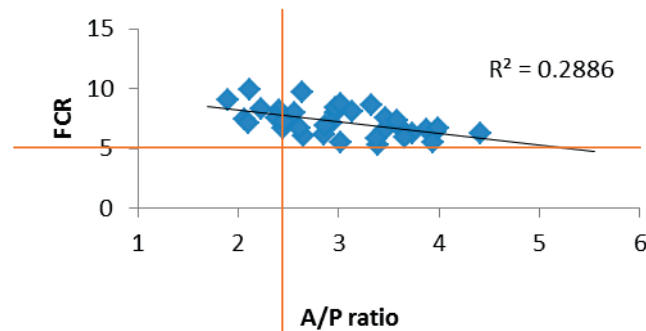
Variables data measured were TDN/CP ratio, rumen acetate to propionate ratio and feed conversion ratio. The data were analysed by correlating the value of A/P ratio to the FCR and TDN/CP to A/P ratio (Steel and Torrie, 1993). The strength of relationship among parameters was determined based on the correlation coefficient which was classified medium (0.40 to 0.599); strong (0.60 to 0.799); and very strong (0.80 to 1).

## Results and Discussion

The results are presented in Figure 1 and Table 2. The results showed that A/P ratio had medium negative correlation to FCR ( $r = 0.54$ ). The higher A/P ratio resulted in the lower FCR, the increase of A/P ratio caused FCR to decrease. A/P ratio of rumen liquid increased as the increase of acetate molar proportion and the decrease of propionate proportion (Li et al., 2017). Available energy (TDN) result rumen VFA concentration and the pattern of rumen VFA production depends on diet composition (Bergman, 1990). The diets containing high concentration of non-fiber carbohydrates results in ruminal fermentation with high propionate production, whereas diets containing a high fiber content lead to high acetate production. The



high concentration of non-fiber carbohydrates can be indicated by the high of total digestible nutrients.



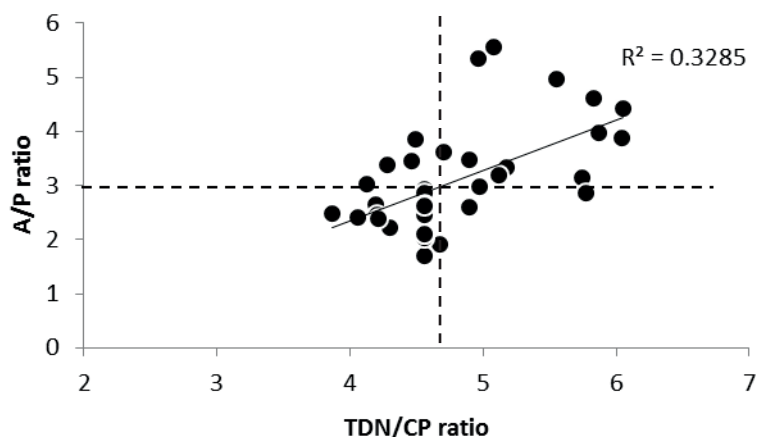
**Figure 1.** Correlation of A/P ratio to FCR

**Table 2.** Correlations and equations of variables

Correlation	X	y	Equations	R <sup>2</sup>	r
A/P to FCR	A/P ratio	FCR	$y = -0.9645x + 10.092$	0.2886	0.54
TDN/CP to A/P	TDN/CP	A/P ratio	$y = 0.9329x - 1.3943$	0.3285	0.57

In this study, high A/P ratio caused decrease FCR. A lower FCR could indicate that the cattle required less feed per kg of gain. It was due to propionic acid had a positive relationship with energy utilisation efficiency (Cherdthong et al., 2014). The propionate pathway requires metabolic hydrogen, and hence less hydrogen is lost in the form of methane (Cho et al., 2014). Propionate has a major impact on tissue distribution of nutrients (Nagaraja et al., 1997) because propionate is the most important VFA precursor of glucose synthesis in the ruminant (Geay, 1992).

The equations to predict FCR from A/P ratio is presented in Table 2. The results showed that every 1 point increase of A/P ratio resulted in 0.96 point decrease of FCR. The optimum acetate:propionate ratio for efficient fattening cattle should be 3 or less. Based on the equations, the optimal conditions of A/P ratio (3 or less) results 7.19 of FCR. It means that at least it required 7.19 kg DM of feed to reach 1 kg body weight gain.



**Figure 2.** Correlation between TDN/CP ratio and A/P ratio

Figure 2 shows that there was a positive and medium correlation between TDN/CP and A/P ratio ( $r = 0.57$ ). An increase in TDN/CP ratio resulted in increased A/P ratio. The most considered nutrients contained in fattening beef cattle diets are TDN and protein. Total digestible nutrients represent the energy density of the feedstuff. According to a research by Cho et al. (2014), increasing energy level in diet elevated propionate production. High energy content in the diet resulted in low A/P ratio. However, the high energy level should be accompanied by protein supply. The supply of energy precursors and nitrogen (N) is a primary limiting factor in the synthesis of microbial protein to maintain ruminal fermentation in the rumen (Sauvant et al., 1995).

Based on the second equation, the increasing 1 point of TDN/CP ratio will be accompanied by 0.9329 point increase of A/P ratio. From this study, at least it requires 4.72 of TDN to CP ratio to get 3 or less of A/P ratio. Therefore by those TDN to CP ratio, it can be obtained a good feed efficiency, which is 7.19. From the results, if dietary CP content is 12%, TDN content in the diets should be 56.6% at least.

## Conclusion

It can be concluded that A/P ratio of rumen liquid has negative and medium relationship with feed conversion ratio, energy to protein ratio has positive and medium to A/P ratio. Feed for beef cattle should contain 4.72 of TDN/CP ratio to reach a good A/P ratio less than 3 and feed conversion ratio 7.19 or less.

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## Dietary Protein Requirement for Maintenance and Growth of Southern Thai Indigenous Cattle

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### Abstract

This experiment was conducted to study protein requirements for maintenance and growth of Southern indigenous cattle. Sixteen indigenous male cattle initial average body weight of  $134.2 \pm 30.52$  kg with about 2 years old were given a difference levels of protein in feed at 7.3, 8.3, 10.3 and 12.5% with the same amount of energy intake ( $219.2 \pm 4.59$  kcal/kgW<sup>0.75</sup>/day) for 90 days. The dietary intakes of DM and GE were not significantly different ( $p > 0.05$ ). The nitrogen intake was significantly different ( $p < 0.01$ ) ranged from 1.05 to 1.86 g N/kg W<sup>0.75</sup>/day. The average dairy gain (ADG) was significantly ( $p < 0.01$ ) increased with an increase level of protein, ADG were 393.06, 497.50, 756.94 and 791.03 g/d in animal given 7.3, 8.3, 10.3 and 12.5% CP, respectively. Thus, increasing protein intake to 65% (from 7.3 to 12.5% CP) was efficient in term of feed conversion rate (FCR) and body weight gain ( $p < 0.01$ ). The relationship between the N intake and ADG was:  $N \text{ intake} = 0.3564ADG + 3.2633$  ( $R^2 = 0.662$ ). The protein requirement for maintenance and growth rate at 1 g /kgW<sup>0.75</sup>/day of growing male indigenous cattle was 0.522 g N/kg W<sup>0.75</sup>/day (3.26 g CP/kg W<sup>0.75</sup>/day) and 0.0569 g N/ kg W<sup>0.75</sup>/day (0.356 g CP/kg W<sup>0.75</sup>/day), respectively. Lower than previous study

**Keywords:** Thai southern indigenous cattle, protein requirement

### Introduction

Livestock, particularly ruminant production in southern Thailand is grossly insufficient in meeting the local demands for meat. Until very recently, cattle in southern of Thailand were kept mainly to fighting and to date, the bulk of the cattle are still owned by the traditional farmers in the villages. Nutrition is a major input in commercial animal production and in many instances the dominant economic factor which determines the success or failure of a particular livestock enterprise. Knowledge of the nutritional requirements of the animals is needed so that feed resources can be efficiently used to optimize output. The appropriate of feed requirement for beef cattle in Thailand were not yet clearly defined, so that much more elucidation is required. There has been little scientific evaluation of whether either of these nutrient requirement systems is suitable for Thai southern indigenous cattle. ARC (1996) suggested that growing *Bos indicus* cattle require about 10% less metabolizable energy requirement for maintenance as compared to *Bos Taurus* cattle. The protein requirement of Thai Native cattle would be lower than Thai crossbred (14%) and lower than *Bos Taurus* cattle 40 and 50% as recommended by NRC (2000) and ARC(1984), respectively (Chaokaur and Sommart, 2008). However, the accuracy of protein requirement for Thai southern indigenous cattle has not been yet evaluated. Therefore the experiment for Thai southern indigenous cattle reared under farm condition should be carried out to provide additional information for development of beef cattle production in Thailand.



## Material and methods

Sixteen growing males southern indigenous cattle, with an average initial body weight of  $134.20 \pm 30.52$  kg were used. The animals were housed in individual stalls at a private farm and fed with total mixed ration (TMR) 60% oil palm frond (OPF) and 40% concentrate (DM basis). The chemical compositions of TMR are shown in Table 1. The energy content of diets was formulated to contain gross energy intake which was 2 times higher than in maintenance (M) requirement (assuming  $M = 101.58 \text{ kcal/kgW}^{0.75}/\text{day}$ ) established earlier for Malaysian indigenous cattle (Liang et al, 1988). The protein levels were 7.3, 8.3, 10.3 and 12.5% in TMR. The animals were weighed at the beginning and every two weeks until the end of the experimental period. The experiment consisted of 90 day feeding periods. The experimental design was CRD. Each animal was assigned to four feeding treatment levels (crude protein). The animals were fed twice a day at 08:00 h and 16.00 h and had free access to drinking water. The refusal (feed ort) was collected, recorded daily and was sampled separately from the feed at the end of experimental period for dry matter (DM), gross energy (GE) and crude protein analyses (AOAC, 1984). Crude protein intakes were calculated as DM intake multiplied by CP content in TMR. The average CP intakes ( $\text{g CP/kgW}^{0.75}/\text{day}$ ) of each head of cattle were used to estimate dietary protein requirement. The values of ADG ( $\text{g/kgW}^{0.75}/\text{day}$ ) were plotted vs. CP intake according to the model suggested by Steen et al. (1997). The maintenance protein requirement predicted from the protein intake regression when ADG equals to zero.

The proximate nutritional values of feeds throughout this study were determined according to AOAC procedures (1984). The acid detergent fiber (ADF) and neutral detergent fiber (NDF) contents were determined by the method of Goering and Van Soest (1970).

## Statistical Analysis

The effect of protein feeding levels on ADG was analysed by two way analysis of variance using SAS program (SAS, Institute Inc., North Carolina, 1988). Orthogonal polynomials were used to describe the response of ADG by linear and quadratic effects to levels of protein intake. Responses in ADG was regressed against their respective CP intake using linear regression analyses.

## Results and Discussion

The total mix ration composed of 60% OPF and 40% concentrate (Table1). All the TMR (L1 to L4) seem to have the same amount of gross energy per kg. On the other hand, protein composition increases from L1 to L4. L1 had the lowest CP (7.3%), followed by L2, L3 and L4 (8.3, 10.3 and 12.5 %, respectively).

The animals used in this study were still growing as their weights ( $134.17 \pm 11.71$  kg) have not reached maturity weights of 300 kg. Body weights, average daily gain and feed consumed by Thai southern indigenous cattle are shown in Table 2. According to the concept of this study, there was a significant ( $p < 0.01$ ) increase in protein intake of the cattle as the crude protein concentration in the feeds increased. There were no significant differences in DM and GE intake among cattle receiving different protein feeding levels. The average daily gain was significantly ( $p < 0.01$ ) affected by the different protein intake. The highest average daily gain was shown by the cattle that was fed with 12.5% CP (791.03 g/day) followed by 10.3% (756.94 g/day), 8.3 % (497.50 g/day) and 7.3% (393.06 g/day). The average daily gain of the cattle fed with L4 was significantly ( $p < 0.01$ ) higher than for all the other diets. The values of ADG and protein intake were regressed linearly for the determination of dietary protein requirement for growth. The regression equation relationship between ADG and protein intake is protein intake ( $\text{g/kgW}^{0.75}/\text{day}$ ) =  $0.3564 \times \text{ADG}(\text{g/kgW}^{0.75}/\text{day}) + 3.2633$  ( $R^2 = 0.662$ ). The protein requirement for maintenance was determine by the intercepts of the respective equations were 3.26 g



CP/kgW<sup>0.75</sup>/day or 0.522 gN/ kgW<sup>0.75</sup>/day. The slopes of the regression line indicated the increase 0.356 g CP/kgW<sup>0.75</sup>/day per unit of increase 1 g/ kgW<sup>0.75</sup>/day or 0.0569 gN/ kgW<sup>0.75</sup>/day.

**Table 1.** Chemical compositions of total mixed ration (TMR)

(Composition, DM basis)	TMR offered			
	L1	L2	L3	L4
DM	90.1	88.7	89.9	89.7
Ash	5.0	4.7	4.8	4.9
CP	7.3	8.3	10.3	12.5
NDF	58.0	55.5	57.0	56.7
ADF	43.6	41.9	43.3	43.2
ADL	4.0	4.0	4.0	4.0
GE (cal/g)	2332.1	2303.0	2305.7	2303.6

**Table 2.** The effect of varying the protein levels on the ADG of Thai southern indigenous cattle.

Item	Levels of protein intake				SEM	Significance level	
	L1	L2	L3	L4		L	Q
Body weight, kg	143.38	118.08	142.33	132.90	32.04	NS	NS
Average daily gain, g/d	393.06	497.50	756.94	791.03	97.00	**	NS
DM intake, kg/d	3.72	3.27	3.95	3.68	0.88	NS	NS
FCR, g DMI/g gain	9.73	6.60	5.17	4.62	1.56	**	NS
N intake, g/day	43.58	43.11	64.78	73.05	15.02	*	NS
N intake, g	1.05	1.20	1.56	1.86	0.09	**	NS
CP/kgW <sup>0.75</sup> /day							
CP intake, g/d	272.35	269.43	404.87	456.54	93.92	*	NS
CP intake, g/kgW <sup>0.75</sup> /d	6.56	7.52	9.75	11.61	0.61	**	NS
GE intake, kcal/kgW <sup>0.75</sup> /d	208.79	212.96	221.84	218.47	14.87	NS	NS

SEM: standard error of the means, L, Q: Linear and quadratic effect of different levels CP intake  
NS: Not significantly different ( $p > 0.05$ ), \* ( $p < 0.05$ ) and \*\* ( $p < 0.01$ )

The protein requirement for maintenance from this study 3.26 gCP/kgW<sup>0.75</sup>/day was close to 4.34 gCP/kgW<sup>0.75</sup>/day of average protein requirement for maintenance of overall breed of cattle in Thailand (Department of livestock development, 2008). However, the value was lower than 5.03 gCP/kgW<sup>0.75</sup>/day of Native breed as reported by Department of livestock development (2008). The value was in the range of 2.13-4.36 gCP/kgW<sup>0.75</sup>/day for native cattle in Northeast part of Thailand as reported by Senarath et al. (2008); Kawashima et al. (2000) and close to 3.58 gCP/kg W<sup>0.75</sup>/day for Brahman cattle in Thailand (Chaokaur et al., 2008). The protein requirement for growth every 1 g of kg W<sup>0.75</sup> from this study is 0.356 gCP/kgW<sup>0.75</sup>/day or 35 gCP for every 100 g of W<sup>0.75</sup>, was lower than 0.38, 0.56, 0.59 gCP/kgW<sup>0.75</sup>/day for native cattle, Brahman and Brahman crossbred, respectively as reported by Chaokaur et al. (2008). The value also lower than for native cattle, Brahman and Brahman crossbred as 0.43, 0.60 and 0.64 gCP/ kg W<sup>0.75</sup>/day (Department of livestock development, 2008). However if compared to European





cattle, the cattle in Thailand was required lower protein than NRC (2000) and ARC (1984) by 65.28 and 68.97%, respectively. The value of protein requirement for maintenance and growth of Thai indigenous cattle predicted from the protein intake regression vs. ADG were quite low. The value could be explained by feed utilization and low quality of feed digestion of indigenous cattle or the nutrient recycle, similar to buffalo in situation of poor nutrient and limited resources of feed or depended on breed of animal. It therefore need more experiment to explain.

## Conclusion

The relationship between the protein intake and ADG for Thai southern indigenous cattle in this experiment was found to be  $\text{protein intake} = 0.356 \times \text{ADG} + 3.26$  ( $R^2 = 0.662$ ). The value of protein requirement for maintenance is  $3.26 \text{ gCP/kgW}^{0.75}/\text{day}$  and the value for growth is  $0.356 \text{ g CP/kgW}^{0.75}/\text{day}$  for every 1g of metabolic weight. The both values were lower than other experiment especially .....% lower than value reported by Department of livestock development (2008).

## Acknowledgements

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## The Effect of Dietary Protein Intake on Body Protein Growth in Thin Tailed Lambs

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### Abstract

A study was carried out to examine the effect of dietary protein intake on the growth of body protein on thin tailed lambs fed diet with different protein level. The study used 12 lambs of 3-4 months old with  $15.02 \pm 2.01$  kg (CV = 0.86%) of body weight. The lambs were reared intensively in individual pen and fed a complete feed containing 14, 16 and 18% crude protein (CP), 60% total digestible nutrients (TDN), for 9 weeks. Protein content of the lambs' body was measured by urea space methods. The results showed that the body protein growth rate increased with dietary protein intake, up to 162 g/d protein intake. However, when the dietary protein intake was higher than that point, the body protein growth rate decreased. It is concluded that best diet to obtain the highest body protein growth rate should contain 15.4% protein.

**Keywords:** body protein, crude protein, lambs

### Introduction

Fattening lambs could give many advantages, such as shortening time of raising, reducing feed cost and reducing the total waste from livestock (Purnomoadi et al., 2016). The success of fattening could be evaluated from the increase of body weight which was caused by the changes in body composition including fat, water and protein (Restitrisnani et al., 2013). The body component that is expected to change largely is the body protein. Body protein can reflect the muscle tissue that is formed; the greater body protein content the greater muscle tissue is formed and the more meat can be produced.

The growth rate of the body protein is dominantly influenced by intake of dietary protein. The dietary protein has an important role in the metabolism process in the cell and for the formation of body tissues especially muscle tissue (Costa et al., 2013). If dietary protein intake is less than the requirement of lambs, then the process of formation of body tissue will not be reached maximally. On the other hand, if dietary protein intake is very high, the feed cost may increase; while the excessive protein cannot be utilized by livestock and wasted through feces and urine, which potentially cause of environmental pollution (Lohakare et al., 2006). Therefore, it becomes very important to pay attention to the amount of dietary protein that should be given to lambs, so that lambs growth rate can be achieved optimally and efficiently, in an environmentally friendly way.



## Materials and Methods

This study used 12 lambs of 3-4 months of age with  $15.02 \pm 2.01$  kg (CV = 0.86%) body weight. The lambs were raised in individual cages equipped with feed and drinking bunk. They were given complete feed in form of pellet containing 14, 16, 18% crude protein (CP) and 60% total digestible nutrient (TDN). Lambs were reared intensively in individual pen for 9 weeks. The parameters observed were dietary protein intake, and changes of body composition. Body composition was measured by urea space method in accordance with Restitrisnani et al. (2013). Measurement of body composition was done in weeks 0 and week 9. Data were analyzed using regression correlation. Value of correlation based on r value, which consists of no relationship ( $r = 0.00$ ), weak ( $r \geq 0.00-0.25$ ), moderate ( $r \geq 0.25-0.50$ ), strong ( $r \geq 0.50-0.75$ ), very strong ( $r \geq 0.75-0.99$ ), perfect ( $r = 1.00$ ).

## Results and Discussion

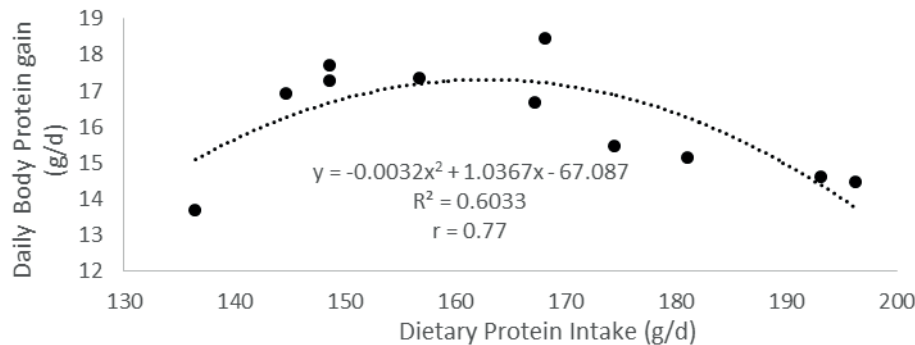
The dry matter intake, protein intake, body protein gain, daily body protein and protein conversion gain of lambs are presented in Table 1. The 9 week fattening lambs showed that dietary protein intake was ranged at 136.49 - 168.43 g/day and averaged gave 168.43 g/day. During the fattening time, the body protein gained around 860.60-1319.49 g (1063.91 g in average). Daily body protein gain was 13.66 – 18.42 g/day (16.34 g/day in average). Protein conversion was ranged at 8.39 – 13.58 (10.4 in average).

**Table 1.** Dry matter intake, protein intake, body protein gain, daily body protein and protein conversion gain of lambs.

Parameter	Range	Average	Standard Deviation
Dry Matter Intake (g)	793.8 – 1206.2	1053	103.1
Protein Intake (g)	136.49 – 196.2	168.4	18.5
Body Protein 0 weeks (g)	932.1 – 1544.8	1207.5	189
Body Protein 9 weeks (g)	2660.3 – 1997.1	2255.7	201.8
BPG (g)	860.6 – 1319.4	1063.9	122.1
DBPG (g/d)	13.6 – 18.4	16.3	1.4
Protein Conversion	8.3 -13.5	10.4	1.7

BPG = Body Protein Gain, DBPG = Daily Body Protein Gain

The body protein gain and protein conversion to body protein gain in lambs was higher than the sheep as reported by Wati et al. (2015) who found that sheep consumed 133.8 g/d protein had body protein increase of 10.16 g/day with protein conversion of 12.2. Higher body protein growth of lambs in this study was attributed to the fact that the lambs were in the fast muscle growth phase (Owens et al., 1993). The correlation between body protein content and dietary protein intake is presented in Figure 1.



**Figure 1.** Correlation between body protein content and dietary protein intake

Based on Figure 1, dietary protein intake was highly correlated with body protein gain ( $r = 0.77$ ). From the equation presented in Figure 1, it is predicted that the optimal daily body protein gain of 16.87 g/day can be achieved when dietary protein intake is 162 g/d or the feed should contain 15.4% of CP. It is shown in Figure 1 that beyond such level of dietary protein intake, the body protein gain would decrease. This might be caused by the capacity of muscle to enlarge is limited, so that when the dietary protein intake was excessive, it cannot be used by the muscle to grow more than the capacity. Hood and Allen (1973) stated the muscle tissue in lambs grows with cell enlargement process (hypertrophy), the enlargement of cells have limitations to grow optimal and affected by breed, sex and age. The results of this study was lower than the results of Haddad (2001) who reported the optimal protein content in feed was 16% for fattening Awassi lambs. This difference might be caused by the breed used. This study used thin tailed lambs which a small breed, while Awassi breed is a medium breed. Bello et al. (2016) stated that the small breed needed lower protein requirement.

## Conclusion

From the current study, it can be concluded that the best dietary protein content for highest body protein growth rate was 16.87 g/d, which can be obtained by a diet containing 15.4% protein.

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## Effect of Yeast Fermented Cassava Pulp (YFCP) Supplementation on Feed intake, Digestibility and Rumen Fermentation in Beef Cattle

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### Abstract

The objective of this experiment was to investigate the effects of yeast fermented cassava pulp (YFCP) included in concentrate diets on feed intake, digestibility and rumen fermentation in beef cattle. Four, 2 to 3 years old male Thai native beef cattle with  $180 \pm 5$  kg of body weight were randomly assigned according to a  $4 \times 4$  Latin square design to receive 4 dietary treatments with 4 levels of YFCP in the concentrate mixture (0, 100, 200, and 300 g/h/d). Animals were offered the concentrate at 0.5%BW and rice straw was fed *ad libitum* with available water at all times. As the result of this experiment, it was found that, rice straw intake and nutrient digestibility were linearly increased ( $P < 0.05$ ) with increasing levels of YFCP especially, in cattle receiving 300 g/h/d of YFCP supplementation.  $\text{NH}_3\text{-N}$  and BUN concentrations in the rumen were linearly increased ( $P < 0.05$  and  $P < 0.01$ , respectively) when increasing levels of YFCP while ruminal pH were similar among treatments ( $P > 0.05$ ). Total volatile fatty acid and propionic acid were higher while acetic acid and acetic to propionic acid ratio concentration were lower when increase YFCP level ( $P < 0.01$ ). Population of bacteria was linearly increased ( $P < 0.01$ ) and protozoal population was linearly decreased ( $p < 0.01$ ). Based on this experiment, it could be concluded that YFCP could improve rumen fermentation, dry matter intake, nutrient digestibility, population of bacteria while decrease protozoal population in beef cattle.

**Keywords:** yeast fermented cassava pulp, feed intake, digestibility, rumen fermentation, beef cattle

### Introduction

As cassava starch production increase, a large volume of waste by-product is also generated; cassava pulp is the solid and comprises approximately 10-15% of the original root weigh (Khempaka *et al.*, 2009). In Thailand, at least 1 million tons of cassava pulp is generate annually. After drying, some of the by-product are used to produce fertilizer or are included in diets for ruminant and swine. According to composition values, cassava pulp contains 69.89% starch, 1.7% ash, 1.6% CP, 27.8 CF, and 0.1% EE on a DM basis (Sriroth *et al.*, 2000). Although cassava pulp, the residue obtained after the extraction of starch from cassava root, is low in crude protein (Lounglawan *et al.*, 2010). Chauynarong *et al.* (2009) reported the major limitation of using cassava root meal in animal feed is its low protein content and deficiency in essential amino acid. Therefore using microorganism fermentation to improve the nutritive value of cassava pulp is possible.

Yeast cells are a rich source of vitamins, enzymes and some unidentified cofactors that are helpful in increasing microbial activity in the rumen (Dawson *et al.*, 1990). In addition, dietary yeast can be used as a ruminant feed especially *Saccharomyces cerevisiae*. Querioze *et*



*al.* (2004) reported that *Saccharomyces cerevisiae* has been used in animal diets for several decades and is considered sources of high quality proteins, B-complex vitamins, selenium and zinc. Yeast products are beneficial by enhancing dry matter (DM) intake and overall animal performance in ruminants (Denvev *et al.*, 2007). Several benefits of yeast product supplementation to ruminant nutrition have been demonstrated; an increase in nutrient digestibility, reduction in ruminal ammonia and increase of ruminal microbial population (Chaucheyras-Durand *et al.*, 2008). Polyorach *et al.* (2010) reported that yeast fermented cassava chip (YEFECAP) can increase crude protein content from 3.4 to 32.5%. Therefore, the objective of this experiment was to investigate effects of fermented cassava pulp (YFCP) in concentrate on feed intake, digestibility and rumen fermentation in beef cattle fed on rice straw.

## Materials and Methods

### Preparation of YFCP

Preparation was adapted from the method Polyorach *et al.* (2012) and some details are as follows:

- Twenty g of yeast were weighed into a flask, 24 g of sugar were added and 100 ml distilled water were mixed well and the mixture was incubated at room temperature for 1 h. (A).
- To prepare medium, 24 g of molasses were dissolved in the solution of 100 ml distilled water with 48 g urea and then pH of medium solution was adjusted using H<sub>2</sub>SO<sub>4</sub> to achieve final pH at 3.5-5 (B).
- Mix (A) and (B) were mixed at the ratio 1:1 and then flash with air for 18 h.
- After 18 h, transfer yeast medium solution was transferred and mixed with cassava pulp at a ratio 50ml:100g.
- These were fermented for 3 days, and then sun-dried. Final product was stored in the plastic bag before mixing with concentrate.

### Animal, diets and experimental design

Four, 2 to 3 years old male Thai native beef cattle with 180±5 kg of bodyweight were randomly assigned according to a 4×4 Latin square design to receive 4 dietary treatments with 4 levels of YFCP in concentrate (0, 100, 200, and 300 g/h/d ; T1, T2, T3, and T4, respectively). Ingredients of concentrate and chemical compositions of YFCP used in the experiment are shown in Table 1.

Cattle were housed in individual pens with respective dietary treatments. The cattle were offered the concentrate at 0.5%BW and rice straw was fed *ad libitum* twice daily at 07:00 and 16:00 h with available was at all time. Concentrate and roughage intakes were measured separately. The experiment was run for 84 days, the diets were fed for 21 days each period. During each experimental period, 14 days were as dietary adaptation while 7 days were for sample collections of feces (rectal sampling). Rumen fluid samples were collected at the last day of each period.

### Data collection and sampling procedures

Feed and fecal samples were collected during the last seven days of each period. Samples were dried at 60 °C for analysis of dry matter (DM) and crude protein (CP) content (AOAC, 2012), and neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest *et al.*, 1991). At the end of each period, rumen fluid and jugular blood samples were collected at 0 and 4 h after feeding. Rumen fluid was taken from the rumen by a stomach tube connected with a vacuum pump. Rumen fluid was immediately measured for pH and temperature. There were separated for two part, the first part was for NH<sub>3</sub>-N and VFAs by using HPLC. Rumen fluid





samples were used for  $\text{NH}_3\text{-N}$  analysis using the Kjeltach Auto 1030 Analyzer. The second part, for analyze Microbial population (Bacteria and Protozoa) using total direct count method according to Galyean (1989) by Haemocytometer.

### Statistical analysis

The data analyzed in a 4×4 Latin square design by analysis of variance using the GLM procedure of SAS (SAS, 1998). The results were presented as mean values and standard error of means. Treatments trends were compared by using Orthogonal polynomials. Differences among means with  $P < 0.05$  were accepted as representing statistically significant differences.

## Results and Discussion

### Effect on feed intake and nutrient digestibility

Feed intake and nutrient digestibility are shown in Table 2. The highest DM intake of rice straw was found in the 300 g/h/d YFCP treatment. Nutrient digestibility (DM, OM, CP and NDF) were significantly improved by higher levels of YFCP. Thus higher intake could be attributed by higher digestibility. In agree with Wohlt *et al.* (1991) and Gomez-Alarcon *et al.* (1990) who reported that supplementation of *Saccharomyces cerevisiae* increased the digestibility of protein, cellulose and fiber. Similarly, Weidmeier *et al.* (1987) reported that supplementation of yeast culture increased crude protein and hemicellulose digestibility in ruminant.

### Characteristics of ruminal fermentation and blood metabolites

Rumen fermentation characteristics and blood urea nitrogen (BUN) are shown in Table 3. These results could be reflected by effect of YFCP in improving  $\text{NH}_3\text{-N}$  and BUN concentrations, with increasing levels of YFCP ( $P < 0.05$ ) and ( $P < 0.01$ ), respectively. Ruminal  $\text{NH}_3\text{-N}$  obtained in this study was closer to optimal level at 15-30mg/100 ml (Leng, 1990). Moreover, increase levels of YFCP in the concentrate linear increased population of bacteria while linear decreased protozoa population in the rumen ( $P < 0.01$ ). Nguyen *et al.* (2005) reported that the higher bacteria growth efficiency in the absence of the protozoa in the rumen is probably related to the fact that removal of protozoa engulf and digest bacteria. Furthermore, fermentation end product VFA as well as individually VFA including those of propionate and proportion of C2:C3 concentration were significantly enhanced as influenced by higher level of YFCP supplementation were highest at 300 g/h/d. Total VFA and propionate production were significantly increased while acetate production were significantly decreased when YFCP supplementation level was increased. Acetate to propionate ratio was reduced from 2.9 to 2.0 in the 0 g/h/d and the 300 g/h/d YFCP supplementation, respectively. The inverse of relationship of acetate to propionate (C2:C3) ratio and amount of concentrate in the diet has explained by the tendency of fiber fermented bacteria to produce C2 and starch fermented bacteria to produce C3 (Styter, 1976).

## Conclusions and recommendations

Based on this experiment it could be concluded that inclusion levels of YFCP at 300 g/h/d could improve rumen fermentation, dry matter intake, nutrient digestibility, increased population of bacteria and decreased protozoa population in beef cattle. However, future study should be investigated in productive ruminants species such as lactating dairy cow and fattening beef cattle.



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**Table1.** Ingredients of concentrate and chemical composition of yeast fermented cassava pulp (YFCP) used in the experiment.

Items	Concentrate	YFCP <sup>a</sup>
Ingredients, %		
Cassava chip	58.0	-
Rice bran	1.0	-
Coconut meal	16.0	-
Palm meal	20.0	-
Urea	1.5	-
Molassese	2.0	-
Sulphur	0.5	-
Mineral mixed	0.5	-
Salt	0.5	-
Chemical composition, %		
DM	87.65	89.8
-----% of DM-----		
OM	92.9	98.2
CP	12.1	23.3
NDF	25.5	39.6
ADF	15.3	19.9

<sup>a</sup> yeast fermented cassava pulp (YFCP)

**Table2.** Effect of yeast fermented cassava pulp (YFCP) supplementation in concentrate on feed intake and digestibility of nutrients in beef cattle.

Item	YFCP supplementation (g/h/d)				SEM <sup>1</sup>	Contrasts <sup>2</sup>		
	0	100	200	300		L	Q	C
Rice straw DM intake								
kg/d	2.5	2.5	2.7	2.79	0.03	*	ns	ns
% of BW	1.4	1.4	1.5	1.5	0.21	*	ns	ns
g/kgBW <sup>0.75</sup>	52.4	51.5	57.0	56.6	0.79	*	ns	ns
Concentrate intake								
kg/d	0.9	0.9	0.9	0.9	0.01	ns	ns	ns
% of BW	0.5	0.5	0.5	0.5				
g/kgBW <sup>0.75</sup>	18.3	18.3	18.3	18.3	0.04	ns	ns	ns
Total intake								
kg/d	3.5	3.5	3.6	3.7	0.03	**	ns	ns
% of BW	1.9	2.0	2.1	2.2	0.02	**	ns	ns
g/kgBW <sup>0.75</sup>	70.7	72.0	79.7	81.6	0.76	**	ns	ns
Nutrient digestibility, %								
DM	61.6	61.4	68.3	70.5	2.32	*	ns	ns
OM	57.8	59.1	66.1	68.6	1.21	**	ns	ns
CP	42.0	47.1	58.2	64.5	1.86	**	ns	ns
NDF	42.8	53.2	61.8	63.8	1.71	*	ns	ns
ADF	54.7	54.8	57.0	62.1	2.08	ns	ns	ns

<sup>1</sup>SEM=standard error of the means; <sup>2</sup>L=linear, Q=quadratic, C=cubic; \*=P<0.05, \*\*P=<0.01, ns=non-significance (P>0.05).



**Table3.** Effect of yeast fermented cassava pulp (YFCP) supplementation in concentrate on rumen fermentation characteristics, blood urea nitrogen (BUN) and total direct count in beef cattle.

Item	YFCP supplementation (g/h/d)				SEM <sup>1</sup>	Contrasts <sup>2</sup>		
	0	100	200	300		L	Q	C
Ruminal temperature (°C)	39.1	38.9	38.8	38.5	0.13	ns	ns	ns
Ruminal pH	6.8	7.0	6.9	7.0	0.01	ns	ns	ns
NH <sub>3</sub> -N, mg/100 ml	13.7	15.1	16.1	16.8	1.33	*	ns	ns
BUN, mg/100 ml	13.0	13.0	13.2	13.5	0.06	**	ns	ns
Total direct count, cells/ml								
Bacteria, ×10 <sup>10</sup>	3.3	4.6	5.4	5.8	0.51	**	ns	ns
Protozoa, ×10 <sup>5</sup>	11.0	9.7	8.0	7.1	1.08	**	ns	ns
Molar proportion of VFA, mmol/l								
Total VFA, mmol/l	103.9	106.7	112.9	114.9	0.8	**	ns	ns
Acetate (C2), %	63.5	61.9	58.8	56.8	0.7	**	ns	ns
Propionate(C3), %	22.3	23.6	26.6	28.6	0.4	**	ns	ns
Butyrate (C4), %	14.2	14.5	14.6	14.6	0.8	ns	ns	ns
C2:C3 ratio	2.9	2.7	2.3	2.0	0.2	**	ns	ns

<sup>1</sup>SEM=standard error of the means; <sup>2</sup>L=linear, Q=quadratic, C=cubic; \*=P<0.05, \*\*P=<0.01, ns=non-significance (P>0.05).



## Influences of Yeast Fermented Potato Peel and Cassava Peel on Gas Kinetics and Digestibility Using *In Vitro* Gas Technique

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### Abstract

This study aimed to investigate the effect of yeast media solution ratios fermented potato peel and cassava peel on gas kinetics and digestibility using *in vitro* gas techniques. The experimental design was a 2×2 factorial arrangement in a completely randomized design. Factor A was two kind of substrates (potato peel and cassava peel) and factor B was two ratios of yeast media solution fermented substrates (Subs:YMS; 1:0 and 1:1). Results revealed that yeast medium solution fermented substrates at the ratio 1:1 improved CP contents both potato peel and cassava peel and influenced the gas kinetics fraction a, b, (a+b) and c (P<0.05) especially when fermented with potato peel. Cumulative gas production (96h) was increased when yeast medium solution was incorporated (P<0.01) and the highest volume was found in yeast medium solution fermented potato peel. *In vitro* true digestibility at 24 and 48h was significantly higher in the ratio 1:1 of yeast fermented with both potato peel and cassava peel (P<0.05). It could be concluded that ratio of yeast medium solution fermented potato peel and cassava peel at 1:1 improved gas production and *in vitro* true digestibility, especially when fermented with potato peel. These results revealed a potential use of yeast fermented potato peel and potentially improved ruminant production efficiency in further *in vivo* experiment.

**Keywords:** yeast media solution, potato peel, cassava peel, *in vitro* digestibility, gas kinetics, *in vitro* gas technique

### Introduction

In Thailand, the farmers always use rice straw and others crop residues as roughage sources fed to ruminants in the dry season. However, there are limitation of using due to the low quality in terms of protein content and digestibility. Therefore, supplementation of protein sources for ruminants are very valuable regime to meet the nutrients requirement and to serve productivity for ruminants. Cassava root is widely use as energy sources for ruminant feeding especially in tropical region (Wanapat and Kang, 2015). Some of residuals such as cassava peel from cassava powder factories and potato peel from potato chips production factories are also have high potential to use as ruminants feed. Recently, incorporation of microbial additives such as a culture of *Saccharomyces cerevisiae* to the diet has become common practice in ruminant nutrition



(Campanile et al., 2008). In addition, Boonnop et al. (2009) reported that fermentation of dry cassava chips and fresh cassava root with yeast culture could increase CP content from 3.4 to 32.5 % DM and 3.2 to 21.1 %DM, respectively. However, there are limited studies on the use of yeast media solution fermented with potato peel and cassava peel using *in vitro* gas technique. Therefore, the aim of this research was to determine the effect of yeast media solution ratios fermented potato peel and cassava peel on gas kinetics and digestibility using *in vitro* gas techniques.

## Materials and methods

*In vitro* technique of Menke et al. (1979) was conducted to determine the effect of yeast media solution ratios fermented with potato peel and cassava peel using beef cattle rumen fluids. The experimental design was a 2×2 factorial arrangement in a completely randomized design. Factor A was two kind of substrates (potato peel and cassava peel) and factor B was two ratios of yeast medium solution fermented substrates (Subs:YMS; 1:0 and 1:1). Bakers' yeast (*Saccharomyces cerevisiae*) was prepared for making the solution. The formulation of activated yeast was prepared by using 20 g of bakers' yeast and 20 g cane sugar mixed with 100 mL distilled water, then mixed well and incubated at room temperature for 1 h (A). Liquid media was prepared using 8 g molasses and 100 mL distilled water, followed by addition of 64 g urea, then adjusting the pH of the solution using H<sub>2</sub>SO<sub>4</sub> to achieve a final pH 3.5 to 5 (B). Mixed (A) and (B) at 1:1 ratio then flushed with air for 66 h at room temperature using an air pump (600 W). After 66 h, substrates were mixed with yeast medium solution at the ratio of 1:0 and 1:1, Subs:YMS ratios at 1:0 would be added by the water to adjust similar total volume of the condition and then fermented under shade for 2 days and followed by sundry for 2-3 days. The gas production was measured at 0, 1.5, 3, 6, 9, 12, 24, 36, 48, 72 and 96 h of incubation according to Foiklang et al. (2016). Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979). Nutrient compositions of substrates were analyzed according to the standard methods (AOAC, 1995). At 24 and 48 h post inoculation a set of samples were determined for *in vitro* true dry matter digestibility (IVTDMD) according to Van Soest and Robertson (1985). Data were analyzed by using the GLM procedures of the statistical analysis system institute (SAS, 1996).

**Table 1.** Chemical composition of yeast media solution ratios fermented potato peel and cassava peel used in the experiment

Item	Potato peel		Cassava peel	
	0YMS	1YMS	0YMS	1YMS
Chemical composition				
Dry matter, %	12.9	14.2	13.4	12.2
	----- % of dry matter -----			
Organic matter	91.2	92.9	93.9	94.6
Crude protein	18.2	51.1	5.2	44.3
Neutral detergent fiber	30.9	25.3	21.7	17.1
Acid detergent fiber	12.8	10.9	18.9	10.7

YMS= yeast media solution

## Results and discussions

Potato peel fermented with YMS at the ratio 1:0 and 1:1 contained 18.2 and 51.1% of CP, respectively while cassava peel fermented with YMS at the ratio 1:0 and 1:1 contained 5.2 and



44.3% of CP, respectively as shown in Table 1. Foiklang et al. (2017) stated that the CP contents may come from yeast cells in the solution, yeasts will use both urea and sugar to synthesize protein in their cells.

Gas kinetics were different between YMS ratios ( $P < 0.01$ ; Table 2). Fermentation of substrates with YMS affected the immediately soluble fraction (a), insoluble fraction (b), potential extent of gas production (a+b) and the gas production rate (c) ( $P < 0.05$ ), especially when fermented with potato peel excepted fraction a. Cumulative gas production (96h) was higher ( $P < 0.01$ ) when yeast medium solution was incorporated and the highest volume was found in yeast media solution fermented potato peel (1:1). Marrero et al. (2015) reported that yeast addition with alfalfa hay (*Medicago sativa*) as substrate could increase accumulated gas production. *In vitro* true dry matter digestibility at 24 and 48h was significantly higher in the ratio 1:1 of yeast fermented with both potato peel and cassava peel ( $P < 0.05$ ) while types of substrates did not influence on *in vitro* true dry matter digestibility.

These results were agreed with Tang et al. (2008) who supplemented yeast culture in rice straw and maize stover that could improve the rate of gas production, DM and OM disappearances. In addition, *in vitro* true digestibility at 24 and 48 h of incubations were shown to have high correlation with gas volume which was significantly higher in supplementation of YMS at the ration of 1:1 ( $P < 0.05$ ) both in potato peel and cassava peel.

**Table 2.** Effect of yeast media solution ratios fermented potato peel and cassava peel on gas kinetics and *in vitro* true digestibility.

Substrates	Yeast media solution ratios	Gas Kinetics <sup>1</sup>				Gas <sup>2</sup> (96 h)	IVTDMD <sup>3</sup> , %	
		a	b	c	a+b		24h	48h
Potato peel	0 YMS	-9.1 <sup>b</sup>	113.6 <sup>ab</sup>	0.058 <sup>b</sup>	104.5 <sup>bc</sup>	102.1 <sup>bc</sup>	65.3 <sup>b</sup>	78.5 <sup>b</sup>
	1 YMS	-7.2 <sup>b</sup>	146.6 <sup>a</sup>	0.078 <sup>a</sup>	139.4 <sup>a</sup>	139.3 <sup>a</sup>	75.2 <sup>a</sup>	85.2 <sup>a</sup>
Cassava peel	0 YMS	-4.1 <sup>ab</sup>	95.7 <sup>c</sup>	0.049 <sup>b</sup>	91.6 <sup>c</sup>	90.4 <sup>c</sup>	62.5 <sup>b</sup>	75.3 <sup>b</sup>
	1 YMS	-1.8 <sup>a</sup>	108.9 <sup>b</sup>	0.074 <sup>a</sup>	107.1 <sup>b</sup>	109.6 <sup>b</sup>	74.1 <sup>a</sup>	84.5 <sup>a</sup>
SEM		0.57	5.43	0.003	6.96	6.87	2.60	2.37
Comparison								
Substrates		*	**	ns	*	*	ns	ns
YMS ratios		*	*	*	**	**	*	*
Interaction		*	*	ns	*	*	ns	ns

<sup>1</sup>a= the gas production from the immediately soluble fraction, b= the gas production from the insoluble fraction, c= the gas production rate constant for the insoluble fraction (b), a+b = the gas potential extent of gas production. <sup>2</sup>Cumulative gas production at 96 h of incubation (mL/0.2g DM of substrate). <sup>3</sup>IVTDMD = *in vitro* true dry matter digestibility. YMS= yeast medium solution. \* $P < 0.05$ , \*\* $P < 0.01$ , ns = non-significant. SEM=standard error of the means.

## Conclusions and recommendations

Based on this study, it could be concluded that the ratio of yeast media solution fermented potato peel and cassava peel at 1:1 improved gas production and *in vitro* true digestibility, especially when fermented with potato peel. These results revealed a potential use of yeast fermented potato peel and potentially improved ruminant production efficiency in further *in vivo* production trials in beef cattle and lactating dairy cows.





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## Effect of Yeast Fermented Dehulled Rice (YEFEDER) Levels with Different Kind of Roughage on Gas Production and *In Vitro* Degradability Using *In Vitro* Gas Production Technique

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### Abstract

The objective of this study was to investigate the effect of yeast-fermented dehulled rice (YEFEDER) with different kind of roughage on gas production and *in vitro* degradability using *in vitro* gas production technique. The treatments were arranged according to a 2x4 factorial arrangement in a Completely randomized design (CRD) with two roughage sources (R); rice straw (RS) and sweet grass hay (*Pennisetum purpurium* cv. Mahasarakham) (SGH) and four ratios of R to YEFEDER (100:0, 75:25, 50:50 and 25:75). Under this study, results showed that the cumulative gas production (at 96 h) and gas production kinetics fraction (b) was not significantly different among treatments ( $P>0.05$ ). However, there was the interaction between roughage sources and R to YEFEDER ratios of kinetics gas (c) were significantly different among treatments ( $P<0.01$ ). In addition, the effect on degradability was found significantly different by influenced by R ( $P<0.001$ ), while SGH was higher digestibility than RS and at 50:50 and 25:75 of the ratios of R to YEFEDER were the highest among treatments ( $P<0.05$ ). Moreover, future research should be conducted using YEFEDER with SGH especially to replace concentrate for *in vivo* production trials in fattening beef and dairy cattle.

**Keywords:** YEFEDER, rice straw, sweet grass hay, degradability, *in vitro* gas production

### Introduction

Roughage source is important for ruminant and rice straw is the main crop-residue which farmers usually use as ruminant feed. However, rice straw is low in nutritive value with low protein (2-3%DM), high fiber composition and low dry matter digestibility (Polyorach et al., 2014). Sweet grass hay (*Pennisetum purpurium* cv. Mahasarakham) has the high CP and NFC content (15.2 and 12.1 % respectively), also high DM digestibility (Mapato and Wanapat, 2016). Concentrate feed is source of energy and protein which was supplemented when ruminants lack of nutrients but it is high price which leading to increase livestock production cost. In the previous study dry cassava chips and fresh cassava root fermented with *Saccharomyces cerevisiae* had been increased protein content (Boonnop et al., 2009). Boonnop et al. (2010) using the yeast fermented cassava chip (YEFECAP) to replace feed for soybean meal in concentrate could enhance the part of rumen fermentation and nutrient digestibility in beef cattle.



Paddy rice (*Oryza sativa* L.) is an important staple food in many countries in Asia, which provides not only essential nutrients but also carbohydrates for energy (Zhao et al., 2017). By the end of 2016, the price of paddy rice was lowest in ten years' time; there is a need to develop new innovation for increasing the utilization of paddy rice. Nevertheless, yeast fermented dehulled rice (YEFEDER) has not yet been used in ruminant feeding especially when fed on different roughage sources. Therefore, the objective of this research was to study the effect of yeast fermented dehulled rice with different kind of roughage and roughage to YEFEDER ratio on gas production and *in vitro* digestibility using *in vitro* gas production technique.

## Materials and Methods

### Preparation of yeast-fermented dehulled rice (YEFEDER)

Yeast solution preparation was done according to Wanapat et al. (2011) and Polyorach et al. (2014) procedure was follow: (1) Weigh yeast 20 g into a beaker, add 20 g sugar, distilled water 100 ml mixed become to the solution and incubate at room temperature for 1 h. (2) Preparation medium: weigh 24 g molasses, 100 ml distilled water, 48 g urea -and mixed carefully after then adjust pH of medium solution by H<sub>2</sub>SO<sub>4</sub> until pH 3.5–5. Mix (1) and (2) at the ratio 1:1 of solution flush with air for 60 h. After 60 h, take yeast medium solution to mix with paddy rice at a ratio of 1 ml: 2 g, then dry under shade for 3 days, followed by sun-drying for 2 days. Paddy rice was cracked to get the dehulled rice. The last production is kept in big plastic bag for mixing with the dehulled rice. The YEFEDER, SGH and RS were dried in a hot air-oven at 60°C for 48 hour and ground through sieve 1 mm apertures before using in the experiment. The method used for *in vitro* fermentation was based on the technique. (Menke and Steingass, 1988). Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979). *In vitro* degradability was determined *in vitro* dry matter degradability (IVDMD) (Tilley and Terry, 1963). All obtained data were subjected to the General Linear Models (GLM) Procedure of the Statistical Analysis System Institute (SAS, 1998) according to a 2×4 Factorial arrangement in a Completely randomized design (CRD). The statistical model included roughage sources (R), ratio of R:YEFEDER, R ratio × R:YEFEDER. For all parameters, differences between treatments means were contrasted by Duncan's new multiple range test (Steel and Terrie, 1980).

## Results and Discussions

The chemical compositions of concentrate, YEFEDER, RS and SGH are presented in Table 1. The CP content of YEFEDER was 28 % of DM. Increased protein of rice with yeast solution can improve protein content of the experimental feeds. This value was closely to those reported by Boonnop et al. (2009); Wanapat et al.(2009) and Polyorach et al.(2014). SGH contained CP content at 14 %DM which was closely the value reported by to Mapato and Wanapat (2016) (15.1 %DM). Under this study, it was found that the cumulative gas production (at 96 h) and gas production kinetics fraction (b) were not significantly different among treatments (P>0.05). The interaction between roughage sources and R to YEFEDER ratios of kinetics gas (c) were significantly different among treatments (P<0.01). Moreover, it was found that R and R:YEFEDER ratio of IVDMD were significantly different, while the diet contained SGH greater than RS to YEFEDER (P<0.05) (Table 2). These findings could be due to the nutritive value of SGH was lower in NDF and ADF contents than RS. In agreement with Ramin and Huhtanen (2013) who reported that lower NDF and ADF in feedstuff would lead to higher digestion. These results were found similar to Wanapat et al. (2011) revealed that yeast fermented cassava chips replaced soybean meal in concentrate for dairy cows and improved nutrient



digestibility. Due to the increasing of crude protein levels would provide more readily available energy, enhance of microbes, and increase degradability were found by Boonnop et al. (2010), who indicated that yeast fermented cassava chips could replace soybean meal and has beneficial to cattle in terms of rumen fermentation efficiency, microbial protein synthesis, and nutrient digestibility.

## Conclusion

Based on this study, it could be concluded that use of SGH and R:YEFEDER improved *in vitro* kinetics gas (c) and *in vitro* DM degradability. Therefore, this study revealed that SGH and R:YEFEDER at 50:50 or 25:75 were beneficial for ruminant feeding . However, future research should be conducted especially using YEFEDER to replace concentrate with SGH using for *in vivo* trials.

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**Table 1.** Chemical composition of yeast fermented dehulled rice (YEFEDER), rice straw and Sweet grass hay used in the experiment.

Items	YEFEDER	Sweet grass hay	Rice straw
DM, %	71.5	91.8	90.3
-----%DM-----			
CP	28.6	14.7	3.3
NDF	65.1	57.4	82.7
ADF	36.8	34.9	58.2

**Table 2.** Effect of yeast fermented dehulled rice (YEFEDER) with rice straw or Sweet grass on gas production, on *in vitro* gas production and *in vitro* degradability.

Trts	Dietary treatments		Gas production kinetics (ml)					<i>In vitro</i> degradability (%)
			a	b	c	a+b	Gas <sup>e</sup>	
RS : YEFEDER								
T1	100	0	1.7	57.3	0.01	58.9	43.2	32.7
T2	75	25	0.1	63.0	0.01	63.9	47.6	41.1
T3	50	50	0.9	46.4	0.03	44.4	42.5	55.3
T4	25	75	2.0	55.0	0.02	54.1	47.3	57.8
SGH : YEFEDER								
T5	100	0	2.0	49.6	0.07	41.8	47.6	46.7
T6	75	25	2.3	62.0	0.05	58.3	58.4	57.9
T7	50	50	2.8	46.7	0.06	44.4	45.1	68.9
T8	25	75	3.6	48.3	0.04	46.2	44.6	66.6
SEM			0.68	6.3	0.003	5.9	3.73	6.5
R			***	ns	***	ns	ns	*
R:YEFEDER			ns	ns	**	*	ns	*
Interaction			*	ns	**	ns	ns	ns

\*P<0.05; \*\*P<0.01; \*\*\*P<0.001; ns, not significant; a, the gas production from the immediately soluble fraction; b, the gas production from the insoluble fraction; c, the gas production rate constant for the insoluble fraction ratio; a+b, the gas potential extent of gas production. <sup>e</sup>Cumulative gas production at 96 h (ml/0.2 g DM substrate); RS, rice straw; YEFEDER, yeast fermented dehulled rice; SGH, sweet grass hay; R, roughage sources; SEM, standard error of means.



## ***Session 8-Arawan I***

ANN-01-0017

### **The Effect of Liquid Smoke on Methane Emission from Faeces**

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#### **Abstract**

A study was undertaken to assess the effect of liquid smoke on methane emission from faeces. The study used 10 kg fresh faeces (equivalent to 2.2 kg dry matter) of Madura. The faeces were placed in a transparent container with a capacity of 50 litres and the cover was designed by equipment to give aerobic condition. The experimental design used in this study was a completely randomized design (CRD) with 4 treatments and 5 replications. The treatment applied was the amount liquid smoke addition on the faeces, i.e. T0 (faeces without liquid smoke); T9 (9 ml liquid smoke/kg DM faeces); T22 (22 ml liquid smoke/kg faeces) and T36 (36 ml liquid smoke/kg DM faeces). The parameter observed in this study was methane emission from the faeces, measured by methane analyser (Horiba Ltd., Japan) and equipped with airflow meter. The data were recorded automatically by IBM PC at 3 hours intervals until the level of methane was similar to the methane at the open air. The results showed that liquid smoke reduced the faecal methane production ( $P < 0.001$ ). Faecal methane production decreased with the increasing concentration of liquid smoke. The faecal methane production of T0, T9, T22 and T36 were 0.23, 0.09, 0.06 and 0.05 mg CH<sub>4</sub>/kg DM faeces, respectively. Addition liquid smoke on faeces could reduce faecal methane production by 60-79%. From the equation it was predicted that the concentration of liquid smoke that effectively reduce methane from faeces is 22 ml liquid smoke per kg DM faeces. It can be concluded that liquid smoke can be used to reduce methane faeces as much as 60-79% and the optimum concentration of liquid smoke to reduce methane emissions is 22 ml/kg DM faeces.

**Keywords:** methane emission, faeces, liquid smoke

#### **Introduction**

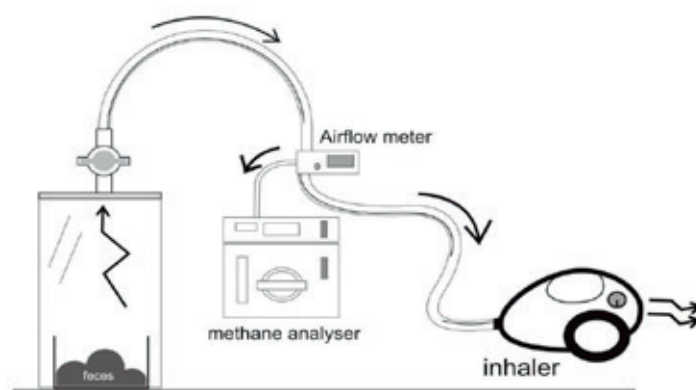
Methane is the greenhouse gases that have a destructive power greater than the other greenhouse gases. It takes more than 4 years to repair the damage caused by methane (McCourt, 2006; Jiao et al., 2014). Livestock is one of the major sources of methane in the atmosphere, i.e. 89% (Steinfeld et al., 2006; Shibata, 1994). In ruminants, methane is a by-product of the fermentation process in the rumen and is also produced by faeces (ZhongRong-Zhen et al., 2016). Increased economic sector will increase demand for livestock products and will result in an increased livestock population, which in turn will increase total methane emissions in the atmosphere. Priano et al. (2014) stated that faeces contribute 1% of total methane in the atmosphere. Many studies focused on reducing methane emissions in the rumen, but the study



on production of methane from faeces is limited. Most of conventional farms do not process their waste due to the limited funds to build the plantation processing for their livestock waste. Therefore, it is necessary to find other ways that cheap and environmentally friendly to mitigate methane emission from faeces. Liquid smoke is a product that is widely used to conserve food, especially to preserve meat and fish (Ariestya et al., 2016; Soazo et al., 2016). This proves that liquid smoke is safe to use and have not a negative impact on the environment. On the other hand, liquid smoke also has a bioactive content antibacterial (Saloko et al., 2014). That might be able to reduce methanogenic bacteria. The purpose of this study was to examine the effect of liquid smoke on methane production from faeces.

## Materials and Methods

Faeces used in this study were collected from Madura bulls in 7 consecutive days. The bulls were raised under feeding regimes containing 12.87% of crude protein (CP) and 58.63% total digestible nutrients (TDN). The study used a completely randomized design (CRD) with 4 treatments and each treatment had 5 replicates. The faeces collected were blended and homogenized, and treated with liquid smoke (T0= faeces without liquid smoke; T9= 9 ml liquid smoke/kg DM faeces; T22= 22 ml liquid smoke/kg DM faeces and T36= 36 ml liquid smoke/kg DM faeces). Each treatment had 5 replicates of 10 kg fresh, which was equivalent to 2.2 kg dry matter (DM) faeces. The treated faeces were placed in transparent container with a capacity of 50 litres and the cover is slightly open in order to give aerobic condition.



**Figure 1** Methane Measurement from Faeces (Purnomoadi et al., 2016).

The production of methane from faeces was measured using methane analyser (Horiba Ltd., Japan) to measure methane concentration and connected on airflow meter to measure gas volume which was then used to calculate total methane production. The data were recorded automatically by IBM PC at 3 hours intervals until the level of methane was similar to the methane at the open air. This method was a modification of the method established by Kawashima et al. (2001). An overview of equipment is illustrated at Figure 1. The faecal methane production obtained was the total methane production from 10 kg of faeces (equivalent

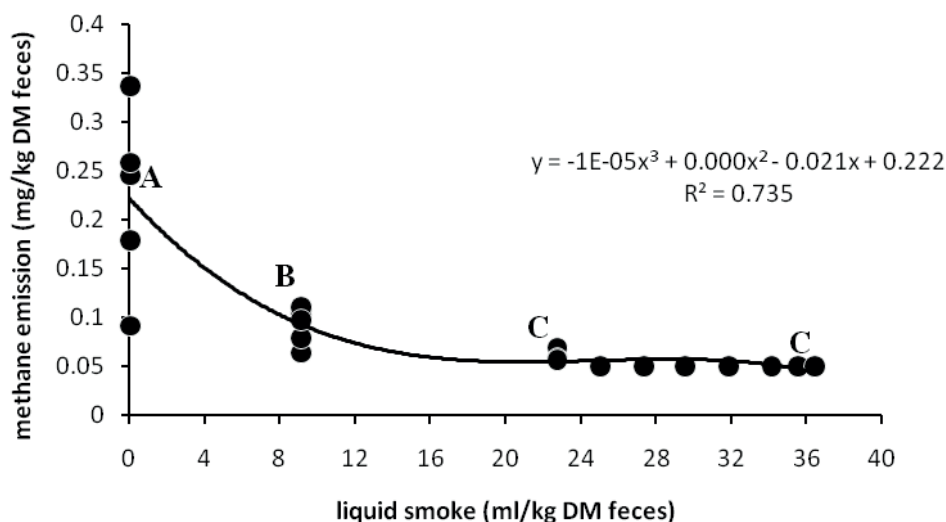




to 2.2 kg/DM faeces) from each treatment. Results obtained were converted into weight units (1 liter CH<sub>4</sub> = 0.714 g). The data were analysed by two-way ANOVA. When there were significant differences ( $P < 0.05$ ) among the treatments, Duncan's multiple range test was then carried out.

## Results and Discussion

The data showed that the addition of liquid smoke reduced ( $P < 0.001$ ) methane production from the faeces. Faecal methane production in this study (FLS0, FLS9, FLS22, and FLS36) was 0.23, 0.09, 0.06 and 0.05 mg CH<sub>4</sub>/kg DM faeces, respectively. This study showed that the addition of liquid smoke on faeces could reduce faecal methane production for 60-79%. The methane production from faeces in this study decreased as the amount of liquid smoke in the faeces increased. This indicated that the addition of liquid smoke reduced the amount and activity of methanogenic bacteria in the faeces. Liquid smoke contains phenols that act as antibacterial agents and can reduce the number of microbacterias (Jody et al., 2014).



**Figure 2** Graph of liquid smoke effect on fecal methane emissions (The letter indicated significance  $P < 0.01$ )

By using the equation obtained, it can be predicted that the 22 ml liquid smoke/kg DM faeces addition of liquid smoke is the most effective and efficient to reduce faecal methane production. The decrease in faecal methane production contributes on reducing total methane emission in the atmosphere. The population of cattle in Indonesia is 16,092,561 heads (BPS, 2016). If every cow excretes 4.5 kg DM faeces, and 1 kg DM faeces produces 0.1 milligram methane, then it can be calculated that methane produced by cattle in Indonesia is 2.64 ton/year. With the addition of liquid smoke on faeces, then the methane emission can be reduced as much as 60-79% (equivalent to 1.58 – 2.08 ton/year).



## Conclusion

Based from the results, it can be concluded that liquid smoke can be used to reduce faecal methane production by 60-79%. The maximum concentration of liquid smoke which can be applied on farm and significantly reduce methane emission is 22 ml/kg DM faeces. Further study is needed to investigate the utilization of liquid smoke to reduce methane emission from rumen fermentation either faeces.

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## Effects of Sunflower Oil and Nitrate Supplementation on Methane Production and Rumen Fermentation by Using *In Vitro* Gas Production Technique

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### Abstract

This study was aimed to determine the effect of sunflower oil levels and nitrate supplementation on methane production, rumen fermentation and nutrient digestibility in meat goats using *in vitro* gas production technique. There were two factors; Factor A was sunflower oil levels (0, 3, 6 % basal DM of total diet) and factor B was nitrate levels (0, 1, 2, and 3% basal DM of total diet). The result showed that the potential had greater at 3% of sunflower oil and ED, OMD, ME were higher at 6% of sunflower oil with 3% of nitrate. Volatile fatty acids and propionate production were decreased when increased nitrate level, however, the ratio of acetate: propionate did not affect by dietary treatments. The protozoa population did not affect by the interaction. Therefore, an interaction between 6% of sunflower oil with 3% of nitrate was decreased by 38 % methane production when compared to without sunflower oil and nitrate. Therefore, based on the experiment data concluded that the use of sunflower oil at 3% increase potential of gas production, but added 6% of sunflower oil can be increased ED, OMD, and ME. The combination 6% of sunflower oil with 3% potassium nitrate could decrease methane by 38 %.

**Keywords:** nitrate, sunflower oil, goat, gas production technique

### Introduction

Methane is one the end products of rumen fermentation and represents a loss of 2-12 % of total dry matter intake (Johnson and Johnson, 1995). Enteric methane emissions are predicted to grow by over 30% from 2000 to 2020 (O'Mara, 2010). The impact of diet composition on methane production, Leng (2008) also reported that nitrate can replace carbon dioxide as an electron acceptor with the generation of another reduced to nitrite and then to ammonia. Also, animal studies have reported that nitrate administration decreased *methanogenesis in vivo* (Takahashi and Young, 1991; Hulshof et al. 2012). Hao et al. (2009) revealed that nitrate can be fed safely to goats at levels that source of protein for the rumen microbial protein synthesis in diets low in true protein. In addition, supplementation with dietary lipids are potent modifiers of ruminal fermentation and may offer a nutritional strategy for bacterial protein. So that enhancing the efficiency of protein utilization and alleviating N losses in the ruminant animals (Hristov and Jouany, 2005). For example, methane production was reduced by 22% when fed sunflower oil (400 g/d, approximately 5% of DMI) (McGinn et al. 2004). Which, polyunsaturated fatty acids



(PUFA) consisting double or triple bonds have the potential to be used as hydrogen sinks since these bonds will obtain saturated with hydrogen and less hydrogen will be accessible for methane production. Strategies for enhancing production through the efficient utilization of available feed resources might also nourish to reduce methane emissions from ruminants. Therefore the present study was designed to study the effect of sunflower oil and nitrate supplementation on methane production, and rumen fermentation in goats.

## Materials and methods

Factor A was the levels of sunflower oil (0, 3 and 6 % DM in diet) and factor B was the levels of nitrate (0, 1, 2 and 3 % DM in diets). All animals were fed a diet containing roughage to concentrate ratio (R:C) of 60:40 at 1.5% BW/d and pangola grass hay was used as a roughage source. The diet of R:C was offered to the animals twice per day in the morning (07:00) and afternoon (16:00) feeding time. Roughage and concentrate were ground in a Retsch mill (SR200 model, Retsch, Haan, Germany) to pass a 1 mm mesh prior to analyzing for chemical compositions and *in vitro* gas production measurements. The incubation substrate consisted of roughages (Pangola grass hay and concentrate were mixed at a ratio of 60:40 (w/w, on DM basis) and stored until incubation. Sunflower oil was bought from the supermarket. The DM was determined by drying at 135 °C for 4 h followed by equilibration in a desiccator and OM was calculated as weight loss upon ignition at 600 °C. Roughage and concentrate were dried at 60°C and analyzed using the standard methods of AOAC (1995) for DM, CP, and ash, while neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed according to Van Soest et al. (1991).

Data were statistically analyzed according to 3×4 factorial arrangements in the completely randomized design of each forage using the PROC GLM procedure (SAS, 1996). Significant differences ( $P<0.05$ ) among treatments were determined using Duncan's News Multiple Range test according to Steel and Torrie (1980).

## Results and Discussions

### In Vitro gas production characteristics in incubated

The levels of sunflower oil had affected on gas production characteristic, total gas production (ml/g DM) efficiency digestibility (ED) organic matter digestibility (OMD) and metabolizable energy (ME) (MJ/kg DM) except for the gas production rate constant for the insoluble fraction (c). Levels of NO<sub>3</sub> had effected on asymptotic (b) (ml) and the gas production rate constant for the insoluble fraction (c) but did not effect on ED, OMD, and ME (Table 2). The results showed that sunflower oil 6% and 3% of nitrate increased effective degradability, metabolizable energy and *in vitro* digestibility organic matter degradability. In addition, the fat in rumen diet improves energy efficiency due to lower methane production and direct use of long chain fatty acids in the metabolic pathway of fat synthesis (Farra and Satter, 1971). The potential extent of gas production was higher at 3% of sunflower oil in diets, but with inclusion of 6% a little decrease may be because of sunflower oil had affected to substrate availability and possible toxicity for bacteria agree with McGinn et al. (2004) who reported sunflower oil decreased methane emissions by 22% compared with the control.

### The effect of sunflower oil and nitrate on volatile fatty acids, total volatile fatty acids, and methane production

The levels of sunflower oil had effected on the concentration of acetate and butyrate but did not affected on propionate. Moreover, the levels of nitrate had affected acetate when



increasing levels of nitrate have been to increase concentration of acetate but did not affect the concentration of propionate. However, there were significantly affected in cubic on butyrate. The total volatile fatty acids (mM/L) had effected when increasing levels of sunflower oil but did not affected on acetate: propionate ratio. The combination of nitrate and sunflower oil decreased the molar percentage of acetate at 6 h after feeding when increased levels of nitrate. Propionate decreased the molar percentage by 3% of nitrate at 3 h after feeding but butyrate was decreased at 3% of nitrate. Moreover, sunflower oil had effected on methane, it had been reducing methane production when increase level of sunflower oil. In addition, nitrate had effected on methane emission when an increased level of nitrate at 3 and 24 h after feeding. Results from this research, the levels sunflower oil increased the proportion of acetate and decreased propionate Farra and Satter (1971) observed a shift in the VFA profile from propionate to acetate when fed with nitrate dietary. The results show a trend of an increased proportion of acetate and decreased the proportion of propionate on the increased nitrate containing diet. Alaboudi and Jones (1985) and Sar et al. (2005) observed that upon supplying nitrate, the proportion of butyrate decreased, probably due to the electrons operated with nitrate was reduced. Supplement nitrate increased the percentage of acetate but decreased the percentage of butyrate due to decreased concentrations of NADH and increased concentrations of NAD<sup>+</sup>, which in turn approval production of acetate overproduction of more reduced VFA (Allison and Reddy, 1983). Methane production had effect at 6% of sunflower oil and 3% of nitrate can be decreased by 38 % without sunflower oil and nitrate.

## Conclusion

In conclusion, the rumen fermentation characteristic had affected at 6% of sunflower oil increasing the efficiency digestibility and metabolizable energy. Volatile fatty acid had at 3% of sunflower oil but at 6% was tendency decreased a little bit when compared to with 3%. Methane production was reduced by a combination of 6% sunflower oil and 3% of nitrate supplementation when to compare without sunflower oil and nitrate.

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**Table 1.** The ingredient and chemical composition of concentrate, panggola grass hay used in the experiment

Items	Concentrate	Panggola grass hay
Ingredient, % dry matter		
Cassava distillers dried meal	32.0	-
Soybean meal	20.0	-
Corn distillers dried grains	17.5	-
Rice bran	10.0	-
Wheat bran	10.0	-
Molasses	8.0	-
Mineral and vitamin mixture <sup>1</sup>	2.5	-
Chemical composition		
Dry matter, %	92.2	87.5
	% of dry matter	
Ash	7.0	8.4
Crude protein	14.6	7.3
Ether extract	4.0	1.9
Crude fiber	17.1	32.0
Neutral detergent fiber	42.5	73.4
Acid detergent fiber	26.3	35.9
Acid detergent lignin	10.9	4.0
TDN (%) <sup>2/</sup>	60.2	50.9
NFC <sup>3/</sup>	31.7	8.8

<sup>1/</sup>Mineral and vitamin mix : provided per kg of concentrate including vitamin A, 5,000 IU; vitamin D3, 2,200 IU; vitamin E, 15 IU; Ca, 8.5 g; P, 6 g; K, 9.5 g; Mg, 2.4 g; Na, 2.1 g; Cl, 3.4 g; S, 3.2 g; Co, 0.16 mg; Cu, 100 mg; I, 1.3 mg; Mn, 64 mg; Zn, 64 mg; Fe, 64 mg; Se, 0.45 mg.

<sup>2/</sup>Total digestible nutrients, TDN = tdNFC + tdCP + (tdFA x 2.25) + tdNDF – 7 (NRC, 2001)

<sup>3/</sup>Non Fiber Carbohydrate, NFC= 100-(CP-NDF-EE-ash)



**Table 2** Effect of sunflower oil and nitrate supplementation *in vitro* on gas production characteristics, total gas production (ml/g DM), efficiency digestibility, ED (%), organic matter digestibility, OMD (%) and metabolizable energy, ME (MJ/kg DM).

Items	Levels of sunflower oil (% of diets)				Level of potassium nitrate (% of diets)				SEM	Contrast
	0	3	6		0	1	2	3		
a (ml)	9.46 <sup>a</sup>	7.93 <sup>b</sup>	3.67 <sup>c</sup>		7.14	4.79	6.50	9.63	0.14	NS
b(ml)	26.58 <sup>c</sup>	63.99 <sup>b</sup>	65.26 <sup>a</sup>		48.58 <sup>d</sup>	50.79 <sup>c</sup>	53.90 <sup>b</sup>	54.50 <sup>a</sup>	0.20	C
c (h <sup>-1</sup> )	0.09	0.08	0.10		0.10 <sup>a</sup>	0.10 <sup>a</sup>	0.09 <sup>b</sup>	0.07 <sup>c</sup>	0.01	C
a + b (ml)	36.03 <sup>c</sup>	71.92 <sup>a</sup>	68.92 <sup>b</sup>		55.71	55.59	60.41	64.12	0.30	NS
G24 (ml/g DM)	27.65 <sup>c</sup>	51.68 <sup>b</sup>	54.80 <sup>a</sup>		47.47	42.93	45.40	43.03	0.16	NS
G96 (ml/g DM)	36.17 <sup>c</sup>	71.85 <sup>a</sup>	68.92 <sup>b</sup>		55.69	55.58	60.31	64.33	0.30	NS
ED (%)	30.17 <sup>c</sup>	59.09 <sup>a</sup>	58.06 <sup>b</sup>		46.71	46.64	50.41	52.67	0.21	NS
OMD (%)	60.85 <sup>c</sup>	62.40 <sup>b</sup>	74.69 <sup>a</sup>		57.90	60.62	71.67	73.72	0.57	NS
ME(MJ/kg DM)	8.08 <sup>b</sup>	8.32 <sup>b</sup>	10.21 <sup>a</sup>		7.62	8.04	9.75	10.06	0.09	NS

<sup>a,b,c,d</sup> Mean within the same row for the main effects of levels of sunflower oil and nitrate having different letters are different at  $P < 0.05$ . \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; NS= not significantly different ( $P > 0.05$ ); SEM=standard error of the mean; L= linear; Q=quadratic; C= cubic.



**Table 3** Effect of sunflower oil and nitrate supplementation *in vitro* on proportion volatile fatty acid (VFA).

Items	Levels of sunflower oil (% of diets)			SEM	Contrast	Level of nitrate (% of diets)			SEM	Contrast
	0	3	6			0	1	2		
Acetate (C2) (mol/100 mol)										
0h	75.75 <sup>b</sup>	81.65 <sup>a</sup>	80.95 <sup>a</sup>	0.22	L	76.85	80.25	81.17	0.13	NS
3h	80.29 <sup>a</sup>	77.35 <sup>b</sup>	77.02 <sup>b</sup>	0.12	Q	76.86 <sup>b</sup>	78.80 <sup>a</sup>	78.27 <sup>a</sup>	0.07	L
6h	83.57 <sup>a</sup>	79.18 <sup>c</sup>	81.09 <sup>b</sup>	0.15	Q	81.41	80.71	81.07	0.04	NS
Propionate (C3) (mol/100mol)										
0h	3.10	2.95	2.56	0.02	NS	2.84	2.84	2.72	0.01	NS
3h	2.98	3.98	3.44	0.03	NS	3.34	3.80	3.25	0.02	NS
6h	3.25	2.90	2.98	0.01	NS	2.95	3.13	2.99	0.01	NS
Butyrate (C4) (mol/100mol)										
0h	33.14 <sup>a</sup>	30.84 <sup>b</sup>	30.01 <sup>b</sup>	0.11	Q	31.98	31.16	30.83	0.04	NS
3h	30.18 <sup>c</sup>	34.62 <sup>a</sup>	32.09 <sup>b</sup>	0.15	Q	33.80 <sup>b</sup>	35.27 <sup>a</sup>	32.11 <sup>b</sup>	0.23	C
6h	33.84 <sup>a</sup>	24.79 <sup>c</sup>	28.75 <sup>b</sup>	0.31	L	31.04	31.23	27.57	0.17	NS
Total volatile fatty acid (mM/L)										
0h	75.75 <sup>c</sup>	81.65 <sup>a</sup>	80.95 <sup>b</sup>	0.22	L	76.85	80.25	81.17	0.13	NS
3h	80.29 <sup>a</sup>	77.35 <sup>b</sup>	77.02 <sup>b</sup>	0.12	Q	76.86 <sup>b</sup>	78.80 <sup>a</sup>	78.27 <sup>a</sup>	0.07	L
6h	83.57 <sup>a</sup>	79.18 <sup>c</sup>	81.09 <sup>b</sup>	0.15	Q	81.41	80.71	81.07	0.04	NS
Methane (mol/mol VFA) 1/										
0h	33.14 <sup>a</sup>	30.84 <sup>b</sup>	30.01 <sup>b</sup>	0.11	Q	31.98	31.16	30.83	0.04	NS
3h	30.18 <sup>c</sup>	34.62 <sup>a</sup>	32.09 <sup>b</sup>	0.15	Q	33.80 <sup>b</sup>	35.27 <sup>a</sup>	32.11 <sup>c</sup>	0.23	C
6h	33.84 <sup>a</sup>	24.79 <sup>c</sup>	28.75 <sup>b</sup>	0.31	L	31.04	31.23	27.57	0.17	NS

<sup>a,b,c</sup> Mean within the same row for the main effects of levels of sunflower oil and nitrate having different letters are different at  $P < 0.05$ . \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ; NS= not significantly different ( $P > 0.05$ ); SEM=standard error of the mean; L= linear; Q=quadratic; C= cubic.



## ***In Vitro* Rumen Microbial Population and Fermentation with The Addition of *Sapindus rarak* Extract and Sesame/Canola Oils Microencapsulation**

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### **Abstract**

The aim of this study was to analyze the effect of combination between defaunation by using *Sapindus rarak* extract and microencapsulation of canola/sesame oils on *in vitro* rumen microbial population and fermentation characteristics. The research was conducted by using rumen fluid obtained from fistulated Ongole grade beef cattle. This research design used was block randomized design with 6 treatments and 5 replications. The treatments were T0 = control (forage:concentrate = 70:30), T1 = T0 + *Sapindus rarak* extract 1 mg/ml, T2 = T1 + sesame oil 10%, T3= T1 + sesame oil microencapsulation 10%, T4= T1 + canola oil 10%, T5=T1 + canola oil microencapsulation 10%. Variables observed were total bacteria and protozoal population, ammonia –nitrogen concentration, VFA total and partial production, and methane estimation. The result showed that the addition of *Sapindus rarak* extract 1 mg/mL and its combination with canola oil microencapsulation very significant decreased ( $P < 0.01$ ) protozoal population and increased bacterial population,  $\text{NH}_3\text{-N}$  concentration, dry matter and organic matter digestibility compared to the the other treatments. Furthermore, pH value, total and proportional VFA,  $\text{CH}_4$  estimation were similar among treatments. It's concluded that the addition of *S. rarak* extract 1 mg/mL and combine with canola oil microencapsulation 10% decreased protozoa population, increased rumen bacterial growth,  $\text{NH}_3$  concentration, dry matter and organic matter digestibility but did not affect VFA total and partial.

**Keywords:** canola oil, microencapsulation, rumen fermentation, *Sapindus rarak* extract, sesame oil

### **Introduction**

The production of beef cattle held by farmers in the villages in Indonesia is still low and need to be improved both in numbers and quality to meet the meat requirement. The main problem behind such low productivity of beef cattle would be the less sufficiency of feed resources and based on high forage ration with low quality (native grass). The efficiency of feed utilization based on high forage ration is relatively low resulting in low beef cattle productivity and tends to produce high methane gas. In addition, the high forage based ration usually deficient in nitrogen (protein), thereby decreasing the synthesis of microbial proteins which are the primary source of protein in ruminants (Suharti et al., 2015). Meanwhile, the protein supply form microbial protein will decrease with the presence of protozoa in the rumen because of the bacteria predation by protozoa (Gutierrez, 2007). Our previous study showed that inhibition of rumen protozoa by using saponins increased the growth of rumen bacteria. The extract of



*Sapindus saponaria* contains high saponin up to 84.5% and proven to suppress the growth of protozoa, as well as increase the proportion of *Ruminococcus albus* and *Prevotella ruminicola* (Suharti et al. 2011).

An appropriate strategy need to be done to enhance beef cattle production which fed based on high forage ration. Moreover, the increasing of beef cattle production strategy should be balanced by an improvement of meat quality especially unsaturated fatty acid content. Some of consumers still have concerns about high saturated fatty acid content in the beef (red meat). Therefore, efforts should be made to reduce the content of saturated fatty acids in beef meat and also increase the content of unsaturated fatty acids that are more safe for human health. Supplementation of oils as unsaturated fatty acid sources could improve the quality of meat which have high content of unsaturated fatty acid. However, the used of oils need to be protected to avoid rumen biohydrogenation by rumen microbe that convert unsaturated fatty acid to be saturated fatty acid. Microencapsulation is one of strategy to protect unsaturated fatty acid of feed from rumen microbe biohydrogenation.

This research was aimed to analyze the synergism effect combination of *Sapindus rarak* extract and microencapsulation of canola/sesame oils on rumen fermentation in vitro including population of bacteria and protozoa, concentration of NH<sub>3</sub>-N, production of VFA total and its proportional, dry matter and organic matter degradability, and methane estimation.

## Materials and Methods

Preparation of Sesame/Canola oil microencapsulation according to the Carneiro et al. (2011) method and preparation of *Sapindus rarak* extract according to the Wina et al., 2006.

The experiment was designed as a 6 x 5 block randomized design with 6 treatments include: T0= Napier grass: concentrate=70:30 (control); T1 = control + sapindus extract 1mg/ml; T2=T1+Sesame oil 10% from concentrate; T3=T1+microencapsulation sesame oil 10%, T4=T1+Canola oil 10%, and T5= T1+microencapsulation Canola oil 10%. The *Sapindus rarak* extracts and oils were mixing with concentrate. Concentrate mixture (cassava by product, wheat pollard, soybean meal, coconut cake meal, molasses, CaCO<sub>3</sub>, premix, urea, and sesame/canola oil with or without protection by microencapsulation) with 15%-17% CP and 69%-74% TDN. Variables observed were population of bacteria and protozoa, concentration of NH<sub>3</sub>, production of VFA total and its proportional, dry matter and organic matter degradability, and methane estimation.

*In vitro* fermentation was conducted according to Tilley and Terry (1963). Into each 100 mL fermentation tube, 500 mg substrate according to the each treatment, 40 mL McDougall buffer, and 10 mL rumen fluid were added and incubated at shaker water bath with temperature 39° C. The rumen fluid for this experiment was collected after 3 h morning feeding from the 3 rumens fistulated Ongole crossbred beef cattle with Ethical Approval from Animal Care and Use Committee (AUAC) 01-2013b IPB.



Samples from aliquot were taken after 4 h incubation for pH, VFA,  $\text{NH}_3\text{-N}$ , protozoa, total bacterial analysis and after 48 h incubation for dry matter and organic matter digestibility analysis. The numbers of protozoa in the rumen fluid were counted under a microscope according to Ogimoto & Imai (1981). The 0.5 mL rumen fluid was mixed with 0.5 mL Trypan Blue Formalin Saline (TBFS) which consist of 100 mL formaldehyd 35%, 2 g trypan blue, 8 g NaCl, and 900 mL distilled water and diluted 5 times. The population of protozoa was counted directly on 5 divisions by using a counting chamber ( $0.1 \text{ mm} \times 1 \text{ mm}^2$ ) under a microscope (40x) and calculated by the following formula:  $P = (n/5) \times 10^4 \times d$ , where P= number of ciliates per 1 mL rumen contents, n= number of division that counted in the counting chamber, d= diluted multiple of the sample. Population of total bacteria were counted according to Ogimoto & Imai (1981) by using roller tube method and Rumen-Fluid Glukosa Cellobiosa Agar (RGCA) Modification. The RGCA solution consist of 15 mL mineral solutin I, 15 mL mineral solution II, 0.1 mL Resazurin 0.1% solution, 40 mL distilled water, 2 g bacto agar, 30 ml rumen fluid, 0.2 g glucose, 0.2 g cellobiose, 0.1 g cysteine.HCl.H<sub>2</sub>O, 1 mL Na<sub>2</sub>CO<sub>3</sub> 8% solution, 1 g bacto casiton, 0.3 g yeast extract, 0.2 g yeast extract, 0.2 g starch soluble, 0.4 g NaHCO<sub>3</sub> and 1 mL sodium lactate. Forty-five mL of anerobic dilution solution and 0.5 mL of rumen sample place in the hungate tube. The sample the diluted until 10 times dilution. The 0.5 mL sample from dilution 6 to 10 placed into petri dish that contain RGCA media, then rotated to form a figure eight in order to hold the sample mixed homogeneously. Samples were incubated for 48 hours at a temperature of 37-40°C. The calculation of the bacteria population by using the following formula:  $\text{BP} = C \times 10^n \times 2$ , whih is BP= bacteria population, C= number of colony forming unit, n= number of dilution

Ammonia (N-NH<sub>3</sub>) concentrations were determined by using the micro diffusion method (Conway, 1962). Analysis of total VFA concentration and proportional of VFA by using gas chromatography (Chrompack CP9002, Netherlands, flame ionized detector, Capillary column type WCOT Fused Silica 25 m x 0.32 mm, oven temperature: conditioning at 60°C and running at 115°C and nitrogen as gas carrier). The pH of rumen aliquot supernatant obtained from the screening was adjusted to 3-4 by added with H<sub>2</sub>SO<sub>4</sub>. Before injected to the GC machine, the 1.5 ml rumen supernatant mixed with 30 mg sulfosalicylic acid (C<sub>7</sub>H<sub>6</sub>O<sub>6</sub>S.2H<sub>2</sub>O) and centrifuged with refrigerated centrifuge (7°C) at 12,000 rpm for 10 min. Thus, the solution was injected to the GC around 0.5  $\mu\text{L}$  (Suharti et al., 2011). Methane estimation was calculation from molar proportion of VFA according to Moss et al. (2000) by using the formula:  $0.45(C_2) - 0.275(C_3) + 0.4(C_4)$  which is C<sub>2</sub>= acetate, C<sub>3</sub>= propionate and C<sub>4</sub>= butyrate.

Data were tested using Analysis of Variance (ANOVA) and the differences among treatments' means were examined by Duncan Multiple Range Test (Steel & Torrie, 1995).

## Results and Discussion

### Rumen Microbe Population

The addition of *Sapindus rarak* extract and its combination with canola/sesame oil microencapsulation or pure canola/sesame oil very significant reduced ( $P < 0.01$ ) protozoal population compared to the control treatment. Moreover, total bacterial population very



significant increased ( $P < 0.01$ ) with the addition of *S. rarak* extract combined with canola oil microencapsulation compared to the other treatments (Table 1).

**Table 1.** Total protozoa and bacteria population with the addition of *S. rarak* extract and its combination with Sesame/Canola Oil pure or microencapsulated

Treatment	Variables	
	Protozoa	Bacteria
Control (C)	$4.52 \pm 0.02^A$	$6.85 \pm 2.87^B$
C+ <i>S.rarak</i> extract (T1)	$4.03 \pm 0.04^B$	$6.94 \pm 2.70^B$
T1+SO	$3.89 \pm 0.05^E$	$6.69 \pm 2.82^B$
T1+MSO	$3.98 \pm 0.03^C$	$6.79 \pm 2.82^B$
T1+CO	$3.75 \pm 0.03^F$	$6.74 \pm 2.68^B$
T5+MCO	$3.92 \pm 0.03^D$	$7.43 \pm 3.02^A$

Note: SO=Sesame oil, MSO=Microencapsulation Sesame Oil, CO=Canola Oil, Microencapsulation Canola Oil. The different superscripts with capital letters indicates very significant different ( $P < 0.01$ )

The reduction of protozoal population due to saponin content in the *S.rarak* extract up to 84.5% (Suharti et al., 2011). Saponin could inhibit the growth of protozoa because will bind the steroid membrane of protozoa cell wall and caused lysis cell. The addition of sesame/canola oil without protection also decreased protozoal population because those oil contain unsaturated fatty acid which toxic for protozoa (Machmuller dan Kreuzer 1999, Newbold dan Chamberlain 1988). In addition, microencapsulation of sesame/canola oil have lower defaunation activity compare to those oil without protection because the coating material in the microencapsulation product blocked the interaction between unsaturated fatty acid with protozoa.

The addition of *S.rarak* extract did not affect bacteria population although protozoa population decreased. This might be caused by the protozoa which inhibited by *S.rarak* extract did not those predator for bacteria so only resulting the slight increasing of bacteria population. This result in contrast with Suharti et al. (2011) which stated that the used of *S.rarak* decreased protozoa population and increased bacteria growth.

The increasing bacterial population with the addition of *S. rarak* extract combine with microencapsulation canola oil might be caused by the reduction of protozoal population and stimulation of bacteria growth. Moreover, microencapsulation of canola oil also could protect the unsaturated fatty acid content in the oil so that did not toxic for rumen bacteria anymore.

### Rumen Fermentation Characteristic

The used of *S. rarak* extract and its combination with sesame/canola oil with or without microencapsulation significantly decreased ( $P < 0.05$ ) rumen pH value. In addition, the used of sesame/canola oil microencapsulation tend resulting the lower pH value compared to the used of sesame/canola oil without protection (Table 2). This result indicating that the used of *S. rarak* extract and its combination with sesame/canola oil microencapsulation could increase rumen fermentation activity by rumen microbe so that the pH value decreased. The increasing of rumen fermentation activity will reduce rumen pH.



Concentration of  $\text{NH}_3$  significantly decreased ( $P < 0.05$ ) with the addition of *S. rarak* extract and its combination with sesame/canola oil without protection. In contrast, the used of *S. rarak* extract and Sesame/Canola oil microencapsulation significantly increased ( $P < 0.05$ )  $\text{NH}_3$  concentration. Total VFA production, proportional VFA and methane estimation were similar among treatments. Dry matter and organic matter digestibility very significantly increased ( $P < 0.01$ ) with the addition of *S. rarak* extract and its combination with sesame/canola oil without protection or microencapsulation. In addition, the used of canola oil microencapsulation resulted the highest ( $P < 0.01$ ) dry matter and organic matter digestibility (Table 2).

The reduction of  $\text{NH}_3$  with the addition of *S. rarak* extract and its combination with sesame/canola oil without protection indicating that the decreasing of feed protein degradation. This might be due to toxic effect of unsaturated fatty acid in the oil to rumen microbe and caused the reduction of feed protein degradation by rumen microbe (bacteria and protozoa).

The addition of *S. rarak* extract significantly increased dry matter and organic matter digestibility might be due to the reduction of protozoa and slight increasing of bacteria population. Those change of rumen microbe composition caused the enhancement of dry matter and organic matter digestibility. The contrast result when we combined *S. rarak* extract with canola/sesame oil without protection decreased dry matter and organic matter digestibility caused by the reduction of protozoa and bacteria population. Moreover, the addition of *S. rarak* extract combined with canola/sesame oil microencapsulation improved dry matter and organic matter digestibility because microencapsulation of canola/sesame oil could protect those oil in the rumen so can reduce the toxic effect of unsaturated fatty acid of those oil to rumen microbe.

## Conclusion

The addition of *S. rarak* extract 1 mg/mL and combine with canola oil microencapsulation 10% decreased protozoa population, increased rumen bacterial growth,  $\text{NH}_3$  concentration, dry matter and organic matter digestibility but did not affect VFA total and partial.

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**Table 2.** *In vitro* rumen fermentation characteristic with the addition of *S. rarak* extract and its combination with Sesame/Canola oil with or without protection

Variables	Control	Treatments				
		C+S. <i>rarak</i> extract (T1)	T1+SO	T1+MSO	T1+CO	T5+MCO
pH	6.60 ± 0.45 <sup>c</sup>	6.56 ± 0.43 <sup>abc</sup>	6.56 ± 0.43 <sup>abc</sup>	6.52 ± 0.46 <sup>a</sup>	6.58 ± 0.44 <sup>bc</sup>	6.54 ± 0.47 <sup>ab</sup>
N-NH <sub>3</sub> (mM)	4.88 ± 0.46 <sup>A</sup>	4.23 ± 0.46 <sup>B</sup>	3.21 ± 0.44 <sup>C</sup>	5.26 ± 0.45 <sup>A</sup>	3.07 ± 0.46 <sup>C</sup>	5.00 ± 0.44 <sup>A</sup>
VFA total (mM)	52.61 ± 4.43	57.77 ± 9.83	54.11 ± 12.51	54.67 ± 4.34	61.81 ± 15.99	68.17 ± 17.52
Proportional VFA (mM/100mM)						
Acetate (C2)	60.12 ± 5.20	61.32 ± 7.44	64.65 ± 2.38	59.11 ± 5.31	60.94 ± 5.83	59.71 ± 8.12
Propionate (C3)	25.43 ± 2.98	26.22 ± 5.27	21.52 ± 2.38	26.90 ± 2.85	25.60 ± 4.07	27.26 ± 5.43
Butyrate (nC4)	11.04 ± 2.37	9.51 ± 2.23	10.65 ± 4.27	10.81 ± 2.31	9.79 ± 2.19	9.95 ± 2.64
Iso Valerate (iC5)	1.84 ± 0.29	1.59 ± 0.41	1.75 ± 0.64	1.57 ± 0.18	1.84 ± 0.21	1.61 ± 0.41
Valerate (nC5)	1.57 ± 0.32	1.36 ± 0.30	1.43 ± 0.46	1.61 ± 0.35	1.83 ± 0.22	1.48 ± 0.34
C <sub>2</sub> : C <sub>3</sub>	2.40 ± 0.51	2.45 ± 0.86	3.02 ± 0.24	2.23 ± 0.41	2.45 ± 0.63	2.30 ± 0.79
CH <sub>4</sub> (mM) estimation	12.82 ± 0.46	13.72 ± 0.26	12.80 ± 0.27	14.93 ± 4.00	14.71 ± 2.12	15.47 ± 2.24
Dry matter digestibility (%)	55.07 ± 2.90 <sup>C</sup>	59.04 ± 3.26 <sup>B</sup>	53.56 ± 3.87 <sup>D</sup>	59.18 ± 2.97 <sup>B</sup>	56.03 ± 3.27 <sup>C</sup>	67.32 ± 2.87 <sup>A</sup>
Organic matter digestibility (%)	54.62 ± 2.61 <sup>C</sup>	58.38 ± 3.15 <sup>B</sup>	52.84 ± 3.90 <sup>D</sup>	58.70 ± 2.99 <sup>B</sup>	55.42 ± 3.34 <sup>C</sup>	66.40 ± 2.46 <sup>A</sup>

Note: SO=Sesame oil, MSO=Microencapsulation Sesame Oil, CO=Canola Oil, Microencapsulation Canola Oil. The different superscripts with capital letters indicates very significant different (P<0.01) and lowercase indicates significant different (P<0.05).



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## Effect of Dragon Fruit (*Hylocercus Undatus*) Peel Powder and Roughage to Concentration Ratio on Gas Production Kinetics, Digestibility, and Fermentation Using *In Vitro* Gas Production Technique

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### Abstract

This study aimed to investigate the effect of Dragon fruit peel powder (DFPP) levels and roughage to concentrate ratio on gas kinetics, digestibility and fermentation by using *in vitro* gas technique. The experimental design was a  $3 \times 5$  factorial arrangement in a Completely randomized design (CRD). There were two experimental factors, Factor A was three ratios of roughage to concentrate (100:0, 70:30, and 30:70) and Factor B was five levels of DFPP supplementation (0, 1, 2, 3, and 4 % of substrate) on a dry matter basis. The results revealed that gas production kinetics (the immediately soluble fraction (a), insoluble fraction (b), the gas production rate (c) and potential extent of gas production (a+b) were different among R:C ratios ( $P < 0.01$ ) in R:C at 30:70 than others ratios. Cumulative gas production was higher in the R:C ratio at 30:70 ( $P < 0.01$ ). *In vitro* true dry matter digestibility at 12h and 24h were different by R:C ratio ( $P < 0.01$ ). Moreover, ammonia nitrogen concentration was not influenced when supplementation with DFPP, while R:C was ( $P < 0.01$ ). The pH value was significantly different among treatments ( $P < 0.01$ ). It could be concluded that supplementation of DFPP with roughage to concentrate ratio can improve *in vitro* true digestibility and rumen fermentation. There were no interaction between R:C ratio and DFPP. DFPP significantly influenced total gas production but not the digestibility.

**Keywords:** dragon fruit peel powder, gas production kinetics, *in vitro* digestibility, *in vitro* gas technique



## Introduction

Global warming is an important factor which affects the environment and livestock production. Goodland and Anhang (2009) reported that livestock production and its by-products are responsible for at least 51 percent of global warming gases, accounting for at least 32.6 billion tons of carbon dioxide per year. Methane (CH<sub>4</sub>), carbon dioxide (CO<sub>2</sub>), and nitrous oxide (N<sub>2</sub>O) are a highly important for greenhouse gases in the atmosphere, and their global atmospheric concentrations have considerably increased (Monteny et al., 2006). Recently, researchers have attempted to reduce methane by using plant secondary compounds.

Dragon fruit (*Hylocereus undatus*) is an exotic fruit also known as pitaya or pitahaya in Latin America (Le Bellec et al., 2006). The dragon fruit peel is the major by-product left over from either fresh consumption or fruit processing (Liaotrakoon, 2013). Tannins, saponin, flavonoids, phenols hydroquinone, steroids, and triterpenoids were the type of phytochemical compounds contained in red dragon fruit peel extract and flavonoids in red dragon fruit extract had the correlation with antioxidant activity (Manihuruk et al., 2016). Mahata et al. (2010) studied the effect of diet with varying levels of red dragon fruit peel in broiler and found that protein content and organ development were maintained after adding 15% of red dragon fruit peel to diets. However, there are limited studies on using dragon fruit peel powder (DFPP) as a feed supplement on rumen fermentation characteristics and digestibility. Therefore, the aim of this study was to investigate the effect of dragon fruit peel powder and roughage to concentrate ratios on gas kinetics, rumen fermentation, and digestibility using *in vitro* gas production technique.

## Materials and methods

The experimental design was a 3×5 factorial arrangement in a Completely randomized design (CRD). The dietary treatments were three rations of Roughage to Concentrate (R:C) with five levels of dragon fruit peel powder (DFPP) supplementation (0,1,2,3, and 4% of substrate) on dry matter basis. Therefore, fifteen treatments were as follows: T1 = R:C (100:0) with 0% DFPP, T2 = R:C (100:0) with 1% DFPP, T3 = R:C (100:0) with 2% DFPP, T4 = R:C (100:0) with 3% DFPP, T5 = R:C (100:0) with 4% DFPP, T6 = R:C (70:30) with 0% DFPP, T7 = R:C (70:30) with 1% DFPP, T8 = R:C (70:30) with 2% DFPP, T9 = R:C (70:30) with 3% DFPP, T10 = R:C (70:30) with 4% DFPP, T11 = R:C (30:70) with 0% DFPP, T12 = R:C (30:70) with 1% DFPP, T13 = R:C (30:70) with 2% DFPP, T14 = R:C (30:70) with 3% DFPP, T15 = R:C (30:70) with 4% DFPP. The sample of DFPP and RS were dried at 60°C, then ground to pass through a 1-mm sieve (Cyclotech Mill, Tecator, Höganäs, Sweden) and used for chemical analysis.



The samples were chemically analyzed according to AOAC (1998) for DM, crude protein (CP) and ash. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) in the samples were estimated according to Van Soest et al. (1991).

### **Animals and diet**

Two rumen-fistulated dairy steers with live weight of  $400 \pm 30$  kg were used as rumen fluid donors. The animals were individually penned, clean fresh water and mineral blocks are available at all times. Rice straw as a roughage was fed *ad libitum* and concentrate (16% CP, 11.0 MJ/kg of metabolizable energy, consisting of 70% cassava chip, 6% rice bran meal, 7% coconut meal, 10% palm kernel meal, 1% sulfur, 4% urea, 1% mineral premix and 1% salt on a DM basis) was fed at 0.5% of body weight (BW) in two equal portions at 07.00 and 16.30 hours.

### **Sampling and analysis**

The gas production was measured at 1, 2, 4, 6, 8, 12, 24, 36, 48, 72 and 96 h of incubation. Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979). Inocula ruminal fluid was collected at 0, 2, 4 and 6h of incubation time to measure pH and  $\text{NH}_3\text{-N}$  using the micro-Kjeldahl method (AOAC, 1998). At 12 and 24h post-inoculation a set of samples were determined for *in vitro* true DM digestibility (IVTDMD) according to Van Soest and Robertson (1985) as follows:

$$\begin{aligned} \% \text{IVTDMD} &= \text{true digested DM substrate (g)} \\ &\times 100 \% \text{ weight of sample taken for incubation (g)} \end{aligned}$$

### **Statistical analysis**

All data were statistically analyzed as a  $3 \times 5$  factorial arrangement in a CRD using the general linear procedure in PROC GLM of SAS (1998). All parameters, differences among treatments means were contrasted by Tukey's Multiple Comparison Test (Crichton, 1999).

### **Results and discussion**

Chemical composition of the dragon fruit peel powder and roughage sources are shown in Table 1. Rice straw contained CP at 3% DM, while the CP content of DFPP was 5.4 % DM. Gas production kinetics (the immediately soluble fraction (a), insoluble fraction (b), the gas production rate (c) and potential extent of gas production (a+b) were different between R:C ratios which were significantly higher ( $P < 0.01$ ) in R:C at 30:70 than others ratios. Cumulative gas production was higher in the R:C ratio at 30:70 ( $P < 0.01$ ) as shown in Table 2. *In vitro* true dry matter digestibility (IVTDMD) at 12h and 24h were improved by



R:C rations ( $P < 0.01$ ) as shown in Table 2. Moreover, ammonia nitrogen ( $\text{NH}_3\text{-N}$ ) concentration was not influenced when supplementation with DFPP, while R:C was ( $P < 0.01$ ) and the range was between 15.4 to 25.2 mg/dL. According to Wanapat and Pimpa (1999) was reported that  $\text{NH}_3\text{-N}$  concentrations between 15 to 30 mg/dL were suitable for ruminal microbial activity. The pH value was significantly differenced among treatments ( $P < 0.01$ ) and the range between 6.74-6.93 which did not fall below the normal range (pH range 6.5-7.0) for rumen microbial growth as shown in Table 3.

## Conclusions and Recommendations

Based on this experiment, it could be concluded that supplementation of DFPP could improve *in vitro* rumen fermentation. These results revealed a potential use of DFPP 4% of substrate and roughage to concentrate ratio (30:70) to improve rumen fermentation for ruminant feeding. However, further research should be conducted especially using DFPP for *in vivo* trials.

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**Table 1.** Chemical composition of Dragon fruit peel powder (DFPP) and

Item	RS	Concentrate	DFPP
Chemical composition			
Dry matter, %	90.6	87.5	77.4
	-----% of dry matter -----		
Crude protein	3.0	14.3	5.4
Neutral-detergent fiber	71.3	27.6	33.0
Acid-detergent fiber	55.5	18.2	29.6
Ash	12.3	5.8	1.5

roughage sources used in the experiment

RS, rice straw; DFPP, dragon fruit peel powder

**Table 2.** Effect of Dragon fruit peel powder (DFPP) and roughage to concentrate rations on gas kinetics and *in vitro* true dry matter digestibility (IVTDMD) from *in vitro* incubation with rumen fluid

Treatments	R:C	DFPP, %	Gas kinetics				Cumulative gas (mL) at 96h	IVTDMD,%	
			a (mL)	b (mL)	c (mL/h)	a+b (mL)		12h	24h
1	100:0	0	-0.1	27.1	0.061	27.0	26.9	36.0	41.3
2		1	0.2	27.0	0.060	27.2	27.3	36.2	42.7
3		2	0.4	28.7	0.064	29.1	29.2	37.5	44.5
4		3	0.9	28.6	0.066	29.5	29.6	37.3	45.2
5		4	0.5	26.3	0.068	26.8	27.1	37.8	46.4
6	70:30	0	-0.1	34.5	0.111	34.5	35.0	41.0	51.6
7		1	0.7	37.5	0.102	38.1	39.0	42.9	54.5
8		2	1.4	34.3	0.091	35.7	36.4	44.6	57.4
9		3	2.6	25.0	0.045	27.5	26.8	48.4	57.1
10		4	0.8	41.0	0.051	41.8	40.7	51.7	60.7
11	30:70	0	6.0	51.9	0.086	57.9	59.1	58.0	64.0
12		1	3.5	54.3	0.093	57.8	59.6	58.7	69.8
13		2	4.5	51.7	0.100	56.2	58.0	59.3	71.2
14		3	2.6	32.4	0.074	58.1	59.3	59.2	72.1
15		4	4.8	33.5	1.903	57.8	56.6	59.5	75.6
SEM			0.45	1.98	0.004	2.07	3.18	0.83	0.89
Interaction									
	R:C		**	**	**	**	**	**	**
	DFPP		ns	*	**	*	*	ns	ns
	R:C*DFPP		ns	ns	**	ns	ns	ns	ns

R:C, ration of roughage and concentrate; DFPP, dragon fruit peel powder; a, the gas production from the immediately soluble fraction; b, the gas production from the insoluble fraction; c, the gas production rate constant for the insoluble fraction; a+b, the gas potential extent of gas production level of DFPP in concentrate; IVTDMD, *in vitro* true dry matter digestibility.

\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; ns, not significant



**Table 3.** Effect of Dragon fruit peel powder (DFPP) and roughage to concentrate

Treatments	R:C	DFPP (%)	pH	NH <sub>3</sub> -N, mg/100mL
1	100:0	0	6.69	15.6
2		1	6.69	15.4
3		2	6.67	16.1
4		3	6.66	16.3
5		4	6.67	15.9
6	70:30	0	6.65	16.5
7		1	6.63	16.8
8		2	6.64	16.4
9		3	6.61	21.0
10		4	6.60	21.2
11	30:70	0	6.57	19.6
12		1	6.57	19.5
13		2	6.54	21.8
14		3	6.55	19.6
15		4	6.54	21.0
SEM			0.002	0.87
Interaction				
	R:C		**	**
	DFPP		**	ns
	R:C*DFPP		ns	ns

ratio on pH and NH<sub>3</sub>-N from in vitro incubation with rumen fluid

R:C, ration of roughage and concentrate; DFPP, dragon fruit peel powder.

\*, P < 0.05; \*\*, P < 0.01; ns, not significant.



## Hematology and Physiological Responses as Indicator of Heat Tolerance in Purebred Brahman and Red Angus x Brahman Crossbred Steers in Thailand

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### Abstract

The objectives of this study were to examine and compare hematology and physiological responses of purebred Brahman (B) and Red Angus (RA) x Brahman (B) crossbred cattle (18-months-old) with different breed fraction. Forty steers of B and RA x B crossbred cattle were randomly allotted to 4 groups with varying percentage RA breeding (0%RA x 100% B, n = 10; 25%RA x 75% B, n = 10; 50%RA x 50% B, n = 10; 75%RA x 25% B, n = 10) in completely randomized design. All steers were raised in individual pens until ad libitum access to rice straw, water, and mineral block for 10 months (Jan-Oct). Concentrate (CP 11%) feed was offered twice daily at 1.3 % body weight (BW). Some physiological responses and blood samples, in terms of rectal temperature (RT), respiratory rate (RR), sweating rate (SR), heat tolerance coefficient (HTC), packed-cell volume (PCV), hemoglobin (Hb), red blood cells (RBC) white blood cell (WBC) and mean corpuscular volume (MCV) platelet estimate, differentiate white blood cells (WBCs) type were determined at the same time. Data were analyzed for the effect of hematology and physiological responses. The results show that varying percentage of RA breeding by 0%RA x 100% B, 25%RA x 75% B, 50%RA x 50% B and 75%RA x 25% B has similar ( $P > 0.05$ ) effect on RT, RR, PCV and SR while 75%RA x 25% B crossbred cattle had increased RT, RR and decreased PCV, SR, RBC, Hb, and MCV when compared to other groups ( $P < 0.05$ ). Heat tolerance coefficient (HTC) decreased ( $P < 0.05$ ) linearly with increasing percentage of RA breeding. Varying percentage RA breeding had no ( $P > 0.05$ ) effect on platelet estimate, WBC, neutrophils (N), lymphocytes (L), monocytes (M), eosinophils (E) and N: L ratio ( $P > 0.05$ ). These data indicate that varying percentage RA breeding in 25%RA x 75% B and 50%RA x 50% B has similar on heat tolerance when compared to purebred Brahman.

**Keywords:** hematology, physiological responses, Brahman, red Angus, heat tolerance



## Introduction

The tropical and temperate climate had affected the animal production by the environment, such as temperature, relative humidity, wind speed and solar radiation, may cause stress. These factors interact causing greater on affecting in the reduction of productivity and animal development. This involves a series of adaptations and for cattle production in hot regions, the hematology and physiological responses has shown to be important in determining the tolerance of each breed to their environment. Long ago, the genetic resources from the temperate climate and tropical countries used for the improvement beef cattle of Thailand. Brahman is the main specialized beef breed imported because it was characterized by rusticity, thermal tolerance, resistance to parasites as well as capacity to use high roughage diets, characteristics of great importance in tropical beef production systems. The Thailand show great potential for the development of the fattening beef cattle production. The Brahman, Charolais, Angus, and Simmental were the crosses of specialized beef cattle breeds between *Bos indicus* (Brahman) and European cattle (*Bos taurus*) for the fattening beef cattle production in Thailand. Red Angus breed had its beginning in Europe. The cows are hardy and grow quickly. They produce marbled meat like that of the Black Angus, and their meat is also highly desired in butchers, supermarkets, restaurants, and in the home. Its influence can improve carcass quality whilst still maintaining the adaptive quality of the Brahman. However, much of the research focusing on heat tolerance of pure breed *Bos indicus* compared to *Bos taurus* (Finch, 1985; Hammond et al., 1998; Gaughan et al., 1999; Hansen, 2004) but little information has been published with regard to *Bos indicus* and *Bos taurus* crossbred in the tropical countries where rice straw was fed as base diet. The hypothesis is that suitable percentage purebred Brahman (B) and Red Angus x Brahman (RA) crossbred cattle with different breed fraction must display heterosis effect in terms of heat tolerance. The primary objective of this study was to investigate the rectal temperature (RT), respiratory rate (RR), sweating rate (SR), heat tolerance coefficient (HTC), packed-cell volume (PCV), hemoglobin (Hb), red blood cells (RBC) white blood cell (WBC) and mean corpuscular volume (MCV) platelet estimate, differentiate white blood cells (WBCs) of varying percentage RA breeding (0%RA x 100% B, 25%RA x 75% B, 50%RA x 50% B, 75%RA x 25% B) under fattening fed rice straw based diet.



## Materials and methods

### Animal breed and experimental design



(A)

(B)

(C)

(D)

**Figure 1.** Breed fraction of purebred Brahman (B) and Red Angus (RA) x

Brahman (B) crossbred cattle (18-months-old), (A) = 0%RA x 100% B, (B) = 25%RA x 75% B, (C) = 50%RA x 50% B and (D) = 75%RA x 25% B

Forty steers of varying percentage RA breeding (0%RA x 100% B, n = 10; 25%RA x 75% B, n = 10; 50%RA x 50% B, n = 10; 75%RA x 25% B, n = 10) (Fig. 1.) from beef cattle farm of Faculty of Natural Resources, Rajamangala University of Technology Isan, Sakon Nakhon Campus were used in a completely randomized design (CRD) to determine the effect of hematology and physiological responses. Steers were approximately 18 months old at the start of the trial.

### Animal management and feeding

Steers were raised in individual pens until ad libitum access to rice straw, water, and mineral block for 10 months (Jan-Oct). Concentrate feed was offered twice daily at 1.3 % body weight (BW) per day.



### **Data collection and sampling**

Measurements of ambient conditions included dry bulb temperature and wet bulb temperature. Dry bulb temperature and wet bulb temperature were measured using a mercury thermometer. Measurements were taken 2 times daily at 08:00 am and 03:00 pm and calculated in daily average. A temperature humidity index (THI) was calculated according to equation of (McDowell, 1972) as follows:  $THI = 0.72 (\text{Dry bulb temperature in } ^\circ\text{C} + \text{Wet bulb temperature in } ^\circ\text{C}) + 40.6$ . Steer measurements were conducted every 15 days a crossed experimental period. Measurements included an order of handling, rectal temperature, respiration rate, body weight and blood sampling. Rectal temperature (RT) measurements were made with electronic thermometers and respiration rates (RR) were obtained by observing flank or rib cage movements while steers were in the pen. Rectal temperature and RR were measured two times daily at 08:00 and 15:00 and were calculated in daily average. Blood was collected by jugular venipuncture into tubes containing lithium heparin (Becton Dickinson Pty. Ltd., NSW, Australia). The blood tubes were placed on ice until they were transported to the laboratory. Sweating rate (SR) was measured at 120-min intervals by a modification of the method of Schleger and Turner, 1965 and Pereira et.al. 2010, which is based on the time taken for paper discs impregnated with cobalt chloride to change from blue to pink color. Chromatography paper was prepared by immersion in a 10% cobalt chloride solution and then dried at room temperature on a sheet of glass. The paper was oven-dried (500C), and discs (53 mm in diameter) were punched out and redried. Three discs were then mounted on the midline of a 75-mm strip of 20-mm cellulose adhesive tape, which was then fixed to a 75× 25-mm glass slide and stored in a desiccator. The strips were prepared approximately 18 h before use. Any amounts over 24 h old were discarded. The loin area was clipped immediately prior to testing. An adhesive strip with discs was removed from the slide and placed on the skin, which had been wiped dry. The mean time taken for the three discs to change color was recorded with SW using the following equation:  $SW [g/(m^2 \cdot h)] = 3.84 \times 10^4 / s$  (Schleger and Turner, 1965). The heat-tolerance coefficient for each cow was determined by converting



rectal temperature (0C) to a heat-tolerance coefficient by using following equation of  $HTC = 100 - [18(RT - 38.6)]$  where RT represents the rectal body temperature and HCT the heat-tolerance coefficient.

### **Measurement**

Blood samples were evaluated hemoglobin concentration (Hb: g/100 ml) haemoglobinometer Coulter, Packed Cell Volume (PCV: % vol.) by the microhematocrit method, red blood cells (RBC:  $10^6/\text{mm}^3$ ), white blood cells (WBC:  $10^3/\text{mm}^3$ ) and differentiate WBC type number by microscopic counting in a Burker's chamber. Mean Corpuscular Volume (MCV: fl) were calculated according to Schalm (1965). Neutrophil to Lymphocyte ratio was calculated according to the method of Stull and Rediek (2000).

### **Statistic analysis**

Data were analyzed using Proc. GLM (SAS, 2001). The following models were used to determine treatment mean differences using Duncan's New Multiple Range Test.

## **Results and discussions**

### **Condition**

Ambient environmental conditions during trials are given in Table 1. The Temperature Humidity Index (THI) has been widely used as heat stress index in beef and dairy industries (Stokka et al., 1996; West, 1999; Mader et al., 2002). Temperature humidity index values of 70 or less are considered comfortable, 75 to 78 stress full, and values greater than 78 cause extreme distress and animal are unable to maintain thermoregulatory mechanisms or normal body temperature (Silanikove, 2000). Therefore, In this trial, THI was substantially higher during the April and September measurement dates (80.8 to 82.4), conditions in January to March and October were consistent with those associated with mild heat stress in cattle (THI = 72.8 to 79.8).

**Table 1.** Condition during experimental period, Jan-Oct

Date	Min. Temp., °C	Max. Temp., °C	Temp-humidity index (THI) <sup>a</sup>
Jan	18.27	28.66	72.76
Feb	16.62	26.22	68.88
Mar	21.94	33.21	77.53
Apr	25.53	35.35	82.37
May	25.10	32.06	80.75
Jun	25.40	32.38	81.42
Jul	25.42	32.21	81.64
Aug	25.77	31.82	81.57
Sep	23.92	32.87	80.86
Oct	23.97	36.55	79.84

<sup>a</sup> THI= 0.72 (Dry bulb temperature in °C + Wet bulb temperature in °C) + 40.6 (McDowell, 1972)

### Physiological Responses for heat tolerance indicator

Effects of breed on rectal temperature (RT), respiration rate (RR), packed-cell volume, (PCV), sweating rate (SR) and heat tolerance coefficient (HTC) are shown in Table 2.

Rectal temperature increased ( $P < 0.05$ ) linearly with increasing percentage of RA breeding. The most common index of heat tolerance in cattle is core body temperature as measured by rectal temperature, which is moderately heritable. The Higher rectal temperature indicated low heat tolerance (Shvartz et al., 1977). Some estimates of heritability of rectal temperature in beef cattle are 0.11 (da Silva, 1973), 0.25 (Turner, 1982) and 0.33 (Turner, 1984).



**Table 2.** Effect of different breed fraction on Physiological Responses for heat tolerance indicator under hot condition (THI > 80) in Purebred Brahman and Red Angus x Brahman Crossbred Steers

Item	Breed fraction			
	0%RA x 100% B	25%RA x 75% B	50%RA x 50% B	75%RA x 25% B
Rectal Temp., °C	39.2b	39.6ab	39.7ab	39.8a
Respiration rate, breath per minute	19.9b	20.5ab	20.9ab	21.3a
Packed-cell volume (PCV), %	32.6A	31.7 A	32.8 A	26.6 B
Sweating rate, g/m <sup>2</sup> /h	230.7a 90a	159.7 ab 83.2b	149.9ab 82.0b	106.0b 78.3c
Heat tolerance coefficient (HTC) <sup>1</sup>				

HTC = 100 - 18 (Body temperature in °C - 38.6)

a, b, c Within rows not sharing common superscripts are significantly different (p<0.05)

A, B Within rows not sharing common superscripts are significantly different (p<0.01)

Hammond et al. (1998) reported that on the hottest date, rectal temperature was not different between B and RA. However, the rectal temperature in Angus was greater than the rectal temperature in Brahman during on the hottest summer date (Hammond et al., 1996). Our result also found that influence of B breed on the rectal temperature of beef cattle was found at 50% Brahman or higher. Another possible lower rectal temperature in a higher percentage of RA crossed breeding may be due to Angus steers had lower plasma cortisol concentrations than Brahman x Angus steers (Blecha et al., 1984). The stress of handling cattle can cause an increase in circulating glucocorticoids (Venkateshu and Estergreen, 1970; Crookshank et al., 1979). Environmental heat exposure can cause a transient increase in circulating glucocorticoids that may subsequently





decrease even though body temperature remains elevated during chronic heat exposure (Christison and Johnson, 1972; Alvarez and Johnson, 1973; Rhynes and Ewing, 1973). The HTC values showed for the purebred Brahman were better than those found for the Red Angus x Brahman Crossbred Steers indicated that purebred Brahman was potentially heat tolerant. Rectal temperature was the most useful index of heat tolerance (Hammond et al., 1996), but respiration rate can also signal heat stress when it causes panting (Bennett et al., 1985). However, there is individual (Bianca, 1963) and breed (Hammond et al., 1996) differences in respiration rate that can make interpretation difficult. Purebred Brahman cattle have higher PCV and RACI number compared with British Bos taurus breeds (Hammond et al. 1996) and this suggests a greater oxygen-carrying capacity consistent with lower respiration rate. Consistent with this concept, the relationship between respiration rate, PCV, and erythrocyte in cattle was evident also in this trial. Respiration rate was lower ( $p < 0.05$ ) and PCV tended to be higher ( $p < 0.01$ ) in B than in the RA crossbred steers and highest respiratory rate and lowest PCV were found in 75%RA x 25% B. Greater respiration rates in 75%RA x 25% B may be the result of higher body temperatures. Therefore 75%RA x 25% B cattle increased respiration rate to remove excess heat. Lower PCV in 75%RA x 25% B suggests the influence of RA, as reported by Hammond et al. (1996) that PCV was lower in RA than in B. Another reason of higher PCV in RA crossed breeding than B is possible due to that steer had low heat tolerance and could operate a mechanism of body heat regulation under the hot climate by increased water intake while the water outside was absorbed into the blood vessels gradually, which caused diluted red blood cell or increasing plasma volume so the PCV was lower. Results from this study showed that crossbred of 25%RA x 75% B and 50%RA x 50% B had SR similar to that of 0%RA x 100% B indicated crossbred cattle with B influence more than or equal 50% B had higher sweating rate (heat tolerance) than cattle with 25% B or no B breed fraction. . This consisted with recently researched of Gaughan et al. (1999) who used a new heat load index (HLI) for feedlot cattle to predict the heat balance of cattle, they found that HLI threshold of 100% B, 75% B, 50% B and 25% B were 96, 94, 93 and 90,



respectively. Animal has higher HLI threshold mean that they were higher heat tolerance.

## Hematology

Effects of breed on hematology are shown in Table 3. Red blood cells, hemoglobin, and MCV tended to decrease linearly with increasing percentage of RA breeding (Table 3). The value of hemoglobin concentration has a positive correlation with the number of red blood cell due to hemoglobin is an important component of red blood cell. Likewise PCV, when the ambient temperature was increased the steer obtained heat stress, steer body could operate a mechanism of body heat regulation under the hot climate by sweating and increase water intake, which increased water volume or plasma volume of blood circulatory ways so the value of hemoglobin concentration became lower (Raghavan and Mullick, 1962).

**Table 3.** Effect of different breed fraction on hematology for heat tolerance indicator under hot condition (THI > 80) in Purebred Brahman and Red Angus x Brahman Crossbred Steers

Item	Breed fraction			
	0%RA x 100% B	25%RA x 75% B	50%RA x 50% B	75%RA x 25% B
WBC count, x10 <sup>3</sup> cell/ $\mu$ l	10.1	10.6	11.8	10.7
RAC count, x10 <sup>6</sup> cell/ $\mu$ l	6.2 <sup>a</sup>	6.3 <sup>a</sup>	6.1 <sup>a</sup>	5.6 <sup>b</sup>
Hb, g/dl	10.1 <sup>a</sup>	10.0 <sup>b</sup>	10.0 <sup>a</sup>	8.6 <sup>b</sup>
MCV, fl	56.6 <sup>A</sup>	53.4 <sup>A</sup>	53.7 <sup>A</sup>	46.8 <sup>B</sup>
Differentiate WBCs types	38.1	38.2	38.3	34.5
Neutrophils (N)				
Lymphocytes (L)	54.1	55.1	56.9	58.2
Monocytes (M)	1.8	1.7	1.5	2.1
Eosinophils (E)	5.6	4.8	3.9	5.1
N:L ratio	0.7	0.7	0.7	0.6

Within rows not sharing common superscripts are significantly different (p<0.01)



## Conclusion

The varying percentage RA breeding in 25%RA x 75% B and 50%RA x 50% B has similar on heat tolerance when compared to purebred Brahman. All steers were raised suitably in an environment of Thailand. When increasing percentage of Red Angus breeding up to 75%RA x 25% B or higher could lower heat tolerance.

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## Effects of Supplementation of *Piper Sarmentosum* Leaves Powder in Concentrates on Feed Efficiency, Rumen Fermentation and Protozoa in Thai Native Beef Cattle

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### Abstract

This study was to evaluate the effect of *Piper sarmentosum* (PS) supplementation on feed intake, digestibility, rumen ecology and urinary purine derivative excretion in Thai native beef cattle fed rice straw. Four males Thai native beef cattle were randomly assigned according to a 4 x 4 Latin square design to receive 4 levels treatments of PS at 0, 0.6, 1.2 and 2.4 g/h/d. Animals were offered concentrate mixture at 0.5 % of BW. Rice straw was a roughage source and fed *ad libitum*. Measurements of feed intake and collection of feeds, feed refusals, feces, rumen fluid, and blood samples were taken. As a result of this experiment, it was found that pH and temperature were similar among treatments ( $P>0.05$ ). DM intake of concentrates and rice straw were increased by addition of PS at 2.4 g/h/d ( $P<0.05$ ). Supplementation of PS did not affect nutrient digestibility. However, nutrient digestibility tended to be higher in cattle supplemented at 2.4 g/h/d as compared to other treatments. Rumen microbes were similar among treatments. In contrast, protozoa were lower in cattle fed with supplemental PS at 2.4 g/h/d ( $P<0.05$ ). Base on study, it could be concluded that PS could be used as a supplement in concentrate for Thai native beef cattle for the improvement of rumen fermentation, feed efficiency and decreasing protozoa populations at levels 2.4 g/h/d.

**Keywords:** *Piper sarmentosum*, rumen fermentation, protozoa, cattle, Thai herb

### Introduction

*Piper sarmentosum* Roxb. (PS), which is widely abundant in tropical and subtropical regions including Thailand, is often used as a food flavoring agent, in



traditional medicine, and for pest control. Piper species contain several bioactive compounds, such as alkaloids, amides, pyrones, dihydrochalcones, flavonoids, phenylpropanoids, lignans and neolignans (Fernandez et al., 2012). *P. sarmentosum* extract (PSE) contains antibacterial, antiinflammatory and antioxidant activities (Fernandez et al., 2012). Furthermore, the positive effects of flavonoid-rich plant extracts (PE) on methane emission and methanogens population *in vitro* as well as *in vivo* have been examined (Becker et al., 2014). In addition, flavonoid supplementation could improve ruminal fermentation of dairy cows with increasing milk yield, protecting ruminal acidosis (Balcells et al., 2012), reducing methane emission and changing microbial populations such as protozoa and methanogen. Protozoa are an important key in methanogenesis in the rumen as methanogens attach to their surface. Flavonoid-rich PE reduced the ciliated-associated methanogens population and hence decreased the methane emission (Kim et al., 2015). Patra and Saxena (2010) reported that flavonoids gave direct effects against methanogens, and reduced protozoa related with ruminal methanogenesis.

Supplementation of PS extract as feed additive to improve growth performance, antioxidant capability and immune response of weaned piglets has been elucidated by Wang et al. (2016). Nevertheless, there is a few information on effect of PS as feed additive in ruminants.

Therefore, the objective of this study was to evaluate the effect of PS leaves powder supplementation on feed efficiency, rumen fermentation and protozoa population in Thai native beef cattle fed n rice straw.

## **Materials and methods**

### **Animals and dietary treatments**

Four males Thai native beef cattle were randomly assigned as a 4 x 4 Latin squared design, with similar weight and age at  $150 \pm 20$  kg and 2-3 years, respectively. Experimental treatments consisted of four level of PS leaves powder supplement at 0 (control), 0.6, 1.2 and 2.4 g/head/d, respectively. PS leaves powder was chopped, sun-dried and then oven dried at 60°C before grinding through a 1 mm screen.

Each cattle was housed in individual pens and offered concentrates (Table 1) at 0.5% BW with rice straw fed *ad libitum* twice daily at 07.00 and 16.00 h. Fresh clean water and mineral block were available at all time. The experiment was conducted for four periods and each of the four periods lasted for 21 d in length.



## Data collection and sample analysis

Feed samples were collected twice a week for DM analysis. During the last 7 d of each period, samples of the concentrate mixture, rice straw, refusals, and feces were collected daily. Each of the feed samples during the collection days were pooled by period while feces and urine samples were pooled by each animal in each period and stored at  $-20^{\circ}\text{C}$  for later chemical analyses. All samples were divided into two parts, which the first was for DM analyses, while the second was kept for analyses of ash, CP, aNDF and ADF. The data was used for calculating digestibility of nutrients. Rumen fluid was taken from the rumen by a stomach tube connected to a vacuum pump for measured ruminal pH, temperature, and ammonia nitrogen ( $\text{NH}_3\text{-N}$ ). For determination of protozoal population, the fluid was fixed using a solution of 10% formalin in sterilized 0.9% saline, and was measured using the direct counting microscopic method based on the use of a haemocytometer (Boeco, Hamburg, Germany). All data were analyzed according to a  $4 \times 4$  Latin square design using the SAS (1996) GLM procedure.

## Results and discussions

### Chemical composition of diets

The concentrates and rice straw contained 13.6% and 3.3 % CP, respectively. The PS leaves powder consisted of 26.2% DM, 8.9% OM, 17.2% Ash, 70.6% NDF and 38.6% ADF (Table 1). Moreover, the PS leaves powder contained 26.2% CP, thus it could be potentially protein source for cattle. However, the location may occur influence to the nutritional of plant. As the previously report by Khalid et al. (2008) who determined that PS leaves water contained 23 to 28% CP. Moreover, PS consisted of high Ca,  $\beta$ -carotene and volatile oil, ligands, alkaloids, bioflavonoids and polyphenols, which is antioxidants substances (Kratchanova et al., 2010) and these plant compounds may affect on rumen microorganism.

### Feed intake and digestibility

Supplementation of PS leaves powder on feed intake and digestibilities are shown in Table 2. DM intake of concentrates and rice straw were different by adding PS which was highly increased in supplemented at 2.4 g/h/d ( $P < 0.05$ ). This could possibly be that PS has specified flavors and some of essential oil (Qin et al., 2010) which may increase intake of the diet. Palatability can be improved by using ingredients preferred by animal or by using feed additives, such as flavors, that make the diet more acceptable and encourage greater feed intake. Flavors are feed additives that attempt to enhance the taste and smell of feed to stimulate feed intake. Taste and smell are the senses associated with feed intake. Because smell is the first sensation detected by the animal, aroma of the diet becomes the initial stimulus that drives the animal to eat. Similar findings with





Wang et al. (2016) who reported that supplementation with 50 mg/kg PSE in piglets had the highest average daily feed intake.

Supplementation of PS leaves powder did not adversely affect digestibility of DM, OM, CP, NDF and ADF (Table 1). However, digestibility of nutrients tended to be increased in cattle supplemented at 2.4 g/h/d as compared to other treatments. This result could be attributed to reduced number of protozoa in rumen, leading to increase number of cellulolytic bacteria in rumen (Wanapat et al., 2008), which can increase feed digestibility in animal. Wanapat et al. (2008) demonstrated that supplementation of lemongrass (*Cymbopogon citratus* Stapf.) powder could increase fiber digestibility. Similarly, Ando et al. (2003) reported that supplementation of essential oil from herbs (Peppermint source) could significantly increase nutrient digestibilities.

### **Rumen ecology and microorganisms**

Supplementation of PS leaves powder on rumen ecology, bacteria and protozoal population are in Table 3. PS supplementation did not affect rumen ecology, namely rumen pH range from 6.9-7.1, temperature range from 39.28 to 40.08°C and these ranges were considered as an optimal level for microbial activity (Cherdthong et al., 2014). NH<sub>3</sub>-N concentration were ranged from 10.86 to 13.31 mg/dl were close to those previously reported by Wanapat et al. (2008). Population of total rumen bacterial were similar among treatments (P>0.05). In contrast, protozoal numbers were significant different among treatments which was lower in animals fed PS at 2.4 g/h/d (P<0.05). It is probably due to the presence of essential oil and alkaloids in the PS which is responsible for its antimicrobial activity (Fernandez et al., 2012). Furthermore, the flavonoid-rich plant might be reduced protozoal population and could decreased ruminal methanogenesis (Kim et al., 2015; Patra and Saxena, 2010). Similarly, Supapong et al. (2017) showed that supplementation of *Delonix regia* seed which contained flavonoid-rich resulted in reduced protozoal population and CH<sub>4</sub> production in beef cattle.

### **Conclusion**

Base on study, it could be concluded that supplementation of PS did not effect on nutrient digestibilities and rumen ecology. However, animal fed with 2.4 g/d PS could improve feed intake and decrease protozoal populations. Furthermore, these findings should be further suggested in dairy cow experiments to elucidate the actual effect of PS leaves powder addition on production trial.

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**Table 1.** Ingredient and chemical composition of concentrate, rice straw and *Piper sarmentosum* (PS) leaves powder used in experiment.

Items	Concentrate	Rice straw	PS leaves powder
Ingredients, kg DM			
Cassava chips	55.0		
Rice bran	11.0		
Coconut meal	12.9		
Palm kernel meal	13.5		
Urea	2.6		
Pure sulfur	1.0		
Mineral premix <sup>a</sup>	1.0		
Molasses, liquid	2.0		
Salt	1.0		
Chemical composition			
Dry matter, g/kg	91.3	92.1	26.2
Organic matter, g/kg DM	87.0	80.3	8.9
Acid insoluble ash, g/kg DM	4.3	11.7	17.2
Crude protein, g/kg DM	13.6	3.3	19.1
Neutral detergent fiber, g/kg DM	80.5	72.8	70.6
Acid detergent fiber, g/kg DM	53.9	30.4	38.6

<sup>a</sup>Minerals and vitamins (each kg contains): vitamin A (10,000,000 IU), vitamin E (70,000 IU), vitamin D (1,600,000 IU), Fe (50 g), Zn (40 g), Mn (40 g), Co (0.1 g), Cu (10 g), Se (0.1 g), I (0.5 g)



**Table 2.** Effect of various levels of *Piper sarmentosum* (PS) leaves powder on feed intake, and apparent digestibility in Thai native beef cattle.

Items	Supplementation of PS, g DM/head/day				SEM	Pr > F
	0	0.6	1.2	2.4		
DM intake						
Rice straw						
kg/day	2.30 <sup>b</sup>	2.32 <sup>b</sup>	2.36 <sup>b</sup>	2.45 <sup>a</sup>	0.02	0.01 <sup>**</sup>
g/kg BW <sup>0.75</sup>	54.19	54.85	56.02	58.07	2.51	0.71
Concentrate						
kg/day	0.81 <sup>c</sup>	0.74 <sup>b</sup>	0.64 <sup>a</sup>	0.82 <sup>d</sup>	0.07	0.01 <sup>**</sup>
g/kg BW <sup>0.75</sup>	17.36	15.76	13.67	17.52	0.71	0.01
PS						
kg/day	0.00 <sup>a</sup>	0.60 <sup>b</sup>	1.20 <sup>c</sup>	2.40 <sup>d</sup>	0.01	0.01 <sup>**</sup>
g/kg BW <sup>0.75</sup>	0.00 <sup>a</sup>	0.14 <sup>b</sup>	0.28 <sup>c</sup>	0.56 <sup>d</sup>	0.01	0.01 <sup>**</sup>
Total intake						
kg/day	2.96 <sup>b</sup>	3.00 <sup>b</sup>	3.14 <sup>ab</sup>	3.22 <sup>a</sup>	0.06	0.04
g/kg BW <sup>0.75</sup>	69.73	70.27	73.72	75.59	3.13	0.51
Digestibility, %						
Dry matter	65.05	65.33	66.52	68.85	3.32	0.93
Organic matter	72.64	73.03	73.54	75.83	3.50	0.84
Crude protein	54.05	55.34	55.95	59.88	2.78	0.71
Neutral detergent fiber	50.36	51.92	52.00	54.17	2.63	0.55
Acid detergent fiber	34.23	35.64	35.35	36.51	1.78	0.44

<sup>a,b,c,d</sup>Means in the same row with different superscripts differ (P<0.05)

**Table 3.** Rumen ecology, and microorganism in Thai native beef cattle fed different levels of *Piper sarmentosum* (PS)

Items	Supplementation of PS, g DM/head/day				SEM	Pr > F
	0	0.6	1.2	2.4		
Rumen ecology						
Ruminal pH	7.05	7.00	7.10	6.98	0.06	0.58
Ruminal temperature	40.08	39.86	39.96	39.28	0.49	0.68
NH <sub>3</sub> -N concentration, mg/dl	10.86	13.31	11.56	12.26	0.11	0.49
Ruminal microbes, cell/ml						
Bacteria, x10 <sup>9</sup>	1.07	1.07	1.06	1.05	0.40	0.97
Protozoa, x10 <sup>6</sup>	9.38 <sup>a</sup>	5.65 <sup>b</sup>	6.52 <sup>ab</sup>	3.35 <sup>b</sup>	1.04	0.01 <sup>**</sup>

<sup>a,b</sup>Means in the same row with different superscripts differ (P<0.05)



## ***Session 9-Orchid ballroom I***

ANN-01-0061

### **Determination of Level Protein Intake to Control Fat and Protein in Carcass of Fattened Lambs**

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#### **Abstract**

Twenty four thin tailed lambs aged approximately 3 months old with an average body weight of  $14.19 \pm 2.41$  kg (CV 16.98%) were used in this study to determine the level of dietary protein (percent protein intake per body weight; CPI in %BW) required to achieve a low fat content (less than 5%) in lamb meat preferred by consumer. The lamb were fed the diets containing 14, 16 and 18% crude protein (CP) and 60 and 70% total digestible nutrients (TDN). The lambs were slaughtered after 3 months raising under the feeding treatments which reached average slaughter weight  $24.48 \pm 3.45$  kg. The carcass was weighed to obtain carcass weight, and was then separated into meat, fat and bone. The longissimus dorsi (LD) and Biceps femoris (BF) were used to determine the fat and protein content of carcass by proximate analysis. The data of level CPI (in %BW) was then correlated to fat and protein in carcass, and was analyzed to determine the level protein intake required to control carcass fat. The results showed that the level of CPI (in %BW) were significantly correlated ( $P < 0.05$ ) with carcass fat, meat fat and carcass protein, being 0.11, 0.40 and 0.63 respectively. This study found that level of protein intake per body weight (CPI in %BW) for lamb should be less than 0.7%BW to obtain a consumer preferred low fat content (less than 5%) in lamb meat.

**Keywords:** lambs, percent protein intake per body weight, carcass fat, meat fat, meat protein



## Introduction

In recent years, consumer realized that the high proportion of lipid consumption of meat give some effects on human health such as coronary heart disease, cancer and arthritis. Along with the increasing public awareness to healthy living, consumption of low meat fat increased (Scollan et al., 2006 ; World Cancer Research Fund/American Institute for Cancer Research, 2007 ; Bezerra et al., 2016) and the quality of meat can be identified through its flavor and tenderness (Jaworska et al., 2016 ; Malva et al., 2016 ; Zinder et al., 2017). Intramuscular fat (IMF) is being a factor to determine juiciness and flavour of meat (Lambe et al., 2017). Savel ad Cross (1998) claimed that a minimum level of IMF in meat lambs that can be accepted by consumer is 3%. Lambe et al. (2017) reported a preferred level of IMF of lamb meat is less than 5%.

In the postnatal period, adipose cells in the body of lamb is still carrying out to the hyperplasia process, adipose tissue develops its size of cell (hypertrophy) and increases the amount of cell (hyperplasia) (Hood and Allen, 1973). The increasing of adipose cells occurs as long as the live of lamb and fat accumulation in the meat of lambs will continue (Wangko and Wangko, 2010). Protein is one of nutrient contents that can affect fat proportion in meat. The excess of amino acids from protein would be utilized for fat synthesis in the carcass of lambs and being low quality carcass (Ponnampalam et al., 2003). Therefore, it is necessary to determine the level protein intake to get best quality of meat lambs.

## Materials and Methods

Twenty four thin tailed lambs were used with an average body weight of 14.19 kg  $\pm$  2.41 (CV 16.98%) aged  $\pm$  3 months. The diets contained 14, 16 and 18% of crude protein (CP) and 60 and 70% of total digestible nutrients (TDN). The feed ingredients consisted of rice brand, cassava peel, sugarane top, cassava flour, soybean meal, fish meal, molasses and minerals. The feed and water were given ad libitum. The lambs were reared for 3 months and slaughtered. Lambs were fasted 6 hour prior to be slaughtered. The carcass of lambs was aging at 18°C for 10 hours, then was weight to determine carcass weight. Then carcass was separated between meat, fat and bone to obtained the percentage of carcass fat. The longissimus dorsi (LD) and Biceps femoris (BF) were used to determine the proximate composition (fat and protein).



The data was obtained and analyzed using correlation analysis. The relationship between the two variables was evaluated using the magnitude of the correlation value described by Steel and Torrie (1960).

## Results and Discussion

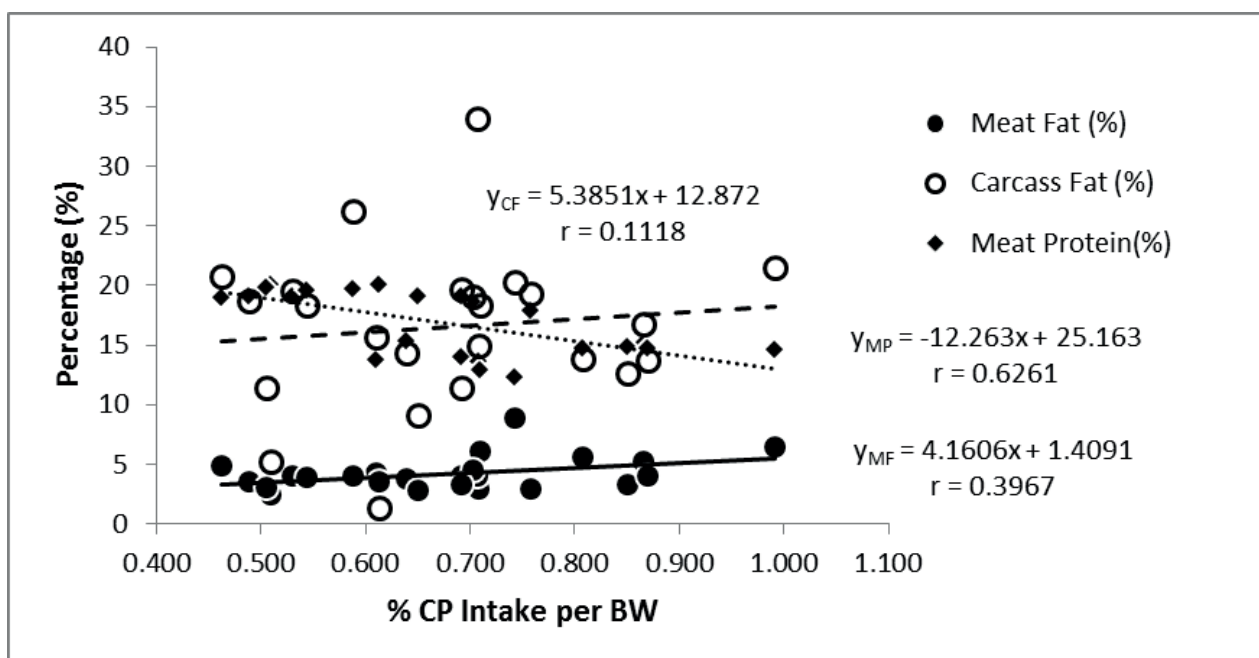
Percent protein intake per body weight, carcass fat (%), fat of meat (%) and protein of meat (%) are presented in the Table 1. The dietary protein intake was 0.46 – 0.99 % per body weight and 0.67% as an average. The carcass fat (%) was around 1.41 - 33.96 % and 16.51% as an average. Fat of meat (%) was around 2.41 – 8.88 % and 4.22 % as an average. Protein of meat (%) was around 12.25 – 20.02% and 16.864% as an average).

**Table 1** Protein intake per body weight (%), carcass fat (%), meat fat (%) and meat protein (%) of lambs.

Parameter	Range	Average	Standar Deviation
Protein intake / BW (%)	0.462 – 0.992	0.677	0.137
Carcass Fat (%)	1.413 – 33.964	16.516	6.597
Meat Fat (%)	2.410 – 8.880	4.225	1.436
Meat Protein (%)	12.250 – 20.020	16.864	2.682

Francisco et al. (2015) found that carcass fat of lamb was 20,9% with protein intake 0,7% per BW. Based on fat class and conformation (Speedy, 1980), lambs in this study is included as class 2 of fat. It was due to its carcass weight was around 14.18 kg (fat 16.71%, muscle 62.21% and bone 18.51%). The meat fat of lambs in this study was higher than those of previous study that taken by Francisco et al. (2015), the meat fat of lambs were 2%. According to Purbowati et al. (2010) the protein of lamb meat were 18,32% which is higher than this study. The growth of animals starts from nerves growth, bone growth, muscle growth and fat growth (Owens et al., 1993). Savel and Cross (1998) claimed that a minimum level of IMF in meat lambs that can be accepted by consumer is 3%. According to Lambe et al ( 2017), a preferred level of IMF of meat lamb is less than 5%. Protein content of meat is about 16 – 22% and the largest component was 75 – 80% of dry matter (Lawrie, 1995).





**Figure 1** Correlation between % CP intake per BW to carcass fat (CF), meat fat (MF) and meat protein (MP).; %meat fat (●), %carcass fat (○), %meat protein (◆)

The result showed that protein intake per body weight (%) was linearly correlated with carcass fat (%), meat fat (%) and meat protein (%) ( $P < 0.05$ ). Protein intake per body weight (%) has weak correlation with carcass fat (%) ( $r = 0.1118$ ) and has medium correlation with meat fat (%) ( $r = 0.3967$ ). However, protein intake per body weight (%) has strong correlation with meat protein (%) ( $r = 0.6261$ ). Figure 1 showed that every increasing of protein intake per body weight (%) increases the carcass fat (%) and meat fat (%) but decreases the meat protein (%). It is due to the muscle has maximum capacity to receive protein intake. Therefore, the excessive protein intake would be used to grow fat in form of adipose cells by hyperplasia process. Muscle tissue in lambs grows as cell enlargement process (hypertrophy) but adipose cells in the body of lambs is still carrying out to be hyperplasia process where is adipose tissue develops size of cell (hypertrophy) and increases of the amount of cell (hyperplasia) during growth period. (Hood and Allen, 1973). The excess of amino acids from protein would be utilized for fat synthesis in the carcass of lambs and being low quality carcass (Ponnampalam et al., 2003).



If lamb with average body weight  $\pm 25$  kg consumed 3.5% dry matter intake/BW it can be calculated that lamb consumed 0.875 kg DMI. From this research it is known that the best protein intake to get low fat of meat is 0.7%/BW. Then, protein consumed by lamb with the average body weight  $\pm 25$  kg is 175 g. Therefore, protein content on diet that should be given to the lamb to get low fat meat is less than 20% .

## Conclusions

Based on this study, it can be concluded that protein intake per body weight of lamb to achieve low fat meat (<5%) which is preferred by consumer as healthy meat is 0.7%. Then, it can be suggested that protein content on diet for lamb should be given less than 20%.

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## Effect of Yeast-Fermented Cassava Pulp Levels on Growth Performance of Growing Goats fed Napier Pakchong 1 Grass

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### Abstract

The aim of this study was to investigate effect of concentrate replacement by yeast-fermented cassava pulp (YFCP) on growth performance of growing goat fed Napier Pakchong 1 grass. Twelve of goats (male and female) were randomly assigned to receive three dietary treatments according to a Randomized Complete Block design. The three treatments were supplementation with concentrate at 2.0% BW, YFCP at 1.0% BW, and YFCP at 2.0% BW. All goats were kept in individual pen and received chopped Napier Pakchong 1 grass in *ad libitum* during 3 months trial. It was found that total feed intake of goat in the control group was higher than goat received 1.0% BW YFCP but not different from goat received 2.0% BW YFCP ( $P<0.05$ ); however, Napier Pakchong 1 grass intake was lowest in 2.0% BW YFCP fed group. Crude protein intake of the control was higher than others ( $P<0.05$ ). Growth performance was significantly different between groups ( $P<0.05$ ) whereas the control was higher than 1.0% BW YFCP fed group but comparable with 2.0% BW fed group. According, the control had feed conversion rate greater than 1.0% YFCP fed group but not different from 2.0% YFCP fed group ( $P<0.05$ ). However, goat received YFCP replaced concentrate had lower feed cost per BW gain than the control ( $P<0.05$ ). Therefore, utilization of YFCP as supplement could productivity for rising growing goat with Napier Pakchong 1 grass.

**Keywords:** *Saccharomyces cerevisiae*, fermented cassava pulp, Napier Pakchong 1, growth performance, goat



## Introduction

In Thailand, the goat population was 539,583 heads, 515,093 and 24,490 heads for meat and milk goats, respectively, of which 50.4 % were in southern where Muslim community (Department of Livestock Development, 2015). Although population of goat is less in the north-east region, low humidity there is suitable for evaporative cooling and health of goat (Rahal et al., 2014). During the past 10 years, the farmers are more interested in meat goat business because they are easy to rise, bred relatively fast and fed by a wide range of roughage. However, in situations where concentrate feed supplements are expensive, farmers should be finding the alternative feed particularly local source for reducing the feeding cost (Wanapat, 1999). Cassava pulp has prevalent use as feedstuff for livestock production. It comprises 15.8-23.4% dry matter with 1.2-2.8% crude protein, 55.0-74.4% nitrogen free extract, 0.1-2.4% fat, 17.9-24.0% crude fiber, and 1.7-2.8% ash, on dry matter basis (Yimmongkol, 2009). An improved nutrient composition of cassava pulp especially crude protein content by fermentation with microorganisms has been reported (Khampa et al., 2009). Addition of molasses and urea increases crude protein though reduced fiber in yeast-fermented cassava pulp (Khampa et al., 2010). Moreover, enzymatic treatment can disrupt the fibrous structure of pulp, allowing more starch granules to be fermented by micro-organism during the fermentation process (Dissaro, 2000; Pilajun and Wanapat, 2016). Fermentation of cassava pulp with microorganism was also for palatability improvement, nutrient preservation, anti-nutritional detoxification, and increase degradability (Aro et al., 2008). Therefore, the aim of this study was to investigate the effect of different additives on chemical composition, *in vitro* gas production and dry matter disappearance of cassava pulp.

## Materials and Method

Twelve of goats, 10 months old of male and female of Thai native x Anglo Nubian crossbred goat with a body weight of  $16.5 \pm 4.67$  kg were randomly assigned to receive three dietary treatments according to a Randomized Complete Block design. The three treatments were supplementation with concentrate at 2.0% BW (1), yeast-fermented cassava pulp (YFCP) at 1.0% BW (2), and YFCP at 2.0% BW (3). YFCP was prepared by modified the method of Khampa et al. (2012) using yeast (*Saccharomyces cerevisiae*), urea and molasses as major components at 0.33%, 3.0%, and 4.5% DM, respectively. All goats were received chopped Napier Pakchong 1 grass in *ad libitum*. Chemical compositions of experimental diets are presented in Table 1. All goats were raised in individual pens with fresh water was available free access, and fed with their respective treatment by divided between two daily feeds (07.00 and 16.00).



Body weight of individual goat was determined before and 2 weeks interval during 3 months trial. Feed intake was investigated daily while samples of feeds and refusals were collected weekly. Samples were dried in a forced-air oven at 60°C for 96 h, ground through a 1-mm stainless steel screen, and analyzed according to the Association of Official Analytical Chemists (AOAC, 1995) for dry matter (DM), total ash and crude protein (CP). The method of Van Soest et al. (1991) was used to determine neutral detergent fiber (NDF) and acid detergent fiber (ADF) on an ash-free basis. Feed utilization efficiency was evaluated using total feed intake to average daily gain ratio and cost of feed per gain

All data were statistically analyzed according to a Randomized Complete Block design using the GLM procedure of SAS (1996) with initial body weight as covariate. Differences between dietary treatment means were determined by Fisher's Least Significant Difference (LSD). Differences between means with  $P < 0.05$  were accepted as representing statistically significant differences.

**Table 1.** Chemical composition of experimental diets

Chemical composition, % DM	Napier Pakchong 1	Yeast- fermented cassava pulp	Concentrate
Dry matter	15.9	15.6	87.4
Organic matter	91.2	96.8	94.7
Crude protein	11.5	11.5	14.1
Ether extract	1.62	0.53	3.61
Neutral detergent fiber	59.4	48.8	16.4
Acid detergent fiber	37.0	22.3	7.07

## Results and Discussion

Napier Pakchong 1 grass and yeast-fermented cassava pulp (YFCP) contained similar proportion of crude protein (11.5% DM) while fiber fractions of YFCP slightly lower than grass (Table 1). The result agreed with Wijitphan and Lowilai (2011) who reported CP content in Napier Pakchong 1 range between 11.1-12.3; however, Kaewthong (2011) reported that Napier Pakchong 1 contained crude protein 15.9% DM. This could be due to many factors especially soil component, fertilizer application, and water management. CP content of YFCP was close to Khampa et al. (2012) who reported CP in cassava pulp was increased to 12.1% DM when fermented with yeast (*Saccharomyces cerevisiae*). In addition, CP content of fermented cassava pulp is mostly related with level of nitrogen source which is urea.

Effect of concentrate replacement by YFCP on feed intake and growth performance of growing goat are showed in Table 2. Total feed intake of goat in the control group was higher than goat received 1.0% BW YFCP but not different



from goat received 2.0% BW YFCP ( $P < 0.05$ ); however, Napier Pakchong 1 grass intake was lowest in 2.0% BW YFCP fed group. CP intake of the control was higher than others ( $P < 0.05$ ) due to higher of CP content in concentrate than YFCP. Khampa et al. (2009) revealed that yeast-fermented cassava pulp increased feed degradation by rumen microbes therefore able to replace concentrate in beef cattle. Lower roughage intake in YFCP fed group may related with gut capacity due to high moisture content of YFCP. In addition, CP intake of goat in this study, 61.1-92.5 g/d, is in range of NRC (1989) recommendation which was 44-83 g/d for 10-20 kg BW goat. However, Kearl (1982) recommended that growing goat could have 3.30% BW of total intake to meet their sufficient nutrient thus 1.0% BW of YFCP fed group may receive insufficient nutrient due to low intake (3.13% BW).

Body weight gain and average daily gain were significantly different between groups ( $P < 0.05$ ), the control was higher than 1.0% BW YFCP fed group but comparable with 2.0% BW fed group. According, the control had feed conversion rate greater than 1.0% YFCP fed group but not different from 2.0% YFCP fed group ( $P < 0.05$ ). However, goat received YFCP replaced concentrate had feed cost per BW gain lower than the control ( $P < 0.05$ ).

**Table 2.** Voluntary feed intake, growth performance, and feed efficiency of growing goat received different dietary treatments

Items	2% BW concentra te	1% BW YFCP	2% BW YFCP	SEM	P- value
Total feed intake, g DM/h/d	736 <sup>a</sup>	531 <sup>b</sup>	643 <sup>ab</sup>	68.6	0.013
Concentrate	311	-	-	-	-
YFCP	-	160	297	-	-
Napier Pakchong 1	425 <sup>a</sup>	371 <sup>ab</sup>	346 <sup>b</sup>	39.7	0.041
Total feed intake, % BW	3.97 <sup>a</sup>	3.13 <sup>b</sup>	3.60 <sup>ab</sup>	0.24	0.023
Crude protein intake, g/h/d	92.5 <sup>a</sup>	61.1 <sup>b</sup>	74.0 <sup>b</sup>	6.51	0.006
Initial body weight, kg	16.5	16.6	16.5	2.34	0.998
Final body weight, kg	20.8	18.6	19.6	2.70	0.442
Body weight gain, kg	4.29 <sup>a</sup>	1.98 <sup>b</sup>	3.07 <sup>ab</sup>	0.66	0.018
Average daily gain, g/h/d	70.3 <sup>a</sup>	32.5 <sup>b</sup>	50.3 <sup>ab</sup>	10.8	0.018
Feed conversion rate	10.8 <sup>a</sup>	16.3 <sup>b</sup>	12.7 <sup>ab</sup>	1.51	0.027
Feed cost, Bath/kg BW gain	68.6 <sup>a</sup>	51.5 <sup>b</sup>	44.3 <sup>b</sup>	6.84	0.032

YFCP, yeast-fermented cassava pulp

<sup>a-b</sup> Values on the same row with different superscripts differed ( $P < 0.05$ )

SEM, standard error of sample mean





Greater growth rate in the control could be due to higher protein content in concentrate than YFCP and resulted in higher feed and protein intakes. Appropriate nutrient for growth of microorganism in the rumen may lead to greater of nutrient digestibility, feed intake, and growth performance, respectively. However, growth rate of growing goat in the present study (50-70 g/d) quiet low when compared to the previous studies. Saichuer et al. (2012) reported that daily growth of goat was 109 g/d when supplemented with 2.0% BW of concentrate. Department of Livestock Production (2008) also found that daily gain at 93.9-103 g/d would be achieved with 2.0-2.5% BW of concentrate supplementation to growing goat. Besides, lower feed cost (35.0%) in YFCP fed goat could provide significantly profit to the owner. Rahman et al. (2014) found feed cost per gain of goat was 20.7% decreased when using soy waste replaced commercial pellet. Moreover, replacement 75.0% DM soybean meal in concentrate for meat goat with fermented cassava pulp decreased 26.7% feed cost have been reported by Kaewwongsa (2011). Thus, using of fermented cassava pulp as a main diet in goat could decrease feed cost 20-35%.

## Conclusion

Concentrate replacement by YFCP decreased roughage and crude protein intakes but did not affect growth rate of growing goat. YFCP supplementation at 2.0% BW decreased feed cost per gain of goat when compared with regular concentrate. Therefore, YFCP have capability to use as supplement feed for growing goat rising with Napier Pakchong 1 grass.

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## Effect of Mangosteen Peel Liquid Protected Soybean Meal on Methanogen and Microbial Population Using *In Vitro* Gas Production Technique

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### Abstract

An *in vitro* gas production technique was conducted to investigate the effect of mangosteen peel liquid protected soybean meal on methanogen and microbial population. The treatments were arranged according to a 6x2 factorial arrangement in a Completely randomized design by using 6 levels of mangosteen peel liquid (MSPL) supplement (0, 2, 4, 6, 8, 10% of total soybean meal), 2 different treated soybean meal (heat at 140°C and pelleting). The gas production kinetics were affected by heat treated soybean meal and MSPL supplementation. Cumulative gas at 96 h post incubation were decrease with increasing MSPL. Quantitative polymerase chain reaction (qPCR) based on 16S RNA revealed that total bacteria, *F. succinogenes*, and *R. albus* numbers were similar among treatments. However, increasing MSPL levels resulted in decreasing *R. flavefaciens* and methanogenic bacteria ( $P < 0.05$ ). Based on this study, it could be concluded that heat treated soybean meal and MSPL supplementation could depress on gas production and methanogenic bacteria.

**Keyword:** gas production, mangosteen peel, methanogen, real-time PCR, soybean meal



## Introduction

Soybean meal (SBM) is the most commonly used protein supplement in beef and dairy diets. It is very palatable and has a good amino acid balance and high availability. Its bypass essential amino acid index is just next to ruminal microbial protein beating all other undegradable protein sources. SBM are excellent sources of RDP and can be extensively degraded ( $\geq 57.4$  to 69.6%) by ruminal microbes (NRC, 2001). Various methods of treating SBM have been studied to alter the rate and extent of protein degradation in the rumen. Various methods of treating proteins have been used to reduce their degradation in the rumen and they can be categorized into chemical and physical treatments. Some of the techniques, e.g., extrusion, roasting, expeller, lignosulfonate and formaldehyde have been used to protect SB and SBM from ruminal degradation. Treating SBM by these methods increases its ruminal bypass protein content up to 70% (Waltz and Stern, 1989).

Mangosteen (*Garcinia mangostan*) peel is a fruit by-product contains both condensed tannins and crude saponins, which exert a specific effect against rumen protozoa, while the rest of the rumen biomass remains unaltered. Supplementation of MSP as well as soap berry fruit pellet has been shown to alter rumen fermentation by decreasing the protozoa population and CH<sub>4</sub> production (Poungchompu et al., 2009). Ngamsaeng et al. (2006) suggested that supplementation of MSP (100 g DM/day) in cattle can increase rumen bacteria and decrease the protozoal population, and maintain the fungal zoospore population. Moreover, supplementation of mangosteen peel in swamp buffalo effected on methanogen population (Wanapat et al., 2014)

Therefore, the objective of the present study was to investigate the effect of mangosteen peel liquid protected soybean meal on methanogen and microbial population using in *in vitro* gas production technique.

## Materials and Methods

The experimental design was a 6×2 factorial arrangement in a Completely randomized design (CRD). The dietary treatments were 6 levels of mangosteen peel liquid supplement (0, 2, 4, 6, 8 and 10% of total soybean meal), 2 different treated soybean meal (heat at 140°C and pelleting). Samples were prepared and weighed (total substrate mixture 200 mg of DM) into 60 ml bottles for various



time incubations. Strict anaerobic techniques were used in all steps during the rumen fluid transferring and incubation periods according to Menke et al. (1979). During the incubation, the gas production was recorded at 1, 2, 4, 6, 8, 12, 24, 48, 72 and 96 h. The rumen inoculums mixtures were sampled at 4, 8, and 12h of fermenting post inoculation analyzed for microbial population. Rumen inoculums were immediately prepared for DNA extraction for methanogenic bacteria, total bacteria and predominant cellulolytic bacteria populations using real-time PCR technique. Community DNA was extracted from 0.5g of rumen content by the RBB + C method (Yu and Morrison, 2004). In brief, the RBB + C method employs two rounds of bead beating in the presence of NaCl and SDS, followed by sequential ammonium acetate and isopropanol precipitations. The precipitated nucleic acids were then treated with RNase A and proteinase K, and the DNA was purified using columns from QIAGEN DNA Mini Stool Kit (QIAGEN, Valencia, CA), according to manufacturer's recommendations.

All obtained data were subjected to the General Linear Models (GLM) procedures of SAS (1998) according to a 6×2 factorial arrangement in CRD. For all parameters, differences among treatments means were contrasted by Tukey's Multiple Comparison Test (Crichton, 1999).

## Results and Discussion

Gas production kinetic including the gas production from the immediately soluble fraction (a), the gas production from the insoluble fraction (b), the gas production rate constant for the insoluble fraction (c), and the potential extent of gas production (a+b) and accumulate gas production were affected by treated soybean meal and MSPL supplementation ( $P < 0.05$ ) and there was an interaction between treated soybean meal and MSPL (Table 1). Accumulate gas production decreased with the increasing level of MSPL supplementation. Previous study by Pongchompu et al., (2009) reported that the reduction of gas production by dietary saponins and tannins from mangosteen peel without adversely affect on digestion were obtained.

Min et al. (2005) reported that quebracho condensed tannin consistently decreases the rate of ruminal gas production and inhibit the growth of



microorganisms. Moreover, saponins have a potent anti-protozoal activity by forming complex with sterols in protozoal cell membranes (Goel and Makkar, 2012).

Rumen microorganism populations revealed that application of quantitative PCR to quantify total bacteria and cellulolytic bacteria treatments did not change population of *F. succinogenes* and *R. albus* ( $P > 0.05$ ). However, increasing MSPL levels resulted in decreasing *R. flavefaciens* and methanogenic bacteria ( $P < 0.05$ ) (Table 2). These results could be due to the effect of a high level of condensed tannin contained in MSPL supplementation. The result of this study agreed with Wanapat et al. (2014), who found methanogen population were significantly decreased when buffaloes were supplemented at 100 g MSP.

## Conclusion

In conclusion, the gas production kinetics were affected by heat treated soybean meal and MSPL supplementation. Increasing level of MSPL supplementation could depress on *R. flavefaciens* and methanogenic bacteria. However, research should be conducted especially using *in vivo* production trials.

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**Table 1.** Gas production kinetics and gas production from *in vitro* incubation

Treatment <sup>1</sup>	Gas kinetics <sup>2</sup>				Gas
	a	b	c	a+b	
T1, SBM-MSPL 0% - Pelleting	-5.7	67.2	0.04	61.5	60.1
T2, SBM-MSPL 2% - Pelleting	-4.5	64.1	0.03	59.6	58.7
T3, SBM-MSPL 4% - Pelleting	-3.8	59.6	0.03	55.8	54.2
T4, SBM-MSPL 6% - Pelleting	-2.6	55.8	0.03	53.2	52.5
T5, SBM-MSPL 8% - Pelleting	-2.5	49.5	0.02	47.0	46.8
T6, SBM-MSPL10% - Pelleting	-2.1	41.7	0.02	39.6	39.2
T7, SBM-MSPL 0% - Heat	-3.7	55.4	0.03	51.7	51.4
T8, SBM-MSPL 2% - Heat	-3.4	50.2	0.03	46.8	46.2
T9, SBM-MSPL 4% - Heat	-2.7	45.1	0.03	42.4	41.9
T10, SBM-MSPL 6% - Heat	-2.2	39.4	0.02	37.2	37.1
T11, SBM-MSPL 8% - Heat	-1.9	32.2	0.02	30.3	30.2
T12, SBM-MSPL 10% - Heat	-1.4	30.8	0.02	29.4	29.3
SEM	1.37	1.02	0.03	2.13	1.57
Comparison					
Methods	ns	*	*	*	*
MSPL level	ns	*	*	*	**
Interaction	ns	*	*	*	*

<sup>1</sup>SBM = soybean meal; MSPL= mangosteen peel liquid.

\*P<0.05, \*\*P< 0.01, ns = non- significant.

<sup>2</sup>a= the gas production from the immediately soluble fraction, b= the gas production from the insoluble fraction, c= the gas production rate constant for the insoluble fraction (b), a+b = the gas potential extent of gas production.



**Table 2.** The effect of mangosteen peel liquid protected soybean meal on total bacterial, predominant cellulolytic bacterial and methanogenic bacteria from in vitro incubation with rumen fluid

Treatment <sup>1</sup>	Real-time PCR technique, copies/mL of incubation				
	Total bacteria (× 10 <sup>8</sup> cell/ml)	<i>F.succinogenes</i> (× 10 <sup>6</sup> cell/ml)	<i>R. flavefaciens</i> (× 10 <sup>5</sup> cell/ml)	<i>R. albus</i> (× 10 <sup>5</sup> cell/ml)	Methanogenic (× 10 <sup>3</sup> cell/ml)
T1, SBM-MSPL 0% - Pelleting	6.9	5.0	4.4	1.7	4.5
T2, SBM-MSPL 2% - Pelleting	6.5	4.3	3.5	1.8	4.1
T3, SBM-MSPL 4% - Pelleting	6.7	4.9	2.7	1.9	3.5
T4, SBM-MSPL 6% - Pelleting	5.8	4.7	1.4	1.5	2.4
T5, SBM-MSPL 8% - Pelleting	6.7	4.5	1.3	1.6	2.1
T6, SBM-MSPL10% - Pelleting	6.5	4.1	1.0	1.6	2.0
T7, SBM-MSPL 0% - Heat	6.8	5.1	3.6	1.9	4.8
T8, SBM-MSPL 2% - Heat	5.4	4.7	3.2	1.4	4.1
T9, SBM-MSPL 4% - Heat	5.9	4.6	2.5	1.5	2.7
T10, SBM-MSPL 6% - Heat	5.7	4.8	2.0	1.4	2.3
T11, SBM-MSPL 8% - Heat	5.5	4.5	1.8	1.3	2.1
T12, SBM-MSPL10% - Heat	5.8	4.6	1.2	1.0	1.8
SEM	1.2	2.6	0.8	0.3	0.7
Comparison					
Methods	ns	*	*	ns	*
MSPL level	ns	ns	*	ns	*
Interaction	ns	ns	ns	ns	*

<sup>1</sup>SBM = soybean meal; MSPL= mangosteen peel liquid.

\*P<0.05, \*\*P< 0.01, ns = non- significant.



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## Fermentation Quality and *In Vitro* Digestibility of Fermented Total Mixed Ration with Difference Roughage and Fermentation Period

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### Abstract

The study on effects of roughage sources in fermented total mixed ration (FTMR) on quality and *in vitro* digestibility were randomly design into completely design . Dietary treatments are consisted 2 factors as the roughage sources )rice straw, sugarcane leave, Napier grass, Pangola grass, Guinea grass, peanut hull and peanut hay (and fermentation period (7, 14, 30 and 45 days). FTMR were mixed and ensiling in plastic bag with differences fermentation period and consequently to chemical analyses and *in vitro* digestibility evaluation .The results reveal that roughage and fermentation period had affected on chemical composition which high moisture and ammonia in fresh grass .Gas production kinetic and *in vitro* digestibility were not significantly different but low moisture roughage trend to be greater .Therefore, the roughage with low moisture content could increase quality and digestibility of FTMR .Furthermore, it should be conducted on roughage source of FTMR to ruminant animal production.

**Keywords** :roughage sources, FTMR feed, rumen fermentation, gas production

### Introduction

In the recent year feeding regime on beef or dairy farm has been overcoming conventional practical into total mixed ration (TMR) .It has been great interested by farmer due to its expected benefits in the nutrition content, feeding management and production planning .The benefit of a TMR are include increased feed intake, enhanced use of low-cost alternative feed ingredients, ability to control the forage concentrate ratio, lower incidence of metabolic and



digestive disorders (Cao et al., 2012; Kim et al., 2012). Typically, the low-quality roughage such a tropical forage is normally contained of high fibrous content that prevents assess of ruminal fermentation and degradation (Wanapat, 2003). During recent years, cassava starch industry has been produced large amount of cassava pulp byproduct and consequently encouraging to use as animal feed. However, limiting effect, cassava starch byproduct in generally had high moisture content that affected on TMR quality and spontaneous influence to aerobic fermentation cause to TMR spoiled. It is conducted to improve the nutritive value and utilization efficiency of low-quality roughages and industrial by-product as fermented total mix ration (FTMR) are efficient to overcome on this problem (Khejornsart et al., 2015). However, there is limit available information about FTMR prepared from cassava starch byproduct with different roughage sources. Therefore, this experiments were investigated the difference roughage sources and fermentation period on nutritive value and *in vitro* gas production and digestibility of fermented total mix ration.

## Materials and methods

### Dietary treatments and design

The experiment was assigned into 4x7 factorial arrangement in completely randomized design (CRD). The dietary treatments are included 7 roughages as rice straw, sugarcane leave, Napier grass, guinea grass, pangola grass, peanut hull, peanut hey and fermentation time by 7, 15, 30 and 45 days, respectively. The FTMR diet were mixed with as formulation in table 1. FTMR diet were mixed and then put into plastic bag and were kept at room temperature.

### Animal donor and in vitro experiment

Two male, crossbred beef cattle (Charolaise x Native beef cattle x Brahman (with average body weight of 258±30 kg) were fed on rice straw *ad libitum* and used as rumen fluid donors. After feeding 14 days, 1000 ml rumen liquor was obtained from each animal before the morning feeding. The rumen fluid was filtered through four layers of cheesecloth into pre-warmed thermos flasks and then transported to the laboratory.

An *in vitro* ruminal experiment was conducted according to the methods described by Menke and Steingass (1988). FTMR diet were freeze-dried and ground to pass through a 1-mm screen. The samples were analyzed for DM, ash and CP using the procedures of AOAC (2012), and NDF and ADF composition by Van Soest et al. (1991) method. For each dietary treatments, approximately 0.5 g of the sample was weighed into six 60mL glass vials, of which three were used to determine DM degradability, and the other three was used for analyses of microbial composition by real-time PCR, as described below. Rumen fluid



collection, donor cattle, and post-sampling were followed with the paper. Fifty milliliters of this medium was dispensed into each vial and these vials were covered with rubber cap and an aluminum ring and then incubated in a water bath at 39°C. The volume of gas was measured at 0, 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 48, 72 and 96 h post incubation. Cumulative gas production data were then fitted to the model of Ørskov and McDonald (1979). At 48 h post incubation, contents of a vial were filtered through pre-weighed filter paper and residues from each vial were freeze-dried to determine DM degradability in the *in vitro* rumen fermentation. All data obtained were analyzed statistically using PROC GLM of SAS. Multiple comparisons among treatment means were performed by Duncan's New Multiple Range Test (DMRT) (Steel and Torrie, 1980).

## Result and discussion

### Chemical composition of experimental diets

The chemical composition of FTMR were significantly different content of DM, OM, CP, NDF and ADF ( $P < 0.05$ ), which fresh roughage had lower DM content than those dry roughage (Table 2), and high NDF or ammonia content were found in pangola grass or peanut hull, while ensiled pH was not effect. Increases ammonia content in FTMR may have occurred because of the contribution of protein of roughage. The fermentation processed by lactic acid bacteria might be transformed water-soluble carbohydrate in to organic acid, as a result, lactic acid increase while DM decrease, which is consistent with previous study (Khejornsart et al., 2015). In the fiber content, increase value during ensiling were different between the types of roughage, whereas this may have been in part due to the differences in nutritional composition of roughage on time of fermentation. This study found the negative effect of fermentation period with CP content decline, it may have been due to the ensiling process of fermentable carbohydrate of roughage, as resulted increasing of the ammonia content .

### Gas production kinetics and *in vitro* degradability

The roughage sources and fermentation period were not effect on gas production kinetic and trend to be rapid degradation in Napier grass or high potential gas production when fermentation for 15 days (Table 3). *In vitro* DM and OM digestibility was not different between the roughage sources and fermentation period ( $P > 0.05$ ), while low moisture roughage tends to be higher in digestibility when compared to low moisture roughage ( $P = 0.7$ ), which may be due to a higher soluble carbohydrate content. On the other hand, Ando et al., (2006) found that DM, OM and CP digestibility of guinea grass silage had higher than rice straw base silage. The ensiling process for FTMR affects *in vitro* rumen fermentation characteristics by activating microbes that fiber-degrading bacteria,



as increased digestibility. In this study, there was no interaction between roughage and fermentation period.

## Conclusion

It can be concluded that the FTMR diet with low moisture content as rice straw, sugarcane leave, peanut hull or hay resulted in the fermentation quality and *in vitro* DM digestibility. Further research using FTMR with high quality roughage diets to improve rumen fermentation and feed efficiency in ruminants are recommended.

## Acknowledgements

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**Table 1.** Dietary ingredients of FTMR feed

Items	T1	T2	T3	T4	T5	T6	T7
Cassava pulp	46.4	46.4	42.6	42.6	42.6	46.4	46.4
Cassava peel	31.8	31.8	30.5	30.5	30.5	31.8	31.8
Roughage sources <sup>1</sup>	8.7	8.7	13.8	13.8	13.8	8.7	8.7
Cassava chip	2.8	2.8	2.8	2.8	2.8	2.8	2.8
Rice bran	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Palm kernel cake	5.5	5.5	5.5	5.5	5.5	5.5	5.5
Molasses	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Urea	0.7	0.7	0.7	0.7	0.7	0.7	0.7
Mineral mixed	0.2	0.2	0.2	0.2	0.2	0.2	0.2
di-Calcium phosphate	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Salt	0.2	0.2	0.2	0.2	0.2	0.2	0.2

<sup>1</sup> T1 =rice straw, T2 =sugarcane leave, T3 =Napier grass, T4 =guinea grass, T5 = pangola grass, T6 =peanut hull, T7 =peanut hey

**Table 2.** Fermentation quality characteristics and chemical composition of FTMR after ensiling

Treatments	DM	OM	CP	NDF	ADF	pH	NH <sub>3</sub> -N
Forage sources <sup>1</sup>							
T1	55.73 <sup>bc</sup>	84.22 <sup>b</sup>	8.52 <sup>ab</sup>	44.59 <sup>c</sup>	25.15 <sup>d</sup>	3.78	16.74 <sup>bc</sup>
T2	55.40 <sup>cd</sup>	84.54 <sup>b</sup>	8.09 <sup>b</sup>	47.32 <sup>bc</sup>	28.09 <sup>b</sup>	3.85	12.11 <sup>c</sup>
T3	45.67 <sup>cb</sup>	84.34 <sup>b</sup>	9.19 <sup>a</sup>	44.74 <sup>c</sup>	26.34 <sup>bcd</sup>	3.84	15.06 <sup>c</sup>
T4	45.99 <sup>ab</sup>	84.00 <sup>bc</sup>	9.11 <sup>a</sup>	44.31 <sup>c</sup>	25.61 <sup>d</sup>	4.09	33.30 <sup>ab</sup>
T5	46.16 <sup>ab</sup>	84.00 <sup>bc</sup>	8.75 <sup>ab</sup>	50.10 <sup>b</sup>	27.92 <sup>bc</sup>	3.92	34.71 <sup>ab</sup>
T6	54.98 <sup>d</sup>	85.16 <sup>a</sup>	6.36 <sup>c</sup>	52.54 <sup>a</sup>	38.25 <sup>a</sup>	3.91	22.45 <sup>abc</sup>
T7	56.43 <sup>a</sup>	83.52 <sup>c</sup>	9.17 <sup>a</sup>	46.71 <sup>c</sup>	25.81 <sup>cd</sup>	3.83	21.45 <sup>abc</sup>
Fermentation time, day							
7	55.44 <sup>b</sup>	84.55 <sup>a</sup>	10.12 <sup>a</sup>	43.60 <sup>b</sup>	27.15 <sup>b</sup>	4.00	19.71
15	55.26 <sup>b</sup>	84.73 <sup>a</sup>	8.24 <sup>b</sup>	44.19 <sup>b</sup>	26.17 <sup>b</sup>	3.85	24.00
30	56.12 <sup>a</sup>	83.87 <sup>b</sup>	8.10 <sup>b</sup>	51.34 <sup>a</sup>	29.53 <sup>a</sup>	3.83	22.16
45	56.24 <sup>a</sup>	83.87 <sup>b</sup>	6.80 <sup>c</sup>	49.61 <sup>a</sup>	29.82 <sup>a</sup>	3.89	23.44
SEM	0.09	0.12	0.16	0.55	0.38	0.03	2.04
Forage sources	**	**	**	**	**	ns	*
Time	**	**	**	**	**	ns	ns
Forage*Time	ns	ns	**	ns	ns	ns	ns

<sup>1</sup> T1 =rice straw, T2 =sugarcane leave, T3 =Napier grass, T4 =guinea grass, T5 = pangola grass, T6 =peanut hull, T7 =peanut hey

<sup>a,b,c,d</sup> Means within the row superscript with significant different) P<0.05





**Table 3.** Effect of roughage sources and fermentation period of FTMR on gas production kinetics and *in vitro* digestibility.

Treatments <sup>1</sup>	a	b	c	a+b	gas (96 h)	IVDMD	IVOMD
T1	0.28	104.61	0.05	104.90	112.59	52.45	59.95
T2	-0.45	98.57	0.06	98.12	104.49	49.07	54.89
T3	-1.34	100.43	0.06	99.08	108.89	49.55	57.77
T4	0.20	96.38	0.06	96.58	102.97	48.29	53.92
T5	-1.35	104.11	0.06	102.75	106.98	51.38	56.29
T6	-0.45	103.79	0.06	103.35	110.17	51.68	58.33
T7	-0.48	105.07	0.05	104.90	110.34	52.30	58.67
Fermentation time, day							
7	4.54	110.16	0.05	114.70	113.79	57.35	60.93
15	4.68	106.97	0.05	111.65	110.77	55.83	59.06
30	3.99	102.00	0.05	105.98	105.14	53.00	55.22
45	2.39	101.00	0.05	103.39	102.56	51.70	53.28
SEM	0.522	1.538	0.002	1.618	1.550	0.809	1.007
Forage sources	ns	ns	ns	ns	ns	ns	ns
Time	ns	ns	ns	ns	ns	ns	ns
Forage sources							
Forage* Time	ns	ns	ns	ns	ns	ns	ns

<sup>1</sup> T1 =rice straw, T2 =sugarcane leave, T3 =Napier grass, T4 =guinea grass, T5 =pangola grass, T6 =peanut hull, T7 =peanut hey  
<sub>a,b,c,d</sub> Means within the row superscript with significant differ (P<0.05).



## Effect of Replacement Soybean Meal by Yeast Waste on Feed Intake and Rumen Ecology in Thai Native Beef Cattle

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### Abstracts

The objective of this research was to study the effect of substitution soybean meal by yeast waste powder on feed intake, rumen ecology and microorganisms in Thai native beef cattle fed on rice straw. Four males Thai native beef cattle were randomly assigned as a 4 x 4 Latin squared design, with similar weight and age at  $120 \pm 20$  kg and 1-2 years, respectively. Experimental treatments consisted of four level of yeast waste replace soybean meal in concentrate feed at 0% (T1), 33% (T2), 66% (T3) and 100% (T4), respectively. Yeast waste obtained from KSL Green Innovation Public Company Limited, Khon Kaen province, Thailand. The yeast waste used in this study contained 26.4% CP. Increasing level of yeast waste in concentrate diets from 0 to 100% replaced soybean meal were not altered rice straw intake and total intake ( $P > 0.05$ ). Rice straw intake was ranges from 53.8 to 56.0 g/kg BW<sup>0.75</sup>. Total intakes were 2.7 to 2.8 kg/d and 71.1 to 72.9 g/kg BW<sup>0.75</sup> which was in the normal range for requirement of beef cattle. Ruminant pH and temperature in cattle fed various levels of yeast waste were not significantly different amount treatments ( $P > 0.05$ ). Replacement soybean meal by yeast waste did not changed bacteria and protozoa population ( $P > 0.05$ ). Thus, the inclusion of yeast waste as feed ingredient in cattle diets up to 100% replacing soybean meal is recommended since it reduced cost of feed, control environmental pollution and hazards that accrue from inadequate waste disposal.

**Keywords:** soybean meal, yeast waste, feed intake, rumen microorganisms, ruminant

### Introduction

Feed is the main costs of ruminant production, thus the use of potentially residues from industry is a way to reduce feed costs. *Saccharomyces cerevisiae* play an essential role in bioethanol production by fermenting a wide range of sugars to ethanol and the residue generated in this process (yeast waste) has been reported to contain about 60-70% of yeast cells and contain 25-30% CP (Díaz et al., 2017). Therefore, utilization of yeast waste as alternative protein source could be beneficial in order to reduce feed cost and reduce environmental pollution. Live yeast cultures from *S. cerevisiae* have been shown to manipulate rumen fermentation and improve ruminant performance, but such effects can be influenced by both the yeast culture and the diet fed to animals, among other factors. Polyorach and Wanapat (2015) reported that live cell yeast is a probiotic source in ruminants which can improve the fermentation in the rumen and increase productivity in ruminants. Moreover, Robinson and Erasmus (2009) reported that yeasts affect yield such as milk yield, feed intake, the result is not clear, but the effect on the



digestibility of the nutrients is positive. However, feeding of yeast waste from bioethanol plant has not studies in ruminant animals. Thus, the objective of this research was to study the effect of substitution soybean meal by yeast waste powder on feed intake, rumen ecology and microorganisms in Thai native beef cattle fed on rice straw.

## Materials and methods

### Animals and dietary treatments

Four males Thai native beef cattle were randomly assigned as a 4 x 4 Latin squared design, with similar weight and age at  $120 \pm 20$  kg and 1-2 years, respectively. Experimental treatments consisted of four level of yeast waste replace soybean meal in concentrate feed at 0% (T1), 33% (T2), 66% (T3) and 100% (T4), respectively. Yeast waste obtained from KSL Green Innovation Public Company Limited, Khon Kaen province, Thailand. The experiment was conducted for four periods and each periods lasted for 21 d in length, with the first 14 d as feed intake measurement and feed adaptation, the last 7 d for sample collection. Each cattle was housed in individual pens (3x5 m) and offered concentrates (Table 1) at 0.5% BW with rice straw fed ad libitum twice daily at 07.00 and 16.00 h. Intake of concentrate and rice straw were measured separately and refusals were recorded daily.

### Sample collection and data

Each of the feed samples during the collection days were pooled by period while feces samples were pooled by each animal in each period and stored at  $-20^{\circ}\text{C}$  for later chemical analyses. Examples were analyzed for DM, ash, CP, aNDF, and ADF.

Rumen fluid was collected at 0 and 4 h post feeding, in the end of each period. Rumen fluid samples were filtrated through 4 layers of cheesecloth. Rumen fluid was immediately measured for pH and temperature using a portable pH meter and subsamples 1 ml of rumen fluid which was transferred to a plastic bottle to which 9 ml of 10 ml/L formalin solution (1:9 v/v, rumen fluid: 10 ml/L formalin) had been added and then stored at  $4^{\circ}\text{C}$  for measurement of bacteria and the protozoal population and was measured using the direct counting microscopic method based on the use of a haemocytometer (Boeco, Hamburg, Germany). All data were analyzed according to a 4x4 Latin square design using the GLM procedure of SAS (1996).

**Table 1.** Ingredient and chemical composition of concentrate.

Items	Level of yeast waste (% DM)			
	0%	33%	66%	100%
Ingredients, kg DM				
Cassava chips	56.0	56.0	56.0	56.0
Soybean meal	10.0	6.66	3.33	0.0
Yeast waste	0.0	3.33	6.66	10.0
Rice bran	14.5	14.5	14.5	14.5
Coconut meal	7.0	7.0	7.0	7.0
Palm kernel meal	7.0	7.0	7.0	7.0
Urea	1.5	1.7	1.9	2.1
Pure sulfur	1.0	1.0	1.0	1.0
Mineral premix	1.0	1.0	1.0	1.0
Molasses, liquid	1.0	1.0	1.0	1.0
Salt	1.0	1.0	1.0	1.0
Chemical composition				
DM, %	91.4	92.1	91.8	92.0
OM, %DM	87.1	87.3	87.3	87.4
CP, %DM	13.6	13.7	13.7	14.0



## Results and discussions

### Chemical composition of the diets

The yeast waste used in this study contained 26.4% CP with low of fiber contents, thus it would be beneficial protein source for ruminant animals. Yeast waste or yeast cream was generated from bioethanol production and contain about 60-70% of yeast cells, thus inclusion of yeast waste in concentrate diet may provide more protein source and essential amino acids to animal (Limtong, 2006). Soybean meal was included 10 kg DM for control group while replacing by yeast waste at 10 kg DM for the highest level and urea was used as N source to balance isonitrogenous content. The concentrates contained crude protein about 13.6-14% which was fed to meet protein requirements for beef cattle.

### Feed intake

Feed intake is regulated and limited by the physical and metabolic requirements of animals. Physical fill is thought to be mainly determined by the rate of digestion of feed, the passage rate and the fill effect of the diet. Effects of yeast waste replace soybean meal on feed intake are in Table 2. Increasing levels of yeast waste in concentrate diets from 0 to 100% replaced soybean meal were not altered rice straw intake and total intake ( $P>0.05$ ). Rice straw intake was ranges from 53.8 to 56.0 g/kg BW<sup>0.75</sup>. Total intakes were 2.7 to 2.8 kg/d and 71.1 to 72.9 g/kg BW<sup>0.75</sup> which was in the normal range for requirement of beef cattle. This result indicated that yeast waste could be substitute soybean meal and no adversary affect feed intake of cattle. Similar to Cherdthong et al. (2014) who revealed that replacement of residue from slaughterhouse for soybean meal in concentrate diets of Thai cattle were not altered on total DM intake and ranged from 2.8 to 3.0 kg/day.

**Table 2.** Effect of yeast waste on feed intake in Thai native beef cattle.

Items	Level of yeast waste (% DM)				SEM	Pr > F
	0%	33%	66%	100%		
DM intake						
Concentrate						
kg/day	0.6	0.6	0.6	0.6	0.01	0.88
g/kg BW <sup>0.75</sup>	16.7	16.6	16.7	16.8	0.11	0.91
Rice straw						
kg/day	2.1	2.1	2.1	2.1	0.04	0.43
g/kg BW <sup>0.75</sup>	54.3	54.6	56.0	53.8	1.22	0.63
Total intake						
kg/day	2.7	2.7	2.8	2.7	0.04	0.74
g/kg BW <sup>0.75</sup>	71.2	71.3	72.9	71.1	1.23	0.69

### Rumen ecology and microorganisms

Table 3 present the data for rumen ecology and microorganisms in Thai native beef cattle fed different levels of yeast waste in concentrate diets. Ruminal pH and temperature in cattle fed various levels of yeast waste were not significantly different among treatments ( $P>0.05$ ). Ruminal pH at 0, 4 h post feeding and mean values were ranges from 6.6 to 6.8, 6.5 to 6.6 and 6.6 to 6.7, respectively. In addition, ruminal temperatures at 0, 4 h post feeding and mean values were ranges from 38.4 to 38.7, 38.4 to 38.9 and 38.5 to 38.7°C, respectively. Similarly, Polyorach and Wanapat (2015) who supplemented yeast in diets resulted in normal levels of ruminal pH and temperature ranges of 6.5-7.0 and 38.0-39.0 °C, which is considered normal and suitable for microbial activity in rumen. Rumen microbes can be assigned to different functional groups, such as bacteria, protozoa and fungi, etc., which degrade the wide variety of feed components or further metabolize some of the products formed by other



microbes. Replacement soybean meal by yeast waste did not changed bacteria and protozoal population ( $P>0.05$ ). At 0, 4 h post feeding and mean values, bacterial population were ranges at  $4.0$  to  $4.5 \times 10^{11}$  cell/ml,  $3.9$  to  $4.1 \times 10^{11}$  cell/ml and  $4.0$  to  $4.3 \times 10^{11}$  cell/ml, respectively. Protozoal population were  $3.1$  to  $3.8 \times 10^6$  cell/ml,  $2.3$  to  $4.1 \times 10^6$  cell/ml and  $3.0$  to  $3.6 \times 10^6$  cell/ml, respectively. These results revealed that yeast waste had no contained antibacterial substance and did not have any negative effect on concentration of bacterial and protozoa in the rumen of beef cattle.

## Conclusion

The replacement of soybean meal by yeast waste in concentrate mixture has emerged as a feasible alternative for Thai native beef cattle farmers. The inclusion of yeast waste as feed ingredient in cattle diets up to 100% replacing soybean meal is recommended since it reduced cost of feed, control environmental pollution and hazards that accrue from inadequate waste disposal.

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**Table 3.** Rumen ecology and microorganisms in Thai native beef cattle fed different levels of yeast waste

Items	Level of yeast waste (% DM)				SEM	Pr > F
	0%	33%	66%	100%		
Rumen ecology						
Ruminal pH						
0 h post feeding	6.6	6.8	6.8	6.6	0.07	0.07
4 h post feeding	6.5	6.6	6.7	6.6	0.07	0.34
Mean	6.6	6.7	6.7	6.6	0.06	0.07
Ruminal temperature, °C						
0 h post feeding	38.4	38.5	38.7	38.4	0.17	0.62
4 h post feeding	38.4	38.9	38.8	38.7	0.12	0.49
Mean	38.5	38.7	38.5	38.6	0.09	0.24
Ruminal microbes, cell/ml						
Bacteria, $\times 10^{11}$						
0 h post feeding	4.3	4.5	4.3	4.0	0.53	0.92
4 h post feeding	4.1	4.0	3.9	4.0	0.59	0.99
Mean	4.2	4.3	4.1	4.0	0.33	0.94
Protozoa, $\times 10^6$						
0 h post feeding	3.5	3.1	3.8	3.8	0.28	0.40
4 h post feeding	2.9	4.1	3.4	2.3	0.64	0.30
Mean	3.2	3.5	3.6	3.0	0.33	0.59



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## Chemical Composition and *In Vitro* Gas Production of the Local Thai and India Moringa (*Moringa oleifera* Lam.) for Ruminant

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### Abstract

This preliminary study was designed to determine the chemical composition, organic matter digestibility (OMD) and metabolisable energy (ME) of the local Thai and Indian Moringa (*Moringa oleifera* Lam.). From the study, crude protein (CP), ether extract (EE), crude fiber (CF), neutral detergent fiber (NDF), acid detergent fiber (ADF), and acid detergent lignin of local Thai Moringa were 15.34, 4.90, 23.38, 45.15, 27.36, and 5.82% DM, while the Indian Moringa foliage contained 16.47% CP, 3.97% EE, 24.50% NDF, 48.56% NDF, 29.39% ADF, and 5.36% ADL, respectively. The Indian Moringa had a higher potential of *in vitro* gas production than the local Moringa (70.44 vs. 65.28 ml), although the rate of gas production of both subspecies was the same (0.05 vs. 0.05% hr<sup>-1</sup>). In addition, the Indian Moringa had higher *in vitro* organic matter digestibility (IVOMD) and estimated ME than the local Moringa (73.68 vs. 70.33 %DM of IVOMD and 9.80 vs. 9.35 MJ ME/kgDM). In conclusion, both local Thai and Indian Moringa foliage could be supplemented to the ruminants. This was due to the high CP, ME, and IVOMD in the leaves of both subspecies.

**Keywords:** local Thai and Indian Moringa foliage, chemical composition, *in vitro* gas production

### Introduction

Moringa (*Moringa oleifera* Lam.) or Horse Radish or Ma Rum a common Thai name, is a well-known non-legume tree that usually found in the tropical Asia and Africa. Moringa leaf and pod were used for food and local medicinal treatment, while the seed was extracted for medicinal oil. Report from Makkar and Becker (1997) and Gidamis et al. (2003) pointed that Moringa foliage contained high crude protein, essential vitamins and minerals. Thus, Moringa is only useful for human consumption, but it is valuable to use as a high protein supplement to the ruminants when the roughage quality situation is poor (Moyo et al., 2011; Nouala et al., 2006). An example works from Soliva et al. (2005) using Moringa leaves and extract Moringa leaves on ruminal turnover and other fermentation traits, could be concluded that the Moringa leaves from both experimental treatments had high potential to use as an alternative to soybean meal and rapeseed meal as protein sources. In milking goat, Dolrudee (2013) found about 66.64% of raw milk increased in goats received 200 g of Moringa supplement than those received only roughage diet. In addition, the milk protein had significantly higher in milking goats fed with



200 g of Moringa supplementation ( $P < 0.01$ ). Furthermore, Moringa could be replaced Napier grass to goats up to 75% (Sultanat et al., 2015). This was in agreement with Babeker and Bdalbag (2015) who reported that Moringo leaf meal could be fed to the small ruminants without any adverse effect on the productive performance and blood indices at the 20% diet inclusion level.

In Thailand, local Moringa is commonly used leaf and pod for food and the leaf is also used for the medical treatment, and the Indian Moringa was mainly extracted the seed for the medicinal oil. Since the Moringa is commonly applied in Thai life, thus the leaf is also an opportunity to use as an alternative protein source for livestock, particularly for the ruminants (Aderinboye et al., 2016; Babeker and Bdalbag, 2015; Sultana et al., 2015). Nevertheless, less information about the use of Moringa foliage for ruminant feed had done in Thailand. Therefore, the aim of this preliminary study was to provide a basic information about the chemical composition, organic matter digestibility and metabolisable energy of the local Thai and India Moringa for ruminant feed formulation. Therefore, results gain from this work could benefit to use in feed formulation and further study.

## **Materials and methods**

### **Sample collection**

Fresh leaf together with petiole, stem, and soft rachis of India Moringa were harvested from about one year old trees at the Palm Oil Refinding and Bio-diesel Integrated Production Plant Project, Chaipatana Foundation, Petchaburi province in May 2017. At the same time, fresh local Thai Moringa foliage of about one year old tree was harvested from the Small Ruminant Research and Development Center, Faculty of Natural Resources, Prince of Songkla University (PSU), Songkhla province. Both freshly harvested foliage were sub-sampled and initially weighted fresh on the field and transferred to the Animal Nutrition Laboratory, Faculty of Natural Resources, PSU, for dry matter determination and chemical analysis, whilst *in vitro* gas production of both dry Moringa samples were determined at Rajamangala University of Technology Isan (RMUTI), Nakornratchasina province.

### **Chemical analysis**

Proximate composition was determined according to AOAC (2000), while fiber analysis such as neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL) were determined according to Van Soest et al. (1991).

### ***In vitro* gas production**

The *in vitro* gas production was determined according the method described by Menke et al. (1979). The rumen fluid used in this study was collected in the morning before feeding (roughage and concentrate at the ratio of 40 to 60) Approximately 200 mg DM of ground Moringa samples with a 1:2 (v/v) mixture of rumen fluid and buffer medium was placed in a grass syringe. Then the mixed samples were included at 39° C in water bath and shaken at regular times. This incubation was performed in triplicates. The volume of gas released was recorded at 2, 4, 6, 8, 12, 24, 48, 72, and 96 hours of incubation period. The *in vitro* degradability data were fitted to the non-linear equation model of Orskov and McDonald (1979), while sample replicated were terminated at 48-incubation period for the *in vitro* organic matter digestibility (IVOMD) determination according to Menke et al. (1979).

### **Gross and metabolisable energies determination**

The gross energy of both subspecies samples was determined using Automatic Leco Adiabatic Bomb Calorimeter model AC 500. The metabolisable energy (ME) was estimated based on an equation described by Menke et al. (1979).





## Statistical Analyses

The data from both subspecies of Moringa were calculated for mean and standard deviation using computer software SPSS version 16.0 (SPSS, 2007).

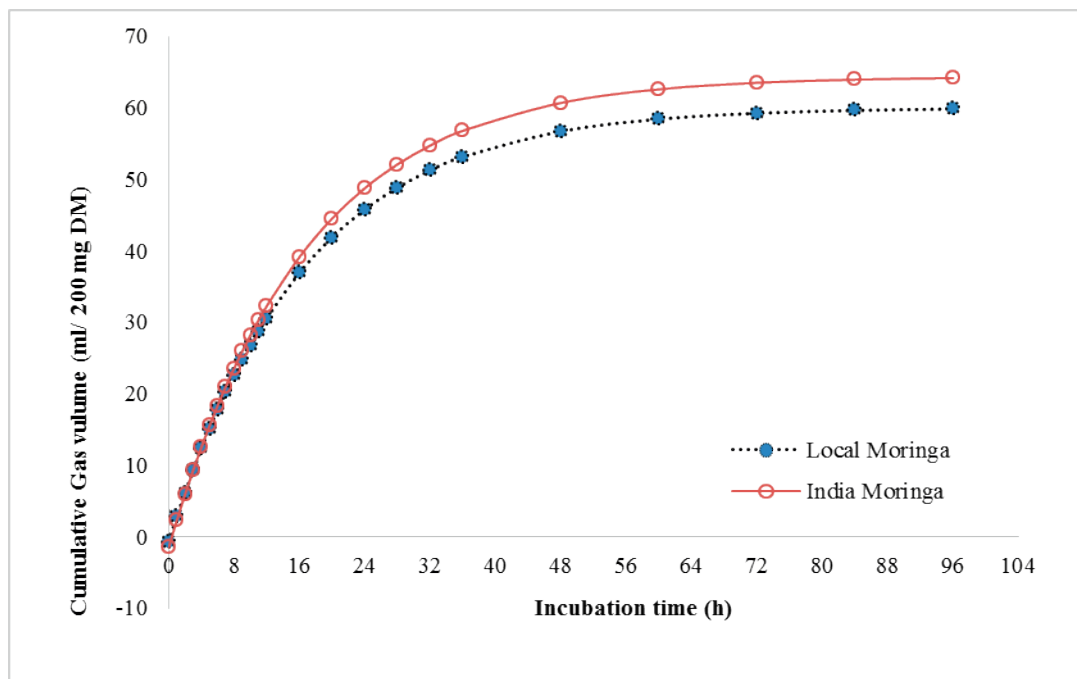
## Results and discussion

### Chemical composition

The chemical composition of local Thai and Indian Moringa foliage is shown in Table 1. From the results, both Moringa subspecies had about 19.99 to 21.20% of DM, 85.79 to 87.37% DM of OM, 15.34 to 16.47% DM of CP, 3.97 to 4.90%DM of EE. Higher NDF (48.65 vs. 45.15% DM) and ADF (29.39 vs. 26.36% DM) contents were indicated from the Indian Moringa than the local Moringa, while lignin content seems to be higher in the local Moringa (5.82 vs. 5.36% DM). Nevertheless, when compare with the results of Moringa foliage from Sultana et al. (2015), it was noticed that our present data of DM, OM, CP, and ash was slightly lower. This was probably related with the harvesting age, cutting interval, and the combination of leaf, petiole, stem, and soft rachis in the foliage samples. This was in an agreement with Humphreys (1991).

When determine the gas production, it was indicated that the cumulative gas production of both subspecies had rapidly increased with the increasing incubation time from 2 to 48 hours before remained constant. The Indian subspecies showed higher gas production from 4 hours until 96 hours of incubation time (Figure 1). From Table 1, the asymptotic gas production (b) of the local and Indian Moringa was 65.22 and 70.44 ml, while the potential gas production was 64.00 ml for the local Moringa and 68.36 ml for the Indian Moringa. In addition, the rate of gas production (c) of the local and Indian Moringa was about the same (0.05 ml/hour). Results from this study could be concluded that the Indian Moringa had higher potential of gas production than the local Moringa, although the rate of gas production of both subspecies was the same. The higher potential of gas production might be related to the higher fermentable carbohydrate fraction that available in the foliage sample. Thus, better IVOMD of the Indian than the local Moringa was indicated (73.68 vs. 70.33 %DM).

From Table 1, GE of the local Moringa was higher than the Indian Moringa, but the estimated ME of the Indian Moringa foliage was higher than the local subspecies. Nevertheless, the ME content of both subspecies (9.35 MJ/gDM for the local and 9.80 MJ/gDM for Indian Moringa) was similar to the report of Sultana et al. (2015) (9.60 MJ/gDM). This ME content was higher than the Napier grass (8.20 MJ/kgDM) (Sultana et al., 2015), oil palm frond silage (4.67 to 5.02 MJ/kgDM) (Mhudmhan et al., 2009), and rice straw (6.11) (Sallam et al., 2007).



**Figure 1** *In vitro* gas production from local Thai and Indian Moringa at vary incubation times

Higher content of IVOMD, ME and CP in both subspecies illustrated the better of utilization of this foliage.

## Conclusions

Both local and Indian Moringa contained high CP, ME, and IVOMD, but had low CF and ADF content. Although the Indian Moringa showed a better gas production potential, IVOMD, and estimated ME than the local Moringa, but both subspecies could be supplemented to the ruminants. This was due to higher crude protein and low fiber content. Nevertheless, more information about the effect of Moringa foliage on the productive performance of Thai ruminants may need to investigate.

## Acknowledgements

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**Table 1.** Chemical composition and gas production kinetics of local Thai and India Moringa foliage.

	Local Thai Moringa	Indian Moringa
Chemical composition		
DM (% as fed basis)	21.20±1.01	19.99±0.89
DM (%)	95.78±0.25	94.25±0.48
OM (%DM)	87.37±0.11	85.79±0.15
CP (%DM)	15.34±0.16	16.47±0.10
EE (%DM)	4.90±0.27	3.97±0.30
Ash (%DM)	8.41±0.20	8.98±0.08
CF (%DM)	23.38±0.24	24.50±0.56
NDF (%DM)	45.15±1.35	48.65±2.58
ADF (%DM)	27.36±0.23	29.39±0.15
ADL (%DM)	5.82±0.35	5.36±0.66
Gas production characteristics		
a (ml) <sup>1</sup>	-1.28±0.75	-2.08±0.76
b (ml) <sup>2</sup>	65.28±3.33	70.44±2.43
c (%/hr) <sup>3</sup>	0.05±0.02	0.05±0.01
a + b (ml) <sup>4</sup>	64.00±2.72	68.36±2.48
Gas production volume (ml/200 mg)		
0 h	-0.72±0.71	-1.50±0.78
2 h	6.81±0.52	5.91±0.87
4 h	12.29±0.37	12.51±1.02
6 h	17.72±0.39	18.36±1.21
8 h	22.52±0.33	23.56±1.38
10 h	26.79±0.43	28.17±1.55
12 h	30.57±0.56	32.23±1.70
24 h	45.77±1.20	48.75±1.47
48 h	56.69±2.00	60.69±3.05
60 h	58.41±2.17	62.58±3.18
72 h	59.23±2.26	63.50±3.26
96 h	59.82±2.35	64.15±3.33
IVOMD (%) <sup>5</sup>	70.33±1.32	73.68±1.80
GE (MJ/kgDM)	17.98±0.25	17.62±0.71
ME (MJ/kgDM) <sup>6</sup>	9.35±0.23	9.80±0.32

<sup>1</sup> a = the gas production from the immediately soluble fraction (ml); <sup>2</sup> b = the gas production from the insoluble fraction (ml); <sup>3</sup> c = the gas production rate constant for the insoluble fraction b (h); <sup>4</sup> a+b = the potential gas production (ml); <sup>5</sup> IVOMD (%) of Moringa calculated by using equation of Menke et al. (1979) as follows: IVOMD (%) = 14.88+0.889GP+0.45CP+0.0651XA where XA = Ash content (%); <sup>6</sup> ME (MJ/kg DM) content of legume hays was calculated using equation of Menke et al. (1979) as follows: (MJ/kg DM) = 1.06+0.157 GP+0.0084 CP+0.0022EE-0.0081XA where GP was 24-h net gas production (ml/200 mg), CP = Crude protein and EE = Ether Extract.



## ***Session 10-Arawan I***

ANN-01-0013

### **Use of Body Measurements to Predict Intermuscular Fat in Thin-Tailed Lambs**

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#### **Abstract**

The objective of this study was to evaluate the using of body measurements to predict intermuscular fat in Thin-tailed lambs. Live body measurements (chest girth, depth of body, body length) and intermuscular fat were performed in twenty-two Thin-tailed lambs with an average age of 7 to 8 months and an average slaughter weight of  $24.28 \pm 2.43$  kg. Intermuscular fat was weighed after carcasses were chilled at  $18^{\circ}\text{C}$  for 10 hours. The study revealed a strong correlation between chest girth and intermuscular fat ( $r = 0.64$ ,  $p < 0.01$ ), while the depth of body and body length showed low correlations with intermuscular fat ( $r = 0.24$  and  $0.20$ ). The results of this study indicated that chest girth had favorable correlations with intermuscular fat. Thus, intermuscular fat prediction using chest girth is a valuable tool to evaluate the carcass quality and quantity in Thin-tailed sheep.

**Keywords:** body measurements, intermuscular fat, prediction, thin-tailed lambs

#### **Introduction**

In recent years, the interest to manipulate the fat composition has been increasing. This is because meat is seen to be a major source of fat in the diet and especially of saturated fatty acids, which has been implicated in diseases associated with modern life, especially in developed country (Wood et al., 2003). For consumers in many countries, fat is an unpopular component of meat because being considered unhealthy. Dietary fat has been hypothesized to increase the risk of colorectal cancer and cardiovascular disease (Webb and O'Neill, 2008). In the other side, fat becomes central to the nutritional value of meat and contributes importantly to the meat quality and price. Fat determines the flavour, smell, juiciness, and tenderness, which have a direct impact on the meat value (Wood et al., 2008), but an excessive increase in fat deposits has low commercial value because it reduces carcass quality, and there may be consumer rejection (Costa et al., 2017).

Among ruminants, it is observed that sheep have a high ability to accumulate internal fat, which acts as an energy reserve for times of food and water scarcity. The body fat in sheep is distributed in the form of visceral, subcutaneous, intermuscular, and intramuscular fat deposits (Costa et al., 2017). Many studies have been trying to evaluate the quality and quantity of these deposits on the live animals using different methods such as modern image analysis techniques, live animal allometric measurements, and neural modeling methods (Slosarz et al., 2001; Font-i-Furnols et al., 2014; Ermias and Rege, 2003; Stelzleni et al., 2003). This evaluation will become



increasingly important to seed stock and commercial beef producers for producing a consistently high-quality meat (Stelzleni et al., 2003).

However, the use of sophisticated evaluation, usually high cost, has become the methods of choice for providing levels of accuracy and precision that are acceptable for most purposes. Nonetheless, cost, operational complexity and lack of widespread availability limit the use of the techniques in developing countries. Thus, there is need to develop techniques which combine cost-effectiveness, acceptable precision, local availability and ease of application (Ermias and Rege, 2003). Nogalski et al. (2017) reported that body measurements reveal a positive correlation with subcutaneous and intramuscular fat depositions, and can be a valuable tool in the process of selecting young beef quality traits and determining the slaughter value of young beef cattle. The prediction of intermuscular fat deposition in lambs is better than intramuscular fat because visceral and subcutaneous fat as a non-edible fat has been deposited, while the intramuscular fat has not been excessively grown. There is limited information about the correlation between body measurements and intermuscular fat deposition in lambs, the objective of this study, therefore to evaluate the using of body measurements to predict intermuscular fat in Thin-tailed lambs, which are the commonest sheep for meat production in the northern dry tropics and western humid areas (Food and Agriculture Organization of the United Nations, 1991).

## Methodology

The experiment was carried out at the Faculty of Animal and Agriculture Sciences, Diponegoro University, Semarang, Indonesia. Twenty-two Thin-tailed lambs were used with an average age of 7 to 8 months and an average slaughter weight of  $24.28 \pm 2.43$  kg. Two lambs were slaughtered every day. Before slaughtered, lambs were fasted for 6 hours and weighed to get the average slaughter weight. Chest girth (CG) was measured behind the scapula by measuring tape. Body length (BL) was measured as the distance between the point of shoulder and the pin bone. The depth of body (DB) was measured as the vertical distance from sternum to withers (Riva et al., 2004). Slaughter was performed according to standard procedures. After slaughtering and dressing, carcasses were chilled at  $18^{\circ}\text{C}$  for 10 hours. Later on, intermuscular fat (ITF) was removed from the carcass and weighed. The data was analyzed by correlating each body measurements (x) with ITF (y). Based on Christmann and Badgett (2009), the formula was equated as  $y = ax + b$ , while coefficient of correlation was interpreted as very weak (0.000-0.200); weak (0.201-0.400); moderate (0.401-0.600); strong (0.601-0.800) and very strong (0.801-1.000).

## Results and Discussions

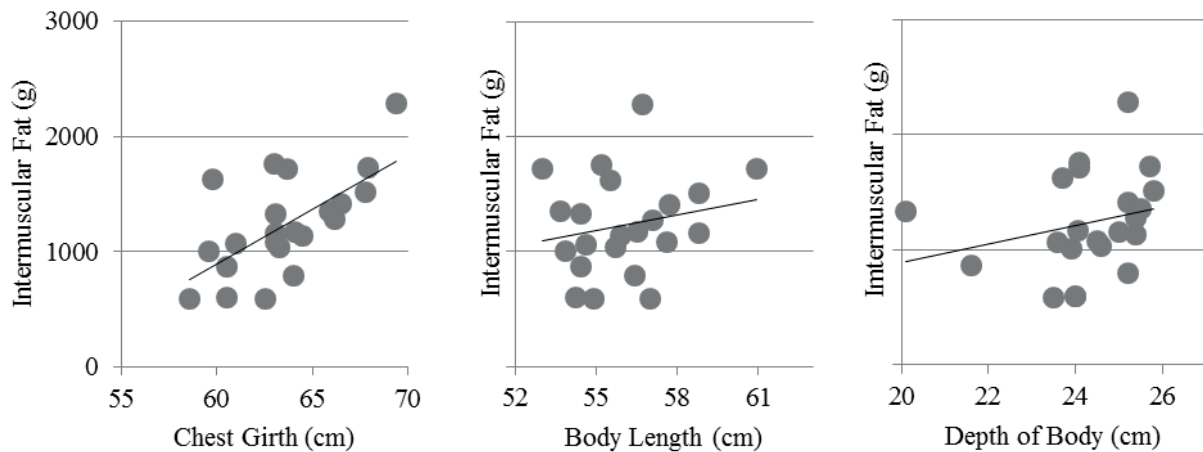
The body measurements and intermuscular fat statistics are presented in Table 1. The intermuscular fat was significantly varied and had the highest coefficient of variants (CV) among the variables, which can be attributed mainly to the growth rate. Body measurements grow earlier, so if the body measurements reach their inflection point, they will grow slower. Fat grows later in life and affected by feed consumed between the examined animals (Owens et al., 1993). The feeding system, whether using concentrate feeding or pasture feeding or both, influence the growth and carcass characteristic of animals. Animals fed concentrate diets tend to have higher fat value compared to range-fed animals (Webb and O'Neill, 2008).



**Table 1.** Body measurements and intermuscular fat of twenty-two Thin-tailed lambs

Variables	Mean	Range	SD	CV (%)
CG (cm)	63.76	58.60-69.40	3.01	4.73
BL (cm)	56.11	53.00-60.95	1.94	3.46
DB (cm)	24.33	20.10-25.80	1.34	5.49
ITF (g)	1,336	589.9-3,669	658.3	49.27

CG = chest girth; BL = body length; DB = depth of body; ITF = intermuscular fat



**Figure 1.** Correlation between body measurements and intermuscular fat

Figure 1 shows the correlation between body measurements and ITF, while the data in Table 2 represents the coefficient of correlation ( $r$ ), the coefficient of determination ( $R^2$ ), and equation ( $y$ ) from regression analyses involving body measurements and ITF in Thin-tailed lambs. It was observed that CG had strong positive correlations with ITF ( $r = 0.64$ ,  $p < 0.01$ ). BL and BD were poorly related (0.20 and 0.24) to ITF. CG contributed 41%, while each BL and DB only represented 4% and 6% of the variation in ITF deposit of Thin-tailed lambs. Nogalski (2017) also reported that thickness of subcutaneous back fat and thickness of subcutaneous rump fat were best estimated by CG rather than by other body measurements.

**Table 2.** Coefficient of correlation ( $r$ ), coefficient of determination ( $R^2$ ), and equation ( $y$ ) from regression analyses involving body measurements and intermuscular fat in Thin-tailed lambs

Independent variables (x)	Y	r	$R^2$	Level of significance
CG	$y = 94.79x - 4793$	0.64	0.41	*
BL	$y = 45.83x - 1338$	0.20	0.04	ns
DB	$y = 79.70x - 705.0$	0.24	0.06	ns

CG = chest girth; BL = body length; DB = depth of body; \* =  $p < 0.01$ ; ns = Not-significant

CG became the independent variable with the highest coefficient of correlation because it represents ITF deposit that covers the entire chest of the animals. The size of CG will vary based on the fat deposit of the body. The greater the number of ITF deposit, the larger the size of CG. BL had the lowest influence on ITF because it reflects the body frame, not the fat deposit of the animals. This agrees with that obtained by Agamy et al. (2015) who reported that bone weight of Ossimi ram-lambs showed significant correlation with BL. The increased BL is due to skeletal growth, while increases in girth are due to muscle development plus the accumulation of adipose



tissue (Assan, 2013). Since DB can determine either increment or impairment of the intermuscular thickness in the chest of the lambs, it had the higher coefficient of correlation rather than BL.

## Conclusion

The results of this study indicated that chest girth had favorable correlations with intermuscular fat. Thus, intermuscular fat prediction using chest girth is a valuable tool to evaluate the carcass quality and quantity.

## Acknowledgements

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## Effect of Inclusion of Enzyme from Fermented Tomato Pomace with *A. Niger* on Feed intake and Growth Performance of Beef Cattle

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### Abstract

Twelve crossbred Brahman x Native bulls with initial BW of  $200 \pm 16$  kg, were used in a completely randomized design. Animals were received either total mixed ration (TMR) with or without fibrolytic enzyme from fermented tomato pomace with *Aspergillus niger*. The experiment was lasted for 90 days. Feed intake was similar among treatments. Cattle fed TMR with enzyme supplementation had greater ( $P < 0.05$ ) in average daily gain when compared with cattle fed TMR solely. Digestibilities of dry matter, organic matter, neutral detergent fiber and acid detergent fiber were higher ( $P < 0.05$ ) in cattle fed TMR with enzyme supplementation when compared with cattle fed TMR solely, while crude protein digestibility was not significantly difference between treatments. Based on the experimental data, it could be concluded that enzyme from fermented tomato pomace with *Aspergillus niger* can improve average daily gain and fiber digestion in beef cattle fed total mixed ration containing rice straw as main roughage source.

**Keywords:** enzyme, *Aspergillus niger*, average daily gain, feed intake, digestibility

### Introduction

Agricultural and industrial by-products are widely available for use as animal feeds such as rice straw, pineapple wastes, sugarcane tops and corn stover. However, their nutritive values are less nutritious and high fiber portion, which influences both intake and digestibility (Wanapat et al., 1999). In non-ruminant animals, exogenous enzymes, such as amylase, glucanase and xylanase, are widely used to improve nutrient digestion and performances. It is well known that fibrolytic enzyme supplementation has been shown to improve fiber digestion and enhanced productive performance of ruminants. Beauchemin et al. (2003) reported that fibrolytic enzyme supplementation improve fiber digestibility of forage diet. Similar resulted with Colombatto et al. (2003) demonstrated that enzyme supplementation increased neutral detergent fiber digestibility. In addition, inclusion of fibrolytic enzyme in fermented total mixed ration (FTMR) had not improved fiber digestion in dairy cows, but fibrolytic enzyme supplementation in total mixed ration (TMR) did increase fiber digestion (Khanh et al., 2012). Yuangklang et al. (2017) has shown that supplementation of fibrolytic enzyme to rice straw based diets, enhanced fiber digestibility in meat goats. The objective of present experiment was aimed to investigate the effect of supplemental enzyme from fermented tomato pomace with *Aspergillus niger* on feed intake and growth performance of beef cattle.



## Materials and methods

Twelve crossbred Brahman x Native bulls with initial BW of  $200 \pm 16$  kg, were used in a completely randomized design. Animals were received either total mixed ration (TMR) with or without fibrolytic enzyme from fermented tomato pomace with *Aspergillus niger*. The enzyme from fermented tomato pomace was prepared by solid-state fermentation. Briefly, tomato pomace was adjusted to 50% moisture content and then thoroughly mixed with *A. niger*. The mixtures were then incubated at 30 C for 3 days and after that the mixtures were dried at 50 C for 5 days and final product was crushed and kept in sealed plastic bags before use. The enzymes were consisted of amylase, protease, cellulose and xylanase at 55,658, 3,706, 46,144 and 34,299 unit/gDM. Total aflatoxin was 1.42 ug/kg. The experiment was lasted for 90 days. Feed intake was similar among treatments. The ingredients of total mixed ration were demonstrated in Table 1. Enzyme was added at 100 mg per kg DM. Animals were housed in individual pens. On day 85 to 90, total feces samples were quantitatively collected and weighed. Feces samples were analyzed for dry matter, ash, crude protein (AOAC, 1990) and neutral detergent fiber (NDF) and acid detergent fiber (ADF) (Van Soest et al., 1991).

## Statistical analysis

All data were subjected to one way ANOVA and Turkey's t test (SPSS, 1997) was used to separate means between control and test group. Differences between treatments were considered statistically significant when  $P < 0.05$ .

## Results

### Growth performance and feed intake

The effect of enzyme supplementation on performance and feed intake are presented in Table 2. Cattle offered TMR with enzyme supplementation had greater final BW and ADG ( $P < 0.05$ ). However, there was no difference between treatments in TMR intake expressed as gDM/d and %BW.

### Digestibility of nutrients

The effect of enzyme supplementation in TMR had greater in nutrient digestibility except for crude protein digestion. Dry matter digestibility was 60.40 and 65.14 % in TMR and TMR with enzyme supplementation, respectively ( $P < 0.05$ ). Organic matter digestibility was 62.41 and 66.77% in TMR and TMR with enzyme supplementation, respectively ( $P < 0.05$ ). Neutral detergent fiber and acid detergent fiber digestibilities were 58.94 and 61.63% and 53.47 and 58.11% in TMR and TMR with enzyme supplementation, respectively ( $P < 0.05$ ). Crude protein was 75.66 and 76.09% in TMR and TMR with enzyme supplementation, respectively ( $P > 0.05$ ).

## Discussion

### Body weight, average daily gain and feed intake

It has been repeatedly reported that enzyme supplementation improved growth rate (Beauchemin et al., 1995). In this study, average daily gain improved when enzyme supplementation. It can be explained that supplemental enzyme in the diet stimulated nutrient digestibility, leading to more nutrient for animal growth. In accordance with Beauchemin et al. (1995) found that added enzyme in timothy hay improved ADG while inclusion of enzyme in barley silage did not show any effect on ADG. TMR intake was not different between treatments. It is well known that intake is restricted by rumen capacity (Van Soest, 1994). So that in this study, TMR was used as diet form and it is well agreed that total mixed ration can enhance feed intake when compare with separate feeding due to the stability of rumen fermentation process.



### Digestibility of nutrients

It is well known that fibrolytic enzyme supplementation has been shown to improve fiber digestion. In this study, all nutrients except protein digestibility were enhanced by inclusion of enzyme. Beauchemin et al. (2003) reported that fibrolytic enzyme supplementation improve fiber digestibility of forage diet. Similar resulted with Colombatto et al. (2003) demonstrated that enzyme supplementation increased neutral detergent fiber digestibility. In addition, inclusion of fibrolytic enzyme in fermented total mixed ration (FTMR) had not improved fiber digestion in dairy cows, but fibrolytic enzyme supplementation in total mixed ration (TMR) did increase fiber digestion (Khanh et al., 2012). Yuangklang et al. (2017) has shown that supplementation of fibrolytic enzyme to rice straw based diets, enhanced fiber digestibility in meat goats.

### Conclusion

Based on experimental data, it can be concluded that supplementation of enzyme from fermented tomato pomace can be improved nutrient digestion and increased growth rate of beef cattle fed total mixed ration containing rice straw as main roughage.

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**Table 1.** Ingredients and analyzed compositions

Ingredient compositions	g/kg
Rice straw	200
Cassava chip	350
Dried tomato pomace	200
Soybean meal	150
Molasses	50
Tallow	20
Urea	20
Dicalcium phosphate	5
Sodium chloride	5
Analyzed composition	g/kg
Dry matter	880
Ash	105
Crude protein	156
Ether extract	351
Neutral detergent fiber	412
Acid detergent fiber	234

**Table 2.** Body weight, average daily gain and feed intake

Items	Experimental treatments		SEM	P-value
	TMR	TMR + Fibrolytic enzyme		
Initial BW, kg	220	216	2.08	0.07
Final BW, kg	250	252	0.72	0.04
ADG, kg/d	0.80	0.88	0.10	0.04
TMR intake, gDM/d	6.96	7.04	0.04	0.19
%BW	2.93	2.94	0.02	0.20

FPE = enzyme from fermented tomato pomace with *Aspergillus niger*; SEM = standard error of the means

**Table 3.** Digestibility of nutrients

Items	Experimental treatments		SEM	P-value
	TMR	TMR + Fibrolytic enzyme		
Dry matter	60.40	65.14	0.82	<0.001
Organic matter	62.41	66.77	0.73	<0.001
Crude protein	75.66	76.09	0.25	0.21
NDF	58.94	61.63	0.49	<0.001
ADF	53.47	58.11	0.73	<0.001

NDF = neutral detergent fiber; ADF = acid detergent fiber; SEM = standard error of the means



ANN-01-0100

## **Influence of Tropical Roughages Combined with Urea and Bamboo Grass (*Tiliacora Triandra*, Diels) Supplementation on Gas Production and *In Vitro* Degradability**

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### **Abstract**

The objective of the present study was to evaluate the Influence of the tropical roughages combined with urea and Bamboo grass (*Tiliacora triandra*, Diels) supplementation on gas production and in vitro degradability using in vitro gas production technique. Two male, rumen fistulated dairy steers were used as rumen fluid donors. The treatments were in a 4 × 2 × 2 factorial arrangement in a Completely randomized design (CRD) with four roughage sources namely rice straw (RS), Ruzi grass (*Brachiaria ruziziensis*; RZ), Signal grass (*Brachiaria brizantha*; SN) and Sweet grass (*Pennisetum purpureum* cv. Mahasarakham; SG) with two levels of Bamboo grass (*Tiliacora triandra* Diels; BG) supplementation at 0% and 4% and with the two levels of urea supplementation at 0% and 2%. Under this investigation, the results revealed that SG has the highest nutritive values (15.2 % CP), and lowest fiber content (34.9 % ADF and 59.4 %NDF). Cumulative gas production was highest (P<0.001) in the diets with supplementation at 2% of urea. For the roughage, it was highest (P<0.001) in diets containing SG. The IVDMD at 12 and 24h were significantly different among roughage sources (P<0.001) between BG supplementation (P<0.05) and urea level (P<0.05). Based on this study it could be concluded that the diets with greater nutritive values resulted in the highest gas production and nutrient degradability, especially, the use of the diet contained Sweet grass combined with urea and Bamboo grass supplementation. However, further study should be conducted using *in vivo* feeding trials.

**Keywords:** roughage, degradability, plant secondary compound, *Tiliacora triandra*, diels

### **Introduction**

In tropical areas, especially during the dry season, the productivity of cattle is reduced due to the low quantity and quality of the forage crops. However, some tropical grasses, such as Ruzi grass (*Brachiaria ruziziensis*; RZ), Signal grass (*Brachiaria brizantha*; SN) and Sweet grass (*Pennisetum purpureum* cv. Mahasarakham; SG) are popular tropical grass as for animal production and can produce large yields per area and can be easily grown in tropical terrain (Pholsen et al., 2014; Mapato and Wanapat, 2016). In addition, dietary protein plays an important role in the nutrition of ruminants, since it is also a source of nitrogen for the synthesis of microbial protein and rumen by-pass protein (Nocek and Russell, 1988). Urea is a non-protein N (NPN) which is used as an alternative protein source in ruminant diets, because of its low cost compared with other protein feeds, such as soy bean meal, with high rumen degradability (Wanapat, 2009; Cherdthong et al., 2011).



Currently, natural products are interesting to use as a feed additives in response to improvements in livestock nutrition and livestock production (Wanapat, 2000). Dietary supplements have the potential to improve nutrient utilization by altering the population of microorganisms, fermentation and digestion in the rumen (McSweeney et al., 2001). Therefore the use of supplementation of these plant secondary compounds metabolites have high potential for improving rumen ecology and subsequently productivity of ruminants (Wanapat et al., 2012). Bamboo grass (*Tiliacora triandra* Diels; BG) is ivy plant that can be found in the forests of Thailand. In the leaves of Bamboo grass has antioxidants, including beta - carotene xanthophyll, vitamin C, vitamin E (Sriket, 2014). Moreover Wanapat et al, (2012) has reported that the leaves of the Bamboo grass is also a compound of condensed tannins (2.3% DM). However, knowledge of the intrinsic values of this Bamboo grass and how they affect the rumen digestion and fermentation efficiency are still needed to be determined. Therefore, the aim of this experiment was to investigate the effects of tropical grasses combined with bamboo grass and urea levels on rumen degradability, gas production kinetics, and rumen fermentation using the *in vitro* gas production technique.

## Materials and methods

The four roughages sources were rice straw (RS), Ruzi grass (*Brachiaria ruziziensis*; RZ), signal grass (*Brachiaria brizantha*; SN) and sweet grass (*Pennisetum purpureum* cv. *Mahasarakham*; SG). There were collected all of grass at 45±3 days of regrowth. All samples were collected to analyze for dry matter (DM), crude protein (CP) and ash by using the technique of AOAC, (2012). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed by the technique of Van Soest et al. (1991). A subsample of each roughage was mixed with cassava chip powder combined with 0% and 2% level of urea and 0% and 4% level of Bamboo grass for use as substrate. The feed ingredients of the concentrate are shown in Table 1. The experimental design was 4 × 2 × 2 Factorial arrangement in a Completely randomized design (CRD). Determination of gas production using the technique of Menke and Steingass (1988) with some modification. Cumulative gas production data were fitted to the model of Orskov and McDonald (1979). At 12 and 24 h post incubation, a set of samples were tested for *in vitro* degradability using the technique of Tilley and Terry (1963). All obtained data were subjected to Statistical Analysis System Institute (SAS, 2004) according to a 4 × 2 × 2 factorial arrangement in a completely randomized design. The statistical model included Roughage sources, Urea level, Bamboo grass level, Roughage sources × Bamboo grass level, Urea level × Bamboo grass and Roughage sources × Urea level × Bamboo grass level interactions. For all parameters, differences among treatments means were contrasted by Duncan's new multiple range test (Steel and Terrie, 1980).

## Results and discussion

### Chemical composition of experimental diets

The chemical compositions of rice straw (RS), Ruzi grass (RZ), Signal grass(SN), Sweet grass (SG), Bamboo grass (BG) in this study are presented in Table 1. The protein content was highest in BG (16.2 % DM) which was similar to Wanapat et al. (2012) who reported at 16.4 % DM and the lowest in RS (2.1% DM,). ADF and NDF contents were highest in RS (55.1 and 75.1 % DM) and lowest in SG (34.9 and 59.4 % DM). The RZ and SN were similar in CP content (8.9 and 8.3 % DM). Ash content was highest in RZ and lowest in BG. However, condensed tannins that contained in BG was 3.1% DM which higher than Wanapat et al. (2012) (2.3 % DM).

### Effects on gas production kinetics and *in vitro* degradability





Gas production from the immediately soluble fraction (a) was significantly affected by roughage source, it was highest ( $P < 0.001$ ) in diets containing RZ, while gas production from the insoluble fraction (b) was also significantly affected by roughage source and it was highest ( $P < 0.001$ ) in diets containing SG and BG supplementation ( $P < 0.05$ ) and urea level ( $P < 0.01$ ). The gas production rate constant for the insoluble fraction was significantly affected by urea level ( $P < 0.01$ ). The gas potential extent of gas production (a+b) was significantly affected by roughage sources, it was highest ( $P < 0.001$ ) in diets containing SG and BG grass supplementation ( $P < 0.05$ ) and urea levels ( $P < 0.001$ ). Gas production level was highest in the diets with supplementation at 2% of urea ( $P < 0.001$ ), as it is also a good source of nitrogen used for the synthesis of microbial protein (Nocek and Russell, 1988) therefore, the effect on gas and degradability was increased. For the roughage, it was highest in diets containing SG ( $P < 0.001$ ), which could be due to SG is grass which has potentially high nutritive value (15.2 %DM of CP) more than other roughage sources, it can be a good feed source for microorganisms to grow and produce gas (Mapato and Wanapat, 2016). The IVDMD at 12 and 24h were significantly different between the roughage sources ( $P < 0.001$ ), 4% of BG supplementation ( $P < 0.05$ ) and 2% of urea level ( $P < 0.05$ ). Using high quality roughages allow rumen microbes to increase the digestion of roughage, providing more nutrients to the host animals (Wanapat et al., 2006), while BG supplementation was significantly increased (Table 2.;  $P < 0.05$ ). These could be due to BG is one of tropical plants or feed resources containing plant secondary compounds such as condensed tannins and/or saponins effect on rumen ecology, fermentation, and productivity. Their direct addition or supplementation as a part of concentrate mixtures could improve rumen fermentation efficiency (Wanapat et al., 2012). However, further research in in vivo feeding trials should be conducted.

## Conclusion

Based on this study, it could be concluded that the use of tropical roughages which has potentially high nutritive value, such as Sweet grass combined with urea and Bamboo grass supplementation resulted in the highest gas production and nutrient degradability. It may be one of the approaches to increase rumen fermentation and to reduce the need for concentrate supplementation for ruminant feeding. However, further research in in vivo feeding trials should be conducted.

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**Table 1.** Chemical compositions of concentrate and roughages

Items	DM %	CP	ADF	NDF	Ash	OM	CT
	-----% of DM-----						
Rice straw	90.8	2.1	55.1	75.1	13	87	-
Ruzi grass ( <i>Brachiaria ruziziensis</i> )	25.3	8.9	36.2	67.8	14.1	85.9	-
Signal grass ( <i>Brachiaria brizantha</i> )	27.5	8.3	30.7	62.9	10.4	89.6	-
Sweet grass ( <i>Pennisetum purpureum</i> cv. Mahasarakham)	13.4	15.2	34.9	59.4	10	90	-
Bamboo grass ( <i>Tiliacora triandra</i> Diels)	20.4	16.2	40.3	61.9	5.5	94.5	3.1
Cassava chip	89.4	3.4	7.7	6.1	4.2	95.8	-
Urea 46% N	99	288	-	-	-	-	-

DM, Dry matter; CP, Crude protein; ADF, Acid detergent fiber; NDF, Neutral detergent fiber; OM, Organic matter; CT, Condensed Tannins

**Table 2.** Gas production kinetics and *in vitro* degradability as influenced by dietary treatments

Roughages	BG	Urea	Gas production kinetics				Gas <sup>d</sup>	IVDMD (%)	
			a	b	c	a+b		12 h	24 h
Rice straw	0 %	0%	0.89	23.2	0.09	24.1	25.6	22.4	39.9
	0%	2%	1.40	30.1	0.07	31.5	31.8	31.9	42.4
	4%	0%	0.19	28.6	0.07	28.8	28.3	27.2	40.6
	4%	2%	1.76	31.2	0.02	32.9	30.2	29.0	43.5
Ruzi grass ( <i>Brachiaria ruziziensis</i> )	0 %	0%	2.26	25.0	0.07	27.3	29.0	36.1	57.6
	0%	2%	1.03	28.9	0.08	29.9	31.7	45.4	60.9
	4%	0%	1.52	28.6	0.07	30.1	30.6	43.8	58.7
	4%	2%	-0.001	30.6	0.10	30.5	31.5	44.7	61.9
Signal grass ( <i>Brachiaria decumbens</i> )	0 %	0%	-0.05	27.6	0.06	27.5	28.0	38.2	59.7
	0%	2%	-1.31	39.1	0.08	37.8	39.0	39.8	60.4
	4%	0%	-0.47	36.7	0.09	36.2	32.0	38.7	60.8
	4%	2%	-0.64	37.7	0.07	37.0	37.2	41.3	62.5
Sweet grass ( <i>Pennisetum purpureum</i> <i>Mahasarakham</i> )	0 %	0%	-0.40	35.2	0.10	34.8	35.5	30.0	59.3
	0%	2%	0.28	43.8	0.06	44.1	44.1	39.6	62.7
	cv. 4%	0%	0.59	38.4	0.08	39.0	41.6	37.7	63.1
	4%	2%	0.807	40.3	0.06	41.2	44.9	40.9	61.7
SEM			0.4	1.57	0.0008	1.64	1.76	1.86	1.23
Comparison									
Roughages			***	***	ns	***	***	***	***
BG			ns	*	ns	*	ns	*	*
Urea			ns	***	**	***	***	*	*
Interactions									
Roughages* BG			ns	ns	*	ns	ns	ns	ns
Roughages*Urea			**	ns	***	ns	*	ns	ns
BG*Urea			ns	**	ns	*	*	*	ns
Roughages*BG*Urea			ns	ns	ns	ns	ns	ns	ns

a, the gas production from the immediately soluble fraction; b, the gas production from the insoluble fraction; c, the gas production rate constant for the insoluble fraction ratio; a+b, the gas potential extent of gas production; d, Cumulative gas production at 96 h (mL/0.2 g DM substrate); SEM, Standard error of the means; \*, P<0.05; \*\*, P<0.01; \*\*\*, P<0.001; ns, nonsignificant (P>0.05); IVDMD, In vitro dry matter degradability and BG, bamboo grass



## Effect of Bamboo Grass (*Tiliacora Triandra*) Pellet Supplementation on Feed Intake, Nutrient Digestibility and Rumen Microbial Population in Thai Native Beef Cattle

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### Abstract

The objective of this study was to investigate the effect of Bamboo grass pellet supplementation on feed intake, nutrient digestibility and rumen microbial population in Thai native beef cattle. Four Thai native beef cattle ( $190 \pm 2\text{kg}$ ) were randomly and assigned to receive four dietary treatments in a  $4 \times 4$  Latin square design. Four treatments were different levels of Bamboo-Cass supplementation at 0, 50, 100, and 150 g/hd/d, respectively. Rice straw was fed *ad libitum* and the concentrate was offered at 0.5% of body weight. Results indicated that nutrient intake, nutrient digestibility, ruminal pH and protozoa population were not significantly different among treatments by supplementation of Bamboo-Cass ( $P > 0.05$ ). However, rice straw intake, total intake, and microbial population were significantly different among treatments ( $P < 0.05$ ). Supplementation of Bamboo-Cass at 150 g/hd/d could enhance feed intake, maintaining rumen pH and microbial population and also tended to increase nutrient intake, nutrient digestibility, and reducing protozoa population. Therefore, using Bamboo-Cass as a supplement could improve feed intake and digestibility at 100 and 150 g/hd/d in Thai native cattle fed rice straw based diet.

**Keywords:** *Tiliacora Triandra*, bamboo-cass, nutrient digestibility, microbial population

### Introduction

Feed sources for ruminant diets in tropical countries are mainly crop residues, rice straw and agro-industrial by-products. It is important to improve the fermentative digestion of rumen microorganisms to provide the utmost fermentative end-product for animal host for growth and production (Wanapat, 2000). Agricultural by-product for ruminant's feed is commonly not enough requirements due to low nutritive value and high lignin. Otherwise, current researches have demonstrated that the use of natural products as feed additives would response to animal nutrition and livestock production problems (Wanapat et al., 2000). These products in tropical regions contain of high to medium secondary plant compounds which have positive effect on protein metabolism and reducing protozoa population in the rumen (Wang et al., 2000). It is also shown a better way to increase animal productivities as well as improve rumen ecology, dry matter intake, milk and meat quantity and quality (Wanapat, 2009; Devendra and Leng, 2011).

Naturally, Bamboo grass (*Tiliacora Triandra*) is a flowering and climbing plant with green leaves and yellow flower in a forest belong to *Menispermaceae* family. It has been known as a medical local resource plant to mainland Southeast Asia in Northeast Thailand and Loa PDR for medicine, cosmetics perfumes, and household products (Rattana et al. 2010; Singthong et al.



2014). According to Sriket (2013) revealed that Bamboo grass is rich in Phenolic compounds that would be useful to utilize as a source of bioactive compounds. Moreover, Kanpukdee and Wanapat (2008) indicated that Bamboo grass contained the variable of condensed tannins and saponins that would be a potential feed source for ruminant production. Therefore, the objective of this study was to determine the effect of Bamboo grass pellet (Bamboo-Cass) on feed intake, nutrient digestibility and rumen microbial population in Thai native beef cattle fed on a rice straw based diet.

## Materials and Methods

### Animals, Feeds and Experimental Design

Bamboo grass (*Tiliacora triandra*) was harvested fresh leaves from the climbing tree and sundried for using in the experiment. Bamboo grass meal made as pellet by combining between bamboo grass (90%), cassava chip (9%) and molasses (1%) was called Bamboo-Cass for feeding to experimental animals. According to Wanapat et al. (1996), procedures was shown: (1) Bamboo grass leaves were collected and then sun-dried about 3 days; (2) Bamboo grass leaves were ground to pass 1 mm sieve in the feed mill using Cyclotech Mill, Tecator, Sweden; (3) mixing Bamboo grass leave meal with cassava chip powder and molasses in respective ratio;(4) mixed well all of ingredient; (5) adding water with the ratio 0.8:1(water and Bamboo grass leave meal, respectively); (6) Bamboo-Cass was produced by using the pellet machine then continuing sun-dried for 3 days.

Four Thai native beef cattle ( $190\pm 2\text{kg}$ ) were randomly assigned to receive four dietary treatments in a  $4 \times 4$  Latin square design and kept into a house with the permanent roof. At the initial stage, all animals were dewormed and injected with vitamin ADE to ensure a good health and similar condition, and added mineral blocks at free choice. Animals were kept in individual pens and fed *ad libitum* with rice straw and water supply. The BW of experimental animals was weighted at the beginning and the end of each period for feed intake measurement. The 14% CP of concentrate was formulated and provided to all animals at 0.5 % BW. All animals were received four different levels of Bamboo-Cass supplementation at 0, 50, 100, and 150 g/hd/d, respectively. Each of periods, first 14 d was for measurement of intake, while the last 7 d experimental animals were moved to animals to metabolism crates for sample collection respectively.

### Data collection and sampling procedures

Animals were fed twice daily at 08:00 h in the morning and 16:00 h in the afternoon. Feed offered and refusal feed were recorded throughout the experimental period for calculating feed intake. Feed samples were collected twice a week for analyzing DM and fecal samples were collected by total collection during placing animals to metabolism crates at last 7 days of each period then stored at  $-20^{\circ}\text{C}$  for later chemical analysis. The samples were divided into two parts, the first part was analyzed for DM, and the second part was kept for analysis of Ash, CP according to AOAC (1990), NDF, and ADF according to Van Soest (1991).

Rumen fluid was taken at 0 and 4 hour at last day of each period post morning feeding. Rumen fluid was measured immediately for pH (HANNA Instrument HI 8424 microcomputer, Singapore) and quickly fixed with 10% of formalin solution (1:9 v/v, rumen fluid: 10% formalin) stored at  $4^{\circ}\text{C}$  for measurement of the microbial population. The total direction counts of bacteria and protozoa of rumen fluid were calculated by according to the method of Galyean (1989) based on the use of a haemocytometer (Boeco).

### Statistical analysis



All data were subjected to the ANOVA for a 4×4 Latin square design using the General Linear Models (GLM) Procedure, Statistical Analysis System Institute (SAS, 1998). Mean separations with a significantly different value ( $P < 0.05$ ) for treatment means were compared by using the orthogonal polynomial (Steel and Torrie, 1980).

**Table 1.** Feed ingredients and chemical compositions of concentrates, Bamboo grass, Bamboo-Cass and rice straw.

Items	Concentrate	Rice straw	Bamboo grass	Bamboo-Cass
Feed ingredients (g/kg of dry matter)				
Cassava chip	61.0			
Rice bran	1.0			
Coconut meal	12.0			
Palm meal	20.0			
Urea	2.5			
Molasses	2.0			
Sulphur	0.5			
Mineral mixed	0.5			
Salt	0.5			
Total	100			
Chemical compositions				
DM	87.5	88.5	89.7	87.0
-----g/kg of dry matter-----				
OM	94.2	87.9	92.5	94.7
CP	14.3	2.1	16.0	14.7
NDF	27.6	75.1	61.9	56.6
ADF	18.2	55.6	40.3	36.74
Condensed tannins	-	-	3.1	-

## Results and discussions

Chemical composition of concentrate, rice straw, Bamboo grass, and Bamboo-Cass are presented in Table 1. The protein content of Bamboo grass was 16 %DM which was similar to Wanapat et al. (2012) (16.4 %DM) but lower than the result of Kanpukdee and Wanapat (2008) (17.1 %DM). However, condensed tannins in bamboo grass was 3.1 %DM which was higher than Wanapat et al. (2012) (2.3 %DM) and Kanpukdee and Wanapat (2008) (2.2 %DM).

The result of feed intake and digestibility of Thai native beef cattle is presented in Table 2. Feed intake was linearly increased ( $P < 0.05$ ) when increasing levels of Bamboo-Cass supplementation. This result was similar to Chanthakhoun et al. (2010) who reported that supplementation of higher protein increased dry matter intake. Moreover, the levels at 100 and 150g are similarly effect on feed intake which was consistent to Manasri et al. (2012) reported that dietary supplementation containing tannins sources (<50 g/kg DM) were no effect on DMI in beef cattle. However, nutrient intake and apparent digestibility were not significantly different ( $P > 0.05$ ) but tend to increase as an effect of Bamboo-Cass supplementation which were resulted from the increasing of microbial population (Table 3).

Microbial population was affected by Bamboo-Cass supplementation for all four treatments (Table 3). The result showed that no effects of Bamboo-Cass supplementation on ruminal pH among treatments and the values stable similarity (6.7-6.8) which are the normal range of rumen ecology and fermentation by microbes reported by Gunun et al. (2016) and Chanjula et al. (2004).



The total direct counts of protozoa was not affected by dietary treatments, while bacteria was significantly different among treatments ( $P < 0.05$ ). However, protozoa population tended to decrease as the effect of Bamboo-Cass supplementation similarly at 100 and 150g. This result was similar to Kang et al. (2012) and Tan et al. (2011) reported that the increase of microbial population while the decrease of protozoa appeared when fed with the plants containing condensed tannins.

**Table 2.** Effect of Bamboo-Cass on feed intake and nutrient digestibility in Thai native beef cattle.

Items	Bamboo-Cass (g/hd/d)				SEM	Orthogonal Polynomials		
	0	50	100	150		L	Q	C
Rice straw intake								
kg/d	2.6	3.3	3.4	3.5	0.15	ns	ns	ns
%BW	1.3	1.6	1.7	1.7	0.03	*	ns	ns
g/kg BW <sup>0.75</sup>	48.8	61.1	63.9	64.2	1.19	*	ns	ns
Concentrate intake								
kg/d	1	1	1	1	-	-	-	-
%BW	0.5	0.5	0.5	0.5				
g/kg BW <sup>0.75</sup>	18.8	18.9	18.8	18.9	-	-	-	-
Total intake								
kg/d	3.6	4.3	4.5	4.7	0.06	*	ns	ns
%BW	1.8	2.1	2.3	2.3	0.03	*	ns	ns
g/kg BW <sup>0.75</sup>	67.6	80.8	84.6	85.9	1.19	*	ns	ns
Nutrients intake, kg/d								
DM	4.0	4.3	4.4	4.6	0.10	ns	ns	ns
OM	3.6	3.8	4.0	4.1	0.09	ns	ns	ns
CP	0.3	0.3	0.3	0.3	0.01	ns	ns	ns
NDF	2.4	2.6	2.8	2.8	0.07	ns	ns	ns
ADF	1.5	1.6	1.7	1.8	0.04	ns	ns	ns
Apparent digestibility, %								
DM	62.5	65.6	66.5	66.1	1.13	ns	ns	ns
OM	71.0	70.0	73.0	72.0	1.12	ns	ns	ns
CP	61.5	63.2	65.0	64.3	2.47	ns	ns	ns
NDF	50.5	52.7	56.6	52.1	1.87	ns	ns	ns
ADF	40.4	43.2	46.3	42.2	1.93	ns	ns	ns

\*,  $P < 0.05$ ; SEM, standard error of mean; ns, not significant ( $P > 0.05$ ); L, Linear; Q, Quadratic; C, Cubic.

## Conclusion

Based on this study it could be concluded that supplementation of Bamboo-Cass enhanced rice straw intake, maintained rumen pH, and the microbial population. In addition, there were tendencies to increase nutrient intake, nutrient digestibility, and reducing protozoa population. Therefore, using of Bamboo-Cass could impact when supplementation at 100 and 150 g/hd/d for Thai native cattle fed rice straw based diet.





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**Table 3.** Effect of Bamboo-Cass on rumen pH and microbial population in Thai native beef cattle

Items	Bamboo-Cass (g/hd/d)				SEM	Orthogonal Polynomials		
	0	50	100	150		L	Q	C
Rumen pH								
Hour 0	6.7	6.8	6.7	6.8	0.02	ns	ns	ns
Hour 4	6.7	6.7	6.6	6.7	0.02	ns	ns	ns
Mean	6.8	6.8	6.7	6.8	0.02	ns	ns	ns
Protozoa, $\times 10^5$								
Hour 0	2.8	2.6	2.6	2.4	0.12	ns	ns	ns
Hour 4	3.9	2.3	2.3	2.3	0.43	ns	ns	ns
Mean	3.3	2.4	2.4	2.3	0.24	ns	ns	ns
Bacteria, $\times 10^{10}$								
Hour 0	28.0	28.3	28.4	28.6	0.14	ns	ns	ns
Hour 4	29.7	30.9	35.8	36.9	0.17	**	ns	*
Mean	28.9	29.6	32.1	32.7	0.08	**	ns	**

\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; SEM, standard error of mean; ns, not significant ( $P > 0.05$ ); L, Linear; Q, Quadratic; C, Cubic.

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## ***In Vitro* Fermentation and Methane Production Influenced by Leucaena Silage and Mangosteen Peel Powder**

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### **Abstract**

The present study aimed to investigate the effect of mangosteen peel powder (MPP) on *in vitro* fermentation and methane production in different Leucaena silage to concentrate ratio (Ls:C). All treatments were randomly arranged according to a 3×2 Factorial arrangement in a completely randomized design. The first factor was three different ratios of Ls:C (40:60, 60:40, and 80:20) and the second factor was two levels of MPP supplementation (0 and 2% of total substrate). The result showed that gas production were affected by different ration of Ls:C ( $P<0.05$ ) while MPP supplementation did not alter ( $P>0.05$ ). Total volatile fatty acid did not change by different ratios of Ls:C; however, it was increased in MPP supplemented group ( $P<0.05$ ). Increasing level of Leucaena silage could increase acetate and propionate profile while butyrate was similar among treatments. However, MPP supplementation decreased acetic acid but could increase propionic acid. Methane production was reduced by increasing level of Leucaena silage and MPP supplement ( $P<0.05$ ). Based on this experiment, it could be concluded that Leucaena silage and MPP could enhance rumen fermentation and reduce methane production. This study recommended to use Leucaena silage as high quality roughage at 60:40 (Ls:C) in cooperated with 2%MPP supplement for rumen mitigation. However, further researches of feeding trial on growth performance and productivity should be conducted.

**Keywords:** Leucaena silage, Mangosteen peel powder, Gas production, Fermentation efficiency, Methane production

### **Introduction**

High silage quality is a key factor to minimize cost of production and sustained animal health and it is making increased considerably from the 1960s as predominant method of forage preservation in temperate areas of the world (Cheli et al., 2013). Manipulation of the rumen microbial ecosystem for enhancement of fibrous feed digestibility, fermentation efficiency, while reducing methane (CH<sub>4</sub>) emission by ruminants are some of the most important goals for animal nutritionists (Guglielmelli, et al., 2010). According to Garcia et al. (1996), Leucaena (*Leucaena leucocephala*) contained protein at high level of 29.2% in leaf and 22.03% in stem and leaf. Moreover, it contains plants secondary compounds such as tannin content (Gupta et al., 1992) and this compound has positive effect without reducing dry matter (DM) intake if animal is fed small amount. Leucaena silage (Ls) can improve feed intake and nutrients digestibility in ruminant and reduce 90% mimosine (Sunagawa et al., 1989) after 14-21 days of silage. On the other hand, Leucaena has abundant and highest biomass productivity in the rainy season;



therefore, it should be harvested and stored as important protein source for animal feeding in dry season. In addition, mangosteen peel is a fruit by-product and many researchers have been interested to use it as a dietary supplement to improve rumen ecology and rumen productivity due to its condensed tannins and crude saponins (Ngamsaeng et al., 2006). It was reported that supplementation of mangosteen peel powder (MPP) could decrease protozoa populations and CH<sub>4</sub> production. Therefore, this experiment was to investigate the effect of MPP on *in vitro* fermentation and CH<sub>4</sub> production in different Leucaena silage to concentrate ratio (Ls:C).

## Materials and methods

### Dietary feed and experimental design

Leucaena were collected from the tree with young branch and leaf. It was then chopped to the length of 2-3 cm length, then packed in plastic bags and pressed well to remove air as anaerobic condition. The silage bags were kept in ambient temperature (about 25-30 °C) at least 30 days before used in the experiment. Silage was done in triplicates at 0.5kg of each. All treatments were arranged according to a 3×2 factorial arrangement in a completely randomized design (CRD) with three replications per treatment including triplicates of blank (medium only) in three incubation runs. Factor A was different ratios of Ls:C (40:60, 60:40, and 80:20) and factor B was MPP supplementation (0 and 2% of total dietary substrate). Dietary samples were dried at 60°C, ground to pass a 1-mm sieve (Cyclotech Mill, Tecator, Sweden) and kept for chemical composition analysis and *in vitro* gas study. Dietary of Ls:C was used as a dietary substrate. Feed ingredients and chemical composition of Leucaena silage, concentrate and MPP are shown in Table 1.

Table 1. Feed ingredients and chemical compositions of experimental diets

Items	Concentrate	Leucaena silage	Mangosteen peel powder
Ingredients, % of dry matter			
Cassava chip	60.0		
Rice bran	10.0		
Corn meal	10.0		
Soybean meal	13.0		
Urea	3.0		
Molasses	2.5		
Mineral premix	0.5		
Salt	0.5		
Sulphur	0.5		
Chemical compositions			
Dry matter, %	87.8	42.1	91.2
	-----% Dry matter-----		
Organic matter	94.6	93.3	95.9
Crude protein	17.3	28.2	3.3
Crude fibre	3.5	12.7	25.9
Ash	5.4	6.7	4.1

### Animals and preparation of rumen inoculums

Two rumen-fistulated crossbred beef cattle (Local × Haryana) with body weight of 450±10 kg and 4 years old were used as rumen fluid donors. The animals were fed 70% rice straw and 30% Mulato II grass at 3% of body weight and supplemented with rice brand at 0.5% of BW before collecting rumen fluid. Animals were placed on a routine for at least 20 days and



kept in individual pens with free choice of clean fresh water and mineral blocks. On day 21<sup>st</sup>, about 500 ml of rumen liquor was obtained from both animals before the morning feeding. The rumen fluid was filtered through four layers of cheesecloth into pre-warmed thermo flasks and then transported to the laboratory.

### ***In vitro* fermentation of substrates, data collection, sampling procedures and analysis**

Samples of 200 mg of Ls:C were weighed into 60 ml serum bottles included MPP supplementation, respectively. All bottles were then sealed with rubber stoppers and aluminium caps and pre-warmed in a water bath at 39°C for 1 h before filling with 30 ml of the rumen inocula mixture (Menke and Steingass 1988). The method used for *in vitro* fermentation was based on the technique described by Menke et al. (1979). Gas production kinetics was collected during the incubation at 1, 2, 4, 6, 8, 12, 24, 36, and 48 hour. Cumulative gas production data were fitted to the model of Orskov and McDonald (1979) as follows:  $y = a + b(1 - e^{-ct})$ ; Where  $a$  = the gas production from the immediately soluble fraction,  $b$  = the gas production from the insoluble fraction,  $c$  = the gas production rate constant for the insoluble fraction ( $b$ ),  $t$  = incubation time,  $(a+b)$  = the potential extent of gas production, and  $y$  = gas produced at time “ $t$ ”. Fermentation liquor was sampled at 6 hour post inoculations and then filtered through four layers of cheesecloth. The first portion (20 mL) was kept into plastic bottles to which 5 ml of 1 M H<sub>2</sub>SO<sub>4</sub> was added to stop fermentation process of microbe activity and then centrifuged at 3,000 x  $g$  for 10 min and the supernatant was stored at -20°C before volatile fatty acid (VFA) analysis by High performance liquid chromatography (Samuel et al., 1997). Methane production (mM/L) was calculated based on the equation of Moss et al. (2000) = 0.45 (C<sub>2</sub>)-0.275 (C<sub>3</sub>)+0.4 (C<sub>4</sub>). All the obtained data were subjected to General Linear Model (GLM) procedures of SAS (2013) according to a 3 × 2 factorial arrangement in a completely randomized design. Differences among treatment means were contrasted by the Tukey’s multiple comparison test (Crichton 1999). Comparison between Ls:C ratio was tested by orthogonal contrast.

## **Results and discussion**

### **Gas production kinetics**

Result of gas production kinetics affected by Ls and MPP supplement are shown in Table 1 and Figure 1. Increasing the ratio of Ls:C resulted in the reduction of gas kinetics and accumulative gas production. Gas production from the immediately soluble fraction ( $a$ ) were found the highest in group 80:20 of Ls:C ( $P < 0.05$ ). However, the present findings found that there was no effect of MPP supplementation on gas production kinetics ( $P > 0.05$ ).

Table 2. Effect of Leucaena silage and mangosteen peel powder on gas production kinetics from *in vitro* incubation

Trts	LS:C <sup>1</sup>	MPP <sup>2</sup>	Gas production kinetics <sup>4</sup>				Gas <sup>5</sup>
			a	b	c	a+b	
T1	40:60	0	-1.4	49.7	0.10	48.3	47.9
T2	60:40	0	-2.5	46.1	0.10	43.6	43.2
T3	80:20	0	0.7	34.0	0.09	34.7	34.1
T4	40:60	2	-4.7	53.0	0.10	48.3	47.8
T5	60:40	2	-1.4	42.4	0.10	41.0	40.7
T6	80:20	2	0.6	32.0	0.09	32.6	32.2
SEM			0.84	1.84	0.003	1.43	1.44
Interactions							
	LS:C		0.010	0.001	0.009	0.001	0.001
	MPP		0.365	0.644	0.690	0.269	0.281
	LS:C*MPP		0.096	0.272	0.344	0.731	0.767



<sup>1</sup> Leucaena silage to concentrate ratio; <sup>2</sup> Mangosteen peel powder (% of total substrate); <sup>3</sup> a, the gas production from the immediately soluble fraction; b, the gas production from the insoluble fraction; c, the gas production rate constant for the insoluble fraction (b); a+b, the gas potential extent of gas production; <sup>4</sup> Cumulative gas production at 48 hour (ml/0.2g DM substrate).

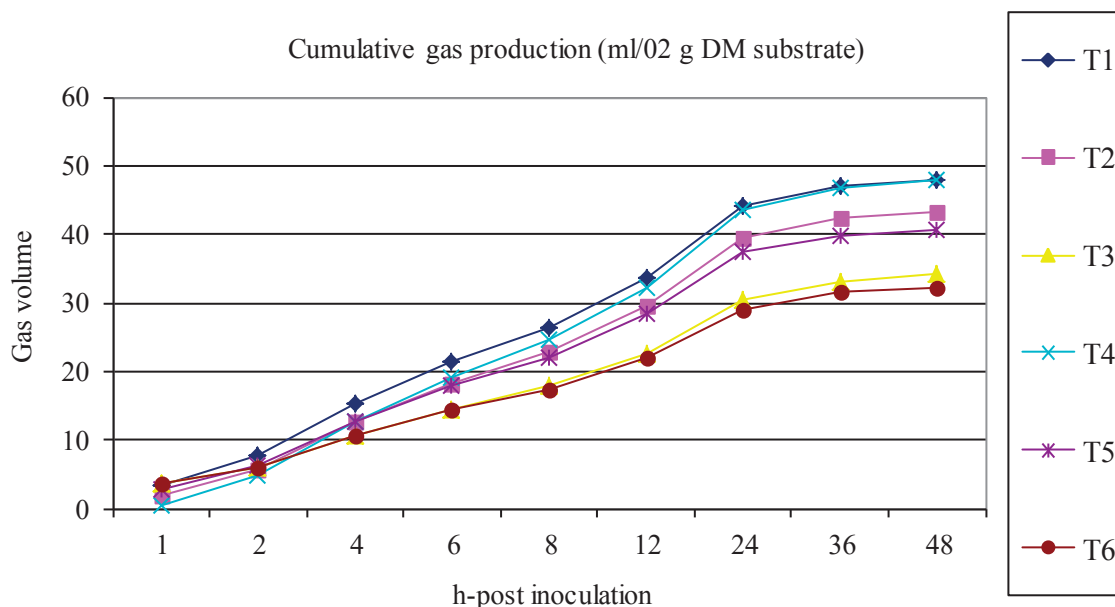


Figure 1. Effect of Leucaena silage and mangosteen peel powder on cumulative gas production at different times of incubation beef cattle rumen fluid.

T1 = Leucaena silage to concentrate ratio (LS:C) 40:60; T2 = LS:C 60:40; T3 = LS:C 80:20; T4 = LS:C 40:60 + Mangosteen peel powder (MPP) 2% of total substrate; T5 = LS:C 60:40 + MPP 2% of total substrate; T6 = LS:C 80:20 + MPP 2% of total substrate.

### Rumen parameter and methane production

Table 3 presents the result on *in vitro* fermentation efficiency and methane production affected by Ls and MPP supplementation. Leucaena silage and MPP did not influence on total VFA and butyric acid concentration ( $P < 0.05$ ). Increasing the ratio of Ls:C with MPP supplement decreased the concentration of acetic acid and  $\text{CH}_4$  production while propionic acid was enhanced ( $P < 0.05$ ).

### Conclusions and recommendations

In summary, Leucaena silage could be used as high quality roughage for ruminant feeding at 60:40 (Ls:C). Leucaena silage in cooperated with 2%MPP supplement could enhance rumen fermentation and reduce methane production. However, more research should be conducted on growth performance and productivity of ruminant.

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Table 3. Effect of *Leucaena* silage and mangosteen peel powder on pH, *in vitro* fermentation efficiency and methane production

Trts	LS:C <sup>1</sup>	MPP <sup>2</sup>	pH	NH <sub>3</sub> -N (mg/dL)	TVFA (mmol/L)	C <sub>2</sub>	C <sub>3</sub>	C <sub>4</sub>	C <sub>2</sub> /C <sub>3</sub>	CH <sub>4</sub> <sup>3</sup>
						mol/100 mol				
T1	40:60	0	6.85	18.8	43.3	72.3	19.7	7.9	3.7	13.1
T2	60:40	0	6.80	19.9	39.7	70.3	22.5	7.2	3.1	11.3
T3	80:20	0	6.85	16.8	38.1	73.7	19.9	6.4	3.7	11.5
T4	40:60	2	6.75	18.8	40.1	73.0	21.0	5.9	3.5	11.9
T5	60:40	2	6.80	17.6	42.6	68.5	23.7	7.8	2.9	11.7
T6	80:20	2	6.80	16.2	47.2	64.8	27.7	7.5	2.3	11.6
SEM			0.03	0.36	1.21	0.68	0.69	0.68	1.13	0.36
Interactions										
	LS:C		0.729	0.029	0.675	0.018	0.029	0.807	0.032	0.048
	MPP		0.134	0.167	0.080	0.005	0.005	0.935	0.009	0.026
	LS:C*MPP		1.000	0.339	0.030	0.006	0.025	0.297	0.026	0.049

<sup>1</sup> *Leucaena* silage to concentrate ratio; <sup>2</sup> Mangosteen peel powder (% of total substrate); <sup>3</sup> Methane production (mM/L) calculated by Moss et al. (2000) = 0.45 (C<sub>2</sub>)-0.275 (C<sub>3</sub>)+0.4 (C<sub>4</sub>); NH<sub>3</sub>-N, ammonia nitrogen; TVFA, total volatile fatty acid; C<sub>2</sub>, acetic acid; C<sub>3</sub>, propionic acid; C<sub>4</sub>, butyric acid; C<sub>2</sub>:C<sub>3</sub>, acetic acid: propionic acid ratio.

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ANN-01-0106

## Influence of Bamboo Leaf Meal Supplementation on *In Vitro* Gas Production and Digestibility

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### Abstract

The of this study was to determine the effect of bamboo leave meal (BLM) supplementation on gas production and digestibility using *in vitro* gas production techniques. All treatments were arranged according to a 2×2×4 factorial arrangement in a completely randomized design (CRD). Factor A was different ratios of roughage to concentrate (R:C; 70:30 and 30:70) and factor B was different type of bamboo leave meal (BLM; green and brown) while factor C was BLM supplementation (0, 2, 4 and 6% of total dietary substrate). The result showed that accumulation gas and gas kinetics were increased in treatments with R:C at 30:70 while there was no different effect of BML supplement and type of BLM found in the present study. Increasing level of BLM supplement decreased protozoa while there was no difference between bamboo type leave meal ( $P>0.05$ ). On the other hand, DM digestibility was increased in R:C at 30:70 with green BLM supplemented at 4-5% of total substrate ( $P<0.05$ ). It could be concluded that BLM could enhance DM digestibility and reduce protozoa population supplemented at 4-6% of total substrate.

**Keywords:** bamboo leaf meal, gas production, digestibility, *in vitro* techniques

### Introduction

Methane (CH<sub>4</sub>) has a 21 times higher global warming potential than carbon dioxide (IPCC, 2007). It is estimated that the world's population of ruminants produces about 15% of total CH<sub>4</sub> emissions. As reported, CH<sub>4</sub> production resulted from fermentation of feed in the gastrointestinal tract of ruminants represents a substantial loss of 2–15% of gross energy intake (Johnson and Johnson, 1995), which reduces the potential conversion of feed energy to metabolizable energy. Whilst numerous chemical additives and antibiotics have been tested and used for this purpose, plants containing bioactive products such as essential oils, saponins (SP) and condensed tannins (CT) (Guglielmelli et al. 2011) with antimicrobial properties may be exploited in ruminant production to reduce CH<sub>4</sub> emissions and improve fermentation efficiency. At appropriate dose, CT and SP containing plants have been shown to suppress protozoal and methanogenesis population while increase bacteria and fungi population, propionate production, partitioning factor, and yield and efficiency of microbial protein synthesis; hence, improve performance in ruminants (Patra and Saxena 2009, Wanapat et al. 2012). Bamboos belong to the grass family, Poaceae, subfamily Bambusoideae. Most species are perennial and spread rapidly via underground rhizomes. Pandas are the primary animal species associated with bamboo consumption, but other animal species may be better adapted to digesting its nutritive constituents (Dierenfeld et al., 1982). Numerous bamboos are fed to horses, cattle, and sheep worldwide (Farrelly, 1984). It is reported that bamboo leave hay contain crude protein at 20.5%



(Gebreziabhear, 2016) or 7.6% (Bersalona et al., 2015) and it could enhance the growth performance of chicken and sheep. Moreover, Coffie et al. (2014) confirmed that bamboo leaves both wet and dry contained tannins and saponins. Therefore, the objective of this study was to evaluate the effect of bamboo leave meal supplementation on gas production and digestibility using *in vitro* techniques.

## Materials and Methods

### Dietary feed and experimental design

Bamboo leave were collected and sundried for about 1 or 2 days until air dry. All treatments were arranged according to a 2×2×4 factorial arrangement in a completely randomized design (CRD) with three replications per treatment including triplicates of blank (medium only) in three incubation runs. Factor A was different ratios of roughage to concentrate (R:C; 70:30 and 30:70) and factor B was different type of bamboo leave meal (BLM; green and brown) while factor C was BLM supplementation (0, 2, 4 and 6% of total dietary substrate). Dietary samples were dried at 60°C, ground to pass a 1-mm sieve (Cyclotech Mill, Tecator, Sweden) and kept for chemical composition analysis and *in vitro* gas study. Dietary of R:C was used as a dietary substrate. Feed ingredients and chemical composition of dietary treatment are shown in Table 1.

Table 1. Feed ingredients and chemical compositions of experimental diets

Items	Concentrate	Rice straw	Green bamboo leaf meal	Brown bamboo leaf meal
Ingredients, % of dry matter				
Cassava chip	60.0			
Rice bran	7.0			
Palm kernel meal	15.0			
Coconut meal	12.0			
Urea	1.5			
Molasses	3.0			
Mineral premix	0.5			
Salt	0.5			
Sulfur	0.5			
Chemical compositions				
Dry matter, %	87.2	93.4	96.2	95.7
	-----% Dry matter-----			
Organic matter	91.9	87.4	88.2	83.5
Crude protein	14.2	2.3	11.2	4.8
Neutral detergent fiber	21.5	74.1	59.2	65.1
Acid detergent fiber	12.9	58.5	40.0	41.5
Ash	8.1	12.6	11.8	16.5

### *In vitro* fermentation of substrates, data collection, sampling procedures and analysis

Two rumen-fistulated dairy steers (180±15 kg BW) were used as rumen fluid donors. The rumen fluid was filtered through four layers of cheesecloth into pre-warmed thermo flasks and then transported to the laboratory. Samples of 200 mg of R:C were weighed into 60 ml serum bottles included BLM supplementation, respectively. All bottles were then sealed with rubber stoppers and aluminium caps and pre-warmed in a water bath at 39°C for 1 h before filling with 30 ml of the rumen inocula mixture (Menke and Steingass 1988). The method used for *in vitro* fermentation was based on the technique described by Menke et al. (1979). Gas production



kinetics was collected during the incubation at 1, 2, 4, 6, 8, 12, 24, 36, 48, 72, 96 and 120 hour by extraction using glass syringes. Cumulative gas production data were fitted to the model of Orskov and McDonald (1979) as follows:  $y = a+b (1-e(-ct))$ ; Where a = the gas production from the immediately soluble fraction, b = the gas production from the insoluble fraction, c = the gas production rate constant for the insoluble fraction (b), t = incubation time, (a+b) = the potential extent of gas production, and y = gas produced at time “t”. The 1 mL of fermentation liquor was collected and kept in a plastic bottle to which 9 mL of 10% formalin solution (1:9 v/v, rumen fluid: 10% formalin) were added and stored at 4°C for total counts of protozoa according to the method of Galyean (2010) based on the use of a haemocytometer (Boeco, Hamburg, Germany). *In vitro* DM digestibility was analyzed according to Van Soest and Robertson (1985). In brief, the content of the bottle was transferred and filtered through pre-weighed Gooch crucibles. The DM of the residue was weighed. All the obtained data were subjected to General Linear Model (GLM) procedures of SAS (2013) according to a 2×2×4 factorial arrangement in a completely randomized design. Differences among treatment means were contrasted by the Tukey’s multiple comparison test (Crichton 1999). Comparison between Ls:C ratio was tested by orthogonal contrast.

Table 2. Effect of bamboo leaf meal supplementation on gas production kinetics from *in vitro* incubation

Trts	R:C <sup>1</sup>	BLM <sup>2</sup>	SL <sup>3</sup>	Gas production kinetics <sup>4</sup>				Gas <sup>5</sup>
				a	b	c	a+b	
T1	70:30	Dry	2	5.4	49.5	0.0	54.9	54.7
T2			4	3.3	55.2	0.0	58.5	58.2
T3			6	3.8	58.9	0.0	62.7	62.4
T4			8	3.9	57.9	0.0	61.8	61.4
T5		Fresh	2	4.1	60.1	0.0	64.2	63.8
T6			4	3.7	55.4	0.0	59.0	58.7
T7			6	4.1	56.9	0.0	61.0	60.5
T8			8	5.2	61.6	0.0	66.7	66.0
T9	30:70	Dry	2	-2.0	69.3	0.1	67.2	67.2
T10			4	-2.1	67.3	0.1	65.2	65.2
T11			6	-0.8	72.8	0.1	71.9	71.9
T12			8	-0.4	68.0	0.1	67.5	67.5
T13		Fresh	2	-0.2	65.1	0.1	64.8	64.8
T14			4	-0.9	66.6	0.1	65.7	65.7
T15			6	0.6	63.7	0.1	64.2	64.2
T16			8	0.3	64.6	0.1	64.9	64.9
SEM				0.891	2.055	0.003	1.977	1.969
Interactions								
	R:C			***	***	***	***	***
	BLM			NS	NS	**	NS	NS
	SL			NS	NS	0.06	NS	NS
	R:C*BLM			NS	**	0.09	**	**
	BLM*SL			NS	0.08	NS	0.08	0.08
	R:C*BLM*SL			NS	NS	NS	NS	NS

<sup>1</sup> Roughage to concentrate ratio; <sup>2</sup> Bamboo leave meal; <sup>3</sup> Supplementation level (% of total substrate); <sup>4</sup> a, the gas production from the immediately soluble fraction; b, the gas production from the insoluble fraction; c, the gas production rate constant for the insoluble fraction (b); a+b, the gas potential extent of gas production; <sup>5</sup> Cumulative gas production at 120 hour (ml/0.2g DM substrate).



## Result and discussion

### Gas production kinetics

Table 3 shows the effect of BLM on gas production. The accumulation gas and gas kinetics were affected by different R:C ( $P<0.05$ ) while there was no different effect of BML supplement and type of BLM found in the present study on gas production ( $P<0.05$ ). Gas production was increased in R:C at 30:70.

### Protozoa and digestibility

Effect of BLM supplementation on digestibility and protozoal population is presented in Table 3. It was found that protozoal population was decreased by either R:C and BML supplementation ( $P<0.05$ ). Increasing level of BLM supplement decreased protozoa while there was no difference between bamboo type leave meal ( $P>0.05$ ). On the other hand, DM digestibility was increased in R:C at 30:70 with green BLM supplemented at 4-5% of total substrate ( $P<0.05$ ).

Table 3. Effect of bamboo leaf meal supplementation on digestibility and protozoal population from *in vitro* incubation

Trts	R:C <sup>1</sup>	BLM <sup>2</sup>	SL <sup>3</sup>	Protozoa ( $\times 10^5$ cell/mL)	<i>In vitro</i> DM digestibility, %
T1	70:30	Dry	2	5.3	44.9
T2			4	2.5	52.6
T3			6	2.5	50.0
T4			8	2.3	37.3
T5		Fresh	2	3.5	51.6
T6			4	1.8	56.8
T7			6	1.5	59.5
T8			8	1.5	39.3
T9	30:70	Dry	2	6.3	59.9
T10			4	4.3	62.1
T11			6	4.3	70.6
T12			8	3.0	69.8
T13		Fresh	2	4.8	67.2
T14			4	3.8	77.3
T15			6	3.8	69.0
T16			8	2.8	62.5
SEM				0.726	2.227
Interactions					
R:C				*	***
BLM				NS	**
SL				**	***
R:C*BLM				NS	NS
R:C*SL				NS	NS
BLM*SL				NS	NS
R:C*BLM*SL				NS	NS

<sup>1</sup> Roughage to concentrate ratio; <sup>2</sup> Bamboo leave meal; <sup>3</sup> Supplementation level (% of total substrate).

## Conclusions and recommendations

Based on this experiment, it could be concluded that BLM supplementation could enhance DM digestibility and reduce protozoa population. This study suggests the use of BLM as rumen enhancer at 4-6% of total substrate.



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## ***Session 11-Orchid ballroom I***

ANN-01-0075

### **Effect of Banana Flower Powder Contained in High Quality Feed Block and Roughage to Concentrate Ratio on *In Vitro* Gas Production Kinetics, Digestibility and Fermentation**

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#### **Abstract**

This study aimed to investigate the effect of various levels of banana flower powder in high quality feed block (HQFB) and roughage to concentrate ratio on gas kinetics, digestibility, and fermentation using *in vitro* gas production techniques. The experimental design was a 2 × 5 factorial arrangement in a completely randomized design. Factor A was two ratio of roughage to concentrate (R:C; 70:30 and 30:70) and factor B was five levels of banana flower powder (BAFLOP) incorporated in HQFB (0, 10, 20, 30 and 40 g/kg DM). Results revealed that all gas kinetics (the immediately soluble fraction (a), insoluble fraction (b), the gas production rate (c) and potential extent of gas production (a+b)) were significantly different between R:C ratios and were significant higher (P<0.01) at 30:70 of R:C ratio while BAFLOP incorporated in HQFB did not affect only the gas production rate (c). Cumulative gas production (120 h) was higher in the R:C ratio at 30:70 (P<0.01) especially when supplemented BAFLOP at 20g/kgDM in HQFB. *In vitro* true dry matter digestibility at 12h and 24h were improved by either R:C or BAFLOP supplementation (P<0.001). The pH declined when R:C ratio at 30:70 was tested as a result of using high concentrate ratio; however, supplementation of BAFLOP could buffer the pH. BAFLOP supplementation did not influence the ammonia nitrogen (NH<sub>3</sub>-N) concentration, while R:C did (P<0.01) and the range was between 17.3 to 24.3 mg/dL. The suitable BAFLOP level in HQFB is 20 g/kg DM. However, *in vivo* feeding trial should be subsequently conducted.

**Keywords:** banana flower powder, high quality feed block, roughage to concentrate ratio, gas kinetics, *in vitro* technique

#### **Introduction**

There is increasing interest in exploiting natural products as feed additives to solve problems in animal nutrition and livestock production (Wanapat et al., 2015). The use of tropical plant and agricultural by-products containing secondary compound such as condensed tannins and saponins have resulted in improving rumen fermentation by enhancing the efficiency of utilization of feed energy while inhibiting rumen methane production (Foiklang et al., 2016). High-quality feed block (HQFB) has been used as strategic supplements for ruminants in the



tropics, especially when fed with rice straw and other low quality roughages-based diets (Foiklang et al., 2011; Cherdthong et al., 2014). These enhancements were similar to those reported by Foiklang et al. (2011) who found that supplementation of HQFB as lick-blocks for swamp buffalo could improve feed intake, nutrient digestibility and rumen fermentation efficiency. Banana flower powder is one of natural agricultural plant containing secondary metabolites and high in mineral elements which are suitable for manipulate rumen fermentation when ruminants consume high levels of concentrate without harmful to animals (Kang et al., 2016). However, there are limited studies on the effect of banana flower powder contained in high quality feed block and roughage to concentrate ratio in ruminants nutrition study. Therefore, the aim of this study was to investigate the effect of banana flower powder contained in high quality feed block and roughage to concentrate ratio on rumen gas kinetics and digestibility of nutrients using *in vitro* gas production technique.

## Materials and Methods

An *in vitro* study based on the technique described by Menke et al. (1979) was conducted to evaluate various levels of banana flower powder in high quality feed block (HQFB) and roughage to concentrate ratio. The experimental design was a  $2 \times 5$  factorial arrangement in a completely randomized design. Factor A was two ratio of roughage to concentrate (R:C; 70:30 and 30:70) and factor B was five levels of banana flower powder (BAFLOP) incorporated in HQFB (0, 10, 20, 30, 40 g/kg DM). HQFB were mixed using local ingredients (rice bran, molasses, urea, white cement, sulphur, premix, salt and tallow) and used at 0.03g in the substrate. The gas production was measured at 0, 1.5, 3, 6, 9, 12, 24, 36, 48, 72, 96 and 120 h of incubation according to Foiklang et al. (2016). Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979). Nutrient compositions of substrates were analyzed according to the standard methods (AOAC, 1995). Inoculum's ruminal fluid was collected at 0, 2, 4 and 6 h of incubation for pH and  $\text{NH}_3\text{-N}$  analysis. *In vitro* true digestibility (TD) measurement based on Van Soest and Robertson (1985). Data were analyzed by using the GLM procedures of the Statistical Analysis System Institute (SAS, 1996).

## Results and Discussions

Rice straw contained 2.1% of CP, 85.6% of NDF and 53.2% of ADF while Concentrate contained 14.2% of CP, 20.3% of NDF and 14.6% of ADF while BAFLOP contained 15.2% of CP and 11.4% of CT (did not show in Table). HQFB contained 39.7-40.4 % of CP which close to the previous reports of Foiklang et al. (2011) and Cherdthong et al. (2014). All gas kinetics (the immediately soluble fraction (a), insoluble fraction (b), the gas production rate (c) and potential extent of gas production (a+b)) were different between R:C ratios which were significant higher ( $P < 0.01$ ) at 30:70 of R:C ratio while BAFLOP incorporated in HQFB did not affect only the gas production rate (c) as show in Table 1. Similarly with Kang and Wanapat (2013) who reported that BAFLOP as a feed supplement in concentrate diet influenced the gas kinetics. Cumulative gas production (120 h) was higher in the R:C ratio at 30:70 ( $P < 0.01$ ) especially when supplemented BAFLOP at 20g/kg DM in HQFB. *In vitro* true dry matter digestibility at 12h and 24h were improved by either R:C or BAFLOP supplementation ( $P < 0.001$ ) as show in Table 2. These





similar results were also agreed with Kang and Wanapat (2013) who supplemented BAFLOP that could improve the rate of gas production, DM and OM disappearances. The pH declined as a result of using high concentrate ratio; however, supplementation of BAFLOP could buffer the pH which led to an improvement of ruminal efficiency. BAFLOP supplementation did not influence the ammonia nitrogen (NH<sub>3</sub>-N) concentration, while R:C did ( $P < 0.01$ ) and the range was between 17.3 to 24.3 mg/dL. The NH<sub>3</sub>-N concentration was close to the report of Wanapat and Pimpa (1999) (15 to 30 mg/dL) which was reported to be suitable for ruminal microbial activity.

**Table 1.** Effect of banana flower powder contained in high quality feed block and roughage to concentrate ratio on gas kinetics and cumulative gas from *in vitro* incubation with rumen fluid

R:C ratio	BAFLOP in HQFB (g/kg DM)	Gas Kinetics				Cumulative gas (mL) produced at 120 h
		a (mL)	b (mL)	c (mL/h)	a+b (mL)	
70:30	0	-0.7	86.9	0.02	86.3	86.0
	10	-0.6	103.3	0.03	103.2	103.4
	20	-0.6	110.2	0.02	109.6	110.5
	30	-1.2	105.3	0.02	104.1	104.2
	40	-1.7	111.9	0.02	110.2	110.5
30:70	0	-0.9	118.2	0.03	117.7	117.7
	10	-0.9	114.9	0.03	114.3	114.3
	20	-0.8	126.4	0.04	125.6	125.6
	30	-0.6	107.2	0.05	106.7	106.7
	40	-0.9	113.6	0.05	112.7	112.7
SEM		0.14	2.24	0.004	2.21	2.63
Comparison						
R:C ratio		*	**	*	*	**
BAFLOP levels		ns	*	*	*	*
Interaction		ns	ns	*	*	*
Orthogonal polynomials						
BAFLOP (Linear)		ns	*	*	*	*
BAFLOP (Quadratic)		ns	*	ns	*	*
BAFLOP (Cubic)		ns	ns	ns	ns	ns

\*  $P < 0.05$ , \*\*  $P < 0.01$ . R:C ratio, roughage to concentrate ratio; BAFLOP, banana flower powder. a, the gas production from the immediately soluble fraction; b, the gas production from the insoluble fraction; c, the gas production rate constant for the insoluble fraction; a+b, the potential extent of gas production. SEM, standard error of mean; ns, not significant.

## Conclusion and Recommendation

Based on this study, it could be concluded that BAFLOP incorporated in HQFB could improve gas production, *in vitro* true digestibility and rumen fermentation. The suitable BAFLOP level in HQFB is 20 g/kg DM. However, *in vivo* feeding trial should be subsequently conducted.



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**Table 2.** Effect of banana flower powder contained in high quality feed block and roughage to concentrate ratio on *in vitro* true dry matter digestibility (IVTDMD) and rumen fermentation parameters

R:C ratio	BAFLOP in HQFB (g/kg DM)	IVTDMD		Rumen parameters		
		12h	24h	Temp.	pH	NH <sub>3</sub> -N
70:30	0	35.9	61.8	38.8	6.82	17.3
	10	36.4	61.5	38.7	6.91	18.1
	20	38.5	65.3	39.0	6.90	18.0
	30	38.3	65.9	38.7	6.95	17.9
	40	38.4	68.3	39.1	6.93	17.7
30:70	0	50.4	82.9	38.5	6.09	23.1
	10	50.6	86.5	38.4	6.43	24.0
	20	51.0	88.7	38.6	6.50	23.5
	30	52.7	88.4	39.2	6.57	24.3
	40	51.8	88.5	38.8	6.62	24.2
SEM		1.95	3.83	0.7	0.02	0.82
Comparison						
R:C ratio		**	**	ns	***	***
BAFLOP levels		ns	ns	ns	***	ns
Interaction		ns	ns	ns	***	ns
Orthogonal polynomials						
BAFLOP (Linear)		ns	ns	ns	***	ns
BAFLOP (Quadratic)		ns	ns	ns	ns	ns
BAFLOP (Cubic)		ns	ns	ns	ns	ns

\*  $P < 0.05$ , \*\*  $P < 0.01$ , \*\*\*  $P < 0.001$ . R:C ratio, roughage to concentrate ratio; BAFLOP, banana flower powder. SEM, standard error of mean; ns, not significant.

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## Feed Intake and Blood Metabolite of Goats Fed Urea-Calcium Hydroxide Treated Oil Palm Frond

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### Abstract

This study was aimed to study the effects of urea-calcium hydroxide treated oil palm frond (UCOPF) in total mixed ration (TMR) on feed intake and blood metabolites of goats. Four male goats with an average initial weight of  $30.0 \pm 1.00$  kg were randomly assigned according to a  $4 \times 4$  Latin square design. Four dietary treatments containing 40% of fermented oil palm frond (FOPF), 5% urea treated oil palm frond (UOPF 5%), 5% calcium hydroxide treated oil palm frond (COPF 5%) and 2.5 + 2.5% urea-calcium hydroxide treated oil palm frond (UCOPF 2.5%) were used as main roughage sources. The diets were offered *ad libitum* in total mixed ration (TMR) at 40:60 ratio of roughage to concentrates (DM basis). The result revealed that voluntary feed intake, blood glucose and PCV of each goat were not significantly different among treatments. Ruminal pH was unchanged by dietary treatments, except FOPF was lower ( $P < 0.05$ ) than other treatments. The concentration of  $\text{NH}_3\text{-N}$  and BUN were found highest ( $P < 0.05$ ) in UOPF 5%. Based on this study, UCOPF at 40% in TMR diet did not affect on feed intake and blood metabolites of goats.

**Keywords:** oil palm frond, urea-calcium hydroxide, blood metabolite, goats, feed intake

### Introduction

Oil palm frond (OPF), a cheap and abundant by-product of the oil palm industry, particularly have been given emphasis lately with great potential to be utilized as a roughage source or as a component in complete feed for ruminants in many tropical countries such as Indonesia, Malaysia and Thailand. However, the use of OPF in livestock production is limited for their complex biological structure, low protein content, metabolizable energy values (Ishida and Abu Hassan 1997), and as up to 20-20.5% of their dry biomass is lignin contents (Abdul Khalil et al. 2006), thus resulting in low voluntary feed intake. Various treatment methods have been used to improve nutritive value of agricultural co-products such as rice and wheat straw including physical, biological and chemical treatments. It was found that using urea treatment could increase nutritive value of rice straw (Wanapat, 1994) and sugarcane bagasse (Ahme et al., 2013). However, the cost of urea treatment was remarkably expensive which resulted on higher cost of production. Similarly, Wanapat (1994) reported that the use of urea-treated (5%) rice straw with increased nutritive value especially protein content and fiber degradation but the cost was relatively high due to increasing price of urea. Fadel Elseed et al. (2003) suggested that



when amount of urea was reduced and combined with calcium hydroxide  $\text{Ca(OH)}_2$ , it could improve rumen digestibility. The concentrated alkaline agents can chemically break the ester bonds between lignin and hemicellulose and cellulose, and physically make structural fibres swollen (Wanapat et al., 2009). Therefore, the aim of this experiment was to determine effects of various treated OPF on feed intake and blood metabolite in goats.

## Materials and Methods

### Animals, treatments, and experimental design

Four male crossbred (Thai native x Anglo Nubian) goats at ages about 16 months old with  $30.0 \pm 1.00$  kg body weight were randomly assigned according to a 4x4 Latin square design to investigate the effects of urea-calcium hydroxide treated oil palm frond. Dietary treatments were as follows: fermented oil palm frond (FOPF), 5% urea treated OPF (UOPF 5%), 5%  $\text{Ca(OH)}_2$  calcium hydroxide treated OPF (COPF 5%), and 2.5 + 2.5% urea- $\text{Ca(OH)}_2$  treated OPF (UCOPF 2.5%). OPF was collected from the Faculty of Natural Resources, Prince of Songkla University, Hat Yai Campus, Songkhla, Thailand. OPF treatments were prepared by adding urea and  $\text{Ca(OH)}_2$  (as hydrated lime) according to respective ratio using 100 L of water to 100 kg air dry OPF. OPF was then packed in plastic container (50 L) for a minimum of 30 days before feeding to the animals. Four experimental diets consisting of 40:60 ratio of roughage to concentrates (DM basis) were offered *ad libitum* in a total mixed ration. The diets were formulated to provide the nutrient allowances to meet or exceed the NRC (1981) requirements of growing goats.

All goats were kept individually in pens (0.50x1.20m) under well-ventilated sheds where water and mineral salt were available at all time. The experiment was conducted for 4 periods, and each period lasted for 21 d. During the first 14 d of each period, all animals were fed by respective diets for *ad libitum* intake, whereas during the last 7 d, the animals were moved to metabolism crates for total collection during the time goats with restriction to 90% of the previous voluntary feed intake to ensure total feed intake. Feeds were provided twice times in two equal portions daily at 0800 and 1600 h. For determination of daily DMI, refusals were collected and weighed daily before feeding. Feed samples obtained each time were oven dried at  $60^\circ\text{C}$  for 72 h, grounded to pass through a 1-mm sieve, and composited by period on an equal weight basis, and analyzed for DM, ether extract, ash, and CP content (AOAC, 1995). Goats were individually weighed before the morning feeding at the beginning and ending of each experimental period. At the end of each period, rumen fluid was collected from all goats by using a stomach tube at 0 and 4 h-post feeding during the digestibility trial. This was strained through 4 layers of cheese cloth and pH measured immediately using a pH meter (HANNA instruminals HI 98153 microcomputer pH meter, Singapore) fitted with a combined electrode. The ruminal fluid was then acidified with 3 mL of 1 M  $\text{H}_2\text{SO}_4$  added to 30 mL of ruminal fluid. The mixture was centrifuged at  $16,000 \times g$  for 15 min, and the supernatant was stored at  $-20^\circ\text{C}$  before  $\text{NH}_3\text{-N}$  analysis by using the micro-Kjeldahl methods (AOAC, 1995). Blood samples (about 10 mL) were collected from a jugular vein (at the same time as ruminal fluid sampling) into tubes containing of 12 mg of EDTA. Plasma was separated by centrifugation at  $2500 \times g$  for 15 min at  $5^\circ\text{C}$  and stored at  $-20^\circ\text{C}$  until analysis. Plasma glucose and packed cell volume (PCV) were measured by using commercial kits (No. 640, Sigma Chemical Co., St. Louis, USA). All data were subjected to the analysis of variance by using Proc. GLM and treatment means were performed and compared by using Duncan's New Multiple Range Test a level of  $\alpha = 0.05$  to determine the significance between the treatments.



## Results and discussion

The results showed that (Table 1) overall mean of feed intakes of each goat in terms of total DMI (%BW, and g/kg BW<sup>0.75</sup>) were not significantly ( $P>0.05$ ) affected by dietary treatments, ranging from 1.05-1.13 kg/d and greater values for the goats fed COPF 5% was observed. However, Wanapat et al. (2009) who found that the treatment of urea 5.5% and 2.2% urea + 2.2% calcium hydroxide could increase ( $P<0.05$ ) dry matter intake (from 4.4 to 6.3 kg/h/day) and digestibility in dairy cows (from 49.5 to 61.6% DM) when compared with untreated rice straw. This could be due to the differences in animals and their physiological stage. Indeed, these effects may be related with smell and low palatability of OPF treated with urea and calcium hydroxide as compared with urea-treated rice straw. Similarly, Paengkom et al. (2006) reported that intake of OPF by goats increased quadratically, ( $P<0.01$ ) with increasing urea supplementation up to 30 g/kg OPF and thereafter, decreased ( $P<0.05$ ) with 40 and 50 g urea/kg OPF, probably due to low palatability of the diet containing a high concentration of urea.

**Table 1.** Effect of various treated oil palm frond on feed intake in goats.

ITEM	Treatment <sup>1</sup>				SEM <sup>2</sup>
	FOPF	5.0% UOPF	5.0% COPF	2.5% UCOPF	
Total DMI, kg/d	1.10	1.05	1.13	1.11	0.04
DMI, %BW	2.98	2.81	3.16	2.99	0.16
DMI, g/kg W <sup>0.75</sup>	73.52	69.39	77.09	73.08	3.69

<sup>1</sup>Treatment FOPF = Fermented oil palm frond, UOPF= Urea treated oil palm frond, COPF= calcium hydroxide treated oil palm frond, UCOPF= Urea-calcium hydroxide treated oil palm frond.

<sup>2</sup>SEM = Standard error of the mean (n=4).

DMI = Dry matter intake.

Table 2 presents the effect of UCTOPF on ruminal pH, NH<sub>3</sub>-N and blood metabolite. In current study, Ruminal pH, NH<sub>3</sub>-N, and BUN were found higher ( $P<0.05$ ) in the treated group as compared to the control. This increase was partially due to urea treatment enhanced its nitrogen content of OPF which contributed to the addition of nitrogenous substrate (Ahmed et al., 2013). These results were similar to previous work by Polyorach and Wanapat (2014) reported that higher in the treated group as compared to the control. Ruminal pH and NH<sub>3</sub>-N ranging from 6.49 to 6.72 and 15 to 30 mg/dl were reported an optimal range for the improvement of fermentation, microbial growth, and feed intake in ruminants fed urea-treated rice straw (Wanapat and Pimpa, 1999). Furthermore, BUN of goats consumed treated OPF ranged from 14.39 to 27.53 mg/dl, which was reported in the normal range in normal goats, which has been reported in the range of 11.2 to 27.7 mg/dL (Lloyd, 1982). No significance ( $P>0.05$ ) of by dietary treatments was detected for blood glucose and PCV and all were within the normal ranges 50-75 mg/dL and 22-38 mg/dl, respectively (Lloyd, 1982).

## Conclusions

In conclusion, treatment of OPF with urea and/or Ca(OH)<sub>2</sub> had no effects on feed intake and blood metabolites of goats but could improve rumen fermentation mainly ruminal pH, NH<sub>3</sub>-N, and BUN. However, further researches on feeding trial of treated OPF are recommended to investigate its effects on animal performances and production such as meat and milk.

**Table 2.** Effect of various treated oil palm frond on ruminal pH, NH<sub>3</sub>-N and blood metabolites in goats.

Items	Treatments <sup>1</sup>				SEM <sup>2</sup>
	FOPF	5.0% UOPF	5.0% COPF	2.5% UCOPF	
Ruminal pH					
0 h-post feeding	6.59 <sup>b</sup>	6.69 <sup>ab</sup>	6.732 <sup>ab</sup>	6.94 <sup>a</sup>	0.08
4 h-post feeding	6.38	6.53	6.51	6.50	0.08
Mean	6.49 <sup>b</sup>	6.61 <sup>ab</sup>	6.62 <sup>ab</sup>	6.72 <sup>a</sup>	0.04
NH <sub>3</sub> -N mg/dL					
0 h-post feeding	12.14 <sup>b</sup>	17.50 <sup>a</sup>	11.43 <sup>b</sup>	13.57 <sup>b</sup>	0.91
4 h-post feeding	12.86 <sup>c</sup>	22.86 <sup>a</sup>	13.21 <sup>c</sup>	16.43 <sup>b</sup>	0.64
Mean	12.50 <sup>c</sup>	20.18 <sup>a</sup>	12.32 <sup>c</sup>	15.00 <sup>b</sup>	0.60
BUN, mg/dL					
0 h-post feeding	14.74 <sup>b</sup>	27.04 <sup>a</sup>	14.00 <sup>b</sup>	24.92 <sup>b</sup>	0.96
4 h-post feeding	14.81 <sup>b</sup>	28.01 <sup>a</sup>	14.79 <sup>b</sup>	25.31 <sup>a</sup>	0.92
Mean	14.78 <sup>b</sup>	27.53 <sup>a</sup>	14.39 <sup>b</sup>	25.12 <sup>a</sup>	0.90
Glucose, mg/dL					
0 h-post feeding	62.25	64.75	60.00	63.00	1.28
4 h-post feeding	61.50 <sup>b</sup>	64.25 <sup>ab</sup>	62.50 <sup>ab</sup>	65.00 <sup>a</sup>	0.91
Mean	61.88	64.50	61.25	64.00	0.92
PCV, %					
0 h-post feeding	29.00 <sup>a</sup>	27.00 <sup>b</sup>	27.75 <sup>ab</sup>	26.50 <sup>b</sup>	0.40
4 h-post feeding	26.00	26.25	27.00	25.75	1.21
Mean	27.50	26.62	25.37	26.12	0.76

<sup>a-c</sup>Means within a row were compared which were significantly ( $P < 0.05$ ) different.

<sup>1</sup>Treatment FOPF = Fermented oil palm frond, UOPF= Urea treated oil palm frond, COPF= calcium hydroxide treated oil palm frond, UCOPF= Urea-calcium hydroxide treated oil palm frond.

<sup>2</sup>SEM = Standard error of the mean (n=4).

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## Blood Chemistries of Dairy Cow During Pre-Calving, at Calving and Post Calving Period

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### Abstract

The aim of this research was to evaluate blood chemistries in dairy cows during 1 week pre-calving, calving and 1 week post-calving. Twenty clinically healthy Holstein dairy cows from the faculty's farm had blood collected and were given concentrate feed two times a day (1.3% BW per day). Animals were fed rice straw ad libitum for 1 week before expected calving. Post calving dairy cows were offer total mixed ration (TMR) feed. The TMR consisted of concentrate to roughage (rice straw) at 70:30. The blood samples collection from coccygeal vessel were done by using heparinized vacutainer tubes. All biochemical variables ( $\beta$ -hydroxybutyrate, BHB; non esterified fatty acids, NEFA; total proteins, TP; albumin, ALB; glucose, GLU; blood electrolytes, BE; calcium, Ca; and blood urea nitrogen, BUN) were determined using automated analyzers. There were significant lower means of the NEFA and BHB in the pre-calving as compared to calving and post-calving periods ( $P < 0.05$ ), while others biochemical variables did not differ among the time relative to calving. High prevalence of elevated NEFA and BHB during dry period through first 1 weeks of post-calving indicated that periparturient dairy cows suffered some degree of negative energy balance and ketosis. These resulted indicated that dairy cows during periparturient in this study suffered with negative energy balance and ketosis. Blood metabolites, namely NEFA and BHB, were suitable used for indicators to predict dairy cow health during 1 week pre-calving, calving and 1 week post-calving

**Keywords:** serum metabolites, periparturient, pre-calving, post-calving, dairy cow

### Introduction

The use of blood metabolites for herd-level health assessment during the period around calving (transition period) has been an area of study for many years (Ingraham and Kappel, 1988; Oetzel, 2004). Blood metabolites profiles can be used not only to detect sick animals, but also to detect herd health (Ingraham and Kappel, 1988).

Dry matter intake decreases around parturition, whereas energy demands for lactation increases, these results in negative energy balance (NEB) (Herdt, 2000). Measures of NEFA and BHB in blood of dairy cow during transition period are useful for detection of cow health. Increased concentrations of NEFA and BHB in cow serum during transitional indicated that cow increased risk of disease (Suthar et al., 2013).

Aspartate aminotransferase (AST) is liver enzyme which release to blood steam when the liver metabolism has changed. Changing in liver metabolism caused from high productivity. Ketosis exceeded during first 2 weeks postpartum in high production dairy cow caused to hepatic cell damage and resulting to higher level of serum AST (Meikle et al., 2004).



The objective of this research was to evaluate blood chemistries metabolites to predict disease in dairy cow during 1 week pre-calving, calving and 1 week post-calving.

### Materials and methods

Data were collected from randomly selected Holstein crossbred dairy cows on the faculty farm. Twenty clinically healthy cows were used as animal subjects. Blood samples were collected weekly from each cow, during transitional period (1 week before expected calving to 1 week postpartum). The blood samples collection from coccygeal vessel were done by using of heparinized vacutainer tubes. Blood samples were centrifuged immediately (5000 rpm, at 4 °C for 15 minutes) to obtain plasma. Plasma samples were stored at -20°C until analysis for NEFA, BHB, total proteins (TP), albumin (ALB), glucose (GLU) blood electrolytes (BE), calcium (Ca) and blood urea nitrogen (BUN) using automated analysers (Vitallab Flexor E).

Statistical analyses (the GLM procedure) by SAS were performed. The significance level was declared at  $P < 0.05$ . NEFA concentrations  $> 0.40$  mmol/L indicate problems with energy balance (Oetzel, 2004).

### Results and discussion

Concentration of TP, ALB, GLU, BE, Ca and BUN did not vary during 1 week prepartum (Table 1) throughout 8 week postpartum. There were significant differences in the comparison of means of NEFA and BHB among the pre-calving, calving and post-calving periods ( $P < 0.05$ ). NEFA and BHB values were significantly lower ( $P < 0.05$ ) at the 1 week before calving than the BHB values at calving date or the 1 week post-calving.

**Table 1.** Comparison of blood metabolites determined in 1 week pre- , calving and 1 week post-calving healthy cows

Parameter	Weeks relative to calving			P value
	-1	0	1	
Non-esterified fatty acids, mmol/l	0.8 <sup>a</sup>	1.3 <sup>b</sup>	1.0 <sup>ab</sup>	*
Beta-hydroxybutyrate, mmol/l	0.7 <sup>a</sup>	0.9 <sup>b</sup>	0.8 <sup>ab</sup>	*
Total proteins, g%	6.9	6.5	6.4	ns
Albumin, g%	3.3	2.3	3.1	ns
Glucose, mg%	48.5	50.5	50.8	ns
Blood urea nitrogen, mg%	16.5	17.8	16.3	ns
Creatinine	1.6	1.5	1.1	ns
SGOT (AST)	78.4	81.0	80.0	ns
SGPT (ALT)	7.1	6.0	6.0	ns
ALP Alkaline phosphatase	65.6	70.5	59.3	ns
Blood electrolytes, mEq/l	143.0	143.6	141.0	ns
Na	4.6	4.3	4.8	ns
K	107.1	106.0	106.5	ns
Cl	7.7	9.0	8.3	ns
Calcium, mg%	6.4	5.0	6.0	ns
Phosphorus, mg%	20.0	16.0	18.3	ns

\*significant at  $P < 0.05$ , ns= non-significant at  $P < 0.05$

Serum BHB concentrations above 1.2 mmol/L indicated that cow suffer with subclinical ketosis, while clinical ketosis is associated with BHB concentrations above 2.6 mmol/L



(Duffield, 2000; Oetzel, 2004). In recent studies, concentration of NEFA in blood plasma of cow at 1 week before calving throughout 1 week post-calving were higher than the above reference values. Higher level of NEFA could indicate negative energy balance (NEB). NEFA are derived from lipolysis, which occurs during energy deprivation; thus high NEFA in dairy cows are a biological indicator of NEB. The NEB could be due to low feed intake and high milk production during transitional period, resulting in mobilizing energy reserves. Concentration of NEFA in blood plasma indicated the rate of fat mobilization. The fat mobilization occurred when cow suffer with negative energy balance (Ingvarsten, 2006).

## Conclusion

These results indicated that dairy cows during periparturient in this study were suffering with NEB and ketosis. Blood metabolites, namely NEFA could be used as blood chemistry indicators to predict disease in dairy cow during 1 week pre-calving, calving and 1 week post-calving.

## Acknowledgment

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## The Study on Determination of Feed Digestibility Using Frequency of Defecation on Thin Tailed Lamb

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### Abstract

The purpose of this study was to evaluate the relationship between consumption and frequency of defecation as well as the frequency of defecation and digestibility on thin tailed lambs. This study used 32 lambs aged 3 months old with an average body weight of  $13.69 \pm 0.70$  kg (CV=17.76). Fed with pelleted complete feed and was ad libitum. Frequency of defecation and digestibility were analyzed by correlation regression analysis. The correlation between feed intake and defecation frequency have medium ( $r=0.59$ ) positive correlation and significant ( $P<0.05$ ). The correlation between digestibility and frequency of defecation have strong ( $r=0.76$ ) negative correlation and significant ( $P<0.05$ ). The data showed frequency of digestibility could be used to observed digestibility.

**Keywords:** digestibility, consumption, defecation frequency and thin tailed lambs

### Introduction

Lambs are in the phase of growing, especially in the digestive track. One of important factors in fattening Lambs is feed. The good quality of feed can be obtained from different sources that have different nutritional contents as well. digestibility is the most widely used method of feed evaluation and a practical way of formulating rations (Sauer and Ozimek, 1986). The total collection method is usually used to evaluate the digestibility (Peiretti et al., 2006). This commonly used way is still not practical in its application because it spends a lot of work and time. Therefore, it is necessary to study the frequency of defecation to the dry matter digestibility rate in livestock in this case the lamb, where can be estimate if digestibility level to be low it has high frequency defecation rate. Defecation of animals describes undigested nutrient in digestive track of animals (Owens et al., 2010). The purpose of this study is to evaluate the correlation between feed intake, frequency of defecation and the level of digestibility in lambs.

### Materials and Methods

This study used 32 heads of lambs aged 3 months old and an average of body weight of  $13.69 \pm 0.70$  kg (CV=17.76). The animals were given complete feed in pellet form and raised in individual cage. Parameters of this study were monitored and recorded manually every five minutes for 3x24 hours. Data were observed in three days. Collected data were averaged and

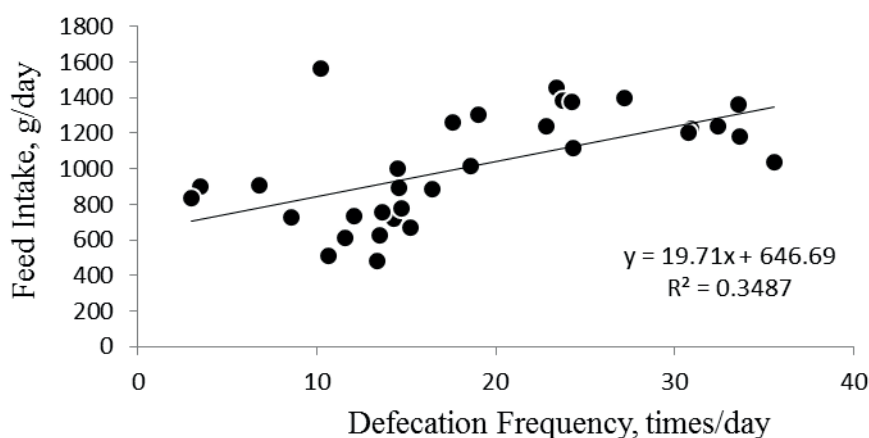


expressed in minutes time day. The dietary contained 14%, 16% and 18% of crude protein and 60% and 70% of TDN.

Frequency of defecation and digestibility were analyzed by correlation regression analysis. The correlation coefficient was evaluated by value described with 0.00-0.19 (very low), 0.20-0.39 (low), 0.40-0.59 (medium), 0.60-0.79 (strong) and 0.80-1.00 (very strong) (Sugiono, 2008). The equation of regression were evaluated by T test. The less difference value and statistically significant, the strong accuracy will get.

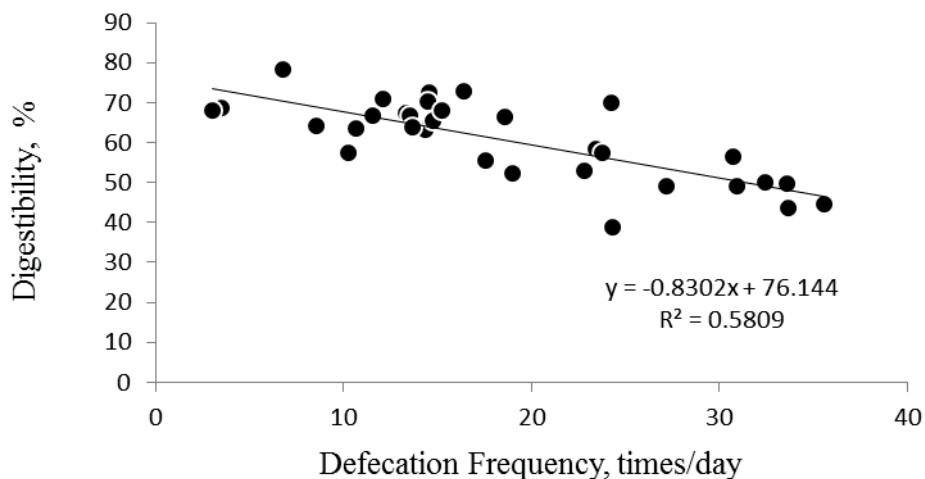
## Result and Discussion

### The relationship between feed intake and defecation frequency



**Figure 1.** Relationship between Consumption and Frequency of Defecation

The correlation between feed intake and frequency of defecation was shown in Figure 1. The correlation between feed intake and frequency of defecation is positive and medium ( $r = 0.59$ ;  $P < 0.05$ ). This relationship indicates that the increasing value of feed intake impact on increasing the frequency of defecation. It is due to high feed intake, the feed will be pressed in the rumen so that the feed will quickly be pressed to enter the next digestive tract. Lee et al. (2010) showed that the higher frequency of defecation, the higher feed intake. Kim et al. (2013) claimed the increasing of feed intake will increase the frequency of defecation.



**Figure 2.** Correlation between Frequency of defecation and dry matter digestibility

### The relationship between digestibility and defecation frequency

The correlation between the dry matter digestibility and the frequency of defecation is shown in Figure 2. The correlation shows negative results with strong relation ( $r = 0.76$ ;  $P < 0.05$ ). This relationship shows that the higher the frequency of defecation, the lower the digestibility. This is due to the high consumption value will have an impact on the high feed rate because of pressure, so that feed is not digested in the rumen to the maximum and reduce the level of digestibility. This will have an impact on increasing the frequency of defecation. Although most of the feed undergoes fermentation, small amount may pass unchanged through the rumen into the omasum and abomasum (Rounds and Dennis, 2012). Ki et al. (2003) said the level of feed digestibility will decrease due to retention by microbes in the rumen too short.

### Conclusion

Based on the result, it can be inferred that frequency of defecation can be used to evaluate the digestibility in lambs fed complete feed. It may suggested that frequency of defecation is a good alternative to estimating digestibility in lambs fed complete feed.

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## Effect of *Terminalia Chebula* RETZ. Meal on Nutrient Intake, Digestibility and Microbial Population of Goats

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### Abstract

Eight, male crossbred (Thai Native×Anglo Nubian) goats with 13±3 kg of body weight (BW) were randomly assigned according to double 4×4 Latin square design. The dietary treatments were supplementation of *T. chebula* meal at 0%, control; 0.8%, 1.6% and 2.4% of total DM intake. All animals were fed rice straw *ad libitum* while additional concentrate was fed at 1.3% body weight daily. The results showed that nutrient intake (DM, OM, CP, NDF and ADF) was similar among treatments ( $P>0.05$ ). Apparent digestibility of CP, NDF and ADF were increased in goats when supplemented with *T. chebula* meal at 0.8% of total DM intake ( $P<0.05$ ). In the other hands, the numbers of bacteria and fungi zoospore were not affected by the addition of *T. chebula* meal. While, the population density of protozoa in the rumen was decreased when supplemented with *T. chebula* meal ( $P<0.05$ ). Based on this experiment, it could be concluded that supplementation of *T. chebula* meal at 0.8% of total DM intake resulted in improving digestibility and reduced protozoal populations without affected on nutrient intake of goats.

**Keywords:** *Terminalia chebula* RETZ. , nutrient intake, digestibility, microbial population

### Introduction

*Terminalia chebula* Retz. (samor-Thai) is one kind of Thai herb which contains condensed tannins and saponins about 8.4 and 9.9% dry matter (DM) (Anantasook et al., 2016). Patra et al. (2006) reported that *T. chebula* have potential use for manipulating rumen fermentation. A previous study found that *T. chebula* pulp has a potential to be used for mitigation of enteric methane production in *in vitro* fermentation (Anantasook et al., 2016). In addition, Patra et al. (2006) found that total protozoa counts were significantly decreased with supplementation of *T. chebula* at 0.50mL. However, little information is available on the use of *T. chebula* in animal diets. Therefore, the aim of this experiment was to evaluate the effect of *T. chebula* meal on nutrient intake, digestibility and microbial population of goats *in vitro* gas technique.



## Materials and Methods

### Animals, diets and experimental design

Eight, male crossbred (Thai Native×Anglo Nubian) goats with  $13\pm 3$  kg of body weight (BW) were randomly assigned according to double  $4\times 4$  Latin square design. The dietary treatments were *T. chebula* meal supplementation at 0, 0.8, 1.6 and 2.4% of DM feed intake. The concentrate was formulated to be at 14.0% crude protein (CP) and 2.3 Mcal/ME (NRC, 1981) (Table 1). Animals were fed the concentrate diets at 1.3% BW and rice straw as a roughage source was fed *ad libitum*. Goats were housed individually in ventilated pens with wooden slotted flooring in an open goat barn raised above the ground. Clean fresh water and feed blocks were available at all times.

### Data collection and chemical analysis

The experiment was run in four periods, each period lasted for 21 days, the first 14 days for treatment adaptation and feed intake measurements whilst the last 7 days was for sample collection of feeds and fecal. Feeds, refusals and faecal samples were dried at 60 °C and ground (1 mm screen using the Cyclotech Mill, Tecator, Sweden) and analysed using the standard methods of AOAC (1997) for DM, ash and CP content, neutral detergent fiber (NDF) and **acid detergent fiber** (ADF) (Van Soest et al., 1991).

At the end of each period, approximately 60 ml of rumen fluid were collected at 0 and 4 h post-feeding by a stomach tube connected to a vacuum pump. Rumen fluid samples were then strained through four layers of cheesecloth. About 1 ml rumen fluid was collected and 9 ml of 10% formalin solution (1:9 v/v, rumen fluid: 10% formalin) was added and stored at 4 °C for measuring bacteria, protozoa and fungal zoospores according to the method of Galyean (1989).

### Statistical analysis

The data were analysed in a double  $4\times 4$  Latin square design by analysis of variance run in the GLM Procedure (SAS, 1998). Differences between treatment means were determined by Duncan's New Multiple Range Test (Steel and Torrie, 1980). Significant effects were identified at  $P<0.05$ .

## Results and Discussions

Experimental feed and their chemical compositions are shown in Table 1. The mixture of concentrate contained 14.0% CP and 2.3 Mcal/ME. The *T. chebula* meal contained 8.4 and 9.9% of condensed tannins (CT) and saponins (SP), respectively. However, the actual levels of CT recovered were below target, 1.4 and 2.3% DM, respectively. The effect of *T. chebula* meal supplementation on nutrient intake are presented in Table 2. The result showed that there were no effects of *T. chebula* meal supplementation on DM, OM, CP, NDF and ADF intake among all treatments. In contrast, apparent digestibility of CP, NDF and ADF were increased when supplemented with *T. chebula* meal at 0.8% of total DM intake ( $P<0.05$ ). Patra et al. (2006) also indicated that DM and OM digestibility were significantly suppressed by the addition of *T. chebula* at about 6%. A depression in feed degradability by extracts of *T. chebula* could be due to phenolic compounds such as tannins, gallic acids, ellagic acids and tannic acids. Tannins have been implicated for their inhibitory effect on feed digestion in many experiments (Hristov et al., 2003). Microbial population in the rumen of goats fed with treatment diets are shown in Table 2.





The total bacteria and fungi zoospore were not affected by the addition of CT in *T. chebula*. While, the population density of protozoa in the rumen was decreased when supplemented with *T. chebula* meal ( $P < 0.05$ ). Similarly, Patra et al. (2006) reported that total protozoa counts were significantly decreased with supplementation of *T. chebula* at 0.50 mL; also, entodiniomorphs are also tended to reduce.

## Conclusion and Recommendations

Based on this experiment, it could be concluded that supplementation of *T. chebula* meal at 0.8% of total DM intake resulted in improving digestibility and reduced protozoal populations. Further research should be study on rumen fermentation and methane production.

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## Effect of Bamboo Grass Pellet (Bamboo-Cass) Levels on Gas Production Kinetics and *In vitro* Degradability

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### Abstract

The objective of this study was to evaluate the effects of roughage to concentrate (R:C) ratio and levels of bamboo grass pellet (Bamboo-cass) on *in vitro* gas production and digestibility. Rumen fluid was collected from two rumen-fistulated dairy steers fed on rice straw-based diet with concentrate supplement to maintain normal rumen ecology. The treatments were arranged according to a 3 × 5 Factorial arrangement in a Completely randomized design. First factor was differences of R:C ratio (100:0, 70:30, and 30:70 ratio) and the second factor was Bamboo-cass supplementation at 1, 2, 3, and 4% of dietary substrate, respectively. Under this investigation, R:C ratio increased gas production kinetics ( $P < 0.001$ ) while Bamboo-cass supplementation did not ( $P > 0.05$ ). In addition, supplementation of Bamboo-cass increased *in vitro* digestibility ( $P < 0.001$ ). Based on this study, it could be concluded that supplementation of Bamboo-cass increased *in vitro* degradability. It is suggested that R:C ratio at 70:30 with Bamboo-cass at 4g/kg of total dietary substrate could improve the *in vitro* degradability.

**Keywords:** bamboo grass pellet, roughage to concentrate ratio, *in vitro* digestibility, gas kinetics

### Introduction

Rice straw is a common roughage source for ruminants despite its low in crude protein and high in neutral-detergent fiber (NDF). In Asian region, there are many local feed resources which can be used as feeds to ruminants by improving rumen ecology and hence ruminant productivity (Devendra and leng, 2011; wanapat, 1990).

Currently, natural products are highly important as feed additives to response in animal nutrition and livestock production issue (Wanapat et al., 2000). Feed additive is a potential way to improve nutrients utilization by modifying microbial population, fermentation, and digestion in the rumen (McGuffey et al., 2001). A number of plants containing plant secondary compound have been reported affect rumen ecology (Busquet et al., 2006) Bamboo grass or *Tiliacora Triandra* is a climbing plant with deep green leaves and yellowish flowers. Bamboo grass contains high levels of flavonoids (Condensed tannins, CT and saponins, SP), beta-carotene and minerals (Singthong et al., 2009). However, there were no reports about these feed resources containing secondary plant compounds which are affected to rumen ecology, fermentation, and productivities. Therefore, this study was to investigate the effect of Bamboo-cass on gas production and digestibility using *in vitro* gas technique.



## Materials and Methods

### Dietary substrate, treatments, and experimental design

Fresh bamboo grass was collected in Khon Kaen province, Thailand and was sun-dried for 3 or 4 days. Bamboo-cass was formulated according to the respective ingredients shown in Table 1. All pellet ingredients were ground to pass a 1-mm screen using the Cyclotech Mill (Tecator, Höganäs, Sweden) and mixed well with water at ratio of 0.5:1 (water: pellet meal). All treatments were arranged according to a  $3 \times 5$  Factorial arrangement in a Completely randomized design (CRD). Factor A was different roughage to concentrate ratio (R:C) at 100:0, 30:70 and 70:30, while factor B was different levels of Bamboo-cass supplementation (0, 1, 2, 3 and 4 % of dietary substrate).

Dietary samples were dried at 60 °C, ground to pass a 1mm sieve (Cyclotech Mill, Tecator, Sweden), and kept for chemical composition analysis and *in vitro* gas study. All samples were analyzed for dry matter (DM), ash, crude protein (AOAC, 2012), and neutral detergent fiber (NDF) and acid detergent fiber (ADF) Van Soest et al., 1991). Condensed tannins (CT) were estimated by the vanillin-HCl method modified by (Wanapat and Pongchompu, 2001) and saponins were measured by using method extraction (Kwon et al., 2003).

### Rumen and substrate inocula

Two rumen-fistulated dairy steers ( $300 \pm 15$  kg BW) were used as source of rumen inocula, and 1000 ml of rumen fluid was collected before morning feeding. The method in this study was based on the technique described by Menke et al. (1979).

*In vitro* degradability: At 12 and 24 h post inoculation, *in vitro* digestibility was measured based on the following equation according to Van Soest et al. (1991): True digestibility (TD) = ((DM of feed taken for incubation - NDF residue)  $\times$  100)/DM of feed taken for incubation.

### Medium solution preparation

Medium solution of 4000 ml was prepared for 116 bottles per each run (Makkar et al., 1995). Reducing medium (4000 ml) consists of 1900 ml distilled water, 960 ml rumen buffer solution (35.0 g NaHCO<sub>3</sub> and 4 g NH<sub>4</sub>HCO<sub>3</sub> made up to 1 l with distilled water), 960 ml macro-mineral solution (6.2 g KH<sub>2</sub>PO<sub>4</sub>, 5.7 g Na<sub>2</sub>HPO<sub>4</sub>, 2.22 g NaCl, and 0.6 g MgSO<sub>4</sub> · 7H<sub>2</sub>O made up to 1 l with distilled water), 0.48 ml micromineral solution (10.0 g MnCl<sub>2</sub> · 4H<sub>2</sub>O, 13.2 g CaCl<sub>2</sub> · 2H<sub>2</sub>O, 1 g CoCl<sub>2</sub> · 6H<sub>2</sub>O, 8.0 g FeCl<sub>3</sub> · 6H<sub>2</sub>O and made up to 100 ml with distilled water), 4.88 ml resazurine (0.1 g made up to 100 ml with distilled water), and 198 ml freshly prepared reduction solution (190 ml distilled water, 1344 mg Na<sub>2</sub>S · 9H<sub>2</sub>O, and 8 ml 1 M NaOH). Rumen fluid was mixed with the reducing medium at ratio of 2:1 (reducing medium: rumen fluid). The mixture was kept stirred under CO<sub>2</sub> pumping at 39 °C using a magnetic stirrer fitted with a hot plate. The portion of 30 ml medium solution was transferred into each bottle and incubated in the water bath at 39 °C.

### Samples collection and chemical analysis

Gas production kinetics: During the incubation, gas production was recorded at 1, 2, 4, 6, 8, 12, 24, 48, 72, 96 and 120 h by extraction using glass syringes. Cumulative gas production data were fitted to the model of Orskov and McDonal (1979).

### Statistical analysis

All the obtained data were subjected to General Linear Model (GLM) procedures of SAS (2013) according to a  $3 \times 5$  factorial arrangement in a completely randomized design. Differences among treatment means were contrasted by the Tukey's multiple comparison test (Crichton, 1999).



## Results and discussion

Gas production from the immediately soluble fraction ( $a$ ,  $b$ ,  $c$ ,  $a+b$  and cumulative gas production at 120 h) were not affected by the dietary supplementation while gas production from the ratio of roughage to concentrate were increased by high ratio of concentrate (Table 2;  $P < 0.001$ ). These results were in agreement with Anantasook and Wanapat (2012), who reported that supplementation of rain tree pod meal containing condensed tannins and saponins at 4, 8 and 12 mg/0.2 g DM had no effect on cumulative gas production. In contrast, Paengkoum et al. (2015) reported that cumulative gas production was decreased by supplementation of mangosteen peel with condensed tannins at 2–6% DM. Vieira and Borba (2011) also suggested that supplementation of quebracho tannins at 2.5% and 5% DM suppressed cumulative gas production. The difference in the effect of condensed tannins on cumulative gas production and rumen fermentation characteristics could be attributed to the substrate used in the incubation. On the other hand Bamboo-cass supplementation increased DM and NDF *in vitro* digestibility. It could be explained that CT and SP contents and mineral elements in Bamboo-cass could enhance microbial activity in the rumen (Kang and Wanapat 2013).

## Conclusion

Based on this study, it could be concluded that gas production kinetics were highly influenced by ratio of R:C ( $P < 0.01$ ). Whilst DM and NDF *in vitro* degradabilities were significantly affected by R:C ratio, and Bamboo-cass at 24h. The highest values were found at higher level of R:C and Bamboo-cass supplementation.

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**Table 1.** Feed ingredient and chemical composition of experiment diets

Items	Concentrate	Rice straw	Bamboo-cass
<b>Ingredients %</b>			
Cassava chip	65.0	-	-
Rice bran	13.7	-	-
Coconut meal	15.0	-	-
Palm kernel meal	9.0	-	-
Urea	2.5	-	-
Molasses	3.0	-	-
Sulfur	0.5	-	-
Salt	1.0	-	-
Mineral premix	1.0	-	-
<b>Chemical composition</b>			
Dry matter (%)	87.5	90.0	87.0
	.....% dry matter.....		
Crude protein	14.8	2.2	16.4
Organic matter	94.2	96.4	94.5
Neutral-detergent fiber	28.9	71.7	39.9
Acid-detergent fiber	17.2	47.7	31.8
Condensed tannins	-	-	2.3
Crude saponins	-	-	1.3



**Table 2.** Effect of R:C ratio and Bamboo-cass supplementation on gas production kinetics using *in vitro* technique

Trts	R:C <sup>a</sup>	Bamboo-cass <sup>b</sup>	Gas production kinetics <sup>c</sup>				Gas <sup>d</sup>
			<i>a</i>	<i>b</i>	<i>c</i>	<i>a+b</i>	
T1	100:0	0	2.3	32.8	0.03	35.1	35.1
T2	100:0	1	1.1	30.2	0.02	31.3	31.8
T3	100:0	2	2.1	36.5	0.03	37.2	37.2
T4	100:0	3	1.7	36.9	0.06	36.1	38.6
T5	100:0	4	1.5	26.4	0.04	27.9	27.9
T6	70:30	0	2.6	33.7	0.06	36.3	36.4
T7	70:30	1	1.7	32.9	0.07	34.0	34.0
T8	70:30	2	2.1	32.8	0.08	34.9	35.1
T9	70:30	3	3.7	30.5	0.07	34.3	34.3
T10	70:30	4	3.7	34.2	0.06	37.5	37.5
T11	30:70	0	3.3	46.3	0.14	43.3	43.4
T12	30:70	1	-2.9	46.5	0.14	43.1	43.2
T13	30:70	2	-3.4	45.7	0.14	42.5	42.6
T14	30:70	3	-3.3	41.5	0.11	39.6	38.3
T15	30:70	4	-4.7	44.5	0.17	39.8	39.9
SEM			0.394	1.12	0.0032	0.651	0.17
Comparison							
R:C ratio			**	**	**	**	**
Bamboo-cass			ns	ns	ns	ns	ns
Interaction			ns	ns	ns	ns	ns

\*\* P<0.01. <sup>a</sup> Roughage : Concentrate ratio at 100;0,70:30, and 30:70

<sup>b</sup> Bamboo-cass levels at 0, 1,2,3, and 4 g/kg of total substrate

<sup>c</sup> *a*, the gas production from the immediately soluble fraction; *b*, the gas production from the insoluble fraction; *c*, the gas production rate constant for the insoluble fraction; *a+b*, the gas potential extent of gas production

<sup>d</sup> Cumulative gas production at 120 h (ml/0.2 g DM substrate)

SEM, standard error of mean; ns, not significant (P>0.05).



**Table 3.** Effect of roughage to concentrate ratio and Bamboo-cass supplement on *in vitro* degradability

Trts	R:C <sup>a</sup>	Bamboo-cass <sup>b</sup>	<i>In vitro</i> degradability (%)			
			12 h		24 h	
			DM	NDF	DM	NDF
T1	100:0	0	24.4	11.2	29.4	13.3
T2	100:0	1	25.2	11.5	29.6	13.9
T3	100:0	2	26.4	11.5	29.8	14.1
T4	100:0	3	26.4	11.8	30.3	14.7
T5	100:0	4	27.0	11.8	30.5	14.9
T6	70:30	0	34.3	21.3	40.5	23.6
T7	70:30	1	34.7	21.5	40.5	23.8
T8	70:30	2	34.8	21.9	43.1	24.0
T9	70:30	3	35.6	21.7	43.8	24.3
T10	70:30	4	35.0	21.8	46.4	24.5
T11	30:70	0	54.5	26.7	62.2	39.8
T12	30:70	1	55.3	27.2	63.3	40.3
T13	30:70	2	56.0	27.7	63.6	41.7
T14	30:70	3	57.5	28.0	63.5	42.8
T15	30:70	4	57.7	28.0	63.6	42.7
SEM			0.191	0.029	0.099	0.017
Comparison						
R:C ratio			***	ns	***	***
Bamboo-cass			***	ns	***	***
Interaction			**	ns	***	***

\*\* P<0.01, \*\*\* P<0.001. <sup>a</sup> Roughage : Concentrate ratio at 100;0,70:30, and 30:70

<sup>b</sup> Bamboo-cass levels at 0, 1,2,3, and 4 g/kg of total substrate.

SEM, standard error of mean; ns, not significant.





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## Effect of Fresh Cassava Root and Feed Block Containing High Sulfur on Gas Kinetics and Rumen Fermentation Using *In Vitro* Gas Production Technique

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### Abstract

The aim of this study was to evaluate the effect of fresh cassava root and feed block containing high sulfur (FBS) on gas kinetics, rumen fermentation and digestibility of nutrients using *in vitro* gas production technique. The experimental design was a 4×2 Factorial arrangement in a completely randomized design (CRD), with three replications per treatment. Factor A was fresh cassava root and roughage ratio at 100:0, 60:40, 40:60 and 0:100 and used as substrate at 488 mg in the inoculum. Factor B was feed block containing high sulfur (FBS) at 2 and 4% and supplement in substrate at 12 mg. Cumulative gas production (96 h) was influenced by different ratio of fresh cassava root and rice straw. In addition, FBS containing sulfur supplementation at 4% could increase cumulative gases when compared to 2% groups. The rate of gas production (b) and (c) were significant different ( $P < 0.01$ ) among ratio of fresh cassava root and rice straw while level of sulfur in feed block were not altered on these parameters. Ammonia-nitrogen concentration were significant different ( $p < 0.01$ ) among treatment which was reduced when decreasing levels of cassava root. *In vitro* dry mater digestibility, NDF and ADF digestibility were significantly different ( $p < 0.01$ ) and was increased when increasing ration of fresh cassava root to rice straw. Furthermore, inclusion of sulfur at 4% in feed block was significantly higher *in vitro* dry mater digestibility when compared to 2% sulfur. In addition, levels of sulfur in feed block were not significantly different in total VFA and VFA profiles ( $P > 0.05$ ) except propionic acid which was higher in 4% of sulfur than those of other groups. Similarly, increasing level of fresh cassava root in the ration could increase concentration of propionic acid ( $P < 0.05$ ). In conclusion, using of fresh cassava root with feed block containing high sulfur 4% could enhanced kinetics of gas, propionic acid concentration and *in vitro* digestibility.

**Keywords:** fresh cassava root, sulfur, feed block, rumen fermentation, gas production

### Introduction

Cassava (*Manihot esculenta*, Crantz) is a major crop which importance in the tropics. It is grown mainly by smallholder farmers within existing farming systems primarily for the starchy root which is used for human food or as an energy source for non-ruminant or ruminant livestock feed (Khang and Wiktorsson, 2005; Khampa et al., 2006; Wanapat and Khampa, 2007). Use of fresh cassava root (CR) as an energy supplement is attractive in ruminant diets, because of its low cost compared with cassava chip, and the farmers can grow cassava in their area (Cherdthong et al., 2011). However, feeding of CR in ruminant is limited since it contains a high of hydrocyanic acid (HCN) which is responsible for chronic toxicity. Nguyen et al. (1997) reported that CR contains HCN at about 114 mg/kg fresh basis. In ruminants, HCN can be



rapidly detoxified by rhodanese and  $\beta$ -mercaptopyruvatesulfurtransferase by rumen microbes. Rhodanese is a sulfur transferase that catalyzes the deformation of cyanide and thiosulphate or other suitable sulfur donor to less toxic thiocyanate which is excreted in the urine. The main source of sulfur for HCN detoxification is sulfur amino acid, cysteine and methionine or elemental sulfur (S).

Feed block has been developed to contain local feed ingredients, particularly those from different energy sources (e.g. molasses, rice bran), essential minerals (S, Na, P) and non-protein nitrogen (NPN) source. Cherdthong et al. (2014) demonstrated that supplementation of feed block improved feed intake, N utilization, and blood biochemistry in Thai native beef cattle fed on rice straw.

Therefore, the aim of this study was to evaluate the effect of fresh cassava root with feed block containing high sulfur (FBS) on gas kinetics, rumen fermentation and digestibility of nutrients using *in vitro* gas production technique.

## Materials and methods

The experimental design was a 4 $\times$ 2 Factorial arrangement in a completely randomized design (CRD), with three replications per treatment. Factor A was fresh cassava root and roughage ratio at 100:0, 60:40, 40:60 and 0:100 and used as substrate at 488 mg in the inoculum. Factor B was feed block containing high sulfur (FBS) at 2 and 4% and supplement in substrate at 12 mg. Fresh cassava root was collected and ground to pass a 1-mm sieve. The FBS was prepared according to Cherdthong et al. (2014). Samples of FBS and rice straw were dried at 60 °C, then ground to pass a 1-mm sieve (Cyclotech Mill, Tecator, Sweden) and used for chemical analysis and in the *in vitro* gas test. Substrates were prepared and weighed to 500 mg of DM into 50 ml serum bottles. Dietary treatments were tested in the *in vitro* gas production technique (Menke and Steingass, 1988). During the incubation, data of gas production was measured immediately after incubation at 0, 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 48, 72 and 96 h by using a pressure transducer and a calibrated syringe. Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979). Fermentation liquor was sampled for measured pH, NH<sub>3</sub>-N, VFA, digestibility of NDF and ADF were measured. *In vitro* digestibility was determined after termination of incubation at 12 and 24 h, when the content was filtered through pre-weighed Gooch crucibles (40 mm of porosity) and residual DM was estimated. The percentage of weight loss was determined and presented as *in vitro* DM digestibility (IVDMD) (Tilley and Terry, 1963). The protozoal population was measured using the direct counting microscopic method (150 $\times$ ) based on the use of a haemocytometer (Boeco, Hamburg, Germany). All data from the experiment were analyzed as a completely randomized design using the GLM procedure of SAS (1996).

## Results and discussion

### Gas production, kinetic analysis of gas production

Cumulative gas production for each of the substrate treatments was presented as gas production curves (Figure 1) and values for the estimated parameters obtained from the kinetics of gas production models for substrates studied are given in Table 1. Cumulative gas production (96 h) was influenced by different ratio of fresh cassava root and rice straw. This could be due to high content of rumen fermentable starch contain in cassava root led to providing more substrate for producing gas. In addition, sulfur supplementation at 4% in FBS could increase cumulative gases when compared to those in 2% level. The rate of gas production (b) and (c) were significant different ( $P < 0.01$ ) among ratio of fresh cassava root and rice straw while level of sulfur in feed block were not altered on these parameters. This high gas production result indicated the higher values in *in vitro* true digestibility (Table 3). Similarly, Promkot et al. (2007)



reported that supplementation of sulfur on fresh cassava foliage was able to increased rate of gas production (c) with the level of sulfur inclusion in the substrate fresh cassava foliage.

### **Ruminal pH, ammonia-nitrogen and protozoa**

The pH value of the inoculum was very similar in all treatments (Table 2). However, these values did not necessarily reflect the values which could be generated in an *in vivo* system, due to the buffering activity of the inoculum. The pH values in this experiment represent a monitor of the fermentation and did not fall below the normal range (pH range 6.5-7.0) for rumen microbe growth especially for cellulolytic bacteria (Promkot et al., 2007).

The concentration of the ammonia-nitrogen has been used as a qualitative reference to understand the adequacy of the rumen environment according to the microbial activity on fibrous carbohydrates. Ammonia-nitrogen concentration were significant different ( $p < 0.01$ ) among treatment which was reduced when decreasing levels of cassava root. Ammonia-nitrogen was generate from protein digestion in the rumen, thus reduction protein content from fresh cassava root may reduce concentration of ammonia-nitrogen. The results of this study confirm those of Khang and Woiktorsson (2004) who found out that rumen ammonia-nitrogen concentration increased with the level of supplementation of fresh cassava foliage. High rumen ammonia-nitrogen concentration in those researches could be due to high CP intake. In addition, population of protozoa in fluids were not altered with level of fresh cassava root or sulfur ( $P > 0.05$ ).

### ***In vitro* digestibility and volatile fatty acid**

Effect of fresh cassava root and FBS using *in vitro* gas on digestibility are shown in Table 3. *In vitro* dry mater digestibility, NDF and ADF digestibility were significantly different ( $p < 0.01$ ) and was increased when increasing ration of fresh cassava root and rice straw. Rice straw contains high fiber content which may limit digestibility rate of diet. Furthermore, inclusion of sulfur at 4% in feed block was significantly higher *in vitro* dry mater digestibility when compared to 2% sulfur. Sulfur is an essential element for bacteria in rumen, growth and normal cellular metabolism as metabolism is closely related to nitrogen metabolism. The animal relies on microorganisms in the rumen to convert sulfate to hydrogen sulfite which is used to synthesize methionine and cysteine for microbial growth. Therefore, the continuous availability of sulfur could improve microbial populations and increase digestibility. Promkot and Wanapat (2009) reported that sulfur supplementation at 0.4% (DM) in the ration was beneficial to cows consuming fresh cassava foliage or cassava hay (10% diet), improved rumen microbial protein synthesis and reduced HCN concentration in substrate. Reduction of HCN in substrate could be due to influence of sulfur addition. HCN can be rapidly detoxified by rhodanese by rumen microbes. Rhodanese is a sulfur transferase that catalyzes the deformation of cyanide and thiosulphate or other suitable sulfur donor to less toxic thiocyanate which is excreted in the urine. Even current study did not measured HCN content in *in vitro* gas, assuming that HCN was reduced due to no adversary affect gas production and digestibility. However, concentration of HCN in *in vitro* fermentation should be further determined.

The affected of sulfur level and fresh cassava root ratio on total volatile fatty acid production and individual VFAs is given in Table 4. There were not altered in total VFA and acetic acid concentration as affect by treatments. In addition, levels of sulfur in feed block were not significantly different in total VFA and VFA profiles ( $P > 0.05$ ) except propionic acid which was higher in 4% of sulfur than those other. Increasing level of fresh cassava root in the ration could increase concentration of propionic acid ( $P < 0.05$ ). Therefore, in our study it was assumed that propionate level increased due to an increase in starch degradability which contain in



cassava root. Agreed with Promkot et al. (2007) demonstrated that sulfur supplementation at 0.5% DM with fresh cassava foliage significantly increased total VFA production.

## Conclusion

Base on this study, using of fresh cassava root with feed block containing high sulfur 4% could enhanced kinetics of gas, propionic acid concentration and *in vitro* digestibility. However, these findings should be applied further in *in vivo* studies.

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**Table 1.** Effect of fresh cassava root with feed block containing high sulfur on kinetic of gas

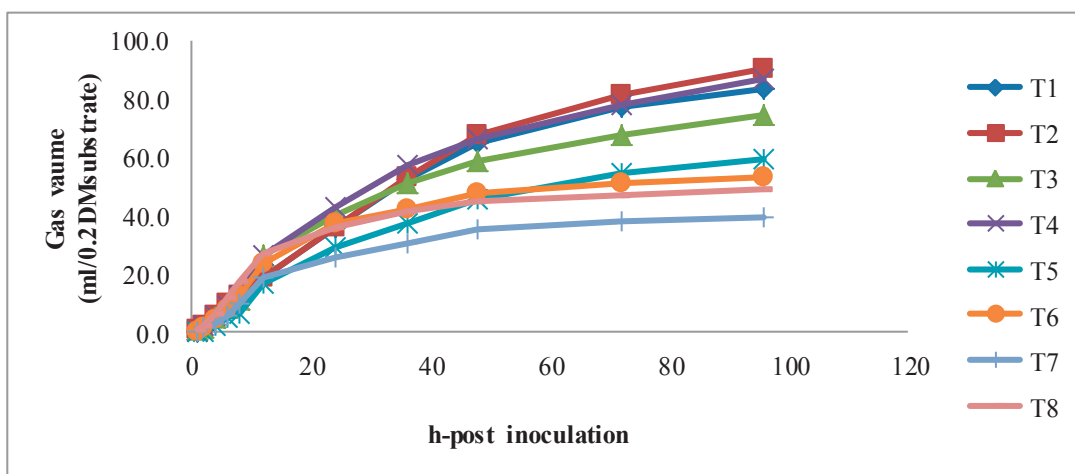
Treatment	C:R <sup>b</sup>	FBS <sup>b</sup>	Gas production kinetic <sup>c</sup>				Gas(96h) ml/0.5g
			a	b	c	a+b	
T1	100:0	2	-2.10	101.47	0.021	99.37	83.3
T2		4	-1.20	106.9	0.025	105.70	90.2
T3	60:40	2	-3.08	96.03	0.030	92.95	74.3
T4		4	-1.19	70.73	0.018	69.54	87.3
T5	40:60	2	-2.86	69.03	0.025	66.17	59.1
T6		4	-3.49	55.98	0.030	52.49	53.1
T7	0:100	2	-2.80	55.04	0.017	52.24	39.6
T8		4	-2.54	54.30	0.020	51.97	48.9
SEM			0.10	0.90	0.001	0.90	1.9
Interaction							
C:R			ns	**	**	**	**
FBS			ns	ns	ns	ns	*
C:R*FBS			ns	ns	ns	ns	ns

<sup>a</sup> Fresh cassava root : rice straw ratio

<sup>b</sup> Level of sulfur in feed block.

<sup>c</sup> a, the gas production from the immediately soluble fraction; b, the gas production from the insoluble fraction; c, the gas production rate constant for the insoluble fraction.

(b) ; a+b, the gas potential extent of gas production \* p<0.05; \*\* p<0.0001.



**Figure 1.** Effect of fresh cassava root with feed block containing high sulfur on cumulative gas during 0 to 96 h incubation



**Table 2.** Rumen parameters and protozoal number as affect by fresh cassava root and sulfur in feed block

Treatment	C:R <sup>a</sup>	FBS <sup>b</sup>	pH	NH <sub>3</sub> -N (mg/dl) <sup>c</sup>	Protozoa (×10 <sup>5</sup> cell/ml)
T1	100:0	2	6.7	30.8	5.5
T2		4	6.7	34.7	6.0
T3	60:40	2	6.6	22.9	5.0
T4		4	6.8	23.3	4.5
T5	40:60	2	6.6	19.8	6.5
T6		4	6.6	20.2	6.0
T7	0:100	2	6.6	18.1	4.5
T8		4	6.6	18.3	5.0
SEM			0.08	0.20	1.0
Interaction					
C:R			ns	**	ns
FBS			ns	ns	ns
C:R*FBS			ns	ns	ns

<sup>a</sup> Fresh cassava root : rice straw ratio. <sup>b</sup>Level of sulfur in high containing feed book. NS = Non-significant, \*p<0.05, \*\*p<0.001

**Table 3.** Effect of fresh cassava root with feed block containing high sulfur on nutrients digestibility

Treatment	C:R <sup>a</sup>	FBS <sup>b</sup>	Dry mater (%)	<i>In vitro</i> dry mater digestibility (%)	Neutral detergent fiber (%)	Acid detergent fiber (%)
T1	100:0	2	45.4	90.7	69.8	44.5
T2		4	36.2	92.4	70.4	46.9
T3	60:40	2	37.6	75.3	53.8	39.0
T4		4	39.8	77.6	52.2	37.6
T5	40:60	2	29.2	55.3	53.1	44.9
T6		4	26.1	58.2	53.1	23.9
T7	0:100	2	27.0	50.0	34.1	32.6
T8		4	32.0	54.0	41.2	28.5
SEM			0.7	1.3	0.9	0.8
Interaction						
C:R			*	**	**	*
FBS			ns	*	ns	ns
C:R*FBS			ns	ns	ns	ns

<sup>a</sup> Fresh cassava root : rice straw ratio. <sup>b</sup>Level of sulfur in high containing feed book. NS = Non-significant, \*p<0.05, \*\*p<0.001



**Table 4.** Effect of fresh cassava root with feed block containing high sulfur on volatile fatty acid concentration

Treatment	C:R <sup>a</sup>	FBS <sup>b</sup>	Total VFA mmol/L	C2	C3	C4	C2:C3
				mol/100mol			
T1	100:0	2	110.7	65.9	27.2	15.5	2.7
T2		4	114.1	64.3	29.2	16.4	2.8
T3	60:40	2	114.3	65.9	26.3	15.5	2.9
T4		4	112.2	65.6	28.3	17.0	3.0
T5	40:60	2	110.2	66.7	25.7	16.9	3.1
T6		4	109.8	65.4	27.8	17.6	3.0
T7	0:100	2	105.9	67.4	22.1	16.5	2.8
T8		4	106.5	68.5	24.9	16.6	3.0
SEM			3.2	1.3	0.5	0.2	0.01
Interaction							
C:R			ns	ns	*	**	**
FBS			ns	ns	*	ns	ns
C:R*FBS			ns	ns	ns	ns	ns

a Fresh cassava root : rice straw ratio. bLevel of sulfur in high containing feed book. VFA=Total volatile fatty acid; C2=Acetic acid; C3=Propionic acid; C4=Butyric acid; C2:C3 = Acetic acid: Propionic acid ratio. NS = Non-significant, \*p<0.05, \*\*p<0.001.



## **Inclusion of Yeast Waste as Protein Source to Replace Soybean Meal in Concentrate Diet on Ruminal Fermentation and Kinetics of Gas Using a Gas Production Technique**

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### **Abstract**

This experiment was conducted to investigate the utilization of yeast waste as protein source to replace soybean meal on kinetic of gas, rumen ammonia-nitrogen and digestibility of nutrients by using *in vitro* gas production technique. The experimental design was a completely randomized design (CRD) and the dietary treatments were replacing soybean meal (SBM) with yeast waste (YW) (SBM:YW) in concentrate at 100:0, 75:25, 50:50, 25:75 and 0:100. Yeast waste obtained from KSL Green Innovation Public Company Limited, Thailand. The gas production was recorded at 0, 0.5, 1, 2, 4, 6, 8, 12, 16, 24, 48, 72 and 96 h of incubation. The yeast waste contained 26.4% CP. Gas production from soluble fractions (*a*), gas production from the insoluble fraction (*b*), potential extent of gas production (*a+b*) and gas production rate constants for the insoluble fraction (*c*) were not altered when increasing concentration of yeast waste replacing soybean meal ( $P>0.05$ ). Cumulative gas production (at 96 h of incubation) was ranges from 69.3 to 72.8 ml which was similar when compared between inclusion yeast waste and control group. Ruminal  $\text{NH}_3\text{-N}$  concentration in bottles serum did not altered when inclusion various levels of yeast waste replacing soybean meal which ranges from 15.4 to 18.2 mg%. *In vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) did not changed by increasing levels of yeast waste in the diets ( $P>0.05$ ). In conclusion, yeast waste could be replace soybean in concentrate diet which no negatively effect on gas kinetics, rumen fermentation and *in vitro* digestibility, and therefore its use in animal feeding would contribute to reduce environmental pollution.

**Keywords:** fermentation, digestibility, soybean meal, yeast waste

### **Introduction**

Bioethanol has been identified as the mostly used biofuel worldwide since it significantly contributes to the reduction of crude oil consumption and environmental pollution (Azhar et al., 2017). Microorganisms such as yeasts play an essential role in bioethanol production by fermenting a wide range of sugars to ethanol. *Saccharomyces cerevisiae* is the most commonly used microorganism to ferment sugarcane juice and molasses for bioethanol production, and the residue generated in this process (yeast waste) has been reported to contain about 60-70% of





yeast cells (Díaz et al., 2017). The yeast waste is produced in large amounts and has a high pollution potential, and therefore its use in animal feeding would contribute to reduce environmental pollution.

*S. cerevisiae* have been shown to manipulate ruminal fermentation and to have beneficial effects on ruminant performance (Robinson and Garrett, 1999). However, the most of the studies on yeast as feed additives have been conducted with live yeast cultures, and the potential of yeast waste as ruminal fermentation modifiers has received much less attention.

Therefore, the aim of the study was to evaluate the effect of replacing soybean meal with yeast waste powder on gas kinetics, rumen fermentation and digestibility using an *in vitro* gas production technique.

## Materials and Methods

The experimental design was a completely randomized design (CRD) and the dietary treatments were replacing soybean meal (SBM) with yeast waste (YW) (SBM:YW) in concentrate at 100:0, 75:25, 50:50, 25:75 and 0:100. Yeast waste obtained from KSL Green Innovation Public Company Limited, Khon Kaen province, Thailand. The sample of yeast waste, rice straw and concentrate were dried at 60°C for 48 h, then ground to pass through a 1-mm sieve and used for chemical analysis. Dietary treatments were tested in the *in vitro* gas production technique (Menke and Steingass, 1988). During the incubation, data of gas production was measured immediately after incubation at 0, 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 48, 72 and 96 h by using a pressure transducer and a calibrated syringe. Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979). Fermentation liquor was sampled for measured pH, NH<sub>3</sub>-N, *in vitro* dry matter degradability (IVDMD) and *in vitro* organic matter degradability (IVOMD). All data were analyzed as a completely randomized design using the GLM procedure of SAS. Ingredient and chemical composition of concentrates, yeast waste and rice straw used in the experiment shown in Table 1.

## Results and discussions

### Chemical composition of the diets

The chemical compositions of concentrate, YW and rice straw are shown in Table 1. Concentrate and rice straw consisted of 13.8-14.0% and 2.8 % of CP, respectively. The yeast waste contained 26.4% CP with low of fiber contents, thus it would be beneficial protein source for ruminant animals.

### Gas kinetics and cumulative gas production

Table 2 show the values for the estimated parameters obtained from the kinetics of gas production models for substrates studied. It was found that gas production from soluble fractions (*a*) were ranges from 3.4 to 4.44 ml/ 0.5 g and was not significantly different among treatments ( $P>0.05$ ). The gas production from the insoluble fraction (*b*), potential extent of gas production (*a+b*) were also not altered when increasing concentration of yeast waste replace soybean meal ( $P>0.05$ ). The gas production rate constants for the insoluble fraction (*c*) ranged from 0.06 to 0.07 ml/ 0.5 g which was similar among yeast waste group replacing soybean meal. Cumulative gas production (at 96 h of incubation) was ranges from 69.3 to 72.8 ml which was similar when compared between inclusion yeast waste and control group (Figure 1). The results from current study revealed that replacing soybean meal with yeast waste did not show negatively effect on kinetics of gas and fermentation.

### Ruminal NH<sub>3</sub>-N, *in vitro* digestibility

Effect of replacing soybean meal with yeast waste on ruminal NH<sub>3</sub>-N, *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) given in Table 3. Ruminal NH<sub>3</sub>-N concentration in bottles serum did not altered when inclusion various levels of



yeast waste replace soybean meal which ranges from 15.4 to 18.2 mg%. Similar in ruminal NH<sub>3</sub>-N concentration due to the dietary treatments contained similar crude protein contents. However, these values were close to the optimum level for rumen microorganism growth (15–30 mg%), as stated by Wanapat and Pimpa (1999).

*In vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD) did not changed by increasing levels of yeast waste in the diets ( $P>0.05$ ). IVDMD at 12 and 24 h of incubation were ranges from 53.6 to 56.7% DM and 65.7 to 70.7% DM, respectively. In addition, IVOMD were 73.6 to 76.5% and 74.8 to 78.0% DM for incubation times at 12 and 24 h, respectively. The results indicated that inclusion of yeast waste at 100% replaced soybean meal could be used as protein source in concentrate diet and had no adversary effect on digestibility.

## Conclusion

Based on this experiment, it could be concluded that yeast waste could be replace soybean in concentrate diet which no negatively effect on gas kinetics, rumen fermentation and *in vitro* digestibility, and therefore its use in animal feeding would contribute to reduce environmental pollution. However, these finding should be applied for further *in vivo* experiment in order to increase animal performance and reduce environmental pollution from waste.

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**Table 1.** Ingredient and chemical composition of concentrates, rice straw and yeast waste used in the experiment (kg dry matter (DM)).

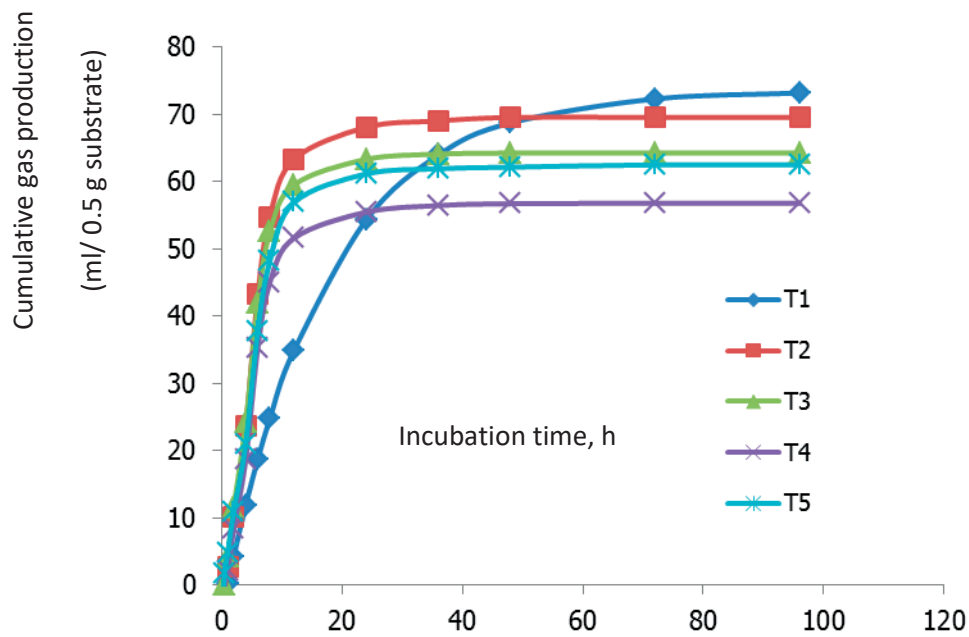
Item	Soybean meal: Yeast waste					Rice straw	Yeast waste
	100 : 0	75 : 25	50 : 50	25 : 75	0 : 100		
Ingredients, kg							
DM	56.0	55.8	55.5	55.3	55.0		
Cassava chips	10.0	10.0	10.0	10.0	10.0		
Soybean meal	15.0	11.3	7.5	3.8	0.0		
Palm kernel meal	7.0	7.0	7.0	7.0	7.0		
Coconut meal	7.0	7.0	7.0	7.0	7.0		
Yeast waste	0.0	3.8	7.5	11.3	15.0		
Urea	1.0	1.3	1.5	1.8	2.0		
Mineral premix	1.0	1.0	1.0	1.0	1.0		
Molasses, liquid	1.0	1.0	1.0	1.0	1.0		
Pure sulfur	1.0	1.0	1.0	1.0	1.0		
Salt	1.0	1.0	1.0	1.0	1.0		
Chemical composition							
DM, %	94.6	94.1	94.6	94.7	94.8	95.9	95.0
%DM							
OM	88.9	88.2	87.7	86.0	85.9	78.6	69.6
Ash	11.1	11.7	12.3	13.9	14.0	21.4	30.4
CP	14.0	14.0	13.8	13.8	13.9	2.8	26.4
NDF	19.1	18.9	19.2	19.2	19.3	78.9	8.2
ADF	7.2	7.3	7.3	7.4	7.5	58.4	4.6

**Table 2.** Effect of replacing soybean meal with yeast waste on gas kinetics and cumulative gas at 96 h after incubation

Treatments	Kinetics of gas, ml/ 0.5 g				Cumulative gas, ml
	a	b	c	a+b	
100:0	-4.3	77.7	0.06	73.5	72.1
75:25	-4.4	74.6	0.07	70.2	72.8
50:50	-4.0	68.9	0.07	64.9	69.3
25:75	-3.4	60.7	0.07	57.3	70.0
0:100	-3.6	67.7	0.07	64.1	71.8
SEM	0.38	5.7	0.003	5.4	1.01
P-value	ns	ns	ns	ns	ns

**Table 3.** Effect of replacing soybean meal with yeast waste on ruminal NH<sub>3</sub>-N, *in vitro* dry matter digestibility (IVDMD) and *in vitro* organic matter digestibility (IVOMD)

Treatments	IVDMD, %DM		IVOMD, %DM		Ruminal NH <sub>3</sub> -N, mg%
	12 h	24 h	12 h	24 h	
100:0	55.1	69.5	74.8	77.0	16.9
75:25	53.7	70.7	76.5	77.2	18.2
50:50	54.1	66.6	76.0	78.0	17.5
25:75	56.7	69.5	73.6	76.1	15.4
0:100	53.6	65.7	73.9	74.8	16.1
SEM	1.85	2.41	0.91	0.88	0.78
P-value	ns	ns	ns	ns	ns



**Figure 1.** Effect of replacing soybean meal with yeast waste on cumulative gas during incubation times



## Using of Urea and Molasses Fermented Cassava Pulp on Rumen Fermentation and Methane Production

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### **Abstract**

This study aimed to investigate the using of fermented cassava pulp with urea and molasses on rumen fermentation and methane production in beef cattle. All treatments were into a 4x4 replicated Latin square design. Four feed treatments were cassava pulp (control), cassava pulp fermented with urea 4%, cassava pulp fermented with molasses 4% and cassava pulp fermented with urea 4% and molasses 4%. The results shown that fermented cassava pulp did not affect pH and temperature. Moreover, NH<sub>3</sub>-N was highest in cattle fed cassava pulp fermented molasses and cassava pulp fermented urea and molasses. In this study, there were differences (P<0.05) in acetic acid and propionic acid concentrations when beef cattle were fed with different treatment. The value of total VFA and C4 were found no difference (P>0.05) among treatments. Moreover, CH<sub>4</sub> production was lower than control when supplement with cassava pulp fermented urea/molasses. Based on the present findings, it could be concluded that fermented cassava pulp with urea and molasses can increase propionic acid and reduce CH<sub>4</sub> production.

**Keywords:** fermented cassava pulp, rumen fermentation, methane emission, beef cattle

### **Introduction**

Livestock contributes to about 18% to the global anthropogenic greenhouse gas (GHG) emissions, accounting for about 37% of the total anthropogenic methane (CH<sub>4</sub>) and 65% of global anthropogenic nitrous oxide. CH<sub>4</sub> is produced normally during the fermentation of feed by methanogenic bacteria (Hristov et al., 2013). The removal of ruminal protozoa can also reduce CH<sub>4</sub> production as some population of methanogens remains attached to protozoa (Cieślak et al., 2016).

Cassava pulp, a fibrous by-product of the cassava processing industry, has recently become attractive as a cellulosic biomass due to its nature as a cheap, abundant, and renewable agricultural product. At present, cassava pulp is generally used as low-value animal feed. In Thailand, more than 60% of the cost of milk production is the cost of feeds particularly concentrates. There are many attempts to reduce cost of feeds through the utilization of cheap raw materials, such as agro-industrial by product. Although cassava pulp, the residue obtained after the extraction of starch from cassava roots, is low in crude protein (Lounglawan et al., 2010). Cassava pulp offers an alternative to high-starch grains, and can be used as an energy source in the diets. Molasses has risen in price in Mexico to the point where there is interest in reducing the level consumed by the animals, and for this purpose high levels of urea have been found to be a useful mechanism for achieving this affect (Silvestre et al., 1977a; Ferreiro and



Preston 1976). Urea has been utilized as a supplemental nitrogen source for beef cattle rations when protein sources are expensive. Ensiling is a crop preservation method based on natural lactic acid fermentation under anaerobic conditions where anaerobic microbes build up organic acids mainly lactic acid by using fermentable carbohydrates (Gollop et al., 2005; Ki et al., 2009). To improve silage preservation and guarantee animal feed quality, silage additives such as chemicals, enzymes, and bacterial agents can be employed. Addition of carbon and nitrogen sources could improve the quality of silage. Wanapat et al. (2013) reported that supplementation of urea and molasses could improve the quality of whole crop rice silage in terms of nutritive value and rumen degradation. Addition of carbon and nitrogen sources could improve the quality of silage and have subsequent effects on rumen degradation characteristic and production in ruminants. The aim of this research was to investigate the using of fermented cassava pulp with urea and molasses on rumen fermentation and CH<sub>4</sub> production in beef cattle fed on rice straw.

## **Materials and Methods**

### **Animals, diets, experimental design, and animal management**

Four beef cattle with an average body weight of 300±30 kg were used in this experiment. All animals were housed in individual pens and given feed twice a day, with roughage *ad libitum*, concentrate at 1.5% body weight and cassava pulp at 0.5% body weight. Clean, freshwater, and mineral salt block were offered freely. The experiment was a 4x4 Latin square design (LSD). The four treatments were un-supplementation (control), supplementation with cassava pulp fermented urea at 4%(CU), supplementation with cassava pulp fermented molasses at 4%(CM), and supplementation with cassava pulp fermented urea at 4% and molasses at 4%(CUM). Chemical composition are presented in Table 1. The experiment was conducted for four periods; each experimental period lasted for 21 days. During the first 14 days, all animals were fed with their respective diets, and samples were collected during the last 7 days of each period.

### **Data collection, sampling procedures, and statistical analysis**

Roughage and concentrate were sampled daily during the collection period and were composited for analysis. Feed and fecal samples were collected during the last 7 days of each period. Composite samples were dried at 60°C in a forced-air oven for 48 h and ground at 1 mm screen using Cyclotech Mill, Tecator, Sweden for analysis of dry matter, ash, ether extract, crude protein (AOAC, 1990), and neutral detergent fiber (NDF), acid detergent fiber (ADF) (Van Soest et al., 1991). During the final day of collection period, rumen fluid and blood samples were collected at 0, 3 and 6-h post-feeding. Rumen fluid were immediately measured for temperature and pH (Hanna Instruments HI 8424 microcomputer, Singapore). Rumen fluid samples were then kept for analysis of ammonia nitrogen (NH<sub>3</sub>-N) using the micro-Kjeldahl methods AOAC (1990) and volatile fatty acids (VFA) using high pressure liquid chromatography (HPLC) according to Samuel et al. (1997).

### **Statistical analysis**

All data were statistically analyzed according to a 4x4 Latin square design using the ANOVA procedure of SAS (1996). Differences between treatment means were determined by Duncan's New Multiple Range Test (Steel and Torrie, 1980). Differences between means with P<0.05 were accepted as representing statistically significant differences.



## Results and Discussion

Experimental feed and their chemical compositions are shown in Table 1. Concentrate diet was formulated by using available local feed resources and had a high quality in terms of high CP and low NDF and ADF content. Cassava pulp fermented urea/molasses quality were improved in terms of CP by treatment of CU, CM and CUM (15.69, 5.09 and 9.06 % dry matter). Rice straw was used as roughage source; however, it contained low CP and high NDF and ADF.

### Rumen fermentation and blood metabolites

Ruminal temperature, pH was similar among treatments and the values were quite stable at 38.01-38.17°C and pH (6.7-6.82), respectively (Table 2). These values were optimal for normal rumen fermentation (Wanapat, 1990). However, more fermentation can increase acid production and an expectation of lower rumen pH may have observed. Ruminal pH is a key determinant of the profile of nutrients available for absorption (Beauchemin et al., 1991). However, ruminal pH and cellulolytic activity were depressed by diets containing high cereals (Franzolin and Dehority, 1996), which may induce acute or chronic acidosis; thereby, causing reduction of feed intake, nutrients absorption and animal performance depression (Owens et al., 1998). Treatments with CM were found lower in concentration of ruminal NH<sub>3</sub>-N than with CU. Both treatments with urea have higher concentration of NH<sub>3</sub>-N (T<sub>2</sub>=12.96 and T<sub>4</sub>=16.03 mg/dl) than in the treatments control and with molasses (T<sub>1</sub>=8.64 and T<sub>3</sub>=10.94 mg/dl). The available rumen NH<sub>3</sub>-N would be used in microbial protein synthesis by the rumen microbes. Increasing levels of U in the diet was associated with higher ruminal NH<sub>3</sub>-N but did not affect physiology and was adequate for microbial growth. It is a potential approach to exploiting the use of local feed resources. Moreover, Chanjula et al. (2007b) also reported that cassava was a good source of ruminal degradable starch in replacing corn grain and had the potential to improve goat performance. In this study, there were differences (P<0.05) in C<sub>2</sub> and C<sub>3</sub> concentrations when beef cattle were fed with different treatment. Moreover, Wanapat et al. (2011b) compared from 4 sources of protein in concentrate diets, SBM, CH, *Leucaena leucocephala* (LL) and YEFECAP in lactating dairy cows and found that propionic acid was found the highest in cows receiving CH and YEFECAP. As shown, the value of Total VFA and C<sub>4</sub> were found no difference (P>0.05) among treatments. Moreover, CH<sub>4</sub> production was lower than control went supplement with cassava pulp fermented urea/molasses. It may be because of H-sink in rumen fermentation use for produce C<sub>3</sub> concentration thus reduce CH<sub>4</sub> production.

## Conclusion

Based on the present study, it is concluded that NH<sub>3</sub>-N was highest in cattle fed cassava pulp fermented molasses and cassava pulp fermented urea and molasses. Fermented cassava pulp with urea and molasses can increase propionic acid and reduce CH<sub>4</sub> production.

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**Table 1.** Chemical compositions of concentrate, cassava pulp fermented with urea, cassava pulp fermented with molasses and cassava pulp fermented with urea and molasses

<b>Ingradients</b>	<b>Concentrate</b>	<b>C<sup>1</sup></b>	<b>CU<sup>2</sup></b>	<b>CM<sup>3</sup></b>	<b>CUM<sup>4</sup></b>	<b>RS<sup>5</sup></b>
Chemical composition						
Dry matter, %	91.08	88.83	47.32	48.17	48.27	90.45
		.....% dry matter.....				
Organic matter	88.25	95.03	98.15	97.08	97.15	78.80
Crude protein	15.95	3.34	15.69	5.09	9.06	2.69
Neutral detergent fiber	48.76	33.16	30.79	35.66	45.00	84.09
Acid detergent fiber	17.13	25.40	24.10	17.41	16.11	44.94

<sup>1</sup>C= Cassava pulp <sup>2</sup>CU= Cassava pulp fermented with urea; <sup>3</sup>CM = Cassava pulp fermented with molasses, <sup>4</sup>CUM = Cassava pulp fermented with urea and molasses, <sup>5</sup>RS= Rice straw

**Table 2.** Effect of urea/molasses fermented cassava pulp on ruminal pH, temperature and NH<sub>3</sub>-N concentration

<b>Items</b>	<b>Control</b>	<b><sup>1</sup>CU</b>	<b><sup>2</sup>CM</b>	<b><sup>3</sup>CUM</b>	<b>SEM</b>
Ruminal pH,	6.70	6.79	6.82	6.82	0.04
Ruminal temperature, °C	38.17	38.03	38.01	38.07	0.39
NH <sub>3</sub> -N, mg/dL	8.64 <sup>d</sup>	12.96 <sup>b</sup>	10.94 <sup>c</sup>	16.03 <sup>a</sup>	0.36

<sup>abc</sup>Values on the same row with different superscripts differed (P<0.05)

<sup>1</sup> CU = Cassava pulp fermented with urea; <sup>2</sup> CM = Cassava pulp fermented with molasses, <sup>3</sup> CUM = Cassava pulp fermented with urea and molasses

SEM=standard error of the means



**Table 3.** Effect of urea/molasses fermented cassava pulp on volatile fatty acid (VFA) concentration

Items	Control	<sup>1</sup> CU	<sup>2</sup> CM	<sup>3</sup> CUM	SEM
Total VFA, mmol/L					
0 h-post feeding	125.44 <sup>ab</sup>	110.23 <sup>b</sup>	127.94 <sup>a</sup>	130.90 <sup>a</sup>	4.63
3 h-post feeding	129.18	128.56	131.12	130.34	5.75
6 h-post feeding	128.03 <sup>ab</sup>	135.99 <sup>a</sup>	111.80 <sup>b</sup>	120.45 <sup>ab</sup>	6.16
mean	127.55	124.93	123.62	127.23	2.64
Acetic acid, % of total VFA					
0 h-post feeding	68.21 <sup>a</sup>	64.36 <sup>c</sup>	66.21 <sup>b</sup>	62.04 <sup>d</sup>	0.29
3 h-post feeding	68.35 <sup>a</sup>	63.87 <sup>ab</sup>	63.92 <sup>ab</sup>	59.24 <sup>b</sup>	1.35
6 h-post feeding	67.61	65.22	67.26	65.29	2.62
mean	68.06 <sup>a</sup>	64.48 <sup>ab</sup>	65.80 <sup>ab</sup>	62.19 <sup>b</sup>	1.19
Propionic acid, % of total VFA					
0 h-post feeding	21.79 <sup>c</sup>	25.77 <sup>b</sup>	24.69 <sup>b</sup>	28.50 <sup>a</sup>	0.64
3 h-post feeding	21.86 <sup>b</sup>	27.15 <sup>a</sup>	27.74 <sup>a</sup>	31.42 <sup>a</sup>	1.39
6 h-post feeding	21.74	24.74	24.65	26.35	2.20
mean	21.79 <sup>b</sup>	25.89 <sup>a</sup>	25.69 <sup>a</sup>	28.76 <sup>a</sup>	1.02
Butyric, % of total VFA					
0 h-post feeding	10.00	9.87	9.11	9.45	0.81
3 h-post feeding	9.78	8.98	8.35	9.34	0.83
6 h-post feeding	10.66	10.03	8.09	8.36	0.88
mean	10.15	9.63	8.51	9.05	0.76
CH <sub>4</sub>	28.69 <sup>a</sup>	25.75 <sup>b</sup>	25.95 <sup>b</sup>	23.70 <sup>b</sup>	0.74

<sup>abcd</sup> Values on the same row with different superscripts differed (P<0.05)

<sup>1</sup> CU = Cassava pulp fermented with urea 4%; <sup>2</sup> CM = Cassava pulp fermented with molasses 4%, <sup>3</sup> CUM = Cassava pulp fermented with urea 4% and molasses 4%

SEM=standard error of the means



## Meat Quality of Bali Beef fed With Supplement Blocks Contain Different Liquid Smoke levels

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### Abstract

An experiment was carried out to study the effect of liquid smoke levels in feed block supplement on Bali beef quality. The addition of liquid smoke together with coconut water into the feed block; Urea Coconut Water Liquid Smoke Multi-nutrient Block (UCSMB) in fattening Bali cattle is one of the innovations to enhance the growth of livestock and meat quality after aging. This research conducted on 9 Bali cattle aged two years fattened for 45 days. After the slaughtering, the *Longissimus dorsi* muscles were dissected and aged for eight days. This study used an entirely randomized design of factorial pattern of 3 x 5, where factor 1 was liquid smoke levels (0, 1, 2%) on 10% concentration and factor 2 was aging time (0, 2, 4, 6, and eight days). All treatments replicated for three times. The variables measured were meat cooked shear force (MCSF) value, cooking loss, and meat color (L\*, a\*, b\*). The results of this study showed the increase in the level of liquid smoke in feed block resulted in MCSF value which is more or less the same. Similarly, cooking loss on meat cooked 80°C for 30 minutes yields approximately the same value as increasing liquid smoke levels. The longer aging time decreased shear force value on cooked meat and cooking loss. The meat color (L\*, a\*) increased, and b\* decreased as the level of liquid smoke increasing. Meanwhile L\*, a\*, and b\* increases with increasing maturation time. The result of this study concluded that the liquid smoke in UCSMB could improve the quality of the meat of Bali cattle.

**Keywords:** aging, Bali cattle, liquid smoke, *longissimus dorsi*, meat quality, UCSMB

### Introduction

Handling post-slaughter meat of good and proper cattle needs attention. Postharvest livestock muscle undergoes biochemical changes resulting in muscle quality during live cattle will decrease as a post-mortem. But by improving the quality of muscle during livestock is still alive through the provision of quality feed and application of postmortem aging will improve the quality of meat. It is well-known that quality feed will provide good livestock growth characterized by increased body weight and high meat quality. In general, concentrate on livestock will give a high body weight gain, but require a high cost. The cost of feed becomes very valuable in the maintenance of cows that can reach 80%. In this case, the researchers overcame these high costs through the use of easily available feed materials at lower prices through the application of feeding technology. One such technology is the utilization of coconut water in feed supplement block as a substitute for molasses on urea molasses multi-nutrient blocks. Simultaneously added additional ingredients that act as antioxidants which in addition to



inhibiting oxidation of feed also inhibits oxidation of postmortem meat of livestock. One ingredient that is liquid smoke has been studied as an antioxidant in fresh meat and processed meat products (Abustam et al., 2012; Abustam et al., 2015b). The functional properties of meat such as the ability to bind water, cooking loss and meat texture will be better because of the ability of liquid smoke as a binder. Some previous studies reveal that an additional liquid smoke on Bali beef meatballs is capable of increasing the water holding capacity and decreasing the cooking loss of meatball product (Abustam et al., 2015a). Similarly, the functional property of Bali beef is increased by adding post-rigor liquid smoke on *Longissimus dorsi* muscle (Abustam et al., 2012). The use of liquid smoke in flour in Bali beef and buffalo meatballs produces the same sensory characteristics on different smoke flour types; however, the level of firmness and elasticity of Buffalo meatballs is higher than that of Bali beef meatballs (Abustam et al., 2015b).

The use of liquid smoke in mice was conducted by Budijanto et al. (2008) in which in 14 days of observation, the body weight of mice increased at all levels of liquid smoke exposures. Meanwhile, the liquid smoke as an antioxidant in catfish was studied by Ernawati et al. (2012). Budaraga (2017) concluded that protein of tilapia (*Oreochromis niloticus*) increased to 79% with the use of liquid smoke concentration of 5% as a preservative. The utilization of liquid smoke in fattening Bali cattle feed is new and innovative, and it is expected to increase the yield and meat quality to reduce the amount of meat used in the processing and simultaneously improves the durability of the product.

## Materials and Methods

This study utilized the post-rigor *Longissimus dorsi* (LD) muscle, from 9 males of 2-year-old cattle fed with Urea Coconut Smoke Multi-nutrient Blocks (UCSMB) and elephant grass (*Pennisetum purpureum*) as a source of roughages for 45 days. *Longissimus dorsi* muscle-derived from Bali cattle kept in Maiwa Breeding Center Hasanuddin University, 180 km north of Makassar capital of South Sulawesi. Muscle extraction and retrieval were performed at the slaughterhouse Tamangapa Makassar. The liquid smoke concentration of 10% added to the UCSMB feed at 1 to 2% (w/w) functioned as the binder and preservative. *Longissimus dorsi* muscles (LD) were dissected from all of the nine male cattle after the slaughtering and aged for eight days with two days interval the measurement.

Urea, coconut water, liquid smoke multi-nutrient block (UCSMB) is a modification of UMMB (urea-molasses block multi-nutrient) where molasses replaced with coconut water, and liquid smoke was added as an antioxidant and binder (Abustam et al., 2015c). There were three kinds of UCSMB used with liquid smoke in concentration 10% treatment adapted to the levels of 0% (control), 1%, and 2% in the formulation (w/w). The composition of the feed material in UCSMB shows in Table 1.

This study used an entirely randomized design of factorial pattern of 3 x 5. The first factor was the levels of liquid smoke in the feed block (0, 1%, and 2%) and the second factor was the aging time (0, 2, 4, 6 and 8 days) at a temperature of 2<sup>0</sup> - 5<sup>0</sup>C replicated for three times. Thus, the variables measured included shear force value of cooked meat (CMSF) at 80<sup>0</sup>C for 30 minutes, cooking loss (CL), and meat color (L\*, a\*, b\*).

Every animal received feeds in the form of 500 g of UCSMB, and 20 kg of *Pennisetum purpureum* per day for 45 days.

Shear force measurement is intended to examine the degree of meat tenderness after cooking at a temperature of 80<sup>0</sup>C for 30 minutes at 50 g sample. The shear force measured by using CD Shear Force, in which the meat samples in cylinder form with 1 cm length and a diameter of 0.5 inches placed in the hole on CD shear force. Thus, the samples have torn by



using CD shear force knife with 1 mm thickness. The bigger the weight to tear the meat samples, the harder the meat tenderness. SF value was in kg/cm<sup>2</sup> unit (Abustam, 2012).

The measurement of cooking loss is performed based on the ratio between the weights before and after the meat is cooked at a temperature of 80<sup>o</sup> for 30 minutes (Soeparno, 2005; Abustam, 2012).

**Table 1.** Composition of feed materials in UCSMB<sup>1)</sup>

Feed materials	Composition (g/kg) at Levels of liquid smoke concentration of 10%		
	0%	1%	2%
1. Coconut water	30	29	28
2. Urea	5	5	5
3. Rice bran	30	30	5
4. Corn meal	10	10	10
5. Copra meal	10	10	10
6. Cement	10	10	10
7. Cow Mineral	2	2	2
8. Table salt	3	3	3
9. Liquid smoke	0	1	2

<sup>1)</sup> Abustam et al. (2015c)

Meat color measurement refers to the Hunter system of L\*, a\*, b\* wherein L\* is the level of lightness, a\* is the degree of redness and b\* is the level of yellowness ranging from 0% to 100%. The higher the percentage of reflection, the more the color becomes lighter, darker red and darker yellow (AMSA, 2012). Meat color measurements using a portable colorimeter TES-135 Digital Color.

Processing of the data by utilizing analysis of variance (ANOVA) and testing between average uses LSD based on Steel and Torrie (1991) with SPSS (SPSS 16.0, SPSS Ltd., West Street Woking, Surrey, UK).

## Results and discussion

The properties of meat quality of *Longissimus dorsi* affected by liquid smoke levels in UCSMB and aging time shown in Table 2.

**Table 2.** Effects of liquid smoke levels in UCSMB and aging time of *Longissimus dorsi* on the properties of meat quality of Bali cattle (means and SE)

Treatments	CMSF (kg/cm <sup>2</sup> )	CL (%)	Meat color		
			L*(%)	a*(%)	b*(%)
LS Levels:	Sig:NS	Sig:NS	Sig:0.001	Sig:0.001	Sig:0.001
0%	2.70 <sub>±</sub> 0.66	12.38 <sub>±</sub> 1.19	42.14 <sup>a</sup> <sub>±</sub> 5.27	15.85 <sup>a</sup> <sub>±</sub> 3.60	4.14 <sup>a</sup> <sub>±</sub> 2.47
1%	2.66 <sub>±</sub> 0.52	12.20 <sub>±</sub> 1.04	42.59 <sup>a</sup> <sub>±</sub> 4.80	15.13 <sup>ab</sup> <sub>±</sub> 1.17	4.86 <sup>ab</sup> <sub>±</sub> 1.66
2%	2.89 <sub>±</sub> 0.64	12.24 <sub>±</sub> 1.53	45.37 <sup>b</sup> <sub>±</sub> 3.48	14.53 <sup>b</sup> <sub>±</sub> 2.94	5.05 <sup>b</sup> <sub>±</sub> 2.95
Aging:	Sig:0.001	Sig:0.001	Sig:0.001	Sig:0.039	Sig:0.001
0 days	2.44 <sup>a</sup> <sub>±</sub> 0.63	14.43 <sup>a</sup> <sub>±</sub> 0.84	37.85 <sup>a</sup> <sub>±</sub> 4.56	11.07 <sup>a</sup> <sub>±</sub> 2.44	1.03 <sup>a</sup> <sub>±</sub> 2.08
2 days	2.71 <sup>a</sup> <sub>±</sub> 0.33	11.27 <sup>b</sup> <sub>±</sub> 0.43	41.67 <sup>b</sup> <sub>±</sub> 4.92	16.79 <sup>b</sup> <sub>±</sub> 1.51	4.98 <sup>b</sup> <sub>±</sub> 1.11
4 days	3.37 <sup>b</sup> <sub>±</sub> 0.51	11.68 <sup>bc</sup> <sub>±</sub> 0.44	44.80 <sup>c</sup> <sub>±</sub> 1.35	15.98 <sup>bc</sup> <sub>±</sub> 1.00	5.41 <sup>bc</sup> <sub>±</sub> 1.07
6 days	2.94 <sup>abc</sup> <sub>±</sub> 0.42	11.84 <sup>cd</sup> <sub>±</sub> 0.38	46.11 <sup>cd</sup> <sub>±</sub> 3.74	15.84 <sup>bd</sup> <sub>±</sub> 2.62	5.09 <sup>bd</sup> <sub>±</sub> 1.70
8 days	2.28 <sup>a</sup> <sub>±</sub> 0.49	12.15 <sup>ce</sup> <sub>±</sub> 0.55	46.40 <sup>ce</sup> <sub>±</sub> 1.53	16.17 <sup>be</sup> <sub>±</sub> 1.21	6.91 <sup>e</sup> <sub>±</sub> 0.71



CMSF, .....; CL, .....; L\*, .....; a\*, .....; b\*, .....

<sup>a-e</sup> Numbers with different superscripts in the same column stated a significant difference ( $P < 0.05$ ) and a highly significant difference ( $P < 0.001$ )

LS Levels: Liquid smoke levels

The liquid smoke level in UCSMB has no significant effect on CMSF and cooking loss (Table 2) states that liquid smoke concentration of 10% to 2% level of UCSMB material composition has not been able to decrease CMSF and cooking loss. The previous research on the addition of liquid smoke directly on fresh meat shows that the concentration of liquid smoke does not significantly affect the shear force of cooked meat at a temperature of 80°C for 15 minutes. There is a tendency of SF values to decrease with increasing levels of liquid smoke (Abustam et al., 2014b). There is no decrease in CMSF on increasing the level of liquid smoke to the question of whether the cooked meat observed in this study experienced protein oxidation. As Bhattacharya et al. (2016) stated that if the protein undergoes oxidation, it can decrease the tenderness of the flesh. Rowe et al. (2004) suggest that the protein oxidation will change the WHC and tenderness of the meat.

The longer time of aging decreased the value of meat cooked shear force reaching 0.16 point from the first day of aging indicate that liquid smoke during aging increase the tenderness of the meat despite the increase in CMSF on the second and sixth days of maturation. Similarly, cooking loss decreases with increasing maturation time reaching 2.28 point from the first day of aging indicating that liquid smoke in UCSMB feed can decrease cooking loss during maturation.

Increasing the level of liquid smoke in UCSMB feed increases the lightness of L\* color, decreases the reddish color of a\* and increases the yellowish color of b\*. Previous research has shown that the administration of liquid smoke in the form of flour in fresh meat has no significant effect on color lightness (L\*) and reddish color (a\*) (Abustam et al., 2014a). Antioxidant activity on liquid smoke was able to inhibit the oxidation of proteins so that the color of the L\* and b\* of meat had increased. Bhattacharya et al. (2016) stated that when meat protein undergoes oxidation the functional properties of meat, then it will decrease.

The lightness level (L\*) of Bali beef in this study (Table 2) met the range that typically occurs in beef that is between 35 %- 60% (AMSA, 2012). Similarly the value for redness level (a\*) of beef Bali in this study fulfilled the range that typically occurs in beef, i.e., between 2% - 30% (AMSA, 2012).

Aging time increased the lightness of the color (L\*), the level of redness (a\*) and the degree of yellowness (b\*). Increasing the aging time color L\* and a\* grown in the 8.55 and 5.1 points, while the color of b\* increased 5.88 points. These results suggest that the color of the flesh during maturation does not undergo protein oxidation. Bhattacharya et al. (2016) stated that when meat protein undergoes oxidation the functional properties of meat, then it will decrease.

## Conclusions

The current study revealed that the increase in the level of liquid smoke in feed block resulted in MCSF value which is more or less the same. Similarly, cooking loss on meat cooked 80°C for 30 minutes yields approximately the same value as increasing liquid smoke levels. The longer aging time decreased shear force value on cooked meat and cooking loss. The meat color (L\*, a\*) increased, and b\* decreased as the level of liquid smoke increasing. Meanwhile L\*, a\*, and b\* increases with increasing maturation time. The result of this study concluded that the liquid smoke in UCSMB could improve the quality of the meat of Bali cattle.



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## **Effect of *Flemingia (Flemingia macrophylla)* as a Protein Replacement of Soybean Meal on Feed intake, Digestibility of Nutrients and Microbial Population in Thai Native Beef Cattle**

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### **Abstract**

The objective of this study was to determine the effect of *Flemingia (Flemingia macrophylla)* as a protein replacement for soybean meal on feed intake, digestibility of nutrients and microbial population in Thai native beef cattle. Four Thai native beef cattle with initial bodyweight (BW) of  $130 \pm 10$  kg were randomly assigned according to a  $4 \times 4$  Latin square design to receive four replacement levels of soybean meal (SBM) by *Flemingia* hay meal (FHM) at 0, 30, 60, and 100 % on dry matter (DM) basis. All cattle were fed rice straw ad libitum while additional concentrate was fed at 0.5 % BW daily. The experimental findings revealed that total feed intake, intake of rice straw and digestibility of DM, OM, and ADF were not affected ( $P > 0.05$ ) by feed supplementation. However digestibility of CP and NDF were increased ( $P < 0.05$ ) with increase in the replacement level of SBM by FHM. No differences ( $P > 0.05$ ) were found in ruminal pH and  $\text{NH}_3\text{-N}$  concentration in the rumen fluid of cattle fed with FLM when compared to those on control diet. Protozoa and fungal population were not altered by FLM supplementation while bacterial population was significantly increased in cattle receiving FLM at 60 or 100 %DM. Populations of total bacteria, cellulolytic bacteria, proteolytic bacteria and amylolytic bacteria were not affected by dietary treatments ( $P > 0.05$ ). The present study suggested that replacement of SBM by FHM at 100 % in concentrate ration resulted in improvement of nutrient digestibility, rumen fermentation and bacteria populations for Thai native beef cattle fed on rice straw in Thai native beef cattle.

**Keywords:** *Flemingia macrophylla*, soybean meal, rice straw, bacteria population, beef cattle

### **Introduction**

Ruminants raised in the tropics largely depend on seasonal feed resources which are relatively low in quality in terms of low crude protein (CP) but high in crude fiber (CF); hence, the manipulation of rumen efficiency through the uses of local feed resources would be an advantage (Wanapat, 2000). *Flemingia macrophylla* is a drought-tolerant, perennial multipurpose shrub legume especially suited to low-input smallholder production systems in the subhumid and humid tropics. *Flemingia* foliage has high crude protein (CP) at 25.8 % and condensed tannin (CT) at 5.8 % (Kang et al., 2016) with fresh edible biomass of 45 to 64 tons/ha/year (Binh et al., 1998). Moreover, the total tannin content of *Flemingia* was 30.8 g/kg DM (Mui et al., 2002). The major benefit of tannins in feed is protection of plant protein from digestion in the rumen, making it available for digestion and utilization in the abomasum and small intestine (Waghorn, 1990; Norton, 1999). Kang et al. (2016) reported that



supplementation of *Flemingia* hay meal (FMH) could enhance *in vitro* fermentation and reduced CH<sub>4</sub> production. It was proposed that a small addition of *Flemingia* to the diet can reduce protein degradation in the rumen, increasing the supply of rumen undegradable protein in the gut resulting in higher live weight gains as well as potentially decreasing rumen CH<sub>4</sub>.

More important, low quality of proteins has been found in the seasonally dry of feed during the dry season was determined by low livestock productivity (Leng, 1990). Soybean meal (SBM) has long been used as a prominent source of CP for ruminants, however, with its increasing price, the use results in higher cost of production. Concentrate supplemental feeds used to improve overall efficiency of animal production (Wanapat and Cherdthong, 2009). Use of alternative protein sources may help to increase livestock productivity by providing a high protein supplement. Therefore, the objectives of this study were to investigate the effect of *Flemingia* (*Flemingia macrophylla*) as a protein replacement for soybean meal on feed intake, digestibility of nutrients and microbial population in Thai native beef cattle.

## Materials and Methods

Four Thai native beef cattle with initial body weight (BW) of  $130 \pm 10$  kg were randomly assigned to receive four dietary treatments according to a 4 x 4 Latin square design. All animals were received concentrate diet at 0.5% of body weight and rice straw was fed *ad libitum*. The experimental treatments consisted of four dietary containing different levels of *Flemingia* hay meal (FHM) (at 0, 30, 60, and 100 % of dry matter (DM) as a replacement for SBM, respectively. Feed ingredients and chemical composition are presented in Table 1. Roughage and concentrate were sampled for feeds analysis daily during the collection period. The experiment was conducted for four periods; each experimental period lasted for 21 days. First 14 days were used as adaptation period in which, all animals were fed with their respective diets followed by a 7-day collection period. During the collection period, cattles were kept in metabolism crates for the daily feces collection. Feed and fecal samples were collected each day during a 7-day collection period. Composited samples were dried at 60°C in a forced-air oven for 48 h and ground to 1-mm screen using Cyclotech Mill (Tecator, Sweden) to determine DM, OM, CP according to AOAC (1990), NDF and ADF according to Van Soest et al. (1991). During the final day of collection period, rumen fluid were collected at 0 and 4-h post feeding. Rumen fluid pH was immediately measured for pH (HANNA Instrument HI 8424 microcomputer, Singapore). Rumen fluid samples were then kept for analysis of ammonia nitrogen (NH<sub>3</sub>-N) using the micro-Kjeldahl methods AOAC (1990). Other part of rumen fluid was collected and prepared for three groups of bacteria (cellulolytic, proteolytic, and amylolytic bacteria) determined using roll-tube technique (Hungate, 1969). All data were analyzed according to a 4x4 Latin square design using Proc GLM/Proc Mix (SAS, 1996). Data were analyzed using the model  $Y_{ijk} = \mu + M_i + A_j + P_k + \epsilon_{ijk}$  where  $Y_{ijk}$  is the observation from animal  $j$ , receiving diet  $i$ , in period  $k$ ;  $\mu$ , the overall mean;  $M_i$ , effect of treatment ( $i=1$  to 4);  $A_j$ , the effect of animal ( $j=1$  to 4);  $P_k$ , the effect of period ( $k=1$  to 4); and  $\epsilon_{ijk}$ , the residual effect. Treatment means were statistically compared by Duncan's New Multiple Range Test (Steel et al., 1997). Differences among means with  $P < 0.05$  were accepted as representing statistically significant differences.



## Results and Discussion

The chemical compositions of four concentrate diets, FHM and rice straw are shown in Table 1. On a DM basis the CP contents in concentrate mixtures were similar among treatments while NDF and ADF contents were increased with increasing FHM inclusion. Table 2 presents the data for feed intakes and apparent digestibility of nutrients. Total feed intake, intake of rice straw and digestibility of DM, OM, and ADF were not affected ( $P>0.05$ ). However, digestibility of CP and NDF were increased ( $P<0.05$ ) with increasing level of FHM replacement. The decreasing of feed intake and apparent nutrient digestibility could relate to formation of tannin–protein complex preventing feed to be attached by rumen microbes (Horigome et al. 1988). On the other hand, Fagundes et al. (2014) reported that feeding *Flemingia* foliage resulted in lower digestibility of goat. Calabrò et al. (2011) and Guglielmelli et al. (2011) reported that if CT in the feed exceeded 6 % of DM, feed intake and digestibility would be dramatically reduced while CT level between 2 and 4 % DM, it would help to protect protein from rumen digestion, there by increasing bypass protein or rumen undegradable protein. Table 3 showed effect of *Flemingia* (*Flemingia macrophylla*) as a protein replacement for soybean meal on ruminal pH, ruminal temperature,  $\text{NH}_3\text{-N}$  concentration and microbial population in cattle. No differences ( $P>0.05$ ) were found in ruminal pH and  $\text{NH}_3\text{-N}$  concentration in the rumen fluid of cattle fed with level of FHM replacement. Rumen pH at 0 and 4 h post-feeding were unchanged by dietary treatments and the values were quite stable at 6.6-6.8. These values were optimal for normal rumen fermentation (Wanapat,1990). In this study found that an average value of 15.5 mg/d  $\text{NH}_3\text{-N}$  concentration in the ruminal fluid for the different treatments, a value that is close the optimal concentration of 15 to 30 mg/dl recommended by Wanapat and Pimpa (1999). Protozoa and fungal population were not altered by FLM replacing SBM while bacterial population was significantly increased when SBM was replaced with FLM at 60 or 100 %DM. Populations of total bacteria, cellulolytic bacteria, proteolytic bacteria and amylolytic bacteria were not affected by treatment supplementation ( $P>0.05$ ). Condensed tannin in the diet has beneficial effects which are dedicated by protein–tannin complexation, decreasing availability of feed protein for ruminal degradation and ammonia nitrogen release (Makkar,2003). However, inhibitory activity of tannins against bacteria has been implicated owing to the ability of tannins to form complexes with the cell wall and membrane of bacteria causing morphological changes of the cell wall and the extracellular enzymes secreted (Jones et al., 1994).

## Conclusions and recommendations

Based on this study, it could be concluded that FHM could be used as protein replacement for soybean meal which could enhanced nutrient digestibility, rumen fermentation and bacteria populations. This study suggested a replacement of SBM by FHM at 100 % in concentrate ration for Thai native beef cattle fed on rice straw. However, further research should be conducted to investigate on the productivity of both beef and dairy cattles.

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**Table 1.** Ingredient and chemical composition of concentrates, Flemingia hay meal (FHM) and rice straw

Ingredients	Levels of LHM replacing SBM,%DM				FLM	Rice straw	
	0	30	60	100			
Ingredient (kg of DM)							
Cassava chip	60.0	60.0	58.0	58.0			
Soybean meal (SBM)	20.0	14.0	8.0	0.0			
Flemingia hay meal (FLM)	0.0	6.0	12.0	20.0			
Rice bran	5.0	4.6	4.0	4.0			
Coconut meal	4.0	4.0	5.0	5.0			
Palm kernel meal	4.0	4.0	5.3	4.8			
Urea	2.0	2.4	2.7	3.2			
Molasses	2.0	2.0	2.0	2.0			
Mineral premix	1.0	1.0	1.0	1.0			
Salt	1.0	1.0	1.0	1.0			
Sulfur	1.0	1.0	1.0	1.0			
Chemical composition							
DM, %	85.5	84.2	85.3	86.1	84.6	90.2	
		% of DM					
CP	18.1	18.3	18.2	18.6	25.6	2.4	
OM	92.7	91.4	93.2	94.1	92.3	89.7	
NDF	18.2	21.3	24.5	26.8	53.1	85.2	
NDF	12.3	14.5	16.8	18.9	31.2	56.8	



**Table 2.** Effect of *Flemingia (Flemingia macrophylla)* as a protein replacement for soybean meal on voluntary feed intake and nutrient digestibility.

Items	Replacement levels FLM replacing SBM, %DM				SEM	P-value
	0	30	60	100		
Rice straw intake,						
Kg DM	1.7	1.6	1.7	1.8	1.81	0.14
%BW	1.1	1.0	1.1	1.2	0.63	0.19
g/kg BW <sup>0.75</sup>	40.2	37.1	41.7	42.6	0.05	0.13
Total DM intake,						
kg DM	2.5	2.7	2.7	2.8	0.87	0.27
%BW	1.6	1.5	1.5	1.7	5.06	0.97
g/kg BW <sup>0.75</sup>	58.3	52.8	54.3	61.2	0.18	0.10
Apparent digestibility, %						
DM	60.8	60.03	62.25	63.5	3.45	0.04
OM	63.2	67.2	66.5	67.0	3.58	0.42
CP	74.0 <sup>b</sup>	72.8 <sup>ab</sup>	72.1 <sup>a</sup>	71.2 <sup>a</sup>	0.38	0.04
NDF	55.1 <sup>a</sup>	55.6 <sup>a</sup>	57.2 <sup>ab</sup>	59.8 <sup>b</sup>	0.54	0.03
ADF	46.3	44.2	43.7	45.4	3.71	0.53

<sup>a, b</sup>, Means in the same row with different superscripts differed (P<0.05)  
SEM, standard error of the means





**Table 3.** Effect of *Flemingia (Flemingia macrophylla)* as a protein replacement for soybean meal on ruminal pH, ruminal temperature, NH<sub>3</sub>-N concentration and microbial population in cattle

Items	Replacement levels FLM replacing SBM, %DM				SEM	P-value
	0	30	60	100		
Ruminal pH	6.9	6.8	6.7	6.8	0.34	0.12
Temperature	39.0	39.1	38.6	38.9	0.40	0.09
NH <sub>3</sub> -N, mg/dl	16.4	15.5	15.8	15.1	0.44	0.08
Ruminal microbes, cells/ml						
Bacteria, ×10 <sup>11</sup>	6.4 <sup>a</sup>	6.8 <sup>a</sup>	8.5 <sup>ab</sup>	9.2 <sup>b</sup>	0.38	0.04
Protozoa, ×10 <sup>6</sup>	4.6	4.3	4.0	3.5	0.86	0.89
Fungal zoospore, ×10 <sup>4</sup>	1.5	2.5	2.4	2.9	0.33	0.23
Rolltube technique, (CFU/ml)						
Total viable bacteria, x10 <sup>8</sup>	9.2	8.6	8.2	9.1	0.43	0.30
Cellulolytic bacteria, x10 <sup>8</sup>	8.2	7.9	8.1	8.3	1.45	0.26
Proteolytic bacteria, x 10 <sup>7</sup>	7.2	7.4	6.1	7.3	1.43	0.31
Amylolytic bacteria, x 10 <sup>7</sup>	7.4	7.2	8.4	8.1	0.46	0.32

<sup>a, b</sup>, Means in the same row with different superscripts differed (P<0.05)

SEM, standard error of the means



## Nutrient Utilization and Rumen Ecology of Thai Indigenous Cattle Given Hay and Sago Palm Pith with Different Levels of Soybean Meal

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### Abstract

This study aimed to determine nutrient utilization and rumen ecology of Thai indigenous cattle given hay and sago palm pith (SPP) as an energy source with different levels of soybean meal (SBM) as a protein source. Four ruminally fistulated Thai indigenous bulls, with an average BW of  $299 \pm 14$  kg were arranged in a 4 x 4 Latin square design. Plicatum hay was offered *ad libitum* with SPP supplementation at 0.75% of BW. The treatments were 4 different levels of SBM: 0.25, 0.50, 0.75 and 1.0% of BW (T1, T2, T3 and T4, respectively). Total DMI and OMI were significantly ( $p < 0.05$ ) higher for T3 than T1 group. The CPI was lowest in T1, followed by T2, T4 and T3, respectively. No significant differences among treatments regarding the apparent digestibility of DM, OM, NDF and ADF were found. N balance was significantly lower in T1 ( $P < 0.05$ ) than T3 and T4 ( $P > 0.05$ ). Ruminal pH of all groups were not significantly different ( $P > 0.05$ ). Average ruminal  $\text{NH}_3\text{-N}$  and BUN concentration were increased according to the level of SBM supplementation. However, VFA concentration and microbial population in the rumen fluid were not significantly different among the treatments ( $P > 0.05$ ). Therefore, supplementation of SBM at 0.75% of BW with SPP at 0.75% of BW resulted in a positive effect on nutrient utilization and rumen ecology of Thai indigenous cattle given plicatum hay.

**Keywords:** soybean meal, sago palm pith, Thai indigenous cattle, nutrient utilization, rumen ecology

### Introduction

Low quality forages are important sources of nutrients used to maintain Thai indigenous cattle during dry and heavy rainy season when fresh forages are not available. To optimize the utilization of these forages and maintain acceptable animal performance, it is generally desirable to enhance intake and digestion via the provision of supplemental nutrients. Generally, protein is considered to be the dietary component that is first limiting to the utilization of low quality forage. Supplementation of protein has been shown to increase intake, digestion and performance of ruminant fed low quality roughages. However, providing supplemental protein to cattle consuming low quality forage may or may not increase forage dry matter intake (DMI) depending on the energy to protein ratio of the forage (Hammond, 1983). In our previous study, supplementation of sago palm pith (SPP) as an energy source at 0.75% of BW for Thai indigenous cattle given low quality hay and a protein source from soybean meal (SBM), increased total DMI, OM intake (OMI) and digestibility due to improvement of protein and energy balance (Ngampongsai and Chanjula, 2009). Therefore, this study is aimed at examining the effects of different levels of SBM supplementation on nutrient utilization and rumen ecology of Thai indigenous cattle given hay with SPP.



## Materials and Methods

Four ruminally fistulated Thai indigenous bulls with average body weight (BW) of  $299 \pm 14$  kg were randomly assigned according to a 4 x 4 Latin Square Design. Each animal was kept in an individual pen and received free access to water. Plicatum hay (*Paspalum plicatum* Michx.) was offered to each animal *ad libitum* with SPP supplementation at 0.75% of BW. The treatments were SBM supplementation at 0.25, 0.50, 0.75 and 1.00 % of BW (T1, T2, T3 and T4, respectively). The diet was offered daily at 08.00 and 16.00 hours.

The experiment consisted of 4 periods. Each period lasted for 20 days, with the first 14 days for an adaptation period and the last 6 days for the sample collection period with intake was restricted to 90% of the previous voluntary intake of hay. Feed intake was measured on a daily basis during the adaptation period. Dietary feed offered,orts, and 24 hours feces and urine voided by individual animals during the collection period were recorded and representative samples were taken for chemical composition analysis. Representative samples of feed and feces were analyzed according to AOAC (1990) for proximate analysis and fiber components (Van Soest et al., 1991). Urine nitrogen (N) was analyzed by the method of the AOAC (1990). On the last day of the sample collection period, rumen fluid sample was collected at 0 and 4 hours post-feeding via the fistula. Samples were divided into two portions. One portion was acidified with  $H_2SO_4$  (1 M) and stored at  $-20^\circ C$  for analysis of ammonia nitrogen ( $NH_3-N$ ) (AOAC, 1990) and volatile fatty acid (VFA) (Josefa et al., 1999). Another portion was fixed with 10% formalin solution in normal saline (0.9% NaCl) for total direct count of the bacteria, protozoa, and fungi zoospores (Gaylean, 1989). Blood samples were collected via the jugular vein into heparinized tube at the same time as rumen fluid sampling. The blood samples were centrifuged and supernatants were separated and frozen at  $-20^\circ C$  prior to blood urea nitrogen (BUN) determination.

All data were subjected to analysis of variance for 4x4 Latin square design and treatment means were compared using Duncan's Multiple Range Test (DMRT) (Steel and Torrie, 1980).

## Results and Discussion

The effects of SBM supplementation on feed intake, apparent nutrients digestibility and N balance of Thai indigenous cattle are presented in Table 1. The DMI from plicatum hay and SPP+SBM was statistically unaffected by dietary treatments and there was a trend of increasing with an increasing levels of SBM supplementation up to 0.75% of BW. A positive effect of protein or N supplementation on the intake or utilization of low quality hay has been recognized (Guthrie and Wagner, 1988). Total DMI and OMI were significantly ( $p < 0.05$ ) higher for T3 than the T1 group. The CP intake (CPI) was lowest in T1, followed by T2, T4 and T3, respectively. No significant differences among treatments regarding the apparent digestibility of DM, OM, NDF and ADF were found. This was similar to the findings of Kawashima et al. (2000) in Thai native cattle which any of fiber fraction digestibilities of ruzi hay were not improved by the supplementation of SBM. It showed that the animal has an ability to digest fiber well even without protein supplementation. Furthermore, Casper et al. (1994) hypothesized that identifying a correct non- structural carbohydrate and ruminal degradable protein ratio may increase efficiency of rumen fermentation process. The lack of response in total tract DM digestibility of the higher SBM levels supplementation may have been due to low non-structural carbohydrate and ruminal degradable protein ratio resulting from excess ruminal degradable protein or not enough degradable carbohydrate. The digestibility of CP showed a similar trend as that of CPI, although the values in T2, T3 and T4 showed no significant differences ( $P > 0.05$ ). The improved CPI and CP digestibility associated with SBM supplementation likely reflects increased N supply for ruminal microbial and synthesis. Diets with high CP or ruminal degradable protein



concentrations usually have greater apparent digestibility of OM and CP due to increase intake of more digestible feeds and increase  $\text{NH}_3\text{-N}$  synthesis for incorporation into microbial protein (Olmos Colmenero and Broderick, 2006).

**Table 1.** Nutrient intake, apparent digestibility and N balance of Thai indigenous cattle given plicatulum hay and SPP with different levels of SBM

Attributes	SBM level (% BW)				SEM
	T1 (0.25%)	T2 (0.50%)	T3 (0.75%)	T4 (1.00%)	
DM intake (kg/d)					
SPP+SBM	3.08	3.83	4.04	3.77	0.275
Plicatulum hay	1.91	1.96	2.31	2.31	0.150
Total	4.99 <sup>b</sup>	5.79 <sup>ab</sup>	6.35 <sup>a</sup>	6.08 <sup>ab</sup>	0.329
OM intake (kg/d)	4.62 <sup>b</sup>	5.35 <sup>ab</sup>	6.14 <sup>a</sup>	5.57 <sup>a</sup>	0.235
CP intake (kg/d)	0.42 <sup>c</sup>	0.81 <sup>b</sup>	1.12 <sup>a</sup>	1.09 <sup>a</sup>	0.057
NDF intake (kg/d)	2.39	2.52	2.84	2.65	0.136
Apparent digestibility (%)					
DM	64.63	67.41	69.58	65.25	3.378
OM	67.98	70.32	73.61	68.26	3.101
CP	55.46 <sup>b</sup>	69.57 <sup>a</sup>	76.17 <sup>a</sup>	72.73 <sup>a</sup>	3.523
NDF	49.73	49.03	54.45	46.62	5.540
ADF	21.62	27.57	36.03	29.40	3.68
N utilization					
N intake (g/d)					
SPP+SBM	67.85 <sup>c</sup>	128.87 <sup>b</sup>	168.41 <sup>a</sup>	179.15 <sup>a</sup>	10.031
Plicatulum hay	10.23	10.95	12.34	12.34	0.801
Total	78.08 <sup>c</sup>	139.32 <sup>b</sup>	180.75 <sup>a</sup>	191.48 <sup>a</sup>	10.117
N excretion (g/d)					
Faeces	34.39	42.49	46.31	50.53	4.740
Urine	23.94 <sup>b</sup>	46.48 <sup>ab</sup>	64.39 <sup>a</sup>	49.97 <sup>ab</sup>	9.332
Total	58.33 <sup>b</sup>	88.77 <sup>a</sup>	110.70 <sup>a</sup>	100.50 <sup>a</sup>	8.214
N balance (g/d)	19.74 <sup>b</sup>	50.55 <sup>ab</sup>	70.03 <sup>a</sup>	90.98 <sup>a</sup>	13.694

Total N intake was significantly increased according to the levels of SBM supplementation. Increasing SBM levels tended to increase urinary N excretion, however did not affect faecal N excretion. Total N excretion also showed a similar trend as N intake, however, there was no difference among T2, T3 and T4. N balance was significantly lower in T1 ( $P < 0.05$ ) than T2, T3 and T4 ( $P > 0.05$ ). It is now well established that N balance depends not only the intake of N, but also the amount of fermentable carbohydrate in the diet (Sarwar et al., 2003). The lack of N balance improvement when SBM level in the diet was beyond 0.25% of BW could be explained by an imbalance of energy yielding nutrients for microbial uptake for  $\text{NH}_3\text{-N}$  resulting in insufficient utilization and absorption of nutrient or that these levels are higher than the overall requirements of the animal (Gabler and Heinrichs, 2003).

The pattern of rumen fermentation of Thai indigenous cattle given plicatulum hay and SPP with different levels of SBM are given in Table 2. Average ruminal pH for individual treatment ranged from 6.83-6.91, which falls within the range of acceptability for ruminal bacteria to effectively digest fiber and protein (Hoover, 1986). Average ruminal  $\text{NH}_3\text{-N}$  concentration were increased according to the level of SBM supplementation. Likewise, BUN concentration were significantly ( $P < 0.05$ ) higher as higher levels of SBM were incorporated into diet. The concentration of BUN are highly correlated to protein intake and reflect the level of



ruminal  $\text{NH}_3\text{-N}$  production (Preston et al., 1965; Lewis, 1975). This would indicate that available ruminal  $\text{NH}_3\text{-N}$  could be used and/or absorbed in the rumen for further synthesis. Differences in  $\text{NH}_3\text{-N}$  concentration between dietary treatments were likely due to the increasing degradable protein from SBM (Bodine, 2000).

**Table 2** Rumen ecology and BUN concentration of Thai indigenous cattle given plicatulum hay and SPP with different levels of SBM

Attribute	SBM (%BW)				SEM
	T1(0.25%)	T2(0.50%)	T3(0.75%)	T4(1.00%)	
Rumen pH	6.88	6.86	6.83	6.91	0.175
$\text{NH}_3\text{-N}$ (mg/dl)	10.63 <sup>b</sup>	12.15 <sup>ab</sup>	16.61 <sup>a</sup>	16.97 <sup>a</sup>	2.450
TVFA (mmol/L)	96.72	85.20	97.36	89.37	8.767
C <sub>2</sub> (%)	71.93	71.40	72.25	72.79	1.390
C <sub>3</sub> (%)	18.15	18.53	18.69	16.76	1.259
C <sub>4</sub> (%)	9.91	10.08	9.26	10.14	0.580
C <sub>2</sub> : C <sub>3</sub>	4.39	3.92	3.97	4.61	0.317
Microbial population					
Bacteria ( $\times 10^{11}$ cell/ml)	1.55	1.71	1.84	1.76	0.158
Protozoa ( $\times 10^6$ cell/ml)	1.86	1.87	1.88	2.11	0.106
Fungi ( $\times 10^5$ cell/ml)	0.66	0.64	0.67	0.65	0.023
BUN (mg/dl)	16.09 <sup>b</sup>	24.67 <sup>a</sup>	29.03 <sup>a</sup>	27.84 <sup>a</sup>	3.228

Overall means of total VFA and C<sub>2</sub>, C<sub>3</sub>, C<sub>4</sub> concentrations and the C<sub>2</sub> to C<sub>3</sub> ratio in the rumen were not different among dietary treatments ( $P > 0.05$ ). The total VFA concentrations in all treatments were presented at normal range of 70-30 mmol/l (France and Siddons, 1993). The C<sub>2</sub> to C<sub>3</sub> ratio tended to be slightly decreased by the supplementation of SBM up to 0.75% of BW. Effect of any feed supplementation on ruminal fermentation are consider positive either when is reported an increase on total VFA, and C<sub>3</sub> production or when is found a decrease on C<sub>2</sub> to C<sub>3</sub> ratio (Castillejos et al., 2008). Populations of rumen microbes were variable and were not affected by SBM supplementation levels.

## Conclusion

In conclusion, under the conditions of this study, providing SBM at 0.75% of BW and SPP at 0.75% of BW, might be an appropriate ratio for Thai indigenous cattle consuming low quality hay.

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## Comparison Between Hay and Silage of *Pennisetum Purpurium* cv. Mahasarakham Feeding on Feed intake, Nutrient Digestibility, and Rumen Fermentation in Thai Native Beef Bulls

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### **Abstract**

Eight Thai native beef bulls were used in a completely randomized design to evaluate the feeding value of Sweet grass (*Pennisetum purpurium* cv. Mahasarakham) preservation (silage or hay) on feed intake, digestibility and rumen fermentation. The animal received both of forage *ad libitum*. Based on this experiment, there were no significant differences ( $P>0.05$ ) between treatments regarding DMI, digestibility of CP, NDF and ADF. Likewise, mean ruminal  $\text{NH}_3\text{-N}$ , TVFAs, proportion of VFAs, protozoa population and BUN were not affected ( $P>0.05$ ) by silage and hay feeding, DM digestibility, however, appeared to be significantly higher ( $P<0.05$ ) in silage treatment but ruminal pH was significantly lower ( $P<0.05$ ). Based on this study, Sweet grass can be fed as a silage or hay which can be preserved with no negative effect on the animals.

**Keywords:** silage, hay, forage, preservation, beef bulls

### **Introduction**

In the present, Elephant or Napier grass (*Pennisetum purpurium*) is a favorite forage crop due to its high yield and good nutrient composition. There are two varieties namely tall and short (Mott or dwarf) which are different in production and nutritive values. Tall variety can produce large amount of yield but has lower nutritive value than the short variety due to its lower in leaf to stem ratio (LSR). Sweet grass (*Pennisetum purpurium* cv. Mahasarakham) is a short variety which is better in nutritive value, good palatability, wide adaptability, ease of management and harvesting compared to the tall variety (Mukhtar et al., 2003; Halim et al., 2013; Kabirizi et al., 2015; Negawo et al., 2017). It is a leafier grass with high potential to be a good quality forage for the ruminants. However, research information of this grass is limited. Therefore, the objective of this experiment was to comparison between hay and silage as the preservation of Sweet grass on feed intake, nutrient digestibility, and rumen fermentation in Thai native beef bulls.

### **Materials and Methods**

#### **Experimental animals, diets, sample collection and analysis**

Eight Thai native beef bulls (27 months of age) with an initial bodyweight (BW) of  $317\pm 4$  kg were used in this study. Animals were kept individually in pens (4 x 3 m) under well-ventilated sheds where water and mineral salt were available at all times. Sweet grass (*Pennisetum purpurium* cv. Mahasarakham) was harvested at  $45\pm 7$  days of regrowth, chopped into small pieces (2.0-3.0 cm) and collected to prepare as hay or silage. All harvested material were separated for two parts: the first part was made hay by sun-dried for 3-5 days, then stored



for subsequent feeding and the second part was made silage by sun-dried for 5-6 hours then mixed with 3 % of molasses before storing in the plastic barrel under anaerobic condition for 21 days before feeding. The concentrate was mixed by using local feed ingredients (Table 1) and fed at 1.0 % of bodyweight.

Animals were separated for two groups to receive Sweet grass hay or silage *ad libitum*. The experiment was conducted for 21 days. During the first 14 days as animal adaptation period and the last 7 days for data collection including feed intake, feed and feces sample. Feed and feces samples obtained were oven dried at 60 °C for 72 h, grounded to pass through a 1-mm sieve, and composited by period on an equal weight basis, and analyzed for dry matter (DM), crude protein (CP) content (AOAC, 2012). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) were analyzed by the technique of Van Soest et al. (1991). On the last day, rumen fluid and blood sample to collected pH, NH<sub>3</sub>-N measurements (AOAC, 2012), volatile fatty acids analysis (Samuel et al., 1997), and protozoa population (Galyean, 1989)] and blood urea nitrogen (BUN; Crocker, 1967), respectively. All data obtained from the experiment were subjected to ANOVA (SAS, 2004) by Duncan's New Multiple Range Test (DMRT) (Steel and Torrie, 1980).

**Table 1.** Feed ingredients of concentrate and chemical composition of experimental diets.

Items	Concentrate	Sweet grass silage	Sweet grass hay
Feed ingredients			
Cassava chip	62.0		
Rice bran	5.0		
Coconut meal	18.0		
Palm meal	10.0		
Urea	1.5		
Molasses	2.0		
Sulfur	0.5		
Mineral mixed	0.5		
Salt	0.5		
Total	100		
Chemical composition			
Dry matter, %	89.4	33.2	85.7
	-----% of dry matter-----		
Crude protein (CP)	14.3	15.7	15.3
Neutral Detergent Fiber (NDF)	20.1	58.1	61.7
Acid Detergent Fiber (ADF)	11.5	32.7	33.1
pH	-	3.9	-

## Results and Discussion

In this study, DMI, digestibility of CP, NDF, ADF were not significant different between silage and hay feeding ( $P>0.05$ ). However, DM digestibility of Sweet grass silage was higher than hay ( $P<0.05$ ). This result might be influence of ADF content in silage was lower than hay resulted in has higher digestibility of DM. In agreed with Ramin and Huhtanen (2013) reported that forage crop with lower ADF content showed higher digestion and rate of fermentation. However, ruminal pH was difference among treatment ( $P<0.05$ ) which was higher in Sweet grass hay due to its high in DM and NDF contents affect salivary output during eating by affecting the eating rate resulted in increased rumen pH (Beauchemin et al., 2008). Nevertheless, feeding





of silage and hay were not effect on  $\text{NH}_3\text{-N}$ , TVFAs, proportion of VFAs, protozoa population and blood urea nitrogen ( $P>0.05$ ).

**Table 2.** Effect of Sweet grass silage and hay on feed intake and nutrient digestibility of Thai native beef bulls.

Items	Silage	Hay	SEM
Forage intake			
kg.DM/h/d	4.0	4.2	0.36
% of BW	1.8	1.9	0.12
Total intake			
kg.DM/h/d	6.0	6.4	0.37
% of BW	2.8	2.9	0.10
Nutrient digestibility, %			
DM	69.0 <sup>a</sup>	67.8 <sup>b</sup>	0.11
CP	75.6	75.2	0.26
NDF	55.4	54.7	0.30
ADF	50.5	50.2	0.32

<sup>ab</sup>Means within rows followed with different superscript letters are statistically different ( $P<0.05$ ); \*SEM, Standard error of the mean; DM, dry matter.

**Table 3.** Effect of Sweet grass silage and hay of on rumen fermentation patterns and blood urea nitrogen in Thai native beef bulls.

Items	Silage	Hay	SEM*
pH	6.4 <sup>a</sup>	6.7 <sup>b</sup>	0.06
$\text{NH}_3\text{-N}$ , mg%	22.8	21.8	0.49
TVFAs, mg%	93.8	94.7	0.56
Acetic acid, %	63.9	64.8	0.50
Propionic acid, %	25.6	25.0	0.49
Butyric acid, %	10.5	10.2	0.30
Protozoa population, $\times 10^5$ cell/mL	12.9	13.0	0.67
Blood urea nitrogen, mg%	15.3	14.9	0.29

<sup>a,b</sup>Means within rows followed with different superscript letters are statistically different ( $P<0.05$ ). \*SEM, Standard error of the mean; TVFAs, Total volatile fatty acids

## Conclusion

Overall, it could be concluded that conserving Sweet grass as silage increased DM digestibility while hay increased ruminal pH. However, there were not adversely effect on animal utilization.

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## Increasing Productive Performance of Native Chickens by Herbs in Rural Community

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### Abstract

The objectives of this study were to: 1) study the context of raising native chickens in rural community. 2) transfer the knowledge of using herbs in increasing productive performance of native chickens to the farmers. 3) study the effect of herbs on productive performances of native chickens; and 4) study the understanding and satisfactions of farmers on utilization of herbs for increasing productive performances of native chickens. The target group was 16 farmers in Ban Talunglek, Tambon Talunglek, Amphur Muang, Buriram Province who participated in training workshop on transfer of using herbs for increasing productive performances of native chickens. After the training, the knowledge was implemented by study the effect of dietary supplementation of herbs on productive performances of native chickens done by farmers. White kwao krue (*Pueraria mirifica*), turmeric (*Curcuma longa* Linn), and kariyat (*Andrographis paniculata* (Burm. F.) Nees) were used in single and combination form. Twenty-one days old of 320 crossbred native chickens and average body weight  $245 \pm 51.23$  g/chick were used. The chickens were divided into 8 treatments with 4 replications and each replication consisted of 10 birds. The experiment was conducted for 12 weeks. Data of average daily gain, feed conversion ratio, and carcass percentage were collected for data analysis by using ANOVA and compared the average by Duncan's New Multiple Range test (DMRT). It was found that farmers raised the native chicken in backyard, using feed source in household and selling the native chickens as extra income. There was only one farmers raised the native chicken as main income. From the transfer knowledge, it had shown that the farmers gained knowledge and understanding as the highest level and could apply to raise their native chickens. The effects of dietary supplementation of herbs on productive performance of native chickens had shown that white kwao krue, turmeric, kariyat could be used for increasing productive performance of native chickens raised in rural community. In addition, chickens' health, there was very low mortality rate among groups. Farmers showed highest level of satisfaction on using herbs for increasing productive performance of native chickens because they could raise the chickens without antibiotics or commercial anthelmintic. It was an alternative way for produce herbal native chickens.

**Keywords:** white kwao krue, turmeric, kariyat, productive performances, native chickens, knowledge transfer



## Introduction

Rural native chicken farming is being practiced in many areas throughout Thailand. Rural backyard poultry production plays a vital role in the rapidly growing economy. It provides livelihood security to the family in addition to security the availability of food (Padhi. 2016). Native chicken meat is one of the most commonly used as animal protein source. The meat has lower fat and cholesterol content but the disadvantages of native chickens are lower growth efficiency and low productive performance is attributable to lack of proper feed, management, and sanitation programs (Jaturasitha et al. 2008). At present, the consumers need natural products that have high nutritive value, no contamination with microbes, chemicals and good quality. Native chicken are generally raised without using antibiotics or chemicals but some farmers used the antibiotic when they found the chickens infected some disease by they do not know the proper way use of those antibiotics. These antibiotics can be a remained in the meat and caused some side effects to the consumers. Therefore, the objectives of the study were to study the context of raising native chickens in rural community; transfer the knowledge of using herbs in increasing productive performance of native chickens to the farmers and implemented the knowledge by study the effect of herbs on productive performances of native chickens, and study the understanding and satisfactions of farmers on utilization of herbs for increasing productive performances of native chickens.

## Materials and Methods

In this study, we had divided the study into 4 parts as follow:

1. Study of the context of raising native chicken in Ban Talunglek, Tambon Talunglek, Amphur Muang, Buriram Province by survey and using in-depth interview.

2. Transfer of knowledge in using herbs for increasing productive performance of native chickens by training workshop for farmers in Ban Talunglek.

3. Utilization of herbs for increasing productive performance of native chickens according to sufficiency economy philosophy with 16 farmers in Ban Talunglek, Tambon Talunglek, Amphur Muang, Buriram Province. Three herbs used in this study were white kwao krue (*Pueraria mirifica*), turmeric (*Curcuma longa* Linn), and kariyat (*Andrographis paniculata* (Burm. F.) Nees) in single and combination forms. Twenty-one days old of 320 crossbred native chickens and average body weight was  $245 \pm 51.23$  grams were used in this study. The chickens were divided into 8 treatments with 4 replications and each replication consisted of 10 birds. The experimental diet were control diet (T1) without supplementation of any herbs, T2 was diet which supplemented with white kwao krue 1%, T3 was diet which supplemented with turmeric 0.1%, T4 was diet which supplemented with kariyat 0.2%, T5 was diet which supplemented with white kwao krue 1% and turmeric 0.1%, T6 was diet which supplemented with white kwao krue 1% and kariyat 0.2%, T7 was diet which supplemented with turmeric 0.1% and kariyat 0.2%. T7 was diet which supplemented with white kwao krue 1% and turmeric 0.1%, and kariyat 0.2%. The experiment was conducted for 12 weeks. Data of average daily gain (ADG), Feed conversion ratio (FCR), and carcass percentage were collected for data analysis by using ANOVA and compared the average by Duncan's New Multiple Range test (DMRT).

4. Study of farmer's satisfactions and understanding on utilization of herbs for increasing the productive performance of native chickens by using 5-scale rating questionnaire and In-depth interviewing.

## Results and discussions

It was found that farmers raised the native chicken in backyard, using feed source in household and selling the native chickens as extra income. There was only one farmers raised the



native chicken as main income and most of farmers were female. These information were consistent with Laenoi et al. (2015) and Haitook, Tawfik, and Zöbisch (2003). After transfer knowledge of using local herbs for increasing productive performances of native chickens, it had shown that the farmers gained knowledge and understanding as the highest level and could apply to raise their native chickens by study the effects of dietary supplementation of herbs on productive performance of native chickens.

**Table 1.** Effects of dietary supplementation of herbs on productive performance of native chickens

Productive performance	week	dietary supplementation of herbs								C.V.
		T1	T2	T3	T4	T5	T6	T7	T8	
ADG (gram/chick/day)	3-7	14.82 <sup>a</sup>	16.51 <sup>a</sup>	14.11 <sup>bc</sup>	13.21 <sup>bcd</sup>	12.86 <sup>cd</sup>	11.68 <sup>d</sup>	11.40 <sup>d</sup>	12.07 <sup>db</sup>	13.31
	8-13	18.76	21.87	18.56	16.57	19.29	15.95	24.62	18.82	19.43
	3-13	15.31 <sup>ab</sup>	17.55 <sup>a</sup>	14.92 <sup>ab</sup>	13.57 <sup>ab</sup>	14.79 <sup>ab</sup>	12.64 <sup>b</sup>	16.87 <sup>ab</sup>	14.24 <sup>ab</sup>	13.89
Feed intake (gram/chick/day)	3-7	9.35 <sup>ab</sup>	9.85 <sup>a</sup>	8.00 <sup>c</sup>	7.65 <sup>c</sup>	8.90 <sup>b</sup>	7.75 <sup>c</sup>	7.55 <sup>c</sup>	7.45 <sup>c</sup>	11.09
	8-13	2.15 <sup>b</sup>	2.36 <sup>a</sup>	2.23 <sup>b</sup>	2.00 <sup>c</sup>	1.95 <sup>c</sup>	1.96 <sup>c</sup>	2.22 <sup>b</sup>	2.00 <sup>c</sup>	7.18
	3-13	3.09 <sup>b</sup>	3.35 <sup>a</sup>	3.03 <sup>b</sup>	2.76 <sup>c</sup>	2.84 <sup>c</sup>	2.74 <sup>c</sup>	2.98 <sup>b</sup>	2.75 <sup>c</sup>	7.07
Feed conversion ratio	3-7	2.95 <sup>ab</sup>	2.69 <sup>a</sup>	2.71 <sup>ab</sup>	2.81 <sup>abc</sup>	3.28 <sup>abc</sup>	3.30 <sup>bc</sup>	3.40 <sup>c</sup>	3.07 <sup>abc</sup>	10.58
	8-13	2.50 <sup>ab</sup>	2.28 <sup>ab</sup>	2.57 <sup>ab</sup>	2.62 <sup>ab</sup>	2.72 <sup>b</sup>	2.77 <sup>b</sup>	2.18 <sup>a</sup>	2.47 <sup>ab</sup>	9.77
	3-13	2.62 <sup>ab</sup>	2.39 <sup>a</sup>	2.60 <sup>ab</sup>	2.67 <sup>ab</sup>	2.87 <sup>b</sup>	2.90 <sup>b</sup>	2.39 <sup>a</sup>	2.61 <sup>ab</sup>	8.70
Carcass percentage	13	69.69 <sup>c</sup>	82.89 <sup>a</sup>	70.00 <sup>bc</sup>	65.65 <sup>c</sup>	82.94 <sup>a</sup>	78.46 <sup>ab</sup>	85.77 <sup>a</sup>	84.94 <sup>a</sup>	10.77

<sup>a,b</sup> mean with different superscript in a row are significantly different (P<0.05)

The results had shown that ADG of chickens received 1.0% white kwao krue was the highest 17.6 g/d, it was different from the chickens received 1.0% white kwao krue combined with 0.2% kariyat that the lowest 12.6 g/d. Feed consumption of chickens received white kwao krue also showed the highest 3.4 g/d and control group was 3.1 g/d. While the chickens received 1.0% white kwao krue combined with 0.2% kariyat was the lowest 2.7 g/d. Feed conversion ratio of chickens received 1.0% white kwao krue and the chicken received 0.1% turmeric and 0.2% kariyat was the lowest 2.4 while the chickens received 1.0% white kwao krue combined with 0.1% turmeric was the highest as 2.9. Carcass percentage of chickens received 0.1% turmeric combined with 0.2% kariyat was the highest 85.8% while the carcass percentage of chickens received mixed all herbs was 82.94% by there was no significantly different from the chickens received 1.0% white kwao krue (82.9%) and the chickens received 1.0% white kwao krue combined with 0.1 turmeric (84.9%) as shown in Table 1. The result from this study was not consistent with the Somkuna et al. (2015) reported that native chickens received turmeric at 0.1% and kariyat at 0.2 % showed better growth performance. This was due to the differences of raising and sanitation management of each farm. However, the dietary supplementation with white kwao krue showed the positive effect on growth performance because it contains some phytoestrogen (Chershewasart et al. 2004) which could stimulate their fat cumulative. Moreover, the active compound in turmeric (curcumin) can reduce the pathogenic bacteria such as *Lactobacillus acidophilus*, *L. plantarum* and *E. coli* that cause diarrhea. In addition, curcumin is considered as natural source of color which can improve the chicken skin color (Jayaprakasha et al. 2005; Nouzarian et al. 2011; Narumon et al. 2015). And in kariyat (andrographolide) can protect and reduce infection of respiratory diseases. In terms of chicken health, there was very low mortality rate among groups. Farmers showed highest level of satisfaction on using



herbs for increasing productive performance of native chickens because they could raise the chickens without antibiotics or commercial anthelmintic. It was an alternative way for produce herbal native chickens.

## Conclusion

It can be concluded that white kwao krue (*Pueraria mirifica*), turmeric (*Curcuma longa* L.), kariyat (*Andrographis paniculata* (Burm. F. Nees) could be used for increasing productive performance of native chickens raised in rural community. It was an alternative way for produce herbal native chickens.

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## **Regression Models for Estimating Fat Carcass Percentage Using Chest Measurement in Thin Tailed Lambs**

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### **Abstract**

This study was conducted to evaluate the possibility of using chest measurement for subcutaneous, intermuscular, and total fat percentage in Thin Tailed Lambs. Twenty one heads of three months old male thin tailed lambs with initial body weight (BW)  $14.57 \pm 2.19$  kg were raised up to 3 months fattening period. Chest girth (CG), chest depth (CD), and chest width (CW) were measured before slaughtered. The fat of the carcass was separated into subcutaneous, intermuscular, and total fat, then weighed. The data was analyzed by linear regression to determine the correlation and equation between chest measurement and fat carcass percentage, then was evaluated by t-test, standard error (SE) and the differences were measured to predict the accuracy of the equation. The results showed that the correlation between CG, CW and CG/CD in intermuscular and total fat percentage was positive and significant ( $P < 0.05$ ) with correlation value moderate to high ( $r = 0.510 - 0.664$ ), while subcutaneous were not significant. SE of each variable showed a low value (0.002-0.020) that indicate the prediction is close to the actual fat percentage value. The lowest differences between prediction and actual value could be found in CG regression equation in total fat (0.368%), while the highest value was 8.918% in intermuscular fat in CW/CG regression. Based on the results of this study, it can be concluded that CG regression is the best equation for estimating intermuscular, and total fat percentage using chest measurement in Thin Tailed Lambs.

**Keywords:** chest measurement, fat percentage, regression, lambs

### **Introduction**

Fat is one of factor that determine carcass grading qualification in animals. Fat carcass consist of subcutaneous, intermuscular and intramuscular. The minimum requirement for either extractable fat in order to achieve acceptable consumer satisfaction for grilling red meat at 5% for sheepmeat (Hopkins et al., 2006). There are some methods to predict the fat before slaughtering the lambs. The ultrasound scanning is one of the methods that can be used for estimating fat carcass percentage of live animals (Grill et al., 2015).

Body Condition Score (BCS) have been used as reflection of animal's fat reserve. However, BCS is a subjective means of assessing an animal's lean body mess and body fat (Summers et al., 2012). Therefore there is a need to have an accurate and objective measurement for estimating fat while the animal is still alive. Body measurement can be used to predict body



weight and carcass characteristics (Agamy et al., 2015). Chest girth is one of variable that have been used to predict body weight. Agamy et al. (2015) reported that chest girth had the highest correlation with body weight in Barki Lambs. The fat thickness of lambs can also indicated in chest depth. This study was conducted to evaluate the possibility of using chest measurement for subcutaneous, intermuscular, and total fat percentage in Thin Tailed Lambs.

## Materials and Methods

Twenty one heads of male thin tailed lambs ( $\pm$  3 months old) with initial body weight (BW)  $14.57 \pm 2.19$  kg (CV= 15.03%) were used in this study. They were fattened by fed the complete feed contained three levels of crude protein (CP; 14, 16 and 18%) and two levels of total digestible nutrients (TDN; 60, 70%) that composed of rice bran, cassava meal, sugar cane top, cassava peel, soybean meal, fish meal, molasses and mineral and was given in pelleted form. All lambs were housed in individual pen and given freely access to feed and water throughout the experimental period.

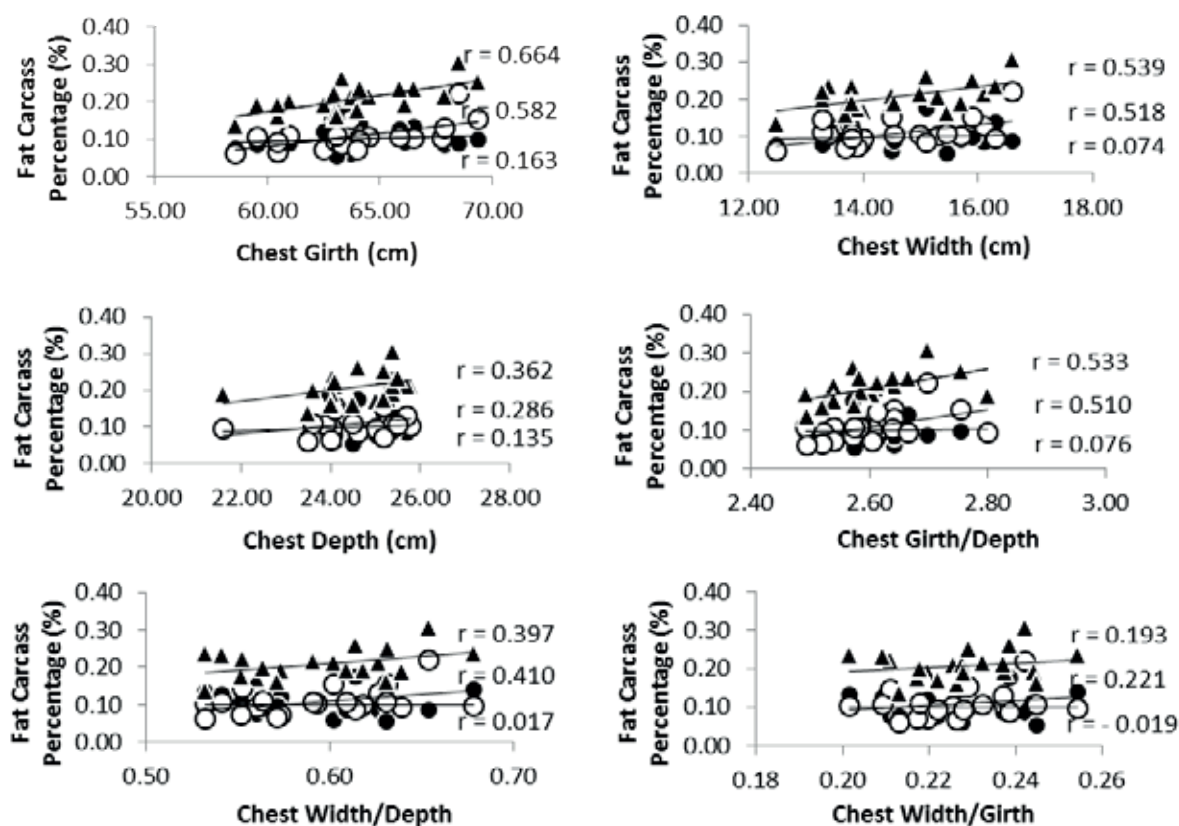
Parameters measured were CG (cm), CW (cm), CD (cm), CG/CD, CW/CD, CW/CG, subcutaneous, intermuscular, and total fat percentage of carcass weight. Those measurements were taken before slaughtered that used tape measures and elaborate calipers. All lambs were slaughtered randomly after 3 months of feeding. Lambs were fasted for 6 hours before slaughtered. The slaughter method was done follow Islamic methods. The carcass was kept in a cold room at 18°C for 10 hours. The fat of carcass were separated into subcutaneous, intermuscular, and total fat (comprises of subcutaneous and intermuscular), then were weighed.

The relationship between body measurements and fat carcass percentage were analyzed by correlation regression analysis. The strength of correlation coefficient was evaluated by the value described by Steel and Torrie (1960), while the accuracy of the equation of regression was evaluated by t-test, standard error (SE) and the differences between predicted value and actual value. The accuracy was also determined if the difference value was low and statistically significant.

## Results and Discussion

The correlation between chest measurement and fat carcass percentage are presented in Figure 1. The percentage of subcutaneous fat in CG, CW, CD, CG/CD, and CW/CD has a positive correlation value (0.163, 0.074, 0.135, 0.076, and 0.017, respectively) while in CW/CG was negative (-0.019). The negative correlation in CW/CG is caused by the growth of subcutaneous fat might be maximum, so it will decrease the growth rate. Owens et al. (1993) reported that fat deposition is starting from intermuscular, subcutaneous and intramuscular. Intermuscular and total fat percentage showed a positive correlation in all chest measurements. The correlation between CG in intermuscular and total fat percentage showed a strong correlation value, being 0.582 and 0.664, respectively, while, in CG/CD showed a medium correlation value, being 0.510 and 0.533, respectively. The bigger chest measurement value will give the more fat carcass percentage. This result agreed to the report of Abd-Alla (2014) that total fat stores weight of carcass were positively correlated with chest girth in Barki Lambs.





**Figure 1.** The correlation between chest measurements and fat carcass percentage; % subcutaneous (●); % intermuscular (○) and % total fat (□).

Table 1 shows the equation regression of chest measurement and fat carcass percentage. The table showed the correlation of intermuscular and total fat in CG, CW and CG/CD were statistically significant ( $P < 0.05$ ), while subcutaneous fat was not significant ( $P > 0.05$ ). The growth of subcutaneous fat might be stable, so it's statistically not significant with chest measurements. Fat deposition is starting from intermuscular, subcutaneous and intramuscular (Owens et al., 1993). Chest girth were reported to have the highest correlation with body weight in Barki Lambs (Agamy et al., 2015). The SE of both variables showed a low value (from 0.002 to 0.020) and have a small differences of the actual value (0.368 - 7.520%). These result means either intermuscular or total fat regression equation in CG and CG/CD can be used. The CG regression is the best indicator of overall value, because it has the lowest differences and strong correlation in intermuscular and total fat percentage. The results of this study are in agreement with Nigm et al. (1995) who concluded that heart girth (or chest girth) was the best single measurement for predicting different carcass traits of Merino sheep.

## Conclusion

This study concluded that chest girth (CG) has a strong correlation to intermuscular and total fat carcass percentage, so that the regression equation using CG has a good accuracy to estimate intermuscular and total fat carcass percentage in thin tailed lambs as shown by the low differences between the predicted and actual value.



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**Table 1** The equation regression of chest measurement and fat carcass percentage.

No	Variable	Equation	r	SE	Diff. of predicted to actual value	
					cm	%
1.	CG					
	Subcutaneous	$y = 0.0015x + 0.0011$	0.163 <sup>ns</sup>	0.005	-0.002	5.178
	Intermuscular	$y = 0.007x - 0.3405$	0.582 <sup>*</sup>	0.018	-0.001	4.943
	Total Fat	$y = 0.0085x - 0.3394$	0.664 <sup>*</sup>	0.020	-0.003	0.368
2.	CW					
	Subcutaneous	$y = 0.0018x + 0.0727$	0.074 <sup>ns</sup>	0.002	-0.000	7.433
	Intermuscular	$y = 0.0164x - 0.1303$	0.518 <sup>*</sup>	0.017	0.000	6.958
	Total Fat	$y = 0.0182x - 0.0577$	0.539 <sup>*</sup>	0.018	0.000	2.420
3.	CD					
	Subcutaneous	$y = 0.0038x + 0.0048$	0.135 <sup>ns</sup>	0.004	-0.001	6.414
	Intermuscular	$y = 0.0103x - 0.1445$	0.286 <sup>ns</sup>	0.010	0.000	8.795
	Total Fat	$y = 0.0141x - 0.1397$	0.362 <sup>ns</sup>	0.013	-0.001	2.520
4.	CG/CD					
	Subcutaneous	$y = 0.027x + 0.0287$	0.076 <sup>ns</sup>	0.002	-0.000	7.520
	Intermuscular	$y = 0.2319x - 0.496$	0.510 <sup>*</sup>	0.016	0.000	6.719
	Total Fat	$y = 0.2589x - 0.4673$	0.533 <sup>*</sup>	0.018	0.000	2.315
5.	CW/CD					
	Subcutaneous	$y = 0.0116x + 0.0922$	0.017 <sup>ns</sup>	0.001	0.000	7.626
	Intermuscular	$y = 0.3581x - 0.1042$	0.410 <sup>ns</sup>	0.014	0.000	7.675
	Total Fat	$y = 0.3698x - 0.012$	0.397 <sup>ns</sup>	0.014	0.000	2.850
6.	CW/CG					
	Subcutaneous	$y = -0.0405x + 0.1083$	-0.019 <sup>ns</sup>	0.001	0.000	7.598
	Intermuscular	$y = 0.5976x - 0.0278$	0.221 <sup>ns</sup>	0.008	0.000	8.918
	Total Fat	$y = 0.557x + 0.0805$	0.193 <sup>ns</sup>	0.008	-0.000	3.211

CG: Chest Girth, CD: Chest Depth, CW: Chest Width



## Development of Near Infrared Spectroscopy for Nondestructive and Rapid Measurement of Chemical Compositions and Somatic Cell Counts in Raw Milk

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### Abstract

Near-infrared spectroscopy (NIRs) is a method of non-destructive analysis of samples. About 150 samples of cow milk from smallholder dairy farms of Dairy collection center at Sakon Nakhon Livestock Breeding and Research Centre were analyzed for calibration and validation of the calibration performed. A wavelength scanning instrument, NIRFlex N-500 (Buchi, Switzerland) equipped with fiber optic probe was used with a scanning range from 4000 to 10 000  $\text{cm}^{-1}$  in diffuse reflectance mode. All the other chemometric analyses including spectral data pretreatment and partial least square regression analyses were performed with The Unscrambler® program. Fat, protein, lactose, solid not fat and total solid were determined by MilkcoscanFT600 and the somatic cell was determined by Fossomatic5000 basic at Veterinary Research and Development Center Upper Northeastern Region. Raw spectra of unhomogenized milk samples are the strong absorbance around 5900 and 7800  $\text{cm}^{-1}$  (1282-1695 nm). The calibration model was created by PLS regression with pretreatment by smoothing (7seg.), MSC and second derivatives (27 seg.). The determined correlation coefficients for calibration ( $R_{\text{cal}}$ ) were for fat 0.97, protein 0.94, lactose 0.79, SNF 0.90, total solid 0.95 and SCC 0.60. The correlation coefficients for validation ( $R_{\text{val}}$ ) were for fat 0.96, protein 0.90, lactose 0.69, SNF 0.84, total solid 0.94 and SCC 0.51 (Table2). The NIR calibration for fat, protein and total solids showed good performance with high  $R_{\text{val}}$  and slightly low SEP. The NIR calibration for fat, protein, and total solids showed good performance with high  $R_{\text{val}}$  and slightly low SEP. Lactose, SNF and SCC showed low  $R_{\text{val}}$ . It concludes that NIRs would allow for screening of milk samples. As part of this analysis, the somatic cell count determination by NIR would allow health screening of cows and differentiation between healthy and mastitis milk samples.

**Keywords:** NIR, milk, fat, protein, somatic cell



## Introduction

Near-infrared spectroscopy (NIRs) is a method of non-destructive analysis of samples, by measuring the amount of light that a sample absorbs. The best results for all measured components were found in the wavelength region 1100- 2400 nm. Promising results were obtained also for the region 700- 1100 nm that is suitable for online application (Pravdova et al., 2001). The components of a material including C-H, N-H, O-H and S-H bonds can be determined based on the selective absorption of electromagnetic waves, and the spectral signature of the material is defined by the absorbance as a function of wavelength (Ku et al., 2015). Using NIR, many substances such as fat, protein, lactose, water, and melamine have been successfully detected in milk (Chen et al., 1994; Laporte et al., 1998; Laporte et al., 1999; Schmilovitch et al., 2000; Kasemsumran et al., 2007). Meanwhile, somatic cell count (SCC) in milk can also be determined by NIR, based on changes of lactose and protein in milk (Tsenkova et al., 2001a; Ku et al., 2015). Mastitis, although an animal welfare problem, is a food safety problem and is the biggest economic problem. Mastitis is characterized by physical, chemical and bacteriological changes in the milk and pathological changes in the glandular tissue of the udder. Several reports in literature about quality determination of milk are available highlighting the benefits of a fast, non-invasive technique allowing the simultaneous determination of physic-chemical parameters in combination with multivariate analysis (MVA) (Benedictis and Huck, 2016)

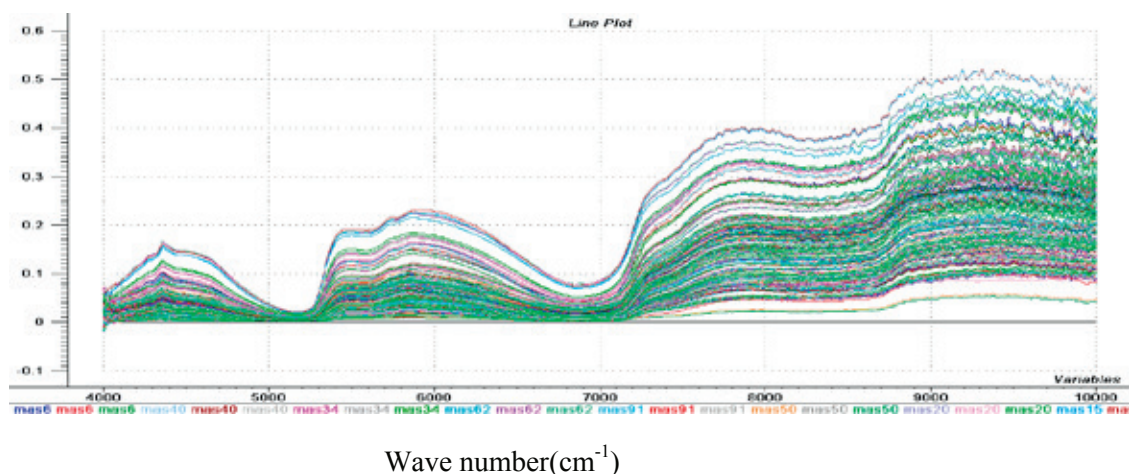
The purpose of this study was to investigate the influence of mastitis milk samples in the data set used for development of milk composition calibration equations in the near-infrared region from 700 to 1100nm on the accuracy of NIRS determination of fat, protein, lactose, solid not fat, total solids and somatic cells content of non-homogenized milk.

## Materials and Methods

About 150 samples of cow milk from smallholder dairy farms of Dairy collection center at Sakon Nakhon Livestock Breeding and Research Centre were analyzed for calibration and validation of the calibration performed. A wavelength scanning instrument, NIRFlex N-500 (Buchi, Switzerland) equipped with fiber optic probe was used with a scanning range from 4000 to 10 000  $\text{cm}^{-1}$  in diffuse reflectance mode. Samples of milk in the standard plastic bottle were warmed to 25°C. Each sample was analyzed three times and the average spectrum was used for calibration. The whole spectrum area has been tested. The same samples were employed for full cross validation by The Unscrambler® program. All the other chemometric analyses including spectral data pretreatment and partial least square regression (PLS) analyses were performed with The Unscrambler® program. Fat, protein, lactose, solid not fat and total solid were determined by MilkcoscanFT600 and the somatic cell was determined by Fossomatic5000 basic at Veterinary Research and Development Center Upper Northeastern Region.

## Results and Discussions

Raw spectra of unhomogenized milk samples are shown in figure 1. The strong absorbance was showed around 5900 and 7800  $\text{cm}^{-1}$  (1282-1695 nm). Melfsen et al., (2012) reported that the fat content in milk dominates the NIR spectra in the selected range of 851-1649 nm. The strong second and third overtone absorption bands of C-Hand scattering effects from fat globules of different size in raw milk are characteristic.



**Figure 1** Near infrared spectra of raw milk sample in reflectance mode.

Total range, mean, and standard deviation of contents of fat, protein, lactose, solid not fat, total solids and somatic cell in the milk samples used in this study are shown in Table 1. Fat content, in particular, varied considerably with values ranging from 0.53% to 14.1%. All milk constituents covered almost the same range in the calibration and validation data sets. Data from all milk constituents were normally distributed. The total protein content in milk from affected udder quarters could be higher due to increased SCC and mastitis (Forsback et al., 2009).

**Table 1** Characteristics of milk content measured by reference methods

Cell/ml		%Fat	% protein	% lactose	% SNF	%TS	SCC ( $\times 10^5$ )
SCC< $5 \times 10^5$ (N=97)	Mean	3.24	3.09	4.93	8.72	11.96	2.41
	Range	0.67-7.69	2.51-4.06	4.41-5.26	8.06-9.50	9.38-16.35	0.07-4.99
	SD	1.21	0.27	0.18	0.32	1.21	1.32
SCC> $5 \times 10^5$ (N=86)	Mean	3.26	3.40	4.68	8.78	12.04	46.12
	Range	0.53-14.09	2.71-7.16	3.17-5.30	7.33-11.70	9.12-23.32	5.01-267.77
	SD	2.18	0.62	0.47	0.57	2.27	61.07

N: number of samples, SD: standard deviation, SNF: solid not fat, TS: total solids, SCC: somatic cell count

The calibration model was created by PLS regression with pretreatment by smoothing (7segment), multiple scatter correction (MSC) and second derivatives (2D) by Savitsky-Golay method (27 segment). The determined correlation coefficients for calibration ( $R_{cal}$ ) were for fat 0.97, protein 0.94, lactose 0.79, SNF 0.90, total solid 0.95 and SCC 0.60. The correlation coefficients for validation ( $R_{val}$ ) were for fat 0.96, protein 0.90, lactose 0.69, SNF 0.84, total solid 0.94 and SCC 0.51 (Table2). The NIR calibration for fat, protein and total solids showed good performance with high  $R_{val}$  and slightly low SEP. Saranwong and Kawano (2008) obtained similar results with transmittance mode in wavelength region 600-1000nm. However, lactose, SNF and SCC showed low  $R_{val}$ . It would allow for screening of milk samples. It is known that mastitis causes a rise in whey protein and a decrease in casein content. Mastitis also changes the ionic concentration in milk. Alteration of the blood-milk barrier leads to an increase of sodium, chloride and a decrease of potassium concentration in milk. All these changes probably



influenced the NIR spectral data and the spectral regions, which are most important for determination of respective components in the set of low SCC and high SCC (Tsenkova et al., 2001b).

**Table 2.** Results of PLS regression for milk content determination

	pretreatment	R <sub>cal</sub>	SEC	R <sub>val</sub>	SEP	Bias
%Fat	Smoothing(7seg.)_MSC_2D(27seg)	0.97	0.62	0.96	0.68	0.002
% protein	Smoothing(7seg.)_MSC_2D(27seg)	0.94	0.23	0.90	0.28	-0.004
% lactose	Smoothing(7seg.)_MSC_2D(27seg)	0.79	0.32	0.69	0.38	0.000
% SNF	Smoothing(7seg.)_MSC_2D(27seg)	0.90	0.27	0.84	0.33	0.000
% TS	Smoothing(7seg.)_MSC_2D(27seg)	0.95	0.77	0.94	0.83	0.001
SCC (x10 <sup>5</sup> )	Smoothing(7seg.)_MSC_2D(27seg)	0.6	54.32	0.51	58.86	-0.35

MSC: multiple scatter correction, R<sub>cal</sub>: the determined correlation coefficients for calibration, R<sub>val</sub> : the correlation coefficients for validation, SEC: standard error of calibration, SEP: standard error of prediction, Bias: the average of differences between reference value and NIR value

## Conclusion

The NIR calibration for fat, protein and total solids showed good performance with high R<sub>val</sub> and slightly low SEP. Lactose, SNF and SCC showed low R<sub>val</sub>., It would allow for screening of milk samples. As part of this analysis, the somatic cell count determination by NIR would allow health screening of cows and differentiation between healthy and mastitis milk samples.

## Acknowledgements

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## Effect of Hydroponic Maize Fodder Supplementation on Production Performance in Graded Murrah Buffaloes of Scarce Rainfall Zone

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### Abstract

Hydroponic fodder production plays significant role in augmenting fodder shortage of small holder dairy production system in scarce rainfall areas. The present experiment was conducted in the adopted villages of Krishi Vigyan Kendra, Yagantipalle, Kurnool dist., A.P. to assess the effect of hydroponic maize fodder (HMF) on production performance in the milch buffaloes. A low cost hydroponic fodder production devise (Krishi Fodder Master) was fabricated and utilized in the experiment. Twenty graded murrah buffaloes of 2<sup>nd</sup> and 3<sup>rd</sup> lactation were equally divided into two groups (T<sub>1</sub>&T<sub>2</sub>). First group fed with 5kg sorghum straw per day along with recommended quantity of concentrates and the second group fed with 5kg sorghum straw and 12kg HMF per day. Data on HMF production, milk yield/day, % fat in milk and 3.5% FCM/day was collected for 60 days trial period. 7.2kg HMF was obtained from one kilogram of maize seed within 7 days without using any nutrients in the irrigated water. The DM, CP, CF, EE, TA and NFE contents in HMF were 14.3, 15.8, 12.62, 3.74, 3.27 and 64.57 respectively. The data revealed that 6.5% increased mean milk yield/day was recorded in T<sub>2</sub> (6.88±0.47) as compared to T<sub>1</sub> (6.46±0.49). The mean % fat and SNF in T<sub>1</sub> and T<sub>2</sub> were recorded as 6.78±0.19, 8.86±0.16 and 7.08±0.23, 9.14±0.12 respectively. The gross income per day was 13.05% more in T<sub>2</sub> (₹250.47±17.31) than T<sub>1</sub> (₹221.55±15.49). The data revealed that saving of ₹15.61/buffalo/day on concentrate feeding and increased net returns of ₹44.58/buffalo/day were recorded on supplementing HMF. These results were found significant at 5% (p<0.5). Feeding of HMF to buffaloes produced under Krishi Fodder Master was found to be economical for medium producing animals in low rain fall areas.

**Keywords:** graded Murrah buffaloes, low cost hydroponic system, hydroponic maize fodder, Krishi fodder master

### Introduction

Kurnool district of Andhra Pradesh falls under scarce rainfall zone with average rainfall of 670mm. Dairy farming is the sustainable livelihood activity for the farmers with 2-5 milch buffaloes per household in rain fed agriculture. Feeding of dairy animals in this area is mostly on grazing for 6-8hours per day with additional dry fodder supplementation. Due to scarcity of water, fodder cultivation is meagre in this zone. At this junction hydroponics technology is an alternative to grow green fodder for farm animals. But the high cost and expenses involved in conventional method of hydroponic fodder production system is not economically suitable for small and marginal farmers. Studies revealed that hydroponics fodder can be produced in low cost greenhouses or devices (Naik *et al.*, 2014 and Krishna Murthy *et al* 2017).



However, only a few reports are available on the feeding of the hydroponic green fodder to lactating cows (Naik et al 2014) but no reports are available on feeding of hydroponic maize fodder (HMF) to milch buffaloes. Therefore, present experiment was conducted under National Initiative on Climate Resilient Agriculture (NICRA) project during 2016-17 with the following objectives.

- To study the effect of feeding HMF on milk production in milch buffaloes.
- To study the economic impact of feeding HMF to milch buffaloes.
- To assess the efficiency of Krishi Fodder Master for green fodder production in scarce rainfall areas.

## Materials and Methods

### Low cost hydroponic device

Krishi Fodder Master is a portable structure with PVC pipes prepared to hold ten hydroponic trays in five rows vertically with 6.5' (H) X 4' (W) X 2' (B) measurements covered with 90% green shade net. The trays used in the system were U.V. stabilized and have protrudes in the bottom for easy drain for excess water. The size of tray was 2.5feetX1.5feet. Irrigation facility for each row was provided with one mister connected to 0.5hp motor controlled by a cyclic timer.

### Cultivation of Hydroponic maize fodder

Clean seeds of maize (*Zeamays*) were soaked in tap water for 12h and kept for sprouting in air tight condition for 36h. The sprouted maize seed spread in the trays @1.5kg/3.75sf<sup>-1</sup>. The first trays were on the top row and change every day to the lower rows replacing with new trays as mentioned by Krishna Murthy et al (2017). The seedlings were allowed to grow for 5 days and on sixth day, entire fodder along with root mat removed and fed to the dairy animals.

### Experimental details

Twenty graded murrah buffaloes of 2<sup>nd</sup> and 3<sup>rd</sup> lactation were selected in Meerapuram village of Banaganapalli mandal and equally divided into two groups. First group (T<sub>1</sub>) fed with sorghum straw (5kg) and recommended quantity of concentrate feed. The second group (T<sub>2</sub>) fed with sorghum straw (5kg) along with 12kg HMF. Data on milk yield, Fat and SNF in the milk, income and expenditure on feeding was recorded for 60 days. The data was statistically analyzed for its significance using ANOVA.

## Results and Discussion

The biomass yield of HMF was recorded as 6.2kg per kg seed. Similarly Naik et al (2014) reported as 5.5kg and Krishna Murthy et al (2017) reported as 4.82kg fresh biomass yield per kg seed. The dry matter content in the fresh HMF was observed as 14.3% whereas Naik et al (2014) reported as 18.3%. Similarly Dung et al (2010) reported DM as 19.7% in hydroponically grown barley fodder.

The CP, CF, EE, Total ash and NFE contents in HMF were 15.8, 12.62, 3.74, 3.27 and 64.57 respectively. Higher CP, CF and Total Ash and less EE and NFE were observed in HMF compared to maize grains. However 13.3%CP, 6.37% CF, 1.75% EE and 75.32%NFE were reported in the earlier studies (Naik et al 2014). Similarly Reddy et al (1988) observed that the hydroponically grown barley fodder was comparable to leguminous fodders and superior to non leguminous fodders. Finney, 1982; Sneath and McIntosh, 2003 have reported in their studies as the sprouted grain are rich source of anti-oxidants and related trace minerals such as selenium and Zn and feeding of the sprouted grains improve the animals' productivity.

**Table 1.** Nutrients details of experiment feeds and fodder

Nutrient	Hydroponic fodder	Maize grain	Sorghum straw
Dry matter	14.3	89.58	89.6
Crude protein	15.8	9.75	3.4
Ether Extract	3.74	2.93	0.84
Crude fiber	12.62	2.95	34.19
NFE	64.57	82.27	52.43
Total Ash	3.27	2.1	9.14

The data on daily milk yield, fat in milk and 6%FCM yield were given in table 2. The data revealed that 6.5% increased milk yield was recorded in T<sub>2</sub> over T<sub>1</sub>. Naik et al 2014 reported as 13.7% increased milk yield on feeding of HMF to cows and Reddy et al 1988 reported as 7.8% milk yield improvement on supplementation of ration containing hydroponic barley fodder to cows. The higher milk yield was due to high protein content in the ration containing HMF. The fat and SNF contents were 6.78± 0.19, 8.86±0.16 and 7.08±0.23, 9.14±0.12 respectively in T<sub>1</sub> and T<sub>2</sub>. The data revealed that 4.42% improvement in milk fat was recorded in T<sub>2</sub> over T<sub>1</sub>. The 6%FCM was also high in T<sub>2</sub> (8.38±0.59) compare to T<sub>1</sub> (7.64±0.56). The results were found significant at 5% level (p>0.5).

**Table 2.** Average milk yield and milk composition of experimental animals.

Particulars	T <sub>1</sub>	T <sub>2</sub>
Milk yield(kg/day)	6.46±0.49	6.88±0.47
Fat (%)	6.78±0.19	7.08±0.23
SNF (%)	8.86±0.16	9.14±0.12
6%FCM (kg/day)	7.64±0.56	8.38±0.59

The gross income, cost of feeding and benefit cost ratio were given in table 3. The average daily gross income was 13.05% more in T<sub>2</sub> (₹250.47±17.31) as compared to T<sub>1</sub> (₹221.55±15.49). The cost of feeding in T<sub>1</sub> and T<sub>2</sub> were ₹68.11±4.12 and ₹52.5±3.01 respectively. The data revealed that saving of ₹15.61/buffalo/day on concentrate feed by supplementing 12kg HMF and it was reflected in net income and also benefit cost ratio. Increased net profit of ₹44.58/buffalo/day was recorded on supplementing HMF. Naik et al (2014) reported in their studies as higher net profit of ₹12.67/cow per day due to feeding of hydroponic maize fodder.

**Table 3.** Economics of milk production per day (Average of 60 days)

Particulars	T <sub>1</sub>	T <sub>2</sub>
Gross income	₹221.55±15.49	₹250.47±17.31
Expenditure on feeding	₹68.11±4.12	₹52.5±3.01
Net income	₹153.42±11.46	₹197.98±16.36
B:C ratio	1:3.25	1:4.77



## Conclusion

The fodder shortage in the scarce rainfall areas can be overcome with Krishi Fodder Master with minimum expenditure. Feeding of hydroponic maize fodder effected on increased milk production, fat and SNF in milk due to its high nutritive value. The cost of concentrate feed can be reduced on supplementation of HMF. Hence it is concluded that hydroponic fodder production with low cost devices are more apply suitable for low rain fall areas to meet the green fodder demand of Small and marginal farmers with higher net returns.

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## Effect of Fermented Total Mixed Ration with Microbial Culture on Fermentation Quality and *In Vitro* Digestibility

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### Abstract

This study was aim to investigate effect of culture microorganisms in fermented total mix ration (FTMR) on quality and *in vitro* digestibility. The experiment was completely randomized design. The treatments included 11 inoculations according to microbial mixed culture as; *Rhizopus oligosporus* TISTR 3001, *Aspergillus niger* TISTR 3013, *Lactobacillus bruchnari*, and *Saccharomyces Cereverceae*. An *in vitro* gas technique at various incubation times were investigated. The result indicated that FTMR with single microbial inoculation by *Rhizopus oligosporus* or mix culture between *Lactobacillus bruchnari* and *Saccharomyces Cereverceae* or *Rhizopus oligosporus* could be improved chemical composition and higher in gas production than those other mixed culture. Gas production kinetic and *in vitro* dry matter digestibility were not different but rice straw trend to be greater. It is indicating the microbial mix culture moisture could increase quality and dry matter digestibility of FTMR. Furthermore, fermented total mix ration with inoculation and varying roughage source on ruminant productivity are recommended.

**Keywords:** microbial culture, FTMR, rumen fermentation, gas production

### Introduction

The cost of feed is rising in the beef cattle production, particularly in dietary dependency on feed resources. Conventional feeding practice and using with different roughages are noticeably necessary to overcome feed cost. Total mixed ration (TMR) has been great interested by beef production farmer due to its expected benefits in the nutrition content, feeding management and production planning. The benefit of a TMR are include increased feed intake, enhanced use of low-cost alternative feed ingredients, ability to control the forage concentrate ratio, lower incidence of metabolic and digestive disorders (Cao et al., 2012; Kim et al., 2012). The tropical forage is normally contained of high fiber content that prevents assess of ruminal hydrolytic enzymes to cellulose and hemicellulose (Wanapat, 2009). During recent years, mixed microbial culture and their fibrolytic enzymes have been used to improve the nutritive value and utilization efficiency of low-quality roughages and industrial by-product. However, there is no available information about FTMR prepared from cassava starch residue including microbial mix culture. Therefore, this experiments were investigated on the microorganism culture on nutritive value and *in vitro* gas production of fermented total mix ration.



## Materials and methods

### Dietary substrate and microbial culture

The experiment was completely randomized design (CRD). The treatments included 11 microbial cultures. The culture was prepared according the method of TISTR. In brief, the production processes for fermented feed product is as following: (1) *Rhizopus oligosporus* TISTR3001, (2) *Lactobacillus buchneri*, (3) *Aspergillus oryze* TISTR3019, and (4) *Saccharomyces cerevisiae* were obtained from TISTR (Thailand Institute of Science and Technology Research). The organisms were cultivated in the PDA slant. Spore suspension were prepared by adding 5 ml of 0.05 M phosphate buffer (pH 7.0) into the culture slant and diluted until the number of spore is  $10^5$  spore/ml. Preparation of yeast inoculum were done by adding 5 ml of 0.1 % peptone into the culture slant. The organism was cultivated by transferring into a 250 ml Erlenmeyer flask each containing 100 ml of medium using streamed rice bran as a carbon source. The flask was incubated at 30°C for 40 hrs. before inoculated with yeast ( $1 \times 10^7$  cell/ml). Fermented total mix ration were assorted with 15% of cassava chip, 34% of cassava pulp, 12% of rice bran, 6% soybean meal, 8% of leucaena leave meal, 10% of palm kernel meal, 3% of molasses, 1% urea, 1% of mineral mixed and 10% of chopped rice straw. FTMR diet were inoculated with  $10^5$ - $10^6$  CFU/g) of culture mixed microbial and put into plastic bag and were kept at 25°C for 14 days.

### Animal donor and preparation rumen inoculums mixed

Two male, crossbred beef cattle with body weight of  $258 \pm 30$  kg were used as rumen fluid donors. Beef cattle rumen fluid was collected from animals fed with rice straw *ad libitum*. The animals received the diets for 14 d before the rumen fluid was collected. On day 15, 1000 ml rumen liquor was obtained from each animal before the morning feeding. The rumen fluid was filtered through four layers of cheesecloth into pre-warmed thermos flasks and then transported to the laboratory.

Preparation of artificial saliva were done according to Menke and Steingass (1988). The artificial saliva and rumen fluid were mixed in a 2:1 ratio to a rumen inoculums mixed. Three bottles of each sampling time contained only rumen inoculums mixed solution were included with each run and the mean gas production value of these bottles were termed the blank value. The blank value was subtracted from each measurement to give the net gas production.

### Sampling and chemical analysis

Samples of FTMR dried by using lyophilizer, then ground to pass a 1-mm sieve and use for chemical analysis and in the further *in vitro* gas production evaluation. The samples were analyzed for DM, ash and CP using the procedures of AOAC (2012), and NDF and ADF content by Van Soest et al. (1991) method. Gas production was recorded at 0, 0.5, 1, 2, 4, 6, 8, 12, 18, 24, 48, 72 and 96 h of incubation. Cumulative gas production data were fitted to the model of Ørskov and McDonald (1979). Rumen fluid samples were then filtered through four layers of cheesecloth. A liquid sample were analyzed for  $\text{NH}_3\text{-N}$  analysis using the micro-Kjeldahl and VFA analysis using HPLC. The *in vitro* degradability was determined after termination of incubation, when the contents were filtered through pre-weighed Gooch crucibles and residual dry matter was estimated. The percent loss in weight was determined and presented as *in vitro* dry matter degradability (IVDMD).



### Statistical analysis

All data obtained were analyzed statistically using PROC GLM using microbial culture as main factors. Multiple comparisons among treatment means were performed by Duncan's New Multiple Range Test (DMRT) (Steel and Torrie, 1980).

## Results and discussion

### Chemical composition of experimental diets

Chemical composition of FTMR with microbial culture were shown in table 1. DM and CP content were highest in *Rhizopus oligosporus*, *Aspergillus oryze* and *Lactic acid buchnari* inoculation (61.67, and 15.21 %, respectively) and lowest in three mix culture of ARL or control (45.97, 8.9 and 12.16 %, respectively). ADF and NDF content were highest in ARS (54.42 and 36.00%, respectively) and lowest in RLS (39.03 and 20.14%, respectively). In this study consistent with Cao et al. (2012), ensiled reducing in DM content, which microbial contribute to silage fermentation (e.g., *Lactobacillus* species) utilized these readily available carbohydrates and converted these to lactate and acetate.

### Rumen fermentation characteristic, gas production kinetics and *in vitro* degradability

Gas production kinetic as immediately soluble fraction (a), insoluble fraction (b) and potential extent of gas production (a + b) are shown in Table 2. It is not significantly effect on gas production kinetic and trend to be rapid degradation in ARL or high potential gas production with L inoculation. Cumulative gas production (96 h) was not influenced by microbial inoculation sources. IVDMD was significantly different between the microbial inoculation sources ( $P < 0.05$ ). Moreover, in this study found that microbial culture mix were higher than those single microbial inoculations. The increasing cell wall digestibility could result of shortening by the action of extracellular enzyme from silage microflora, resulting in a greater susceptibility to enzymatic attack in the rumen (Morrison, 1988). In this study, relatively high DM digestibility for FTMR possibility because of greater NDF content compared with non-inoculation.

Ruminal fermentation of FTMR with microbial inoculation were statistically different among treatment. ALS were highest in ammonia content and lowest in control group (25.6 vs 12.1 mg/dL). The optimum range of rumen  $\text{NH}_3\text{-N}$  concentration for an efficient digestion has been estimated to be 3.56 mM to 4.99 mM (Satter and Slyter, 1974). However, the values obtained for the FTMR with inoculation were higher than those the reported. Total VFA and individual VFA production were significantly different as affected by microbial culture inoculation ( $P < 0.05$ ). FTMR with high in rapidly fermentable carbohydrates lead to populations of bacteria which produce relatively more propionate and butyrate than acetate (McDonald et al., 1995), that indicate significantly higher butyrate concentration in microbial mix inoculation.

According to Cao et al. (2012) studied in *in vitro* it was found that DM digestibility of FTMR had higher than fresh TMR. In addition, FTMR was 4.2% increase in DM disappearance and 2.0% increase in ADF. This finding may be illuminated by the nutrient in FTMR is initially fermented during the period of fermentation. It has already been seen that the low pH can be weaken the binding between lignin and hemicellulose and cellulose fractions. Bureenok et al. (2012) also reported on lactic acid bacteria inoculation to improve the quality of fermented total mixed ration which dry matter and crude protein contents of FTMR treated LAB were higher than the untreated FTMR.



## Conclusion

Based on this study, it was revealed that fermented feed with *Rhizopus oligosporus* only or mixed culture between *Lactobacillus bruchnari* and *Saccharomyces cereverceae* or *Rhizopus oligosporus* could be higher in gas production than those mixed culture. However, these findings should be further conducted in *in vivo* experiment.

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**Table. 1** Effect of microbial culture inoculation on chemical composition of FTMR

Treatments*	Nutritive value of FTMR with fermented feed, % DM			
	DM	CP	NDF	ADF
N	55.85 <sup>ab</sup>	12.16 <sup>b</sup>	41.00 <sup>bc</sup>	32.06 <sup>ab</sup>
A	58.07 <sup>ab</sup>	13.95 <sup>a</sup>	49.50 <sup>abc</sup>	29.00 <sup>ab</sup>
R	59.06 <sup>a</sup>	13.54 <sup>ab</sup>	53.30 <sup>a</sup>	30.00 <sup>ab</sup>
L	51.42 <sup>ab</sup>	14.59 <sup>a</sup>	48.60 <sup>abc</sup>	36.00 <sup>a</sup>
S	55.88 <sup>ab</sup>	15.16 <sup>a</sup>	52.00 <sup>a</sup>	30.00 <sup>ab</sup>
AR	55.08 <sup>ab</sup>	14.30 <sup>a</sup>	50.50 <sup>ab</sup>	33.00 <sup>ab</sup>
AL	53.39 <sup>a</sup>	15.21 <sup>a</sup>	40.00 <sup>c</sup>	21.00 <sup>b</sup>
AS	55.22 <sup>ab</sup>	14.79 <sup>a</sup>	49.50 <sup>abc</sup>	30.00 <sup>ab</sup>
RL	53.31 <sup>ab</sup>	12.15 <sup>b</sup>	42.03 <sup>bc</sup>	30.43 <sup>ab</sup>
RS	51.67 <sup>a</sup>	13.96 <sup>ab</sup>	52.50 <sup>a</sup>	28.19 <sup>ab</sup>
LS	51.84 <sup>a</sup>	14.48 <sup>a</sup>	51.12 <sup>a</sup>	28.15 <sup>ab</sup>
ARL	45.97 <sup>b</sup>	14.63 <sup>a</sup>	50.10 <sup>ab</sup>	28.65 <sup>ab</sup>
ARS	55.02 <sup>ab</sup>	14.67 <sup>a</sup>	54.42 <sup>a</sup>	29.00 <sup>ab</sup>
ALS	54.84 <sup>ab</sup>	13.92 <sup>ab</sup>	45.11 <sup>b</sup>	26.01 <sup>ab</sup>
RLS	55.46 <sup>ab</sup>	14.72 <sup>a</sup>	39.03 <sup>c</sup>	20.14 <sup>b</sup>
ARLS	54.86 <sup>ab</sup>	15.02 <sup>a</sup>	45.23 <sup>c</sup>	25.67 <sup>ab</sup>
SEM	5.102	0.961	1.055	4.338

\*A = *Aspergillus oryze*, R = *Rhizopus oligosporus*, L = *Lactobacillus buchnari*, S = *Saccharomyces Cereveceae*

**Table. 2** Effect of microbial culture inoculation on *in vitro* rumen degradability of FTMR

Treatments*	<i>In vitro</i> gas kinetic and DM digestibility <sup>1</sup>					
	a	b	c	a +b	IVDMD	Gas (96 hr.), ml.
N	-2.26	98.56	0.06	96.30	55.6 <sup>c</sup>	88.10
A	-1.07	105.69	0.06	104.62	59.6 <sup>c</sup>	96.42
R	-2.66	113.06	0.05	108.40	64.5 <sup>ab</sup>	100.20
L	-0.32	116.67	0.06	116.36	64.9 <sup>ab</sup>	108.16
S	-0.44	105.43	0.05	104.99	64.2 <sup>b</sup>	96.79
AR	-1.99	95.59	0.06	95.60	68.0 <sup>a</sup>	89.60
AL	-2.37	103.60	0.06	101.22	64.8 <sup>ab</sup>	93.02
AS	-1.76	111.29	0.07	109.53	64.2 <sup>b</sup>	101.33
RL	-0.95	106.86	0.06	105.91	63.9 <sup>b</sup>	97.61
RS	-0.73	104.42	0.07	103.69	63.9 <sup>b</sup>	95.49
LS	-0.07	101.88	0.05	101.81	62.5 <sup>bc</sup>	93.61
ARL	-3.02	108.88	0.05	105.86	67.3 <sup>a</sup>	97.76
ARS	-0.48	103.87	0.04	103.38	65.6 <sup>abc</sup>	95.28
ALS	-2.68	110.80	0.07	108.11	65.8 <sup>ab</sup>	99.89
RLS	-2.20	100.19	0.05	96.99	62.4 <sup>abc</sup>	96.99
ARLS	-0.99	109.27	0.06	108.28	68.8 <sup>a</sup>	99.18
SEM	0.952	3.538	0.012	3.618	1.55	3.409

\*A = *Aspergillus oryze*, R = *Rhizopus oligosporus*, L = *Lactobacillus buchnari*, S = *Saccharomyces Cereveceae*

<sup>1</sup>a=gas production from the immediately soluble fraction, b=gas production from the insoluble fraction, c=gas production rate constant for the insoluble fraction (b), (a+b)= potential extent of gas production.

**Table 3.** Effect of microbial culture inoculation on *in vitro* ruminal fermentation characteristic

Treatments*	NH <sub>3</sub> -N (mg/dL)	TVFAs, mmol/L	VFAs, %		
			Acetate (C2)	Propionate (C3)	Butyrate (C4)
N	12.1 <sup>b</sup>	86.6 <sup>bc</sup>	60.3 <sup>b</sup>	28.6 <sup>a</sup>	11.1 <sup>b</sup>
A	16.7 <sup>b</sup>	96.8 <sup>ab</sup>	63.6 <sup>ab</sup>	25.4 <sup>a</sup>	11.0 <sup>b</sup>
R	14.1 <sup>b</sup>	98.6 <sup>b</sup>	60.6 <sup>b</sup>	27.4 <sup>a</sup>	12.2 <sup>b</sup>
L	15.1 <sup>b</sup>	82.1 <sup>cd</sup>	59.6 <sup>b</sup>	25.5 <sup>a</sup>	14.9 <sup>a</sup>
S	23.3 <sup>ab</sup>	85.3 <sup>c</sup>	61.7 <sup>b</sup>	23.2 <sup>b</sup>	15.1 <sup>a</sup>
AR	24.7 <sup>ab</sup>	87.7 <sup>bc</sup>	61.4 <sup>b</sup>	25.8 <sup>a</sup>	12.7 <sup>b</sup>
AL	22.5 <sup>ab</sup>	105.2 <sup>a</sup>	66.0 <sup>a</sup>	22.7 <sup>b</sup>	11.3 <sup>b</sup>
AS	21.5 <sup>ab</sup>	81.7 <sup>cd</sup>	58.8 <sup>bc</sup>	29.4 <sup>a</sup>	11.7 <sup>b</sup>
RL	19.1 <sup>b</sup>	94.2 <sup>b</sup>	61.3 <sup>b</sup>	28.0 <sup>a</sup>	10.7 <sup>b</sup>
RS	24.0 <sup>a</sup>	74.3 <sup>d</sup>	58.4 <sup>c</sup>	28.8 <sup>a</sup>	12.8 <sup>a</sup>
LS	22.2 <sup>ab</sup>	90.8 <sup>bc</sup>	60.9 <sup>b</sup>	27.9 <sup>a</sup>	11.2 <sup>b</sup>
ARL	23.4 <sup>ab</sup>	98.9 <sup>b</sup>	57.8 <sup>c</sup>	28.6 <sup>a</sup>	13.6 <sup>a</sup>
ARS	19.6 <sup>ab</sup>	97.6 <sup>ab</sup>	56.4 <sup>c</sup>	28.7 <sup>a</sup>	14.9 <sup>a</sup>
ALS	25.6 <sup>a</sup>	91.6 <sup>bc</sup>	61.2 <sup>b</sup>	25.9 <sup>ab</sup>	12.9 <sup>bc</sup>
RLS	22.9 <sup>b</sup>	93.3 <sup>bc</sup>	61.7 <sup>b</sup>	26.7 <sup>a</sup>	11.6 <sup>b</sup>
ARLS	21.4 <sup>a</sup>	87.7 <sup>bc</sup>	58.7 <sup>bc</sup>	28.9 <sup>a</sup>	12.5 <sup>b</sup>
SEM	2.042	3.677	2.042	1.043	0.963

\*A = *Aspergillus oryze*, R = *Rhizopus oligosporus*, L = *Lactobacillus buchnari*, S = *Saccharomyces Cereveceae*



## Effect of Addition of Siamese Neem Foliage on pH and Number of Lactic Acid Bacteria in Napier Grass Silage

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### Abstract

This study was aimed to investigate the effect of Siamese neem (SN) foliage addition as the source of tannins on pH and number of lactic acid bacteria (LAB) in Napier grass ensiling process. Napier Pak Chong 1 grass silage added with varied levels of Siam neem (SN) foliage was established in a Completely Randomized Design with five replicates. Four levels of Siam neem foliage addition at 0, 10, 20 and 30 percent (fresh weight) into Napier grass silage were prepared. pH and number of LAB of Napier grass silage were measured. The results showed that addition of SN foliage at varied levels (10, 20, 30 % fresh weight) did not effected on LAB population of ensiled grass opened at 60 days after ensiling and pH of ensiled grass opened at 60 and 90 days after ensiling. This results indicated that addition of SN foliage has no detrimental effected on silage quality, however, study on effect of tannins in SN foliage on chemical composition and protein degradation during ensiling process is further needed.

**Keywords:** tannins, protein degradation, ensiling, Napier grass, silage additive, LAB

### Introduction

Using of Napier Pak Chong 1 (*Pennisetum purpureum* x *Pennisetum americanum*) silage as roughage source for ruminants feeding has recently greater interested in Thailand. However, it is generally found that nutritive value of ensiled grass is decreased by extensive protein degradation that occurs during ensiling process. Improving the characterization of grass silage quality by using several additives such as formic acid (Nagel and Broderick, 1992), formaldehyde (Henderson, 1993) and particular tannins (Tabacco et al., 2006) has been increasing established and demonstrated that these additives could effectively reduce protein degradation and non-protein nitrogen formation in grass silage. Siamese neem (*Azadirachta indica* A. Juss. var. *Siamensis* Valetton) is tropical available plant species that high tannins content, and has potentially use as the tannins source to use as silage additive in order to improve quality of Napier grass silage. However, as tannins able to inhibit activity of microbes, and the inhibitory effect of tannins in Siamese neem on fermentative parameter and bacteria involved in ensiling process are not yet established. Therefore, this study was aimed to evaluate the effect of



levels of Siam neem foliage addition as ensiling additive on pH and number of lactic acid bacteria (LAB) in Napier grass silage.

## Materials and Methods

### Plant materials and silage preparations

Napier Pak Chong 1 grass silage added with varied levels of Siam neem (SN) foliage was established in a Completely Randomized Design with five replicates. Napier Pak Chong 1 grass was harvested at 60 days of re-growth from Nongkai Animal Nutrition Research and Development Center, Nongkai, Thailand and chopped into a 2-3 cm of length and wilted under shading for 18 h prior to use for silage making. Siam neem foliage was harvested from Muang District, Udon Thani Province, Thailand, chopped into a 2-3 cm of length and wilted under shading for 12 h prior to use as silage additive. Four levels of Siam neem foliage addition at 0, 10, 20 and 30 percent (fresh weight) into Napier grass silage were prepared. Three kilograms of each mixtures of Napier grass and Siam neem foliage were placed into a double layers black plastic bag, packed and removed the air by using vacuum pump prior to cap to ensure an oxygen-free fermentation condition. All silage bags were kept under shading until opened at 60 and 90 days after ensiling for further analysis.

### Analytical Procedures

#### *pH of silage juice*

Extraction of silage juice for pH measurement was conducted using the procedure as described by Bureenok et al. (2007) and Guo et al. (2008) with minor modified. A 25 g of sample from each silage bag opened both at 60 and 90 d after ensiling was placed in a blender jar, diluted with 100 mL of distilled water and macerated for 30 sec, and stored at 4 °C for 12 h. The extracted juices were filtered through two layers of cheesecloth, and the pH of filtrated silage juices were immediately measured by using pH meter.

#### *Number of LAB*

A 10 g of sample from each silage bag opened at 60 d after ensiling was placed into a sterilized 250 mL bottle contained 90 mL of 0.85 % NaCl in water, capped and gently well-mixed. An aliquots were diluted into  $10^{-4}$  to  $10^{-6}$  folds. One mL of silage juice from each dilutions was spread into a plate contained MRS agar and incubated in an anaerobic incubator at 35 °C for 3 d, and LAB were counted and expressed as colony forming unit (CFU).

### Statistical Analysis

All the data collected were subjected to analysis of variance with significant differences between means tested by Duncan's Multiple Range Test (Steel and Torrie, 1980). Mean differences were considered significant at  $P < 0.05$ . All data were analyzed using the General Linear Model of Statistical Analysis System (SAS, 1998).

## Results and Discussion

Number of lactic acid bacteria (LAB) and pH of Napier grass silage opened at 60 and 90 day after ensiling as effected by Siamese neem (SN) addition are presented in Table 1. Although addition of SN foliage as the source of tannins which is well known that this compound has an antimicrobial properties, however, different level of SN foliage addition in this study did not effected on the number of LAB ( $P < 0.05$ ). The number of LAB ranged from 7.83 to 8.12  $\log_{10}$  CFU/g of silage. In the other hand, SN foliage addition at 10 % fresh weight tend to increase LAB population in Napier grass ensiled, this result has agreed with the report by Hara-Kudo et



al. (2005) and Hara (1997) who demonstrated that tea catechins not affected on LAB. The pH of all ensiled grasses did not effected by SN foliage addition and ranged in the normal value of good condition of silage, and this result was also related to the number of LAB.

**Table 1.** Effect of levels of Siam neem (SN) foliage addition in ensiling on pH and number of LAB of Napier grass silage.

Items	Treatments [Level of SN foliage, % (w/w)]				SEM	P-value
	T1 (0 %)	T2 (10 %)	T3 (20 %)	T4 (30 %)		
LAB, log <sub>10</sub> CFU/g	7.91	8.12	7.83	7.89	0.075	0.0907
pH						
- 60 d after ensiling	4.76	4.80	4.77	4.72	0.097	0.9536
- 90 d after ensiling	4.79	4.88	4.87	4.82	0.046	0.5030

SEM = standard error of the means

## Conclusion

In term of using of tannins-containing plant species as ensiling additives in order to improve silage quality such as decreasing of protein degradation, this results indicated that addition of Siamese neem foliage in Napier grass ensiling has no detrimental effected on silage quality such as pH and growth of lactic acid bacteria, and probably use as the silage additive. However, further research is needed to investigate the effect of tannins in Siamese neem on protection of protein from degradation by the microbial during the ensiling process.

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## Effects of Microflora-Treated Rice Straw on Rumen Fermentation and Digestibility Using *In Vitro* Gas Production Technique

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### Abstract

This study aimed to investigate the effect of microflora- treated rice straw on rumen fermentation and digestibility using *In vitro* gas production technique. The experimental design was a completely randomized design (CRD) and the treatments were; T1=Rice straw (control), T2= Rice straw treated *Aspergillus niger*, T3= Rice straw treated Effective microorganism (EM), T4= Rice straw treated Mixed microbes PD1 (from the Land Development Department, Ministry of Agriculture and Co-operative of Thailand), and T5= Rice straw treated *Saccharomyces cerevisia*. It was found that microflora-treated rice straw could improve the rice straw quality especially, crude protein by *Aspergillus niger* group followed by *Saccharomyces cerevisiae*, Mixed microbes PD1 and effective microorganism group while untreated rice straw were the lowest ( $P<0.01$ ). Moreover, microflora treated groups could decrease NDF and ADF of rice straw ( $p<0.05$ ). Gas kinetics in microflora-treated rice straw affected the insoluble fraction (b) and potential extent of gas production (a+b) ( $P<0.01$ ), but did not affect on the immediately soluble fraction (a) and gas production rate (c) ( $P>0.05$ ). Cumulative gas production (96 h) was highest in the microflora-treated rice straw group by *Aspergillus niger* treated group followed by EM, *Saccharomyces cerevisiae*, Mixed microbes (PD1) group and the lowest was untreated rice straw group ( $p<0.01$ ). In addition, *in vitro* true degradability (IVDMD and IVOMD) at 48 h of incubations were shown to have high correlation with gas volume which was significantly higher in microflora-treated group and the highest ( $p<0.05$ ) was *Aspergillus niger* treated group. In conclusion, potential used of microflora could improve nutritional values of rice straw and *in vitro* true degradability. However, further researches for *in vivo* trial could be conducted.

**Keywords:** microflora, rice straw, digestibility, effective microorganism (EM), *Aspergillus niger*, *Saccharomyces cerevisia*, mixed microbes PD1





## Introduction

Rice generates a relatively large amount of crop residues known as straw. The farmers in Thailand use rice straw and others crop residues as roughage sources fed to ruminants in the dry season. However, there are limitation of using due to the low quality in terms of protein content and digestibility. Therefore, improvements of rice straw quality for ruminant feed become important. In addition, numerous methods of physical, chemical and biological treatments have been investigated, including supplementation with other feed stuffs or components in order to improve the utilization of rice straw by ruminants. Recently, the silage microflora plays an active role in the successful outcome of fermentation processes there were Lactic acid bacteria (LAB), enterobacteria, Clostridia, Yeast, Mold, etc. (Oladosu et al., 2016). However, there are limited studies on the use of Microflora to improve nutritional value of rice straw. Therefore, the aim of this study was to study effects of Microflora-treated rice straw on rumen fermentation and digestibility using *In vitro* gas production technique.

## Materials and Methods

### Microflora treated rice straw preparation

The formulation of activated microflora (*Aspergillus niger*, EM, Mixed microbes PD1 and *Saccharomyces cerevisiae*) was prepared by using 20 g of microflora and 20 g cane sugar mixed with 100 mL distilled water, then mixed well and incubated at room temperature for 1 h (A). Liquid media was prepared using 4 g molasses and 100 mL distilled water, followed by addition of 2 g urea, Mixed (A) and (B) at 1:10 ratio. After that the rice straw was mixed with microflora solution and fermented for 1 week.

### Experimental design and dietary treatments

This study was conducted using an *in vitro* gas production technique at various incubation time intervals. The experimental design was a completely randomized design (CRD) with three replications per treatment. The treatments were T1=Rice straw (control), T2= Rice straw treated *Aspergillus niger*, T3= Rice straw treated Effective microorganism (EM), T4= Rice straw treated Mixed microbes PD1 (from the Land Development Department, Ministry of Agriculture and Co-operative of Thailand), and T5= Rice straw treated *Saccharomyces cerevisiae*.

Rice straw was samples and dried at 60 °C, then ground to pass a 1-mm sieve (Cyclotech Mill, Tecator, Sweden) and used for chemical analysis and in the *in vitro* gas test. The samples were analyzed for dry matter (DM), ash and crude protein (CP) using the procedures of AOAC (1998), neutral detergent fiber (NDF) and acid detergent fiber (ADF) according to Van Soest et al. (1991).

### Animals and preparation of rumen inoculums

Animals rumen fluid was collected from animals fed with concentrate (14.0% CP and 80.6% TDN) at 0.5% of BW in to equal portions, at 07.00 h and 16.00 h and rice straw (follow treatments) was fed on *ad libitum*. The animals were kept in individual pens and clean fresh water and mineral blocks were offered as free choice. The animals received the diets for 20 d before the rumen fluid was collected. On d 20, 1,000 mL rumen liquor was obtained from each animal before the morning feeding. The rumen fluid was filtered through four layers of cheesecloth into pre-warmed thermo flasks and then transported to the laboratory.



### ***In vitro* fermentation of substrates**

Samples of each total mixed substrate (500 mg), following respective treatments were weighed into 50 mL serum bottles. For each treatment, three replications were prepared. Ruminant fluid from each animal was mixed with the artificial saliva solution of Menke and Steingass (1988) in a proportion 2:1 (mL/mL) at 39°C under continuous flushing with CO<sub>2</sub>. Thirty milliliters of rumen inoculum mixture were added into each bottle under CO<sub>2</sub> flushing. Bottles were sealed with rubber stoppers and aluminium caps and incubated at 39°C (96 h) for *in vitro* gas test. Thirty minutes after starting the incubation, the bottles were gently mixed and then mixed three times every 3 h. For each sampling time, three bottles containing only the rumen inocula were included within each run and the mean gas production values of these bottles were used as blanks. The blank values were subtracted from each measured value to give the net gas production.

### **Sample and analysis**

During the incubation, data of gas production was measured immediately after incubation at 0, 2, 4, 6, 8, 12, 18, 24, 48, 72, and 96 h by using a pressure transducer and a calibrated syringe. Cumulative gas production data were fitted to the model of Orskov and McDonald (1979) as follows:

$$y = a + b (1 - e^{-ct})$$

Where a = the gas production from the immediately soluble fraction, b = the gas production from the insoluble fraction, c = the gas production rate constant for the insoluble fraction (b), t = incubation time, (a+b) = the potential extent of gas production. y = gas produced at time "t".

The percent loss in weight was determined and presented as *in vitro* dry matter degradability (IVDMD). The dried feed sample and residue left from above was ashed at 550°C for determination of *in vitro* organic matter degradability (IVOMD) (Tilley and Terry, 1963).

### **Statistical analysis**

All data were analyzed as a Completely randomized design (CRD) using the PROC GLM of SAS (1998). Multiple comparisons among treatment means were performed by Duncan's New Multiple Range Test (DMRT) (Steel and Torrie, 1980). Differences among means with  $p < 0.05$  were accepted as representing statistically significant differences.

## **Results and Discussion**

The result showed that microflora-treated rice straw could improve the rice straw quality especially, crude protein by *Aspergillus niger* group followed by *Saccharomyces cerevisiae*, Mixed microbes PD1 and effective microorganism group while untreated rice straw were the lowest ( $P < 0.01$ ) as showed in Table 1. Moreover, microflora treated groups could decrease NDF and ADF of rice straw ( $p < 0.05$ ). Crude protein of *Saccharomyces cerevisiae* treated rice straw was lower than reported by Foiklang et al. (2017) who reported that yeast medium solution (YMS) fermented rice straw at ratio 1:0.5, 1:1 could increase rice straw CP at 11.2 and 15.7%CP, respectively with short form rice straw

**Table 1.** Chemical composition of cassava products (% of dry matter).

Treatments	Dry matter	Organic matter	Crude protein	Neutral detergent fiber	Acid detergent fiber
Rice straw	87.0 <sup>a</sup>	83.7 <sup>c</sup>	2.1 <sup>c</sup>	77.3 <sup>a</sup>	54.8 <sup>a</sup>
<i>Aspergillus niger</i>	41.5 <sup>b</sup>	89.7 <sup>a</sup>	9.3 <sup>a</sup>	70.2 <sup>b</sup>	49.2 <sup>b</sup>
Effective microorganism	39.9 <sup>a</sup>	88.3 <sup>ab</sup>	7.8 <sup>b</sup>	72.1 <sup>b</sup>	50.3 <sup>b</sup>
Mixed microbes PD1	41.2 <sup>b</sup>	87.6 <sup>ab</sup>	8.4 <sup>ab</sup>	75.7 <sup>a</sup>	54.2 <sup>a</sup>
<i>Saccharomyces cerevisiae</i>	40.9 <sup>b</sup>	87.3 <sup>b</sup>	8.8 <sup>a</sup>	76.2 <sup>a</sup>	54.7 <sup>a</sup>
SEM	0.55	0.63	0.25	0.74	0.81
P-value	**	**	**	*	*

<sup>a,b,c</sup> Value on the same row with different superscripts differ ( $P < 0.05$ ), \* $P < 0.05$ , \*\* $P < 0.01$ , ns = non-significant different, SEM=Standard error of the mean

Gas kinetics were different among treatments ( $P < 0.01$ ; Table 2, Figure 1). Microflora treated rice straw affected the insoluble fraction (b) and potential extent of gas production (a+b) ( $P < 0.01$ ), but did not affect on the immediately soluble fraction (a) and gas production rate (c) ( $P > 0.05$ ). Cumulative gas production (96 h) was higher in the microflora treated rice straw group by *Aspergillus niger* group was the highest follow by Effective microorganism, *Saccharomyces cerevisiae*, Mixed microbes (PD1) group and the lowest was untreated rice straw group. These results were similar to reported by Oladosu et al. (2016) who showed that microflora could improve quality of rice straw.

In addition, *in vitro* true degradability (IVDMD and IVOMD) at 48 h of incubations were shown to have high correlation with gas volume which was significantly higher in microflora treated group and the highest ( $p < 0.05$ ) was *Aspergillus niger* treated group.

## Conclusions and Recommendations

Based on this study, it could be concluded that using microflora-treated rice straw could improve nutritional values of rice straw and enhance *in vitro* nutrient digestibility. These results revealed a potential use of microorganism to improve nutritional value of cassava products, leading to improve rumen fermentation efficiency and a possible productivity in ruminants. However, further researches for *in vivo* trial could be conducted.

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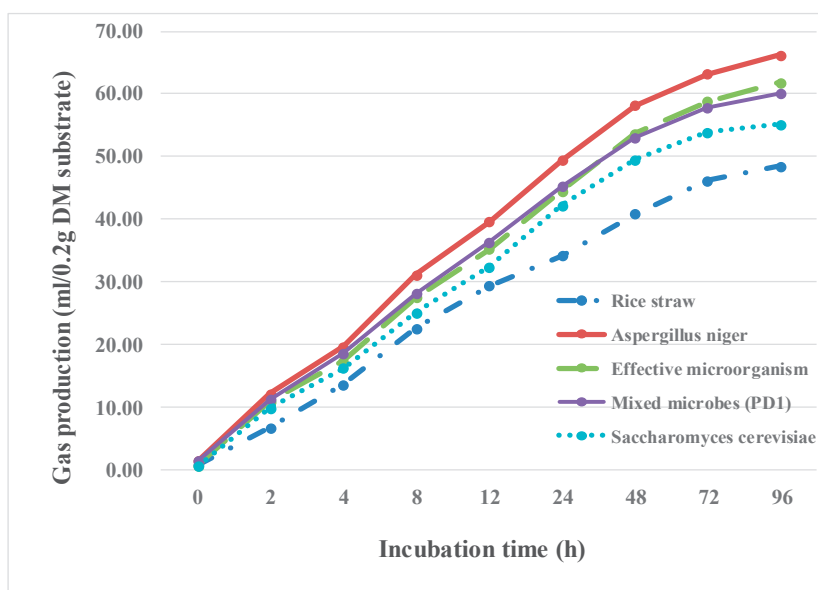
Resources, Rajamangala University of Technology-Isan, Sakon Nakhon Campus for providing experimental animals and laboratory.

**Table 2.** Effect of protein source with roughage to concentrate ratio on gas production kinetic and degradability from in vitro incubation with rumen fluid

Treatments	Gas kinetics <sup>1</sup>				Gas (96 h) ml/0.2 g DM substrate	<i>In vitro</i> degradability (%)	
	a	b	c	a+b		IVDMD	IVOMD
Rice straw	1.9	43.8 <sup>d</sup>	0.066	45.7 <sup>d</sup>	48.5 <sup>d</sup>	52.8 <sup>b</sup>	74.8 <sup>ab</sup>
<i>Aspergillus niger</i>	3.7	59.5 <sup>a</sup>	0.070	63.2 <sup>a</sup>	66.2 <sup>a</sup>	62.1 <sup>a</sup>	77.5 <sup>a</sup>
Effective microorganism	3.3	55.8 <sup>ab</sup>	0.066	59.1 <sup>b</sup>	61.8 <sup>b</sup>	54.8 <sup>ab</sup>	75.6 <sup>ab</sup>
Mixed microbes (PD1)	2.5	51.0 <sup>c</sup>	0.070	53.5 <sup>c</sup>	55.2 <sup>c</sup>	48.7 <sup>bc</sup>	70.2 <sup>bc</sup>
<i>Saccharomyces cerevisiae</i>	3.5	54.1 <sup>bc</sup>	0.070	57.6 <sup>b</sup>	60.2 <sup>b</sup>	41.1 <sup>c</sup>	69.0 <sup>c</sup>
SEM	0.49	1.21	0.002	1.25	1.34	2.36	1.36
P-value	ns	**	ns	**	**	*	*

<sup>a,b,c,d</sup> Value on the same row with different superscripts differ ( $P < 0.05$ ), \* $P < 0.05$ , \*\* $P < 0.01$ , ns = non-significant different, SEM=Standard error of the mean.

<sup>1</sup>a= The gas production from the immediately soluble fraction, b= The gas production from the insoluble fraction, c= The gas production rate constant for the insoluble fraction (b), a+b = The gas potential extent of gas production.



**Figure 1.** Effects of microflora- treated rice straw on cumulative gas production



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## Effects of Fresh Purple Napier Grass (*Pennisetum Purpureum* ‘Prince’) and silage on Ruminal Gas Production- *In Vitro* Study

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### Abstract

The objectives of this study was to investigate the effect of fresh purple napier grass and silage on *in vitro* ruminal gas production. Purple napier grass (*Pennisetum Purpureum* ‘Prince’) was selected to use in this study. Fresh and silage conditions of purple napier grass had effect on *in vitro* gas kinetic parameters and gas productions ( $P < 0.01$ ). Moreover, fresh and silage of purple napier grass affected on the insoluble fractions and methane production at 24 h of incubation. The results suggested that different condition of grass had different effect on *in vitro* gas and methane production; however, others factors affected on biological properties of anthocyanin, such as molecular weight and structure should be further investigated.

**Keywords:** purple napier grass, *Pennisetum purpureum* ‘prince’, gas production, *in vitro*, rumen fermentation

### Introduction

Small ruminants are important animal resources in the tropics, where they play a predominant role in the sustenance of the livelihoods of impoverished families especially in the rural areas. Feed resources for ruminant livestock in the tropics are natural pastures which are limited in supply during the dry season. While enough amount of roughage can be produced in the rainy season, ruminant production has to very much rely on rice straw in the dry season. Feed shortage in terms of quantity and quality in the dry season is a key constraint on further development of dairy production as well as beef production. Napier grass may be utilized as small ruminant feed in the dry season. Napier grass could be a promising roughage for feeding small ruminant because of its high yield under the severe environment (Kawashima et al., 2001). The use of napier grass as small ruminant feed has been demonstrated in other countries (Preston, 1988). In addition, purple napier grass is a member of napier grass family but containing secondary compounds such as anthocyanin which is natural pigment (antioxidant property). Therefore, the objectives of this study were to characterize the purple napier grass in fresh and silage conditions and their effects on *in vitro* gas production study.

### Materials and Methods

#### Determination of *in vitro* gas production

Purple napier grass (*Pennisetum Purpureum* ‘Prince’) was harvested from the area of Muang District, Nakhon Ratchasima, Thailand, by cutting from the youngest fully leaves and stems from purple grass. All samples were dried at 50°C using a forced air oven prior to grinding through a 1.0 mm sieve. Sample of each purple napier grass was used in the study. The treatment



in this experiment include: fresh grass (control group) compared with grass silage (grass was fermented for 21 days). This procedures were performed with 10 replicates in 2 separated runs.

Three matured rumen fistulated Saanen male goats fed with 2.5 % of body weight (% BW) DM/day containing dried-ground Pangola (*Digitaria eriantha*) and commercial concentrate (14 % of CP) (60:40) were used as donors of rumen fluid. Ruminant fluid and artificial saliva and *in vitro* gas production procedure were prepared and run according to Menke & Steingass (1988). The total gas produced were measured at 0, 2, 4, 6, 8, 12, 24, 48, 72 and 96 h of incubation, and net gas production values were corrected by subtracting blank values from the samples (Menke & Steingass, 1988). Cumulative gas production data were fitted to the model of Orskov & McDonald (1979) described as  $Y = a+b(1 - e^{-ct})$ ; where  $Y$  represents the cumulative gas production at time  $t$ ,  $a$  is the gas production from the immediately soluble fraction,  $b$  is the gas production from the insoluble fraction,  $c$  is the rate of gas production (/h) and  $(a+b)$  is the potential gas production. At 12 and 24 h of incubation, the methane (CH<sub>4</sub>) volume was measured by absorbed produced CO<sub>2</sub> into the content by added 4.0 mL of 10 M NaOH, therefore, the gas volume remaining in the syringe considered to be CH<sub>4</sub> (Fievez et al., 2005).

### Statistical analysis

Data of *in vitro* gas production were analyzed using the PROC GLM of SAS (1998). Multiple comparisons among treatment means were identified using Duncan's New Multiple Range Test (Steel & Torrie, 1980). Mean differences were considered significant at  $P<0.05$ .

## Results and Discussion

### *In vitro* gas productions

The kinetics of gas production models and gas production at 12 and 24 h of incubations of each treatments are presented in Table 1. Different of purple napier grass in fresh and silage conditions had no effect on the gas production from the immediately soluble fraction ( $a$ ) ( $P>0.05$ ). While the gas production from the insoluble fractions ( $b$ ), the gas production rate ( $c$ ) and the potential extent of gas production ( $a+b$ ) were affected by fresh and silage of purple napier grass ( $P<0.001$ ). However, it was found that fresh and silage of purple napier grass had effect on  $b$  value (67.50 and 22.0). Fresh and silage of purple napier grass had effect on total gas (TG) and methane (CH<sub>4</sub>) productions at 12 and 24 h of incubations ( $P<0.01$ ) (Figure 1 and 2) but had effect on CH<sub>4</sub>/TG ratio ( $P<0.001$ ). For methane production at 24 h, purple napier grass silage (4.64 mL/g DM of substrate) had methane production less than fresh purple napier grass (7.05 mL/g DM of substrate) about 34.18%.

Different gas kinetics and gas productions of fresh purple napier grass and silage probably due to different of their chemical compositions. Fresh purple napier grass showed a highest gas productions and methane production compared with purple napier grass silage. Gas production from the insoluble fractions and methane production at 24 h of incubation may due to condition of purple napier grass silage had effect on gas kinetics and gas productions.

## Conclusion

It can be concluded that the fresh purple napier grass showed the highest gas productions and methane production compared with purple napier grass silage. Gas production from the insoluble fractions and methane production at 24 h of incubation may due to condition of silage purple napier grass had effect on gas kinetics and gas productions.



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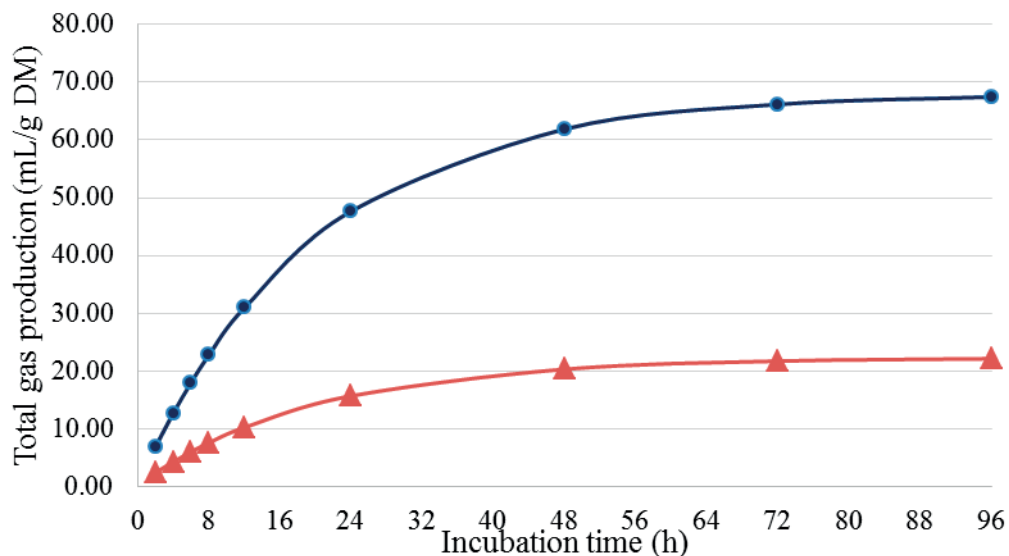
**Table 1.** Effects of fresh purple napier grass (FPN) and silage (PNS) on *in vitro* gas and methane productions

	Treatments <sup>1</sup>		SEM	P-value
	FPN	PNS		
<b>Gas kinetics<sup>2</sup></b>				
<i>a</i>	0.50	0.34	0.40	0.7780
<i>b</i>	67.50 <sup>a</sup>	22.00 <sup>b</sup>	1.62	0.0001
<i>c</i>	0.05	0.05	0.00	0.00
<i>a+b</i>	68.00 <sup>a</sup>	22.33 <sup>b</sup>	2.00	0.0001
<b>Total gas production (mL/g DM of substrate)</b>				
12-h	30.95 <sup>a</sup>	10.26 <sup>b</sup>	1.10	0.0002
24-h	47.67 <sup>a</sup>	15.71 <sup>b</sup>	1.50	0.0001
<b>Methane (CH<sub>4</sub>) production (mL/g DM of substrate)</b>				
12-h	4.10 <sup>a</sup>	2.44 <sup>b</sup>	0.17	0.0024
24-h	7.05 <sup>a</sup>	4.64 <sup>b</sup>	0.27	0.0032
<b>CH<sub>4</sub>/total gas ratio (v/v)</b>				
12-h	0.13 <sup>b</sup>	0.24 <sup>a</sup>	0.00	0.0001
24-h	0.15 <sup>b</sup>	0.30 <sup>a</sup>	0.00	0.0001

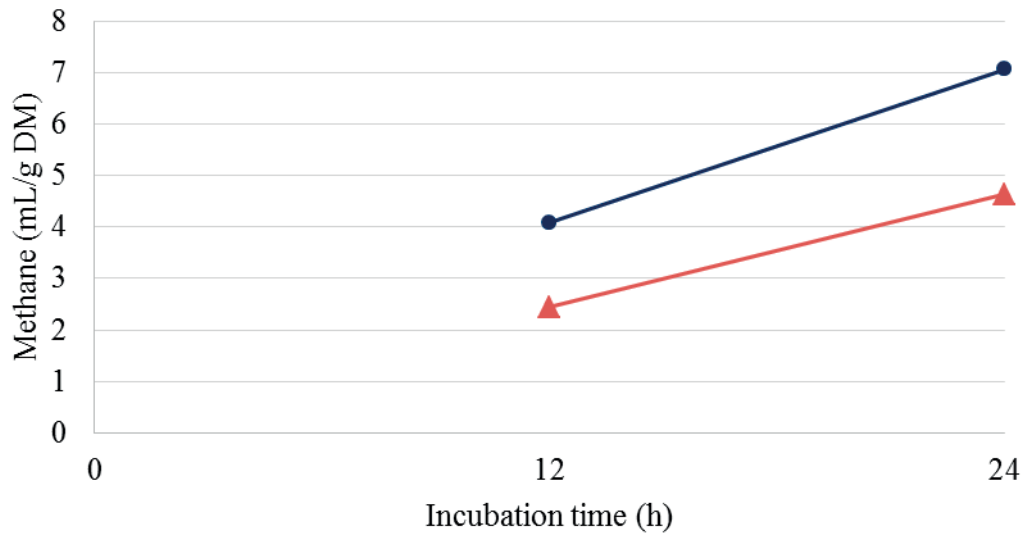
<sup>a,b,c,d</sup> Within rows, values followed by different letters are significantly different ( $P < 0.05$ )

<sup>1</sup>FPN: Fresh purple napier grass, PNS: purple napier grass silage.

<sup>2</sup>*a*: the gas production from the immediately soluble fraction, *b*: the gas production from the insoluble fraction, *c*: the gas production rate constant, *a+b*: the potential extent of gas production.



**Figure 1.** Total gas production of fresh purple napier grass (O) and purple napier grass silage (Δ).



**Figure 2.** Methane production of fresh purple napier grass (O) and purple napier grass silage (Δ).



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## Effect of Thapra Stylo Silage Treated with Dried Mao Pomace and Lactic Acid Bacteria on Feed Intake and Digestibility of Goats

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### Abstract

This study was conducted to evaluate the effects of various additive silages on carcass characteristics and meat quality of growing goats. Twenty male goats were divided into 4 groups and individually penned for 30 days. Each group were fed an untreated silage (C), dried Mao pomace treated silage (DMP), fermented juice of epiphytic lactic acid bacteria treated silage (FJLB) or DMP plus FJLB (DMP+FJLB). Dry matter intake was not significantly different among treatments. The digestibility of DM and OM in goats fed with Stylo silage treated with DMP and FJLB were higher ( $P < 0.05$ ) than the other treatments. Digestibility of NDF and ADF in goats fed with Stylo silage treated with FJLB were higher than the other treatments ( $P < 0.05$ ).

**Keywords:** dried Mao pomaces, lactic acid bacteria, Thapra Stylo, silage, goat

### Introduction

Dried Mao pomace (DMP), a by-product of Mao wine or juice processing from Mao Luang seed (*Antidesma bunius* Linn.). DMP is acidic and contains some organic acid and sugar that may have the potential as a silage additive to inhibit the growth of undesirable bacteria and stimulate LAB (lactic acid bacteria) growth during the ensiling process (Bureenok et al., 2012). Thapra Stylo is one of the most promising legume available for ruminant production in tropical area which contain high protein and grow over variety of soil types (Phaikaew and Hare, 2005). Thapra Stylo silage without any additives was difficult to make good quality silage, with high pH value and  $\text{NH}_3\text{-N}$  content (Liu et al., 2011, 2012). Recently, Bureenok *et al.* (2011) demonstrated that the treatment of silage with fermented juice of epiphytic lactic acid bacteria (FJLB) resulted in stable silage ( $\text{pH} < 4.1$ ) and improved nutrient digestibility of silages. Therefore, aim of this study was to investigate the effect of applying DMP alone and combined with FJLB on feed intake and digestibility of Thapra Stylo legume silages in goats.

### Materials and methods

Thapra Stylo legumes were chopped into 2-3 cm. length and mixed with the silage additives. Silages were untreated (control) or prepared with DMP, or DMP plus FJLB (DMP+FJLB). DMP and FJLB were applied at 100 g/kg of fresh matter (FM) and 5.58 cfu/g FM, respectively. The experiment forages were tightly packed in 100-litre plastic drums with clamp lid and stored until the start of the feeding trial which started 30 days later.

Twenty male crossbred Boer x Saanen goats ( $13.4 \pm 1.9$  kg body weight) were randomly assigned to one of the four dietary treatment silages in a Randomized complete block design (RCBD). The experiment was lasted for 28 days which consisted of 21 days for feed intake



measurement and followed 7 days for total collections. All goats were received concentrate (14% CP) at 1.5% of body weight (BW) and *ad libitum* silage.

### Chemical analysis

Silage samples (20 g fresh material) were macerated with 70 ml of distilled water and stored in a refrigerator at 4 °C for 12 h. Then, the extract was filtered (filter paper no.5; Whatman, England) and the pH of the extract was recorded. The filtrate was stored at -20 °C until the analysis of lactic acid, volatile fatty acids (VFAs). Feed and silages were sampled once a week and kept for chemical analysis. Faeces samples were collected at the end of each period and analysed for DM, CP, EE, ash according to standard methods AOAC (1995). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were determined by the method of Van Soest et al. (1991). The total phenolic content was determined according to the method described by Waterhouse (2005). The results are expressed in gallic acid equivalents (mg gallic acid/ g DM).

### Statistical analysis

The data were analysed using the General Linear Model procedure by SAS. Data of feeding trial were analysed by using the procedures of SAS for a Randomized complete block design.

### Results and discussion

Chemical composition of experimental diets was shown in Table 1. All treated silages were well-preserved with lower pH (pH <4.2) and higher lactic acid content. Dry matter intake of silage was not significantly different among silages (Table 2). The digestibility of DM and OM in goats fed with Stylo silage treated with DMP and FJLB were higher ( $P<0.05$ ) than the other treatments. The goats fed with DMP+FJLB treated silage had lower ( $P<0.05$ ) digestibility of CP than the other diets. Bureenok et al. (2011) reported that the addition of FJLB improved the CP digestibility. However, in this study the CP digestibility was decreased when addition with DMP plus FJLB. Addition of DMP increased total phenolic content in diets. Previous reports indicated that the polyphenols could restrict proteolysis in ensiled forage and reduced the protein solubility through protein-phenol binding (Salawu et al., 1999; Guo et al., 2008, Lee et al., 2008). Some LAB such as *L. plantarum* could degrade phenolic compounds (Landete et al., 2008; Rodríguez et al., 2009). Therefore, the LAB in FJLB may degrade phenol content which decreasing the protein-phenol binding. Digestibility of NDF and ADF in goats fed with Stylo silage treated with FJLB were higher than the other treatments ( $P<0.05$ ). Yahaya et al. (2004) found that addition of FJLB increased digestibility of DM and NDF compared with the control and silage treated with acetic acid. These results also agreed with Weinberg et al. (2007) who reported that some LAB inoculants applied at ensiling or added directly to the rumen fluid had the potential to increase the DM and NDF digestibility. Addition of 100 g/kg of DMP did not effect on silage intake in goats. The nutrient digestibility of goats were improved by feeding of DMP and FJLB treated silages. In this regard, DMP can provide the goat feeding with an inexpensive alternative feed ingredient, while reducing the environmental impact of waste disposal in the Mao juice industry.

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**Table 1.** Chemical composition in experimental diets

Item	Concentrat	DMP+FJL			
	e	CO	DMP	FJLB	B
DM (%)	89.51	34.5	40.0	36.5	38.50
CP (%DM)	16.05	10.7	11.6	12.7	10.89
NDF (%DM)	25.75	68.4	64.9	66.5	74.12
ADF (%DM)	45.65	3	6	1	74.12
Ash (%DM)	7.02	45.1	40.4	41.4	53.22
Total phenolic content (mg gallic acid/g DM)	-	5	1	6	53.22
		11.2	14.1		
		5	8	13.3	17.46
Silage profiles					
pH	-	4.41	3.95	4.25	4.02
Lactic acid (%DM)	-	0.69	1.15	3.87	2.78
Acetic acid (%DM)	-	0.99	1.89	2.37	3.61
Propionic acid (%DM)	-	0.13	0.11	0.16	0.67
Butyric acid (%DM)	-	0.63	0.20	0.43	0.46

DMP = dried Mao pomace, FJLB = fermented juice of lactic acid bacteria, DM = dry matter, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber.

**Table 2.** Feed intake and nutrient digestibility of Thapra Stylo legume silages in goats

Item	Control	DMP	FJLB	DMP+FJLB	SEM	P-value				
Feed intake (%BW)										
Silage	2.64	2.55	2.13	2.29	0.11	0.3428				
Concentrate	1.64	1.68	1.69	1.68	0.02	0.6742				
Total Intake	4.28	4.24	3.82	3.97	0.11	0.4045				
Digestion, (%)										
DM	74.46	b	77.4	ab	84.05	a	72.08	b	1.20	0.0157
OM	76.70	b	78.92	ab	85.58	a	73.55	b	1.12	0.0109
CP	77.81	ab	81.16	a	86.14	a	71.21	b	1.35	0.0094
NDF	65.57	b	69.8	b	79.58	a	66.35	b	1.38	0.0088
ADF	62.85	b	63.84	b	75.63	a	68.24	ab	1.46	0.0272

DMP = dried Mao pomace, FJLB = fermented juice of lactic acid bacteria, DM = dry matter, BW = body weight, OM = organic matter, CP = crude protein, NDF = neutral detergent fiber, ADF = acid detergent fiber, SEM = Standard error of mean.



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## ***In Vitro* and *In Vivo* Evaluation of Malic Acid on Methane Mitigation in Paddy Straw Based Complete Diet for Sustainable Animal Production in Dairy Cattle**

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### **Abstract**

An experiment was conducted to evaluate the *in vitro* and *in vivo* effect of malic acid on rumen methane reduction for dairy cattle. The *in vitro* gas production technique (IVGPT) was carried out by incubating the malic acid at varying levels in paddy straw based complete ration with rumen liquor for a period of 24 hours. The *in vitro* finding was validated by *in vivo* feeding trial in indigenous dairy cattle and the methane emission was estimated by sulfur hexa fluoride (SF<sub>6</sub>) tracer gas technique. The malic acid at 0.39 % was the minimum level resulted a highly significant ( $p < 0.01$ ) reduction of methane by 15.95 % than control. Similarly, there was a highly significant ( $p < 0.01$ ) reduction of methane (ml) per 100 mg of truly digested substrate in minimum level of 0.39 % malic acid supplemented group (15.69 %) when compared to control. The methane emission per animal per day was significantly ( $p < 0.05$ ) decreased by 3.26 % in malic acid supplemented group than control. Similarly the methane emission per kg DMI was also significantly decreased by 3.11 % in malic acid added group than without added group. The methane per kg DDMI was numerically decreased by 2.43 % in malic acid supplemented group than un-supplemented group. The methane emission per kg milk production was also significantly ( $p < 0.05$ ) reduced by 5.43 % in malic acid added group than control. The total volatile fatty acid and propionic acid were significantly ( $p < 0.05$ ) increased by 2.69 % and 11.71 % respectively in malic acid supplemented group than control. The acetic acid and acetate to propionate ratio were significantly ( $p < 0.05$ ) decreased by 4.04 % and 14.86 % respectively in malic acid added group when compared to control. It was concluded that the supplementation of malic acid at 0.39 % of paddy straw based complete diet was significantly ( $p < 0.05$ ) reduced the methane emission.

**Keywords:** malic acid, *in vitro*, *in vivo*, methane reduction, dairy cattle

### **Introduction**

Methane is normally emitted by dairy cattle. Among global methane production, 15–20 % of the CH<sub>4</sub> was emitted from ruminants (Moss, 2000) and causes global warming. The methane emission also represents a loss of feed energy by 8-12 %, there by it reduces the animal productivity and increases the environmental temperature. There are various feeding strategies are used to reduce the methane emission from dairy cattle for sustainable animal production. The supplementation of malic acid is the potential feeding strategy to reduce the methane emission in dairy cattle (Bharathidhasan, 2016). The malic acid is converted in to propionic acid by utilizing hydrogen ions in the rumen, there by the methane production is decreased. Hence the present research was carried out to study the effect of malic acid on reduction of methane emission by *in*



*vitro* and *in vivo* in indigenous dairy cattle for sustainable animal production in paddy straw based complete diet.

## Materials and methods

### *In vitro* gas production technique (IVGPT)

The *in vitro* gas production technique (IVGPT) (Menke and Steingass, 1988) was carried out by incubating the malic acid at 0, 0.13 %, 0.26 %, 0.39 % and 0.52 % of paddy straw based complete ration with rumen liquor in shaking water bath for a period of 24 hours. The rumen fluid was collected from three cattle maintained on grazing and it was squeezed through four layers of muslin cloth in to an Erlenmeyer flask under continuous flushing with CO<sub>2</sub> and it was maintained at the temperature of 39 °C. Then rumen fluid was mixed with media as described by Menke and Steingass (1988). The paddy straw based complete diet was used as substrate and it was taken as 200 mg in 100 ml calibrated syringes and weighed quantity of malic acid was added to the syringes in triplicate. Then 30 ml of rumen inoculum was anaerobically transferred to glass syringe and it was incubated in a shaking water bath at 39 °C for 24 hrs. At the end of the incubation period the methane was estimated in Gas Chromatography and *in vitro* true dry matter digestibility (IVTDMD) was determined (Van Soest and Robertson, 1988).

### *In vivo* feeding trial

The *in vitro* finding of the effect of supplemental malic acid on methane reduction was validated by *in vivo* in paddy straw based complete ration for indigenous dairy cattle. The methane emission was estimated by sulfur hexa fluoride (SF<sub>6</sub>) tracer gas technique (Johnson and Johnson, 1995). Eight indigenous dairy cattle with uniform size body weight were selected and divided into two groups with four animals each. The animals were fed with and without supplementation of malic acid at 0.39 % in paddy straw based complete diet (Paddy straw (60): Concentrate mixture (40)). The trial was conducted for a period of 30 days and the collection period was 7 days. The dairy cattle were fed with measured quantity of paddy straw and concentrate mixture separately and left over were recorded to study the dry matter intake (DMI) per day per animal. They are allowed to drink free access of water and reared under standard management practices.

**Table 1.** Quantity of paddy straw and concentrate mixture offered to the indigenous dairy cattle per animal per day

Feed ingredients	Without malic acid (Control)	With Malic acid (Treatment)
Paddystraw (60%)	4.2 kg	4.2 kg
Concentrate mixture (40%)	2.8 kg	2.8 kg
Total	7.0 kg	7.0 kg
Malic acid	0	27.30 g (0.39 % of complete feed)

The dung samples were collected during the last week of trial for digestibility study. The rumen liquid was collected for estimation of rumen fermentation characteristics like volatile fatty acids (Chase, 1990) bacterial count (Gall *et al.*, 1949) and protozoal count (Moir, 1951).

### Estimation of methane

Methane concentration was estimated using Gas Chromatography (Perkin Elmer, Claurus 500 model) fitted with Flame Ionization Detector (FID) and capillary column (30 meter length and 250 micrometer diameter). Helium was used as carrier gas with oven temperature at 60° C, injector temperature at 100°C and detector temperature at 110°C.

Methane concentration in samples (%) was calculated using the following formula.

$$\text{Methane concentration (\%)} = \frac{\text{Peak area of sample gas}}{\text{Peak area of standard gas}} \times \text{Methane concentration in standard (\%)}$$





All data collected were statistically analyzed as per the Snedecor and Cochran (1989).

## Results and discussion

### Effect of supplemental malic acid on reduction of methane emission by *IVGPT*

The effect of supplemental malic on methane (ml), *IVTDMD* and methane (ml) per 100 mg of truly digested substrate were presented in Table 2. The methane emission was significantly ( $p < 0.01$ ) decreased in all malic acid added groups than control. The lowest concentration of malic acid that resulted highly significant ( $p < 0.01$ ) reduction by 15.95 % was in treatment 4 than control. Similarly, there was a highly significant ( $p < 0.01$ ) reduction of methane (ml) per 100 mg of truly digested substrate in treatment 4 (15.69 %) when compared to control. The lowest concentration of malic acid that induced highly significant ( $p < 0.01$ ) reduction in methane (ml) per 100 mg of truly digested substrate was 0.39 % and it was identified level for *in vivo* study. The *IVTDMD* was not differed among treatment groups.

**Table 2.** Effect of supplemental malic acid on methane (ml), *IVTDMD*%, methane (ml) per 100 mg of truly digested substrate by *IVGPT* (Mean<sup>#</sup> ± S.E)

Treatment	Inclusion level of standard malic acid (%)	Methane (ml)	<i>IVTDMD</i> % <sup>NS</sup>	Methane (ml) per 100 mg of truly digested substrate
1	0	3.01 ± 0.06 <sup>c</sup>	46.28 ± 0.89	3.25 ± 0.08 <sup>c</sup>
2	0.13	2.83 ± 0.07 <sup>b</sup>	46.11 ± 1.78	3.08 ± 0.16 <sup>b</sup>
3	0.26	2.71 ± 0.08 <sup>b</sup>	46.23 ± 0.84	2.93 ± 0.04 <sup>b</sup>
4	0.39	2.53 ± 0.18 <sup>a</sup>	46.22 ± 2.36	2.74 ± 0.06 <sup>a</sup>
5	0.52	2.51 ± 0.05 <sup>a</sup>	46.20 ± 1.84	2.73 ± 0.07 <sup>a</sup>

<sup>#</sup> Mean of six observations; <sup>NS</sup> Not significant; Means bearing different superscripts in the same column differ significantly ( $p < 0.01$ )

Jalc et al. (2002) also reported that the addition of malic acid decreased the methane emission by 1.9 % than control by *in vitro*. Similarly Li et al. (2009) also observed that the supplementation of 24 mM malate and 24 mM fumarate with linolenic acid was decreased ( $P < 0.0020$ ) the methane ( $\mu\text{mol}$ ) emission by 71.53 % and 84.54 % than control. As methanogenesis is principally a sink for metabolic hydrogen in the rumen and diverting hydrogen away from methane formation would decrease the methane production. Malic acid was a potential precursor for propionate and may act as electron sink competing with methanogen with available hydrogen ultimately reduce in the methane production. (Lopez et al., 1999).

### Effect of supplemental malic acid with and without supplementation on reduction of methane emission in paddy straw based complete diet for indigenous dairy cattle

The effect of malic acid with and without supplementation on DMI, methane emission gram per animal per day, per kg DMI, per 100g DMI, per kg digestible dry matter intake (DDMI), per 100 g DDMI and per kg milk production are presented in Table 3 and the rumen fermentation characteristics are presented in Table 4. There was no significant difference in DMI and DDMI among treatment and control groups. The methane emission per animal per day was significantly ( $p < 0.05$ ) decreased by 3.26 % in malic acid supplemented group than control. Similarly the methane emission per kg DMI or methane emission per 100 g DMI was also significantly decreased by 3.11 % in supplemental malic acid added group than without added group. Foley et al. (2009) also reported that supplementation of malic acid at 3.75 % and 7.5 %



decreased ( $p < 0.001$ ) the methane emission by 6.30 % and 15.77 % respectively than control in beef cattle.

**Table 3.** Effect of malic acid with and without supplementation on DMI, DDMI and methane emission gram per animal per day, per kg DMI, per 100g DMI, per kg DDMI, per 100 g DDMI and per kg milk production (Mean<sup>#</sup> ± SE)

S.No.	Parameters	Without malic acid (Control)	With Malic acid (Treatment)
1	BW (kg) <sup>NS</sup>	263.43 ± 4.83	260.81 ± 7.02
2	DMI (kg/day) <sup>NS</sup>	6.69 ± 0.01	6.68 ± 0.01
3	DMI (g/day) <sup>NS</sup>	6687.76 ± 1.20	6677.04 ± 8.17
4	DMI (g/kg BW) <sup>NS</sup>	25.41 ± 0.47	25.66 ± 0.68
5	DDMI (g/day) <sup>NS</sup>	3771.87 ± 27.97	3739.12 ± 16.36
6	CH <sub>4</sub> g/animal/day	94.77 ± 0.77 <sup>a</sup>	91.68 ± 0.37 <sup>b</sup>
7	CH <sub>4</sub> g/kg DMI	14.17 ± 0.12 <sup>a</sup>	13.73 ± 0.08 <sup>b</sup>
8	CH <sub>4</sub> g/100g DMI	1.42 ± 0.02 <sup>a</sup>	1.37 ± 0.01 <sup>b</sup>
9	CH <sub>4</sub> g/kg DDMI <sup>NS</sup>	25.13 ± 0.39	24.52 ± 0.15
10	CH <sub>4</sub> g/100g DDMI <sup>NS</sup>	2.51 ± 0.04	2.45 ± 0.02
11	Milk production per day <sup>NS</sup>	4.68 ± 0.03	4.79 ± 0.06
12	CH <sub>4</sub> g/kg milk production	20.24 ± 0.24 <sup>a</sup>	19.14 ± 0.29 <sup>b</sup>

<sup>#</sup> Mean of four observations; <sup>NS</sup> Not significant; Means bearing different superscripts in the same row differ significantly ( $p < 0.05$ )

The methane per kg or per 100g DDMI was numerically decreased by 2.43% in malic acid supplemented group than un-supplemented group, but it was not significant. Foley *et al.* (2009) also observed a decrease ( $P < 0.001$ ) in methane (gram) per kg total dry matter intake than control in beef cattle. The methane emission per kg milk production was significantly ( $p < 0.05$ ) reduced by 5.43% in malic acid added group than control.

The total volatile fatty acid was significantly ( $p < 0.05$ ) increased by 2.69 % in malic acid supplemented group than without supplemented group. Li *et al.* (2009) also reported that the supplementation of 24 mM malate with linolenic acid increased the volatile fatty acids in fermented solution as the incubation time increased at 6 hrs and 12 hrs incubation when compared to control. The highly significant ( $p < 0.05$ ) increase in propionic acid by 11.71 % in malic acid added group when compared to control. The acetic acid was significantly ( $p < 0.05$ ) decreased by 4.04 % in malic acid added group than without added group. The A/P ratio was also significantly ( $p < 0.05$ ) decreased by 14.86 % in malic acid added group when compared to control. Foley *et al.* (2009) also reported that supplementation of DL malic acid at 0, 2.5, 5.0 and 7.5 % increased ( $P < 0.001$ ) the propionic acid and decreased ( $P < 0.001$ ) the acetic acid and acetate to propionate ratio than control in beef cattle. The supplementation of sodium salts of malate and fumarate at 0, 8, 16 and 24 mM in 70 % concentrate: 30 % ground alfalfa roughage based diet increased the propionic acid and decreased the acetic acid & acetate to propionate ratio significantly ( $P < 0.0001$ ) in both malate and fumarate added groups than control (Li *et al.*, 2009).

There was no significant difference in bacterial and protozoal population among the treatment groups. On contrary to the present findings the earlier report was dealt with increased bacterial count (Khampa *et al.*, 2009) and decreased protozoal count (Foley *et al.*, 2009) while supplementation of malic acid.

It was concluded that the supplementation of malic acid at 0.39 % of paddy straw based complete diet was significantly ( $p < 0.05$ ) reduced the methane emission per animal per day by



3.26 % and methane per kg milk production by 5.43 % than control in indigenous dairy cattle. The decrease in methane emission in the present study was due to the supplementation of malic acid which was more effective alternate hydrogen sink competing with methanogenesis (Newbold *et al.*, 2002). Also the malic acid was the direct metabolic precursor of propionic acid and it has the potential to decrease the methane emission by directing hydrogen into succinate rather than in to methane production (Lopez *et al.*, 1999). Further the energy saved through decrease in methane emission was used for increasing milk production and it may also decreases the global warming.

**Table 4.** Effect of malic acid with and without supplementation on rumen fermentation characteristics (Mean  $\pm$  SE)

S.No.	Parameters	Without malic acid (Control)	With malic acid (Treatment)
1	TVFA (mg/dl)*	60.75 $\pm$ 0.28 <sup>a</sup>	62.43 $\pm$ 0.48 <sup>b</sup>
2	Acetic acid (%)*	68.58 $\pm$ 0.37 <sup>a</sup>	65.81 $\pm$ 0.97 <sup>b</sup>
3	Propionic acid (%)**	23.15 $\pm$ 0.24 <sup>a</sup>	26.22 $\pm$ 0.63 <sup>b</sup>
4	Butyric acid (%) <sup>NS</sup>	8.27 $\pm$ 0.41	7.97 $\pm$ 0.41
5	A/P ratio**	2.96 $\pm$ 0.04 <sup>a</sup>	2.52 $\pm$ 0.10 <sup>b</sup>
6	Bacterial population ( $\times 10^8$ ) <sup>NS</sup>	4.59 $\pm$ 0.03	4.54 $\pm$ 0.10
7	Protozoal Population ( $\times 10^5$ ) <sup>NS</sup>	3.58 $\pm$ 0.05	3.69 $\pm$ 0.02

# Mean of four observations; <sup>NS</sup> Not significant; Means bearing different superscripts in the same row differ significantly \*( $p < 0.05$ ), \*\*( $p < 0.01$ )

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