3rd AINI INTERNATIONAL SEMINAR
In conjunction to 50th Anniversary Faculty of Animal Science
Andalas University
THE ROLE OF NUTRITION AND FEED IN SUPPORTING SELF SUFFICIENT
IN ANIMAL PRODUCTS, FOOD SAFETY AND HUMAN WELFARE
Padang, 24 – 25 September 2013

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

PROCEEDING
3rd International Seminar and 9th Biennial Meeting of AINI
"The Role of Nutrition and Feed in Supporting Self Sufficiency in Animal Products, Food Safety and Human Welfare"

in conjunction with
the 50th Anniversary of the Faculty of Animal Science
University of Andalas, Padang West Sumatera

Grand Inna Muara Hotel, Padang 24-25 September 2013

THE COMMITTEE

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Khairisma Fedra, S.Pt
Irwanto, A.Md
“The Role of Nutrition and Feed in Supporting Self Sufficient in Animal Products, Food Safety and Human Welfare”

Indry Zelita Suci, S.Kom

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Dr. Ir. Ade Djulardi, MS
Dhr. Yuherman, M.S, Ph.D
Ir. H. Fuad madarisa, M.Sc
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Fauziah
FOREWORD

President AINI

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

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

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

Distinguish guests, participants, ladies and gentleman,

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

I would like also to take this opportunity to share the idea with all you, that AINI as the organization of scientist, to have a international scientific journal is a must. The jurnal deals with all aspects of nutrition and feed issues in tropical conditions. The Management board of AINI has taken the decision for revitalizing the AINI Journal to become the Journal of Nutrition and Feed Science, internationally recognized, by involving the International committee of lecture as the reviewers. To this end, we need
fully your support and encourage the scientists especially the young scientists to publish their work in English. The accomplishment of this task will bring the association be more respected in national and international level.

Distinguish guests, participants, ladies and gentleman,

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

Distinguish guests, participants, ladies and gentleman,

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

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

Wassalamu ‘alaikum Wr. Wb. 

Padang, September 24th, 2013

President AINI

Prof. Ali Agus
Assalamu’alaikum Wr. Wb.,
The Honourable Rector of The University of Andalas,
The Dean Faculty of Animal Science, University of Andalas
Distinguish Guests, Seminar Participants, Ladies And Gentlemen,

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

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

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

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

Good evening.

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

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

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

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

Ladies and Gentlemen,

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

Feed Industries contribute to the fulfillment of animal development in Indonesia because Feed is one most important factors to develop animal production and animal population and with cheap in price of feed will give high benefit could be reached by the
farmer. The development of feed and animal industries needs science and technology, and through this seminar, it is hoped that the scientists from all over the world could contribute the information and technology in disciplines in feed sciences.

**Ladies and Gentlemen,**

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

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

**Wassalammualaikum wr.wb.**

Dr. Werry Darta Taifur, SE, MA  
Rector of Andalas University
WELCOME SPEECH
Governor of West Sumatera

Assalamualaikum. w.w
Your Excellency, Chairman of House of Representative of West Sumatera Province. Ir. Yulteknil, MM
The Honorable, Dr. Mursyid Ma’sum, M.Agr, Director of Animal Feed, Directorat General of Livestock and Animal Health, Ministry of Agriculture.
The honorable, Prof. Dr. Ali Agus DAA, DEA, The Chairman of Indonesian Association of Nutritionist and Feed Scientist (AINI) from Gajah Mada University.
Honorable guest, the Rector of the University of Andalas.
The Honorable guests all keynote speaker.
Honorable guest, the Dean of The Faculty of Animal Science.
Seminar Committees, Participants, Ladies and Gentlemen,

Seminar Committees, Participants, Ladies and Gentlemen,

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

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

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

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

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

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

Ladies and Gentlemen,

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

Bismillahirrahmaanirrahim, I officially open this seminar.  
Wabillahitaufik walhidayah, Wassalamualaikum warahmatullahi wabarakaatuh.

Governor of West Sumatera Province

Prof. Dr. Irwan Prayitno, PSi, MSc
### SEMINAR PROGRAM

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<td>Welcome address by President of AINI (Prof. Dr. Ali Agus)</td>
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<td>Welcome address by Rector of Andalas University</td>
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DAY-1 (WEDNESDAY, SEPTEMBER 25, 2013)

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<td>08:30 – 9:00</td>
<td>Dr. Ir. Mursyid Ma’sum, M.Agr (Director of Animal Feed, Directorate General of Livestock and Animal Health Services, Ministry of Agriculture - Indonesia)Title: “Policy And National Program For Feed Development”</td>
<td>Moderator: Dr. Maria Endo Mahata</td>
<td>OMBILIN HALL</td>
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<td>9:00 – 9:30</td>
<td>Prof Dr. Tamo Fukamizo (Kinki University Japan).Title: “The Mode of Action of Chitinolytic Enzymes: Production of Bioactive Oligosaccharides as Animal Nutrients”</td>
<td>Dr. Maria Endo Mahata</td>
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<td>9:30 – 10:00</td>
<td>Dr. Yuwares Ruangpanit (Kasetsart University, Thailand)Title: “Improving Egg Nutritional Value By Dietary Marine Sources – A Current Update”</td>
<td>Dr. Maria Endo Mahata</td>
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<td>10:00 – 10:30</td>
<td>Prof. Dr. Yose Rizal (Unand, Indonesia)Title: “The Utilization of Juice Waste Mixtures in Poultry Diets: A Review”</td>
<td>Dr. Maria Endo Mahata</td>
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<td>10:30 – 11:00</td>
<td>Prof Dr Abdul Razak Alimon, Universiti Putra Malaysia, MalaysiaTitle: “Utilization Of Herbs As Growth Promoters In Animal Feed”</td>
<td>Dr. Rusmana Ningrat</td>
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<td>11:00 – 11:30</td>
<td>Dr. Robert L. Payne, Ph D, PAS (Evonik – US)Title: “The Role Of Amino Acids In Sustainable Poultry Production”</td>
<td>Dr. Rusmana Ningrat</td>
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<td>Dr. Rusmana Ningrat</td>
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<td>12:00 – 12:30</td>
<td>Prof. Dr. Ali Agus, DAA, DEA (University of Gajah Mada, Indonesia).Title: “Food And Feed Safety Issues In Indonesia: Reducing Mycotoxins Toxicity And Its Carry Over From Feed Into Animal Products”</td>
<td>Dr. Rusmana Ningrat</td>
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<td>12:30 – 13:30</td>
<td><strong>LUNCH BREAK</strong></td>
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### SESSION 1. RUMINANT NUTRITION (ROOM OMBILIN 2-3)

**CHAIR: DR. IRSAN RYANTO H, UNAND, INDONESIA**

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<td>14:00 – 14:10</td>
<td>Meat Physical Properties of Local Lamb Fed Urea-Impregnated Zeolite Ration, Kardaya, D., E. Dihansih, D. Wahyuni, Djuanda University, Bogor, Indonesia</td>
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<td>In vitro study of sardinella lemu oil based calcium-soap supplementation effects on the sheep’s rumen digestibility, Asep Sudarman</td>
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<td>14:20 – 14:30</td>
<td>Milk Composition Of Etawah Crossbred Goat Fed Forage And Leaves Pellet, Suryanindyah, Y. Y., N. Umami, Nurliyani, Y. S. Muthoharotin, Y. P. Oktaviani, UGM, Indonesia</td>
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<td>Rumen fermentability and digestibility of lingzhi (ganoderma lucidum) and organic chromium supplementation in high and low forage rations, Dwierra Evynnie, Toto Toharmat, Sumiati and Dian Astriana</td>
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<td>14:40 – 14:50</td>
<td>Ruminal Degradation Characteristics Of Maize (Zea Mays) Leaves, Rusdi, Mustaring, M. Salman, Animal Husbandry Dept, Tadulako University, Palu Indonesia</td>
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<td>The Effect Of Concentrate Offered In Ratio Based On Rice Straw To The Performance Of Bali Cattle, Trisnadewi, S., T. G. O. Susila, I W. Wijana, Faculty of Animal Husbandry, Udayana University, Bali</td>
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<td>15:00 – 15:10</td>
<td>Enhancing Performance of Sheep by Feeding Corn Leaf Biscuit. Yuli Retnani, Sobri, D. K. Putra, and T. Toharmat, IPB Bogor, Indonesia</td>
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<td>15:20 – 15:30</td>
<td>Supplementation of Solid Ex-Decanter Multi-Nutrient Block on Simbrah Breed Weaned Calves Performances as Integrated Farming System with Palm Fruit Agroindustry, Fariani, A., A. Abrar, G. Muslim, E. D. Y. Astuti and L. Warly, Sriwijaya University, Palembang, Indonesia</td>
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<td>16:50 – 17:00</td>
<td>CLOSING CEREMONY AT OMBILIN 2-3</td>
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FAREWELL DINNER AT MAJOR HOUSE OF PADANG CITY
SESSION 2. NON-RUMINANT NUTRITION (ROOM ANAI 1-2 )

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

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<td>Productivity Of Local Chicken In Growth Periods And Carcass Characteristics By Inclusion Of Moringa Oleifera Leaves Meals In The Diets</td>
<td>Hafsah, S. Sarjuni, T. Riske, J. Kumbok</td>
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<td>The Effect Of Palm Kernel Meal Contain Probiotic To Reduce The Fecal Ammonia Emmission In The Laying House, Yusrizal, F. Manin, Yatno and Noverdiman, University of Jambi, Indonesia</td>
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<td>3</td>
<td>13:50 – 14:00</td>
<td>Contribution Of Lysin And Calcium Of Azolla Microphylla On Egg Shell Calcium Deposition In Arab Hen, Wulandari, E., C., R.H. Prawitasari, N Suthama, W. Murningsih, V.D Yunianto, I. Estiningdriati and H.I. Wahyuni, Agriculture Diponegoro University, Semarang, Indonesia</td>
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<td>4</td>
<td>14:00 – 14:10</td>
<td>Japanese Quail Eggs Qualtyfed Fermented Jatropha Curcas Meal, Sumiarti, R. Mutia, and R. Khalim, IPB Bogor, Indonesia</td>
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<td>5</td>
<td>14:10 – 14:20</td>
<td>Evaluation in the presence of black tea waste extract on different level of energy-protein rations in the performance and carcass parameters of broiler Dilla mareistia fassah¹ *, supadmo² and rusman³</td>
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## SESSION 3. FEED SCIENCE AND TECHNOLOGY, PASTURE AND RANGE LAND, NUTRITION AND REPRODUCTION, SOCIO-ECONOMIS OF FEED AND FOOD (ROOM OMBILIN 1)

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

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

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| P-03 | The Effect Bioprocess of Banana Peels With The Different of incubation length and the source of Local microorganisms (MOL) on Crude Protein, Crude fiber and lignin content. | Tri Astuti and S. Amir |
| P-04 | The content of phytochemical and antibacterial activity of cinnamon leaf (Cinnamomum burmanii) and Noni Fruit and Leaf (Morinda citrifolia L) mixture extract to Replace Antibiotic. | Yuniza, A and Yuherman |
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| P-08 | Effect of totaltimemarketingonmicroorganismsincattle Meat Marketed In Padang Great Market, West Sumatera. | Yuherman, Eva Umar, and John Farlis |
| P-10 | Performances And Hematological Profile Of Broiler Under Heat Stress Fed Diet Containing Carica Papaya L. Leaf Meal And Curcoma Domestica Val. | Dwi Margi Suci, Dewi Api, Tri Astuti, F.Kumala, Dewi and D. Kuncoro Sakti |
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| P-15 | Effect Of Fumaric Acids In Silage Processing From Shrimp Head Waste As Animal Feed. | Mirzah, Montespirit and Suslina A Latif |
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**INVITED SPEAKERS**

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

**Dr. Mursyid Ma’sum, M.Agr**
Director of Animal Feed, Directorate General of Livestock and Animal, Health Services.

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

1. Crystal structure analysis of the chitinase-oligosaccharide complex
2. NMR analysis of the interaction of chitin-binding proteins
3. Calorimetric analysis of the interaction of chitin-binding proteins
4. Conversion of chitinase into a glycosynthase by protein engineering technique
5. Biomass conversion from fungal cell wall by enzymatic digestion

Recently, in collaboration with Dr. Maria Mahata, University of Andalas, he successfully produced partially N-acetylated chitooligosaccharides, which might be used as animal food ingredients, directly from fungal cell wall. In today’s his lecture, mechanism of oligosaccharide production from chitin and chitosan biomass will be presented, and the utilization of the products will be discussed.
The Role of Nutrition and Feed in Supporting Self Sufficient in Animal Products, Food Safety and Human Welfare

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

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

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

Prof. Dr. Abdul Razak Alimon (Malaysia)
obtained his Bachelor of Science and Masters of Science in Agriculture from the Faculty of Rural Science, University of New England, NSW, Australia in 1971 and 1980, respectively, and completed his Ph.D degree in 1989 at University of Reading, United Kingdom. He is currently a Professor of Animal Nutrition at the Department of Animal Science, Faculty of Agriculture, Universiti Putra Malaysia. His current interest is in the utilization of herbs as growth promotants in poultry and also agricultural byproducts as animal feed.
Prof. Dr. Yose Rizal (Indonesia)
graduated from the Faculty of Animal Science, University of Andalas, Padang, West Sumatra, Indonesia with a Sarjana degree in 1981, and completed his Master and Ph.D degrees in Animal Nutrition, at the Department of Animal Science, University of Illinois, USA in 1987 and 1989. He is currently a Professor at the Faculty of Animal Science, University of Andalas, Padang, West Sumatra, Indonesia. Now, he is also responsible for the Quality Assurance at the University of Andalas. His area of interest is in the utilization of agriculture wastes/by-products for poultry feeds.

Prof. Dr. Suhubdy Yasin (Indonesia)
Is highly distinguish professor in ruminant nutrition science, awarded as Ph.D. From the University of Queensland, Australia 2002. He was a fullbright visiting professor at Utah State University, USA 2008/2009. He is Director a Research Center Of Tropical Rangelands and Grazing Animal Production Systems, Indonesia.
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15. QUALITY OF RENNET FROM RABBIT STOMACH DURING COLD AND FROZEN STORAGE

Nurliyani1, Indratiningsih2, Mufti Tri Matra3

Corresponding author e-mail: nurliyani@yahoo.com; Telp/fax 62274-513363/62274-521578; Jl. Fauna 3 Kampus UGM, Bulaksumur, Yogyakarta 55281, Indonesia

1,2,3Department of Animal Product Technology, Faculty of Animal Science Universitas Gadjah Mada, Yogyakarta, Indonesia

Abstract

Problems in cheese production in Indonesia is the use of milk coagulating enzymes (rennet) for the curd formation, which is still imported. Stomach rabbit is waste of rabbit slaughtering can be used as an alternative in the cheese production. The purpose of this study was to determine the quality of rabbits stomach rennet during cold and frozen storage temperatures. The rennet quality are include the milk coagulating ability, curd yield percentage, pH and proteolytic activity of rennet. Rennet quality data were analyzed statistically using a two-way ANOVA, with three times replication. The results showed that the average of milk coagulation time, curd yield and pH were not significantly different between the rennet stored at cold and frozen temperatures. However, during storage 0; 15 and 30 days occurred significant difference (P<0.05) on milk coagulation time, curd yield and rennet pH, a started 0; 15 and 30 days: the average coagulation time was 3.35; 3.91 and 4.63 minutes, the average yield of curd (rendemen) was 11.07; 8.93 and 7.47%, and the average of rennet pH was 5.60; 5.88 and 5.94, respectively. The proteolytic activity of rabbit stomach rennet stored at frozen temperatures was higher (P<0.05) than the rennet stored at cold temperatures. There was a significant change in proteolytic activity of rennet during storage. At storage 0; 15 and 30 days, the average of proteolytic activity of rennet was 25.87; 24.79, and 24.43 mg / ml / min, respectively. In conclusion, the rabbit stomach rennet stored at cold and frozen temperatures has the same quality in its ability to milk coagulate, curd yield and pH value. However, there were slight changes in the quality of rabbit stomach rennet during 30 days of storage at cold and frozen temperatures, which is an increase in the milk coagulating time, decreasing in curd yield, increasing the pH value and decreasing of proteolytic activity of rennet.

Key words: Rennet Quality, Rabbit Stomach, Storage.

INTRODUCTION

The digestibility of organic matter of feeds is lower in rabbits than in other herbivorous animals, primarily due to lower digestion of crude fibre. The degradation of organic matter in the digestive tract of rabbits involves a number of hydrolytic reactions which are catalysed by enzymes of endogenous and/or microbial origin. It is generally assumed that hydrolytic activity and digestive volume correlate positively with the digestion efficiency. Part of the digestive tract of rabbit i.e starting from stomach, small and large intestine and also caecum known to contain proteinase activity, and in the stomach having the highest proteinase activity (Marounek and Vovk, 1995).

The availability of milk clotting enzyme in Indonesia is still a problem for cheese production, so that it still imports. Various sources of plant-protease can be used to produce cheese but usually tasted bitter. Rabbit stomach is an animal
rennet, which is a waste of rabbit slaughtering can be used as an alternative to milk clotting enzyme. Enzymes are proteins that are easily damaged by a variety of factors, such as pH and temperature. Freeze-thawing or freeze-drying has been widely used for the preservation of various kinds of biological materials, in particular, for maintaining their biological activities over a long period of time. It is known, however, that to a certain extent the biological activities suffer damage under certain conditions of freeze-thawing or freeze-drying (Hanafusa, 1967).

Therefore, for sustainability of availability of milk clotting enzyme from rabbit stomach, proper handling and storage required to maintain their enzyme activities. The purpose of this study was to determine the quality of rennet from rabbit stomach during cold and frozen storage temperatures.

MATERIALS AND METHODS

Materials

Materials in this study includes rabbit stomach, acetic acid solution 1.5%, NaCl 5%, NaOH 1 N for rennet extraction. For proteolytic assay using rennet extract, hidrolysate casein, aquadest, tyrosin, HCl, phosphate buffer pH 7 and Trichloroacetic acid (TCA) 5%.

Methods

Rennet extraction of rabbit stomach

Rennet extraction was performed according to Utama (1985) method. After removing the internal contents, rabbit stomach was washed with tape water internally while their veins and fat contents were removed externally, cut in small size and weighed. Solution for extraction was prepared by using mixture of acetic acid solution 1.5% and NaCl 5%. The pieces of stomach was mixed with extractor solution, covered and stirred overnight at room temperature. After stirring, the mixed was filtered with cheese cloth, and measured the pH value using digital pH-meter. NaOH 1N was added, when pH of rennet extract less than 5.6 until reaches pH 5.6. Rennet was stored in refrigerator and freezer for 30 days. At the day 0; 15 and 30 the rennet quality was assessed which includes milk clotting and rendemen evaluation, pH, and proteolytic activity.

Evaluation of milk clotting activity and rendemen

Milk clotting and rendemen evaluation were performed according to Scott (1981) with slight modification. Tubes filled pasteurized milk as much as 5 ml, added 10% rennet extract and incubated in waterbath at 40°C. As the flocculation started the time was noted. Coagulated milk (curd) filtered and weighed.

Measuring pH of rennet

Value of pH was measured by digital pH-meter (HANNA –S 487092) which has been calibrated with a buffer standard.

Proteolytic activity assay

The proteolytic assay of rennet extract (enzyme) from rabbit stomach was assayed by using standard curve of tyrosine solution according to Whitaker (1972) with slight modification. Tyrosine was diluted in small volume of HCl and added with phosphate buffer pH 7.0 until 100 ml. Casein powder (Hammersten) 0.2 g diluted in small volume of NaOH 0.1 N and added phosphate buffer pH7.0 until 100 ml as a substrate (S) for enzyme. Mixture of 3 ml substrat and 1ml enzyme (E) incubate in shaker waterbath at 40 oC for 30 minutes. Enzyme activity was stopped with 5 ml TCA 5%. This mixture of substrat, enzyme and TCA was allowed to stand at room temperature for 60 minutes, and then centrifuged.
The proteolytic enzyme activity =
(volume S + volume E+ Vol TCA)x (% tyrosine/100) x1/t x 10^3 µg/ml/minute

Note: (S: Substrat, E: Enzyme, t: incubation time)

Statistical analysis
The results of this study was analyzed statistically by two way ANOVA, and difference between the means was analyzed by DMRT (Duncan’s Multiple Ranges Test).

RESULTS AND DISCUSSION

Milk coagulation (clotting) time
Coagulation time in this study showed that how much time it takes rennet to coagulate milk. There was no significantly different in coagulation time of rennet after storage at cold and frozen temperature, whereas the increased storage time it will increase the milk coagulation time (P<0.05) (Table1). The time required of rennet at cold or frozen stored for 30 day to reach the flocculation of cow milk was longer than that of rennet stored 0 or 15 day.

Table 1. The average of coagulation time (minute) of rennet from rabbit stomach during cold and frozen storage

<table>
<thead>
<tr>
<th>Storage</th>
<th>Storage time (day)</th>
<th>Average ns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold</td>
<td>0</td>
<td>3.35</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>4.09</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>4.51</td>
</tr>
<tr>
<td>Frozen</td>
<td>3.35</td>
<td>3.91</td>
</tr>
<tr>
<td></td>
<td>3.73</td>
<td>4.76</td>
</tr>
<tr>
<td></td>
<td>4.63</td>
<td>3.95</td>
</tr>
<tr>
<td>Average</td>
<td>3.35a</td>
<td>3.91b</td>
</tr>
<tr>
<td></td>
<td>4.63c</td>
<td>3.97</td>
</tr>
</tbody>
</table>

ns: not significant
a,b,c: different letter in the same row indicated significantly different (P<0.05)

According to Ahmed et al. (2013), milk clotting activity of gastric enzyme Camel was influenced by the pH of the milk at the renneting stage, and the flocculation time increase with the age of the Camel. The pH of the milk for rapid flocculation is very important during cheese making since the acidification by the lactic acid bacteria helps the enzyme activity in which the enzyme is a protease having an optimum activity around pH 5.5. This contributes to the destabilization of the casein micelles.

Curd yield (rendemen)
Curd yield (rendemen) obtained by coagulating milk with fresh rennet (0 day) was highest (11.07%) followed by rennet that storage 5 days (8.93%) and 30 days (7.47%) (Table 2). Curd yield (rendemen) obtained by coagulating milk with fresh rennet (0 day) was highest (11.07%) followed by rennet that storage 5 days (8.93%) and 30 days (7.47%) (Table 2). Curd yield in this study was lower than curd yield in the previous study obtained by coagulating buffalo milk with laboratory made rennet derived from buffalo calves abomasum (fresh and stored rennet) and commercial rennet. The mean values (32.2%±0.24%) of curd obtained from fresh rennet coagulated milk was highest followed by commercial rennet coagulated milk (29.87%±0.11%) and stored rennet (3 month) coagulated milk.

(Eppendorf centrifug 5804R) merk)at 3000 rpm for 15 minutes at 4oC. Supernatant was measured on Spectrophotometer 280 nm (UV-1601PC, UV-VISIBLE Spectrophotometer, SHIMADZU). The control of enzyme activity was prepared with the blank (without enzyme).
The Role of Nutrition and Feed in Supporting Self Sufficient in Animal Products, Food Safety and Human Welfare

(28.24 %±0.13%) (Ahmed et al., 2013). The difference of curd yield maybe caused by different sources of milk and types of rennet. In this study, using rennet from rabbits stomach, whereas in the previous study by Ahmed et al. (2013) using buffalo calves abomasum.

Table 2. The average of curd rendemen(%) produced by rennet coagulation

<table>
<thead>
<tr>
<th>Storage</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>Average ns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frozen</td>
<td>11.07</td>
<td>8.73</td>
<td>7.4</td>
<td>9.07</td>
</tr>
<tr>
<td>Average</td>
<td>11.07a</td>
<td>8.93b</td>
<td>7.47c</td>
<td>9.16</td>
</tr>
</tbody>
</table>

ns : not significant
a,b,c: different letter in the same row indicated significantly different (P<0.05)

According to Mona et al. (2011), cheese yield is affected by many factors including milk composition, amount and genetic variants of casein, milk quality, somatic cell count (SCC) in milk, milk pasteurization, coagulant type, vat design, curd firmness at cutting, and manufacturing parameters (Mona et al., 2011). As compared to cow milk, buffalo milk is richer in fat, lactose, protein, total solids, vitamins and minerals, such as calcium, magnesium and inorganic phosphate (Murtaza et al., 2008). The high protein content of buffalo milk and total solids helped in developing high viscosity curd (Ghadge et al., 2008).

It is a very important parameter: the higher the recovered percentage of solids, the greater the amount of cheese obtained and therefore gain in economic terms. In the cheesemaking process, therefore, it is very important to obtain the maximum possible recovery of substances from milk. Equally important is the calculation of the effects that each milk component, and in particular, fat and casein, can have on cheese yield, in order to adopt a milk quality payment system that could remunerate each parameter for its actual value (Paolo et al., 2008).

Cheese yield is affected by many factors including milk composition, amount and genetic variants of casein, milk quality, somatic cell count (SCC) in milk, milk pasteurization, coagulant type, vat design, curd firmness at cutting, and manufacturing parameters (Mona et al., 2011).

The pH value of rennet

There was no significantly different between the pH of rennet derived from rabbit stomach after cold and frozen storage (Table 3). However, the pH of fresh rennet (0 day) was lower (5.60) (P<0.05) than rennet stored at 15 days (5.88) or 30 days (5.94).

Table 3. The average of pH value of rennet during cold and frozen storage

<table>
<thead>
<tr>
<th>Storage</th>
<th>0</th>
<th>15</th>
<th>30</th>
<th>Average ns</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cold</td>
<td>5.6</td>
<td>5.92</td>
<td>5.97</td>
<td>5.83</td>
</tr>
<tr>
<td>Frozen</td>
<td>5.6</td>
<td>5.84</td>
<td>5.91</td>
<td>5.78</td>
</tr>
<tr>
<td>Average</td>
<td>5.6a</td>
<td>5.88b</td>
<td>5.94b</td>
<td>5.81</td>
</tr>
</tbody>
</table>

ns : not significant
a,b : different letter in the same row indicated significantly different (P<0.05)
The pH of rennet in this study similar to the previous study (Ahmed et al., 2013), showed that the mean pH value of stored rennet (5.75±0.02) was noted greater than that of fresh rennet (5.47±0.02). The clotting activity of rennet decreases as its pH turns towards alkalinity (Ahmed et al., 2013).

Milk clotting activity was influenced by the pH of the milk at the renneting stage. All enzyme preparations exhibited almost a linear curve with an increased pH from 5.8 to 6.6. The optimum pH for clotting camel milk for gastric enzyme Camel was at 5.8, and the flocculation time increased with the age of the Camels. The pH of the milk for rapid flocculation is very important during cheese making since the acidification by the lactic acid bacteria helps the enzyme activity in which the enzyme is a protease having an optimum activity around pH 5.5. This contributes to the destabilization of the casein micelles. In regards to bovine milk, the optimum pH for gastric enzyme camel 6.0 (Saliha et al., 2011).

**Proteolytic activity**

As shown in Table 4, the average proteolytic activity of rennet derived from rabbit stomach after frozen stored was higher (P<0.05) than rennet after cold storage. The longer in storage time showed the lower activity of rennet (P<0.05). The other study showed that total of gastric proteinase activity of 3-month-old rabbits was 4322 (expressed as mg azocase in decomposed/h), whereas total proteinase activity of 4-week-old was 1550. The gastric proteolytic activity of represented 46.3% of the total proteolytic activity of the digestive tract (Marounek and Vovk, 1995).

Table 4. The average of proteolytic activity (µg/ml/minute) of rennet during cold and frozen storage

<table>
<thead>
<tr>
<th>Storage</th>
<th>Storage time (day)</th>
<th>Average</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>15</td>
</tr>
<tr>
<td>Cold</td>
<td>25.87</td>
<td>24.45</td>
</tr>
<tr>
<td>Frozen</td>
<td>25.87</td>
<td>25.15</td>
</tr>
<tr>
<td>Average</td>
<td>25.87&lt;sup&gt;a&lt;/sup&gt;</td>
<td>24.79&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

a, b, c : different letter in the same row indicated significantly different (P<0.05)
p, q : different letter in the same column indicated significantly different (P<0.05)

According to the previous study, shelf life of rennet derived from buffalo calves abomasum that soaked in 12% NaCl solution and added 1% sodium benzoate solution reflected that all samples were active up to three months storage period, while after six months evaluation 15% rennet samples were found inactive (Ahmed et al., 2013).

Proteins can undergo degradation by many mechanisms. However, the primary mechanism of concern with frozen storage is aggregation. The freezing process, however, subjects proteins to other stresses as a consequence of the removal of water as ice. The resulting cryoconcentration and desiccation of protein can be classified as osmotic stresses. Protein structure changes that occur as a consequence of such stresses have a greater probability of being irreversible, and are classified as freeze denaturation (Singh et al., 2009). Upon the fast freezing (e.g., when the freezing rate >20°C/min), small ice crystals and a relatively large surface area of ice–liquid interface are formed, which increases the exposure of protein molecules to the ice–liquid interface and hence increases the damage to the proteins. During thawing, additional damage to proteins is
caused by recrystallization process. Recrystallization exerts additional interfacial tension or shear on the entrapped proteins and hence causes additional damage to the latter (Cao et al., 2003). When the freezing process was varied so as to achieve different freezing rates, the slowest freezing rate caused the highest enzyme leakage (Nilsson and Ekstrand, 1993).

This low-temperature effect (“chill”) is distinct from the effect on protein structure that comes from the actual freezing (e.g., cryoconcentration, phase changes, and ice surface denaturation). A more precise thermodynamic explanation for cold denaturation comes from considering the free energy of protein unfolding. Cold-induced unfolding (cold denaturation) is a physical consequence of the temperature sensitivity of noncovalent electrostatic and hydrophobic interactions, which become weaker at lower temperatures. However, chill-induced unfolding probably makes a molecule more susceptible to freeze-induced stresses, leading to aggregate formation and/or loss of structure (Singh et al., 2009).

**CONCLUSION**

Milk clotting enzyme extracts derived from rabbit stomach has the same quality in cold storage and frozen for 30 days, but there was a slight decrease in the proteolytic activity of rennet are kept cool. The longer it is stored there is little loss of quality, namely an increase in rennet coagulation time, curd yield reduction, increased pH and decreased proteolytic activity.

**REFERENCES**


3rd AINI INTERNATIONAL SEMINAR
In conjunction to 50th Anniversary Faculty of Animal Science
Andalas University

THIS IS TO CERTIFY THAT

NURLIYANI

as PRESENTER

At the 3rd Aini International Seminar “The Role of Nutrition and Feed in Supporting Self Sufficient in Animal Products, Food Safety and Human Welfare”
Padang, 24 – 25 September 2013

Chairman of Committee

Proff. Dr. H. Novirman Jamaran, MSc

Dean Faculty of Animal Science

Dr. Ir. H. Jafriinur, MSP