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Bogor Agricultural University

# PROCEEDING

The 1<sup>st</sup> International Seminar on Animal Industry 2009

“Sustainable Animal Production for  
Food Security and Safety”

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Published:  
Faculty of Animal Science  
Bogor Agricultural University (IPB)  
Bogor - Indonesia

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## PREFACE

It is a great pleasure for us that the Proceeding of the 1<sup>st</sup> International Seminar on Animal Industry has been successfully completed. The proceeding consists of 76 papers, among them 8 papers from invited speakers, 37 papers from oral presentation, and 31 papers from poster presentation. Papers from the invited speakers were not further reviewed and some of them were not in full papers. The papers from participants included in this proceeding were reviewed by experts in the related field. If the reviewed papers required substantial correction, they were sent back to the authors for correction. However, due to time limitation, if the reviewed papers need only non-substantial correction, the reviewing process were considered sufficient after few corrections were done by the reviewers.

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

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

Bogor, March 2010  
On behalf of Editors,

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





## FOREWORD FROM CHAIRMAN OF ORGANIZING COMMITTEE

Assalamu'alaikum warahmatullah hiwabarakatuh and selamat pagi

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

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

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

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

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

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

Wabillahi taufiq wal hidayah  
Wassalamualaikum warahmatullahi wa barakatuh

Bogor, November 23<sup>rd</sup>, 2009  
Chairman,

Prof. Dr. Ir. Muladno, MSA.





## REMARKS FROM THE DEAN OF FACULTY OF ANIMAL SCIENCE

Assalamu'alaikum Warahmatullahi Wabarakatuh

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

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

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

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

Wabillahi ta'ufiq wal hidayah,

Wassalamualaikum warahmatullahi wa barakatuh

Bogor, November 23<sup>rd</sup>, 2009

Dean,

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





## FOREWORD FROM RECTOR OF BOGOR AGRICULTURAL UNIVERSITY

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

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

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

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

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

Bogor, November 23<sup>rd</sup>, 2009  
Bogor Agricultural University  
Vice Rector for Resources and Development

Prof. Hermanto Siregar





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## SEMINAR PROGRAM

Monday, November 23<sup>rd</sup>, 2009

Time	Ballroom 3	
	Event	Speaker
08.00-09.00	Registration	
09.00-10.00	Opening Ceremony <ul style="list-style-type: none"> <li>• Organizing Committee Report</li> <li>• Welcome Address from Rector of the Bogor Agricultural University</li> <li>• Opening and Keynote Speech by Directorate of Livestock Services</li> </ul>	Prof. Dr. Ir. Muladno, MSA. Prof. Dr. Ir. Hery Suhardiyanto, M.Sc. Dr. Tjeppey Sujana
10.00-10.15	Coffee Break and Poster Session	
10.15-10.45	Plenary 1: Breeding for Sustainable Future (AnGR)	Orlando Fernandez, DVM, FPCCP
10.45-11.15	Plenary 2: Nutritional Strategies to Enhance Efficiency and Production of Chickens Under High Environmental Temperature	Ahmad Mujahid, PhD, Ichiro Hagimori, Kazuaki Takahashi and Atsuro Matsuda
11.15-11.45	Plenary 3: Anticipating the Outbreak of Zoonotic Infectious Diseases Related to Animal Industry	Dr. Drh. Retno Dewi Bagja
11.45-12.15	Plenary 4: New Development of Animal Production in Indonesia	Prof. Dr. Ir. Toto Toharmat, M.Agr.Sc.
12.15-13.30	Lunch	
13.30-14.00	Poster Session	

Time	Room C	Room D
	Genetics, Breeding and Reproduction Moderator: Prof. Dr. Ir. Cece Sumantri, M.Agr.Sc.	Feed and Nutrition Moderator: Dr. Ir. Sumiati, M.Sc.
14.10-14.25	<b>Anneke Anggraeni:</b> Genetic Polymorphism of the Kappa-Casein Gene in Holstein-Friesian Dairy Cattle in West Java Province	<b>Retno Murwani:</b> Effect of Mungbean as Local Feed Ingredients to Substitute Soybean Meal in the Diet on the Performance of Broilers
14.25-14.40	<b>Yuni Erwanto:</b> Identification of Pig Using Polymerase Chain Reaction-Retraction Fragment Length Polymorphism for <i>Halal</i> Authentication	<b>Riyadh Abbas Al-kirshi:</b> The Chemical Composition and Nutritive Value of Mulberry Leaf Meal as a Protein Source in Poultry Diets
14.40-14.55	<b>Almira Primasari:</b> Identification of Growth Hormone Releasing Hormone Gene in Local Buffalo ( <i>Bubalus bubalis</i> ) using PCR-RFLP	<b>Lovita Adriani and Andi Mushawwir:</b> The Effect of Ration with Antibiotics ( <i>Virginiamycin</i> ) and Temulawak ( <i>Curcuma xanthorrhiza Roxb.</i> ) on Performances and Income over Feed Cost of Broiler
14.55-15.10	<b>Jakaria:</b> Identification of Growth Hormone (GH) Gene MspI and Alu Locus Polymorphism in Indonesian Cattle Breeds	<b>Atapattu, NSBM:</b> Effect of Dietary Chili Powder on Growth Performance and Serum Cholesterol Contents of Broiler Chicken
15.10-16.00	Coffee Break, Poster Session and Exhibition	

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Time	Room C	Room D
	<b>Genetics, Breeding and Reproduction</b> Moderator: Prof. Dr. Ir. Sri Supraptini Mansjoer, M.S.	<b>Feed and Nutrition</b> Moderator: Prof. Dr. Ir. Wiranda G. Piliang, M.Sc.
16.00-16.15	<b>Tatik Suteky and Dwatmadji:</b> Effects of Work on Reproductive Performance of Bali Cattle under the Oil Palm Plantation in Bengkulu	<b>Dewi Apri Astuti:</b> Physiological Status, Blood Profile and Body Composition of Sheep Fed with Ca-Saponified Lemuru Oil Coated by Herbs
16.15-16.30	<b>Idalina Haris:</b> Performance of Grade-1 Kids as a Result of Grading-up Between Local Goat and Boer Goat	<b>Despal:</b> Comparison of Indirect and Direct Determination of Microbial Growth in the Rumen Simulation Technique (Rusitec)
16.30-16.45	<b>M. Aman and Dasrul:</b> Growth Selection by Evaluation of Exterior Parameter and Nutritional Approach on Local Meat Chicken	<b>Ahmad Salihin Baba:</b> Availability of Browse Plants to Goats Fed with Napier Grass and Concentrate: I. Effects on Voluntary Feed Intake and Body Weight Gain
16.45-17.00	<b>Restu Misrianti</b> Identification of Pituitary-Specific Positive Transcription Factor 1 (Pit1) Gene Polymorphism in Indonesian Swamp Buffalo ( <i>Bubalus bubalis</i> ) and Holstein-Friesian Cows	<b>Muhammad Daud</b> Potential Oligosaccharide of Extract Rumbia Fruit ( <i>Metroxylon sago Rottb.</i> ) as Prebiotic
19.00-21.00	Dinner Party (Dinner Symposia)	

### Tuesday, November 24, 2009

Time	Ballroom 3	
	Event	Speaker
08.00-08.30	Plenary 5: Herbs and Herbals in Animal Nutrition	Prof. Abdul Razak Alimon
08.30-09.00	Plenary 6: BROILER Chicken Welfare: WHAT DO THEY WANT AND WHAT DO WE WANT?	Prof. Dr. Zulkifli Idrus
09.00-09.30	Plenary 7: The global market of organic animal products – chances and risks	Prof. Dr. Gerold Rahmann
09.30-10.00	Coffee Break and Poster Session	
10.00-10.30	Plenary 8: Future of Domestic Ducks in Rice Field	Dr. Lertrak Srikitjakarn
10.30-11.00	Plenary 9: Development of Indonesian policy in contributing sustainable production	Dr. Tjeppy D. Soedjana, M.Sc.

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Time	Room C	Room D
	Pasture Management Moderator: Prof. Dr. Soedarmadi, M.Sc.	Feed and Nutrition Moderator: Dr. Ir. Suryahadi, DEA.
11.10-11.25	<b>Marsetyo:</b> Growth, Production and Nutritive Value of <i>Brachiaria mulata</i> as Affected by Levels of Urea Fertilization	<b>Anita S. Tjakradidjaja:</b> Importance of Phosphorous Supplementation in Improving Fermentability, Microbial Protein, Synthesis and Degradability of Ammoniated Rice Straw
11.25-11.40	<b>Tarsono:</b> Early Growth of <i>Panicum sarmentosum</i> Roxb. - A Promising Grass for Livestock Integration on Coconut Plantation	<b>Elizabeth Wina:</b> Biological Activity of Tannis from <i>Acacia mangium</i> Bark Extracted by Different Solvents
11.40-11.55	<b>Luki Abdullah:</b> Productivity of <i>Brachiaria humidicola</i> as Result of Different Nutrient Source Application	<b>Mohammad Winugroho:</b> Organic Milk Production In Rural Dairy Farms In Lembang, West Java-Indonesia
11.55-12.10	<b>Panca Dewi MHKS:</b> The Use of Soil Potential Microorganism, Humic Acid, Grasses and Legumes Forage in Marginal and Degraded Lands in Indonesia	<b>Indah Wijayanti:</b> Production, Characterization and Purification of Xylanase from <i>Staphylococcus aureus</i> MBXi-K4
12.10-13.30	<b>Lunch Symposia (Lunch and Poster Session)</b>	
	<b>Social, Economic and Policy in Animal Farming</b> Moderator: Dr. Ir. Rachmat Pambudy, M.S.	<b>Feed and Nutrition</b> Moderator: Dr. Ir. Erika B. Laconi, M.S.
13.30-13.45	<b>Tridjoko W. Murti:</b> Profile of Milk Industry in the Province of Central Java. I. A Study on Dairy Cooperatives Profitability	<b>Rusdi:</b> Effect of Polyethylene Glycol (PEG) on in vitro Dry Matter and Nitrogen Digestibility of <i>Leucaena</i> species and Signal grass ( <i>Brachiaria decumbens</i> )
13.45-14.00	<b>Ulrikus R. Lole:</b> Market Structure and Marketing Efficiency of Beef Cattle from NTT (Case in Kupang Regency)	<b>Insun Sangadji:</b> Productivity of Bali Cattle Fed with Ration Containing <i>Pleurotus ostreatus</i> Fermented and Urea-Ammoniated Sago Waste
14.00-14.15	<b>Amiruddin Saleh:</b> The Level of Mass Media Usage and the Role of Communication of Cattle Farmers Group Members in Cattle Supervisory Communication Network	<b>Sri Suharti:</b> Changes in Microbial Population, Fermentation Characteristic and Gas Production from Beef Cattle Rumen in Response to Lerak ( <i>Sapindus rarak</i> ) Extract
14.15-14.30	<b>Irma Badarina:</b> Production Performance of Bali Cattle Supplemented with Concentrate Pellet Diet Based Palm Oil Sludge	<b>Agus Budiansyah:</b> The Characteristics of Phytase Enzyme in Beef Cattle Rumen Liquor from Abattoir
	<b>Animal Production</b> Moderator: Prof. Dr. Ir. Pollung H. Siagian, M.S.	<b>Feed and Nutrition</b> Moderator: Dr. Ir. Asnath M. Fuah, M.S.
14.30-14.45	<b>Mohd Amizi Bin Ayob and Mohd Azid Bin Hj Kabul:</b> Cattle Integration in Oil Palm Plantation through Systematic Management	<b>Iyep Komala:</b> The effect of Garlic ( <i>Allium sativum</i> ) Extract on the Growth of Bacteria Isolated from Uterus Dairy Cattle





Time	Room C	Room D
14.45-15.00	<b>Mohamad Yamin:</b> Increasing Local Sheep Growth Performance through Rapid Selection at Fattening Farm	<b>Taufik Budi Pramono and Dyahruri Sanjayasari:</b> Effect of Protein Level and Energy Protein Ratio on the Growth Broodstock Performance of Senggaringan Fish ( <i>Mystus nigriceps</i> )
15.00-15.15	<b>Agnes Wahyuni:</b> Detection of Enterobacter sp from Dairy Cow's Milk in Boyolali and Sleman	<b>Anita S. Tjakradidjaja:</b> Effectivity of <i>Jatropha curcas</i> Seed Meal Fermented with Various Moulds as Protein Source for Male Mice ( <i>Mus musculus</i> )
15.15-15.30	<b>Prima Puji Raharjo:</b> Lambing Type and Ewe Age on Milk Yield of Local Sheep at UP3 Jonggol (Jonggol Animal Science Teaching and Research Unit)	<b>Syahrhani Syahrir:</b> The effect of Mulberry Leave Extract Fermentation in Feed to Performance of Mice
15.30-15.45	<b>Coffee Break and Poster Session</b>	
	<b>Feed and Nutrition</b> <b>Moderator: Dr. Despal S.Pt., M.Sc.</b>	<b>Feed and Nutrition</b> <b>Moderator: Dr. Ir. Kartiarso</b>
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16.15-16.30	<b>Erika B. Laconi:</b> The Evaluation of Ruminal Metabolism on Fries Holstein (FH) Calves Fed Biofermented Pod Cocoa Using <i>Phanerochaete chrysosporium</i>	<b>N.G.A. Mulyantini:</b> Carcass Composition of Broilers Fed Diets Formulated on Total and Digestible Amino Acid Basis
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Thursday, November 25, 2009

Time	Event
07.00-15.00	<b>Excursion:</b> <ul style="list-style-type: none"> <li>• PT Indolakto</li> <li>• Peternakan Domba "Tawakal Farm"</li> </ul>

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## The Characteristics of Phytase Enzyme from Beef Cattle Rumen Liquor Obtained from Abattoir

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### ABSTRACT

The aim of this experiment is to utilize the liquor of rumen cattle from abattoir as feed additive, as source of phytase enzyme, to increase the quality of broiler ration based on local feed materials. This experiment was conducted in two stages. First stage was to identify enzymes in rumen liquor of cattle. The second stage was to characterize and stabilize enzyme which include determination of optimum temperature and pH of enzyme, examination enzyme resistance to heating at optimum temperature, and studying effect of metal ions and chemical substances. The results of the experiment show 1) optimum precipitation of rumen liquor enzymes from local cattle is reached at the concentration level of 60 % of ammonium sulphate, meanwhile from imported cattle is obtained at the level of 70 % of ammonium sulphate; 2) general characteristics of phytase enzyme from rumen liquor of local cattle differs from that of imported cattle; those from the rumen liquor of local cattle has optimum temperature at 50 °C, optimum pH at 6, resistance to heating up to 180 minutes at optimum temperature and needs mostly methal ions as activator; on the other hand, the enzyme from the rumen liquor of imported cattle has optimum temperature at 39 °C, optimum pH at 6, resistance to heating up to 180 minutes at optimum temperature; the phytase enzyme needs mostly methal ions as activator, some compounds inhibiting the enzyme activity are EDTA and  $\beta$ -mercaptoethanol; and 3) activity of the phytase enzyme from the rumen liquor of local cattle is higher than that of imported cattle.

*Key words:* phytase enzymes, local and imported cattle, rumen liquor, abattoir

### INTRODUCTION

The experiment of rumen cattle liquor utilization as poultry feed in solid material still limited, however, the liquor of rumen cattle is never utilized as feed additive and feed supplement in poultry ration based on local feed. Based on Statistical Data of Animal Husbandry 2007, the numbers of slaughtered cattle every year are not less than 1.75 million heads and it is about 1.5 million heads come from local cattle with average of 300 kg and the rest is from imported cattle. The weight of rumen content is about 14.3 percent from body weight (Hungate, 1968), and a cattle can produce 42.9 kg of rumen content. Thus, the potency of rumen content from slaughtered cattle can reach 75.075 thousand tons per year. The liquor portion of rumen content reaches 31 liters per head (Priego *et al.*, 1977), so that, the potency of rumen liquor reach 52.7 million liter per year. Huge amount of rumen liquor will become a potential pollutant for the

environment if there is no good management in utilization.

This experiment was conducted with the objective to utilize the cattle rumen liquor from abattoir as feed additive, source of phytase enzyme, to increase the quality of broiler ration based on local feed materials. The experiment studying phytase enzyme from rumen liquor may have benefit as feed additive to increase the nutrient digestibility and the value of low quality local feed in increasing the productivity of poultry.

### MATERIALS AND METHODS

#### Rumen enzyme preparation

Rumen content from local and imported cattle were taken from cattle slaughtered from the abattoir in Bogor. Sampling of rumen content from local and imported cattle was taken in two replications and each was taken from 3 – 5 cattles.



Rumen liquor was taken from rumen content by filtration in cold condition. The liquid part of filtration was centrifuged at 10 000 g (4 °C) for 10 minutes to separate supernatant from microbial cells (Lee *et al.*, 2002). Supernatant was then taken as a source of crude enzyme.

#### Enzyme purification using ammonium sulphate

Supernatant containing enzymes was then reacted with different levels of ammonium sulphate and stirred by magnetic stirrer for 1 hour and kept one night at 4 °C. The levels of ammonium sulphate used were 40, 50, 60, 70 and 80 %. Supernatants were then centrifuged again at 10 000 g (4 °C) for 15 minutes. The filtrate was taken and added with phosphate buffer pH 7 at the ratio of 10 : 1 (100 ml supernatant of rumen liquor were dissolved with 10 ml of phosphate buffer pH 7). The precipitates (enzyme source) in phosphate buffer were then kept in freezer for enzyme assay.

#### Phytase enzyme assay

Determination of phytase enzyme activity was measured with Heinones and Lahti (1981) and Greiner *et al.* (1997) methods.

#### Enzyme characterization

Phytase was characterized based on optimum temperature and pH, effect of methal ions and enzyme stabilized based on resistance to heating at optimum temperature.

Determination of optimum pH was done by measuring the enzyme activity in universal buffer at pH 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0. Determination of optimum temperature was carried out by measuring the enzyme activity at 29, 39, 50, 60, 70 and 80 °C in 0.05 M phosphate buffer pH 7. Examination of stabilization enzyme resistance to heating was done by heating the enzyme at optimum temperature for 0, 30, 60, 90, 120, 150 and 180 minutes before adding substrate.

The examination of methal ion effect was conducted by measuring the enzyme activity after treating with some methal ion at 1 mM concentration of  $\beta$ -mercaptoethanol, EDTA, FeCl<sub>2</sub>, MgCl<sub>2</sub>, ZnCl<sub>2</sub>, MnCl<sub>2</sub>, CoCl<sub>2</sub>, CuCl<sub>2</sub> and CaCl<sub>2</sub>. The examination was done by incubating 100  $\mu$ l of enzyme with 100  $\mu$ l methal ions for 10 minutes at room temperature.

#### Data analysis

Data obtained were analyzed by descriptive analysis (Steel and Torrie, 1980).

### RESULTS AND DISCUSSION

#### Enzyme precipitation with ammonium sulphate

Enzyme isolation from cattle rumen liquor was done by salting out method, in which the enzyme was precipitated by adding neutral salt in high concentration (ammonium sulphate) a little by a little. Ammonium sulphate was used because it dissolved faster than other chemicals, did not cause toxic and could stabilize the enzyme (Schwzimmer and Pardee, 1957). Enzyme precipitation by ammonium sulphate was carried out because it has the same polarity as water. Addition of ammonium sulphate in protein solution will attract water molecules around the protein surface, so that, the protein is not protected by water molecules, consequently inter protein molecules are aggregated and precipitated (Scope, 1987). Ammonium sulphate salt will destroy water mantel around the protein and thus protein could be precipitated (Schwzimmer and Pardee, 1957). Choosing of optimum ammonium sulphate concentration was obtained by measuring the highest enzyme activity.

Results of the present experiment of phytase enzyme precipitation by ammonium sulphate shows that, in local cattle rumen liquor, the maximum phytase enzyme activity has been reached at 60% ammonium sulphate concentration with the value of 1.0561  $\mu$ mol/ml/minute, but in imported cattle rumen liquor the maximum activity was reached at 70% ammonium sulphate concentration with the value of 0.9432  $\mu$ mol/ml/minute. Before the addition of ammonium sulphate, phytase enzyme activity in local cattle rumen liquor was 0.3559  $\mu$ mol/ml/minute and imported cattle rumen liquor was 0.2130  $\mu$ mol/ml/minute. The increase of phytase enzyme activity after the treatment with ammonium sulphate in local cattle rumen liquor was 2.97 times, and in imported cattle rumen liquor was 4.43 times. Figure 1 shows the increase in phytase enzyme activity in rumen liquor from abattoir after precipitation with ammonium sulphate. Phytase enzyme activity of local cattle rumen liquor was nearly the same as that of imported cattle rumen liquor, but the





activity in local cattle tended to have higher value than that in imported cattle. Phytase enzyme is a phytate digesting enzyme. Phytate is antinutrition that present in many feedstuffs, mainly in cereals and roughages from leaf of plants.

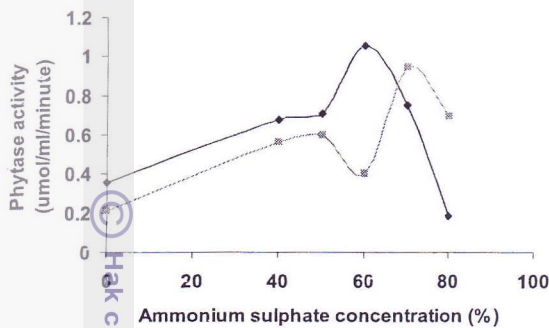


Figure 1. Effect of Ammonium Sulphate Concentration in Enzyme Precipitation of Phytase Enzyme Activity from Local Cattle (—◆—) and Imported Cattle (—■—) Rumens Liquor

### Enzyme characterization

The aim of enzyme characterization was to determine the optimum condition of enzyme activity, so that, the use of enzyme could be adjusted to the optimum condition. Samples of phytase enzyme used for characterization were taken from enzyme that was precipitated with 60% ammonium sulphate concentration for local cattle rumen liquor, and 70% ammonium sulphate concentration for imported cattle rumen liquor.

Reaction of enzyme catalytic was affected by temperature. Palmer (1991) indicates that increasing the temperature would increase energy kinetic of enzyme and would increase the vibration, translation and rotation movement of enzyme and substrate, so that probability of

enzyme and substrate to have contact would be increased. Therefore, enzyme activity increased up to certain temperature, but decreased if optimum temperature has passed over. Thus, increasing of temperature up to certain border line would increase enzyme catalytic activity, but would destroy the enzyme because heating. Each enzyme has a range of optimum pH, where the enzyme showed maximum activity and stability. Lehninger (1995) shows that characteristic of enzyme pH could be indicated by the pH when proton receiver and donor on the enzyme catalytic side were in the required ionization level. Some enzymes need methal ions (cations) as activator for its activity and some chemical substances could decrease enzyme activity. Some of methal ions and chemical substances were examined in the experiment were  $\text{FeCl}_2$ ,  $\text{MgCl}_2$ ,  $\text{ZnCl}_2$ ,  $\text{MnCl}_2$ ,  $\text{CuCl}_2$ ,  $\text{CoCl}_2$ ,  $\text{CaCl}_2$ ,  $\beta$ -mercaptoethanol and EDTA. Results of enzyme characterization are shown in Table 1.

The present results showed that optimum temperature to reach maximum enzyme activity in local cattle was at 50 °C and in imported cattle was at 39 °C. Phytase enzyme from local cattle rumen liquor was more resistance to the change of temperature than that from imported cattle rumen liquor. Figure 2 shows phytase enzyme activity in different incubation temperature.

The result also indicated that the optimum pH to obtain maximum enzyme activity in local cattle rumen liquor has been reached at pH 6, and in imported rumen liquor at pH 7. Figure 3 shows that phytase enzyme activity in different pH. Both enzymes were more stabile in range of pH 6 up to pH 7.

Sariyska *et al.* (2005) demonstrated that optimum temperature of phytase enzyme isolated from *Aspergillus niger* was 55 – 58 °C, and optimum pH at 5.05.

Table. 1. Characteristic of phytase enzyme of cattle rumen liquor from abattoir

Variables	Local cattle	Imported cattle
Optimum temperature (°C)	50	39
Optimum pH	6	7
Resistance to heating (minutes)	180	180
Cations / substances positive effect	$\text{Fe}^{++}$ , $\text{Mg}^{++}$ , $\text{Mn}^{++}$ , $\text{Zn}^{++}$ , $\text{Cu}^{++}$ , $\text{Co}^{++}$ , $\text{Ca}^{++}$ , EDTA and $\beta$ -Mercapto-ethanol	$\text{Fe}^{++}$ , $\text{Mg}^{++}$ , $\text{Mn}^{++}$ , $\text{Zn}^{++}$ , $\text{Cu}^{++}$ , $\text{Co}^{++}$ and $\text{Ca}^{++}$
Cations / substances negative effect	None	EDTA and $\beta$ -Mercapto-ethanol
Cations / substances little effect	None	None



However, Greiner and Farouk (2007) that investigated phytase enzyme isolated from wastewater bacteria in Malaysia, obtained optimum temperature at 65 °C and optimum pH at 4.5. Powar and Jagannathan (1982) showed that optimum pH of phytase enzyme isolated from bacteria *Bacillus subtilis* was at pH 7.5.

Cao *et al.* (2007) indicated that phytase enzyme was very sensitive to temperature, high pressure and heat treatment at 100 °C for 10 minutes had caused loss of enzyme activity. Most of phytase enzyme had optimum temperature at the range of 45 – 60 °C and optimum pH at the range of 4.5 – 6.0, but phytase enzyme from bacteria such as *Bacillus amylolequefaciens* and *Bacillus subtilis* had optimum temperature at 55 – 70 °C and optimum pH at 6.5 – 8.0 (Cao *et al.*, 2007). Cheng *et al.* (1999) showed that phytase enzyme from rumen microbe, *Selenomonas ruminantium*, had active temperature at the range of 20 °C – 55 °C and pH from 3 – 6 depending on the buffer used, and the optimum pH were 4.0 – 5.5 in sodium asetat buffer. On the other hand, Puhl (2006) demonstrated that phytase enzyme from *Selenomonas ruminantium* and its cloning had optimum pH at the range of 4.5 – 6.0, and optimum temperature 55 – 60 °C. This has shown that temperature and pH of phytase enzyme depends on types of microbes.

Yankov *et al.* (1998) had screened the rumen bacteria having phytase enzyme activity which were *Provetella ruminicola*, *Ruminobacter amylophylus*, *Selenomonas ruminantium* and *Streptococcus bovis*. Further examination on the rumen bacteria showed that the bacterium having high phytase enzyme activity were *Selenomonas ruminantium*, followed by *Megasphaera elsdenii*. Other bacteria had also shown phytase enzyme activity, but the phytate enzyme activity could not be detected. Phytase enzyme activity was higher in precipitated bacteria than that in supernatant of rumen liquor and depended on types of feeds. Feeds from high cereal barley (90% barley) had higher phytase enzyme activity than feed containing lower amount of cereal barley (55% barley).

The experimental results in local cattle rumen liquor showed that no methal ions and other chemical substance β-mercaptoethanol and EDTA could decrease phytase enzyme activity. Most of methal ions and chemical substances examined could be used as activator or were needed to increase phytase enzyme activity. The highest phytase enzyme activity in local cattle rumen liquor was obtained when ion Ca<sup>++</sup>

(150.78%) was added and followed by Mg<sup>++</sup> (156,24%), Fe<sup>++</sup> (155,73%), Zn<sup>++</sup> (150.13%), Mn<sup>++</sup> (139.69%), Co<sup>++</sup> (139.23%), EDTA (135.95%), Ca<sup>++</sup> (122.50%) and β-mercaptoethanol (112.27%) respectively. In contrast, in imported cattle rumen liquor, all methal ions did not show to decrease or inhibit phytase enzyme activity, but β-mercaptoethanol and EDTA decreased and inhibited phytase enzyme activity. The highest phytase enzyme activity was obtained when ion Zn<sup>++</sup> (164.10%) was used and followed by ions Cu<sup>++</sup> (150.19%), Ca<sup>++</sup> (142.15%), Fe<sup>++</sup> (141.61%), Mn (130.59%), Co<sup>++</sup> (115.16%), Mg<sup>++</sup> (111.34%), EDTA (82.86%) and Mercaptoethanol (63.47%). Figure 4 shows the effect of methal ions and chemical substances on phytase enzyme activity of cattle rumen liquor from abbatoir.

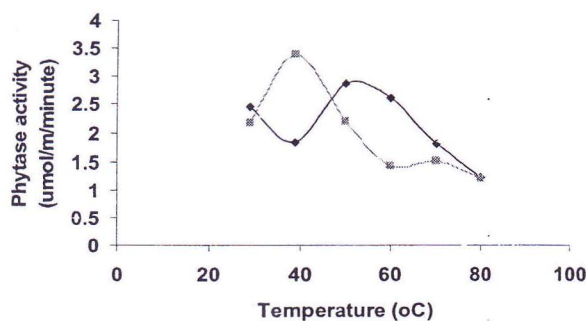


Figure 2. Effect of Temperature on Phytase Enzyme Activity in Local Cattle (—◆—) and Imported Cattle (—■—) Rumen Liquor

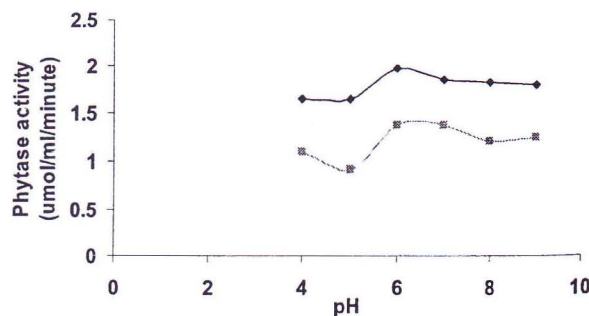


Figure 3. Effect of pH on Phytase Enzyme Activity from Local Cattle (—◆—) and Imported Cattle (—■—) Rumen Liquor



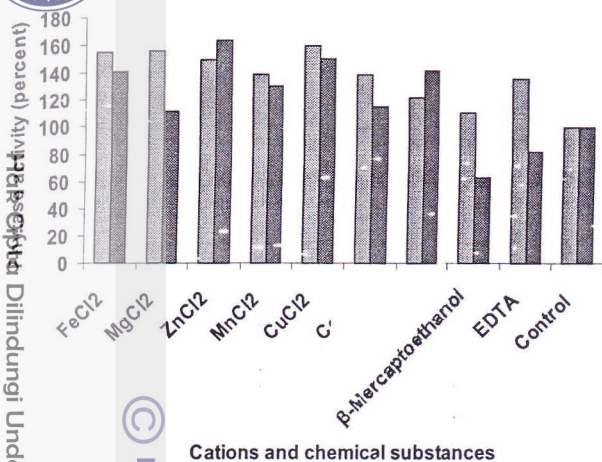


Figure 4. Effect of Cations and Chemical Substances on Phytase Enzyme Activity in Local Cattle (■) and Imported Cattle (▒) Rumen Liquor

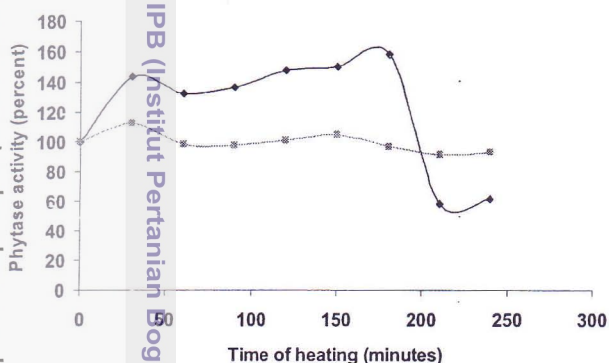


Figure 5. Effect of Heating Time on Phytase Enzyme Activity in Local Cattle (—◆—) and Imported Cattle (—■—) Rumen Liquor

Powar and Jagannathan (1982) indicated that phytase enzyme from *Bacillus subtilis* needed methal ion  $\text{Ca}^{++}$ , and it was the same as phytase enzyme in cattle rumen liquor from abbatoir in this experiment; however, the enzyme activity was inhibited by  $\text{Ba}^{++}$ ,  $\text{Sr}^{++}$ ,  $\text{Hg}^{++}$ ,  $\text{Cd}^{++}$ , and borate. Saryska *et al.* (2005) showed that phytase enzyme from *Aspergillus niger* needed ions  $\text{Ca}^{++}$  and  $\text{B}^{++}$ ; the enzyme also had interesting phenomena because it needs ions  $\text{Pb}^{++}$  and  $\text{Ag}^{++}$  too, but the activity was inhibited by the presence of methal ions  $\text{Hg}^{++}$ ,  $\text{Cu}^{++}$ ,  $\text{Zn}^{++}$ ,  $\text{Fe}^{++}$  and  $\text{Al}^{++}$ . Greiner and Farouk (2007) reported the same finding that, phytase enzyme activity was inhibited significantly by methal ions  $\text{Zn}^{++}$ ,  $\text{Fe}^{++}$ ,  $\text{Fe}^{+++}$ ,  $\text{Mo}^{++}$  and phosphate. Cheng *et al.* (1999) indicated that phytase

enzyme from rumen microorganisms was stimulated by methal ions  $\text{Ca}^{++}$ ,  $\text{Na}^{++}$ ,  $\text{K}^{++}$ , and  $\text{Mg}^{++}$ , but inhibited by methal ions  $\text{Fe}^{++}$ ,  $\text{Zn}^{++}$ ,  $\text{Mn}^{++}$  and was not affected by ions  $\text{Co}^{++}$  and  $\text{Ni}^{++}$ .

Effect of heating on phytase enzyme activity showed that in local cattle rumen liquor, heating at 50 °C temperature (optimum incubation temperature) for 180 minute did not decrease phytase enzyme activity. Phytase enzyme activity was decreased after heat treatment for 210 minutes with the activity was only 58.04%, but it was 61.87% after heat treatment for 240 minutes. The enzyme needs heat stimulation to work effectively. Heating for 30 up to 180 minutes increased phytase enzyme activity. In imported rumen liquor, heat treatment at 39 °C temperature (optimum incubation temperature) for 180 minutes increased phytase enzyme activity, but heat treatment for 210 minutes has slightly decreased the enzyme activity with the value was 91.32%. The value of enzyme activity after heating for 240 minutes was 92.92%. Both phytase enzymes from local and imported cattle rumen liquor were resistance to heat treatment. Figure 5 shows the effect of heating on phytase enzyme activity from cattle rumen liquor.

## CONCLUSIONS

Optimum precipitation of phytase enzyme from rumen liquor is obtained at 60% ammonium sulphate concentration for local cattle and at 70% ammonium sulphate concentration for imported cattle.

General characteristics of phytate enzyme from the rumen liquor of local cattle differ from those from the rumen liquor of imported cattle. Phytase enzyme from local cattle rumen liquor has optimum temperature at 50 °C, optimum pH at 6, resistance to heat treatment up to 180 minutes, needs most of methal ions as activator, but no chemical substances, such as EDTA and β-mercaptoethanol, inhibit enzyme activity. On the other hand, phytase enzyme from imported cattle rumen liquor has optimum temperature at 39 °C, optimum pH at 7, resistance to heat treatment up to 180 minutes, needs most of methal ions as activator; however, chemical substances, such as EDTA and β-mercaptoethanol, inhibit phytase enzyme activity. The activity of the phytase enzyme from rumen liquor of local cattle is higher than that of imported cattle.



## ACKNOWLEDGMENTS

We thank to Directorate General of Higher Education, Department of National Education for funding this research through competitive research grant with Memorandum of Understanding Implementation of the Reasearch No.007/SP2H/PP/DP2M/III/2008, March 6<sup>th</sup> 2008.

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