The 6th ISTAP International Seminar on Tropical Animal Production

“Integrated Approach in Developing Sustainable Tropical Animal Production”

PROCEEDINGS

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Yogyakarta Indonesia

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PREFACE

On behalf of Faculty of Animal Science, Universitas Gadjah Mada, I am pleased to present you the 6th International Seminar on Tropical Animal Production (ISTAP) which is held on October 20 – 22, 2015 at Auditorium drh. Soepardjo, Faculty of Animal Science UGM, Yogyakarta. Under the main theme “Integrated Approach in Developing Sustainable Tropical Animal Production”, we expect that information and ideas on animal production systems in the tropics and its related problems will be shared among participants, thus we can elaborate an integrated approach in developing sustainable tropical animal production. I believe, this can be achieved since more than 250 animal scientists, researchers, students, and producers from more than 15 countries join this seminar.

In this moment, I have to address my great thanks to all people who have contributed for the success of this seminar. First, to all participants, thank you for your contributions, time, and efforts in participating in all sessions in this seminar. We also would like to extend our gratitude to the reviewers and editors for dedicate their expertise and precious time in reviewing and editing the papers. I deeply appreciate the hard work of all members of the Steering Committee, Organizing Committee, and students of Faculty of Animal Science UGM for making this seminar achieved a great success!

I hope all of you enjoy the seminar and Jogja as well!

Dr. Cuk Tri Noviandi

Editor in Chief
REPORT FROM ORGANIZING COMMITTEE

Dear all of the scientists, delegates, participants, ladies and gentlemen,

Praise be to The Almighty for His Merciful and Beneficent to raise up this memorable moment for all of the scientists and delegates from all over the world who were interested in Animal Science field to meet up together.

On behalf of all the members of Board Committee, it is my great pleasure and honor to welcome all of you and impress thankful, and present a high appreciation for your participation in joining the 6th ISTAP in Yogyakarta, one of the Special Region in Indonesia where culture and tradition live in harmony with the modern nuance and educational spirit makes it a beautiful venue of this seminar.

During this event, we have distinguished scientists from all over the world to present plenary papers Livestock Management, Production, and Environment; Feed, Land, and Landscape for Sustainable Animal Production; Livestock Industry and Technology; Economics, Social, and Culture in Livestock Development; and Special issue on Halal Food, Safety and Regulation. It is noted that around 200 scientists as well as livestock producers, companies, graduate and postgraduate students from 15 countries attend the seminar; and more than 160 research papers will be presented. We can see great enthusiasm of all the scientists to solve livestock problems as well as to share valuable information and knowledge for human prosperity all over the world.

The 6th ISTAP Program consists of scientific and technical programs as well as social and cultural activities. The scientific and technical programs offer 4 plenary sessions, field trip, and many scientific sessions (both oral and poster presentation). The social and cultural programs of the 6th ISTAP are very important as the scientific and technical programs since the promotion of friendship and future scientific cooperation are also central to this seminar. Opening Ceremony offers you the Seminar Program a glance. Participants will attend a warm invitation from Dean Faculty of Animal Science UGM in a Welcome Dinner that will give you the most memorable moment to attend. Field trip activity offers a wonderful sightseeing to the most spectacular natural landmark in Yogyakarta, Merapi Lava Tour and Ulen Sentalu Museum. We do hope that you will not miss any of these wonderful opportunities.

Closing Ceremony will be held on October 22nd, 2015, immediately after the last session of presentation. The 6th ISTAP award will be announced for some participant as an appreciation for their valuable research.

Finally, on behalf of 6th ISTAP Committee, I wish all of the participants having a great achievement of success and fulfill the expectation as well as enjoying the interaction with all scientists participating in the seminar.

High appreciation I may acknowledge to the Rector of Universitas Gadjah Mada and Dean Faculty of Animal Science UGM, who have concerned to facilitate the seminar site host.

Special thank to the Steering Committee, Scientific Committee, Reviewers and Editorial Boards for their great contribution to make the seminar successfully organized.

Terima kasih (Thank you).
Sincerely Yours,

Prof. I Gede Suparta Budisatria, Ph.D
Chairman
The Organizing Committee of the 6th ISTAP
WELCOME ADDRESS

Selamat pagi (Good morning)

Dear Rector of Universitas Gadjah Mada, all of Invited Speakers, honorable guests, all of delegates, participants, distinguished guests, Ladies and Gentlemen Attendants of The 6th ISTAP,

It is my great pleasure and honor to extend a warm welcome to all of you at The 6th International Seminar on Tropical Animal Production, which be held on October 20 – 22, 2015 at Auditorium drh. Soepardjo, Universitas Gadjah Mada, Yogyakarta Indonesia. This seminar is proudly organized by Faculty of Animal Science Universitas Gadjah Mada.

The contribution of this seminar to the development of national food security is truly significant for introducing of new scientific knowledge and equipments that is much needed in Indonesia to maintain a safe and secure environment and to look at more effective ways to meet future challenges. We can see great enthusiasm of the entire participant to present their latest research as well as to share valuable information and knowledge for human prosperity all over the world.

In these 3 days of seminar, we have invited some Plenary Speakers and Invited Papers who are qualified as scientists and bureaucrats in animal science field to share their valuable information and knowledge. Other participants can deliver their precious research through oral and poster presentations.

Finally, on behalf of Faculty of Animal Science, we would like to extend our sincere gratitude to the Minister of Rural, Rural Development, and Transmigration, Republic of Indonesia, Mr. Marwan Jafar, for his generosity to be with us here to give Keynote Speech. Then, it is our great honor and pleasure to have qualified scientists and bureaucrats as Plenary Speakers and Invited Papers to share their valuable knowledge during the plenary and concurrent sessions. Moreover, special thank you is for the Steering Committee, Scientific Committee, Reviewers and Editorial Boards for their great contribution to make the seminar a great success. Also, we would like to congratulate and deliver high appreciation to the Organizing Committee as the organizer for their great contribution and generous efforts to make the seminar successfully organized.

And to all of the participants, I hope that this seminar will always success and bring some acknowledgement for all of us. Also, I wish all of the participants having a great achievement of success and fulfill the expectation as well as enjoying the interaction with all participants.

With all of our hospitality, we will try our best to make your brief visit to our country become a wonderful and memorable moments. We are looking forward to meeting you all in the future event.

Wish you all a very pleasant and most enjoyable stay in Yogyakarta, Indonesia, beside you scientific journeys.

Terima kasih (Thank you).

Sincerely Yours,

Prof. Dr. Ali Agus
Dean Faculty of Animal Science UGM
OPENING REMARKS

Dear all of Scientists, distinguished guests, delegates, participants, Ladies and Gentlemen,

On behalf of Universitas Gadjah Mada, I am happy to welcome you and present a high appreciation for your participation in joining the 6th International Seminar on Tropical Animal Production hosted by the Faculty of Animal Science UGM in Yogyakarta from 20 – 22 October 2015.

Under the theme of “Integrated Approaches in Developing Sustainable Tropical Animal Production”, we do hope that this seminar concludes with shared ideas and best practices, technology, and global networks that are required to increase animal production. The increase of animal production as one source of food is crucial to feed the world given that the population is expected to increase from 6 billion to about 8.3 billion in 2030. According to FAO (2008, 2009), the consumption of animal food increased from 10 kg/per annum in 1960, 26 kg/per annum in 200, and it is expected to be 37 kg/per annum. Animal production is an integral part of food production and contributing for the quality of human food supply. Animal and agricultural production is an important component in the integrated farming systems in developing countries as this produces high quality foods, provides job opportunities in rural areas, as well as enriching livelihood.

As a tropical country with high animal biodiversity, Indonesia and other tropical countries, have a variety number of indigenous and local animal genetic resources and germ plasm. This variety of animal germ plasm could be explored and developed not only for animal and food production but also for animal conservation. Apart from being exploited as food resources, it is therefore important to consider animal conservation. Conservation will protect the genetic potency of local bred and their family, and the domesticated animal bred, and this would secure our future food resources.

In these 3 days of seminar, we believe those aforementioned issues will be discussed, and technical solution as well as recommendation will be provided to solve the existing problems in tropical animal production.

Finally, on behalf of Universitas Gadjah Mada, we would like to congratulate and thanks to the Faculty of Animal Science UGM as the organizer for their great efforts to make the seminar successfully organized. To all of participants, I wish all of you have a great discussion and interaction with other scientists participating in the seminar as well as enjoying your time in Yogyakarta.

Thank you

Prof. Ir. Dwikorita Karnawati, M.Sc., Ph.D.
Rector of Universitas Gadjah Mada
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Session 10: Animal Feed and Nutrition 3 (1st Floor Room 8A, Faculty of Animal Science UGM)
Chairperson: Nanung Danar Dono, Ph.D

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Production and Application of Keratinase Enzyme from 4 Strains of *Bacillus* spp. Isolated from Yogyakarta and Garut City

Theresia Galuh Wandita¹, Suharjono Triatmojo¹, Jajang Gumilar², Nanung Agus Fitriyanto*¹

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²Faculty of Animal Husbandry, Padjajaran University, Bandung, Indonesia  
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ABSTRACT: Processing of waste chicken feathers can be used as a biological treatment with keratinase enzyme. Keratinase enzyme can be produced by microorganisms. Keratinase enzyme expected to be produced from *Bacillus* spp. which has been previously isolated from Yogyakarta and Garut City. The purpose of this research was to determine the production of keratinase enzyme produced from *Bacillus* spp. and apply the keratinase enzyme in the process of degradation of chicken feathers. Research consists to measuring the growth of *Bacillus* spp. and investigation of degradation of chicken feathers by *Bacillus* spp. were analyzed descriptively, while data of digested protein by *Bacillus* spp. analyzed using a split plot design, if there are differences followed by Duncan New Multiple Range Test (DMRT). The results obtained bacterial growth and the ability of degradation was found on *Bacillus* sp. TD5B. Increasing of growth rate was followed by faster degradation time. Concentration soluble protein by *Bacillus* megaterium capable of producing higher compared with any others strains. *Bacillus* meganterium has had highest soluble protein (2,030 mg/ml). The longer degradation time followed by highest concentration soluble protein of feathers. The best incubation time at 8 hours that containing 2,256 mg/ml of soluble protein.

Keywords: *Bacillus* spp., Feathers, Keratinase Enzyme

INTRODUCTION

Poultry feathers contain more than 90% of crude protein in keratin form, found as wastes or by-products at poultry processing plants (Howie et al., 1996). Increasing quantities of feathers could effect to the environmental pollution (Rajput and Gupta, 2013). The crude protein content in feather wastes could have a great potential nutrient value and may have some advantage as a protein sources for substitute from more expensive dietary ingredients for animal feed such as poultry and ruminant animal (Xie et al., 2010). Worldwide, commercial poultry processing generates 5 millions of tons of feathers per year, which are currently converted to feather meal through steam pressure and chemical treatment (Freeman et al., 2009). Including in Indonesia, poultry industries are growing faster comparing to the other livestock industry due to the high demand of poultry meat as cheap and high quality protein sources for human consumption. Furthermore, making keratin waste more digestible, established chemical treatment process such as alkali hydrolysis and steam pressure cooking, is both high cost processes and destructive to certain amino acids from feathers such as methionine, lysine, and tryptophan (Tork et al., 2012). The nutritional upgrading of feather as animal feed, especially amino acids content with the treatment of microbial keratinase might lead to a significant increase in the availability of certain amino acids in feather keratin (Joshi et al., 2007).

MATERIALS AND METHODS

Source of Keratin and Preparation of chicken feathers as substrate

Chicken feathers (whole feathers) were collected from chicken slaughterhouse at Yogyakarta district. Feathers were then extensively washed in tap water continued by sterilization with
autoclaved and then dried in hot air oven for 48 h. They were stored at 28°C until used.

**Microbial Culture**

The organisms was grown in basal salt medium (g/L): meat extract, 1.0; biological peptone, 1.0; NaCl, 0.5; and feathers, 1.0. For submerged fermentation, 24 h grown seed culture was used at 5% (v/v) concentration. The cultivation was performed at 28°C at 120 rpm on a shaking incubator for 24 h. Every 6 h, sample was grown by spread plate methods. After 3 d incubation, the bacteria were able to count of colonies.

**Feathers Degradation**

The success rate of substrate degradation was measured by medium turbidity and amount of feathers in medium. Feathers 0.5 g/L to be completed degraded by four strain bacteria in different time. The cultivation was performed at 28°C at 120 rpm on a shaking incubator for 3 d.

**Keratinase Production**

The cultivation was performed at 28°C at 120 rpm on a shaking incubator for 8 h. Every 2 h, sample was centrifuged at 4°C at 3,000 rpm for 15 min. The supernatant was used as crude enzyme source. Crude enzyme protein assayed using Lowry methods.

**Data Analysis**

Data from protein concentration of hydrolyzed feather produced were analyzed using a split plot design. Furthermore, if there are differences between the mean, analyses will be continued with Duncan’s New Multiple Range Test (DMRT).

**RESULTS AND DISCUSSION**

According to the measurement of the growth in liquid medium of four-isolated strains, which confirmed to be belong to Bacillus spp., it was showed different profiles from one to the others. Without the addition of feather as substrate, the growth of Bacillus sp. TD5B showed higher pattern in liquid medium compared to Bacillus sp. TD5K. Furthermore, Bacillus sp. LS2B showed higher growth compare with Bacillus meganterium (Figure. 1).

![Figure1](image.png)

**Figure1.** Comparison the Growth of bacteria Bacillus sp. TD5K (square), Bacillus sp. TD5B

The growth of all strains hours, and continued by stationary phase. Based on the Figure 2, log phase of the growth showed at 2 h until 18 h. In that moment the number of colonies that formed on the agar medium increased significantly. By the addition of poultry feathers in the liquid medium, the growth of Bacillus spp. confirmed faster compared without the addition of poultry feathers as a substrate (Figure. 2). There are differences in the growth profiles of the isolated strain when growing with and without poultry feathers in liquid medium. Medium with the addition of chicken feathers substrate begins with the log phase at 0 h up to 18 h and can reach about 6 x 105 CFU/ml, while the medium without the addition of feather substrate the growth just reach 2 x 105
CFU/ml (Figure 2). This was due to the addition of feather substrate that causes isolates of *Bacillus spp.* regenerate faster against the time. After 24 h incubation, the color in a liquid medium was changes from yellow into a murky brown on medium.

Figure 2 Comparison of bacterial growth a) *Bacillus* sp. TD5K; b) *Bacillus* sp. TD5B; c) *Bacillus* sp. LS2B; d) *Bacillus* meganterium with feathers (square) and without feathers (diamond) in the culture medium

Bacterial growth can be measured by calculation of bacteria growth in the agar medium or colony forming units (CFU). Discoloration on medium with the addition of chicken feathers a sign that chicken feathers contained in the medium hydrolyzed by isolates of *Bacillus spp.* Keratinolitik extracellular enzyme produced by each isolate *Bacillus spp.*, keratin found in chicken feathers will be hydrolyzed into peptides and amino acids that dissolve (Mazzoto *et al.*, 2011).

**Feather substrate degradation by Bacillus spp.**

Results of the feather degradation by *Bacillus spp.* showed the different in the degradation time. *Bacillus* sp. TD5B showed degraded the feathers at about 65 hours, it was more quickly in degrading from *Bacillus* sp. TD5K that completely degraded the feathers at about 68 hours. The substrate degradation by *Bacillus* sp. LS2B was performed at about 71 hours, and *Bacillus* meganterium need about 72 hours to completely degraded the feathers.

In addition of poultry feathers, which completely degraded by the keratinase enzyme, was also indicated by the changed of the color in a liquid medium. It was suggested that the murky yellow which appear in the medium as the result of hydrolysis process of proteins into peptides and amino acids. It was totally different in color medium at 0 hour which appears as clear yellow (Figure 3).

*Bacillus spp.* both can multiply and produce a keratinase enzyme in medium supplemented with chicken feathers, because the feathers are one of the extra nutrients for bacterial cells of as a source of carbon and nitrogen. Carbon and nitrogen are needed by cells of *Bacillus spp.* to produce more keratinase enzyme that can break down keratin contained in chicken feathers (Ali *et al.*, 2011). Keratinase will be produced in large quantities when there is a keratin substrate in the medium (Gupta and Ramnani, 2006).
Figure 3. Degradation of chicken feathers by 4 *Bacillus* strain. a.) *Bacillus* sp. TD5K at 0 hours; a.) *Bacillus* sp. TD5K at 68 hours; b.) *Bacillus* sp. TD5B at 0 hours; b.) *Bacillus* sp. TD5B at 65 hours; c.) *Bacillus* sp. LS2B at 0 hours; c.) *Bacillus* sp. LS2B at 71 hours; d.) *Bacillus* meganterium at 0 hours; d.) *Bacillus* meganterium at 72 hours

Concentration of soluble protein by *Bacillus* spp. keratinase

Investigation of feather digested protein by keratinase from all strains was performed in submerge fermentation in liquid meat extract medium containing poultry feathers. The digested protein in the medium from feathers suggested as the action of keratinase activity against feathers keratin. The result was shown in Figure 4.

Figure 4. Graph of enzyme keratinase production by *Bacillus* sp. TD5K, *Bacillus* sp. TD5B, *Bacillus* sp. LS2B, and *Bacillus* meganterium

The data were then analyzed using split plot design. Based on the results, the different types of *Bacillus* strain effect on concentration of soluble protein (mg/ml) degraded from feather by keratinase enzyme. Furthermore, it has showed significant interaction between the substrate and the addition of different types of *Bacillus* strain. It is stated that the addition of the substrate treatment factors significantly influence the concentration of soluble protein by the strains. *Bacillus* meganterium was significantly different (P>0.05) with *Bacillus* sp. TD5K, *Bacillus* sp. TD5B, and *Bacillus* sp. LS2B. The difference in incubation time effect on concentration of soluble protein (mg/ml), and showed significant interaction between the addition of the substrate and the difference in incubation time. It is stated that the addition of the substrate treatment factors significantly influence the concentration of enzyme keratinase produced by the strains. The incubation time of 2 hours, incubation time of 4 hours, 6 hours of incubation time and incubation time of 8 hours was significantly different (P<0.05). The enzymes can be produced by making more cultures of bacterial isolates. The feather substrate suggested to be a carbon and nitrogen sources for the living of the cells. This indicates that the isolates of *Bacillus* spp. affect the concentration of enzyme produced associated with log phase in the growth phase of each strain. The increasing of incubation times, resulted in the acceleration of microbial activity and the number of microbes (Ali et al., 2011).

CONCLUSIONS

In conclusion, *Bacillus* spp. can be produced keratinase enzyme. Bacterial growth and the ability of degradation was found on *Bacillus* sp. TD5B. Isolates and incubation time work on concentration soluble protein of feathers.
REFERENCES


