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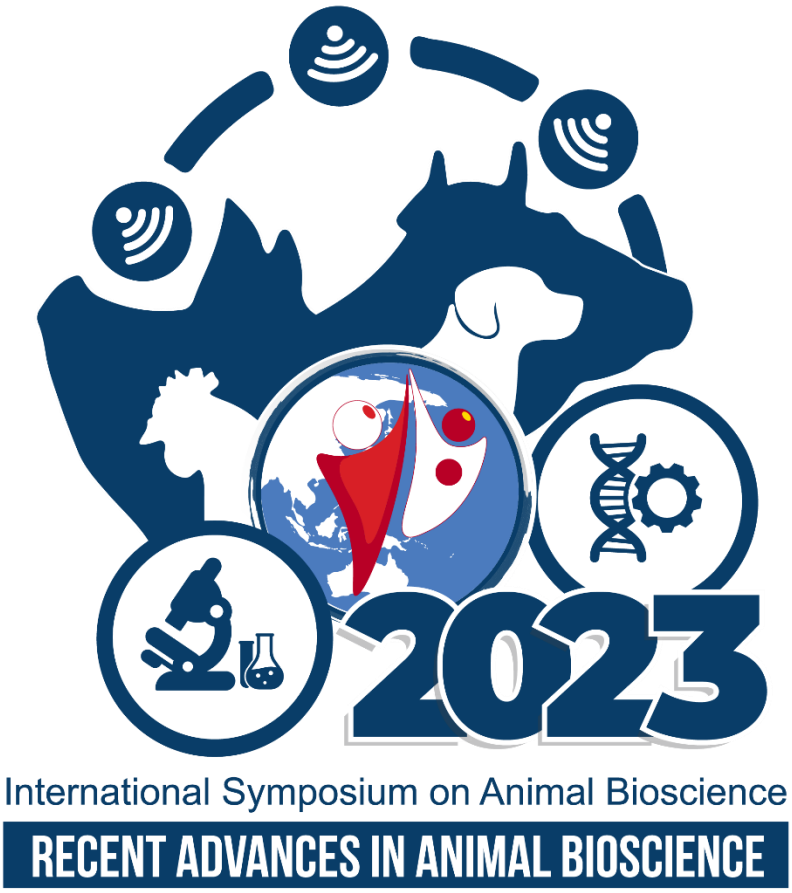

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Program

11:00-11:20 JST (9:00-9:20 VST) Opening ceremony (Venue A)

Prof. Tanjuro GOTO (Dean of the Faculty of Agriculture, Okayama University)
Assoc. Prof. Thong Quang LE (Nong Lam University)
Prof. Hiroaki FUNAHASHI (Chair of ISAB 2023)

11:20-12:50 JST (9:20-10:50 VST) Plenary session (Venue A)

20 min for presentation and 10 min for Q&A
Chairperson: Assoc. Prof. Thong Quang LE (Nong Lam University)
Prof. Naoki NISHINO (Okayama University)

IP-1 **Gut microbiota and disease: Research topics using germ-free and gnotobiotic mice**
Hidetoshi MORITA(Okayama University)

IP-2 **Sustainable livestock farming to adapt methane mitigation: Trends, current status and solutions**
Khang Nguyen DUONG (Nong Lam University)

IP-3 **RNA particle technology – The safe, precise and flexible vaccine for swine**
Ngoc Hoang ĐANG (MSD Animal Health Vietnam)

12:50 ~ 13:30 JST (10:50-11:30 VST) Coffee break

13:30 ~ 15:00 JST (11:30-13:00 VST) Oral presentation Part 1

Venue A

Chairperson: Assoc. Prof. TSURUTA Takeshi (Okayama University)
Assoc. Prof. Thieu Quang NGUYEN (Nong Lam University)

PA-1 **Characterization of antibacterial activity of *Lactococcus lactis* subsp. lactis KM and DH1 isolated from traditional fermented milk in Kenya and Malaysia**
○Thu Nga PHAM, Kensuke ARAKAWA, Ade SUKMA, Hidetoshi MORITA, Taku MIYAMOTO (Okayama University; Andalas University; Minori Inc.; Functional Food Creation Research Institute Co. Ltd.)

PA-2 **Establishment of a cultivation system for methanogenic archaea *Methanobrevibacter smithii* using the hydrogen-producing bacteria *Christensenella minuta* as a source of hydrogen**
○Hiroshi KIRII, Yumika AOKI, Takahiro SHIMOSAKA, Kensuke ARAKAWA, Isafumi MARU, Hidetoshi MORITA (Okayama University; Bizen Chemical Co., Ltd.)

PA-3 **Influence of applying the *Lactobacillus* spp., *Bacillus* spp. and *Saccharomyces cerevisiae* bacterial strains in controlling ammonia and hydrogen sulfide from goat and cattle manures, and improving growth performance**

○Nguyen Khang DUONG, Thuy Binh Phuong LE, Hoang Dao DANG, Son Tinh LE, Thi Thanh Hien NGUYEN (Nong Lam University; HUTECH University; Kim Loi Dai Thanh Co. Ltd.)

PA-4 **Identification of anti-yeast organic acids produced by *Lactiplantibacillus plantarum* 3121M0s isolated from Mongolian fermented mare milk, airag**

○Md. Bakhtiar LIJON, Yuko MATSU-URA, Kensuke ARAKAWA, Takumi UKITA, Hidetoshi MORITA, Taku MIYAMOTO (Okayama University; Minori Inc.; Functional Food Creation Research Institute Co. Ltd.)

PA-5 **Dipeptidyl peptidase 4 activity in the duodenum, ileum, and liver of mice fed various dietary proteins**

○Takuma KIYOCHIKA, Riyan BAEK, Takeshi TSURUTA, Naoki NISHINO (Okayama University)

PA-6 **Effects of fermented soybean meal on growth performance and gut morphology of broiler chickens**

○Quang Thieu NGUYEN, Thi Hong Phuong TO, Nguyen Hoang Huy DANG, Young Ho HONG, Kyung Hoon CHANG (Nong Lam University HCMC; CJ CheilJedang Feed ingredient Division Vietnam; CJ CheilJedang Blossompark Gyeonggi-do, Korea)

Venue B

Chairperson: Assoc. Prof. Toshimitsu HATABU (Okayama University)
Prof. Khang Nguyen DUONG (Nong Lam University)

PB-1 **Insulin-like growth factor-1 regulates the gene expression of Insulin-like growth factor-1 receptors, steroidogenic enzymes, and steroid production of granulosa and theca cells in bovine small follicles**

○Ahmad Farid RAWAN, Hikmatullah LANGAR, Kohei KAWANO, Koji KIMURA (Okayama University)

PB-2 **Detection of polymorphism in *RLA* gene in two goat breeds in Vietnam: Is the mutation A781G in *RLA* gene lethal?**

○Huu Tinh NGUYEN, Minh Lam DANG, Van Hop NGUYEN, Phu Nam Anh BUI (Institute of Animal Sciences for Southern Vietnam; Ho Chi Minh City Open University)

PB-3 **Morphology of bovine uterine glands in various culture conditions**

○Yosuke SUGINO, Taiki SATO, Nozomi FUJIWARA, Koji KIMURA (Okayama University)

PB-4 **Impact of thermal–humidity index on milk yield and reproduction traits on the 2 generations of crossbred cows in Ho Chi Minh City**
○Nguyen Hai Vy HO, Dinh Thuy NGUYEN, Thi Tra Mi BUI (Nong Lam University HCMC)

PB-5 **The effect of 5-aminolevulinic acid supplementation against microbiota composition in laying hen infected with *Eimeria tenella***
○Shota FUJINO, Akihito IMASATO, Shin TANIGUCHI, Makoto MATSUBAYASHI, Hidetoshi MORITA, Toshimitsu HATABU (Okayama University; Hokusatsu Regional Promotion Bureau; Osaka Metropolitan University)

PB-6 **Morphological development of *Toxocara canis* eggs in vitro**
○Han V. N. N, Linh B.N.T, Mai D. C., Mai D. T. (Nong Lam University HCMC)

15:00 ~ 15:30 JST (13:00 ~ 13:30 VST) Coffee break

15:30 ~ 16:45 JST (13:30 ~ 14:45 VST) Oral presentation Part 2

Venue A

Chairperson: Assoc. Prof. Takayuki IBI (Okayama University)
Assoc. Prof. Thieu Quang NGUYEN (Nong Lam University)

PA-7 **Effect of rice bran and chicken manure ratio in diets on growth performance of black soldier fly larvae**
○Thuy Binh Phuong LE, Xuan Tam NGUYEN, Huynh Quang Thong LE, Hoang Dao DANG, Nguyen Khang DUONG (Nong Lam University HCMC; HUTECH University HCMC)

PA-8 **Effect of soya bean waste and mango waste in diets on growth performance of black soldier fly larvae**
○Thi Lieu PHAN, Huynh Quang Thong LE, Duc Luc DO, Nguyen Khang DUONG (Vietnam National University of Agriculture; Nong Lam University HCMC)

PA-9 **Monitoring the digesta- and tissue-associated bacteria inhabiting various gut segments of goats using conventional and viability PCR**
○Dinh Phong TRAN, AO DAOHU, Takeshi TSURUTA, Naoki NISHINO (Okayama University)

PA-10 **Effects of metabolizable energy levels in diets of brown broilers at growing stages and sex**
○Kim Ngan NGUYEN, Duy Dong DUONG, Quang Thieu NGUYEN (Nong Lam University HCMC)

PA-11 **Effect of lard intake on immunoglobulin A coating of gut bacteria**
○Mao TERAOKA, Naoki NISHINO, Takeshi TSURUTA (Okayama University)

Venue B

Chairperson: Prof. Koji KIMURA (Okayama University)
Prof. Khang Nguyen DUONG (Nong Lam University)

PB-7 **A retrospective study on feline hypertrophic cardiomyopathy: prevalence and population characteristics of affected cats**
○Phuong Thao VU, Pongrawee SAENPANYA, Quang Thong LE (Nong Lam University HCMC)

PB-8 **Steroidogenesis and morphological characteristics of granulosa and luteinizing granulosa cells in various cell culture systems**
Hikmatullah LANGAR, Ahmad Farid RAWAN, Kohei KAWANO, Koji KIMURA (Okayama University)

PB-9 **The direct effect of estradiol-17β in bovine oviductal contractility**
○Sayaka KUBOTA, Risa OKAWARA, Koji KIMURA (Okayama University)

PB-10 **Mitochondrial DNA copy number in frozen-thawed bull spermatozoa is negatively correlated with the motility**
○Hai Thanh NGUYEN, Son Quang DO, Hiroshi KOBAYASHI, Takuya WAKAI, Hiroaki FUNAHASHI (Okayama University; Okayama Prefectural Center for Animal Husbandry and Research; Nong Lam University HCMC)

PB-11 **Effects of culture condition based on in vivo ovarian tissue temperature on the in vitro growth and developmental competence of oocytes derived from bovine early antral follicles**
○Kohei KAWANO, Kenichiro SAKAGUCHI, Yojiro YANAGAWA, Seiji KATAGIRI (Okayama University)

16:45 ~ 17:00 JST (14:45 ~ 15:00 VST) Closing ceremony (Venue A)

Symposium summary and Excellent presentation award
Prof. Naoki NISHINO (Okayama University)
Assoc. Prof. Thong Quang LE (Nong Lam University)

IP-01

Gut microbiota and disease: Research topics using germ-free and gnotobiotic mice

○ **Hidetoshi MORITA***

(Graduate School of Environmental, Life, Natural Science and Technology, Okayama University; *Correspondence: hidetoshi-morita@okayama-u.ac.jp)

Keywords: gut microbiota, germ-free mouse, gnotobiotic mouse

Germ-free mice are unique research subjects that have not been naturally exposed to or colonized by any microorganisms, whether bacteria, archaea, or fungi. This characteristic makes germ-free mice invaluable tools in controlled, sterile research environments. Gnotobiotic mice, derived from germ-free mice, are raised in settings where the presence and types of microorganisms to which these mice are exposed are meticulously managed by researchers. The term "gnotobiotic" originates from the Greek words "gnostos" meaning "known" and "bios" meaning "life" The deliberate manipulation of their microbiota enables researchers to study the impact of known microorganisms on various physiological and pathological processes, shedding light on the intricate relationships between these microorganisms and host health.

Approximately 100 trillion symbiotic microorganisms contribute significantly to our health by promoting digestion, catalyzing the synthesis of essential nutrients, and strengthening our immune defenses. This diverse community of symbiotic microorganisms forms complex and intricate interactions that deeply impact our metabolism, immune system, and overall physical and mental well-being. In my presentation, I will introduce research findings regarding the profound influence of these microbial communities on our immune system and overall health.

In my presentation, I will explore the vital roles of gut microbiota in health and disease. We'll begin with the significance of germ-free mice as research tools in understanding the intricate relationship between host health and gut microbiota. By studying immune and inflammation gene-deficient germ-free mice with specific gut microbiota（Sadlack *et al*. 1993）, we uncover the commensal micro-organisms' role in living organisms. Next, I'll highlight a study involving T-cell receptor β chain and p53 double-knockout mice that developed spontaneous adenocarcinoma in the large intestine, revealing the complex interplay between genetics and gut microbiota in cancer development (Kado *et al*. 2001). I will also delve into the intriguing topic of helper T cell differentiation, demonstrating how the types and bacterial count of gut bacteria play a pivotal role in determining the direction of this crucial immune response (Atarashi *et al*. 2013). Lastly, my presentation will touch upon a study involving fecal metabolites from gnotobiotic mice transplanted with gut microbiota from patients with Alzheimer's disease (Fujii *et al*. 2019). This research offers potential insights into the connection between the gut microbiota and neurodegenerative disorders, opening new avenues for Alzheimer's disease research.

[References]

- Sadlack B, Merz H, Schorle H, *et al*., Ulcerative colitis-like disease in mice with a disrupted interleukin-2 gene, *Cell*, **75**: 253-261 (1993).
- Kado S, Uchida K, Funabashi H, *et al*., Intestinal microflora are necessary for development of spontaneous adenocarcinoma of the large intestine in T-cell receptor beta chain and p53 double-knockout mice, *Cancer Res*, **61**: 2395-2398 (2001).
- Atarashi K, Tanoue T, Oshima K, *et al*., Treg induction by a rationally selected mixture of *Clostridia* strains from the human microbiota, *Nature*, **500**: 232-236 (2013).
- Fujii Y, Nguyen TTT, Fujimura Y, *et al*., Fecal metabolite of a gnotobiotic mouse transplanted with gut microbiota from a patient with Alzheimer's disease, *Biosci Biotechnol Biochem*, **83**: 2144-2152 (2019).

IP-2

Sustainable livestock farming to adapt methane mitigation: Trends, current status and solutions

Nguyen Khang DUONG*

(Research and Technology Transfer Center, Nong Lam University HCMC; *Correspondence: khang.duongnguyen.hcmuaf.edu.vn)

Keywords: genetic resource, livestock, methane, prebiotics, probiotics, sustainable farming

The objectives of this report are to contribute to the conference discussion on sustainable livestock farming to adapt methane mitigation by (1) increasing production of livestock and fish in small-holder farming systems basin through management and better use of local feed and animal genetic resources; (2) reducing greenhouse gas emissions from agricultural activities; (3) increasing the access of farmers to renewable sources of energy, black soldier fly/earthworm production; (4) promoting the use of prebiotics and probiotics as replacement for antimicrobial drugs in feed for livestock and aquaculture in target areas, and reducing environmental pollution.

Increase production of livestock and fish in farming systems, reduce greenhouse gas emissions from agricultural activities

The research and application related to (i) the need to increase the productivity of ruminant animals by crossbred apply, and (ii) dietary manipulation to reduce enteric methane production from cattle and goats with special emphasis on the dual role of agricultural by-products, better use local feed available feed resources such as cassava foliage as a source of "bypass" protein and a means of modifying the rumen fermentation, cassava foliage as a means of reducing rumen methane. Increase the access of farmers to renewable sources of energy and BSF/earthworm.

Incorporating household food waste with livestock excreta in biodigesters

The excreta produced by a family does not produce enough biogas needed for cooking. On the other hand, on a world basis, some 1.3 billion tonnes of food waste are unused each year; this reduces the food for human consumption, and equally serious ends up in "land-fills" with production of methane gas that contributes to global warming. Biodigester is the best way to solve pollution, produce renewable energy, and reduce methane production.

Raising and using BSF larvae as a protein source for aquaculture and BSF larvae manure as a organic fertilizer source for crop production at the farms. This will contribute to recommendation of using BSF as aqua-feed to bring the benefit economically to farmers and mitigation for significant negative environmental impact.

Promote the use of prebiotics and probiotics as replacement for antimicrobial drugs in feed for livestock and aquaculture

Improve the environment with probiotics

Livestock and poultry wastes and agricultural by-products that have not been thoroughly treated, leading to environmental pollution. Probiotics has actively produced and applied. Probiotics in treating the breeding environment, creating more organic fertilizer sources and preventing and combating diseases in cattle and poultry has initially brought good results.

Biochar as a prebiotics

Biochar could have equally beneficial effects on the fermentation in biodigesters and in the ruminant animal.

Rice distillers' by-product and biochar as prebiotics in cattle diets

Studies have confirmed that: (i) the cassava root can be used equally successfully as cassava pulp as the basis of intensive fattening of cattle provided it is ground and preserved by ensiling; (ii) that

both rice distillers' and biochar improve the growth rates of cattle fed cassava foliage as the source of protein; and (iii) that there may be synergistic effects from combining rice distillers' by-product with biochar as sources of prebiotics.

IP-03

RNA particle technology – The safe, precise and flexible vaccine for swine

Ngoc Hoang DANG*

(MSD Animal Health Vietnam; *Correspondence: ngoc.hoang.dang@merck.com)

Key words: gene of Interest, RNA particle technology, RNA vaccine

Vaccination is a vital tool to prevent disease in livestock production and has been a major advancement in recent years. Technically, vaccines imitate infection using inactivated or live attenuated antigens to stimulate the immune system. Exposing the body to antigens leads to the production of antibodies specifically directed against them. Memory cells release antibodies and other factors to enable a more rapid and efficient response when exposing to the same antigens again. RNA particle vaccine, a new class of vaccines, provides a different way to present the antigens. For conventional vaccine, the antigens are grown in the lab, have been attenuated or killed and then presented to the body. However, in this evolutionary technology, an electronic gene sequence is utilized. RNA particle technology only uses the known gene of interest, which RNA that encodes a specific protein belong a pathogen. RNA particles are not living organizations but are simply packages containing RNA encoding a gene of interest. Upon vaccination of an animal the RNA particles enter the host dendritic cells, the gene of interest is unpackaged and provides instructions to the dendritic cells to translate the sequence into proteins which act as antigens. The dendritic cells present the antigens to B and T cells, then a targeted immune response is triggered. RNA particles are designed just to deliver the information and not to replicate themselves, safety is maximized. This remarkable technology targets specific pathogens to produce the precise, herd-specific vaccines against both viral and bacterial pathogens. This technology also is able to target multiple pathogens and farm specific strains with a single vaccine injection.

PA-01

Characterization of antibacterial activity of *Lactococcus lactis* subsp. *lactis* KM and DH1 isolated from traditional fermented milk in Kenya and Malaysia

○**Pham Thu NGA[1], Kensuke ARAKAWA[1,*] Ade SUKMA[1,2], Hidetoshi MORITA[1], Taku MIYAMOTO[1,3,4]**

([1]Graduate School of Environmental, Life, Natural Science and Technology, Okayama University; [2]Faculty of Animal Science, Andalas University; [3]Minori Inc.; [4]Functional Food Creation Research Institute Co. Ltd.; *Correspondence: karakawa@okayama-u.ac.jp)

Keywords: lactic acid bacteria, biopreservation, bacteriocin, nisin, *Lactococcus lactis*

[Introduction] Some strains of lactic acid bacteria produce antibacterial peptides "bacteriocins" which are useful candidates for food biopreservation. Among them, nisin A produced by *Lactococcus lactis* subsp. *lactis* strains is the most famous bacteriocin widely used as an approved safe biopreservative in over 60 countries around the world. Nisin Z, a variant of nisin A, is also a famous bacteriocin used in various countries. In our laboratory, *Lc. lactis* subsp. *lactis* KM and DH1 with antibacterial activity had been isolated from traditional fermented milk products, Kenyan "Maziwa Lala" and Malaysian "Dadih", respectively (Miyamoto *et al.*, 1986; Ohhira *et al.*, 1988). This study aimed to characterize antibacterial activity of the two strains for their future use to foods.

[Materials and Methods] *Lc. lactis* subsp. *lactis* KM and DH1 were cultivated in TYLG broth at 25, 30 and 37℃ for 72 h. In addition to them, strains NBRC 100933^T (a non-bacteriocin producer), IFO 12007 (a nisin A producer) and JCM 7638 (a nisin Z producer) of the same species were used as controls. First, time-dependent changes in pH and turbidity of each culture and antibacterial activity of each cell-free culture supernatant (CFS) were measured to determine the optimum incubation temperature and time for their growth and antibacterial productivity. Antibacterial activity of the CFS was assayed with the agar-well diffusion method using *Listeria monocytogenes* VTU 206 as an indicator. Next, effects of pH (2-10), storage (4 and 25℃ for 1 week), heating (65℃ for 60 min, 95 and 110℃ for 20 min, and 121℃ for 15 min), and enzymes (catalase, pepsin, trypsin, α-chymotrypsin, and proteinase K) on antibacterial activity of the CFS were evaluated. After that, antibacterial spectra of the CFS were assayed against 16 bacterial strains. Finally, a structural gene of nisin was amplified with PCR and sequenced.

[Results and Discussion] Strains KM and DH1 grew well at all tested incubation temperature, but antibacterial activity of the CFS incubated at 25℃ was slightly but certainly higher than at 30 and 37℃. In addition, antibacterial activity of the CFS at 25℃ was almost changeless for 8-72 h of incubation. Therefore, 25℃ for 24 h was selected as the optimum incubation conditions. Next, effects of pH, storage, heating, and enzymes on the antibacterial activity were evaluated. Antibacterial activity at pH 2 and 3 was much higher, and that at pH 10 was lower than at the others. All tested storage and heating treatments had little effects under pH 4.0, but clearly decreased the activity except heating at 65 and 95℃ under pH 7.0. Among the enzyme treatments, α-chymotrypsin deceased and proteinase K completely invalidated the activity; meaning that the antibacterial substances in the CFS would be peptides, namely supposed to be bacteriocins. Then, antibacterial spectra of the CFS of KM and DH1 were similar to those of the JCM 7638-CFS containing nisin Z rather than the IFO 12007-CFS containing nisin A. Finally, the structural gene (*nisZ*) of nisin Z was detected with PCR and sequenced in KM and DH1. These results strongly suggested that KM and DH1 should be nisin Z producers. Now purification and identification of nisin Z-like inhibitory substances produced by KM and DH1 are ongoing.

[References]

- Miyamoto T, Gichuru GGS, Akimoto T, Nakae T. Identification and properties of lactic acid bacteria isolated from traditional fermented beverages in East Africa. *Jpn. J. Zootech. Sci.* 57, 265-276 (1986).
- Ohhira I, Miyamoto T, Kataoka K, Nakae T. The isolation of lactic acid bacteria from traditional side-dish fermented foods in Southeast Asia. *Jpn. J. Dairy Food Sci.* 37, 49-59 (1988).

PA-02

Establishment of a cultivation system for methanogenic archaea *Methanobrevibacter smithii* using the hydrogen-producing bacteria *Christensenella minuta* as a source of hydrogen

○**Hiroshi KIRII[1], Yumika AOKI[1], Takahiro SHIMOSAKA[1, 2], Kensuke ARAKAWA[1], Isafumi MARU[2], Hidetoshi MORITA[1,*]**

([1]Graduate School of Environmental, Life, Natural Science and Technology, Okayama University; [2]Bizen Chemical Co., Ltd.; *Correspondence: hidetoshi-morita@okayama-u.ac.jp)

Keywords: archaea, gut microbiota, *Methanobrevibacter smithii*, *Christensenella minuta*

[Introduction] Methanogenic archaea are anaerobic microbes that produce methane and are found in various environments, including human intestines. *Methanobrevibacter smithii*, the most common type in the human gut, uses H_2 to make methane. Previous studies have linked its abundance to age, particularly in elderly people in certain regions (Lu *et al.* 2019). Traditional culture methods involve high-pressure gases, posing safety and equipment challenges. This study aimed to develop a safer culture method using the hydrogen-producing bacterium *Christensenella minuta*, a potential H_2 source for *M. smithii* in the human gut.

[Materials and Methods] *M. smithii* JCM 30028 and *C. minuta* JCM 16072 obtained from the Japan Collection of Microorganisms (JCM) were used in this study. The composition of each liter of medium was 37 g Brain Heart Infusion, 5 g yeast extract, 3.6 g sodium bicarbonate, 0.1 g uric acid, 1 g ascorbic acid, and 0.1 g glutathione, and the pH was adjusted to 7.4. Ampicillin and kanamycin, which are antimicrobial only for bacteria and not for archaea, were added to the medium used to culture for *M. smithii*. An anaerobic chamber (Bactron: Shellab product) was used to enable anaerobic culture. Cultivation was conducted in an incubator located inside the Bactron, and the temperature was set at 37°C.

[Results and Discussion] The medium inoculated with *C. minuta* (300 ml) was placed in a conical flask, and the medium inoculated with *M. smithii* (15 ml) was placed in a test tube and connected with a tube with a rubber stopper. As a result, the growth of *M. smithii* was confirmed and it became possible to culture *M. smithii* in liquid medium with *C. minuta* as a hydrogen source. Next, liquid medium (400 ml) inoculated with *C. minuta* and agar medium inoculated with *M. smithii* were placed together in an anaerobic box and incubated. As a result, colonies of *M. smithii* were observed, confirming the growth of *M. smithii* on the agar medium. The *M. smithii* colonies were much smaller than the *C. minuta* colonies as in the literature. These methods do not require pressurization and culture was possible without the use of special equipment needed to perform pressurization. It has also been suggested that *C. minuta* and *M. smithii* coexist in the human intestine, and it is considered that *M. smithii* uses H_2 produced by *C. minuta* in the actual intestinal tract (Ruaud *et al.* 2020). It is inferred that this culture method has enabled the cultivation of *M. smithii* in an environment like that of the actual intestine. In the future, we plan to isolate *M. smithii* and other methanogenic archaea from Japanese fecal samples using the *M. smithii* culture method established in this study. Furthermore, we intend to conduct a comprehensive analysis of the intestinal microbiota using archaea-specific primers to investigate the presence and significance of *M. smithii* and other methanogenic archaea in the intestinal microbiota unique to Japanese.

[References]

- Wu L, Zeng T, Zinellu A, *et al*., A cross-sectional study of compositional and functional profiles of gut microbiota in sardinian centenarians. *mSystems*, **4**: e00325-19 (2019).
- Ruaud A, Esquivel-Elizondo S, de la Cuesta-Zuluaga J, *et al*., Syntrophy via interspecies H_2 transfer between *Christensenella* and *Methanobrevibacter* underlies their global cooccurrence in the human gut. *mBio*, **11**: e03235-19 (2020).

PA-03

Influence of applying the *Latobacillus spp.*, *Bacillus spp.* and *Saccharomyces cerevisiae* bacterial strains in controlling ammonia and hydrogen sulphide from goat and cattle manures, and improving growth performance

○**Nguyen Khang DUONG[1]*, Thuy Binh Phuong LE[1], Hoang Dao DANG[2], Son Tinh LE[3], Thi Thanh Hien NGUYEN[3]**

([1]Nong Lam University HCMC; [2]HUTECH University HCMC; [3]Kim Loi Dai Thanh Company Limited, Viet Nam; *Correspondence: duongnguyenkhang@gmail.com)

Keywords: lactic acid bacteria, *Enterococcus faecium*, exopolysaccharide, antioxidant activity

[Introduction] Poultry and animal farms are a source of wastes and attract flies, rodents and other pests that create odor smell, pollute water sources and carry diseases to other farms. Current waste management practices include reduction, reuse, recycling, and composting in farms. Many technologies have been developed and investigated in order to reduce odor from farms, including the use of effective microorganisms (Yongzhen and Waijiong, 1994; Nizaha and Syed, 2008). The aim of this study was to evaluate the effectiveness of Digest One product containing the *Latobacillus* spp., *Bacillus* spp. and *Saccharomyces cerevisiae* bacterial strains as a control method to reduce the odor production from goat and cattle manures and improve their growth performance.

[Materials and Methods] The study was conducted at The Research and Technology Transfer Center (RTTC), Nong Lam University of Ho Chi Minh (NLU), Vietnam from February to April 2023. The Digest One product in liquid form available of Kim Loi Dai Thanh Company Limited was applied. Four treatments were with microorganism application of the bedding surface: DO0 (control); DO0.5 (0.5 g/m^2); DO1.0 (1.0 g/m^2), and DO1.5 (1.5 g/m^2). 80 Sindhi crossbred were divided into 4 pens with an area of 42 m^2/pen and 80 Bach Thao x Saanen crossbred goats were divided in 4 pens with an area of 25 m^2/pen. The manures were allowed to accumulate for 3 days before meeting the appropriate amount in order to run this trial. The microbial mixture was spread on the manures of the treated groups on the first day and one additionally on the next day. Atmospheric ammonia and hydrogen sulfide were measured by the detection method of infrared and electrochemical sensors (Geotech BIOGAS 5000) at the end of experiment. The quality of manure was recorded on the score of manure, pH, humidity, ammonia and hydrogen sulfide concentrations were measured. Feed intake, weight gain and feed conversion ratio were measured and calculated.

[Results and Discussion] Our study found some positive influence on the ammonia and hydrogen sulfide levels present in the environment by adding Digest One into the manure. The ammonia and hydrogen sulfide levels were significantly decreased in the treatment group compared to the control group. The single-gas detector showed few hydrogen sulfides in the environment (2ppm) in higher addition of Digest One product at 1.0 and 1.5 g/m^2 of the manure surface. Digest One product handled livestock and poultry manure very well with a dosage of 1g/m^2 of barn surface; it is safe to use, improves weight gain, and does not harm animal health. The litter conditioner, including Digest One product, had a direct action on litter characteristics such as moisture and, consequently, on the improvement of the incidence of pododermatitis and the performances of the animals.

[References]

- Nizaha JM, Syed HSA. A study of the effectiveness of beneficial microorganisms in layer chicken manure management. In: Proc. 29th MSAP Ann. Conf., pp. 120-121 (2008).
- Yongzhen N, Waijiong L. Report on the Deodorizing effect of effective microorganisms (EM) in poultry production. Beijing, China. pp. 402-440 (1994).

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PA-04

Identification of anti-yeast organic acids produced by *Lactiplantibacillus plantarum* 3121M0s isolated from Mongolian fermented mare milk, airag

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Keywords: lactic acid bacteria, biopreservation, anti-yeast activity, Organic acid, *Lactiplantibacillus plantarum*

[Introduction] Food biopreservation using natural antimicrobial substances, particularly produced by lactic acid bacteria (LAB), has recently attracted attention. However, many researchers in this field have targeted undesirable bacteria, not fungi, which cause food spoilage and poisoning. In this study, we aimed to select a LAB strain with anti-yeast activity and to identify the produced anti-yeast substances.

[Materials and Methods] 236 strains of LAB owned by our laboratory were cultivated in MRS broth, and screened with anti-yeast activity that was assayed using the overlay and the agar-well diffusion methods against *Saccharomyces cerevisiae* 4C, *Candida parapsilosis* JCM 1612^T, and *Rhodotorula mucilaginosa* JCM 8115^T. A strain (3121M0s) with the highest activity was selected, and its anti-yeast effect was also confirmed with co-cultivation with *R. mucilaginosa* JCM 8115^T. The species of 3121M0s was identified with 16S rRNA gene sequencing and species-specific multiplex PCR. Next, the optimum growth conditions of 3121M0s were determined with the cell viability and the anti-yeast activity. After that, effects of pH (2.0-10.0), heating (121℃, 15 min), and enzymes (catalase and various proteases) on the activity were examined. At last, anti-yeast organic acids produced by 3121M0s were purified and identified with HPLC and MS after liquid-liquid separation of the culture supernatant with ethyl acetate.

[Results and Discussion] Among the screened 236 strains, 3121M0s that had been isolated from Mongolian fermented mare milk "airag" showed the highest anti-yeast activity; and also effectively inhibited the growth of *R. mucilaginosa* JCM 8115^T on the co-cultivation test. The species of 3121M0s was identified as *Lactiplantibacillus plantarum*, and then the optimum growth conditions to show the high stable activity were determined as at 30℃ for 48 h. The activity of 3121M0s was shown at pH 2.0-4.0, and hardly affected by heating and enzymes except pepsin; suggesting the active substances would be organic acids. Therefore, the organic fraction on liquid-liquid separation of the culture supernatant was analyzed using HPLC and MS to purify and identify the anti-yeast substances. As the result, lactic acid (16.05 g/L; D/L = 2:1), acetic acid (1.44 g/L), 4-hydroxyphenyllactic acid (28.7 mg/L), 4-hydroxybenzoic acid (26.9 mg/L), and 3-phenyllactic acid (99.1 mg/L) were identified as anti-yeast substances. Among them, acetic acid showed the strongest activity, and the others promoted the activity. In addition, each amount of acetic acid, 3-phenyllactic acid, and 4-hydroxyphenyllactic acid from 3121M0s was much higher than those from the type strain (JCM 1149^T) of the same species. These results suggest that *Lactiplantibacillus plantarum* 3121M0s isolated from airag could be useful for biopreservation of fermented foods by inhibiting contaminated yeasts.

PA-05

Dipeptidyl peptidase 4 activity in the duodenum, ileum, and liver of mice fed various dietary proteins

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Keywords: dietary protein, dipeptidyl peptidase 4, gut, mice

[Introduction] Food proteins are highly digestible, and many peptide bonds are susceptible to hydrolysis by various digestive enzymes. Peptide bonds associated with proline are an exception; proteases and peptidases in the digestive enzymes do not hydrolyze the proline-containing peptides. A milk protein β-casein is high in proline content, and β-casomorphin generated during digestion has been claimed to cause digestive discomfort. Meanwhile, DPP4 (dipeptidyl peptidase 4), a cell membrane-associated enzyme, can hydrolyze such proline-containing peptides. DPP4 is expressed in the gut, liver, and kidney and is also present in soluble form in blood. It is not clear how DPP4 is involved in the degradation of dietary proteins with various proline content. In this study, four proteins with different proline content were fed to mice, and differences in duodenal, ileal, and hepatic DPP4 activity were investigated.

[Materials and Methods] Diets containing casein, meat, egg albumin, and soy protein isolate as a single protein source (20%) were prepared and fed ad libitum to 10 male C57BL/6 mice for four weeks. Casein and egg albumin were crude extracts of reagent grades, and meat protein was prepared by lyophilizing and defatting commercially purchased pork. The proline content ranks casein > soy > meat > egg albumin. Blood samples were collected under anesthetic and euthanased, and duodenal, ileal, and liver samples were obtained. To compare the enzyme activity under presence or absence of dietary protein in the gut, ten mice assigned for one dietary treatment were divided into two groups. A feeding group was allowed access to food and water until euthanasia, and a fasting group was deprived of food for 16 h before sampling. DPP4 activity was determined by kinetic assay using Gly-Pro-pNA as substrate.

[Results and Discussion] DPP activity per tissue weight was highest in the liver, and similar levels were seen in the duodenum and ileum. The DPP activity of the liver was greatly lowered when determined per protein. DPP4 activity in the duodenum and ileum did not differ between feeding and fasting groups, whereas that in the liver was substantially increased because of fasting. Although we hypothesized that casein feeding should activate DPP4 in the gut more than others, no differences were observed in the DPP4 activity between casein, meat, egg albumin, and soy protein isolate feedings, either per tissue weight or per protein. The higher DPP4 activity, expressed per protein, in the small intestine than in the liver suggested that DPP4 is involved in the degradation of dietary proteins. However, the fact that DPP4 activity in the gut did not differ between feeding and fasting groups indicated that DPP4 activity was not affected by the levels of proline-containing peptides in the diet.

PA-06

Effects of fermented soybean meal on growth performance and gut morphology of broiler chickens

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Keywords: Broiler chickens, fermented soybean meals, growth performance, gut morphology

[Introduction] Soybean meal (SBM) has long been used in animal diets as it provides an excellent source of both energy and protein in animals. However, SBM contains antinutritional factors (ANFs) that inhibit the activity of proteolytic enzymes, reduce nutrient use, and affect the performance and health of animals, especially young animals (Hong *et al.*, 2004; Li *et al.*, 2014). The fermentation process is thought to reduce residual ANFs and increase the activity of digestive enzymes in chickens (Feng *et al.*, 2007). The objectives of this study were to evaluate the effects of fermented SBM (ST50 and ST55) on the growth performance, microbial flora, and intestinal morphology of chickens.

[Materials and Methods] The trial was conducted in Nong Lam University, Ho Chi Minh City. A total of 480 d-old broilers (Ross 308, mixed sex) were randomly assigned into six treatments with 8 replications of 10 chicks per replicate. There were three feeding phases: 1–14; 15–28 and 29–42 days of age. ST50 and ST55 were replaced SBM in the chick diets of the first phases and switched to the commercial diets for growing and finishing broilers for next 4 weeks. Six treatments were as follows; N-CON, feed without antibiotics and fermented SBM; P-CON, feed contained two kinds of antibiotic without fermented SBM as positive control; ST55-3 and ST55-5, non-medicated feed contained 3% and 5% of ST55, respectively; ST50-3 and ST50-5, non-medicated feed contained 3% and 5% of ST50, respectively. Live body weight, average daily gain, feed intake, feed conversion ratio, villus height and crypt depth were measured. Carcass evaluation on dressing percentage and cut up parts of yield as percentage at 42 days of age. The intestinal samples were collected at 28 days old (5 samples at ileum) and at the end of the trial (10 samples at ileum) to measure the villi height and the crypt depth. Five fecal samples per treatment were collected to isolate total *Escherichia coli*, *Lactobacillus* and *C. perfringens* at14th day of the experiment. Data was statistically analyzed by Minitab 17.0, using ANOVA, Tukey test and Chi-square test to test the factor effects. A significant difference was set at $p \leq 0.05$.

[Results and Discussion] The chicks fed the diet with 3% or 5% Soytide 55 and Soytide 50 showed the similar growth performance to those of medicated or non-medicated control groups. There were no significant differences in the number of *E. coli*, *Lactobacillus* and *C. perfringens* of feces among groups. Feeding of fermented SBM had no impact on the carcass dressing percentages and relative weight of organs in broilers ($p>0.05$), except for leg and liver. At the end of the trial, ileal villi length in ST50-5 group was significantly longer ($p<0.05$) compared with N-CON. ST50-5 group had the highest villi height/crypt depth ratio ($p<0.01$).

[Conclusion] The results indicate that feeding of fermented SBMs during early phase are beneficial to the ileal villi morphology in broiler chicks without improvement of growth performance.

[References]

- Hong KJ, Lee CH, Kim SW. *Aspergillus oryzae* 3.042GB-107 fermentation improves nutritional quality of food soybeans and feed soybean meals. *J. Medicinal Food* 7, 430-434 (2004).
- Feng J, Liu X, Xu ZR, Wang YZ, Liu JX. Effects of fermented soybean meal on digestive enzyme activities and intestinal morphology in broilers. *Poult. Sci.* 86, 1149-1154 (2007).
- Li CY, Lu JJ, Wu CP, Lien TF. Effects of probiotics and bremelain fermented soybean meal replacing fish meal on growth performance, nutrient retention and carcass traits of broilers. *Livest. Sci.* 163, 94-101 (2014).

PA-07

Effect of rice bran and chicken manure ratio in diets on growth performance of black soldier fly larvae

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Keywords: Black soldier fly larvae, rice bran, chicken manure, growth, feed intake

[Introduction] Black soldier fly larvae (BSFL) could consume many kinds of organic material, including animal manure, kitchen scraps, and agricultural waste. BSFL could eat and process the diversity of substrates; the efficiency with which they have done these ways may be the highest among the flies. Another important bioactivity is that the larvae development time of over three weeks was longer than that of house flies. This means a single larva will consume a larger amount of substrate and produce larger pupae (Čičková et al., 2015). Wild black soldier fly larvae are already used to manage manure successfully (Sheppard et al., 2002), reducing odor and pest fly populations. All of these benefits make BSFL practical to rear and a suitable tool for waste management purposes in a small-scale, and possibly a sustainable animal feed source. The aim of this study was to determine the growth performance of black soldier fly larvae fed by different ratios of rice bran (RB) and chicken manure (CM) in the diet.

[Materials and Methods] The study was carried out from April to June 2022 at the chicken farm of Hoan Hao–Vina company, Thanh Hoa district, Long An Province, Vietnam. The experiment was set up in a one-factor completely randomized block design with three replications for each treatment. For each replication, 100 individuals were randomly selected from an experimental tray. A total of 15 trays were used to evaluate the effect of replacing the RB with CM on the growth performance of BSFL between 5 treatments: T1 (100% RB + 0% CM), T2 (75% RB + 25% CM), T3 (50% RB + 50% CM), T4 (25% RB + 75% CM), T5 (0% RB + 100% CM). On the first day of the experiment, each treatment was added 10 grams of eggs of BSFL with the same laying date and 200 grams of concentrate feed. After 5 days, in the experimental trays, the amount of food was added according to the treatment ration formula. The studied parameters were length, width, and weight of black soldier fly larvae in 7, 14, and 21 days after hatching.

[Results] The results showed that replication 0, 25, 50, 75, and 100% of chicken manure affected length of BSFL at day 21 were 18.10, 19.07, 19.70, 19.58, and 18.94mm, respectively (P=0.001). The width of BSFL at day 21 were 3.87, 4.23, 4.46, 4.45 and 4.20mm, respectively (P=0.001). The body weights of BSFL at day 21 were 16.83, 16.40, 17.50, 15.97 and 17.03gr (P=0.554), respectively. The FCR of BSFL at the end of experiment were 1.16, 1.23, 1.54, 1.73 and 2.17 (P=0.001), respectively. In conclusion, BSFL fed a diet with 75% of chicken manure had the best growth rate.

[References]

- Sheppard DC, Tomberlin JK, Joyce JA, Kiser BC, Sumner SM. Rearing methods for the black soldier fly (diptera: Stratiomyidae). *J. Med. Entomol.* 39, 695–698 (2002).
- Čičková H, Newton GL, Lacy RC, Kozánek M. The use of fly larvae for organic waste treatment. *Waste Manag*. 35, 68–80 (2015).

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PA-08

Effect of soya bean waste and mango waste in diets on growth performance of black soldier fly larvae

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Keywords: Black soldier fly larvae, soya bean waste, mango waste, growth, feed intake

[Introduction] Black soldier fly larvae (BSFL) could consume many kinds of organic material and could be used and treated for small-scale waste management by using media substrates such as food waste (Nguyen et al., 2015). The refused feed that BSFL did not consume, combined with their rich nitrogen manure, could be used as fertilizer (Čičková et al., 2015). All these benefits make BSFL practical to rear and a suitable tool to use waste, and possibly a sustainable animal feed source. The proposed solution is to supply BSFL as a sustainable source of protein to livestock and aquaculture farmers to raise rural farmers' income and improve farming practices. The aim of this study was to determine the growth performance of BSFL fed by different ratios of soya bean waste (SBW) and mango waste (MW) in the diet.

[Materials and Methods] The SBW and MW was collected and carried from the local market and the experiment conducting at the Ruminant Research and Technology Transfer Farm, Research and Technology Transfer Center, Nong Lam University of Ho Chi Minh City, Vietnam from Feb to Apr 2023. The experiment was setup in a one factor completely randomized block design with three replications for each treatment. All total of 15 trays were used to evaluate the effect of replacing the soya bean waste (SBW) with mango waste (MW) on the growth performance of BSFL between 5 treatments: T_1 (100% SBW + 0% MW), T_2 (75% SBW + 25% MW), T_3 (50% SBW + 50% MW), T_4 (25% SBW + 75% MW), T_5 (0% SBW + 100% MW). 5 treatment tray was put 10g of eggs and 200g of concentrate feed. After 5 days, the larvae in this tray were randomly divided into 3 trays. Feed was supplied to the larvae one time at 7.30AM into one place of the tray and weighed to measure the feed intake for every time feeding. For each replication, 100 individuals were randomly selected from an experimental tray through days 7, 14 and 21 after hatching for parameter criteria length and width. The live weight of larvae will be weighed at the day of 21 in the end of experiment.

[Results] The results showed that replication 0, 25, 50, 75 and 100% of MW affected length of BSFL at day 21 were 17.76, 18.81, 19.27, 18.20 and 17.63mm, respectively (P=0.001). The width of BSFL at day 21 were 3.83, 3.93, 4.12, 3.88 and 3.61mm, respectively (P=0.001). The body weights of BSFL at day 21 were 15.97, 16.41, 17.56, 16.18 and 15.34gr (P=0.001), respectively. The FCR of BSFL in the end of experiment were 1.18, 1.10, 1.04, 1.21 and 1.37, respectively (P=0.001). Growth rate was improved and FCR were reduced in treatments combined between SBW and MW. The black soldier fly larvae fed a diet with 50% of SBW and 50% of MW had the best growth and performance.

[References]

- Čičková H, Newton GL, Lacy RC, Kozánek M. The use of fly larvae for organic waste treatment. *Waste Manag.* 35, 68–80 (2015).
- Nguyen TTX, Tomberlin JK, Vanlaerhoven S. Ability of black soldier fly (diptera: *Stratiomyidae*) larvae to recycle food waste. *Environ. Entomol.* 44, 406–410 (2015).

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PA-09

Monitoring the digesta- and tissue-associated bacteria inhabiting various gut segments of goats using conventional and viability PCR

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Keywords: digesta-associated bacteria, tissue-associated bacteria, viability PCR, conventional PCR

[Introduction] The productivity and health of ruminant animals heavily depend on the activities of gut microbiota, especially for the rumen; hence, attempts to understand and control gut microbiota have been made intensively and continuously. Although the rumen is not the only habitat, limited research has been performed on microbiota residing in gut segments other than the rumen and rectum. Information on tissue-associated microbiota is also lacking compared to digesta-associated microbiota. The differences and characteristics of gut microbiota in various gastrointestinal segments need to be understood to gain further insights into the relationship between gut microbiota, nutrition, and health. In this study, gut contents and tissue samples were collected from the dorsal rumen, ventral rumen, abomasum, duodenum, cecum, and rectum of goats to investigate their microbiota. In addition, the viability PCR, which combines a photochemical reaction with the application of a photo-reactive propidium monoazide (PMA) showing high affinity to DNA, was employed to detect live bacteria. Evaluation of the effectiveness of the viability PCR in farm animal research was also the aim of this experiment.

[Materials and Methods] Four castrated male goats were used in this study. The goats were fed a diet containing 50 % concentrates and 50 % grass hay *ad libitum*. Digesta and tissue samples were taken from the dorsal rumen, ventral rumen, abomasum, duodenum, cecum, and rectum after feeding the diet for two weeks. Tissue samples were well-washed with ice-cold sterile saline. The bacterial suspension was treated with and without PMA before the DNAs were purified and the subsequent PCR-based assessment. Quantitative PCR (qPCR) was made to measure the total bacterial population. Two-step PCR targeting the V4 region of 16S rRNA genes was performed to generate amplicon libraries for next-generation sequencing.

[Results and Discussion] The total population of digesta-associated bacteria was lower in the abomasum and duodenum than in other gut segments. Although the difference between the dorsal and ventral rumen was small, the population in the rumen appeared lower than in the cecum and rectum. The viable populations were lower by approximately one log order than the total populations in the rumen, cecum, and rectum, and the viability appeared much lower in the abomasum and duodenum. Additionally, compared to other gut segments, the abomasum and duodenum had a reduced number of tissue-associated bacteria. The viability of bacteria in the rumen and cecum, in contrast to digesta-associated microorganisms, appeared to be significantly diminished. In the abomasum and duodenum, most tissue-associated bacteria were regarded as alive. The phylum and family levels showed differences after PMA treatment. Finally, the results showed that after PMA, the rectum had the most balanced gut microbial composition in both digesta and tissue and the most diverse digesta-associated microbiota, while the dorsal part of the rumen displayed the lowest diversity. These findings contribute to our understanding of the gut microbiota and highlight the importance of viability PCR in evaluating the gut microbial composition.

PA-10

Effects of metabolizable energy levels in diets of brown broilers at growing stages and sex

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Keywords: brown broilers, growing stages, sex, metabolizable energy

[Introduction] According to data from the General Statistics Office of Vietnam on January 1, 2021, the number of brown broilers is 327 million, accounting for 81.3% of the total broiler chickens in Vietnam. Although brown broiler groups are said to have delicious meat quality, the growth rate greatly differs between roosters and hens, leading to differences in nutrient needs. Therefore, it is challenging to achieve uniformity when raising male and female brown broilers together. In broiler diets, ME (metabolizable energy) is the factor that regulates feed intake, so ME is the first factor to consider when calculating diets (Khalil et al., 2021). In Vietnam, there are no nutritional standards for brown broilers, so the diets are based on recommendations from foreign imported chickens. This study aims to determine optimum level of ME in diets by age and gender of Vietnamese brown broilers.

[Materials and Methods] The study was arranged in a completely randomized design with one factor, including 2 stages. In stage 1 (1-28 days old), 980 brown broilers regardless of gender were arranged in one of 5 treatments (TM) with 5 ME levels: 2950, 3000, 3050, 3100, 3150 kcal; each TM has 7 replicates. In stage 2 (29-84 days old), 900 brown broilers were arranged into 6 TM: T1, T2, T3 for roosters and TM: M1, M2, M3 for hens; each TM had 12 replicates. The ME levels used in each growing period are different according to age and sex, with the standard ME level being simulated according to the nutritional of Rowan Range broilers supplied by Aviagen company. ME in each TM according to growing period (29-49 and 50-84 days old): T1 (3000, 3100 kcal), T2 (3050, 3150 kcal), T3 (3100, 3200 kcal) and M1(3050, 3100 kcal), M2 (3000, 3050 kcal), M3 (2950, 3000 kcal). The ME in the rooster diet will increase in each TM and conversely in the hen diet will decrease compared to the basic ME level (T1 and M1), the difference between each TM is 50 kcal/kg of feed. Performance indicators: body weight (BW), average daily gain (ADG), feed intake (FI), feed conversion ratio (FCR), European broiler index (EBI), and feed cost for weight gain were monitored to evaluate the influence of ME levels on brown broilers' diets.

[Results and Discussion] In stage 1, diet of ME 2950 kcal/kg improved BW, ADG, feed cost per kilogram weight gain in mixed rearing chicken; this result is similar to results Bui Thi Thom's result on Cay Cum broiler (2017). In stage 2, increasing of ME levels for male diet (period 29-49 days old: 3050, 3100 kcal; 50-84 days old: 3150, 3200 kcal) did not affect BW, ADG, FI, FCR, EBI as well as when reducing the ME level in the female diet (period 29-49 days old: 2950, 3000 kcal; 50-84 days old: 3000, 3050 kcal). However, the feed cost per kilogram weight gain better reduced when using diets with higher ME level for rooster, whereas reducing the ME level in the hen diet increased the cost.

[Conclusion] In 1-28 days old period of brown broiler, the ME level of 2950 kcal/kg should be used to build a diet. In 29-84 days old, it is recommended to increase ME level in the male's diet to save costs in terms of using feed for weight gain and should not reduce ME level in the female's diet.

[References]

- Aviagen. Managing the Rowan Ranger. (2018) < https://aviagen.com/brands/rowan-range/>.
- Bui TT, Tran VP, Nguyen HQ. Effects of energy levels in diets for Cay Cum broiler in Thai Nguyen. *Thai Nguyen Univ. Sci. Technol. Magazine* 11, 153-160. ISSN: 1859-2171 (2017).
- General Statistics Office of Vietnam. Vietnam livestock statistics on the number of heads and poultry products on January 1st, 2021 (2021)
- Khalil MM, Abdollahi MR, Zaefarian F, Ravindran V. Influence of feed form on the apparent metabolisable energy of feed ingredients for broiler chickens. *Anim. Feed Sci. Technol.* 271, 114754 (2021).

PA-11

Effect of lard intake on immunoglobulin A coating of gut bacteria

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Keywords: immunoglobulin A, dietary fat, gut bacteria

[Introduction] Immunoglobulin A (IgA) is a major antibody secreted into the gut and partly contributes to symbiosis with gut bacteria by selective bacterial coating. Lard is widely used in animal fat diet feeding experiment, which derive from pigs' fat. We recently showed that lard-enriched diet (30%w/w) feeding reduces amount of IgA coating gut bacteria in mice. However, it remains unclear how amount and intake period of lard influence IgA coating gut bacteria. In this study, we verify the relationship between amount of lard intake, lard intake period, and IgA coating gut bacteria.

[Materials and Methods] Mice were divided into four groups and fed normal fat diet (control) and high lard diet with three different amounts (10%w/w, 20%w/w, 30%w/w) for 2, 4, and 8weeks, respectively: L10 group, L20 group L30 group. The feces were collected at 2, 4, and 8weeks, and the fecal bacteria was isolated from the feces by centrifugation. The amount of IgA coating a single fecal bacterium (amount of IgA coating: strength of IgA binding to a single fecal bacterium) and IgA coating ratio (IgA coating ratio: proportion of IgA binding fecal bacteria) were detected by FACS analysis using flow cytometry. Separate and collect IgA-coated bacteria and non-coated bacteria by affinity purification using magnetic beads. The bacteria occupancy of IgA-binding and non-binding bacteria was calculated at the phylum level by amplicon sequence analysis targeting the 16S rRNA gene. Calculate the IgA coating index (ICI= Relative abundance of IgA-coated bacteria/ Relative abundance of IgA non-coated bacteria) to evaluate the level of IgA coating to specific bacteria in the phylum level.

[Results and Discussion] It was confirmed that lard intake increased as the lard content of the feed increased. The amount of IgA coating gut bacteria decreased significantly in all lard intake groups from the 2 weeks compared to the control group, and a same trend was observed in the L20 and L30 groups until the 8 weeks. On the other hand, only L30 group at the 2 and 4 weeks showed the significant decrease compared to control group and at the 8 weeks all lard intake groups showed significant decrease compared to the control group in IgA binding ratio. These suggest that the amount of IgA coating a single bacterium decreases at a low intake and in a short period of lard intake, whereas a decrease in the proportion of IgA binding fecal bacteria decrease with high amount or 8 weeks of lard intake is required. L20 and L30 groups decrease significantly compared to control group at 2 and 4 weeks, and L20 group decrease significantly at 8 weeks in the ICI of Bacillota. However, no clear trend was observed in the ICI of Bacteroidota. The ICI of the phylum Pseudomonadota, which includes opportunistic bacteria such as Escherichia coli, showed a significant increase in the L20 and L30 groups at 2 and 4 weeks compared to the control group, and although the increase slowed down at the 8th week, L20 showed significantly higher values compared to the control group. These results suggest that the different amount and intake period of lard had different effects on IgA reactivity to specific bacteria in the phylum level.

PB-01

Insulin-like growth factor-1 regulates the gene expression of Insulin-like growth factor-1 receptors, steroidogenic enzymes, and steroid production of granulosa and theca cells in bovine small follicles

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Keywords: follicles, granulosa cells, theca cells, IGF-1

[Introduction] Insulin-like growth factor-1 (IGF-1) hormone plays a crucial role in follicular growth, antral formation, and steroidogenesis. IGF-1 is known to play a key role in the acquisition and maintenance of functional dominance. However, the biological effect of IGF-1 is a topic of interest, whether it is managed by IGF-1 receptors (*IGF1R*) or IGF-binding proteins.

[Methods and Materials] We examined the expression of *IGF1R* in uncultured granulosa cells (GCs) of the three largest follicles from paired ovaries, the effects of various concentrations (10, 50, and 100 ng/ml) of IGF-1 on the expression of *IGF1R*, *STAR*, *CYP11A1*, *CYP17A1*, *HSD3B* and *CYP17B1* mRNA in cultured GCs and on production of androstenedione (A4) and progesterone (P4) in cultured theca cells (TCs). We also investigated the effects of IGF-1 on the expression of proliferating cell nuclear antigen (*PCNA*) mRNA and on GCs number.

[Results] Small follicles (<6 mm) expressed significantly lower *IGF1R* than mid-sized follicles (7-8 mm) and large follicles (≥9 mm), which expressed the highest levels of *IGF1R* mRNA ($p<0.05$). IGF-1 (10-100 ng/ml) significantly increased *IGF1R* mRNA in cultured GCs. IGF-1 (50-100 ng/ml) increased *STAR*, *CYP11A1* mRNA, and IGF-1 (10-100 ng/ml) enhanced *CYP17A1* mRNA in GCs. IGF-1 had no effect on *CYP17B1* mRNA expression in GCs. IGF-1 (100 ng/ml) increased the *HSD3B* mRNA in GCs of small follicles ($p < 0.05$). IGF-1 (50 ng/ml) and (50-100 ng/ml) increased A4 and P4 production in theca cells, respectively, which serve as the substrate for estrogen (E2) synthesis in GCs. Interestingly, IGF-1 (100 ng/ml) significantly increased the *PCNA* mRNA expression in cultured GCs and GC number of small follicles. These results showed that IGF-1 increased *IGF1R* mRNA in GCs, which may mediate the action of IGF-1 required for the follicular growth. IGF-1 increased the steroidogenic enzyme mRNA and steroid hormone production, which are essential for E2 synthesis and IGF-1 increased the GC number.

[Conclusion] Overall, these findings indicate that IGF-1 is critical for follicular growth and selection.

PB-02

Detection of polymorphism in *RLA* gene in two goat breeds in Vietnam: Is the mutation A781G in *RLA* gene lethal?

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Keywords: RLA, mutation, Co goat breed, Bach Thao goat breed

[Introduction] The *RLA* gene plays an important role in growth regulation and development of goat. Co goat and Bach Thao goat are two popular goat breeds in Vietnam which are mainly bred for meat and milk consumption. The genomic region consisting of part of exon 2, entire intron 2 and a part of exon 3 (422 bp) of *RLA* gene was analyzed for polymorphism in Co and Bach Thao goat breeds. The aim was to detect the polymorphism at A781G locus of *RLA* gene in Co goat and Bach Thao goat, as has been reported in Boer bucks and Chinese goat breeds.

[Materials and Methods] Blood samples (n=112) were collected at farmers' households. Genomic DNA was extracted according to the manufacturer's protocol. Primers used in PCR reactions was previously described. The allele and genotype frequencies were calculated by standard formula (Falconer and Mackay, 1996). The Chi-square test was used to test whether the population was in Hardy-Weinberg equilibrium at the A781G locus of the *RLA* gene.

[Results and Discussion] The locus A781G in exon 2 of the *RLA* gene was amplified from ovine genomic DNA. The size of amplicon obtained was 422 bp. The 422 bp fragment includes parts of exon 2 (126 bp), intron 2 (227 bp) and a portion of exon 3 (69 bp) of *RLA* gene. Restriction digestion of the amplified region by the endonuclease, HaeIII with a recognition sequence–GG/CC, showed polymorphism and revealed the existence of two genotypes, *i.e.*, AA (366 bp and 56 bp) and AB (422 bp, 366 bp and 56 bp), characterized by the presence of two alleles namely A and B (Fig1). BB genotype was absent. Genotypic frequencies for AA and AB were 0.43 and 0.57, respectively. The allele frequencies were 0.71 and 0.29 for A and B alleles, respectively. This variation is due to A781G transition, which caused an amino acid change from serine to glycine. The highly significant ($P<0.001$) Chi-square value (17.91) showed that the population is not under Hardy-Weinberg equilibrium.

The observed departure from Hardy-Weinberg equilibrium is due to the complete absence of the homozygous BB genotype, possibly due to natural selection. Heterozygous animals were present and were all free from physical deformities. This result indicates that the mutation A781G in the *RLA* gene is recessive lethal. A recent publication also reported the absence of BB genotype at A781G locus in 9 sheep breeds but did not conclude anything about the lethal effect of this mutation. This is the first report of lethal mutation (A781G locus) in the *RLA* gene of sheep. There are many studies revealing the existence of high homology between domestic animal and human genome sequences and hence, this mutation should also be explored in humans. The role of the *RLA* gene in oogenesis, follicular development and embryogenesis has been confirmed by earlier reports. It is concluded that this mutation could serve as a molecular marker and thus can be used for MAS in goat populations to avoid embryonic losses.

[References]

- Falconer DS, Mackay TFC. Introduction to Quantitative Genetics. 4th Edition, Addison Wesley Longman, Harlow (1996).

PB-03

Morphology of bovine uterine glands in various culture conditions

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Keywords: 3D culture, bovine, uterine gland, adenogenesis, extracellular matrix

[Introduction] In mammals, successful development and implantation of a conceptus is attributed to secretions from uterine glands (Spencer *et al.*, 2019). However, the detailed secretory functions of uterine glands have been unclear. A 3D-culture system is reported to be able to imitate the *in vivo* morphologies and functions of luminal epithelia. Submandibular glands and mammary glands formed gland-like structures in 3D-culture systems under the various kinds of supplemental factors and extracellular matrices (Steinberg *et al.*, 2005; Nguyen-Ngoc and Ewald, 2013). Here, we evaluated the effects of supplemental factors and extracellular matrices on the morphologies and functions of 3D-cultured bovine uterine glands.

[Materials and Methods] Uterine glands were isolated from bovine endometria by enzymatic digestion and filtration. Uterine gland fragments were embedded in basement membrane extract (Matrigel), collagen I, and gelatin methacryloyl (GelMA) mixed with DMEM/F12 in the presence of Wnt3a, Wnt5a, Wnt7a, EGF, or FGF2, respectively. After the 3-5 days culture, the regeneration rate and morphology of gland-like structures were evaluated. The gland like structures were also stained with a proliferation marker (Ki67), tight junction marker (ZO-1), and phalloidin (F-actin) using immunohistochemical analysis, and then the gene expressions of secreted proteins from uterine glands (*SERPINA14*, *MEP1B*, and *SPP1*) in isolated uterine glands, cysts, and gland like structures were examined using qRT-PCR.

[Results] In the Matrigel culture system, EGF induced aggregate formation from uterine gland fragments and turned into gland-like structures accompanying elongation with twisting after the 2-3 days culture. These morphological changes were not observed in the presence of Wnts and FGF2, respectively. The gland-like structures showed the complex of multiple and simple cell layers and partly expressed ZO-1 at the apical side of the cells. The mRNA expression of *SERPINA14* was significantly suppressed in gland-like structures compared with in cysts, but *MEP1B* and *SPP1* mRNA expressions did not change among isolated uterine glands, cysts, and gland like structures. In the collagen I culture system, gland-like structures were also formed under the EGF treatment, and the complex of multiple and simple cell layers was shown. The length and linearity of elongating parts of gland-like structures were significantly upregulated following the increasing collagen I concentration, however, the localization of Ki67 positive cells did not differ. In the GelMA culture system, few gland-like structures accompanying elongation were observed in spite of the EGF treatments. These data showed that the combination of supplemental factors and extracellular matrices affected the morphology of bovine uterine glands in the 3D-culture system.

[References]

- Spencer TE, Kelleher AM, Bartol FF. Development and function of uterine glands in domestic animals. *Annu. Rev. Anim. Biosci.* 7, 125-147 (2019).
- Steinberg Z, Myers C, Heim VM, Lathrop CA, Rebustini IT, Stewart JS, Larsen M, Hoffman MP. FGFR2b signaling regulates ex vivo submandibular gland epithelial cell proliferation and branching morphogenesis. *Development* 132, 1223-1234 (2005).
- Nguyen-Ngoc KV, Ewald AJ. Mammary ductal elongation and myoepithelial migration are regulated by the composition of the extracellular matrix. *J. Microsc.* 251, 212-223 (2013).

PB-04

Impact of thermal–humidity index on milk yield and reproduction traits on the 2 generations of crossbred cows in Ho Chi Minh City

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Keywords: heat stress, Temperature–Humidity Index (THI), milk production, crossbreed, Holstein Friesian

[Introduction] High-producing dairy cows, such as the Holstein breed, require THI <72 to show their optimum productivity performance. However, South Vietnam has two seasons with more than half a year having high temperatures and humidity. Crossbreeding the Holstein cow has a high potential to improve adaptation to the environment and milk production. Therefore, the aim of this study is to investigate the effect of high temperature and humidity on milk production and reproduction in two generations of Holstein Friesian crossbred (HF) dairy cows in the model farm located in Ho Chi Minh City.

[Materials and Methods] The farm, situated in Ho Chi Minh City, adopts a confinement farming approach to rear generations of crossbred HF dairy cattle. During a 3-month investigation (from March to May), we carefully selected 63 dairy cows responsible for milk production. The milk quantity was recorded from each F1 and F2 hybrid animal participating in the examination. The THI index was calculated by using the mentioned formula after careful monitoring and recording of temperature and humidity data. Moreover, we collected crucial details regarding the production of milk, factors associated with fertility (such as the age at which they first mate, the coefficient of mating, the secondary sex ratio, etc.) from the cows chosen for the survey.

[Results / Results and Discussion] The results show that the THI index ranged from 78.3 to 84.3, showing that the cows were always in a state of mild to severe heat stress with an increase in respiratory rate and rectal temperature; especially at noon. The age of first breeding and first calving age in F1 cow group was earlier than that in the F2 cow group. The fertilization coefficient was 2.28 times higher in the F1 group than in the F2 group. However, the F1 group had slower postpartum reconstitution time than the F2 group. In terms of average milk yield and total milk yield, group F1 was lower than group F2 (19.9 kg/day compared to 22.4 kg/day; 6750 kg/yield compared to 7044 kg/yield, respectively). The number of male calves being born was higher than that of female calves. The results indicate that the F1 group has better fertility indicators than the F2 group. While the milk production productivity in the F2 group is better than in the F1 group. Improving barn temperature and humidity, particularly during noon and afternoon, may ensure that cows have an optimal living environment and enhanced productivity.

[References]

- Atrian P, Shahryar HA. Heat stress in dairy cows (a review). *Res. Zool.* 2, 31-37 (2012).
- Dimov D, Penev T, Marinov I. Temperature-humidity index–an indicator for prediction of heat stress in dairy cows. *Vet. Ir Zootech.* 78, 10–15 (2020).
- Habeeb AA, Gad AE, Atta MA. Temperature-humidity indices as indicators to heat stress of climatic conditions with relation to production and reproduction of farm animals. *Int. J. Biotech. Recent Adv.* 1, 35-50 (2018).

PB-05

The effect of 5-aminolevulinic acid supplementation against microbiota composition in laying hen infected with *Eimeria tenella*.

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Keywords: 5-aminolevulinica acid, *Eimeria tenella*, microbiota, laying hen

[Introduction] Acute avian coccidiosis, caused by *Eimeria tenella* infection, leads to a loss of performance, which results in significant economic losses. *E. tenella* infection destroys epithelial tissues, and bloody feces, diarrhea, and body weight loss are observed as major symptoms. Anti-coccidial drugs such as ionophores have been commonly used for the control of coccidiosis. However, several problems, such as the emergence of drug-resistant parasite strains, have arisen, so there is an urgent need to develop new ways to deal with them. Recently, we have reported that 5-aminolevulinic acid (5-ALA), a precursor of heme biosynthesis, has beneficial effects against laying hens infected with *E. tenella* by the modification of cecal inflammation. However, its mode of action is still unclear. Recently, it has become clear that the microbiota affects various diseases. Several researchers reported that *E. tenella* infection led to disturb the diversity and composition of the intestinal microbiota. Hence, we focused on the microbial flora to elucidate the mechanisms of action of 5-ALA. This study aimed to evaluate the microbial flora in both cecum and feces from *E. tenella*-infected chicks in order to elucidate the mode of action of 5-ALA.

[Materials and Methods] Chicks (White Leghorn) were divided randomly into two groups: control (CT) group and 5-ALA 20 ppm administered (5-ALA) group. The chicks in 5-ALA group were fed 5-ALA supplemented meals from 7 days old. At 14 days post-hatch, the chicks in both groups were orally inoculated with mature sporulated oocysts of *E. tenella* OPU strain (2.5×10^3 oocysts per chick). Feces were collected daily for oocyst counting using the fecal floatation method. Feces (FE) and cecal contents (CE) were collected at 5 days post infection (dpi) for analysis of microbiota. Cecal and Fecal DNA was extracted for by next generation sequencing using the 16s rRNA V4 regions primers. The microbial compositions were evaluated for alpha diversity using Chao1 and Shannon index, beta diversity using Bray Curtis and UniFrac (weighted/unweighted) analysis, and Taxonomy analysis using SILVA (ver. 138).

[Results and Discussion] Total number of fecal oocysts was reduced in the feces from 5-ALA group, but not be a significant difference. According to microbiome analysis, significant differences of alpha diversity index in cecal contents with un-infection and beta diversity in all groups except for cecal contents with infection were observed by 5-ALA administration. 5-ALA administration significantly increased the relative abundance of the phylum *Firmicutes* and decreased that of phylum *Proteobacteria* in cecal contents with infection. 5-ALA administration decreased the relative abundance of the phylum *Cyanobacteria* in feces with un-infection. The abundance of *Lactobacillacease* was significantly higher at 5 dpi in the cecum of the 5-ALA group compared with the CT group. On the other hand, the abundance of *Enterococcaceae* was reduced by *E. tenella* infection in both groups. These results of next-generation sequence analysis indicated that *Lactobacillaceae,* including lactic acid bacteria in cecum, increased in the 5-ALA-administrated *E. tenella* infected group and 5-ALA administration might have a good effect against *E. tenella* infection by increasing them. These results may suggest that the administration of 5-ALA may be effective against avian coccidiosis by improving the intestinal environment.

PB-06

Morphological development of *Toxocara canis* eggs in vitro

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Keywords: *Toxocara canis* egg, morphology, incubation

[Introduction] *Toxocara canis* (Werner, 1782) is a typical gastrointestinal helminth found in canids. *T.canis* is distributed worldwide and is a major zoonotic helminth, causing human toxocariasis. This study aims to observe morphological changes from non-embryonated to infective stages of *T.canis* eggs outside the host.

[Materials and Methods] Rapid detection of the *Toxocara canis present* by flotation method. *T. canis* eggs were collected by using the sedimentation method and counted by using the McMaster method. Then, a total of 200 *T. canis* eggs were incubated in per petri dish containing 5 ml of distilled water at 30–33ºC with regularly aerated and refilled with distilled water equal to the initial water level for 21 days. The observation and record were performed daily until all embryos were developed into L2 larvae (infected larvae) by standard microscopy.

[Results and Discussion] This study showed the developmental stages of *T. canis* egg with one cell, two cells, three cells, four cells, early morula, late morula, blastula, gastrula, tadpole, pre-larva, embryonated larva. Blastula (spherical embryo with one side dark and one side lighter or shrunk into a solid form and concentrated on one side); Gastrula (outer layer of cells grows, the embryo folds into a kidney shape); Tadpole (folded embryos with clear kidney shapes, dark and even color); Pre-larva (the embryo has a "U" shape, the two ends of the "U" grow long and touch each other in a ring shape); Larvae 1 (the larvae are curled into rings, the body is quite rough, thick with dark spots, and actively move immediately after exposure to microscope light); Infective larvae L2 are long and slender; the density of spots of these larvae was much reduced in the esophagus and tail, but the internal structure was still unclear and the larvae had the movement when stimulated by microscope's light. Measurement results showed that there was no difference in the size of eggs containing one embryonic cell and eggs containing infective larvae statistically with a length of 83 ± 5.14 µm and a width of 73.43 ± 3.67 µm. Except for larva L1 and infective larvae L2, observations reveal that each stage lasts roughly 1-2 days. After 24 hours, 100% of one-cell eggs develop into two-cell, three-cell, and four-cell eggs, at rates of 61.15%, 14.10%, and 8.08%, respectively. By day 3, early morula was 0.64%, whereas late morula was 92.01%. The following day, 16.71% of eggs had reached the blastula stage, while 68.54% had achieved the gastrula stage. On day 5, 5.74% of eggs were in the pre-larva stage and 13.34% of eggs were at the tadpole stage. On day 6, the rate of eggs developing into larvae L1 was 97.5%. On day 7 and 8, the proportion of eggs that contained infective larvae L2 was 10.37% and 18.1%, respectively. From day 9 to day 21, 98.01% of eggs matured into infectious larvae L2, with no statistical difference until day 21.

[References]

- Abou-El-Naga IF. Development stages and viability of *Toxocara canis* eggs outside the host. *Biomédica* 38, 189-197 (2018).
- Azam D, Ukpai OM, Said A, Abd-Allah GA, Morgan ER. Temperature and the development and survival of infective *Toxocara canis* larvae. *Parasitol. Res.* 110, 649–656 (2012).
- Cruz LM, Allanson M, Kwa B, Azizan A, Izurieta R. 2012. Morphological changes of *Ascaris* spp. eggs during their development outside the host. *J. Parasitol.* 98, 63-68 (2012).
- Tekele MG. *Aspects of the morphology, life cycle and epidemiology of Toxocara species and Toxoscaris leonina.* Master of Sciences. University of the Free State, South Africa (2003).
- Passey RF, Fairbairn D. The conversion of fat to carbohydrate during embryonation of *Ascaris* eggs. *Can. J. Biochem. Physiol.* 35, 511– 525 (1957).

PB-07

A retrospective study on feline hypertrophic cardiomyopathy: prevalence and population characteristics of affected cats

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Keywords: feline hypertrophic cardiomyopathy, population characteristics, diagnostic imaging

[Introduction] Hypertrophic cardiomyopathy (HCM) is a primary myocardial defect. It is diagnosed when there is increased left ventricular wall thickness of a non-dilated ventricle (i.e., concentric left ventricular hypertrophy) without concurrent conditions affecting the left ventricular wall thickness, including systemic hypertension due to various causes, aortic stenosis, hyperthyroidism, or acromegaly. The disease is considered the most common cardiac disease in cats, accounting for 14.5% to 34% among apparently healthy cat population. Two mutations responsible for HCM have been identified in Maine coon and ragdoll cats, involving the cardiac myosin binding protein C gene (MYBPC3); however, the cause remains unknown for other cat breeds. Feline HCM has been established as a familial disease and has been observed in mix-breed, Persian, and American shorthair cats. The genetic mutations cause cardiac structural changes (disarray, hypertrophy, fibrosis) and functional abnormalities (incompliance, decreased cardiac output, etc.), which eventually lead to clinical manifestation and sudden death. Due to its heterogenous nature, the clinical presentations and characteristics on diagnostic imaging can be variable among individuals. Understanding this, we aim to study the prevalence and characteristics of cats diagnosed with hypertrophic cardiomyopathy. The study was conducted in Thonglor Pet Hospital Rama 9, Bangkok, Thailand.

[Materials and Methods] Electronic medical records of 61 cats diagnosed with hypertrophic cardiomyopathy were collected and classified into four groups: preclinical, clinical without congestive heart failure or arterial thromboembolism, clinical with congestive heart failure, and clinical with arterial thromboembolism. Assessment criteria included breed, age, sex, physical examination findings, and measurements and subjective findings on echocardiographic and radiographic examination.

[Results] Hypertrophic cardiomyopathy cases accounted for 17.3% of the study population and 58.6% of cases diagnosed with cardiac diseases. There were 5/61 (8.2%) asymptomatic cats, 47/61 (77.0%) cats showed clinical signs without congestive heart failure or arterial thromboembolism, 2/61 (3.3%) cats with congestive heart failure, and 7/61 (11.5%) cats suffered arterial thromboembolism. Affected cats were likely to be Thai cats, older than 6 years, and male. Variables significantly associated with the clinical status included fast heart rates, femoral pulse abnormalities, fast respiratory rates, tachypnea and dyspnea, hindlimb paresis on physical examination, increased end-diastolic interventricular septal thickness, large left atrial diameter, increased ratio between left atrial and aortic diameters, presence of thrombus/smoke within the left atrium on echocardiography, and cardiomegaly on radiography.

[Conclusion] Our study showed that hypertrophic cardiomyopathy had high prevalence in certain groups of cats, and several findings on physical examination and diagnostic imaging could aid in determining the clinical status of affected cats. However, because of the retrospective nature of this study, the collected cases were assessed and diagnosed by various clinicians with inconsistent procedures and subjective evaluation. Therefore, it is recommended that a controlled, prospective study about this topic be conducted in the future.

PB-08

Steroidogenesis and morphological characteristics of granulosa and luteinizing granulosa cells in various cell culture systems

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Keywords: granulosa cells, luteinizing granulosa cell, 2D, 3D, suspension cell culture

[Introduction] Cell culture is an invaluable tool for studying molecular physiology. However, over the last decades, 2D static culture has been a common method for granulosa cells (GCs) and luteinizing granulosa cells (LGCs). This study aimed to identify the most suitable cell culture system for bovine GCs and LGCs.

[Materials and Methods] GCs were obtained from small follicles (2–6 mm). To assess the effect of various cell culture systems on the quality of GCs and LGCs, GCs were cultured in 2D (adherent surface), suspension cell culture (hydrophobic surfaces), and a 3D culture system (cell-repellent surface). Cultures were maintained for up to 4 days under each condition, followed by a 24-hour treatment with 2 µg/ml insulin and 10 µM forskolin to mimic *in vitro* luteinization.

[Results] In the 2D cell culture, GCs and LGCs displayed a flattened, epithelial-like morphology, while suspension culture prompted GCs and LGCs clamping, individual cells showed flatted and elongated epithelial-like morphology. In a 3D cell culture, spheroids were formed by both GCs and LGCs, and the cell morphology appeared as oval and circular. Significantly higher levels of P4 (EIA) and gene expression related to steroidogenesis (*CYP11A1, STAR, and HSD3B* gene by RT-PCR) were detected in GCs cultured in suspension and 3D culture compared to the 2D culture system. Induction of *in vitro* luteinization in the suspension culture system led to further stimulation of P4 production and steroidogenic gene expression. Conversely, in the 3D spheroid cell culture system, stimulation of *in vitro* luteinization was associated with decreased P4 production and down-regulation of steroidogenic genes when compared to the 2D culture.

[Conclusion] This study suggests that suspension cell culture enhances E2 in GCs and P4 in LGCs. Therefore, it is recommended that suspension cell culture is an excellent model for synthesizing steroids and studying steroidogenesis in both GCs and LGCs.

PB-09

The direct effect of estradiol-17β in bovine oviductal contractility

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Keywords: cow, oviduct, smooth muscle, contraction and relaxation movement, estradiol-17β

[Introduction] Dysfunction of the oviduct is considered to be one of the causes of human infertility since the transport of gametes and embryos in the oviduct at an appropriate time is important for the establishment of pregnancy. Early embryo and sperm transport through the oviductal isthmus depends on the contraction and relaxation of the smooth muscle layers (Hunter, 2012). Estradiol-17β (E2) is known to indirectly increase contractility by promoting the secretion of oviductal contractile factors such as endothelin. Previous research has shown that the herbal medicine Tokishakuyakusan directly increases bovine oviductal tonus via G protein coupled estrogen receptor 1 (GPER1) (Kubota *et al.*, 2022). In this study, we investigated that the direct effect of E2, the original ligand of GPER1, on the contraction and relaxation of bovine oviductal smooth muscle and its mechanism of the effect.

[Materials and Methods] We used bovine oviductal isthmic tissues at four stages of the estrous cycle: stage I (1-4 days after ovulation), stage II (5-10 days), stage III (11-17 days), stage IV (18-20 days). Isthmic tissues cut into 3-4 pieces of 5 mm length were used for the Magnus method to monitor the longitudinal contractility (contraction frequency, contraction force, and tonus). The effect of E2 (1 or 10 nM) and G protein-coupled estrogen receptor 1 (GPER1) agonist (G-1, 1 or 10 μM) on oviductal contractility were examined. In the same way, the effects of E2 (1 nM) with pre-treatment of GPER1 antagonist (G-15, 25 or 250 nM) and Rho kinase (ROCK) inhibitor (Y-27632, 1 μM) were also examined. Furthermore, the protein expression level of GPER1, RhoA, and ROCK II in the oviductal smooth muscle of each stage was measured by Western blotting.

[Results and Discussion] E2 had no effects on the frequency and contraction force at all stages. However, the tonus was significantly increased by 1 nM E2 in stage I. The tonus in stages II-IV did not change by E2 treatment. Both G-1 treatment groups also increased oviductal tonus similar to E2 at stage I. The treatment of G-15 and Y-27632 significantly suppressed the E2-induced increase of oviductal tonus in stage I. There was no significant difference in GPER1 and RhoA protein expression among the estrous stages. On the other hand, the protein expression of ROCK II in stage I was higher than that in stage II. In conclusion, 1 nM E2 directly affects oviductal contractility by increasing tonus via GPER1 and ROCK II activation in stage I. The difference in the effect of E2 among each stage may be due to differences in the expression level of ROCK II.

[References]

- Hunter RH. Components of oviduct physiology in eutherian mammals. *Biol. Rev. Camb. Philos. Soc.* 87, 244-255 (2012).
- Kubota S, Yamamoto Y, Kimura K. A Chinese medicine, Tokishakuyakusan, increases bovine oviductal tonus via G protein-coupled estrogen receptor 1. *Biol. Pharm. Bull.* 45, 1133-1141 (2022).

PB-10

Mitochondrial DNA copy number in frozen-thawed bull spermatozoa is negatively correlated with the motility

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Keywords: spermatozoa, bulls, mitochondrial DNA, motility, frozen semen

[Objective] Objective of the current study was to investigate the relationship between mitochondrial DNA copy number and motility parameters in commercial frozen-thawed bull spermatozoa.

[Materials and Methods] In the first experiment, mitochondrial DNA copy number (MDCN), mitochondrial content (MC), percentage of spermatozoa with high mitochondrial membrane potential (HMMP), intracellular reactive oxygen species (ROS) and motility parameters of frozen-thawed spermatozoa were examined in five different bulls by using a quantitative real-time PCR (qPCR), flow cytometry and computer-assisted sperm analysis (CASA), respectively, and analyzed the relationships. In the second experiment, to determine if MDCN, MC, HMMP, and motility indicators of frozen-thawed spermatozoa drastically change through life, those were examined in samples prepared at three different points in the lives of four bulls by using qPCR, flow cytometry and CASA, respectively, and analyzed the correlations.

[Results and Conclusion] Results in the first experiment showed that all parameters examined, including MDCN, MC, HMMP, ROS and motility indicators, significantly differed among frozen-thawed spermatozoa from five bulls. Both MDCN and MC were negatively correlated with HMMP and motility indicators, but were positively correlated with ROS, whereas there was a highly positive relationship between MDCN and MC. In the second experiment, MDCN and MC determined in frozen-thawed spermatozoa derived from three different points in the lives of four bulls did not find any trends and correlations through their lives (1.3-14.3 years old). From these results, we conclude that MDCN and MC differ among frozen-thawed spermatozoa prepared from different sires, and those are negatively correlated with HMMP and sperm motility parameters, but positively correlated with ROS, demonstrating that these appear to be useful markers to assess sires' spermatozoa. Furthermore, the current simple method of measuring sperm MC by fluorescence intensity under flow cytometry is a reliable sperm evaluation.

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PB-11

Effects of culture condition based on *in vivo* ovarian tissue temperature on the *in vitro* growth and developmental competence of oocytes derived from bovine early antral follicles

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[Introduction] Numerous developing oocytes exist in mammalian ovaries. *In vitro* growth (IVG) culture system for those immature oocytes enables to produce superior livestock efficiently. In cattle, culture temperature used for the IVG of oocytes is generally 38.5 or 39.0℃, which is close to the normal temperature in the vagina or rectum. However, temperature on the ovarian tissue is reported to be 1℃ lower (37.5℃) than those in the vagina or rectum, therefore, the current temperature condition could be inappropriate for the IVG of bovine oocyte. In the present study, we investigated the effects of culture condition based on *in vivo* ovarian tissue temperature on the *in vitro* growth and developmental competence of oocytes using IVG of oocyte-cumulus granulosa complexes (OCGCs) derived from early antral follicles (0.5-1 mm in diameter).

[Methods] A total of 524 OCGCs were subjected to 12 days of IVG culture at temperature of 37.5℃, 38.5℃, or 39.0℃. The viability of OCGCs and the formation of antrum in the granulosa cells layer were evaluated every 4 days of IVG culture, and half of the culture medium was replaced simultaneously. Estradiol-17β (E_2) and progesterone (P_4) productions during the 1st, 2nd and 3rd 4-day periods were measured by enzyme immunoassay. Some of the viable OCGCs after IVG (n = 94) were subjected to *in vitro* maturation (IVM), and the nuclear status and diameter of oocytes were evaluated; granulosa cells derived from part of these OCGCs (n = 18/94) were used for evaluating mRNA expressions of Heat Shock Proteins (HSPs) 70 and 90 as markers of heat stress. Other OCGCs after IVG (n = 63) were used for the measurement of reduced glutathione (GSH) levels in oocytes, which is an indicator of oxidative stress. Rest of OCGCs after IVG and IVM (n = 178) were subjected to *in vitro* fertilization and embryo culture, and the cleavage rate, blastocyst rate, and cell number in blastocyst were evaluated.

[Results] The viability rate of OCGCs did not differ among the groups, while the rate of antrum formation on day 12 of IVG culture was approximately 13 points higher in the 37.5℃ group (91.7%) than in the other groups ($P < 0.05$). The P_4 production did not differ among the groups, however, E_2 production during days 8-12 tended to be higher in the 37.5℃ group than in the combined 38.5 and 39.0℃ groups ($P < 0.1$). The mRNA expressions of HSPs 70 and 90, and GSH levels of oocytes did not differ among the groups. The oocyte diameter after culture was larger in the 37.5℃ group (mean ± SD; 120.1 ± 2.9 μm) than in the 39.0℃ group (116.9 ± 3.3 μm) ($P < 0.05$), and that in the 38.5℃ group was intermediate between the other two groups (118.1 ± 2.9 μm). The rates of nuclear maturation and cleavage did not differ among the groups. However, the rate of blastocyst was higher in the 37.5℃ group (61.9%) and 38.5℃ (61.2%) than in the 39.0℃ group (41.7%), and the cell number in the blastocyst was higher in the 37.5℃ (mean ± SD; 150 ± 64 cells) and 39.0℃ groups (148 ± 47 cells) than in the 38.5℃ group (122 ± 48 cells) ($P < 0.05$).

[Conclusion] OCGCs in the 37.5℃ group showed healthy morphology and steroidogenesis, and greater growth and developmental competence of oocytes. Therefore, culture condition close to the *in vivo* ovarian tissue temperature is appropriate for the IVG of bovine oocytes derived from early antral follicles.