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Ho Chi Minh City, Vietnam

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Program

10:00-10:20 JST (8:00-8:20 VST) Opening ceremony (Venue A)

Prof. Yoshinobu KIMURA (Dean of the Faculty of Agriculture, Okayama University)
Assoc. Prof. Toan Tat NGUYEN (Vice President, Nong Lam University)
Prof. Hiroaki FUNAHASHI (Executive Director for Academic Affairs, Okayama University)

10:20-12:00 JST (8:20-10:00 VST) Plenary session (Venue A)

Chairperson: Assoc. Prof. Thong Quang LE (Nong Lam University)
Prof. Koji KIMURA (Okayama University)

IP-1. **Livestock production systems respond to the decline in available resources and climate change in Vietnam**
○Khang Nguyen DUONG (Nong Lam University)

IP-2. **Role of gut IgA in the pathogenesis of high fat diet-induced disorders**
○Takeshi TSURUTA, Teresia Aluoch MUHOMAH, Kei SONOYAMA, Qui D. NGUYEN, Mao TERAOKA, Yurika TAKASE, Aoi NISHIJIMA, Shiori HIMOTO, Emiko KATSUMATA, Naoki NISHINO (Okayama University, Hokkaido University)

IP-3. **Herbal products for animal health - a solution for reduction of antibiotic use**
○Thi Tra An VO (Nong Lam University)

IP-4. **From a lab to farms: Cellular functions in bovine reproductive organs**
○Yuki YAMAMOTO, Koji KIMURA (Okayama University)

12:00-12:30 JST (10:00-10:30 VST) Coffee break and video exhibition

12:30-14:00 JST (10:30-12:00 VST) Oral presentation Part 1 (Parallel session at Venues A and B)

Venue A

Chairperson: Assoc. Prof. Yuki YAMAMOTO (Okayama University)
Assoc. Prof. Thieu Quang NGUYEN (Nong Lam University)

PA-1 **Refractile / lipofuscin bodies in human oocytes are present over a year prior to ovulation and associated with lysosome localization**
○Yuto AOKI, Mone TAKESHITA, Hidetaka TASAKI, Mikiya NAKATSUKA, Junko OTSUKI (Okayama University)

PA-2 **Chromosomal missegregation and low developmental rates in female mutant mice upon the failure of crossing over during meiosis**
○Mone TAKESHITA, Nanami SONO, Hidetaka TASAKI, Tetsuo KUNIEDA, Junko OTSUKI (Okayama University, Okayama University of Science)

PA-3 **The effects of supplementing cryopreservation solution with astaxanthin during the process of mouse sperm cryopreservation**
○Moeka ISHIHARA, Natsuho MORI, Hidetaka TASAKI, Junko OTSUKI (Okayama University)

PA-4 **The effect of betaine for mouse sperm cryopreservation**
○Natsuho MORI, Moeka ISHIHARA, Hidetaka TASAKI, Junko OTSUKI (Okayama University)

PA-5 **Risk factors for African swine fever in Phu Tan district, An Giang province**
○ Thi Viet Thu HO, Thi My Trang HIEN (Can Tho University)

PA-6 **Marek's disease in vaccinated free-range chickens in the Mekong delta**
○ Nguyen Tran Phuoc CHIEN, Ho Thi Viet THU (Can Tho University)

Venue B

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

PB-1 **Blood metabolites and rumen and fecal microbiota of Jersey cows throughout a lactation period**
○Yousofi ZABIALLA, Peter Kiiru GATHINJI, NUOMIN, Riyan BAEK, Souta ASHIDA, Ayumi MIYAKE, Takeshi TSURUTA, Naoki NISHINO (Okayama University, Chugoku-Shikoku Dairy College)

PB-2 **Changes in the blood metabolites concentration, milk composition, and milk microbiota of Holstein cows during a lactation period**
○Peter Kiiru GATHINJI, Karin AKADA, Daohu AO, Sayo NIBUNO, Masumi KANADANI, Takeshi TSURUTA, Naoki NISHINO (Okayama University, Okayama Prefecture Livestock Research Institute)

PB-3 **Changes in blood metabolites concentration and fecal microbiota of dairy calves before and after weaning**
○NUOMIN, Daohu AO, Takeshi TSURUTA, Naoki NISHINO (Okayama University)

PB-4 **Bacterial and fungal microbiota of guinea grass silage stored at moderate and high ambient temperatures with and without wilting**
○Jianjian HOU, Naoki NISHINO, Takeshi TSURUTA (Okayama University)

PB-5 **Effects of Empyreal® 75 on egg production and egg quality of Isa Brown layers**
○Thi Diem Thi PHAN, Lê Van THANH, Huynh Ngoc Han NGUYEN, Duy Hoa NGUYEN, Quang Thieu NGUYEN (Nong Lam University, Cargill)

PB-6 **Astaxanthin enhances production performance and egg quality of laying hens**
○Hong-Phuong NGO, Tuan Hue TRAN, Dinh Nam TRUONG (Nong Lam University)

14:00-15:00 JST (12:00-13:00 VST) Lunch and coffee break

15:00-16:30 JST (13:00-14:30 VST) Oral presentation Part 2 (Parallel session at Venues A and B)

Venue A

Chairperson: Prof. Koji KIMURA (Okayama University)
Assoc. Prof. Thieu Quang NGUYEN (Nong Lam University)

PA-7 **Development of a smooth endoplasmic reticulum cluster model employing mice**
○Chihiro KOMATSU, Hidetaka TASAKI, Junko OTSUKI (Okayama University)

PA-8 **Developmental trajectory of monopronucleated zygotes after in vitro fertilization when they include both male and female genomes**
○Xingqiang WEI, Noritoshi ENATSU, Kohyu FURUHASHI, Toshiroh IWASAKI, Shoji KOKEGUCHI, Masahide SHIOTANI, Junko OTSUKI (Okayama University, Hanabusa Women's Clinic)

PA-9 **The mechanics of abnormal cytokinesis and aggregated chromosome in mouse oocytes when cytoplasmic cyclic adenosine monophosphate is elevated during meiosis**
○Wei XIAO, Hidetaka TASAKI, Junko OTSUKI (Okayama University)

PA-10 **Prevalence and genetic characterization of chicken anemia virus in the Mekong delta, Vietnam**
○Dao Huyen TRAN, Nguyen Thanh LAM, Nguyen Van DANG, Le Nguyen Bao CHAU, Nguyen Khanh THUAN, Nguyen Phuc KHANH, Ly Thi Lien KHAI, Tran Ngoc BICH (Can Tho University)

PA-11 **Molecular characterization of foot-and-mouth disease viruses circulating in the Mekong delta, Vietnam**
○Nguyen Phuc KHANH, Tran Ngoc BICH, Nguyen Thanh LAM, Nguyen Khanh THUAN, Tran Duy KHANG (Can Tho University)

PA-12 **The orexigenic activity of pituitary adenylate cyclase-activating polypeptide in the ventromedial hypothalamus through the increment of agouti-related peptide in mice**
○Thanh Trung NGUYEN, Yuki KAMBE, Atsuro MIYATA, Thanh Ha Nguyen THI, Van Phai DAM (Vietnam National University of Agriculture, Kagoshima University)

Venue B

Chairperson: Assoc. Prof. Kensuke ARAKAWA (Okayama University)
Prof. Khang Nguyen DUONG (Nong Lam University)

PB-7 **Effect of lard-, olive oil- and soybean oil-enriched diet on immunoglobulin A coating of gut bacteria**
○Mao TERAOKA, Naoki NISHINO, Takeshi TSURUTA (Okayama University)

PB-8 **Effect of heat-killed *Lactobacillus plantarum* SNK strain on mucosal digestive enzymes activity in small intestine of aged mice**
○Aoi NISHIJIMA, Takumi WATANABE, Akihito IKEDA, Naoki NISHINO, Takeshi TSURUTA (Okayama University, Bio-Lab Co., Ltd.)

PB-9 **The relationship between milk, udder skin, bedding, and fecal microbiota in a dairy farm**
○Daohu AO, Karin AKADA, Peter Kiiru GATHINJI, Takeshi TSURUTA, Naoki NISHINO (Okayama University)

PB-10 **Monitoring antimicrobial sales intended for livestock at veterinary drug shops in Vietnam**
○Ha Le Thi THU, Chalalai RUEANGHIRAN, Giang Nguyen Thi HUONG, Thuy Doan PHUONG, Phu Doan HOANG, Kiet Bach TUAN, Hien Vo BE, Hue Le THI, Pawin PADUNGTOD, Juan J CARRIQUE-MAS, Bao Dinh TRUONG (Kasetsart University,

Oxford University, Bac Giang Agriculture and Forestry University, Nong Lam University, Emergency Centre for Transboundary Animal Diseases, Food and Agriculture Organization of the United Nations)

PB-11 **Minimal bactericidal concentration of aqueous extract from *Pouzolzia zeylanica L.* against *Pasteurella multocida*, *Bordetella bronchiseptica*, *Actinobacillus pleuropneumoniae* from swine**
○Thi Tra An VO, Hai Trieu LY (Nong Lam University)

PB-12 ***Campylobacter hepaticus*, the cause of spotty liver disease in chickens: transmission and routes of infection**
○Phung CANH (RMIT University, National Institute of Animal Science-Vietnam)

16:30-17:00 JST (14:30-15:00 VST) Coffee break and video exhibition

17:00-18:45 JST (15:00-16:45 VST) Oral presentation Part 3 (Parallel session at Venues A and B)

Venue A

Chairperson: Assoc. Prof. Junko OTSUKI (Okayama University)
Assoc. Prof. Thieu Quang NGUYEN (Nong Lam University)

PA-13 **Tokishakuyakusan increases bovine oviductal tonus via G protein-coupled estrogen receptor 1**
○Sayaka KUBOTA, Yuki YAMAMOTO, Koji KIMURA (Okayama University)

PA-14 **The involvement of endoplasmic reticulum stress in bovine corpus luteum regression**
○Maho MUNETOMO, Koko ONISHI, Koji KIMURA (Okayama University)

PA-15 **Formation of bovine uterine gland like structure in *in vivo* 3D culture system**
○Yosuke SUGINO, Taiki SATO, Nozomi FUJIWARA, Yuki YAMAMOTO, Koji KIMURA (Okayama University)

PA-16 **Effects of non-esterified fatty acids and ketone body on cellular functions of bovine endometrial cells**
○Ibuki UMEBARA, Shunsuke SAKAI, Yuki YAMAMOTO, Koji KIMURA (Okayama University)

PA-17 **Metritis prevalence and treatment efficacy on reproductive beef cattle in Tien Giang province**
○Tran Hoang DIEP, Nguyen Trong NGU (Can Tho University)

PA-18 **LAMP-based detection of TL9 mutant allele in commercial pig population in south of Vietnam**
○Huu Tinh NGUYEN, Bao Quoc NGUYEN, Phu Nam Anh BUI, Mai Nghiep NGUYEN (Institute of Animal Sciences for Southern Vietnam, Institute of Agricultural Sciences for Southern Vietnam, Nong Lam University)

Venue B

Chairperson: Assoc. Prof. Takeshi TSURUTA (Okayama University)
Prof. Khang Nguyen DUONG (Nong Lam University)

PB-13 **Rapid thawing by transient exposure to 70℃ water improves the viability and motility, but not acrosome integrity and PLC zeta-1 distribution of frozen bull spermatozoa**
○Hai Thanh NGUYEN, Son Quang DO, Rukmali ATHURUPANA, Takuya WAKAI, Hiroaki FUNAHASHI (Okayama University, Nong Lam University)

PB-14 **Mitochondrial distribution in oocytes and early embryos from naturally aged mice**
○Sayaka NAKATO, Haruna GEKKO, Hiroaki FUNAHASHI, Takuya WAKAI (Okayama University)

PB-15 **Molecular changes in the intestinal barrier function associate with clinical signs in *Eimeria tenella* early infection periods**
○Hung Hoang Son PHAM, Toshimitsu HATABU (Okayama University)

PB-16 **Production optimization and structural characterization of exopolysaccharides from *Pediococcus pentosceus* FFC003**
○Junliang ZHAO, Kensuke ARAKAWA, Daiki NISHIKAWA, HASIQIMUGE, WULIJIDELIGEN, Nobutada MURAKAMI, Tadatoshi MURAKAMI, Hidetoshi MORITA, Taku MIYAMOTO (Okayama University, Functional Food Creation Research Institute Co., Ltd., Kurashiki-Sakuyo University)

PB-17 **Survey on *Escherichia coli*, *Salmonella* contamination and residues of some antibiotic in pork and chicken in some provinces of Nam bo southwest region**
○Hong Phong LE, Minh Chau VO, Minh Hieu NGUYEN, Thị Thi NGUYEN, Thị Kim Cuc NGUYEN, Thi Diem Hang BUI (National Center for Veterinary Hygiene Inspection No. II)

PB-18 **Prevalence and antibiotic resistance of enterotoxigenic *Escherichia coli* (etec) serotypes O8, O9 isolated from cattle in the Mekong delta, Vietnam**
○Khanh Thuan NGUYEN, Thanh Lam NGUYEN, Phuc Khanh NGUYEN, Thi Thanh Tien VO, Van Sac TRAN, Thi Lien Khai LY, Ngoc Bich TRAN (Can Tho University)

PB-19 **Create beeswax candles with the effect of repelling mosquitoes from natural essential oil**
○Thi Kim Phuong PHAM and Ba Trung NGUYEN (An Giang University, Vietnam National University, Ho Chi Minh City)

18:45-19:00 JST (16:45-17:00VST) Closing ceremony
Symposium summary and excellent presentation award
Prof. Naoki NISHINO (Okayama University)
Assoc. Prof. Thong Quang LE (Nong Lam University)

IP-1

Livestock production systems respond to the decline in available resources and climate change in Vietnam

○**Nguyen Khang DUONG**

(Nong Lam University of Ho Chi Minh City)

Decline in available resources, feed/food production and climate change

Fossil fuel energy will be the main depleting resource as the world uses more than peak production levels (Shafiee and Topal, 2009). As oil reserves are depleted, prices will rise continuously with increasing scarcity and additional demand is now coming due to the increase in wealth in many emerging economies. In these countries, the need to produce material wealth for society is greater. As a result, energy use is unlikely to be reduced. Countries must prepare for a significant rate of depletion of their oil reserves and a large increase in natural costs. The world's population growth has been fueled by the availability of cheap oil, which has supported a "Green Revolution" by providing cheap inputs including fertilizers, pesticides, herbicides, traction… Reducing labor demand and reducing the number of people engaged in agriculture and where irrigation water is available. Cheap oil allows for cheap food production but this will change greatly as oil prices rise, creating the risk of major disruptions to food supplies and even starvation. Feed cost increased more than 20% in recent years in Vietnam. Moreover, using fossil fuels also produces large amounts of greenhouse gas emissions!

Peak oil represents a massive change and will affect other resource availabilities. Agriculture has received inexpensive fertilizers on which high crop yields have been predicated including nitrogen, phosphates and potassium fertilizers.

The dependency of the industrialized countries on oil to drive agricultural production, and the fact that most of these cannot meet their own domestic requirements for energy, has seen a development of alternative fuels including bioethanol produced from sugar cane and maize in Brazil and the USA; bio diesel from plant oils in SE Asia. The production of biofuels and its effect on land use and grain availability is caused for the price hike in food prices. Cereal grain availability for industrial livestock production such as pig, poultry and beef will be highly restricted in coming years and the fall in meat production can only be replaced by expanding the production of the forage-fed ruminant particularly based on crop byproducts (Leng, 2005). Ruminants are the logical animals for future meat and milk production but herbivores in general are likely to be used more extensively with time, particularly the rabbit with its dual capabilities of high reproduction rates and the capacity to utilize efficiently forage resources produced locally. But ruminant produces more methane production and greenhouse! Moreover biofuels production diverts land from food production to transportation energy, affecting biodiversity, erosion and the carbon balance of the land area.

Water required for agriculture has also been depleted (Groundwater Decline and Depletion, USA, 2019). Many of the worlds large river systems are being drained for urban and industrial water supplies or for irrigated crops before they reach a sea or a lake (Climate change, 2021). Other water also decline such as glacial melt, ghengetic plains, fossil ground water... People need more oil for the pumping of deep water, will clearly cut back crop production in many areas and cause a return of vast areas of highly productive irrigated crop land back to rain fed cropping, pasture or desert in the future with loss of food productivity.

Soil erosion and fertilizer run off from cropping systems are also major concerns as the present day because of cereal crops only tap the nutrients in the top few inches of soil. Farmers used a lot of chemical fertilizer for soil, killed soil micro-organisms, caused more erosion. Response of crops to fertilizer application has been slowly diminishing. Crop products are poor nutrient to animal and human requirement.

Global warming is accepted as occurring and cannot be ignored in any discussion on future agriculture in many countries. Increasing sea levels will remove considerable areas of fertile delta. Therefore weather will certainly change, leading to more intense drought and or flooding rains. Then, this warming also carries with it the risk of decreased crop production, with high agricultural cost.

How is livestock production systems response in Vietnam?

- Grain for animal production will become increasingly expensive as the competition for resources for food, feed and fuel, develops... Therefore, the animal production industries based on herbivores will need extensive development based on better use the waste byproducts of agriculture or will have to be produced from land not dedicated to food or biofuels production!
- Increase production of livestock and fish in farming systems basin through management and better use of local feed and animal genetic resources.
- Reduce greenhouse gas emissions from agricultural activities.
- Reduce N pollution and P pollution from livestock and aquaculture.
- To reduce post-harvest loss, improve access to markets and improve food safety.
- Better use wastes for development the larvae of black soldier fly, earthworm, biodigester, manure compost… to reduce greenhouse emission.
- Promote the use of prebiotics and probiotics as replacement for anti-microbial drugs in feed for livestock and aquaculture.
- Adapt farming systems to variable climate and unpredictable weather conditions.
- Increase the access of small-holder farmers to renewable sources of energy.
- Establish networks on farming systems with low carbon footprints.
- Strengthen the capacity for research and apply in the development of carbon-negative farming systems.
- Transfer of technologies based on to adapt the value chain.
- Increase the participation of women, young union, farmer association… in the application of the technologies developed.

Conclusion

Oil depletion, produce biofuels, soil fertility decline caused the high cost of chemical fertilizers, decreasing irrigation water and the loss of arable land to erosion, non agricultural purposes, coupled with decreases in crop production from global warming will be difficult for many nations to food supply. The developing countries have to provide by themselves through the maintenance of small farmer practices that integrate food and fuel production, particularly consider to reduce post-harvest loss, improve access to markets and improve food safety.

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IP-2

Role of gut IgA in the pathogenesis of high fat diet-induced disorders

○Takeshi TSURUTA[1]*, Teresia Aluoch MUHOMAH[1], Kei SONOYAMA[2], Qui D. NGUYEN[1], Mao TERAOKA[1], Yurika TAKASE[1], Aoi NISHIJIMA[1], Shiori HIMOTO[1], Emiko KATSUMATA[1], Naoki NISHINO[1]

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Keywords: IgA, gut bacteria, high fat diet, hyperinsulinemia

[Introduction] Immunoglobulin A (IgA) is the most abundant antibody secreted into the gut and plays a crucial role in immune exclusion by neutralizing pathogenic toxins and preventing pathogen attachment and invasion across the mucosal epithelial barrier. Meanwhile, recent studies revealed that gut IgA also contributes to symbiosis with 40 trillion commensal bacteria that inhabit the gut. Gut IgA selectively coats gut bacteria and the IgA coating of gut bacteria influences on commensal bacterial composition by regulating bacterial growth. Excessive consumption of dietary fat alters the commensal gut bacterial composition, defined as gut dysbiosis, which is characterized by a decrease in the phylum *Bacteroidetes* and an increase in both *Firmicutes* and *Proteobacteria*. High-fat diet (HFD) feeding increases movement of unabsorbed bile acid and dietary lipid including saturated fatty acid into the distal gut. Unabsorbed bile acids are hydrolyzed into secondary bile acids by gut microbial bile salt hydrolase. The secondary bile acids and saturated fatty acid have bactericidal properties and relate to alter the gut bacterial composition. In addition to these factors, we hypothesized that HFD feeding may affect the IgA coating of gut bacteria, which relates to HFD-induced gut dysbiosis, because IgA coating of gut bacteria contributes to maintain a stable gut bacterial composition. To test our hypothesis, we evaluated influence of HFD feeding on the amount of IgA coating gut bacteria in Experiment 1. As a result of Exp. 1, we observed that HFD feeding reduces amount of IgA coating gut bacteria as compared to normal-fat diet (NFD) feeding. In Experiment 2, we verified whether HFD-induced reduction of IgA coating is associated with HFD-induced gut dysbiosis by using IgA-deficient mice. Association with HFD-induced other disorders including obesity and type 2 diabetes symptoms were also verified.

[Materials and Methods] Experiment 1> BALB/c mice were allocated into 2 groups and allowed free access to water and to either a NFD or a HFD for 12 weeks. Fecal samples were collected at 6 and 12 weeks. Other 10 mice were divided into 2 groups; one group was fed NFD for 18 weeks, and the other group was fed HFD for the first 12 weeks and then switched to NFD for the following 6 weeks. At 18 weeks, fecal samples were collected. The fecal bacteria was collected from the feces and stained with FITC-labelled anti-mouse IgA and propidium iodide (PI) for IgA and bacterial nucleus staining. The bacterial suspension was analyzed by flow cytometer. The FITC and PI positive population was regarded as IgA-coated bacteria. The average FITC intensity emitted by a single IgA-coated bacterium was defined as the amount of IgA coating fecal bacteria. Experiment 2> IgA-deficient mice (IgA^-, no gut IgA secretion) and the wild type mice (IgA^+) were allocated into 2 groups and allowed free access to water and to either a NFD or a HFD for 12 weeks, respectively. Fecal samples were collected at 12 weeks and the amount of IgA coating fecal bacteria was evaluated by flow cytometer as described above. The bacterial DNA was extracted from the feces at 12 weeks and used for PCR amplification of 16S rRNA genes. The amplicons were pair-end sequenced on an Illumina MiSeq platform. Principle coordinate analysis (PCoA) analysis was performed to evaluate the similarity of bacterial composition among groups using Primer version 7. At 12 weeks, final body weight was measured and mice were sacrificed by CO_2 inhalation after 12 hours fasting. To evaluate the blood lipid profiles and fat

accumulation in the adipose tissue, the triglycerides, non-esterified fatty acid and total cholesterol levels in the serum and parametrial adipose tissue were measured. To evaluate the symptoms of type 2 diabetes, the fasting blood glucose and insulin levels were measured using the serum and the homeostasis model assessment for insulin resistance (HOMA-IR) index was calculated.

[Results and Discussion] Experiment 1> The amount of IgA coating fecal bacteria was significantly lower in HFD-fed mice than in NFD-fed mice at 6 and 12 weeks, suggesting that HFD feeding reduces IgA reactivity to gut bacteria. There were no significant differences in the amount of IgA coating fecal bacteria between NFD-fed mice and mice fed HFD for the first 12 weeks and NFD for the following 6 weeks. This result implies that HFD-induced reduction of IgA coating of gut bacteria may be recovered by decreased fat consumption. Experiment 2> As with the result of Exp. 1, the amount of IgA coating fecal bacteria was significantly reduced in HFD-fed IgA^+ mice as compared to NFD-fed IgA^+ mice. Meanwhile, any fecal bacteria were not coated with IgA in NFD-fed and HFD-fed IgA^- mice. Regarding the gut bacterial composition, PCoA result showed a clear separation between NFD and HFD feeding while that of IgA^+ and IgA^- mice was closely grouped both under NFD and HFD feeding. This result suggests that a reduction of IgA coating of gut bacteria may not contribute to HFD-induced gut dysbiosis. HFD feeding significantly increased the final body weight and adipose tissue weight as compared to NFD feeding, while IgA-deficiency did not influence on them. Similar tendency was observed in lipid levels in the serum and adipose tissue. The triglycerides, NEFA, and total cholesterol concentration in the serum and adipose tissue were significantly increased by HFD feeding. However, IgA deficiency did not induce significant change in lipid levels in the serum and adipose tissue. These results suggest that a reduction of IgA coating of gut bacteria induced by HFD does not contribute to body weight gain, hyperlipidemia and fat deposition that accompanies HFD feeding. The fasting blood insulin level and HOMA-IR index were significantly increased by HFD feeding. Furthermore, IgA deficiency resulted in further increase in their values only under HFD feeding. These results suggest that HFD feeding and a reduction of IgA reactivity to gut bacteria synergistically exacerbate type 2 diabetes symptoms characterized by hyperinsulinemia and increased HOMA-IR index.

[Conclusion] Our study clearly showed that excessive lard intake reduces amount of IgA coating gut bacteria, which is partly associated with exacerbation of type 2 diabetes symptoms. It remains unclear from this study how excessive lard intake alters IgA reactivity to gut bacteria. Lard is enriched in saturated fatty acid including palmitic acid and stearic acid as compared to other dietary fat such as soy bean oil and olive oil. Furthermore, enzymatic ester-exchange method allows to modify fatty acid composition of lard. Recently, we are evaluating the effect of several types of dietary fat and modified lard on IgA reactivity to gut bacteria to verify whether fatty acid composition of dietary fat influence on IgA coating of gut bacteria. Moreover, we are searching for food ingredients that can prevent HFD-induced reduction of IgA coating of gut microbiota as candidate of food ingredient to prevent type 2 diabetes.

[References]

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IP-3

Herbal products for animal health - a solution for reduction of antibiotic use

○**Thi Tra An VO***

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Keywords: herbal product, animal, solution, antibiotic alternative

Vietnam is a member of the Association of South East Asian Nations (ASEAN) and subsequently, a member of ASEAN Free Trade Area (AFTA). Free trade agreements (FTAs) has opened opportunities for Vietnamese product to enter foreign markets, helping the country's economic improvement. However, the products may be failed in a so-called technical barrier to trade. For instance, the meat product should be produced organically or at least with a limited amount of antibiotic to be imported to foreign countries. This is a big challenge for Vietnamese farmers. A publication in 2020 has reported that antibiotic use for animal in Vietnam was 247 mg/kg which was higher than that in European countries (151.1mg/kg). Government has implemented several regulations as national action plan to reduce antibiotic use such as Animal Husbandry Law (2018) to ban antibiotic as growth promotor infeed, Circular on Management veterinary medicines (2020) to restrict use of antibiotic classes in disease prevention. Therefore, scientists and industries must contribute to the process of education and providing alternative measures for farmers to maintain animal health as well as to prevent infections in new situation.

With the advantage of the tropical climate, many plants in Vietnam have important pharmacological properties such as antibacterial, antiviral, anti-inflammatory, antioxidant, liver cell repair, prevention of kidney damage, etc. Therefore, herbal or plant extract is one of the solutions to replace antibiotic in disease prevention and treatment for animals. Moreover, study and application of products from plants and by-products of cultivation and processing will contribute to increasing income for farmers, maintaining sustainable agriculture. More than that, this tendency also contributes to the creation of organic products, clean and safe food for domestic consumption as well as export. This article summarizes the research results as well as the use of popular herbal extracts locally and internationally to protect the health of animals (livestock, poultry, companion and aquatic animals).

Latin name	**Vietnamese name**	**Part used**	**Properties**
Phyllanthus ninuri	Diệp hạ châu	Whole plant	Anti-bacterial, -viral, liver protective
Allium sativum	Tỏi	Cloves	Anti-bacterial, -viral, -protozoal
Curcuma longa	Nghệ	Rhizome	Anti-bacterial, -viral, -fungal, healing
Zingiber officinale	Gừng	Rhizome	Anti-bacterial, -inflammatory, -emetic
Pouzozia zeylanica	Bọ mắm	Leaf, stem	Anti-bacterial, - inflammatory, oxidant
Oenothera biennis	Anh thảo	Seed, flower, root	Anti-bacterial, -viral
Cassia alata	Muồng trâu	Leaf	Anti-bacterial, -viral
Calophyllum inophyllum	Mù u	Leaf, seed	Anti-bacterial, -viral
Tinospora crispa	Kí ninh	Leaf, root	Anti-bacterial, -viral
Momordica charantia	Mướp đắng	Fruit, seed, leaf	Anti-bacterial, -viral, hypoglycemia
Perilla frutescens	Tía tô	Leaf, seed	Anti-bacterial, -inflammatory, -oxidant
Psidium guajava	Ổi	Fruit, leaf	Anti-bacterial, -viral
Azadirachita indica	Nem	Fruit, leaf	Anti-parasitic, bacterial
Ocimum	Húng quế	Whole plant	Anti-bacterial, -viral
Origanum vulgare	Kinh giới cay	Leaf, stem	Anti-bacterial, -tumor, -oxidant
Echiacea purpura	Cúc tím	Flower	Anti-bacterial, -viral, -fungal, -inflammatory
Eucalyptus camaldulensis	Bạch đàn	Leaf	Anti-bacterial
Thymus vulgaris	Xạ hương	Leaf	Anti-bacterial
Cinnamon	Quế	Vỏ thân	Anti-bacterial
Melaleuca alternifora	Tràm	Leaf	Anti-bacterial, -inflammatory
Anethum graveolens	Thì là	Hạt	Anti-bacterial
Annona squamosa	Mãng cầu	Hạt	Anti-bacterial, -parasitic

IP-4

From a lab to farms: Cellular functions in bovine reproductive organs

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Keywords: cell physiology, cattle, ovary, oviduct, reproductive physiology, uterus

[Introduction] Reduction of bovine fertility rate is an important issue in many countries. In Japan, 99% of cattle get pregnant by artificial insemination (AI) and the fertility rate is approximately 40 - 50 %. Cattle which do not get pregnant after three times of AI is called as a repeat breeder, and the causes are unclear. Understanding cellular function is important to find the causes and establish treatments for the issues. We are investigating the regulatory mechanisms of reproductive organ functions including ovary, oviduct and uterus using in vitro methods. Here, we would like to introduce our recent studies on following topics.

[Luteal function in ovary] Corpora lutea (CL) is a transient organ formed after ovulation in the ovary. CL produces progesterone which is a necessary hormone for the establishment and maintenance of pregnancy. When the pregnancy does not occur, CL regresses for next follicular growth and ovulation. To clarify the mechanism of luteal formation and regression as well as progesterone production is important for supporting pregnancy in cattle.

Mechanosensing and luteal cell function: It has been broadly known that cells perceive mechanical stimuli and change their functions. The stiffness of CL changes throughout their life, that is soft at formation period and rigid at developed period. To investigate the effects of stiffness on CL function, bovine luteal cells were isolated and cultured on plastic plate or the substrates of various stiffness. We found that a reactivity to luteinizing hormone (LH), one of the luteotropic hormones which stimulate progesterone production, differs among the groups. It suggests that CL controls its own function by mechanosensing.

Endoplasmic reticulum (ER) stress and apoptosis: During luteal regression, cells undergo apoptosis. ER is an organelle responsible for protein folding following translation, and ER stress caused by accumulation of misfolded proteins in the ER could stimulate apoptosis signal transduction. To investigate the involvement of ER stress in the CL regression, the expression of ER stress related genes in CLs are measured.

[Oviductal transport mechanism] Oviduct is a site for fertilization and early embryonic development as well as a pathway of gametes and embryo connecting an ovary and uterus. In spite of the important organ for reproduction, oviductal function is less understood. Transport of gametes and embryo is propelled by ciliary beating of epithelial cells and rhythmic contraction of smooth muscles. We have investigated the regulatory mechanisms of epithelial and smooth muscle cell functions, that could be helpful information for infertility treatment caused by the failure of oviductal transport in not only domestic animals but also in women.

Ciliated cell functions: Oviductal epithelium is consisted of two kinds of cells, ciliated and non-ciliated cells. It is known that the number of ciliated cells increases around ovulation, however, its mechanism is unknown. We clarified the process of ciliated cell differentiation from non-ciliated cell during the estrous cycle in cattle using immunohistochemistry [Ito *et al.*, 2016, 2020]. In addition,

factors regulating the speed of oviductal fluid flow created by ciliary beating and the mechanism were demonstrated [Yoshimoto *et al.*, 2017].

Spontaneous smooth muscle contraction: Oviduct shows spontaneous rhythmic contraction without any exogeneous stimuli. We identified several ion channels which are involved in the generation and regulation of spontaneous contraction. Furthermore, calcium dynamics in smooth muscle cells are investigated since calcium is an essential ion for muscle contraction, aiming to clarify the detailed mechanisms.

[Endometrial function] Uterus is a site for fetal growth as well as endocrine organ producing hormones involved in the regulation of estrous cycle, implantation and pregnancy. Endometrium consists of epithelium (luminal and glandular) and stroma, the cellular functions have been investigated.

Influences of heat stress: Heat stress has critical influences on bovine reproduction. Our group demonstrated that endocrinological and immunological capacity of endometrium changed in summer season and under high temperature conditions [Sakai *et al.*, 2018, 2020]. The mechanism how temperature influence on the cellular function was investigated, then the involvement of ER stress was identified in our recent study [Sakai *et al.*, in press]. In addition, we investigate the expression and function of TRPV channels which work as a thermo sensor in cells to find the way for modulating the effects of heat stress.

Uterine gland culture: Uterine glands are necessary for growth of conceptus, however, its roles are unclear. To clarify the detailed function, we are establishing the isolation and culture methods of bovine uterine glands. We utilize three-dimensional culture method using Matrigel, and glandular structure was observed in dishes.

[Summary] More information about reproductive physiology is required to improve the current condition on bovine fertility. *In vitro* study is a good way to find new theory of reproduction since it is possible to focus on the specific cells without untargeted environmental effects. Our group aims to clarify the cellular mechanism in mammalian reproduction systems and contribute to establishment of new methods for improving fertility in cattle.

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PA-1

Refractile / lipofuscin bodies in human oocytes are present over a year prior to ovulation and associated with lysosome localization

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Keywords: human oocyte, refractile body, lipofuscin, autofluorescent, lysosome

[Introduction and Objective] A large refractile body (>5μm in diameter) has been considered as one of the main morphological abnormalities in the cytoplasm of human oocytes. It consists of a mixture of a mixture of lipids and dense granular materials and exhibits the typical autofluorescence of lipofuscin[1)]. Lower fertilization rates and lower embryo development rates have been consistently reported when embryos with large refractile bodies have been transferred[2)]. Refractile bodies appear in both mature and immature oocytes and most studies agree that there is a tendency for the recurrence of refractile bodies in the same patient over repeated treatment cycles[3)]. However, the causes of their occurrence are still unknown. Accordingly, we hypothesized that the presence of large refractile bodies may be related to the development of the ovarian follicles, and aimed to explore the possible association of this development with lysosomes.

[Materials and Methods] Ovaries were obtained from 23 Japanese patients with gender identity disorder, undergoing female-to-male sex rearrangement surgery at the Okayama University Hospital between May 2018 and September 2021. Informed consent for the use of their ovaries and institutional review board approval were both obtained for this research (approval number: K1807-035). Primordial oocytes were isolated from the ovaries. The autofluorescence of the refractile bodies in primordial oocytes was confirmed by fluorescent microscopy. The number of primordial oocytes with and without large refractile bodies was recorded until the total number of primordial oocytes exceed 100. The percentage of large refractile bodies was compared among the patients. LysoTracker Red, a fluorescent probe, was used to stain lysosomes, to identify any association with the refractile bodies.

[Results] Small and large refractile bodies were found in primordial oocytes from all 23 patients with gender identity disorder. Both large and small bodies exhibited autofluorescence. The age of the patients was from 23 to 47 years old (mean age ± SD; 30.9 ± 6.5 years old). No correlation between patients' age and the presence of large refractile bodies was detected ($p>0.05$). Refractile bodies were positively stained with LysoTracker Red.

[Discussion] As refractile bodies were present in primordial oocytes prior to ovulation, and these oocytes requiree a full year to reach maturity, the cause of the bodies may not be related to stimulation protocols nor the timing of ovulation, but the environment in each patient's ovaries. Thus, recurrence is likely to occur. As lysosomes were found to be localized in refractile bodies, the origin of refractile bodies could be residual materials of lipophagy/autophagy or endocytosis. Further studies are required to elucidate these findings.

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PA-2

Chromosomal missegregation and low developmental rates in female mutant mice upon the failure of crossing over during meiosis

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Keywords: meiosis, oocyte, *repro57*, chromosomal missegregation, aneuploidy

[Introduction and Objective] N-ethyl-N-nitrosourea (ENU)-induced mutant mice, *repro57*, have the *Rnf212* gene mutation and both female and male *repro57*(-/-) mice are infertile[1)]. *Rnf212* plays an essential role in the progression of recombination[2)] and crossover designation[3)]. In male *repro57*(-/-) mice, many spermatocytes degenerate at prophase and there are no mature spermatozoa in the seminiferous epithelium, suggesting that infertility is caused by arrested spermatogenesis[1)]. However, the precise mechanisms of infertility in female *repro57*(-/-) mice remains elusive. In this study, we aimed to investigate the phenotype of female *repro57*(-/-) mice, the chromosomal and kinetochore patterns of their mature oocytes, and their developmental potential after in vitro fertilization.

[Materials and Methods] Oocytes were collected from the ampulla of 8-10 week old wild-type and *repro57*(-/-) female mice after superovulation. Immunofluorescence analysis for kinetochore, using an anticentromere antibody was performed and the inner-kinetochore distance between each of the paired kinetochores was measured. In vitro fertilization, using the sperm of 8-11 week wild-type male mice was performed, and embryo development was monitored with a time-lapse observation system (PrimoVision, Vitrolife, Japan).

[Results] All of the wild-type oocytes reached metaphase II and were all euploid. However, among the oocytes of *repro57*(-/-) mice, only 45.0% reached metaphase II, and 90.9% were aneuploid. Furthermore, the kinetochore distances among *repro57*(-/-) oocytes were greater than those of wild-type mouse oocytes ($p < 0.01$). Although there were no significant differences regarding fertilization and early embryo development rates between the wild-type and *repro57*(-/-) mice, embryos derived from *repro57*(-/-) mice displayed significantly low morula and blastocyst rates as compared to embryos derived from wild-type mice ($p < 0.01$). 58.0% of embryos derived from *repro57*(-/-) mice showed mitotic arrest, including various types of abnormal cytokinesis, such as reverse cleavage, while only 14.5% of the embryos derived from wild-type mice exhibited this type of abnormality.

[Discussion] This study identifies one phenotype of female *repro57*(-/-) mice. These results suggest that *repro57*(-/-) mice produce aneuploid oocytes with a premature segregation of sister chromosomes and that these defects adversely affect the later stages of embryo development. Further analysis is required to discover the cause of chromosomal segregation errors in older, infertile patients.

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PA-3

The effects of supplementing cryopreservation solution with astaxanthin during the process of mouse sperm cryopreservation

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Keywords: sperm, cryopreservation, astaxanthin, mouse, optimal concentration

[Introduction and Objective] The effects of cooling, osmotic pressure, and oxidative stress during the cryopreservation process damage the DNA, cell membranes, and mitochondria in sperm. This, in turn, leads to decreased fertilization and motility. It is therefore necessary to supplement cryopreservation solution with protective substances during the process of cryopreservation. In this study, we focused on astaxanthin, which has various beneficial biological functions and properties, such as antioxidation, anti-inflammation and working as an anticarcinogenic and antidiabetic. Previous studies on wild boar and canine sperm have shown the significance of adding astaxanthin to cryopreservation solution. However, the optimum concentration of astaxanthin differed across the various experiments. In this study, we aimed to investigate whether the addition of astaxanthin to cryopreservation solution would improve the motility and other parameters of mouse sperm, and the optimal concentration of astaxanthin supplementation in the cryopreservation solution.

[Materials and Methods] Mice aged 9-12 weeks were used in the experiment. Astaxanthin was dissolved in Dimethyl sulfoxide (DMSO) and added to a cryopreservation solution, consisting of 18% raffinose and 3% skim milk. The concentration of astaxanthin was adjusted to 0.5 μM, 1 μM, 2 μM, and 4 μM, respectively and the same amount of DMSO was added to the control group. After thawing, the total sperm motility, straight line velocity (VSL), average path velocity (VAP), straightness (STR), linearity (LIN), and beat cross frequency (BCF) were assessed by computer assisted sperm analysis and compared between the test groups. The group that had the highest sperm motility was further compared with the control group.

[Results] Among the 5 groups, the 1 μM group showed the highest rates of sperm motility and delivered the best results across the other parameters, although no statistically significant difference was detected. However, further experimentation revealed that sperm motility with 1 μM astaxanthin supplementation was significantly higher than sperm motility without astaxanthin supplementation ($P<0.05$).

[Discussion] This study suggests that astaxanthin is effective in protecting ICR mouse sperm from damage during the cryopreservation process. In accordance with the findings of this study, the optimum concentration of astaxanthin for ICR mouse sperm cryopreservation is estimated to be approximately 1 μM. Further study is required to confirm the optimal concentration of astaxanthin and the effects of combining astaxanthin and betaine supplementation, as our laboratory has also found the latter to be effective in mouse sperm cryopreservation.

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PA-4

The effect of betaine for mouse sperm cryopreservation

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Keywords: sperm, cryopreservation, betaine, motility

[Introduction and Objective] Sperm cryopreservation is an effective method for the preservation of male fertility in humans, domestic animals, and laboratory animals. However, various factors, such as ice crystal formation, osmotic stress, and oxidative stress, negatively influence the motility and viability of post-thawed spermatozoa, which in turn causes reduced fertilization rates. Betaine, which works as an osmoprotectant and has protective effects against the formation of ice crystals during cryopreservation, is known to work as a nontoxic cryoprotectant[1)]. Some reports also indicate that betaine has antioxidant properties[2)]. However, the protective effects of betaine for sperm cryopreservation is still unclear. The purpose of this study is to investigate whether betaine has protective effects during the process of mouse sperm cryopreservation.

[Materials and Methods] These experiments used the sperm of male ICR mice (9-13 weeks of age). A sperm cryopreservation solution, consisting of 18% raffinose and 3% skim milk was used as a standard solution. Each cauda epididymis was placed in 100μL of cryopreservation solution, and various incisions were made in the epididymis. The resulting sperm suspension was placed in a cryovial and then frozen with liquid nitrogen. After thawing, the sperm were preincubated in human tubal fluid (HTF) medium for 1 h at 37 ℃, in an incubator with 5% CO_2 and an atmospheric O_2 concentration. The total motility, straight line velocity (VSL), curvilinear velocity (VCL), average path velocity (VAP), linearity (LIN), straightness (STR), amplitude of lateral head displacement (ALH) and beat cross frequency (BCF) were assessed by computer assisted sperm analysis. Ex.1: Spermatozoa were cryopreserved with solutions containing 0, 0.5, 1, 2, and 4% betaine and the sperm parameters of each group were compared. Ex.2: Frozen-thawed spermatozoa were incubated in HTF medium supplemented with 0, 0.5, 1, 2, and 4% betaine for 1 hour, and each sperm parameter was measured. Ex.3: The plasma membrane integrity of the frozen-thawed spermatozoa in each group was evaluated by staining with propidium iodide (PI), to which intact membranes are impermeable.

[Results] Ex.1: The total sperm motility of the groups with a 1% ($p=0.007$) and 2% betaine supplement ($p=0.0021$) was significantly increased, as compared to the 0% group. However, there was no significant difference in the VSL, VCL, VAP, LIN, STR, ALH or BCF. Ex.2: The addition of betaine to the HTF medium during the thawing procedure did not positively affect the distinct parameters of frozen-thawed spermatozoa. Ex.3: The supplementation of betaine in the cryopreservation solution did not improve the plasma membrane integrity of the frozen-thawed spermatozoa.

[Discussion] In this study, betaine was found to be effective in maintaining sperm motility during the freezing procedure. 1% betaine was the optimal concentration in a cryopreservation solution. As betaine did not protect the plasma membrane of the sperm head, it may, alternatively, be effective for mitochondria and/or flagellum of sperm. To clarify these effects, the mitochondrial function of post-thawed sperm and the antioxidant effects of betaine will require further investigation.

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PA-5

Risk factors for African swine fever in Phu Tan district, An Giang province

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Keywords: African swine fever, risk factor, Phu Tan, An Giang

African swine fever (ASF) outbreak firstly appeared in An Giang, Vietnam in July 2019, and caused serious economic losses for country pig production. This study was carried out for evaluation of the risk factors contributing to the African swine fever occurrence in Phu Tan, An Giang, where most of pigs were raised in small holders, and its pig production had big damage due to poor management. A retrospective survey of 160 pig-keeping households in 18 towns, including 80 pig-keeping households outbreaks of ASF and 80 pig-keeping households without outbreak. The results of risk factor analysis showed that high risk factors for ASF included farms located near live animal markets (less than 150 meters, OR = 2,04) and the main roads (less than 100 meters, OR = 2,53), introducing new piglets to the farm (OR = 8,46); other factors such as using of river water resources (OR = 2,54) and feeding leftover food from outside (OR = 3,69); lack of disinfection (OR = 13,5); presence of wild animals (OR = 8,65) and people (OR = 2,75). The results presented here will inform decision making to better control ASF in the circumstance of ineffective treatment or unavailable vaccine, disease prevention and control rely on strict biosecurity based on specific risk factors of ASF introduction within domestic pig populations.

PA-6

Marek's disease in vaccinated free-range chickens in the Mekong delta

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Keywords: vaccinated, free-range chickens, Marek's disease, Mekong delta

[Purpose] Marek's disease virus (MDV) has been shown to be evolving to higher virulence, and vaccines seem to be less effective in disease prevention. With the aim to investigate the real status of Marek's disease (MD) in vaccinated chicken flocks.

[Materials and Methods] A longitude survey was carried out from 10/2020 to 07/2021 from 32 vaccinated free-range chicken flocks in the Mekong delta which were suspected involving with MD. All chicken flocks were vaccinated by bivalent Marek's disease vaccine (HVT FC-126 + Rispens CVI988). The polymerase chain reaction was used to confirm MD from 103 diseased chickens of these flocks.

[Results] The results reported that 25 flocks were positive (78.1%) and epidemiological measures from these MD flocks showed that cumulative morbidity as high as 10.5% and 75.1% of sick chicken died. The highest morbidity was reported in 12-16 week-old chickens. The popular clinical signs were found in sick birds were emaciation, depression, ruffled feathers and pale appearance; anorexia and paralysis occasionally occurred. Post-mortem examination of 81 samples MD chickens revealed visceral lymphomas in one or more of a variety of chicken internal organs, the most frequently was reported from liver (95.0%), followed by kidneys (80.0%), spleen (75.0%) and lung (70.0%). Genetic analysis of 07 isolates MDV serotype 1 strains showed that they had a closed relationship with high virulent strains from China and Japan with 97-98% amino acid sequence homology.

[Conclusion] These results add to previous evidence that the increasing virulence of MDV may pose as a threat to the standard MD prevention strategy, reducing the success of vaccine protection. The best fit vaccination protocols and biosecurity should be considered for an effective MD prevention strategy.

PA-7

Development of a smooth endoplasmic reticulum cluster model employing mice

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Keywords: sERC, smooth endoplasmic reticulum, oocyte, cyclic AMP, IBMX

[Introduction and Objective] Smooth endoplasmic reticulum clusters (sERCs) are one of the dysmorphic phenotypes in human oocytes[1)]. Some studies have reported that the presence of sERCs in oocytes reduce pregnancy rates and cause a comparatively high number of abnormalities in live births[1,2,3)]. However, some reports have shown that healthy babies can be born, without reduced pregnancy rates, from oocytes containing sERCs. Thus, the clinical and scientific significance of oocytes containing sERCs remains controversial. The presence of sERCs has been confirmed in the metaphase II stage of human and chimpanzee oocytes, but has yet to be identified in any other mammals' oocytes. The mechanisms and causes of the appearance of sERCs are still not fully understood. Accordingly, we aimed to induce the appearance of sERCs in mouse oocytes artificially and thus establish a model that could lead to further research. As we previously found that the serum estradiol and progesterone concentrations on the day of hCG administration were significantly higher in sERC positive cycles, we hypothesized that delayed oocyte retrieval may cause an excessive increase in cAMP and inordinate cytoplasmic maturation, which in turn results in the formation of sERCs.

[Materials and Methods] ICR female mice aged 9 to 14 weeks were used for the experiments. 3-Isobutyl-1-methylxanthine (IBMX), a phosphodiesterase inhibitor, which increases cAMP and cGMP by inhibiting their degradation, was used to promote oocyte maturity. GV stage oocytes were collected from the ovaries following after PMSG / hCG administration. The IBMX concentrations were compared by setting up three test groups of 1 mM, 2.5 mM, and 5 mM. The oocytes were denuded immediately after retrieval. After GV breakdown was confirmed, the oocytes were cultured in a medium supplemented with IBMX. The presence of sERCs was confirmed by immunofluorescence staining, using an anti-calnexin antibody. The timing of the appearance of sERCs was analyzed using a time-lapse observation system (PrimoVision, Vitrolife).

[Results] When GV stage oocytes were collected 48 hours after PMSG administration and cultured in HTF medium with 2.5 mM IBMX, sERC-like structures appeared in 14.9 % (11/74) of oocytes, while most oocytes were dead when they were cultured in HTF medium with 5mM IBMX. The sERC-like structures were positively stained with anti-calnexin antibody.

[Discussion] In this study, we were able to produce sERCs in mouse oocytes when the cAMP level was artificially increased by using 2.5mM of IBMX. The results suggest that cytoplasmic overmaturation could be a cause of sERC formation. As a high concentration of IBMX is toxic and oocytes do not develop further after in vitro fertilization and exposure to this medium, we are now exploring a new method, which mimics ovarian stimulation as used in human reproductive medicine.

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PA-8

Developmental trajectory of monopronucleated zygotes after in vitro fertilization when they include both male and female genomes

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Keywords: Monopronucleus, second polar body, cytoplasmic wave, 1PN, fertilization cone

[Objective] Normal fertilization after in vitro fertilization (IVF) occurs in metaphase II oocytes by the fusion of a single sperm with the oocyte membrane. A sperm which has fused with the oocyte membrane sometimes forms an ooplasmic protrusion (FC: fertilization cone) and then forms a male pronucleus (PN). Shortly after PN formation, a female PN develops beneath the 2nd polar body (PB). However, 2.7-5.6% of fertilized zygotes are reported to exhibit only one PN (1PN). Diploid 1PN formation has been considered to be the result of the fusion of paternal and maternal PNs. Likewise, the combining of the female and male genomes when sperm entry was in proximity to the spindle has been deemed a probable cause of diploid 1PN formation. In this study we aim to explore the mechanisms of 1PN formations, which include both maternal and paternal genomes, by focusing on cytoplasmic wave/flare (CW), the FC and the position of the 2nd PB extrusion.

[Materials and Methods] Zygotes in which both the 2nd PB extrusions and the FCs, and /or initial CWs were observed by time-lapse system (iBIS, Astec, Japan) from January to July 2020, were examined. Time-lapse data for 24 1PN and 453 2PN zygotes from 599 patients was compared. The distance between the position of the 2nd PB extrusion and the FC and/or starting position of the CW was measured. The 1PN formation rate after IVF, the time from the initiation of FC protrusion to its depression, and the time from FC protrusion to the initiation of CW were measured. The threshold for the distance between the 2nd PB extrusion and the FC /starting position was calculated by ROC curve analysis.

[Results and Discussion] The 1PN formation rate after IVF was 4.3% (145/3337) of the fertilized oocytes. The average time from the beginning of protrusion to the depression of the FC was 100 (±37) minutes. The CW occurred within 15-30 minutes after the depression of the FC. The cut-off value for the difference in distance between the 2nd PB extrusion and the FC, or the starting position of the CW, was 18.0μm (AUC: 0.972, 95%CI: 0.955-0.988). Consequently, we calculated that the theoretical risk of 1PN formation which included both female and male genomes was approximately 2.7%. As in humans both the fusion of sperm with the oocyte membrane and the entry of sperm into an oocyte might occur anywhere on the surface of the oocyte, the logical conclusion is that babies derived from 1PNs during natural conception may result in live birth. Thus, it follows that embryos derived from diploid 1PNs after IVF could be an option for embryo transfer, rather than discarded.

PA-9

The mechanics of abnormal cytokinesis and aggregated chromosome in mouse oocytes when cytoplasmic cyclic adenosine monophosphate is elevated during meiosis

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Keywords: cyclic adenosine monophosphate, cytokinesis, meiosis, chromosome, aggregation

[Introduction and Objective] The presence of cyclic adenosine monophosphate (cAMP) has been considered to be a fundamental factor in ensuring meiotic arrest previous to ovulation, and is regarded as a key molecule in the regulation of oocyte maturation[1)]. However, it has been reported that increased levels of intracellular cAMP can result in abnormal cytokinesis and some MI oocytes lead to symmetrically cleaved 2-cell MII oocytes[2)]. Consequently, we aimed to investigate the effects of elevated intracellular cAMP levels upon the mechanics of abnormal cytokinesis and oocyte maturation during the meiosis of mouse oocytes.

[Materials and Methods] ICR mouse oocytes were transferred to HTF+5mg/ml BSA media with 6 different concentrations of Isobutylmethylxanthine (IBMX) (0, 0.5, 1.0, 1.5, 2.0, 2.5 mM), which is a competitive non-selective phosphodiesterase inhibitor and raises intracellular cAMP, one hour after germinal vesicle breakdown. They were then cultured for 24h. A time-lapse observation system (PrimoVision, VitroLife) was used to investigate the movement of the spindles. Immunofluorescence staining of oocytes using α-tubulin and DAPI were performed to confirm the spindle formation of the oocytes after the IBMX was removed.

[Results] It was found that the presence of 1.0-2.5mM IBMX in the culture media prevented spindle rotation, which resulted in the formation of 2-cell MII oocytes, and also caused chromosomal aggregation after polar body extrusion. The rates of chromosomal aggregation increased the greater the concentration of IBMX. In addition, aggregated chromosome (AC) formation was found to be reversible. Spindle formation was possible after IBMX was removed.

[Discussion] In human oocytes the chromosomes aggregate following the first and second polar body extrusions (the AC phase), while mouse oocytes do not have this AC phase[3)]. The results of this current study may indicate that the AC phase in human oocytes could be related to elevated levels of intracytoplasmic cAMP.

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PA-10

Prevalence and genetic characterization of chicken anemia virus in the Mekong delta, Vietnam

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[Introduction] Infectious anemia in chickens is caused by chicken anemia virus (CAV). CAV infects bone marrow-derived cells, causing severe anemia and immunosuppression in infected birds. The disease has become widespread and caused large economic consequences for poultry industry worldwide. Although CAV persists in chicken population in many countries including in the Northern Vietnam (Tan C. et al., 2020; Van Dong H. et al, 2019), no study about CAV in the Mekong delta (MD) was reported. This study is carried out to investigate the prevalence and genotype of CAV in a representative province of the MD.

[Materials and Methods] During 2021, a total of 140 suspected chickens with CAV infection from 38 poultry farms in Ben Tre province were sampled. Clinical signs of the obtained chickens were observed on site of collection. Organ specimens including liver, spleen and kidney from these chickens were obtained for gross pathology observation and PCR detection in laboratory. Tissue homogenates of organ specimens were pooled by 2–3 birds per farm and subjected to DNA extraction using Tissue Viral Extraction TopPURE® (TBR, Vietnam). Specific primer pairs of VP1 gene and PCR reaction for detection of CAV were performed following the previous study (Yao S et al., 2019) using Master Mix 2x BIO-25041 (Bioline, UK). To determine genotypes of CAVs detected in this study, PCR amplicons of the VP1 gene were sequenced using the Sanger method and the partial nucleotide sequences of the gene was phylogenetically analyzed using the maximum likelihood method in MEGA 7 with VP1 nucleotide sequences of other reference CAV strains.

[Results and Discussion] Results from this study showed that prevalence of CAV in chicken flocks of Ben Tre province was approximately 42.1% (16 PCR-positive samples out of 38 pools). Most of the infected chickens did not show apparent clinical signs and typical lesions of CAV infection. The phylogenetic tree based on VP1 gene indicated that CAVs detected in this study were clustered into the genotypes II and IV. Additionally, these viruses were genetically related to other CAVs previously detected in Northern Vietnam. Deduced amino acid sequences for the VP1 protein showed that several amino acid substitutions at the positions of I125L, A290P, Q294H (genotype II) and R249P, W250G, A264G (genotypes IV) were identified among CAVs found in this study and other reference CAV trains.

[Conclusion] This is the first study to report CAV and reveals the genotypic diversity of CAVs circulating in the MD. This study also provides cautions about CAV and highlights need for control and prevention of CAV in the MD and Vietnam.

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PA-11

Molecular characterization of foot-and-mouth disease viruses circulating in the Mekong delta, Vietnam

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Keywords: foot-and-mouth disease virus, Mekong delta, VP1

[Introduction and Objective] Foot-and-mouth disease (FMD) is a severe, highly contagious viral disease of cattle that has a significant economic impact. It is a transboundary animal disease that tremendously affects animal production and disrupts regional and international trade in animals and animal products. This study aimed to determine the molecular characterization of foot-and-mouth disease virus (FMDV) strains circulating in the Mekong delta, Vietnam.

[Materials and Methods] Four FMD isolates consisting of FMD-TV1, FMD-TV2, FMD-TV3, and FMD-TV4 were detected in the cattle by using reverse transcription–polymerase chain reaction (RT-PCR) amplified the 5' untranslated region (OIE, 2009). Then, the VP1 sequences of those FMDV strains were amplified by RT-PCR and sequenced by using Sanger sequencing technology.

[Results and Discussion] Phylogenetic and pairwise sequence comparison analysis showed that FMD-TV1, FMD-TV2, FMD-TV3, and FMD-TV4 strains were clustered in the same group. Those strains shared 90.63 – 95.41% nucleotide similarity and shared 93.57 – 96.13% similarity to O/MYA/7/98 (DQ164925). Besides, those strains were distinctly separated from lineages PanAsia and Ind2001 (topotype ME-SA) with 79,07 – 81,88% and 78,02 – 80,64% nucleotide similarities, respectively. Results of entropy of amino acid sequences revealed that VP1 amino acid sequences of FMDVs were different 27/211 positions within a lineage and 39/211 positions with different lineage. In addition, the detected FMDVs differ approximately 4 – 6 positions when compared to the O/MYA/7/98 (lineage Mya-98). Those different positions are mainly caused by substitution mutation. There were no recombinant events in the VP1 region recorded in the FMDV trains circulating in the Mekong delta.

[Conclusion] This is the first study to report CAV and reveals the genotypic diversity of CAVs circulating in the MD. This study also provides cautions about CAV and highlights need for control and prevention of CAV in the MD and Vietnam.

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PA-12

The orexigenic activity of pituitary adenylate cyclase-activating polypeptide in the ventromedial hypothalamus through the increment of agouti-related peptide in mice

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Keywords: pituitary adenylate cyclase-activating polypeptide, ventromedial hypothalamus, appetite, agouti-related peptide, food intake

Pituitary adenylate cyclase-activating polypeptide (PACAP) is highly expressed in hypothalamic regions that centrally regulate appetite. Although it is suggested that food intake is decreased in PACAP (-/-) mice, the detailed mechanism is still under discussion. To address this issue, we attempted to manipulate PACAP signaling, and investigated the polysynaptic Arc regulation by PACAP neurons in the VMH. We demonstrated that effect of the activation of PACAPergic neurons by designer receptors exclusively activated by designer drugs (DREADDs) in the VMH significantly increased food intake as compared with control mice in the diurnal period. Moreover, CNO-mediated inhibition of PACAP neurons in the VMH regions significantly reduced food intake at 2 h compared with control mice after fasting. These results suggested that food intake in mice is triggered by the increase in PACAP expression in the VMH via modulation of AgRP expression, pointing to PACAP inhibition as a clinically important therapeutic strategy against obesity in the future.

PA-13

Tokishakuyakusan increases bovine oviductal tonus via G protein-coupled estrogen receptor 1

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Keywords: Tokishakuyakusan, G protein-coupled estrogen receptor 1, oviduct, tonus, infertility

[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[1]. Although there are many clinical reports regarding effects of Tokishakuyakusan (TSS), a Chinese medicine on human infertility, there is little scientific evidence. TSS is reported to have an estradiol-17β (E2)-like effect[2] and E2 promotes oviductal contractility[3]. Therefore, TSS may improve oviductal transport capacity via the E2 receptor and contribute to supporting gametes and embryos transport. In this study, we investigated the effect of TSS on bovine oviductal contractility and its mechanism.

[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 TSS solution (10, 100 mg/ml) and G protein-coupled estrogen receptor 1 (GPER1) agonist (G-1, 100 or 1000 μM) on oviductal contractility were examined. In the same way, the effect of TSS solution (100 mg/ml) with pre-treatment of G-1 (100 or 1000 μM) and GPER1 antagonist (G-15, 2.5 or 25 μM) were also examined. Furthermore, the protein expression level of GPER1 in the oviductal smooth muscle of each stage was measured by Western blotting.

[Results and Discussion] TSS had no effects on the frequency and contraction force at all stages. However, the tonus was significantly increased by TSS at all stages. A significant increase was observed in both TSS treatment groups in stage I, and only 1000 μg/ml TSS significantly increased the tonus in stages II-IV. Both G-1 treatment groups also increased oviductal tonus similar to TSS at stage I. The addition of TSS following G-1 treatment did not show a significant increase of tonus compared to the TSS treatment group at stage I. The treatment of 25 μM G-15 significantly suppressed the TSS-induced increase of oviductal tonus at all stages. There was no significant difference in GPER1 protein expression among the estrous stages. In conclusion, TSS affects oviductal contractility by increasing tonus via GPER1, and this effect is particularly pronounced at stage I; however, its detailed mechanism is still unclear. TSS may be helpful for the treatment of infertility in women by accelerating gamete and embryo transport by the longitudinal oviductal contractility.

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PA-14

The involvement of endoplasmic reticulum stress in bovine corpus luteum regression

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Keywords: corpus luteum regression, ER stress, bovine

[Introduction] Luteolysis consists of two phases: functional regression characterized by decreased progesterone secretion and structural regression due to loss of luteal tissue[1]. Although structural regression is characterized by apoptosis of luteal cells, the detailed mechanisms have not been cleared[2]. Endoplasmic reticulum (ER) is an organelle in which proteins are folded into functional structure. ER functional overload, including excessive protein synthesis and accumulation of unfolded proteins in ER lumen, results in ER stress[3]. Cells activate the unfolded protein response (UPR) to cope with ER stress. By activating transcription factor 6 (ATF6), inositol-requiring enzyme 1 (IRE1) and protein kinase-like ER kinase (PERK) which are ER transmembrane proteins activated, UPR is mediated[4]. When ER stress is mild, UPR acts on cell protection, whereas when ER stress is severe, UPR induces apoptosis[5]. ER-related apoptosis may also be involved in structural regression of CL. However, the involvement of ER stress-related apoptosis in luteolysis are still unclear. In this study, we measured expression of ER stress-related factors in bovine CL tissue and cultured luteal cells which were treated with luteolysis factors.

[Materials and Methods] Luteal stages were assorted as early (Days 1-4 after ovulation), developing (Days 5-10), mid (Days 11-17), regressed (Days 18-20) by macroscopic observation of the ovary. After determination of the stages, total RNA was isolated from mid and regressed CL tissue samples. The CLs classified in the mid stage were used for cell culture. After treatment of prostaglandin F2α (1 uM), tumor necrosis factor α (50 ng/ml) and interferon γ (50 ng/ml) for 24, 48 and 72 h, we confirmed cell viability by MTT assay and total RNA was extracted from the cells at the end of culture. The total RNA from the tissue and cells was subjected to qRT-PCR for measurement of expression level of ER stress-related factors (ATF6, ATF4, IRE1, HSPA5, CHOP, sXBP1/uXBP1).

[Results] In CL tissue, the expression level of sXBP1/uXBP1 was higher in regressed than mid ($P < 0.05$). On the other hand, ATF6, ATF4, IRE1, HSPA5 and CHOP mRNA expression level did not change. In cultured cell, viability of luteal cells treated with luteolysis factors was significantly lower than control ($P < 0.05$). After treatment of luteolysis factors for 24, 48 and 72 h, expression level of ATF6 mRNA was significantly higher than control ($P < 0.05$). In the treatment group of luteolysis factors, the expression CHOP mRNA was significantly elevated compared with control ($P < 0.05$). However, mRNA expression level of ATF4, IRE1, HSPA5, and sXBP1/uXBP1 did not change. These results suggest that ER stress is involved in the apoptosis of luteal cells in structural regression.

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PA-15

Formation of bovine uterine gland like structure in *in vivo* 3D culture system

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

[Introduction] All mammalians have glands in endometrium that secrete a variety of factors into the uterine lumen and are essential for pregnancy[1]. In ruminants, including cows and sheep, uterine gland develops during the postnatal period, followed by invaginating from luminal epithelium throughout the stromal layer. However, the detailed mechanism has not been cleared in cows. Recently, three-dimensional (3D) culture has been utilized to elucidate the mechanism of tissue formation *in vitro*. It is reported that simultaneous stimulation of Wnt proteins and epidermal growth factor (EGF) induced tubal structure of intestinal epithelial cells in 3D culture that mimicked *in vivo* intestinal crypt[2]. Wnt proteins are also involved in adenogenesis in ovine endometrium[3], therefore it is expected that formation of bovine uterine gland in 3D culture is possible by Wnt proteins and EGF. Here, we evaluated the effect of Wnt proteins and EGF into 3D cultured bovine uterine gland.

[Materials and Methods] Uterine glands were isolated from sponge layer of bovine endometrium after 11-17 days post ovulation by enzymatic digestion. Uterine gland fragments were suspended in DMEM/F12 containing Wnt3a (40 ng/ml), Wnt5a (50 ng/ml), Wnt7a (50 ng/ml), EGF (5 nM), and 50% Matrigel and cultured for 3 days to generate cysts. Cysts were recovered from Matrigel and re-cultured in the same way for another 7 days, and then formation rate and morphology of uterine gland like structure were evaluated. To evaluate the role of Wnt proteins, 3D cultured uterine gland was stimulated by canonical Wnt signal inhibitor (XAV939; 0, 0.1, 1 μM) or Wnt protein secreting inhibitor (ETC-159; 0, 100, 1000 nM) with EGF and evaluated in the same way described above. The expression level of *SERPINA14* (uterine gland specific gene) mRNA in isolated uterine gland, cyst, and uterine gland like structure was analyzed by quantitative RT-PCR.

[Results] Bovine uterine gland fragment in 3D culture established cyst after 3 days in all culture conditions. Regardless of the presence of Wnt proteins, EGF induced cyst to be packed after 1-2 days following re-culture, and uterine gland like structure was formed by 10 days culture. EGF also induced uterine gland like structure formation even though XAV939 and ETC-159 were added, therefore Wnt proteins and their signals might not be involved in development of bovine uterine gland like structure in 3D culture. The *SERPINA14* mRNA was highly expressed in cyst while its expression in isolated uterine gland and uterine gland like structure was low. In conclusion, EGF but not Wnt proteins was essential for the formation of bovine uterine gland like structure in *in vivo* 3D culture system.

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PA-16

Effects of non-esterified fatty acids and ketone body on cellular functions of bovine endometrial cells

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Keywords: NEFA, BHBA, bovine, endometrium, ER stress

[Introduction] Negative energy balance (NEB) is known to occur in the postpartum period with the initiation of lactation in dairy cows, and it affects health and reproductive function of cows. Especially, in high-performance dairy cows, fertility is reduced around the peak of lactation by NEB. In NEB, body fat is mobilized from adipose tissue due to low blood glucose level to meet the animal's energy requirement. Consequently, plasma levels of non-esterified fatty acids (NEFA), but also of ketone bodies such as β-hydroxybutyrate (BHBA) increase. These findings suggest that there is a relationship between increased blood NEFA and BHBA concentrations and reproductive disorders in postpartum cows. However, its details have not been confirmed. In the present study, we investigated the effects of NEFA and BHBA on proliferation, lipid accumulation and endoplasmic reticulum (ER) stress of bovine endometrial cells.

[Materials and Methods] Endometrial epithelial (EP) and stromal (ST) cells were isolated from bovine uteri collected at the local slaughterhouse and used for the experiments. In the present study, stearic acid (SA), palmitic acid (PA), and oleic acid (OA) were used as NEFA, and BHBA was used as a ketone body. In the low concentration NEFA group, 60 μM SA, 60 μM PA and 80 μM OA (normal postpartum condition) were added to the culture medium, and in the high concentration NEFA group (NEB condition), five times concentration of each fatty acid was added. In the low concentration BHBA group (normal postpartum condition), 500 μM BHBA was added, and in the high concentration BHBA group (NEB condition), 1500 μM BHBA was added. In experiment 1, effects of NEFA and BHBA on the proliferation of each type of endometrial cell were analyzed by DNA assay. In experiment 2, the effects of NEFA and BHBA on lipid accumulation in endometrial cells were examined using the Oil Red O staining. According to the results of experiments 1 and 2, in experiment 3, we investigated the effects of NEFA on the gene expression of ER stress markers of EP and ST cells.

[Results] Cell proliferation of ST cells in the high concentration NEFA group significantly decreased after 48 and 72-h compared to the control group and the low concentration NEFA group ($P<0.05$). On the other hand, BHBA had no effect on proliferation of ST cells. In EP cells, there was a significant difference in proliferation between the low and high concentration NEFA groups ($P<0.05$), but BHBA also had no effect on proliferation. When exposed to high concentration NEFA lipid accumulation in ST cells at 24 and 72-h of culture increased significantly compared to the control group and the low concentration NEFA group ($P<0.05$). Furthermore, in EP cells, treatment of high concentration NEFA significantly increased lipid accumulation compared to the control group and the low concentration NEFA group after 72-h of culture ($P<0.05$). However, BHBA-induced lipid accumulation was not observed in ST cells and EP cells. In ST cells, treatment of high concentration of NEFA increased the gene expression of *BiP* and *CHOP* compared to the control group and the low concentration NEFA group ($P<0.05$). Furthermore, there was a significant difference in the splicing of *XBP1* between the low and high concentration NEFA groups ($P<0.05$). In EP cells, treatment of high concentration NEFA increased the gene expression of *ATF6* compared to the low concentration NEFA group ($P<0.05$). These results suggest that fat metabolite, especially NEFA, affects proliferation, lipid accumulation and ER stress of bovine endometrial cells.

PA-17

Metritis prevalence and treatment efficacy on reproductive beef cattle in Tien Giang province

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Keywords: bacteria, beef cattle, metritis, treatment

[Introduction and Objective] Metritis is an acute inflammatory disease that is one of the most frequent disorders affecting dairy cows, and the resultant reproductive performance, calving problems (dystocia, retained placenta, and stillbirth), infertility, and milk loss are frequently observed. Notably, a study by Sheldon et al. (2002) indicated that uterine bacterial contamination was found at a high score on day 7 or day 21 after parturition, affecting ovarian follicle selection and subsequent growth and function. The bacteria that can enter cows' reproductive tract and develop under favorable conditions, including *Escherichia coli, Salmonella* spp., *Staphylococcus* spp., and *Streptococcus* spp. are more commonly isolated in clinical conditions endometritis in cows (Moges et al., 2013). The objective of this study was to isolate bacteria from the uterine discharge, evaluate their antibiotic resistance and test the treatment efficacy.

[Materials and Methods] A total of 2,962 breeding beef cattle in Tien Giang province was investigated to evaluate the prevalence of metritis. Later, 162 uterine discharge samples from cows on heat were collected and preserved at 4°C for further processing within 24 hours. Four common bacteria species, including *E. coli*, *Salmonella* spp., *Staphylococcus aureus,* and *Streptococcus* spp., were isolated. All were confirmed by PCR technique, apart from *E. coli*. Antimicrobial sensitivity testing was performed as described by Kirby-Bauer (1996). For the treatment of metritis, two regimens were applied using either marbofloxacin plus uterine douching by Rivanol 0.2% (R1) or gentamycin plus prostaglandin injection (R2). In both regimens, ADE vitamin and dexamethasone were used for enhancing resistance and relieving inflammation in cows.

[Results and Discussion] In beef cows, the prevalence of reproductive disorders was 21.4%, with dystocia accounting for the highest rate at 8.99%, closely followed by that of metritis (5.47%). Among the bacteria species investigated, the highest prevalence belonged to *Streptococcus* spp. (87.7%), followed by *Staphylococcus aureus* (61.1%), *E. coli* (59.9%), and *Salmonella* spp. (17.3%). Moreover, in most parities (from 1 to 5 calvings), the prevalence of *Streptococcus* spp. (75-100%) was higher than those of *Staphylococcus* spp. (47.4-70.0%), *E. coli* (45.0-68.4%), and *Salmonella* spp. (5.26-66.7%). Furthermore, for the susceptibility test, bacteria in the metritis discharge in cows were most resistant to colistin with the rate of 66.6%, followed by the resistance to ceftiofur (57.9%), ampicillin (45.2%), doxycycline (40.7%), florfenicol (29.2%), gentamicin (24.9%) and marbofloxacin (16.9%). In the treatment of metritis, both regimens were of 100% efficacy in cure rate (no more inflammatory discharge from the vulva, no inflammatory discharge from the uterus until the next heat when the uterus opens). The number of pregnant cows in the next breeding was 80.0% and 66.7% for those in R1 and R2 treatments, respectively. Taken together, the availability of these data is crucial to set up strategies that can assist in conducting more comprehensive studies to improve the prevention and treatment of reproductive diseases in beef cows in Tien Giang province.

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PA-18

LAMP-Based detection of TL9 mutant allele in commercial pig population in south of Vietnam

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Keywords: LAMP, TL9, mummified piglets, florescence

[Introduction] The objective of this study is to investigate the TL9 mutant allele frequency in commercial pig population in Binh Thang breeding farm. TL9 has been recently attributed to have antagonistic effect on embryonic development and positive consequence on growth in swine. The homozygotic TL9 mutants cause mummified piglets and fetal death, while heterozygotes increase growth rates. Although TL9 protein's exact function has not been identified in pigs, TL9 mutants in mice showed embryonic lethality. Loop-mediated isothermal amplification (LAMP) is a novel nucleic acid amplification method that amplifies DNA with high specificity, efficiency, and rapidity under isothermal conditions. This technique has been used by several authors to detect bacterial, parasitic, and viral fish pathogens. In this study, we implemented LAMP to detect TL9 mutant allele in commercial pig population.

[Materials and Methods] The blood samples and DNA of pigs and mummified piglets were collected in Binh Thang pig breeding farm in 2020. LAMP approach was carried out as previously described with modifications. Primers for TL9 locus were designed according to PrimerExplorer® (https://primerexplorer.jp/e/).

[Results] We hypothesized that for three different TL9 alleles, each allele will display a distinct fluorescence curve during LAMP procedure. For the TL9/TL9 genotype, the fluorescence curve will be the highest while for the del/del genotype, there will not be any fluorescence during LAMP procedure. For the TL9/- genotype, the fluorescence curve will be lower than that of the TL9/TL9 genotype. Currently, we have analyzed 30 samples with LAMP. Thus far, we have found the LAMP approach is suitable to investigate the frequencies of TL9 alleles in the commercial pig population.

PB-1

Blood metabolites and rumen and fecal microbiota of Jersey cows throughout a lactation period

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Keywords: blood metabolites, gut microbiota, Jersey cow, lactation

[Introduction] There are 1.38 million dairy cows in Japan, of which 99% are Holstein and only 0.8% are Jersey. Although the annual milk yield is as low as about 6,000 kg for Jersey cows, the high milk protein (~4.0%) and fat (~5.0%) contents are favored for butter and cheese manufacturing. In addition, Jersey cows are known to be more heat tolerant than Holstein cows. It is thus worthwhile to study the characteristics of Jersey cows in detail to prepare measures for sustainable dairy industry under global warming and as a genetic resource to promote dairy breeding in the tropics. In this study, we monitored blood metabolites concentration and rumen and fecal microbiota throughout a lactation period.

[Materials and Methods] Samples were collected from 7 lactating Jersey cows raised at the Chugoku-Shikoku Dairy College in the Hiruzen area of northern Okayama Prefecture, Japan. The cows calved in August 2020, and we took jugular blood, rumen fluid, and rectum content at 1, 2, 4, 6, and 10 months after the calving. Bacterial DNA was extracted by the repeated bead beating plus column method and purified using a commercial kit. Two-step PCR targeting the V4 region of 16S rRNA genes was performed to generate amplicon libraries for next-generation sequencing. The levels of blood metabolites were determined by using the commercial kits.

[Results and Discussion] Milk yield attained nearly the maximum (25-27 kg/day) at 1 month after calving and remained the level until 4 months. The protein (3.4-3.5%), fat (4.5-4.7%), and solid-not-fat (9.0-9.2%) contents were low in early lactation (1-2 months after calving), and the milk components content substantially increased after 10 months when milk yield decreased to 15 kg/day. Likewise, plasma albumin and cholesterol concentrations were lower and NEFA concentration was higher in early lactation compared to middle and late lactation periods. The level of plasma haptoglobin, an indicator of systemic inflammation, was highest at 1 month after calving. Prevotellaceae (17.8-20.8%), Rikennellaceae (8.95-13.9%), and Lachnospiraceae (6.26-13.8%) were the 3 most abundant bacteria in the rumen and Oscillospiraceae (12.4-21.1%), Lachnospiraceae (7.85-20.9%), and Rikenellaceae (7.92-11.3%) were the 3 most abundant bacteria in feces. The abundances of Oscillospiraceae and Lachnospiraceae were low at 1 month and increased after that both in the rumen and feces. In contrast, the abundances of Methanobacteriaceae and RF39 were high at 1 month and decreased after 2 months. These results indicated that the gut microbiota might complete adaptation to diet change after calving within 1-2 months and this adaptation may be reflected in the blood metabolites somehow later.

PB-2

Changes in the blood metabolites concentration, milk composition, and milk microbiota of Holstein cows during a lactation period

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Keywords: dairy cow, environment, microbiota, milk

[Introduction] The demand for raw milk is expected to increase further globally, and many attempts to increase the dairy cow population and their milk yield have been made. Maintaining a long-lived herd and reducing death loss is key to sustainable and profitable dairy farming, but the practices have become more complex under global warming. The main reasons for herd replacement can be reproductive disorders, mastitis, lameness, and digestive diseases. Although good nutrition management is believed essential to prevent these disorders, the relationship between bacterial contamination in milk and cow nutritional status is not clear. From the early to late lactation period, the nutrition and physiological status of dairy cows vary greatly. In this study, we examined changes in blood metabolites concentration, and the yield, composition, and microbiota of milk of Holstein cows during a lactation period.

[Materials and Methods] Samples were collected from 8 Holstein cows reared at the Okayama Prefecture Livestock Research Institute, which operated automatic milking systems (Lely Astronaut A4, Cornes AG. Ltd., Eniwa, Japan) for management. The cows were housed in a free-stall barn and fed total mixed ration silage, which was formulated to have 550 g/kg of dry matter (DM), 160 g/kg DM of crude protein (N × 6.25), and 720 g/kg DM of total digestible nutrients. Sampling was made every two months from August 2019 to April 2020. The contents of protein, fat, and solids-not-fat (SNF), and the somatic cell count (SCC) of the milk were determined using a CombiFoss FT+ analyzer (Foss Allé, Hillerød, Denmark). Bacterial DNA was extracted by the repeated bead beating plus column method and purified using a commercial kit. 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 albumin, BUN, GPT, and GOT concentrations were low in the early compared with the late lactation period. The Ca concentration showed an opposite changing pattern. Although statistical significance was not observed, the total cholesterol concentration was low and NEFA was high in the early compared with the late lactation period. The milk yield was > 30 kg/day throughout the lactation period, but the protein, fat, and SNF contents were the lowest in the early and the highest in the late lactation period. The SCC count was numerically higher in the early lactation period, but the average SCC values remained < 220×10^3 cells/mL. In the milk microbiota, Lactobacillaceae, Lachnospiraceae, Muribaculaceae, Eubacteriaceae, and Yersiniaceae were found as the five most abundant taxa regardless of the lactation period. Milk microbiota was relatively stable and appeared unrelated to the changes in nutritional status during a lactation period.

PB-3

Changes in blood metabolites concentration and fecal microbiota of dairy calves before and after weaning

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Keywords: blood metabolites, calf, gut microbiota, weaning

[Introduction] Millions of microbiota colonize the gastrointestinal tract of a calf immediately after birth. The gut microbiota plays a vital role in extracting nutrients and developing the gut tissues, while the composition may change with the growth and associated diet alterations. In dairy calves, mother milk is given for a short period and milk replacer is fed for about one month. Calf starter, a solid feed, is also provided when the calves receive milk replacer. Weaning is usually made at 3 months of age, when the starter intake increases sufficiently. Because blood metabolites concentration changes before and after weaning, there is no doubt that weaning affects nutrient metabolism in dairy calves. However, weaning of dairy calves does not involve termination of liquid milk feeding; hence, the impact of weaning may be different from that of beef cattle and monogastric animals. This study examined the blood metabolites concentration and fecal microbiota of dairy calves at 2, 4, 6, and 8 months of age. The objective was to understand whether the gut microbiota is involved in the changes in nutrient metabolism.

[Materials and Methods] Samples of rectum content and caudal vein blood were collected from 7 calves at 2, 4, 6, and 8 months of age. The calves were switched from starter to formula feed around weaning, while hay was always provided *ad libitum*. Although the same formula feed was given after weaning until 8 months, grass silage was fed in addition to hay from 6 months. Bacterial DNA was extracted by the repeated bead beating plus column method and purified using a commercial kit. Two-step PCR targeting the V4 region of 16S rRNA genes was performed to generate amplicon libraries for next-generation sequencing. The levels of serum albumin (Alb), urea nitrogen, total cholesterol (T-Cho), non-esterified fatty acids (NEFA), calcium (Ca), and phosphate were determined by using the commercial kits.

[Results and Discussion] The levels of serum Alb, urea-N, and Ca increased and those of T-Cho and NEFA decreased after weaning. The higher and lower levels remained until 8 months, except for urea-N that returned to a 2-months level at 6 and 8 months. Ruminococcaceae, Lachnospiraceae, Bacteroidaceae were the three most abundant bacteria in feces. The abundances were not affected by weaning and the sampling period; hence, the gut (fecal) microbiota was unrelated to nutrient metabolism changes. The principal coordinate analysis also illustrated that the gut microbiota did not change before and after weaning; however, one or two calves showed a distinctively different microbiota compared to others at each sampling month. The fact that these calves showing different gut microbiota displayed normal levels of blood metabolites ensured that nutrient metabolism changes may occur with few associations of the gut (fecal) microbiota. The crude protein (18-20%) and total digestible nutrients (71-75%) contents of the calf starter and following formula feed are not much different; hence, fecal microbiota was unchanged across weaning. Regardless, blood metabolites concentration exhibited distinctive changes before and after weaning.

PB-4

Bacterial and fungal microbiota of guinea grass silage stored at moderate and high ambient temperatures with and without wilting

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Keywords: guinea grass, silage, acetic acid, bacteria, fungi

[Introduction] Guinea grass is a tropical grass exhibiting high biomass production (20–30 tons DM/ha) during the warm and rainy season. Ensiling is a key strategy to stabilize the forage supply for cattle; however, it is difficult to obtain acceptable fermentation quality without any treatments and additives. Acetic acid is often found as the predominant fermentation product when prepared from direct-cut crops. Furthermore, a high storage temperature is a challenge for ensiling in the tropics. This study was aimed to gain insights into the bacterial and fungal populations associated with acetic acid fermentation of guinea grass silage. The objective was to improve high-quality silage production in tropical and subtropical regions.

[Materials and Methods] Direct-cut (without wilting) and wilted guinea grass were ensiled in a laboratory silo and stored at moderate (25°C) and high (40°C) temperatures. The silos were opened at 1, 3, 5, and 7 days, and at 1 and 2 months after the packaging. Silage pH and fermentative products (acids and alcohols) were determined from water extracts using HPLC. The amplicons of bacterial V4 hypervariable region of 16S rRNA genes and fungal internal transcribed spacer region 2 (ITS2) were sequenced using the MiSeq platform.

[Results and Discussion] Lactic acid was the primary fermentative product and a high abundance of *Lactococcus* (19.7~39.7%) was observed in direct-cut silage during the initial ensiling (<7 days). After 1 and 2 months, the lactic acid content was lowered greatly and the acetic acid content was increased substantially. High concentrations of butyric acid and ethanol were also detected in long-stored direct-cut silage stored at 25°C. When stored at 40°C, the acetic acid content was increased slowly and the levels of lactic and acetic acid were similar, indicating that high-temperature storage could have benefits to suppress acetic acid and butyric acid production. Increased abundance of *Lactobacillus, Clostridium* and *Wallemia* spp., and decreased abundance of *Saitozyma, Papiliotrema* and *Sporobolomyces* were observed in the direct-cut silage during the prolonge ensiling. The lactic and acetic acid contents were greatly suppressed by wilting, and low abundances of *Lactobacillus* (1.72~8.64%) and *Wallemia* (0~30.0%) were observed in wilted silages. Unclassified *Enterobacteriaceae* were the dominant bacteria in direct-cut (38.1~64.9%) and wilted (50.9~76.3%) silages, but the genus was unrelated with the acetic acid fermentation. *Lactobacillus, Clostridium* and *Wallemia* spp. appeared to contribute to acetic acid fermentation in guinea grass silage. High ambient temperature could secure tropical grass ensiling.

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PB-5

Effects of Empyreal® 75 on egg production and egg quality of Isa Brown layers

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Keywords: Empyreal® 75, Isa Brown layer, egg production, egg quality

[Introduction] Soybean meal has long been used in animal diets as it provides an excellent source of both energy and protein for poultry and swine. However, soybeans contain compounds that inhibit the activity of the proteolytic enzyme. They also contain other anti-nutrients, including hemagglutinins or lectins, which contribute to reduced nutrient use. According to Leilane *et al.* (2011), the nutritional quality of soybean products for animal feeding is determined not only by the quantity of nutrients such as protein, amino acids and fat, but mainly by nutrient availability for the animals. Empyreal® 75 is a corn protein concentrate with good amino acid profile, high digestibility, and low antinutritional content. Empyreal® 75 also provides additional benefits such as a source of pigmentation and glutamine to poultry especially for layers. Therefore, the hypothesis of this study is Empyreal® 75 could be used in laying hen diets to replace a part of soybean meal and synthesis pigment to improve the performance and egg quality of layers.

[Materials and Methods] A total of 1763 ISA Brown layers, 32 weeks old, were randomly allocated to one of four treatments with different inclusion levels of Empyreal® 75 (0%, 1.5%, 2.5% and 3.5%) in diets. The EMP1.5, EMP2.5 and EMP3.5 treatments consist of 9 replicates, each replicate contained 48 layers in 36 cages (cage dimensions 40 x 60 x 40 cm). The CON treatment also consists of 9 replicates and 48 layers (36 cages) per replicate excepted for replicates 4, 5 and 6 as they were given 59, 59 and 61 layers in 45, 45 and 48 cages, respectively. The experimental diets were formulated either to meet or exceed recommended critical nutrient concentrations by the breeding company and followed the present diets which were used by the layer farm. Layers were fed approximately 120 g daily at 08:00 am and 16:00 pm, and feed residue was recorded at 7:00 am the next day. Drinking water was offered ad libitum during the experiment. Egg number and egg weight were recorded daily for 70 days. Egg quality traits were conducted according to Stino (1982) and El-Wardany *et al.* (1994). These included shell color (Shell color fan), yolk weight (by electronic balance), yolk pigmentation (Roche yolk color fan), eggshell thickness, albumen height (Digital Vernier Caliper) and Haugh unit.

[Results] The results from a ten-week study showed that feeding Empyreal® 75 in diets of laying hens improves egg production, egg mass, feed conversion ratio, egg quality ($p<0,05$) and finally gives better feed cost for egg production.

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PB-6

Astaxanthin enhances production performance and egg quality of laying hens

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Keywords: astaxanthin, laying hens, productivity, egg quality, yolk color

This study was conducted to determine effect of astaxanthin supplementation on production performance, egg quality of laying hens from 30-40 weeks age in a commercial layer farm in Tien Giang Province, Vietnam.

A total of 480 laying hens (Isa Brown) were divided into 4 dietary treatment groups. Each treatment consisted of 10 replications with 12 laying hens per replication. The control group was fed basal diet (T1), treatment group 1 (T2): basal diet with supplemented 0.075% astaxanthin, treatment group 2 (T3) with 0.1% astaxanthin and treatment group 3 (T4) with 0.125% astaxanthin. Birds were raised in an evaporative cooling system house. Water was provided ad libitum and feed was provided according to breed requirement recommendations.

The results showed that: The 3 treatment groups fed with diets supplemented astaxanthin had a higher production performance with productivity were T2 (83%), T3 (82.7%) and T4 (79.6%) compared to the control treatment T1 (75.5%) ($p<0.05$). There were no effects on egg weight, average eggshell thickness, egg sizes, albumen height, and Haugh unit ($p>0.05$). However, diets containing astaxanthin resulted in a significant increase of yolk color. The yolk color score increased from of 8.6 (T1) to 9.1 (T3 and T4) ($P<0.05$). The highest yolk color score was observed in laying hens fed the diet with 0.1% astaxanthin.

Astaxanthin used in this experiment was extracted from shrimp waste (head & shell) which can be considered as a good source of natural pigment that enhances the yolk color score in laying hens. Astaxanthin could be used in the diet of laying hens to improve egg quality and production performance.

PB-7

Effect of lard-, olive oil- and soybean oil-enriched diet on immunoglobulin A coating of gut bacteria

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Keywords: immunoglobulin A, dietary fat, fatty acid composition, 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. We recently showed that lard fat enriched diet (60%/kcal) feeding reduces amount of IgA coating gut bacteria in mice. However, it remains unclear whether a reduction of IgA coating of gut bacteria is induced by fat and oil other than lard. Lard is enriched in saturated fatty acid including palmitic acid and stearic acid as compared to olive oil (oleic acid-rich) and soybean oil (linoleic acid-rich). In this study, we evaluated the amount of IgA coating fecal bacteria in mice fed high lard diet (HL), high olive oil diet (HO) or high soybean oil diet (HS) to compare the effect of fat and oils, which have different fatty acid composition, on IgA reactivity to gut bacteria.

[Materials and Methods] Mice were divided into four groups and fed normal fat diet (control), HL, HO or HS for 27 weeks, respectively. HL, HO and HS contain each fat and oil at 45%/kcal to avoid separation of olive oil and soybean oil from diet. The feces were collected at 27 weeks and the fecal bacteria was isolated from the feces. The IgA coating fecal bacteria were detected by western blot using anti-mouse IgA antibody. The serially diluted serum mouse IgAs were also detected to make standard curve. (1) The amount of IgA coating fecal bacteria in 1 gram of feces (IgA coating amount) was calculated with reference to the standard curve. (2) The amount of IgA, not used for bacterial coating, in 1 gram of feces (IgA amount not for bacterial coating) was measured by enzyme-linked immunosorbent assay (ELISA). (3) The ratio of (1)/ (1) + (2) was calculated to evaluate the ratio of IgA coating gut bacteria in IgA secreted into the gut. The fatty acid intake was calculated based on feed intake and each fatty acid content contained in each diet.

[Results and Discussion] In contrast to our previous study, there was no significant difference in the IgA coating amount between HL and control group. This may be due to the reduced lard content in diet (60% kcal to 45% kcal). Similar with HL group, the IgA coating amount in HO and HS group did not show significant change as compared to control group, suggesting that at least HO and HS contained 45% kcal oil does not affect IgA coating amount. Meanwhile, HL group shows a significantly higher amount of IgA not used for bacterial coating as compared to other 3 groups. Furthermore, HL group showed a significantly lower ratio of IgA coating gut bacteria in IgA secreted into the gut as compared to control group. This suggests that excessive lard intake promotes IgA which binds to antigen except for gut bacteria. Each group showed a characteristic fatty acid intake; palmitic acid and steric acid intake were highest in HL group, oleic acid intake was highest in HO group, and linoleic acid and linolenic acid intake were highest in HS group. Although IgA coating amount in each group seems not to have any relationship with fatty acid intake, IgA amount not for bacterial coating in each group showed a similar trend with palmitic acid intake. Previous study reported that gut IgA secretion is promoted dependent on palmitic acid intake under normal fat diet feeding. Our observation suggests that palmitic acid content in high fat diet affects gut IgA secretion binding antigens except for gut bacteria.

PB-8

Effect of heat-killed *Lactobacillus plantarum* SNK strain on mucosal digestive enzymes activity in small intestine of aged mice

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Keywords: lactic acid bacteria, aging, mucosal enzyme activity, immunity

[Introduction] Aging causes a variety of biological impairments including a decreased digestive function. Previous study reported that the activity of mucosal digestive enzymes including maltase and lactase were decreased in the small intestine of aged rats as compared to that of young rats. This study also revealed that yogurt-supplemented diet increases maltase activity only in aged rats. However, it remains unclear whether lactic acid bacteria affect the mucosal digestive enzymes activity. To verify it, we evaluated the effect of oral administration of *Lactobacillus plantarum* strain SNK (SNK) on mucosal digestive enzymes activity in the small intestine of aged mice. To exclude the effect of metabolites derived from SNK, heat-killed SNK was used for this study. In addition to mucosal digestive enzymes activity, the effect of SNK on Immunosenescence, characterized by lower phagocytosis activity and cellular cytotoxic activity, was also evaluated because our previous study observed that SNK exerts immunostimulatory effects on the splenocytes in young mice.

[Materials and Methods] 70-weeks-old male C57BL/6J mice were divided into two groups (n=5): Control group fed a control chow diet (AIN93G), SNK group fed a SNK-supplemented diet (2.5×10^9 cells/ g diet). Each group was allowed *ad libitum* access to water and diet for 12 weeks. Body weight and food intake were measured once a week. Mice were sacrificed by CO_2 inhalation and the epididymal adipose tissue, gastrocnemius muscle, small intestine, femur and spleen were collected. The adipose tissue and muscle weight were measured. After measurement of the length of small intestine, the small intestine was separated into the jejunum and ileum. The mucosa was collected from 10 cm of the jejunum and ileum by scraping and the mucosal digestive enzymes activity including maltase, sucrase and aminopeptidase were measured by colorimetric methods. A part of the small intestinal tissue was used for H&E staining to measure the villus height. The bone marrow cells were collected from the femur and differentiated into macrophages (BMDM). The phagocytosis activity of BMDM was evaluated by amount of phagocytosis of fluorescence-labeled *Staphylococcus aureus*. The splenocytes were collected from the spleen and the cellular cytotoxic activity against tumor cell line YAC-1 was analyzed.

[Results and Discussion] Although there were no significant difference in the length of small intestine and the villous height between groups, the maltase activity in the jejunum was significantly increased in SNK group as compared to Control group. The aminopeptidase activity in the jejunum and ileum tended to increase in SNK group (jejunum: $P=0.078$, ileum: $P=0.096$). These results imply that bacterial cells of SNK may increase maltase expression in the jejunum and aminopeptidase expression in the jejunum and ileum at an old age. Meanwhile, there were no significant difference in the final body weight, feed intake and proportion of epididymal adipose tissue and gastrocnemius muscle relative to the body weight between groups, suggesting that the increase in maltase and aminopeptidase activities observed in SNK group does not affect skeletal muscle formation and fat accumulation. The phagocytosis activity of BMDM was significantly higher in SNK group than Control group, while there was no difference in the cellular cytotoxic activity of splenocytes between groups. These results suggest that SNK may stimulate macrophage phagocytosis of pathogenic bacteria, but not cellular cytotoxic activity exerted by cytotoxic T lymphocytes and natural killer cells in aged mice.

PB-9

The relationship between milk, udder skin, bedding, and fecal microbiota in a dairy farm

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Keywords: dairy cow, environment, microbiota, milk

[Introduction] The control and prevention of bovine mastitis are crucial in the dairy industry regardless of the nation and region. Pathogens and sources of infection have been demonstrated for both contagious and environmental mastitis. However, our understanding is still lacking on how the pathogenic and non-pathogenic bacteria contaminate udder milk and why a limited number of cows get mastitis in the same environment and feeding management. We previously examined the microbiota of milk, feed, rumen fluid, feces, bedding, and airborne dust, and found that milk microbiota was associated with bedding and airborne dust microbiota. Streptococcaceae was almost absent in feces and bedding, but a certain proportion was found in milk microbiota regardless of the somatic cell count. This finding was different from a typical image that milk microbiota was greatly affected by fecal microbiota. This study aimed to elucidate the relationships between milk, udder skin, and fecal microbiota. The microbiota variations between individual animals and between years were also examined.

[Materials and Methods] Samples were collected from 10 Holstein cows reared at the Okayama Prefecture Livestock Research Institute, which operated automatic milking systems (Lely Astronaut A4, Cornes AG. Ltd., Eniwa, Japan) for management. The cows were housed in a free-stall barn and fed total mixed ration silage, which was formulated to have 550 g/kg of dry matter (DM), 160 g/kg DM of crude protein (N × 6.25), and 720 g/kg DM of total digestible nutrients. Sampling was made between 10:00–12:00 in August 2018 and in August 2020. Milk samples were collected manually from four udders and udder skin samples were taken by tracing the udder surfaces with sterile cotton swabs. Fecal samples were obtained from the rectum. Bacterial DNA was extracted by the repeated bead beating plus column method and purified using a commercial kit. Two-step PCR targeting the V4 region of 16S rRNA genes was performed to generate amplicon libraries for next-generation sequencing.

[Results and Discussion] No cows showed systemic signs of clinical and sub-clinical mastitis. The somatic cell count varied from 10×10^3 to 95×10^3 cells/mL. There were large differences in milk microbiota between years; the three most abundant families were Moraxellaceae (35.3%), Bacillaceae (13.1%), and Lactobacillaceae (8.5%) in 2018, and Muribaculaceae (17.9%), Lactobacillaceae (15.9%), and Lachnospiraceae (14.1%) in 2020. Typical pathogens such as Streptococcaceae (0.5%), Enterobacteriaceae (1%), Staphylococcaceae (0.03%), and Corynebacteriaceae (0.1%) were detected at low abundances. In udder skin, Lachnospiraceae (9.2%), Lactobacillaceae (8.1%), and Muribaculaceae (7.3%) were the three most abundant bacteria. Carnobacteriaceae (15.5%), Corynebacteriaceae (9.7%), and Oscillospiraceae (8.8%) were seen as the three most abundant bacteria in bedding. Oscillospiraceae (19%) was the most prevalent bacteria in feces. Principal coordinate analysis indicated that milk microbiota was separately grouped from udder skin, bedding, and fecal microbiota. The udder skin and bedding microbiota formed the same group regardless of the sampling year. Cow-to-cow differences were small in the milk, udder skin, and fecal microbiota. A year-to-year difference was distinctively seen only for the milk microbiota.

PB-10

Monitoring antimicrobial sales intended for livestock at veterinary drug shops in Vietnam

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Keywords: antimicrobial use, antibacterial sale, mobile applications, veterinary drug shop, Vietnam

[Introduction and Objective] There is a pressing need to establish surveillance systems for antimicrobial use (AMU) intended for animal production particularly in many low- and middle-income countries. This is an extremely challenging task, notably due to the wide range of animal species, production types and antimicrobials available in the market. In Vietnam, farmers commonly buy antimicrobials from veterinary drug shops. Therefore, veterinary drug shops are a potential target for data collection on AMU.

[Materials and Methods] We collected antimicrobial sales data at veterinary drug shops and estimated the amount of AMU in different animal species by antimicrobial active ingredient (AAI) class using different measurement metrics. We compiled information on all antimicrobials licensed in Vietnam and used this information to develop a mobile application to capture sales of antimicrobials intended for use in poultry, pig, and ruminant. We provided tablets with this application to 60 veterinary drug shops in two provinces of the country (Bac Giang in the north, Dong Thap in the south; three districts and 30 shops per province) for data collection over three weeks. Total sales of antimicrobials were extrapolated to one year, and these amounts were related to three different denominator estimates in each province including standing animal body weight, animal biomass, and Population Correction Unit (PCU).

[Results and Discussion] A total of 3,960 transactions (2,577 (median 75.5 per shop) in Bac Giang; 1,383 (median 28.5 per shop) in Dong Thap) of 831 different antimicrobial-containing products were recorded in the three-week period. Sales of 57 AAIs belonging to 17 classes were recorded. In the three Bac Giang districts, we estimated that 242.0kg of AAI were hypothetically sold over one year. Of those, 202.2kg (83.6 %) were intended for poultry, 19.8kg (8.1 %) for pigs, and 20.0kg (8.3 %) for ruminants. In Dong Thap, an estimated 48.3kg of antimicrobials were sold, including 28.9kg (59.7 %) for poultry, 15.9kg (32.9 %) for pigs, and 3.5kg (7.2 %) for ruminants. After being standardized by different animal population denominators, AMU in Bac Giang amounted to 1,129.2 mg/kg standing animal body weight, 480.2 mg/kg biomass, and 636.1 mg/kg PCU. In Dong Thap, AMU figures were 1,211.0 mg/kg standing animal body weight, 595.8 mg/kg biomass and, and 818.5 mg/kg PCU. We discuss the observed differences between species, location and metrics, as well as the potential advantages and limitations (including potential sources of bias) of this methodology and its applicability at country level. Retail level data collection can effectively be integrated into AMU surveillance systems that help identify priority AMU management areas (species, regions, and antimicrobial classes), establish national benchmarks and reduction targets.

PB-11

Minimal Bactericidal Concentratio of aqueous extract from *Pouzolzia zeylanica L.* against *Pasteurella multocida*, *Bordetella bronchiseptica*, *Actinobacillus pleuropneumoniae* from swine

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Keywords: *P. zeylanica*, aqueous extract, MBC, *Pasteurella multocida*, *Bordetella bronchiseptica*, *Actinobacillus pleuropneumoniae*

[Introduction] Antibiotic abuse in swine production is one of the causes of antimicrobial resistance. International and national actions to reduce antibiotic use including finding scientific data for antibiotic alternatives. One of those is a veterinary herbal medicine which has antibacterial properties.

Pouzolzia zeylanica L. is a well-known plant in Vietnam as a folk medicine to treat human respiratory problems. Extract from *P. zeylanica* have been reported to contain flavones, flavonoids, tannin, carotene, carotenoids, ascorbic, tartaric, malic and pectic acids, gum, minerals and their salts. *P. zeylanica* also has anti-bacteria (*Bacillus sereus*, *Bacillus megaterium*, *Bacillus subtilis*, *Staphylococcus aureus*, *Sarcina lutea*, *Escherichia coli*, *Pseudomonas aureus*, *Salmonella paratyphi*, *Salmonella typhi*, *Vibrio mimicus*, *Vibrio parahemolyticus*, *Shigella dysenteriae*), anti-inflammatory and antioxidant activities. However, little information in the veterinary sector was studied about this medicinal plant.

[Materials and Methods] The goal of the study was to determine the minimal bactericidal concentration (MBC) of aqueous extract from *P. zeylanica*, against *Pasteurella multosida*, *Bordetella bronchiseptica*, *Actinobacillus pleuropneumoniae* using the agar dilution method. Tetracycline and gentamicin were used as control antibiotics for the assay. Bacteria isolates of *A. pleuropneumoniae, B. bronchiseptica, P. multocida* were obtained from swine in a previous study. Aqueous extract of *P. zeylanica*, Muller Hinton Agar, Blood Agar and sheep blood were used.

[Results] Results were shown in the Table 1.

Table 1. MBC of *P. zeylanica* against some respiratory bacteria.

Bacteria	*P. zeylanica* (mg/ml)	Gentamicin (µg/ml)	Tetracycline (µg/ml)
P. multocida (n=5)	500-1000	0.281-0.563	64->128
A. pleuropneumoniae (n=3)	250-500	4.5-9	16-32
B. bronchiseptica (n=5)	250-500	18-32	64-228

[Conclusion] This study, in the first time, prove that *P. zeylanica L.* had bactericidal effect against the bacterial isolates from swine respiratory tract including ones that resistant to tetracycline. Therefore, *P. zeylanica* extract can be an antibiotic alternative for prevention respiratory infection for swine production.

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PB-12

Campylobacter hepaticus, the cause of spotty liver disease in chickens: Transmission and routes of infection

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Keywords: *Campylobacter hepaticus*, spotty liver disease, transmission
Link available: https://www.frontiersin.org/articles/10.3389/fvets.2019.00505/full

[Introduction] Spotty Liver Disease (SLD) has been a persistent problem in the Australian and UK poultry industries for several decades and its presence in North America has recently been confirmed [1, 2].The causative agent was characterized and formally named as *Campylobacter hepaticus* (*C. hepaticus*) in 2016 [3]. This study aimed to determine possible transmission routes of *C. hepaticus* in layer farms by investigating the presence of the bacterium in the birds and environmental samples, and by investigating the spread of infection within flocks.

[Materials and Methods] 1,076 chicken and environmental samples were collected during 10 weeks from three layer farms in Victoria and a further 764 chicken and environmental samples were collected from other chicken farms across Australia. DNA Extraction. Polymerase Chain Reaction (PCR). Isolation of *C. hepaticus*. MALDI-TOF MS to Identify Bacterial Species. Whole-Genome Sequencing and Genomic Analysis.

[Results] Clinical SLD outbreaks were observed in two of the three farms that were monitored over 10 weeks. In both farms, SLD occurred during peak-laying age at 26 and 28 weeks of age; this timing agrees with previous reporting of the most common age at which disease is seen [5]. Both outbreaks in the monitored farms occurred during winter. Outbreaks from other unmonitored farms occurred throughout the year. In previous decades the disease had also been referred to as “Summer Hepatitis” because of an apparent tendency to most commonly occur in summer. However, based on our findings from this epidemiological study and other experiences over the last 5 years, it is clear that the disease can occur all year round. Although the first outbreaks of SLD in a flock generally occur as the birds enter peak lay, further outbreaks, within the same flock, can occur at later ages [4]. In one of the *ad hoc* sampled flocks, an SLD outbreak occurred in birds of 60–62 weeks of age. *C. hepaticus* was successfully isolated from an SLD affected bird from this flock. *C. hepaticus* DNA was detected in a variety of environmental samples including wild bird feces, flies, and rat feces from SLD-positive farms. These motile organisms might be vectors for *C. hepaticus* dissemination. A recent study that investigated biosecurity practices on Australian commercial layer farms showed that wild birds were commonly reported to be present in free-range farms (73%) and it was noted that they are potential sources of diseases that can be transmitted to laying hens [5]. The genome sequences of all isolates were examined for plasmid content as plasmids may play an important role in dissemination of antibiotic resistance genes. Two isolates contained plasmids with very high sequence similarity to *C. jejuni* pTet-like plasmids.

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PB-13

Rapid thawing by transient exposure to 70 ºC water improves the viability and motility, but not acrosome integrity and PLC zeta-1 distribution of frozen bull spermatozoa

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Keywords: frozen semen, bovine, viability, motility, PLCZ1

[Objective] The objective of the current study was to evaluate effects of a rapid thawing by transient exposure to 70 ºC water on various post-thawed parameters of frozen bull spermatozoa.

[Materials and Methods] The study consisted of 2 experiments, including 1) recording temperature changes in straws at different thawing water temperatures (37, 39 or 70 °C) and 2) the impact of a combination method of quick thawing by transient exposure to 70 ºC in 8 sec and subsequent stabilization process at 39 °C in 52 sec on post thawed spermatic parameters as compared with controls (thawing at 37 °C in 46 sec followed by 39 °C in 14 sec or at 39 °C in 60 sec). Experiment 1, the temperature inside straws of frozen bull semen during thawing procedures was measured by a two-channel digital record thermometer recorded every two-second interval with 8 replicates per group. Experiment 2, a computer-assisted sperm analysis, flow cytometry and immunocytochemistry were applied to evaluate the post-thawed spermatic characteristics with 6 replicates from six identically frozen straws. The motile and kinematic parameters (the total, progressive and rapid progressive motility, velocity straight line, velocity curved line, average path velocity, linearity, straightness, wobble, amplitude of lateral head displacement and beat cross frequency), viability, high mitochondrial membrane potential (MMP), acrosomal integrity, mitochondrial reactive oxygen species (ROS) level and PLC zeta-1 distribution (PLCZ1) were assessed in this study.

[Results and Conclusion] Firstly, when temperature inside frozen straw was monitored during warming in water at 37, 39 or 70 ºC, the average warming rate during thawing at 70 ºC was significantly ($P < 0.01$) faster and the calculated time to reach more than 35 ºC was significantly ($P < 0.01$) reduced as compared with thawing at 37 or 39 ºC. Secondly, when frozen semen was thawed at 70 °C for 8 sec and stabilized at 39 ºC for 52 sec, the motile parameters, viability, high mitochondrial membrane potential (MMP) and mitochondrial ROS level of thawed spermatozoa were significantly ($P < 0.05$) improved, as compared with controls thawed and stabilized at 39 °C for totally 60 sec or at 37 °C for 46 sec and then at 39 °C for 14 sec; whereas there were no differences ($P > 0.05$) in all post thawed characteristics between two types of controls. Furthermore, acrosomal integrity and PLC zeta-1 distribution were not different among three thawing methods ($P > 0.05$). In conclusion, we found that a rapid thawing procedure with transit exposure to 70 ºC water for 8 sec followed by stabilization at 39 ºC for 58 sec can maintain the viability, motility and MMP of frozen bull semen, as compared with standard thawing procedures (37 and 39 ºC), although acrosome integrity and PLC zeta-1 distribution were not affected by thawing procedures examined.

PB-14

Mitochondrial distribution in oocytes and early embryos from naturally aged mice

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Keywords: mitochondria, mitochondrial dynamics, aging, oocyte, embryo

[Objective] The developmental competence of oocytes following fertilization declines with maternal aging. How aging affects oocyte quality remains poorly understood. Age-associated changes in mitochondrial function have been reported to be involved in oocyte quality. We hypothesized that oocytes from older females feature an aberrant mitochondrial morphology. To test this hypothesis, we investigated the mitochondrial distribution in oocytes and early embryos from young and naturally-aged mice.

[Materials and Methods] Mature oocytes were collected from the oviducts of C57BL/6N and ICR female mice after superovulation. Mitochondrial distributions in oocytes from young mice (2-3 month-old) and aged mice (11-14 month-old) were visualized by immunofluorescent labeling of fixed oocytes. Mitochondrial dynamics during preimplantation development were analyzed by live-cell imaging by expressing mitochondrially targeted green fluorescent protein (mtGFP).

[Results and Discussion] The number of oocytes from aged mice was significantly lower than that from young mice ($P<0.001$). Mitochondria in young oocytes were dispersed throughout the cytoplasm, whereas aged oocytes displayed mitochondrial aggregation. Live-cell imaging of mitochondria demonstrated that mitochondria in aged embryos markedly accumulated surrounding chromosomes during the embryonic cell division. The mitochondrial cluster sizes in aged embryos significantly increased compared with young embryos ($P<0.05$). These results have implications for understanding how oocytes undergo aging-associated functional decline and restoration of mitochondrial morphology may represent a novel therapeutic approach against maternal aging-related abnormalities.

PB-15

Molecular changes in the intestinal barrier function associate with clinical signs in *Eimeria tenella* early infection periods

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Keywords: adherens junction; bloody feces; diarrhea; *Eimeria tenella*; epithelial barrier; tight junction

[Introduction] The major clinical signs of coccidiosis in chickens due to *Eimeria* parasite are diarrhea and bloody feces. Previous studies showed that the impairment of the intestinal epithelial barrier and the elevation of the intestinal permeability are causes of clinical signs associated with coccidia challenges. Nevertheless, the information about molecular changes of the epithelial barrier at the early period of the infection with a specific *Eimeria* species has not been mentioned. Hence, this study aims to elucidate the temporal relationships between epithelial barrier conditions and clinical signs in laying hen infected with *Eimeria tenella* over the time from the earliest stages of infection.

[Materials and Methods] White Leghorn chickens were inoculated with 1×10^4 oocysts of *E. tenella*. Thereafter the chickens were monitored for their daily clinical signs. During 5-to-10-day post infection (dpi), feces were collected for oocysts counting. Chickens were then administrated with fluorescein isothiocyanate-dextran (FITC-d) for gastrointestinal permeability test. Tissues were collected each day for histopathological observation and total RNA extraction. Finally, the mRNA expression levels of the tight junction, adherens junction, and cytokine genes were determined using the quantitative real-time polymerase chain reaction (qRT-PCR).

[Results and Discussion] Clinical signs such as diarrhea and bloody feces were observed concurrently from 3 to 8 dpi. Histopathology changes such as severe inflammation, hemorrhage, and epithelial desquamation were identified in the cecum specimens. In the *E. tenella*-infected group, the FITC-d level was significantly increased, and the expression of Claudin (CLDN)-2 gene was also higher compared to control group. CLDN-2 is considered as the pore-forming claudin. It creates paracellular anion/cation pores and water channels led to increase the solute permeability by allowing the passage of sodium ions. Whereas, CLDN-3 plays an important role in maintaining intestinal barrier integrity and also are sealing proteins, the reduction of CLDN-3 in this study was part of the increased paracellular permeability, resulting in the leakage of blood and other substances through this route. The expressions of IL-1β and IL-22 were significantly increased on the 4 to the 6 dpi in the *E. tenella*-infected chicks compared to control. A direct correlation was found in the mRNA expression levels between Occludin and IL-1β and IL-22 in this study.

[Conclusion] The findings in this study, suggest that the expression of junctional molecule genes are related to clinical signs such as diarrhea and bloody feces in chicks infected with *E. tenella*. The disruption of barrier function via downregulation CLDN-3, E-cadherin, Occludin, and ZO-1, but increased CLDN-2, could contribute to *E. tenella* infection-induced diarrhea. Furthermore, this study, reports a link between the high levels of both IL-1β and IL-22 and junctional molecules related to the epithelial barrier and intestinal permeability. Insights on the inflammation-dependent alterations of junctional gene expressions will provide new ideas in the development of therapeutics in *E. tenella* infection.

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PB-16

Production optimization and structural characterization of exopolysaccharides from *Pediococcus pentosceus* FFC003

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Keywords: lactic acid bacteria, *Pediococcus pentosaceus*, exopolysaccharide, fermented soybean paste

[Introduction] Some strains of lactic acid bacteria (LAB) produce exopolysaccharides (EPS), which are recently remarkable attention due to their various biofunctions as well as physical effects. There are many reports on EPS-producing LAB and the EPS, but food application of them are still limited, because of the low productivity and the unclear structural features. We had previously isolated an EPS-producing LAB strain, *Pediococcus pentosaceus* FFC003, from a traditional fermented soybean paste, Miso, in Fukui Prefecture, Japan. In this study, we aimed to optimize culture conditions for EPS production and to characterize primary structure of *P. pentosaceus* FFC003.

[Materials and Methods] First, *P. pentosaceus* FFC003 was cultivated in MRS broth, reconstituted skim milk and reconstituted cheese whey for 60 h at 25, 30, 37°C. Culture pH, cell viability and spinnability in the three media were measured every 12 h to survey favorable culture conditions for cell growth and EPS production of the strain. Next, the one variable at a time (OVAT) method and the central composition design - response surface methodology (CCD-RSM) were used to find the optimal amount of the nutrients that significantly affected the EPS yield in the first selected medium. After that, the crude EPS was separated with anion-exchange chromatography, and then each EPS division was applied to size-exclusion HPLC and pre-column derivatization HPLC for the molecular weight and the monosaccharide composition analyses, respectively.

[Results and Discussion] First, *P. pentosaceus* FFC003 showed better growth when incubated at 25°C in MRS broth than 30 and 37°C, and in reconstituted skim milk and reconstituted cheese whey. EPS productivity of the strain was also high at 25°C in MRS broth, the highest spinnability was observed at 24 h-incubation; whereas no spinnability was confirmed when incubated in reconstituted skim milk or reconstituted cheese whey. Next, from the OVAT analysis, it was indicated that meat extract, yeast extract, glucose and triammonium citrate in MRS broth were significant on EPS production from *P. pentosaceus* FFC003. Then, the maximum EPS production (362.63 mg/L) was obtained in the modified MRS broth supplemented with the four significant nutrients whose amounts were optimized using CCD-RSM. After that, the crude EPS prepared from the modified MRS culture supernatant of *P. pentosaceus* FFC003 was separated and almost purified to three divisions, one neutral and two acidic EPS, with the anion-exchange chromatography. The three EPS divisions were analyzed with the size-exclusion HPLC, and determined the peak molecular weight as 150-650 kDa. Finally, the monosaccharide composition of each division was analyzed, showing that the neutral EPS consisted of mannose and glucose (10:90); an acidic EPS was composed of mannose, lyxose, ribose, rhamnose, glucose, galactose and arabinose; and another acidic EPS was mannose, lyxose, ribose, glucuronic acid, glucose and galactose. Such complicated monosaccharide compositions have not been reported, and therefore it was found that at least the acidic EPS produced by *P. pentosaceus* FFC003 should be novel. In this study, we optimized the production and characterized the primary structure of the EPS from *P. pentosaceus* FFC003; that would contribute to food application of the strain and the EPS in future.

PB-17

Survey on *Escherichia coli*, *Salmonella* contamination and residues of some antibiotic in pork and chicken in some provinces of Nam bo southwest region

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Keywords: pork and chicken meat, *E.coli*, *Salmonella*, antibiotic residue, southwest region

[Introduction and Objective] Worldwide, around 70 percent of the cases of food poisoning are caused by bacteria. In Asian countries, *Salmonella*, *Listeria monocytogenes*, and *S. aureus* are the major causes of food poisoning (Ono H.K. *et al.*, 2008). In Vietnam, food poisoning is more and more complicated, becoming a hot issue of society and a concern for public health (Trieu Nguyen Trung, 2011). Moreover, according to the report of the Ministry of Health in 2011, the situation of food poisoning is increasing and has a significant impact on public health. During the 5 years from 2011 to 2015, the whole country had 836 cases of food poisoning, with 25,544 cases and 155 deaths. A total of 79% of reported cases is caused by bacteria, 14% by chemicals, 4% by viruses, and 1% by parasites. (Public Health Report, 2015). According to the General Statistics of Vietnam, by the end of 2016, the population of the Mekong is more than 17,660,000 people, especially, the total population of 04 provinces including An Giang, Vinh Long, Dong Thap, and Can Tho at about 6,153,000 people, is a major consumption market of fresh meat in Vietnam. Therefore, these also have a high risk of food poisoning if not under strict control. As a result of this situation, inspection, assessment and monitoring of bacterial contamination and antibiotic residues on food, especially pork and chicken at the slaughterhouse and business establishments, must be done regularly. Therefore, the survey of the rate of bacterial infection and antibiotic residues in pork and chicken in some Southwest provinces aims to have an overview of the situation, as well as give further solutions.

[Research contents] Identification, analysis and evaluation of *E.coli* and *Salmonella* in pork. Identification, analysis and evaluation of *E.coli* and *Salmonella* in chicken. Identification, analysis and evaluation of antibiotic residues in pork and chicken.

[Materials] Pork and chicken were sampled from different slaughterhouses and business establishments in 4 provinces including An Giang, Vinh Long, Dong Thap and Can Tho. Place of testing and analysis: National Center for Veterinary Hygiene Inspection No.2. Implementation period: from May 1st 2018 to November 30th 2018.

[Results] Prevalence of *E.coli* and *Salmonella* in pork taken at the slaughterhouses in 4 provinces are 38,89% and 14,44% respectively; and at the business establishments are 11,11% and 27,78% respectively. Prevalence of *E.coli* and *Salmonella* in chicken taken at the slaughterhouses of 4 provinces are 20% and 15% respectively; and at the business establishments are 62,50% and 37,50% respectively. A survey results of antibiotic residues on 54 samples of pork and 24 samples of chicken showed that two pork samples (3,7%) and two chicken samples (33,33%) are found to contain Enrofloxacin residue levels in excess.

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PB-18

Prevalence and antibiotic resistance of Enterotoxigenic *Escherichia coli* (ETEC) serotypes O8, O9 isolated from cattle in the Mekong Delta, Vietnam

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Keywords: antibiotic genes, antibiotic resistance, cattle, ETEC, the Mekong Delta

[Introduction and Objective] Enterotoxigenic *Escherichia coli* (ETEC) is one of the pathogens causing diarrhea in cattle frequently. Among serotypes, ETEC O8 and O9 are considered as the common serotypes isolated from diarrhea or healthy cattle (Nagy and Fekete, 1999). Moreover, the antimicrobial resistance of *E. coli* has become a global issue, especially in animal husbandry. This study aims to clarify the prevalence of ETEC O8 and O9 in cattle, and the antimicrobial susceptibility as well as antibiotic resistance genes of those serotypes in the Mekong Delta, Vietnam.

[Materials and Methods] A total of 244 cattle feces was collected from December 2020 to March 2021 in the Mekong Delta, Vietnam. The *E. coli* was isolated from the feces following the previously described by Shams *et al.* (2012). ETEC O8, O9 were identified by the PCR method using the primers in the reports of Li *et al.* (2010) and Iguchi *et al.* (2015). The disc diffusion method (CLSI, 2019) was conducted to determine the antimicrobial susceptibility of ETEC O8, O9 isolates against thirteen antibiotics, including ampicillin (Am, 10ug), amikacin (Ak, 30ug), amoxicillin/clavulanic acid (Ac, 20/10ug), ceftazidime (Cz, 30ug), cefuroxime (Cu, 30ug), colistin (Co, 10ug), chloramphenicol (Cl, 30ug), doxycycline (Dx, 30ug), gentamicin (Ge, 10ug), levofloxacin (Lv, 5ug), ofloxacin (Of, 5ug), streptomycin (Sm, 10ug), tetracycline (Te, 30ug). The prevalence of antibiotic resistance genes (*blaTEM*, *cat1*, *qnrA*, *strA*, *sulII*, *tetA*) was determined by using the PCR method as the previous reports (Boerlin *et al.*, 2005; Jouini *et al.*, 2007; Cattoir *et al.*, 2007; Gow *et al.*, 2008; Van *et al.*, 2008; Abdelgader *et al.*, 2018). The results were tested by the Chi-square method with 95% of confidence.

[Results and Discussion] Of 244 feces samples, *E. coli* O8 and O9 were identified at a relatively high rate with 15.98% (39/244) and 8.20% (20/244) respectively. It indicated that those serotypes might be predominant in cattle in the Mekong Delta. Those *E. coli* O8 and O9 isolates showed high susceptibility to the examined antibiotics such as amikacin (98.31%), doxycycline (96.61%), ofloxacin (94.92%), and levofloxacin (93.22%). This result could be due to the less frequent contact of those *E. coli* isolates with the antimicrobial agents in cattle. However, those isolates exhibited resistance against ampicillin (47.46%), streptomycin (44.07%), tetracycline (42.37%). There were 35/59 *E. coli* O8 and O9 isolates (59.32%) that were resistant from two to twelve antibiotics with 25 resistant patterns. The pattern of Am+Ac+Co+Sm+Te+Cl was the most common type (6.78%). Moreover, *tetA* was the most prevalent gene (66.10%) that was detected from those *E. coli* O8, O9 isolates while *cat1* was the least one (5.08%). Forty-five *E. coli* O8 and O9 isolates (76.27%) harbored from two to four antibiotic resistance genes, and the pattern of *strA+tetA+sulII* was detected at the highest rate (23.73%). It indicated that the antibiotic resistance of *E. coli* O8, O9 could become a potential risk in the antibiotic treatment of diseases caused by those serotypes in cattle. Therefore, the prevalence and antibiotic resistance of *E. coli* O8 and O9 should be controlled to protect cattle health as well as human health in far.

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PB-19

Create beeswax candles with the effect of repelling mosquitoes from natural essential oil

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Keywords: *Aedes aegypti*, *Plectranthus hadiensis*, *Schefflera octophylla*, *Citrus limon. L* essential oil

[Introduction] Mosquitoes (*Aedes aegypti*) transmit dengue fever in humans and there is no specific treatment; trend of using environmentally friendly materials such as honey beeswax, plant essential oils, to repel mosquitoes, is a new biological solution that is highly safe for human health (Münstedt *et al.*, 2009; Müller *et al.*, 2008). Therefore, the aim of this study was to extract, determine the chemical composition, sensory evaluation of the essential oils of *Schefflera octophylla* leaves, *Plectranthus hadiensis* leaves, and *Citrus limon. L* peels; and determine the mosquito repellent effect of honey beeswax candles containing these 3 essential oils.

[Materials and Methods] Steam distillation and modern physico-chemical methods have been used to extract, determine the chemical composition of 3 essential oils, respectively. To determine the mosquito repellent effect of honey beeswax candles containing 3 essential oils, one experiment was designed in a completely randomized design, 4 treatments with 4 essential oil content by 0.521; 0.825; 1.65 and 3.3 µg/candle; 20 female mosquitoes 5-8 days old per treatment; 3 replicates, 1 mosquito per experiment unit; percentage of repellency was calculated as Tripathi *et al.*, (2004).

[Results] First, *Plectranthus hadiensis* essential oil is liquid, light yellow, fragrant; 23 components of D-Limonene; Linalool; Trans-p-mentha-1(7),8-dien-2-ol; cis-p-mentha-1(7),8-dien-2-ol, cis-carveol; bornyl acetate; 3-methyl-4-isopropylphenol and caryophyllene are volatile components belonging to groups of hydrocarbons and terpenes containing aldehydes, alcohols, esters, and acids. Second, *Schefflera octophylla* is a liquid, colorless, pungent odor, 10 components of Heptane; Neopentanoic acid; Spiro[2,4]hepta-4,6-diene; Morpholine; (E)-Beta-methoxy-alpha-methylstyrene; Tocainide; Acetaldehyde; and Fluoroethyne. Third, *Citrus limon. L.* is a liquid, light yellow, fragrant with lemongrass flavor, containing 42 components, in which the highest content is D-limonene; besides, components greater than 1 such as: 6,6-Dimethyl-2-methylenebicyclo[3.1.1] heptane; χ-Terpinene; Geraniol group; Citral; 3,7-Dimethyl-2,6-octadienyl esther; α-Terpineol; β-Phellandrene; β-Bisabolene; Terpinen-4-ol … Morover, there are components greater than 0.1 including β-Ocimene compounds; (+)-4-Carene; Linalool; Cis-p-metha-2,8-dien-1-ol; Decalal; Geranyl acetate; Caryophyllene; α-bergamotene; Humulene; gamma.-Elemene; χ- Asarone; β- Asarone; and α- Bisabolol. Finally, distribution of mosquitoes in the essential oil containers decreased gradually when increasing the concentration of essential oil, which was statistically significant ($P < 0.05$). The mosquito repellent effect of *Plectranthus hadiensis* and *Citrus limon. L* is only over 50% at a concentration of 1.65 µg/candle; not more than 86% at the highest dose of 3.3 µg/candle; the outstanding mosquito repellent effect of the *Schefflera octophylla* is not less than 96 % /3 content of 0.825; 1.65 and 3.3 µg/candle.

[Conclusion] 3 essential oils extracted were effective in repelling mosquitoes, especially the essential oil of *Schefflera octophylla*.

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