International Symposium on New Insights on Animal Nutrition, Breeding and Reproduction

PROGRAM

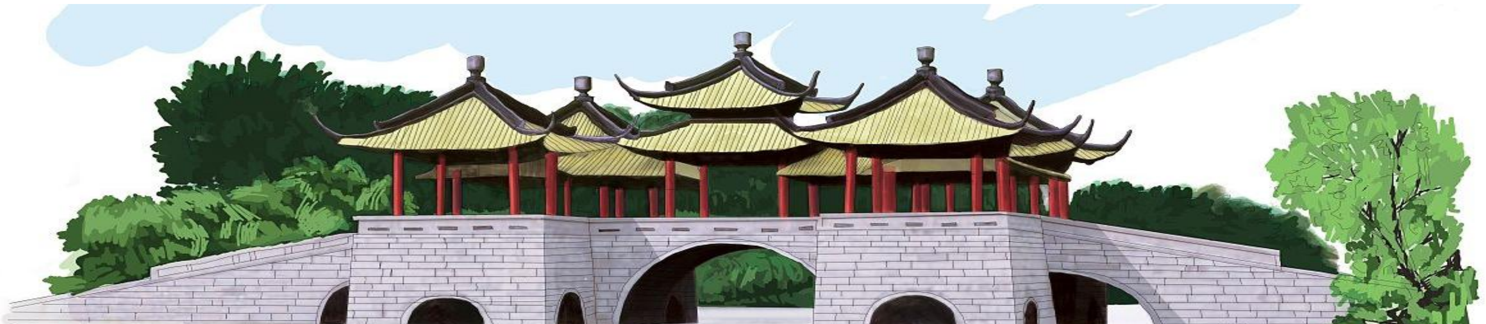
Yangzhou, CHINA

September 26th-27th, 2019

International Symposium on New Insights on Animal Nutrition, Breeding and Reproduction

Agenda

September 26th, 2019	
Opening Ceremony Wei-jing Hall, Metropark Hotel (2nd floor)	
08:30-08:50	Welcome speech from Yangzhou University
08:50-09:00	**Group photo**
Acedemic Speech	
09:00-09:30	**Introduction of International Education and Research Center for Food and Agricultural Immunology (CFAI)**
	Professor Hideki TAKAHASHI (Tohoku University, Japan)
09:30-10:00	**Novel insight into the molecular mechanism of a long noncoding RNA-*FUT3as* regulating *Escherichia coli* F18 susceptibility in weaned piglets**
	Professor Wen-bin BAO (Yangzhou University, China)
10:00-10:30	**Tea Break**
10:30-11:00	**Nutritional Characteristics of Forage Plants and Foraging Behavior of Grazing Animals in Species-rich Vegetation**
	Professor Shin-ichiro Ogura (Tohoku University, Japan)
11:00-11:30	**Toll-like receptor 4 and its Association with Bovine Mastitis and Johne's Disease**
	Professor Niel Karrow (University of Guelph, Canada)
11:30-13:30	**Lunch**



International Symposium on New Insights on Animal Nutrition, Breeding and Reproduction

Acedemic Speech	
13:30-14:00	**The molecular regulatory mechanisms of PEPT1/NHE3 synergistic transport of small peptides from gastrointestinal bacteria in cattle**
	Professor Guo-qi ZHAO (Yangzhou University, China)
14:00-14:30	**Sperm stem cell behaviors in mammalian testis**
	Associate Professor Kenshiro HARA (Tohoku University, Japan)
14:30-15:00	**Uncovering unique features of goose liver and molecular function of the related genes**
	ProfessorTuo-yu Geng (Yangzhou University, China)
15:00-15:30	**Genetic and genomic analyses for meat quality traits in Japanese Black beef**
	Associate Professor Yoshinobu UEMOTO (Tohoku University, Japan)
15:30-16:00	**Tea Break**
16:00-16:30	**Identification of miRNA-mRNA interaction and their potential regulatory role in response to Staphylococcus aureus-induced mastitis in Chinese Holstein cows**
	Professor Yong-jiang MAO (Yangzhou University, China)
16:30-17:00	**Shifts in fermentation end products and bacterial community composition in long-term, sequentially transferred in vitro ruminal enrichment cultures fed switchgrass with and without ethanol as a co-substrate**
	Associate Professor Miao LIN (Yangzhou University, China)
17:00-17:10	**Closing Speech**
September 27th, 2019	
Visiting Labs	
Yangzhou University	

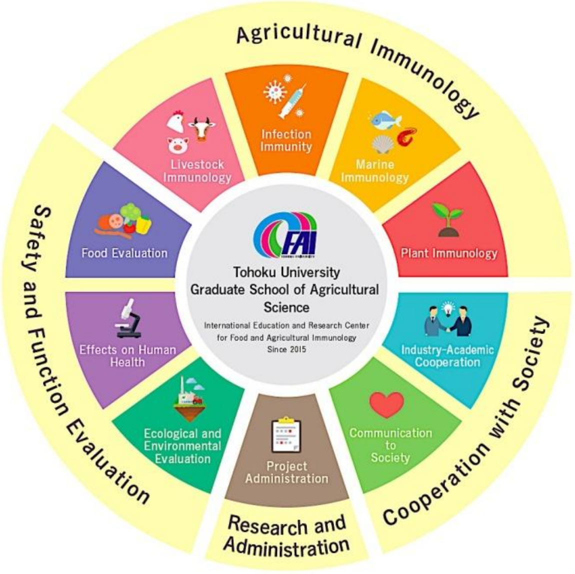
Professor Hideki TAKAHASHI, Tohoku University, Japan

Hideki Takahashi is Professor in Laboratory of Plant Pathology, Director of International Education and Research Center for Food and Agricultural Immunology (CFAI), Vice-Dean of Graduate School of Agricultural Science, Tohoku University and JSPS Core-to-core program Coordinator. He got his PhD degree from Tohoku University on molecular mechanism of symptom expression virus-infected plants in 1993 and studied on salicylic acid-mediated signaling pathway at Waksman Institute, Rutgers University, USA from 1994-1996 as a post-doctoral fellow. He is presently a council member of the Phytopathological Society of Japan. His research focuses NB-LRR class *R*-gene-conferred defense system against virus infection in host plants. Recently, he started to investigate the role of virus latent infection in plants on natural ecosystem in new research field: Neo-virology.

Introduction of International Education and Research Center for Food and Agricultural Immunology (CFAI) in Graduate School of Agricultural Science, Tohoku University

Hideki Takahashi, hideki.takahashi.d5@tohoku.ac.jp

The Center for Food and Agricultural Immunology (CFAI) was established on April 1, 2015. It consists of the following four divisions: ‘Agricultural Immunology’, ‘Safety and Function Evaluation’, ‘Cooperation with Society’ and ‘Research and Administration’. These divisions are sub-divided into 10 units (see illustration). The mission of CFAI is to create the underpinning technical advances and global professional development required for the promotion of safe food and drug-independent health from effective, agricultural systems. This will be based on interdisciplinary research into agricultural immunology in plants, livestock and aquatic organisms. To achieve our mission, we aim to especially promote research exchange with young researchers and graduate students in leading international universities through the financial support by “JSPS Core-to-core program”.



Organization chart of CFAI

Professor Shin-ichiro OGURA, Tohoku University, Japan

Shin-ichiro Ogura graduated Department of Animal Science, Tohoku University. He received his Ph.D. degree in Graduate School of Agricultural Science, Tohoku University in 1997. Following this, he was an Assistant Professor of the Faculty of Agriculture, Miyazaki University, from 1998 to 2003. Since 2003, he has served at the Tohoku University. Now he is a Professor of Laboratory of Land Ecology, Graduate School of Agricultural Science, Tohoku University. He also acts as a Director of Integrated Field Science Center and a Vice Director of Tohoku Agricultural Science Center for Reconstruction, Graduate School of Agricultural Science, Tohoku University. During his career, he had an opportunity of staying in Macaulay Land Use Research Institute, Scotland in 2002. He has focused on the plant-animal interactions in grassland ecosystems, such as pasture and forge crop production and utiization by ruminant animals. Especially, he is interested in diet selection, foraging behavior and nutrient uptake of grazing ruminants. He is now trying to evaluate nutritional characteristics of native plants and its role to animal production, to establish a grazing system in species-rich vegetation.

Nutritional Characteristics of Forage Plants and Foraging Behavior of Grazing Animals in Species-rich Vegetation

Shin-ichiro Ogura, shin-ichiro.ogura.e1@tohoku.ac.jp

In warm and wet climate regions (e.g., south-eastern part of China and Japan), a variety of plant types (monocots, forbs, trees and ferns) and species grow in land ecosystems, including arable lands, grasslands and forests. These plants are available for livestock production because some of them have high palatability and their nutritive values satisfy the requirement of the animals. In Japan, wasted agricultural areas and forests are often used for livestock grazing, as well as pastures. However, there is still little information on nutritional characteristics of the wild plant species, and foraging behavior (diet selection and foraging manner) of grazing animals in such species-rich vegetation.

In my previous researches, grazing animals ingested a wide range of plant species in a species-rich vegatation; they ingested not only herbaceous plants but also trees. The foraging manner of the animals greatly differed among plant types and plant parts ingested. Concentration of nutrients also varied among plant species and seasons; some forbs and trees contained higher mineral elements (e.g., Mg, Ca, Mn, Co) than grasses. In addition, tree leaves have high antioxidative ability than grasses. Thus, daily uptake of some mineral elements and antioxidative components in the grazing animals were higher in species-rich areas than in sown grass pastures.

In this symposium, I will introduce about recent researches on the characteristics of nutritient uptake and foraging behavior of grazing cattle under speicies-rich vegetation. These studis will contribute to clarify the effect of species richness of vegetation on the health and productivity of livestock production.

Professor Guo-qi ZHAO, Yangzhou University, China

Guoqi Zhao graduated from Iwate University, Japan. Now he is professor in College of Animal Science and Technology, Yangzhou University. He also acts as an expert in National Dairy Industry and Technology System, China. During his career, he went to Department of Animal Science, Tohoku University, Japan as a visting scholar for one year. He has been focused on the The molecular regulatory mechanisms of PEPT1/NHE3 synergistic transport of small peptides from gastrointestinal bacteria in cattle. Although high concentrate addition in the diet can improve the cow milk production, it will cause some problems, such as variations of microbiota in the rumen environment and other changes, and then induce nutrition metabolic disorder or disease, like rumen inflammation, laminitis and intermittent diarrhea. The systemic inflammation is mainly caused by the small peptide which released from rumen bacteria. The peptide-transporters SLC15 play an important role in discern and transporting the peptide. However, it is unclearly about the transport mechanism of PEPT1 and NHE3 which are members of SLC15 family. This study aims to illuminate the synergistic effects by PEPT1 and NHE3 in regulating the absorptive pathway and transport mechanism of bacterial peptide using proteomics and immunohistochemical methods. The results will helpful in controlling the risk of inflammation which caused by high concentrate addition in dairy production.

Professor Wen-bin BAO, Yangzhou University, China

Wen-bin Bao, Ph.D. Major in Animal Breeding and Genetics in Department of Animal Sciences and Technology, Yangzhou University, Yangzhou, China. Now he is professor of Key Laboratory for Animal Genetics, Breeding, Reproduction and Molecular Design of Jiangsu Province. He also acts as a secretary-general of Jiangsu pig industry technology alliance and a director of Jiangsu pig healthy breeding technology research center. He has been studying pig disease-resistance breeding for a long time, and mainly focused on the molecular mechanism study of resistance to bacterial diarrhea and viral diarrhea in piglets, including the effects of function genes, long non-coding RNA (microRNA, lncRNA, circRNA), epigenetic modification (DNA methylation, m6A methylation) on piglet's diarrhea, respectively. In recent years, he has presided over 12 projects supported by the National Natural Science Foundation of China, National Key Technology R&D Program of the Ministry of Science and Technology, etc. He successively published more than 100 research papers by the first author or the communication author, SCI includes 40 papers. Besides, he has 4 authorized patents, 1 software copyright, 2 Jiangsu province agricultural standard, etc. In 2017, he has been selected National High-level personnel of special support program ("Ten Hundred Talent Program").

Novel insight into the molecular mechanism of a long noncoding RNA-*FUT3as* regulating *Escherichia coli* F18 susceptibility in weaned piglets

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Escherichia coli F18 (*E. coli* F18) is mainly responsible for post-weaning diarrhea (PWD) in piglets. Now, the genetic basis and regulatory mechanism underlying resistance to *E. coli* F18 infection in piglet are still unclear. Long noncoding RNAs (lncRNAs) modulate various biological processes, but their role in host anti-E. coli F18 infection remains unknown. Here, using RNA sequencing (RNA-seq), we screened out an antisense lncRNA termed FUT3as between E. coli F18-resistant and -sensitive piglets, and functional analysis demonstrated that FUT3as acts as a key positive regulator of *E. coli* F18 infection in weaned piglets. Moreover, iTRAQ proteomics and expression analysis showed that FUT3as knockdown caused the suppression of *FUT3*-mediated glycosphingolipid biosynthesis signaling, leading to promote the resistance to *E. coli* F18 infection in piglets. Interestingly, RNA pull-down, immunoprecipitation (RIP) and immunoprecipitation (ChIP)-qPCR demonstrated that FUT3as also interacts with histone H4, which directly affects the H4K16ac modification level of *FUT3* promoter, thus causes change in gene expression. Besides, functional analysis showed that the decreased Expression of *FUT3* contributes to enhancing E. coli F18 resistance in piglets. In sum, we have identified an lncRNA-based glycosphingolipid biosynthesis signaling regulatory circuit that modulates *E. coli* F18 susceptibility in piglets by probably affecting the histone H4 modifications of *FUT3* promoter. Our results for the first time provide a new insight into the mechanism of lncRNA regulating the anti-E. coli F18 infection in weaned piglets, and provided theoretical guidance for solving the problem of molecular breeding for diarrhea disease resistance in pigs.

Professor Tuo-yu GENG, Yangzhou University, China

Dr. Geng was graduated from Virginia Tech with his major in Poultry Genetics and Genomics. He has worked in the field of animal science for decades, especially in the area of poultry genetics and breeding. He is well equipped with research techniques and skills in molecular biology. He was also exposed to muscle biology and lipid metabolism during his postdoctoral study in Duke University, University of Virginia, and Medical University of South Carolina. After his stay in US for 11 years, he joined in the College of Animal Science and technology, Yangzhou University in 2013. His research interests include identification of genes associated with animal traits, understanding of molecular biology of carbohydrate and lipid metabolisms, discovery of the mechanisms underlying fatty liver, and investigation on the function of genes if interest. Currently, he is focused on finding unique features in goose, especially in the context of fatty liver, and understanding on the molecular function of the genes related to the unique features. So far, he has published more than 30 research papers, including those in Diabetalogia, America Journal of Pathology, Journal of clinical Investiation, and Cell Metabolism.

Uncovering unique features of goose liver and molecular function of the related genes

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Carbohydrate/fat metabolism and energy metabolism are important to animal growth, production and health, but disorder in the metabolisms can lead to animal diseases, such as obesity, fatty liver disease in livestock, and diabetes and nonalcoholic fatty liver diseases (NAFLD) in human. Overfeeding Landes goose (a breed specific for production of foie gras or fatty liver) for 3 to 4 weeks can produce 800-1200 g fatty liver, about 10 times heavier than normal liver. The content of fat in the liver accounts for more than 60%, meaning the liver suffers from severe steatosis. Despite of severe steatosis, goose fatty liver does not show any overt symptoms such as inflammation and fibrosis, which is often seen in human or rodent NAFLD. It suggests that goose may have a unique mechanism by which the liver is protected against the harmful effects associated with severe steatosis. Indeed, it has been reported that the expression of TNFa and complement genes is inhibited in goose fatty liver while the expression of adiponectin receptors, fatty acid desaturase 1 and 2, and mitochondria-related genes is upregulated, which is the opposite to those in NAFLD of human and rodents. The unique feature of goose may be developed during the evolution of its ancestor. As a migratory bird, the ancestor does not store energy in fat tissue but also accumulate a large amount of fat in the liver, so that energy need can be met for its migration. After migration, the fat deposited in the liver is used up and the liver is recovered to normal. Therefore, goose fatty liver is physiological other than pathological. Uncovering the unique feature that goose owns may provide new idea to develop therapeutic approach to treating fatty liver disease in human and other animals.

Recently, our data showed that miR29c expression was comprehensively inhibited in energy homeostasis-related tissues (the liver, fat and muscle) of the overfed vs. normally fed geese, which is different from miR29c induction that occurs in tissues of the diabetic rat. We thus speculate that miR29c and its target genes also may be a component of the unique mechanism underlying goose

fatty liver. To address the function of miR29c, three predicted target genes (i.e., Insig1, Sgk1 and Col3a1) that participate in energy homeostasis or cell growth were validated by a dual-fluorescence reporter system and other in vitro assays. Importantly, expression of Insig1, Sgk1 and Col3a1 was upregulated in goose fatty liver. In line with these observations, treatment of goose hepatocytes with high glucose or palmitate suppressed the expression of miR29c but induced the expression of the target genes, suggesting that hyperglycemia and hyperlipidemia, at least partially, contribute to the suppression of miR29c and induction of the target genes in goose fatty liver. In addition, pharmacological assays indicated that RFX1 was a transcription factor involved in the expression of miR29c. In conclusion, the findings suggest that miR29c may play a role in the regulation of energy homeostasis and tissue growth via its target genes, contributing to the tolerance of the goose to severe hepatic steatosis.

Another interesting research is about endogenous retrovirus (ERV). ERV accounts for 8-10% of the human and mouse genomes and plays an important role in animal life activities. Recent studies have found that the activation of ERV can affect innate immunity and the expression of immune-related genes, and the activity of ERV is affected by many factors including nutritional factors. As it is known that nutrition and immunity are related to each other, we hypothesize that nutritional status can affect the expression of immune-related genes through ERV. To verify this hypothesis, the nutritional status of animals was changed by fasting and feeding, and the expression of intact ERV (ERVK18P, 25P) and immune related genes (DDX41, IFIH1, IFNG, IRF7, STAT3) in the liver was detected. ERV was also overexpressed in primary goose hepatocytes, and the expression of immune related genes was subsequently determined. The data showed that the expression of ERV and immune-related genes was induced in chicken liver but suppressed in goose liver by starvation. Furthermore, the expression of ERV and immune related genes was induced in goose fatty liver by overfeeding. In addition, overexpression of ERV in goose primary hepatocytes can induce the expression of immune-related genes. In conclusion, these findings suggest that ERV mediates the regulation of nutrition/energy levels on the expression of immune-related genes, that the mediation by ERV was varied with avian species, and that ERV and immune-related genes are involved in the formation of fatty liver in geese, which provides a new mechanism for the association between nutrition and immunity.

Professor Niel Karrow, University of Guelph, Canada

Niel Karrow received his PhD degree in the area of immunotoxicology from the University of Waterloo in 1999. He held a post-doctoral fellowship (PDF) in immunotoxicology in the Department of Pharmacology and Toxicology at Virginia Commonwealth University- Medical College of Virginia, and a second PDF in immunogenetics in the Department of Pathobiology, University of Guelph. In 2002 he was hired as an Assistant Professor in the Department of Animal Biosciences, was granted tenure in 2007, appointed to Associate Professor in 2008 and Full Professor in 2017. Since 2016, Dr. Karrow has also been a Visiting Scholar at the College of Animal Science and Technology, Yangzhou University. In 2018, he was also appointed Adjunct Professor in the Animal Nutrition Institute at Sichuan Agricultural University as part of the Technology Innovation and Talent Recruitment Base 111 Project. Dr. Karrow’s research interests focus on immunoregulation, immunotoxicology, and immunogenetics of food production species. This has included identifying genetic markers associated with ruminant inflammatory diseases such as mastitis and Johne's disease; assessing the effects of maternal stress on genetic programming of the fetal neuroendocrine-immune system and the potential for nutritional intervention; promoting healthy neonatal gut development through the use of natural immune modulators; and assessing the immunotoxicity of bacterial and fungal toxin mixtures.

Professor Yong-jiang MAO, Yangzhou University, China

Yongjiang Mao graduated from College of Animal Science and Technology, Yangzhou University. He received his Ph.D. degree with his thesis entitled "The Genetic Diversity, Genetic Differentiation of 6 Cattle Populations in Bovidae in China and the Statistical Methods of Genetic Diversity Research" in 2006. Now he is professor of Animal Breeding, and Genettics College of Animal Science and Technology, Yangzhou University. He also acts as a director of Cattle Society, belonging to Chinese Society of Animal Husbandry and Veterinary Medicine. Meanwhile, he is the member of the Dairy Association of China. During his career, he had an opportunity of staying in Department of Animal Genetics and Breeding, University of Gottingen, Germany for one year. In addition, he went to Department of Animal Science, University of Guelph, Canada as a visting scholar for eight months. He has been focused on the functional genomics and epigenetic mechanisms of important economic traits of cattle, as well as the protection, evalution and exploitation of livestock genetic resources. Especially, he is interested in the regulatory mechanism of non-coding RNAs in mastitis of cow induced by *Staphylococcus aureus* and *Escherichia coli*. He now tries to apply genetic and epigenetic analytical methods and tools for researches on molecular regulation mechanisms of circCD358-miR-370-TLR4 signaling pathway in immune response to bovine masitits induced by *Escherichia coli*.

Identification of miRNA-mRNA interaction and their potential regulatory role in response to *Staphylococcus aureus*-induced mastitis in Chinese Holstein cows

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Cow mastitis is a complex disease, and many prevention treatments for mastitis are not effective. Some studies have shown that miRNAs affect mastitis by targeting certain genes, but the combined miRNA-mRNA analysis for *Staphylococcus aureus* (*S. aureus*) induced mastitis in cows remains blurry. (**Methods)** *S. aureus* was used to artificially infect mammary glands of Chinese Holstein dairy cows for the construction of a mastitis model. The total RNA extracted from *S. aureus*-induced mastitis and healthy mammary gland tissues respectively, were submitted for gene and miRNA expression analysis by Solexa high-throughput sequencing technology. Q-RT-PCR, western blot, luciferase multiplex verification techniques, TAG and cholesterol measurement were used to explore the potential regulatory role of key miRNA-mRNA pairs. (**Result**) A total of 1,230 significant different expressed (DE) mRNAs and 52 significantly DE miRNAs were identified between *S. aureus* induced and healthy mammary glands. Correlation analysis revealed 329 negatively associated miRNA-mRNA pairs, including 31 pairs with up-regulated mRNA and 298 pairs with down-regulated mRNA. In miR-106b-*ABCA1* pair, miR-16b negatively affected lipid metabolism, while *ABCA1* promotes milk fat metabolism in mammary epithelial cells. In addition, miR-145 targeted and negatively related to *FSCN1*, playing immune regulation role in response to *S. aureus* induced-mastitis. Upon *S. aureus* infection of mammary gland, miR-145 expression was down-regulated, which reduced the inhibitory effect on *FSCN1* gene, and the upregulation of *FSCN1* expression promoted mammary epithelial cell proliferation to allow recovery of damaged breast cell tissue. (**Conclusion**) This study revealed the important regulatory role of miRNA-mRNA interaction in the process of *S. aureus* mastitis, providing the laboratory research basis for prevention and treatment of mastitis. Especially, miR-106b-*ABCA1* played important regulatory role in milk lipid metabolism, while miR145- *FSCN1* indicated one possible way of immune mechanism against *S. aureus* infection in dairy cows.

Associate Professor Kenshiro HARA, Tohoku University, Japan

Kenshiro Hara graduated Department of Agriculture, Tohoku University. He received Ph.D. degree in Graduate School of Agricultural and Life Science, University of Tokyo with thesis entitled " Roles of embryonic hindgut endoderm in primordial germ cells migration and differentiation in mice". Now he is Associate Professor of Laboratory of Animal Reproduction and Development, Graduate School of Agricultural Science, Tohoku University. He is studying about the mechanisms of spermatogenesis in mammal. Also, He is challenging to establish the method of reconstitution of in vitro spermatogenesis in domestic animals.

Sperm stem cell behaviors in mammalian testis

Kenshiro Hara, kenshiro.hara.b6@tohoku.ac.jp

Livestock production is supported by continual animal reproduction. Thanks to a high sperm productivity of testis, we can collect huge number of spermatozoa from bull and utilize them efficiently to produce offsprings with good traits by artificial insemination. Thus, high productivity of spermatozoa in testis is a key to achieve industrial animal production. In contrary to oogenesis, sperm production is mainly supported by sperm stem cell function through the balanced self-renewal and differentiation. However, identity and behavior of sperm stem cells remains largely unknown. Elucidation of stem cell behavior and its regulation mechanisms will be important for the establishment of therapeutic treatment for hypospermatogenesis caused by stem cell depletion. In the currest symposium, I will discuss about mammalian sperm stem cell behaviors in normal adult testis, revealed by using mouse as an analytical model.

References

1. Kanamori M, Oikawa K, Tanemura K, Hara K. Mammalian germ cell migration during development, growth, and homeostasis. Reprod Med Biol. 2019 Jun 9;18(3):247-255. Review

2. Hara K, Nakagawa T, Enomoto H, Suzuki M, Yamamoto M, Simons BD, Yoshida S. Mouse spermatogenic stem cells continually interconvert between equipotent singly isolated and syncytial states. Cell Stem Cell. 2014 May 1;14(5):658-72.

Associate Professor Yoshinobu UEMOTO, Tohoku University, Japan

Yoshinobu Uemoto graduated Department of Applied Biosciences, Tohoku University. He received his Ph.D. degree in Graduate School of Agricultural Science, Tohoku University. He had worked in Ministry of agriculture, Forestry and Fisheries (MAFF), National Livestock Breeding Center in Japan (NLBC), and Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh. Now he is Associate Professor of Laboratory of Animal Breeding and Genetics, Graduate School of Agricultural Science, Tohoku University. His research is to understand the genetic control of variation in quantitative traits and to apply the knowledge to livestock breeding by statistical methods. Especially, he is interested in applying genomic information to animal breeding.

Genetic and genomic analyses for meat quality traits in Japanese Black beef

Yoshinobu Uemoto, yoshinobu.uemoto.e7@tohoku.ac.jp

Beef palatability has been shown to be related to water-soluble compounds, such as free amino acids, peptides, nucleotides, and sugars, as well as beef marbling score (BMS) and fatty acid compositions. Improvement of water-soluble compounds can be achieved through manipulation of both genetic and environmental factors. As for genetics factors, information regarding the genetic effects of water-soluble compounds in beef is generally lacking, and thus more comprehensive knowledge is necessary. Here, I introduce the recent studies of our lab for genetic and genomic analyses of water-soluble compounds, especially, inosine 5’-monophosphate (IMP) in Japanese Black beef.

Heritability estimation for water-soluble compounds was firstly performed in Japanese Black beef. Most of the water-soluble compounds yielded low heritability estimates. However, some traits have modelrate heritability, which means genetic factors affect these traits. Especially, nucleotides such as IMP were moderate heritability estimates (Sakuma et al., 2017, Anim Sci J). IMP contributes to *Umami* taste, which is a Japanese term for the fifth basic taste and is an important sensory property of beef palatability.

Our resutls showed that the concentration of IMP in beef is affected by genetic factors, and we secondly performed genomic-wide association studies (GWAS) of nucleotides such as IMP in Japanese Black beef. The results of GWAS using single nucleotide polymporphisms (SNPs) array showed the *ecto-5’-nucleotidase (NT5E)* gene was located on the significant SNPs reigon. The results of candidate gene analysis and functional analysis showed that two non-synonymous SNPs in *NT5E* gene affected the amount of IMP (Uemoto et al., 2017, BMC Genom).

The concentration of IMP in beef is also affected by environmtental factors such as postmortem conditioning and the aging period, and we thirdly evaluated the effect of the aging period on the activity of the NT5E enzyme in different *NT5E* genotypes in Japanese Black beef. The study showed the significant effect of the interaction between the *NT5E* genotypes and the aging period on the degradation rate of IMP in beef (Komatsu et al, 2019, Meat Sci). These detailed characterization of the *NT5E* genotypes would be useful for genetic improvment of beef palatability through marker-assisted selection in Japanese Black cattle.

Associate Professor Miao LIN, Yangzhou University, China

Miao Lin graduated Department of Animal Nutrition, China Agricultural University. She received her Ph.D. degree in College of Animal Science and Technology, China Agricultural University with her thesis entitled “Effect of nitrate on determination of crude proteins in feedstuffs and its influence on rumen fermentation, bacterial community and growth performance of wethers”. Now she is Associate Professor of Institute of Animal Culture Collection and Application, Department of Animal Nutrition and Grass Science, Yangzhou University. During her career, she had an opportunity of staying in Department of Animal Science, University Wisconsion- Madison and US Dairy Forage Research Center for one and a half year. She has focused on the microbiota function of rumen. Especially, she is interested in nitrate and nitrite reduction, volatile fatty acid production in the ruemn. She now tries to evaluate the caproic acid production *in vitro* culture.

Shifts in fermentation end products and bacterial community composition in long-term, sequentially transferred in vitro ruminal enrichment cultures fed switchgrass with and without ethanol as a co-substrate

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In vitro ruminal fermentations resemble *in vivo* fermentations with respect to substrate consumption and dis-tribution of fermentation products in short term (1–5d) incubations. However, little is known regarding changes in *in vitro* fermentations over prolonged incubation or multiple transfers. Gas production, pH, fermentation products, and bacterial community composition were examined in duplicate *in vitro* fermentations of switchgrass plus distillers grains that were transferred at 3–4d intervals over 900d. Additionally, duplicate fermentations inoculated from 160 d-old enrichments into the same medium but supplemented with ethanol, and transferred at 3–4d over a 730 d period were characterized. SWG and SWG +E fermentation showed marked differences in community composition, pH, total product concentrations and ratios, relative to each other and to the original inoculum. The results have implications for the use of ruminal inocula for industrial production of short- and medium-chain fatty acids via the carboxylate platform.

1. Miao Lin, Xiaoxia Dai, Paul J. Weimer (2019). Shifts in fermentation end products and bacterial community composition in long-term, sequentially transferred in vitro ruminal enrichment cultures fed switchgrass with and without ethanol as a co-substrate. Bioresource Technology, 285. https://doi.org/10.1016/j.biortech.2019.121324