PRINT ISSN 2433-6122 ONLINE ISSN 2434-4761

Journal of Integrated Field Science

Vol.18

March. 2021

Field Science Center
Graduate School of Agricultural Science
Tohoku University

Contents

JOURNAL OF
INTEGRATED FIELD SCIENCE
Field Science Center, Graduate School of Agricultural Science, Tohoku University

Symposium mini review

Strengthening the Impact of Immune Regulation in Domestic Animals

Sanggun ROH[1] and Cheol-Heui YUN[2]

[1]Graduate School of Agricultural Science, Tohoku University, Aramaki Aza Aoba 468-1, Aoba-ku, Sendai 980-8572, Japan
[2]Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Sciences, Seoul National University, Seoul 08826, Republic of Korea

Keywords
domestic animals, antibiotics, immunomodulator

Corresponding Author
Cheol-Heui YUN, cyun@snu.ac.kr
Sanggun ROH, sanggun.roh@tohoku.ac.jp

Abstract

Animal farming has been affected by a number of factors including national and international restrictions based on their guidelines and regulations, disease outbreak, welfare issues, balance between demand and supply of the meats and animals together with their by-products or processed-products, and economic and cultural demand. The global population increases coincident with an enhancement of living standards in developing countries, which is likely to create a high demand for animal-derived proteins over time. With that in mind, we are under great pressure and constantly facing a challenge in relation with the issues including climate change (i.e., global warming), banning on the use of antibiotics as a feed additive concordant with disease outbreak for both domestic animals (African swine fever, avian influenza, foot-and-mouse disease) and humans (corona pandemic for instance). Yet, high productivity together with high quality meat from the animal would be the most desirable for producers and animal farming industry. Thus, it is fair to say that current animal farming is under various psychological and physical stressful conditions, which can be categorized into nutritional factors, environmental factors, biological factors and physical factors. Basic, however essential, components of immune system include the recognition of self and non-self, and its remarkable specificity for subtle chemical differences that distinguish one foreign pathogen from another. Domestic animal immunology is seemingly important as we could find answers to the convoluted questions such as finding suitable antibiotic replacements, immunomodulants and vaccines with appropriate adjuvants. In this mini-review, we attempt to categorize aforementioned questions and to provide a direction towards our future of the animal science and biotechnology.

Introduction

Factory livestock systems require not only fast-growing bit also high-yielding animals. Factory farming achieved this with the use of high concentrated feed. This often puts animals at risk of developing painful physiologic problems. Lameness, bone weakness or fractures, infections, and organ failure are common health problems observed in factory livestock. By taking the action against aforementioned problematic factory farming, we are in need of a food and farming revolution, and at the same time we should support to stop an inhumane way of producing food that leads to the cruel abuse of animals.

Behavioral and physiological stresses can prevent animals from achieving normal reproductive success. Stressors associated with intensive livestock management may be responsible for reduced reproductive efficiency. It has been reported that several physiological and biochemical changes that accompany or follow stress in animals, but the obtained responses are various. Parameters used to evaluate stress or welfare are commonly based on sympatho-adrenal measures such as heart rate (Cook and Jacobson, 1996), plasma hormones and metabolites (Bobek *et al.*, 1986) and immune indicators (Agnes *et al.*, 1990). Moreover, factors directly or indirectly affect such action include national and international restrictions based on their guidelines and regulations, disease outbreak, welfare issues, balance between demand and supply of the meats and animals together with their by-products or processed-products, and economic and cultural demand. As

aforementioned, it would be ideal and more ethical to remove or modify the stressors to minimize their outcome rather than to employ drugs to reduce physiological and biochemical aspects of various types of stress in animals (Narayan and Parisella, 2017). The present article attempts to introduce the current and future prospects of the strategies against various stressful stimuli, mainly in domestic animals with recent development.

Challenges in domestic animal industry

Demand for meat is expected to be increased over time together with expanding population, and yet it will be extremely difficult to meet the demand of meat consumption with current platforms and technology. Thus, one of the main goals shall be minimizing stressful conditions together with preventing disease outbreak in animal farming industry.

Environmental factors

The livestock sector is socially, culturally and politically very relevant and signified. It accounts for 40% of the world's agriculture Gross Domestic Product (GDP). It employs 1.3 billion people, and creates livelihoods for one billion of the world's population living in poverty. Climate change including global warming and rearing conditions is seen as a major threat to the survival of many species, and to ecosystems and the financial sustainability of livestock production systems in many parts of the world (Sejian *et al.*, 2018; Summer *et al.*, 2019; Lee *et al.*, 2020). Obviously, the potential problems are even greater in developing countries. Economic studies suggest severe losses if current management systems are not modified to reflect the shift in climate. In short, farmers/ managers need to adapt to the changes accordingly in a short period of time (Moeser *et al.*, 2017; Wickramasuriya *et al.*, 2020). There has been considerable interest in gaining an understanding how domestic livestock respond to climatic stressors. It is somewhat unfortunate that studies have for the most part been undertaken in developed countries. Many studies have provided a wealth of knowledge on the effects of the impact of climatic stress on animal production, reproduction and health.

Antibiotics

We should properly understand the precise role and mechanism of antibiotics and how they were acting as an animal growth promoter (AGP) at the first place. The evidence available in the literature speaks volumes on the beneficial effects obtained from antibiotics used as a feed additive. Antibiotics have beneficial effects on promoting growth of the animals and preventing the disease. With the development of the intensive animal husbandry and formula-based feed industry, antibiotics were widely used around the world. However, instead of being assimilated by animal guts, a high percentage of antibiotics were excreted out as prototypes or metabolites with urine and feces. The volume of antibiotics used for growth promotion in livestock outstrips that used for disease treatment in humans and creates a significant selection pressure for the evolution of antibiotic resistance; a challenge for global health and resource conservation.

The cause of this problem is not just the use of antibiotics to treat the disease. Livestock farmers mix antibiotics into feed to encourage livestock growth and to prevent (although this may not what is happening truly) illness in packed barns and farms. Three-quarters of all antibiotics produced in the world could be used for this purpose (Sejian *et al*., 2018). The use of those antibiotics is very different from that for humans. In humans, the purpose of medication is to treat infections, not preventive ones. This indiscriminate use of antibiotics has been around since the early days of antibiotics and has often been criticized since then. Every time an antibiotic is given, the microbial community is modified as microbiota mutates for self-defense against it (Cromwell, 2002; Hughes and Heritage, 2002). To make matters worse, some of the bacteria in animals are the same as those that cause human pathogenesis, such as Salmonella, Campylobacter, and *Escherichia coli*. Drugs that have become ineffective on the farm often cannot be used to treat humans once the resistance of the particular antibiotics are developed. However, proponents of using antibiotics in animal feed as growth promoters are unsure about the potential to exacerbate antibiotic resistance problems (Wallinga and Burch, 2013; Chattopadhyay, 2014). The adverse effects of inflammation and pro-inflammatory mediators in animals (e.g., reduction in growth, feed intake, reproduction, milk production, and metabolic side effects) are well-known. The anti-inflammatory potential of antibiotics (particularly macrolides) provides a rational basis of their beneficial effects which is independent of their antimicrobial effect (Chattopadhyay, 2014). Hence, there is no doubt about the important role of antibiotics in profitable and efficient production of livestock. AGPs also act directly on host cells and exert anti-inflammatory effects on host physiology including intestinal epithelial cell and gut immune cells. Thinner gut wall and increase of digestive enzyme activities are seen in animals treated with AGPs. The action mode of mechanism of AGP is (1) attenuating the virulence properties of bacteria, (2) beneficial effects to host metabolism, and (3) induction of an anti-inflammatory response in the host.

Disease outbreak

Pigs provide an important source of high-quality protein and production is predicted to increase in future to meet growing global demands for its consumption. However, the supply of pork is threatened by infectious diseases, and amongst African swine fever (ASF) is currently causing greatest concern (Alexander, 2007; Ramos *et al.*, 2017; Dixon *et al.*, 2019; Yun, 2020). ASF has already spread to Southeast Asia and European countries, and cases have been reported in Vietnam, Cambodia, Laos, South Korea, Japan, Myanmar, the Philippines, Poland, Belgium and Bulgaria. In China, which consumes by far the most pork in the world, the impact of such disease is devastating. China lost up to 55% of the pigs it raised during the year 2019. The reality is that pork-producing countries can lose billions of dollars if the disease spreads domestically. The infection destroys the life of livestock farmers and closes the export market. Although ASF is not a threat to humans, there are no vaccines or cures for the disease. Up-to-date information on ASF outbreaks in domestic pigs and cases in wild boar is available on the OIE World Animal Health Information System. This includes daily information on new disease outbreaks, follow-up reports and interactive disease distribution maps for specified time periods are also available.

Immunomodulators

Immunomodulators are medications used to help regulate or normalize the immune system in animals and humans. Lactobacilli, in addition to their role in the development and regulation of immune responses, can effectively enhance antiviral functions in macrophages against avian influenza virus (Shojadoost *et al.*, 2019). The mechanisms of these interactions include enhancement of nitric oxide production, up-regulation of cytokines and immunostimulatory factors, and increased surface expression of co-stimulatory molecules for T cell activation. In-feed *Enterococcus faecium* NCIMB 10415 probiotic increased the production of Salmonella-specific mucosal IgA following immunization with an attenuated *Salmonella enteritidis* vaccine (Beirao *et al.*, 2018). Modulation of the intestinal microbiome is one of the major immune effects; the overall changes in the profile of the microbiome in the "Vaccine+probiotic" group are compatible with reported improvements in live vaccine immunogenicity.

Probiotics have a great potential in effective management for health of ruminant as well. Although feed with probiotics does not affect the growth and meat production directly, it is effective in reducing stress. Several studies have shown that the microbial community in ruminant gastro-intestinal tract (GIT) can be changed by a variety of factors such as diet, probiotics, age, and stress. *In vivo* and *in vitro* studies of the dynamic and functional effects initiated by probiotic therapy can greatly enrich our understanding on when and how these treatments can benefit ruminants. Key areas of future research are to describe the structure and interactions of the intestinal microbiota, and the functional relationship between the microbial community of the intestinal mucosa and host cells. The "metaomics" approach has been used to investigate the dynamic relationship between the GIT microbial community and host metabolism. Such strategy would further identify a key set of to-be-well-defined microbial species for improving health during especially the early development in ruminants.

The GIT microbial diversity and community together with the epithelium is related with the host mucosal innate immune function (Li *et al.*, 2020). A study that focuses on the interaction between mucosal microbial communities and host ruminal epitheliums in particular will facilitate identification of key genes that are important for host immune homeostasis. Ideally, target gene editing technology can be applied to manipulate the genetic composition of entire microbial populations to potentially enable optimal host health and productivity.

Conclusion

Applying the strategy using probiotics and immunomodulators in the host in association with microbial community in domestic animals, often complicated balance is observed between the host immune response and the early colonization of potentially feeding. In addition, considering innate and trained immunity we might be able to find a way to design a set of core microbes with accelerated colonization during the early and crucial time of the animals' life.

References

Agnes, F., P. Sartorelli, B. H. Abdi and A. Locatelli (1990) Effect of transport loading or noise on blood biochemical variables in calves. American Journal of Veterinary Research, 51(10): 1679-1681.

Alexander, D. J. (2007) An overview of the epidemiology of avian influenza. Vaccine, 25(30): 5637-5644.

Beirao, B. C. B., M. Ingberman, C. Favaro, Jr., D. Mesa, L. C. Bittencourt, V. B. Fascina and L. F. Caron (2018) Effect of an Enterococcus faecium probiotic on specific IgA following live Salmonella Enteritidis vaccination of layer chickens. Avian Pathology: journal of the W.V. P. A, 47(3): 325-333.

Bobek, S., J. Niezgoda, K. Pierzchała, P. Lityński and A. Sechman (1986) Changes in Circulating Levels of Iodothyronines, Cortisol and Endogenous Thiocyanate in Sheep during Emotional Stress caused by Isolation of the Animals from the Flock. Journal of Veterinary Medicine Series A, 33(1-10): 698-705.

Chattopadhyay, M. K. (2014) Use of antibiotics as feed additives: a burning question. Frontiers in Microbiology, 5(334).

Cook, C. and L. Jacobson (1996) Heart rate as a measure of adaptation to stress in cattle. Australian Veterinary Journal, 74(6): 471-472.

Cromwell, G. L. (2002) Why and how antibiotics are used in swine production. Animal Biotechnology, 13(1): 7-27.

Dixon, L. K., H. Sun and H. Roberts (2019) African swine fever. Antiviral Research, 165: 34-41.

Hughes, P. and J. Heritage (2002) Food and agriculture organization. Antibiotic growth-promoters in food animals. Retrieved from Leeds, UK

Lee, J. S., S. Kang, M. J. Kim, S. G. Han and H. G. Lee (2020) Dietary supplementation with combined extracts from garlic (Allium sativum), brown seaweed (Undaria pinnatifida), and pinecone (Pinus koraiensis) improves milk production in Holstein cows under heat stress conditions. Asian-Australasian Journal of Animal Sciences, 33(1): 111-119.

Li, W., A. Edwards, M. S. Cox, S. M. Raabis, J. H. Skarlupka, A. J. Steinberger, B. Murphy, A. Larsen and G. Suen (2020) Changes in the host transcriptome and microbial metatranscriptome of the ileum of dairy calves subjected to artificial dosing of exogenous rumen contents. Physiological Genomics, 52(8): 333-346.

Moeser, A. J., C. S. Pohl and M. Rajput (2017) Weaning stress and gastrointestinal barrier development: Implications for lifelong gut health in pigs. Animal Nutrition, 3(4): 313-321.

Narayan, E. and S. Parisella (2017) Influences of the stress endocrine system on the reproductive endocrine axis in sheep (Ovis aries). Italian Journal of Animal Science, 16(4): 640-651.

Ramos, S., M. MacLachlan and A. J. L. D. P. O. Melton (2017) Impacts of the 2014-2015 Highly Pathogenic Avian Influenza Outbreak on the US Poultry Sector.

Sejian, V., R. Bhatta, J. B. Gaughan, F. R. Dunshea and N. Lacetera (2018) Review: Adaptation of animals to heat stress. Animal, 12(s2): s431-s444.

Shojadoost, B., R. R. Kulkarni, J. T. Brisbin, W. Quinteiro-Filho, T. N. Alkie and S. Sharif (2019) Interactions between lactobacilli and chicken macrophages induce antiviral responses against avian influenza virus. Research in Veterinary Science, 125: 441-450.

Summer, A., I. Lora, P. Formaggioni and F. Gottardo (2019) Impact of heat stress on milk and meat production. Animal Frontiers, 9(1): 39-46.

Wallinga, D. and D. G. Burch (2013) Does adding routine antibiotics to animal feed pose a serious risk to human health? Bmj, 347.

Wickramasuriya, S. S., S. P. Macelline, E. Kim, H. M. Cho, T. K. Shin, Y. J. Yi, D. D. Jayasena, S. D. Lee, H. J. Jung and J. M. Heo (2020) Physiological impact on layer chickens fed corn distiller's dried grains with solubles naturally contaminated with deoxynivalenol. Asian-Australasian Journal of Animal Sciences, 33(2): 313-322.

Yun, C. H. (2020) - Editorial - Unforeseen enemy: African swine fever. Asian-Australasian Journal of Animal Sciences, 33(1): 1-3.

JOURNAL OF
INTEGRATED FIELD SCIENCE
Field Science Center, Graduate School of Agricultural Science, Tohoku University

Symposium mini review

Importance of Liver tissue as an Endocrine Organ in Ruminant

Sanggun ROH

Lab of Animal Physiology, Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi-ken 980-8572, Japan

Keywords
ruminant, liver tissue, hepatokine

Corresponding Author
Sanggun ROH, sanggun.roh@tohoku.ac.jp

Abstract

The liver plays a crucial role as a secondary endocrine organ in controlling homeostasis. In ruminant, hepatic function changes dramatically during weaning and parturition through the signaling of hormones and blood metabolites. The management systems, feed composition, and feeding program greatly affect the pathological processes of energy metabolism disorders in the liver. The endocrine system and liver are codependent, and both can alter the quantity and quality of animal production. In this review, we will provide our data on the physiological roles of chemerin and ANGPTL8 (angiopoietin Like 8) as hepatic hormones, and the regulatory factors controlling their production, to better understand hepato-endocrine interplay.

Physiological characteristic function in the liver of ruminant

The liver is an important organ that adjusts metabolism according to the nutrients it takes in, leading to its own biological maintenance and growth. The primary functions of the liver include glucose metabolism, protein metabolism, lipid metabolism, regulation and detoxification of ion gradients, and bile production and secretion; these functions are so diverse that they are often compared to those in chemical factories.

Endocrine research in livestock has significantly contributed to the elucidation of the molecular mechanisms of livestock production traits such as lactation, meat production, and reproduction, which are based on nutrient metabolism. Recent studies have revealed that various organs and tissues other than the classical endocrine glands secrete peptides and cytokines. Therefore, current endocrinology is undergoing a paradigm shift from being a simple model of regulation of metabolic organs by endocrine glands to a metabolic regulation network through hormone secretion by various tissues throughout the body, including the endocrine glands, and this complex network.

In ruminant, shortly after birth, the rumen is physically and functionally underdeveloped (Warner, 1956; Tamate *et al.*, 1962). Like monogastric animals, ruminant take nutrients from milk after birth, but after weaning they take in nutrients produced from solid diets in the rumen. Roughage contributes to rumen development by providing physical stimulation; consequently, short-chain fatty acids (SCFA), which are fermented rumen products, contribute to this development by chemical stimulation.

Hepatokines

The liver has a wide range of functions, and it can be referred to as the largest nutrient-metabolizing organ in the body. Although ruminant livestock such as cattle have metabolic characteristics that differ from those of monogastric animals, they still have functionally important organs wherein nutrients absorbed from the rumen and small intestine are first metabolized. However, recent studies have revealed that the liver also plays a role as an endocrine organ. IGF-1 (Insulin-like growth factor 1) is well known as an endocrine factor derived from the liver (Roberts *et al.*, 1990), but new liver-derived hepatokines such as Fetuin-A, FGF-21 (Fibroblast growth factor-21), Selenoprotein-P, and ANGPTLs have been discovered. Hepatokine has been reported to regulate systemic glucose metabolism, lipid metabolism, and insulin signals, and it is thought that the liver regulates systemic metabolism by issuing endocrine signals according to its own nutritional metabolism status.

It has been suggested that endocrine factors such as insulin and IGF-1 may also be involved in rumen development (Gerrits *et al.*, 1998; Shen *et al.*, 2004). Before weaning, glucose, long chain fatty acids (LCFA, long chain fatty acid), and amino acids are obtained from milk; however, after weaning, short chain fatty acids produced by rumen microorganisms are absorbed in the rumen. Before weaning,

the liver is the main tissue for glycolysis via glucose and ketone body production from LCFA. However, after weaning, gluconeogenesis via propionate becomes more important, and ketone body production decreases due to the decrease in carbohydrate supply from feed and the influence of decomposition by rumen microorganisms. In this paper, I will discuss the physiological roles of chemerin and ANGPTL8 in relation to metabolic regulation in ruminant.

Chemerin

Chemerin is a chemokine-type secretory protein encoded by TIG2 (Tazarotene-induced gene 2), also known as RARRES2 (retinoic acid receptor responder) (Nagpal *et al.*, 1997). To date, three types of chemerin receptors have been identified: CMKLR1 (chemokine like receptor 1), GPR1 (G-protein receptor 1), and CCRL2 (chemokine (C-C motif) receptor-like 2) (Wittamer *et al.*, 2003; Zabel *et al.*, 2005; Barnea *et al.*, 2008). The major gene expression sites of chemerin in mice and humans are the heart, lung, liver, spleen, kidney, pancreas, white adipose tissue, brown adipose tissue, placenta, and uterus; these sites are highly expressed, especially in the liver and white adipose tissue. CMKLR1 is highly expressed in the heart, lung, skeletal muscle, and adipose tissue. In addition, CCRL2 is highly expressed in the spleen and lymph nodes, and GPR1 is highly expressed in skeletal muscle, adipose tissue, and the brain (Fan *et al.*, 1998; Rourke *et al.*, 2014).

The physiological actions of chemerin in relation to the immune system, glucose metabolism, and lipid metabolism are well known. The immune system attracts dendritic cells, macrophages, and NK cells to the site of inflammation. As a metabolic system, insulin sensitivity of peripheral tissues, regulation of glucose uptake, differentiation of mature adipocytes from adipose progenitor cells, and regulation of insulin secretion have been reported (Goralski *et al.*, 2007; Roh *et al.*, 2007; Sell *et al.*, 2009; Ernst *et al.*, 2010; Takahashi *et al.*, 2011).

Chemerin mRNA is highly expressed in adipose tissue, liver, kidney, adrenal gland, spleen, and small intestine, especially in the liver (Suzuki *et al.*, 2016). The chemerin receptors (CMKLR1 and CCRL2) were expressed in various tissues; however, CMKLR1 was highly expressed in the liver, adrenal gland, spleen, and lung, and CCRL2 was highly expressed in adipose tissue, adrenal gland, spleen, large intestine, and lung. In addition, expression of GPR1 was observed only in adipose tissue, liver, lung, and rumen, and its expression was particularly high in the liver. Chemerin was localized in the cytoplasm of bovine hepatocytes. A previous report confirmed that the expression of chemerin was higher in adipocytes than in vascular stromal cells, but the results were consistent (Song *et al.*, 2010). Compared to the expression level in other tissues, it is believed that the liver is the main endocrine organ that produces chemerin in cattle.

Chemerin has been shown to have an insulin secretagogue effect in sheep (Suzuki *et al.*, 2012). Administration of a chemerin analog led to an acute rise in the plasma insulin levels and decreased glucose levels. Plasma NEFA (non-esterified fatty acids) levels were elevated from 60 to 180 min after administration. Chemerin analog administration also transiently elevated the levels of plasma triglyceride and total cholesterol, suggesting increased VLDL (very low-density lipoprotein) secretion from the liver. Plasma HDL (high-density lipoprotein) levels declined after administration. A previous study by our group showed five SNPs in the coding region of the bovine chemerin gene in Japanese Black cattle (Yamauchi *et al.*, 2015). The c.276C>T SNP of the chemerin gene potentially regulates meat quality by affecting the composition of intramuscular fatty acids.

Since the expression of chemerin in the liver during the lactation period was decreased, it is expected that the concentration of chemerin in the blood during this period was decreased. It is possible that the decrease in the expression level of chemerin is involved in the decrease in insulin secretion during lactation. Throughout the lactation and dry stages, the cellular composition within the mammary gland changes significantly. Increased expression of chemerin receptors during the dry period may be caused by immune cells infiltrating the mammary gland tissue during the dry period. The chemerin receptors are expressed in bovine mammary epithelial cells, and chemerin activates the ERK pathway and increases the expression of milk synthesis-related genes. Therefore, it is believed that chemerin may have a lactogenic action or a growth factor-like action in mammary epithelial cells. However, increased expression of the chemerin receptor was found in mammary glands during the dry period. Chemerin is considered to regulate the immune system and metabolic system as hepatokines that are highly expressed in the liver and affect productivity of cattle.

ANGPTL8

ANGPTL8 is a liver-derived secretory protein that is also known as TD26, RIFL, Lipasin, or betatrophin. The expression sites of ANGPTL8 in humans and mice are liver, white adipose tissue, and brown adipose tissue, and its expression in other tissues is extremely low (Zhang, 2012). It has been reported that the physiological effects of ANGPTL8 are involved in the regulation of blood TG (triglyceride) levels, although the receptor for ANGPTL8 has yet to be identified. ANGPTL8 expression in mouse liver decreases with fasting and increases after feeding (Ren *et al.*, 2012). The overexpression of ANGPTL8 resulted in dyslipidemia (elevated blood TG), and knockout mice showed low TG levels (Quagliarini *et al.*, 2012; Wang *et al.*, 2013). In ANGPTL8 KO mice, adipose tissue development is delayed and VDL (very low density) release from the liver is also reduced.

Lipid metabolism in cattle is closely related to growth, energy storage, and supply during lactation in cattle. ANGPTL8 mRNA was highly expressed in liver and adipose tissue and was either slightly or not detected in other tissues of cattle (Nakano *et al.*, 2018). ANGPTL8 protein was detected only in the liver. As a result, the expression of ANGPTL8 in the liver showed no significant change throughout the lactation period or the dry period. The expression of ANGPTL8 in the liver during parturition was examined in the biopsied liver tissue, which showed that the expression of ANGPTL8 in the liver before calving was not changed; however, it was significantly reduced after parturition. The blood TG concentration was observed to be lower one week before calving and it reached its lowest value on parturition; subsequently, it decreased during the first four weeks post-parturition, while the blood NEFA concentration was higher

four weeks after parturition. In dairy cattle, with the start of lactation, NEFA released from adipose tissue is converted to VLDL in its original state or in the liver and subsequently mobilized to the mammary gland (Drackley *et al.*, 2001). Because ANGPTL8 inhibits LPL (Lipoprotein lipase) activity and increases blood TG concentration, it is considered that the changes in ANGPTL8 expression coincide with blood TG concentration during the parturition. Since the regulation of hepatic ANGPTL8 expression by insulin and fasting has been reported, it is considered that the decrease in ANGPTL8 expression after parturition is due to a decrease in blood insulin concentration and a negative energy balance. When the lactation period and the dry period were compared, there was no difference in the expression level of ANGPTL8 in the liver; it is possible that cows used in the dry period were not pregnant. As described above, it was suggested that ANGPTL8 is a factor that regulates lipid mobilization during lactation in dairy cows.

References

Barnea, G., W. Strapps, G. Herrada, Y. Berman, J. Ong, B. Kloss, R. Axel and K. J. Lee (2008) The genetic design of signaling cascades to record receptor activation. Proceedings of the National Academy of Scientists United States of America, 105(1): 64-69.

Drackley, J. K., T. R. Overton and G. N. Douglas (2001) Adaptations of Glucose and Long-Chain Fatty Acid Metabolism in Liver of Dairy Cows during the Periparturient Period. Journal of Dairy Science, 84: E100-E112.

Ernst, M. C., M. Issa, K. B. Goralski and C. J. Sinal (2010) Chemerin exacerbates glucose intolerance in mouse models of obesity and diabetes. Endocrinology, 151(5): 1998-2007.

Fan, P., H. Kyaw, K. Su, Z. Zeng, M. Augustus, K. C. Carter and Y. Li (1998) Cloning and characterization of a novel human chemokine receptor. Biochemical Biophysical Research Communication, 243(1): 264-268.

Goralski, K. B., T. C. McCarthy, E. A. Hanniman, B. A. Zabel, E. C. Butcher, S. D. Parlee, S. Muruganandan and C. J. Sinal (2007) Chemerin, a novel adipokine that regulates adipogenesis and adipocyte metabolism. Journal of Biological Chemistry, 282(38): 28175-28188.

Nagpal, S., S. Patel, H. Jacobe, D. DiSepio, C. Ghosn, M. Malhotra, M. Teng, M. Duvic and R. A. Chandraratna (1997) Tazarotene-induced gene 2 (TIG2), a novel retinoid-responsive gene in skin. The Journal of Investigative Dermatology, 109(1): 91-95.

Nakano, M., Y. Suzuki, S. Haga, E. Yamauchi, D. Kim, K. Nishihara, K. Nakajima, T. Gotoh, S. Park, M. Baik, K. Katoh and S. Roh (2018) Downregulated angiopoietin-like protein 8 production at calving related to changes in lipid metabolism in dairy cows. Journal of Animal Science, 96(7): 2646-2658.

Quagliarini, F., Y. Wang, J. Kozlitina, N. V. Grishin, R. Hyde, E. Boerwinkle, D. M. Valenzuela, A. J. Murphy, J. C. Cohen and H. H. Hobbs (2012) Atypical angiopoietin-like protein that regulates ANGPTL3. Proceedings of the National Academy of Scientists United States of America, 109(48): 19751-19756.

Ren, G., J. Y. Kim and C. M. Smas (2012) Identification of RIFL, a novel adipocyte-enriched insulin target gene with a role in lipid metabolism. American Journal of Physiology Endocrinology Metabolism, 303(3): E334-351.

Roberts, C. A., S. N. McCutcheon, H. T. Blair, P. D. Gluckman and B. H. Breier (1990) Developmental patterns of plasma insulin-like growth factor-1 concentrations in sheep. Domestic Animal Endocrinology, 7(4): 457-463.

Roh, S. G., S. H. Song, K. C. Choi, K. Katoh, V. Wittamer, M. Parmentier and S. Sasaki (2007) Chemerin-a new adipokine that modulates adipogenesis via its own receptor. Biochemical and Biophysical Research Communications, 362(4): 1013-1018.

Rourke, J. L., S. Muruganandan, H. J. Dranse, N. M. McMullen and C. J. Sinal (2014) Gpr1 is an active chemerin receptor influencing glucose homeostasis in obese mice. Journal of Endocrinology, 222(2): 201-215.

Sell, H., J. Laurencikiene, A. Taube, K. Eckardt, A. Cramer, A. Horrighs, P. Arner and J. Eckel (2009) Chemerin is a novel adipocyte-derived factor inducing insulin resistance in primary human skeletal muscle cells. Diabetes, 58(12): 2731-2740.

Song, S. H., K. Fukui, K. Nakajima, T. Kozakai, S. Sasaki, S. G. Roh and K. Katoh (2010) Cloning, expression analysis, and regulatory mechanisms of bovine chemerin and chemerin receptor. Domestic Animal Endocrinology, 39(2): 97-105.

Suzuki, Y., S. Haga, M. Nakano, H. Ishizaki, S. Song, K. Katoh and S. Roh (2016) Postweaning changes in the expression of chemerin and its receptors in calves are associated with the modification of glucose metabolism. Journal of Animal Science, 94(11): 4600-4610.

Suzuki, Y., S. H. Song, K. Sato, K. H. So, A. Ardiyanti, S. Kitayama, Y. H. Hong, S. D. Lee, K. C. Choi, A. Hagino, K. Katoh and S. G. Roh (2012) Chemerin analog regulates energy metabolism in sheep. Animal Science Journal, 83(3): 263-267.

Takahashi, M., Y. Okimura, G. Iguchi, H. Nishizawa, M. Yamamoto, K. Suda, R. Kitazawa, W. Fujimoto, K. Takahashi, F. N. Zolotaryov, K. S. Hong, H. Kiyonari, T. Abe, H. Kaji, S. Kitazawa, M. Kasuga, K. Chihara and Y. Takahashi (2011) Chemerin regulates beta-cell function in mice. Scientific Reports, 1: 123.

Tamate, H., A. D. McGilliard, N. L. Jacobson and R. Getty (1962) Effect of Various Dietaries on the Anatomical Development of the Stomach in the Calf. Journal of Dairy Science, 45(3): 408-420.

Wang, Y., F. Quagliarini, V. Gusarova, J. Gromada, D. M. Valenzuela, J. C. Cohen and H. H. Hobbs (2013) Mice lacking ANGPTL8 (Betatrophin) manifest disrupted triglyceride metabolism without impaired glucose homeostasis. Proceedings of the National Academy of Scientists United States of America, 110(40): 16109-16114.

Warner, A. C. (1956) Criteria for establishing the validity of in vitro studies with rumen micro-organisms in so-called artificial rumen systems. Journal of general microbiology, 14(3): 733-748.

Wittamer, V., J. D. Franssen, M. Vulcano, J. F. Mirjolet, E. Le Poul, I. Migeotte, S. Brezillon, R. Tyldesley, C. Blanpain, M. Detheux, A. Mantovani, S. Sozzani, G. Vassart, M. Parmentier and D. Communi (2003) Specific recruitment of antigen-presenting cells by chemerin, a novel processed ligand from human inflammatory fluids. Journal of Experimental Medicine, 198(7): 977-985.

Yamauchi, E., Y. Suzuki, K. H. So, K. Suzuki, K. Katoh and S. G. Roh (2015) Single Nucleotide Polymorphism in the Coding Region of Bovine Chemerin Gene and Their Associations with Carcass Traits in Japanese Black Cattle. Asian-Australasian Journal of Animal Sciences, 28(8): 1084-1089.

Zabel, B. A., A. M. Silverio and E. C. Butcher (2005) Chemokine-like receptor 1 expression and chemerin-directed chemotaxis distinguish plasmacytoid from myeloid dendritic cells in human blood. The Journal of Immunology, 174(1): 244-251.

Zhang, R. (2012) Lipasin, a novel nutritionally-regulated liver-enriched factor that regulates serum triglyceride levels. Biochemical and Biophysical Research Communications, 424(4): 786-792.

JOURNAL OF
INTEGRATED FIELD SCIENCE
Field Science Center, Graduate School of Agricultural Science, Tohoku University

Symposium mini review

Receptors in Spermatozoa – Their expressions and Functions

Yuki HIRADATE, Kenshiro HARA and Kentaro TANEMURA

Laboratory of Animal Reproduction and Development, Graduate School of Tohoku University,
468-1 Aramaki Aza Aoba, Sendai, Miyagi Japan

Keywords
spermatozoa, neurotensin, acrosome reaction, preimplantation embryo

Corresponding Author
Yuki HIRADATE,
yuki.hiradate.d4@tohoku.ac.jp

Abstract

For stable breeding of livestock, it is necessary to deepen the understanding of sperm having fertility. It is well known that spermatozoa have receptor patterns which are similar to those of nerve cells. Neurotensin (NTS) is initially isolated from hypothalamus, later its multiple functions in several tissues have been elucidated. However, there is still little information about the effects of NTS on the reproductive organs. This paper reviews the mechanism of ligand secretion from the female side as well as the expression of NTS receptors in spermatozoa and their physiological functions. It also describes the contribution of NTS to preimplantation embryo development and focuses on the function of NTS as a cofactor from fertilization to early embryonic development.

Introduction

Neurotensin (NTS), consisting of 13 amino acids, was first isolated from the bovine hypothalamus (Carraway *et al*., 1973) as inducing vasodilatory effect. NTS is processed from its precursor, pro-NTS by protein convertase (Kitabgi, 2006). The action of NTS has a wide variety of biological actions due to the difference in its localization (Carraway *et al*., 1977), including control of fat absorption (Li *et al*., 2016). In reproductive system, a former work reported its NTS induced contractions of smooth muscle, showed a possibility of assisting embryo transport (Reinecke, 1987). However, there is still limited information.

Expression patterns of NTS and NTS receptors and their effects on sperm function

For the first time, we have elucidated the expression and function of NTS and its receptors in a fertilized environment (Hiradate *et al*., 2014). We firstly determined NTS expression patterns using an antibody. The organizational structure of fallopian tube, where fertilization occurs, can be divided into an ampulla and an isthmus part. Spermatozoa pass from the uterus through the lumen and wait in the isthmus for ovulation. The ampulla is the place where the oocytes, which are ovulated from an ovary, stay, and also the place where fertilization occurs. The fact that immunoreactivity of the epithelial cells on both parts were immunostained suggests that NTS is secreted into the lumen. Furthermore, in bovine oviductal epithelial culture model, follicular fluid exposure upregulates *Nts* expression (Hasan *et al*., 2020). These evidence suggest NTS is one of the promising factors that promotes spermatozoa fertility.

Cumulus cells, cell layers surrounding the oocyte, have important functions for fertilization. Cumulus cells are also a source of secretion factors affecting sperm physiological functions as well as epithelial cells. To examine NTS mRNA expression in cumulus cells, qPCR was performed. PMSG following hCG treatment, which induces ovulation. After the treatments, a remarkable increase of NTS mRNA expression level was observed, increasing the possibility that NTS has a specific effect on spermatozoa. Moreover, using an in vitro cumulus cell culture system, NTS secretion levels were compared to determine which ovarian hormone is responsible for NTS expression. FSH and EGF are known as ovulation inducers. E2 and P4 are typical ovarian hormones. As a result, NTS responds to FSH and EGF, but not to E2 and P4, which increase the secretion levels. Furthermore, a specific inhibitor of MEK, U0126 was used to demonstrate NTS expression is regulated downstream of MAPK in the presence of FSH and EGF. Dose-dependent inhibition of NTS expression was observed in both cases, proving that this pathway works.

In contrast, NTS receptor type 1 is expressed in the neck region of spermatozoa. It is known as a calcium storage, named the redundant nuclear envelope. This localization pattern in NTR1 suggests that NTS induces intracellular

calcium mobilization. Because calcium influx into spermatozoa cells is critically important for fertilization, loss of acrosome prior to penetration into the egg membrane is necessary for successful fertilization. Moreover, acrosome reaction is known to be triggered by elevated calcium levels. NTS significantly increased the percentage of acrosome-reacted spermatozoa. Similarly, a recent study discussed the facilitation of acrosome reaction using NTS in the bull and monkey model (Umezu *et al*., 2016; Campbell *et al*., 2020). However, spermatozoa-protein tyrosine phosphorylation is often used as a marker indicating capacitation, defined as spermatozoa which have the ability to fertilize. NTS gradually enhanced tyrosine phosphorylation.

Effects of NTS in preimplantation embryo

Recently, we also revealed the effect of NTS on early embryonic development (Hiradate *et al*., 2020). When the mRNA expression of NTS receptors, *Ntr*1, 2 and 3 was analyzed by qPCR in the preimplantation embryo at each developmental stage, it was found that Ntr1 and 3 were expressed through the blastocyst, suggested NTS can also act on preimplantation embryos. To examine whether NTS affects the development of a pre-implanted embryo, fertilized embryos were cultured in various concentrations of NTS *in vitro*. The ratios of 2-cell and 4-cell embryos were similar, but the Blastocyst formation rate was significantly higher, by as much as 100 nM. The quality of an embryo is also evaluated by counting the number of cells. Comparing this number between the two groups at no supplemented NTS and 100nM NTS added, we found no significant change. Further, the cells of the blastocyst stage can be divided into two types, inner cell mass and trophectoderm. Because ICM cells eventually become the future fetus, The smaller number of ICM makes it difficult for the fetus to develop. Thus, the ratio of the ICM to TE cell number is important. The NTS treatment group showed a higher average, but there was no significant difference. Therefore, these results indicate that the major role of NTS is not proliferation, but rather, differentiation.

Conclusion

We demonstrated that NTS is a novel factor assisting fertilization and early development. This contribution throughout fertilization and early development seems to be a conserved mechanism between species, and it helps to understand the fertility of livestock spermatozoa.

Acknowledgements

I would like to thank the Organizing Committee of the International Virtual Symposium “New Insights on Animal Science”, organized by the International Education and Research Center for Food and Agricultural Immunology (CFAI), and integrated Field Science Center, Graduated School of Agricultural Science, Tohoku University, and JSPS Core-to-Core program.

References

Campbell, G. E., E. L. Jones, P. Comizzoli and D. M. Duffy (2020) Neurotensin stimulates the sperm acrosome reaction and reduces percentages of fertilization in vitro. F & S Science, 1: 27-35.

Carraway, R. E. and S. E. Leeman (1973) The isolation of a new hypotensive peptide, neurotensin, from bovine hypothalami Inflammatory Cytokines View project. The Journal of Biological Chemistry, 248: 6854-6861.

Carraway, R., S. E. Leeman and E. A. Zimmerman (1977) Immunohistochemical Localization of Neurotensin in Endocrine Cells of the Gut. Cell and Tissue Research, 178: 313-321.

Hasan, M. M., J. Vill, F. Lattekivi, J. Ord, Q. U, Ain Reshi, K. Jaager, A. Velthut-Meikas, A. Andronowska, U. Jaakma, A. Salumets and A, Fazeli (2020) Bovine Follicular Fluid and Extracellular Vesicles Derived from Follicular Fluid Alter the Bovine Oviductal Epithelial Cells Transcriptome. International journal of Molecular Science, 21: 53565-53580.

Hiradate, Y., H. Inoue, N. Kobayashi, Y. Shirakata, Y. Suzuki, A. Gotoh, S. G. Roh, T. Uchida, K. Katoh, M. Yoshida, E. Sato and K. Tanemura (2014) Neurotensin Enhances Sperm Capacitation and Acrosome Reaction in Mice. Biology of Reproduction, 91: 1-9.

Hiradate, Y., K. Hara and K. Tanemura (2020) Effect of neurotensin on cultured mouse preimplantation embryos.Journal of Reproduction and Development, 66: 421-425.

Kitabgi, P. (2006) Differential processing of pro-neurotensin/neuromedin N and relationship to pro-hormone convertases. Peptides, 27: 2508-2514

Li, J., J. Song, Y. Y. Zaytseva, Y. Liu, P. Rychahou, K. Jiang, M. E. Starr, J. T. Kim, J. W. Harris, F. B. Yiannikouris, W. S. Katz, P. M. Nilsson, M. Orhomelander, J. Chen, H. Zhu, T. Fahrenholz, R. M. Higashi, T. Gao, L. A. Cassis, T. W. Fan, H. L. Weiss, P. R. Dobner, O. Melander, J. Jia and B. M. Evers (2016) An obligatory role for neurotensin in high-fat-diet-induced obesity. Nature, 533: 411-415.

Reinecke, M. (1987) Neurotensin in the human fallopian tube: Immunohistochemical localization and effects of synthetic neurotensin on motor activity in vitro. Neuroscience Letters, 73: 220-224.

Umezu, K., Y. Hiradate, T. Oikawa, H. Ishiguro, T. Numabe, K. Hara and K. Tanemura (2016) Exogenous neurotensin modulates sperm function in Japanese Black Cattle. Journal of Reproduction and Development, 62: 409-14.

JOURNAL OF
INTEGRATED FIELD SCIENCE
Field Science Center, Graduate School of Agricultural Science, Tohoku University

Symposium mini review

CRISPR/Cas9 Screening Identifies Genes Mediating Porcine Epidemic Diarrhea Virus Replication

Haifei WANG[1], Huan QU[1], Qiufang ZONG[1], Yue CAO[1], Yeyi XIAO[1], Shenglong WU[1,2] and Wenbin BAO[1,2]

[1]Key Laboratory for Animal Genetics, Breeding, Reproduction and Molecular Design, College of Animal Science and Technology, Yangzhou University, Yangzhou 225009, China
[2]Joint International Research Laboratory of Agriculture and Agri-Product Safety, the Ministry of Education of China, Yangzhou University, Yangzhou, Jiangsu 225009, China

Keywords
pig, virus-host interaction, histone modification, gene knockout, resistance, molecular mechanism

Corresponding Author
Wenbin BAO, wbbao@yzu.edu.cn

Abstract

Recently, porcine epidemic diarrhea virus (PEDV) is still identified as the main pathogen causing severe diarrhea in pig farms of many countries. Porcine epidemic diarrhea which is caused by this virus results in substantial economic losses for pig farmers all over the world. Resistance breeding has proven one of the effective strategies to control and prevent the spread of PEDV. Identification of crucial host factors and genetic variants associated with PEDV infection is the prerequisite for implementing resistance breeding. Genetic perturbation enables the generation of marked phenotypes related to PEDV infection, which will advance the identification of host factors crucial for host and PEDV interactions. In this review, we describe the status of PEDV spread in pig farms in recent years and discuss the findings on genes involved in host-PEDV interactions. We also discuss the advantages of genetic screens in identifying host factors that are important for virus replication and how it has been used to expand our understanding of viral pathogenesis. Further studies on host and PEDV interactions using new genetic technologies will advance identifications of key host factors involved in mediating PEDV infections and further contribute to genetic resistance breeding for porcine epidemic diarrhea.

Porcine epidemic diarrhea disease in pig farms

Porcine epidemic diarrhea virus (PEDV) was first recognized in the United Kingdom in 1971 and had spread throughout the world by 2013. PEDV is an enveloped, single-stranded, positive-sense RNA virus that belongs to the family *Coronaviridae*, genus *alphacoronavirus*. The 28 kb genome of PEDV encodes four structural proteins including the spike protein (S), membrane protein (M), envelope protein (E), and nucleocapsid protein (N) and three non-structural proteins (ORF1a/1b and ORF3) (**Fig. 1A**). Among these proteins, the S protein is responsible for the attachment of virus particles to cell surface receptor and for the fusion to host cells (Li, 2015). The PEDV propagates through fecal–oral and nasal cavity pathways to enter the intestinal epithelium (Lin *et al*., 2016; Li *et al*., 2018). PEDV replicates in the cytoplasm of villus epithelial cells and causes villi atrophy, shortening and fusion (**Fig. 1B**), which leads to watery diarrhea, vomiting, and dehydration of infected animals. PEDV can infect pigs at all ages and result in up to 80–100% mortality for suckling pigs, and cause production losses for breeding adult pigs. In recent years, PEDV is still identified as the main pathogen causing severe diarrhea in pig farms (Su *et al*., 2020). Due to the high morbidity and mortality in suckling pigs, porcine epidemic diarrhea disease leads to substantial economic losses to the pig industry of all the world.

Genes involved in mediating host-PEDV interactions

Cell surface receptor is the pivotal determinant for the PEDV to bind and enter host cells. Previous studies suggested that porcine aminopeptidase N (APN) acts as a receptor for PEDV entry into target cells (Li *et al*., 2007; Park *et al*., 2015). However, it is a controversial issue whether APN is

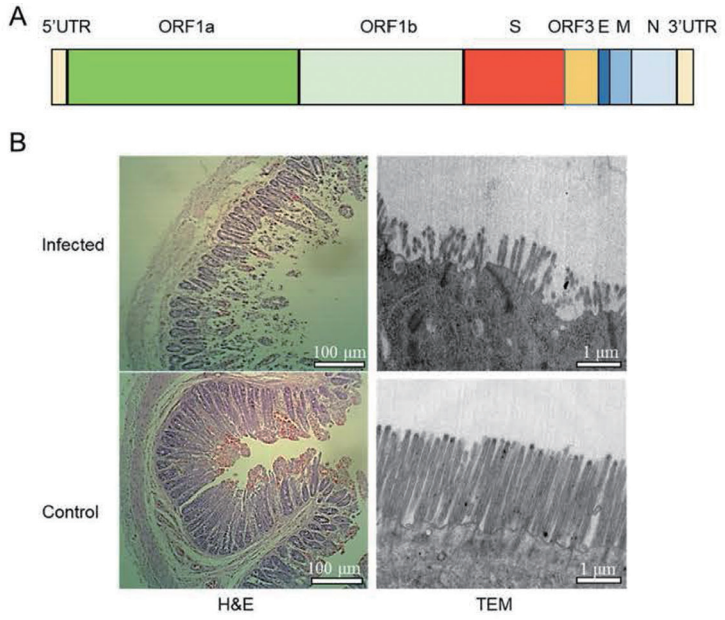

Fig. 1. Genomic structure of PEDV and intestinal pathologies induced by PEDV. (A) Diagram of the PEDV genome. (B) Histopathological analyses of the jejunum tissues derived from PEDV-infected and control animals. H&E: hematoxylin and eosin staining, TEM: transmission electron microscopic.

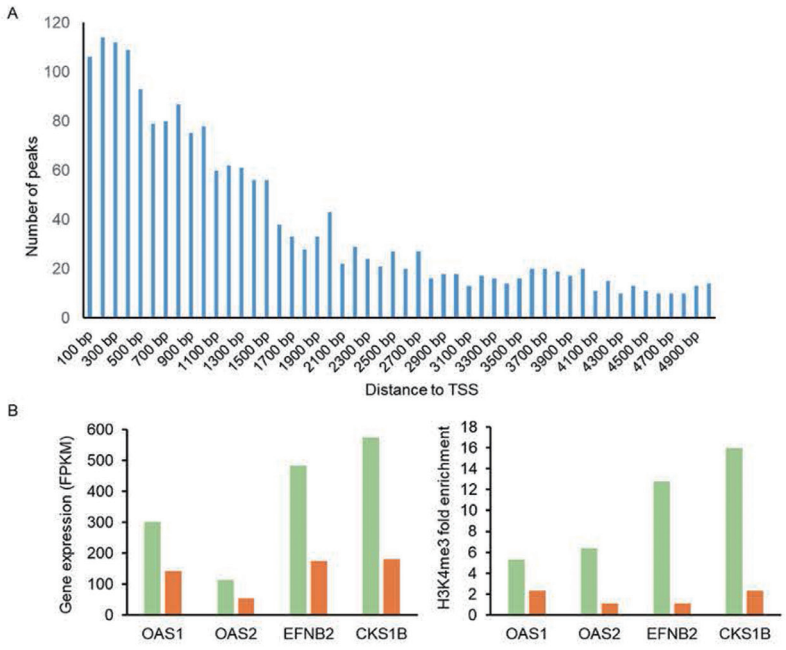

Fig. 2. H3K4me3 peak distribution and associations with gene expression. (A) Distribution of H3K4me3 peaks with the distance to transcription start site. (B) Expression and H3K4me3 fold enrichment of the OAS1, OAS2, EFNB2, and CKS1B genes in PEDV-infected and control animals. Green and oranges bars represent PEDV-infected and control samples, respectively.

the functional receptor for PEDV due to the recent reports that APN is not required for PEDV cell entry (Shirato *et al*., 2016; Ji *et al*., 2018). Therefore, identification of the functional receptor for PEDV cell entry is worthy to be further explored. In addition, researchers have explored the gene expression changes on transcriptomic and proteomic levels and non-coding RNA expression alterations induced by PEDV infection and identified a group of genes such as OAS1, IFIT, and Mx1 potential involved in regulating the interactions between PEDV and host cells (Li *et al*., 2016; Chen *et al*., 2019). Genetic divergence and association analyses on piglet resilience found that the EBI3, MUC16, and TCF3 genes can be related to PEDV infections (Bertolini *et al*., 2017). Mechanistic studies further unraveled that PEDV could avoid the innate antiviral immune responses by restricting production of interferons (Guo *et al*., 2016; Zhang *et al*., 2018). Our studies revealed changes in the patterns of H3K4me3 histone modifications related to PEDV infection (**Fig. 2A**), providing novel insights into PEDV infection from epigenetic layers (Wang *et al*., 2019). Several genes including OAS1, OAS2, EFNB2, and CKS1B demonstrated higher H3K4me3 enrichment and expression levels in PEDV-

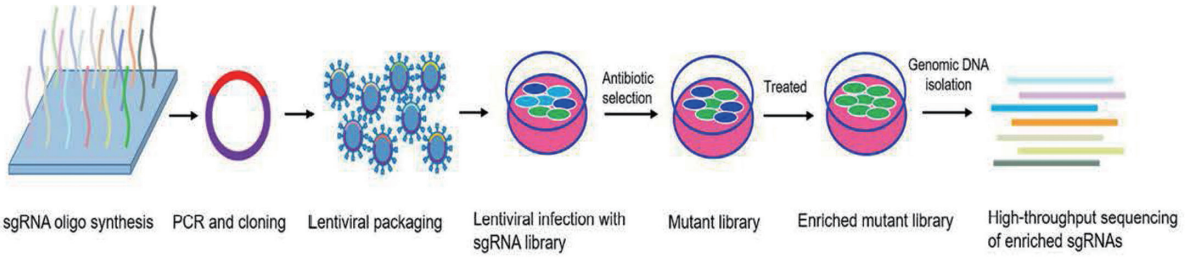

Fig. 3. Schematic of CRISPR/Cas9 screening workflow.

Table 1. sgRNA sequence targeting porcine genes

sgRNA ID	Gene	Gene ID	Chromosome	Sequences
sgTCOF1_1	TCOF1	100516425	chr2	TGGCAGAGGCCAGGAAGCGG
sgTCOF1_2	TCOF1	100516425	chr2	CTACCAGCATCTGCTGCAGG
sgTCOF1_3	TCOF1	100516425	chr2	TACCAGCATCTGCTGCAGGC
sgTCOF1_4	TCOF1	100516425	chr2	GCAGGCGGGCTATGTGCGCG
sgNSRP1_1	NSRP1	100517122	chr12	CGTGAGTGAAAGCCTTCAGA
sgNSRP1_2	NSRP1	100517122	chr12	CCTCTGAAGGCTTTCACTCA
sgNSRP1_3	NSRP1	100517122	chr12	CAGCCCAGATTCTAGGGCAA
sgNSRP1_4	NSRP1	100517122	chr12	CCCAGAGGAGTGTCAAGAGA
sgNSRP1_5	NSRP1	100517122	chr12	CAGATACTTAGCCCGGCAGA
sgSPPL3_1	SPPL3	100154995	chr14	ACACCTGACTGGAATCCACC
sgSPPL3_2	SPPL3	100154995	chr14	CACCTGACTGGAATCCACCA
sgSPPL3_3	SPPL3	100154995	chr14	ACTGGAATCCACCAGGGAAT
sgSPPL3_4	SPPL3	100154995	chr14	CAGGCCCTGTTCCTTCCAAT

infected samples (**Fig. 2B**), suggesting the potential roles of H3K4me3 deposition in promoting their expression. Recent reports have preliminarily revealed the host factors potentially interacting with PEDV, while the key determinant for PEDV cell entry and replication remain poorly understood. Further investigations on the detection of key regulators of host-PEDV interactions and on the underlying molecular mechanisms are required.

Application of CRISPR/Cas9 Screening in virus-host interactions

Loss-of-function genetic detection is an effective strategy in functional genomic studies via stably suppressing or disrupting gene expression in a cell or organism. Programmable CRISPR-associated nuclease Cas9 provides an effective way to cause targeted loss-of-function mutations at specific genomic sites of interest (Cong *et al*., 2013). Cas9 is guided by short RNAs to the specific sites and precisely recognizes and cleaves the target DNA, producing frameshifting indels that results in loss-of-function mutations. Feasibility of genome-wide CRISPR/Cas9 screening was firstly proved and applied in mammal cells (Shalem *et al*., 2014; Wang *et al*., 2014). Emerging studies have proven CRISPR/Cas9 screening as a reliable strategy to identify host factors that are paramount for virus replication (Ma *et al*., 2015; Zhang *et al*., 2016; Savidis *et al*., 2016). Protocols and practical considerations for this strategy can refer to the previous reports (Joung *et al*., 2014). The schematic of CRISPR/Cas9 screening workflow is shown in **Fig. 3**. To unravel host factors that are crucial for PEDV replication, we established a mutant cell library for CRISPR/Cas9 screening. An sgRNA library (~92,000 sgRNAs) targeting the porcine genomic genes (~20,000 genes) was designed, with 3~5 sgRNAs per gene. Examples of sgRNA sequence targeting porcine genes are shown in **Table 1**. A mutant cell library was then obtained after lentiviral infection and antibiotic selection. The mutant cell library was infected with PEDV and survival cells were collected for genomic DNA isolation and high-throughput sequencing. After comparison of sgRNA abundance derived from the PEDV-treated cells with the untreated controls by using the MAGeCK software (Li *et al*., 2014), top ranked genes including ERN1, THEM19, and KDM2B with high potential to repress PEDV replication were screened out. Considering the possible noisy during the screening process, it is required to verify that knockout of the candidate genes confers the phenotype of inhibition of PEDV replication.

Perspectives

With the increased pressure induced by the application of antiviral drugs and vaccine inoculation, frequently appearing mutations bring about variabilities in the viral genome and further alter the pathogenicity of the new PEDV variants. It is difficult to implement selective breeding for disease resistance, as outbreaks are often sporadic and resistant/resilient animals are difficult to identify. Genetic screen technology that can generate marked phenotypes of interest enables identification of host factors crucial for virus replication and it will substantially contribute to our understanding of viral pathogenesis and the development of antiviral therapeutics. Our limited knowledge about the host factors involved in

interaction between PEDV and host cells hinders the control and prevention of porcine epidemic diarrhea. Therefore, investigations on the mechanisms underlying the PEDV-host interactions and detection of crucial genes for PEDV infection to establish strategies to prevent the spread of PEDV.

Acknowledgements

This work was financially supported by grants from the National Natural Science Funds (Grant No. 31972535, No. 31702082), Qinglan Project of Yangzhou University, and the Priority Academic Program Development of Jiangsu Higher Education Institutions.

References

Bertolini, F., J. C. Harding, B. Mote, A. Ladinig, G. S. Plastow, and M. F. Rothschild (2017) Genomic investigation of piglet resilience following porcine epidemic diarrhea outbreaks. Animal genetics, 48(2), 228–232.

Chen, J., C. Zhang, N. Zhang, and G. Liu (2019) Porcine endemic diarrhea virus infection regulates long noncoding RNA expression. Virology, 527, 89–97.

Cong, L., F. A. Ran, D. Cox, S. Lin, R. Barretto, N. Habib, P. D. Hsu, X. Wu, W. Jiang, L. A. Marraffini, and F. Zhang (2013) Multiplex genome engineering using CRISPR/Cas systems. Science, 339(6121), 819–823.

Guo, L., X. Luo, R. Li, Y. Xu, J. Zhang, J. Ge, Z. Bu, L. Feng, and Y. Wang (2016) Porcine epidemic diarrhea virus infection inhibits interferon aignaling by targeted degradation of STAT1. Journal of Virology, 90(18), 8281–8292.

Ji, C. M., B. Wang, J. Zhou, and Y. W. Huang (2018) Aminopeptidase-N-independent entry of porcine epidemic diarrhea virus into Vero or porcine small intestine epithelial cells. Virology, 517, 16–23.

Joung, J., S. Konermann, J. S. Gootenberg, O. O. Abudayyeh, R. J. Platt, M. D. Brigham, N. E. Sanjana and F. Zhang (2017) Genome-scale CRISPR-Cas9 knockout and transcriptional activation screening. Nature Protocols, 12(4), 828–863.

Li, F. (2015) Receptor recognition mechanisms of coronaviruses: a decade of structural studies. Journal of Virology, 89(4), 1954–1964.

Li, B. X., J. W. Ge, and Y. J. Li (2007) Porcine aminopeptidase N is a functional receptor for the PEDV coronavirus. Virology, 365(1), 166–172.

Li, W., H. Xu, T. Xiao, L. Cong, M. I. Love, F. Zhang, R. A. Irizarry, J. S. Liu, M. Brown, and X. S. Liu (2014) MAGeCK enables robust identification of essential genes from genome-scale CRISPR/Cas9 knockout screens. Genome Biology, 15(12), 554.

Li, Y., Q. Wu, L. Huang, C. Yuan, J. Wang, and Q. Yang (2018) An alternative pathway of enteric PEDV dissemination from nasal cavity to intestinal mucosa in swine. Nature Communications, 9(1), 3811.

Li, Z., F. Chen, S. Ye, X. Guo, A. Muhanmmad Memon, M. Wu, and Q. He (2016) Comparative proteome analysis of porcine jejunum tissues in response to a virulent strain of porcine epidemic diarrhea virus and its attenuated Strain. Viruses, 8(12), 323.

Lin, C. M., L. J. Saif, D. Marthaler, and Q. Wang (2016) Evolution, antigenicity and pathogenicity of global porcine epidemic diarrhea virus strains. Virus research, 226, 20–39.

Ma, H., Y. Dang, Y. Wu, G. Jia, E. Anaya, J. Zhang, S. Abraham, J. G. Choi, G. Shi, L. Qi, N. Manjunath, and H. Wu (2015) A CRISPR-based screen identifies genes essential for West-Nile-virus-induced cell death. Cell Reports, 12(4), 673–683.

Park, J. E., E. S. Park, J. E. Yu, J. Rho, S. Paudel, B. H. Hyun, D. K. Yang, H. J. Shin, (2015) Development of transgenic mouse model expressing porcine aminopeptidase N and its susceptibility to porcine epidemic diarrhea virus. Virus research, 197, 108–115.

Savidis, G., W. M. McDougall, P. Meraner, J. M. Perreira, J. M. Portmann, G. Trincucci, S. P. John, A. M. Aker, N. Renzette, D. R. Robbins, Z. Guo, S. Green, T. F. Kowalik, and A. L. Brass (2016) Identification of Zika virus and Dengue virus dependency factors using functional genomics. Cell Reports, 16(1), 232–246.

Shalem, O., N. E. Sanjana, E. Hartenian, X. Shi, D. A. Scott, T. Mikkelson, D. Heckl, B. L. Ebert, D. E. Root, J. G. Doench, F. Zhang (2014) Genome-scale CRISPR-Cas9 knockout screening in human cells. Science, 343(6166), 84–87.

Shirato, K., M. Maejima, M. T. Islam, A. Miyazaki, M. Kawase, S. Matsuyama, and F. Taguchi (2016) Porcine aminopeptidase N is not a cellular receptor of porcine epidemic diarrhea virus, but promotes its infectivity via aminopeptidase activity. The Journal of General Virology, 97(10), 2528–2539.

Su, M., C. Li, S. Qi, D. Yang, N. Jiang, B. Yin, D. Guo, F. Kong, D. Yuan, L. Feng, and D. Sun (2020) A molecular epidemiological investigation of PEDV in China: Characterization of co-infection and genetic diversity of S1-based genes. Transboundary and Emerging Diseases, 67(3), 1129–1140.

T. Wang, J. J. Wei, D. M. Sabatini, and E. S. Lander (2014) Genetic screens in human cells using the CRISPR-Cas9 system. Science, 343(6166), 80–84.

Wang, H., L. Yang, H. Qu, H. Feng, S. Wu, and W. Bao (2019) Global mapping of H3K4 Trimethylation (H3K4me3) and transcriptome analysis reveal genes involved in the response to epidemic diarrhea virus infections in pigs. Animals, 9(8), 523.

Zhang, Q., H. Ke, A. Blikslager, T. Fujita, and D. Yoo (2018) Type III interferon restriction by porcine epidemic diarrhea virus and the role of viral protein nsp1 in IRF1 signaling. Journal of Virology, 92(4), e01677-17.

Zhang, R., J. J. Miner, M. J. Gorman, K. Rausch, H. Ramage, J. P. White, A. Zuiani, P. Zhang, E. Fernandez, Q. Zhang, K. A. Dowd, T. C. Pierson, S. Cherry, and M. S. Diamond (2016) A CRISPR screen defines a signal peptide processing pathway required by flaviviruses. Nature, 535(7610), 164–168.

JOURNAL OF
INTEGRATED FIELD SCIENCE
Field Science Center, Graduate School of Agricultural Science, Tohoku University

Symposium mini review

Immune Evasion Mechanisms of the Zoonotic Protozoan Parasite *Toxoplasma Gondii* in Mammalian Hosts

Hironori BANDO[1,2], Yasuhiro FUKUDA[1], Masahiro YAMAMOTO[2] and Kentaro KATO[1]

[1]Laboratory of Sustainable Animal Environment, Graduate School of Agricultural Science, Tohoku University, 232-3 Yomogida, Naruko-onsen, Osaki, Miyagi 989-6711, Japan
[2]Department of Immunoparasitology, Research Institute for Microbial Diseases, Osaka University, 3-1, Yamadaoka, Suita, Osaka, 565-0871, Japan

Keywords
Toxoplasma, IFN-γ, NLRP3, iNOS, TgGRA15

Corresponding Author
Kentaro KATO,
kentaro.kato.c7@tohoku.ac.jp

Abstract

Toxoplasma gondii is a zoonotic protozoan pathogen that causes toxoplasmosis, an infectious disease that affects most mammals, including domestic animals, wild animals, and humans. Toxoplasmosis in domestic animals causes miscarriages or stillbirths, resulting in economic losses and posing a challenge in animal husbandry. *T. gondii* is thus an important pathogen that causes serious animal and public health issues, yet there is still no vaccine or preventative medicine. Therefore, efforts to develop novel treatments for toxoplasmosis and to understand the interaction between the host immune response and the parasite in host cells are essential. We know that interferon-γ (IFN-γ)-induced tryptophan degradation by indole-2,3-dioxygenase (IDO1) plays an important role in the IFN-γ-induced anti-*T. gondii* response. However, little is known about *T. gondii* virulence mechanisms targeting IDO1. Therefore, we focused on the *T. gondii* effector TgGRA15 and analyzed its virulence function and mechanism to antagonize the IDO1-mediated anti-*T. gondii* response. In this study, we demonstrate that inducible nitric oxide synthase is a key host factor for TgGRA15-dependent disruption of the IDO1-dependent anti-*T. gondii* response.

Introduction

Toxoplasma gondii is an obligate intracellular zoonotic protozoan parasite that causes toxoplasmosis in most mammals, including domestic animals, wild animals, and humans (Boothroyd, 2009; Dubey, 2010). The family *Felidae*, which includes domestic cats, is the definitive host of *T. gondii*. The parasite can easily spread infection through the accidental swallowing of food or water contaminated with oocysts. Accordingly, *T. gondii* is prevalent in most areas of the world (Montazeri *et al*., 2020). Toxoplasmosis in humans and domestic animals can cause congenital disease, miscarriages and stillbirths, leading to not only problems of animal hygiene and public health, but also economic losses to farmers (Stelzer *et al*., 2019). Yet, no effective vaccine or preventive drug has yet been developed. Recently, along with increased overlap of the living space between humans, domestic animals, and wild animals, the number of cases of toxoplasmosis has been increasing annually. In fact, in 2015, we reported that the number of *T. gondii* infected-wild animals is increasing in Japan (Bando *et al*., 2015). Furthermore, *T. gondii* has been ranked among the top five human pathogens that cause life impairment and economic losses in the United States (Batz *et al*., 2012). Therefore, to develop novel therapeutic methods or medicines against *T. gondii*, basic research on the interaction between *T. gondii* and its host is essential.

The host immune resistance responses to *T. gondii* rely on innate and adaptive immunity (Lee *et al*., 2015; Ma *et al*., 2014; MacMicking, 2012). Interferon-γ (IFN-γ), which is produced by $CD4^+$ T cells and natural killer cells and stimulates cell-autonomous responses in both immune and non-immune cells, is the most important molecule for anti-*T. gondii* responses (Suzuki *et al*., 1988). IFN-γ plays a role in the activation of the STAT1 transcription factor and induction of the expression of hundreds of genes (Platanias, 2005). Some studies have shown that IFN-γ-inducible GTPases mediate parasiticidal and parasitostatic responses in mice (Taylor *et al*., 2007; Zhao *et al*., 2009; Yamamoto *et al*., 2012), whereas other recent

studies have reported that these GTPases may not play major roles in IFN-γ dependent anti-*T. gondii* responses in human cells (Ohshima *et al*., 2015; Fisch *et al*., 2019). We have shown that IFN-γ stimulates the expression of indoleamine 2,3-dioxygenase (IDO) and has an essential role in the anti-*T. gondii* responses of various human cell types (Bando *et al*., 2018b). Thus, although IFN-γ has a critical role in the anti-*T. gondii* response of both humans and mice, the IFN-γ-inducible effector mechanisms may differ between these two species.

T. gondii secretes various effector molecules, called rhoptry proteins (ROPs) and dense granule proteins (GRAs), into host cells. These effectors are frequently used to promote parasite growth in host cells (Hakimi *et al*., 2017; Hunter and Sibley, 2012), and their virulence mechanisms, function, and significance have been extensively researched in mouse models (Behnke *et al*., 2011; Etheridge *et al*., 2014; Fentress *et al*., 2010; Reese *et al*., 2011; Rosowski *et al*., 2014; Rosowski and Saeij, 2012; Steinfeldt *et al*., 2010). The *Toxoplasma* effector TgGRA15, one of the dense granule proteins, is secreted into host cells to activate the host transcription factor NF-κB in mice (Gov *et al*., 2013; Jensen *et al*., 2011; Rosowski *et al*., 2011), although it should be noted that most virulence factors suppress the host immune responses (Olias *et al*., 2016; Gay *et al*., 2016). TgGRA15-deficient *T. gondii* has been shown to promote parasite proliferation *in vivo* in mice (Jensen *et al*., 2013; Rosowski *et al*., 2011), meaning that TgGRA15 can support host survival by preventing parasite growth. Thus, the significance of TgGRA15 as a virulence factor remains unclear. In this study, we introduce the virulent mechanism of TgGRA15 targeting the IDO1-dependent anti-*T. gondii* response in human cells.

TgGRA15 promotes *T. gondii* growth when co-cultured in the presence of IFN-γ

The function of TgGRA15 as a virulence factor is unclear; therefore, to explore it in human cells, we generated TgGRA15-deficient (TgGRA15-KO) *T. gondii* by using the CRISPR/Cas9 system. Then, we tested whether TgGRA15 has an important role in the suppression of host immune responses under human cell mono-culture conditions. However, we failed to find any advantageous effect of TgGRA15 on parasite growth in various human cell lines. When *T. gondii* infects its host, the parasite preferentially infects CD11b+ cells such as monocytes, and then the infected cells are carried by the bloodstream to various organs (Courret *et al*., 2006). Several kinds of co-culture models have been established to mimic complex cell-cell interactions by using human tissue or immune cell lines, one of which is the monocyte-hepatocyte co-culture model (Frenkel and Remington, 1980). Because one of the major symptoms of toxoplasmosis is hepatitis, we developed a *T. gondii* infection model using monocyte-hepatocyte co-culture conditions. Human acute monocytic leukemia cell line THP-1 cells were infected with wild-type or TgGRA15-KO parasite, and then both the culture supernatant and infected THP-1 cells were seeded onto human hepatoma cell line Huh7 cells with or without IFN-γ. Interestingly, the parasite numbers under the TgGRA15-KO parasite-infected co-culture condition were significantly reduced compared with the wild-type parasite-infected co-culture condition. These data indicate that TgGRA15 has an advantageous effect on *T. gondii* growth under human cell co-culture conditions.

NLRP3-dependent IL-1β secretion from monocytes is essential for the pro-parasitic effect of TgGRA15 in hepatocytes

We next attempt to reveal the mechanisms of the pro-parasitic effect of TgGRA15 under co-culture conditions. First, to test whether TgGRA15 has an effect on monocytes or hepatocytes, the culture supernatants were collected from wild-type and TgGRA15-KO *T. gondii*-infected THP-1 cells, and then both the parasites and THP-1 cells were removed by filtration. The filtered culture supernatants and newly prepared wild-type or TgGRA15-KO parasites were then added to Huh7 cells with IFN-γ. Then the number of parasites in the Huh7 cells was assessed. The presence of TgGRA15 in THP-1 cells, but not Huh7 cells, led to a reduction in parasite number, suggesting that the presence of TgGRA15 in monocytes and their supernatant is essential for the pro-parasitic effect. Therefore, we next focused on the components of the supernatant from the parasite infected-THP-1 cell culture. Previous studies have reported that *T. gondii* infection induces proinflammatory cytokine IL-1β secretion from THP-1 cells in a TgGRA15-dependent manner (Gov *et al*., 2013). It has also been reported that IL-1β production in monocytes is dependent on Caspase-1 and inflammasome activation (Gov *et al*., 2013; Gov *et al*., 2017). Therefore, to test whether TgGRA15-dependent Caspase-1 and inflammasome activation are important for IL-1β secretion from monocytes, we generated NLRP3-deficient (NLRP3-KO) or Caspase-1-deficient (CASP1-KO) THP-1 cells by using CRISPR/Cas9 systems, and then analyzed IL-1β secretion levels in the culture supernatant. We found that both NLRP3-KO- and CASP1-KO-infected THP-1 cells showed significantly reduced IL-1β secretion. Then, we examined whether IL-1β secretion in THP-1 cells is essential for suppressing the IFN-γ-dependent anti-*T. gondii* response under co-culture conditions. We found that the parasite number in both wild-type parasite-infected NLRP3-KO and CASP1-KO THP-1 cells was significantly reduced compared with that of wild-type THP-1 cells. These results indicate that IL-1β secretion through Caspase-1 and NLRP3 inflammasome activation in THP-1 cells has an important role in the TgGRA15-dependent suppression of the IFN-γ-dependent anti-*T. gondii* response.

The IFN-γ-induced IDO1-dependent anti-*T. gondii* response is downregulated by TgGRA15 in hepatocytes

We previously reported that IDO1-induced tryptophan degradation has an important role in the IFN-γ-dependent anti-*T. gondii* response in various human cell types including hepatocytes (Bando *et al*., 2018b; Bando *et al*., 2019) because tryptophan is an essential amino acid for parasite growth. In fact, we found that the IFN-γ-dependent reduction in parasite numbers in IDO1-deficient (IDO1-KO) Huh7 cells was abolished under TgGRA15-KO parasite-infected co-culture conditions. Therefore, we examined whether IL-1β affects IDO1 expression in Huh7 cells. We found that IL-

1β and IFN-γ co-stimulation severely inhibited IDO1 mRNA and protein levels in Huh7 cells. Then, to examine whether IL-1β-dependent impairment of the IFN-γ-dependent anti-*T. gondii* response was IDO1-dependent, we generated MyD88-deficient (MyD88-KO)— MyD88 is essential molecule for the IL-1 receptor signaling pathway (Adachi *et al*., 1998)—and IL1R1-deficient (IL1R1-KO) Huh7 cells by using CRISPR/Cas9 systems. We found that the pro-parasitic effect of IL-1β in IDO1-KO, MyD88-KO, and IL1R1-KO Huh7 cells was completely abolished. Then, we compared the protein levels of IDO1 under wild-type *T. gondii*- and TgGRA15-KO parasite-infected and non-infected co-culture conditions. We found that the protein levels of IDO1 were significantly reduced under wild-type parasite-infected conditions compared with non-infected conditions. Importantly, the protein levels of IDO1 under TgGRA15-KO parasite-infected conditions recovered to the same levels as those seen under non-infected conditions. These results indicate that the TgGAR15-induced IL-1β-dependent downregulation of IDO1 expression is important for the impairment of the IFN-γ-dependent anti-*T. gondii* response in hepatocytes.

iNOS is essential for TgGRA15-dependent inhibition of the IDO1-dependent anti-*T. gondii* response

Nitric oxide (NO) production is known to strongly downregulate IDO activity transcriptionally, translationally, and post-translationally (Thomas *et al*., 1994). In addition, inducible nitric oxide synthase (iNOS) has been shown to be an important factor for IFN-γ-mediated NO production (Nathan and Xie, 1994). Hence to explain the mechanism of the IL-1β-dependent IDO1 suppression, we focused on iNOS and NO-dependent downregulation of IDO1 activity in hepatocytes. First, we examined the expression level of iNOS mRNA in Huh7 cells. We found that IFN-γ and IL-1β co-stimulation enhanced the expression level of iNOS mRNA and strongly induced NO production in Huh7 cells. Then, to examine the role of iNOS, we generated iNOS-deficient (iNOS-KO) Huh7 cells by using the CRISPR/Cas9 system. We found that NO was not produced from iNOS-KO Huh7 cells upon IFN-γ and IL-1β co-stimulation and that IL-1β-dependent reduction of IDO1 protein levels did not occur in iNOS-KO Huh7 cells. Furthermore, the IL-1β-dependent pro-parasitic effect was completely abolished in the iNOS-KO Huh7 cells under mono-culture conditions, suggesting that IL-1β induces iNOS expression to inhibit the IDO1-dependent anti-*T. gondii* response. Then, we tested whether this mechanism occurs under co-culture conditions. We found that NO production and the reduction of IDO1 was not observed in iNOS-KO Huh7 cells co-cultured with wild-type parasite-infected wild-type THP-1 cells. Moreover, the TgGRA15-dependent pro-parasitic effect was abolished in iNOS-KO Huh7 cells under these co-culture conditions. Finally, we confirmed the GRA15-dependent virulence mechanism in primary human cells. Taken together, our results indicate that iNOS is an essential host factor for the TgGRA15-dependent virulence mechanism under monocyte-hepatocyte co-culture conditions.

Conclusion

In summary, here we showed that IL-1β is produced from monocytes in a *Toxoplasma* effector TgGRA15- and host NLRP3 inflammasome-dependent manner. We further showed that iNOS has an essential role in the *Toxoplasma* TgGRA15-dependent inhibition of the IDO1-induced anti-*T gondii* response in human cells (Bando *et al*., 2018a). Although immune responses in humans and domestic animals are not identical (Guzman and Montoya, 2018), the tryptophan-degrading enzyme IDO and nitric oxide synthases NOS have been found in most mammalian species (Yao *et al*., 2011). Hence, the TgGRA15-dependent virulence mechanism may contribute to *T. gondii* infection in not only humans but also domestic animals. Studies to examine whether the TgGRA15-dependent virulence mechanism has an important role in *T. gondii* infection of domestic animals, and to identify chemical compounds that block iNOS expression or NO production could contribute to the development of novel anti-toxoplasmosis therapies for humans and domestic animals.

Acknowledgments

We thank the staff at the Laboratory of Immunoparasitology, Research Institute for Microbial Diseases, Osaka University and the Laboratory of Sustainable Animal Environment, Graduate School of Agricultural Science, Tohoku University. This study was supported by a Research Program on Emerging and Reemerging Infectious Diseases (17fk0108120h0001) and a fund for the Promotion of Joint International Research (Fostering Joint International Research (B)) (19KK0242) from the Japan Society for the Promotion of Science (JSPS) and the Japanese Initiative for Progress of Research on Infectious Diseases for Global Epidemic (17fm0208018h0001) from the Agency for Medical Research and Development (AMED), by a Grant-in-Aid for Scientific Research on Innovative Areas (17K15677 and 19K16628) from the Ministry of Education, Culture, Sports, Science and Technology, by a Cooperative Research Grant of the Institute for Enzyme Research, Joint Usage/Research Center, Tokushima University, by the Takeda Science Foundation, the Ohyama Health Foundation, the Heiwa Nakajima Foundation, the Cell Science Research Foundation, the Mochida Memorial Foundation on Medical and Pharmaceutical Research, the Uehara Memorial Foundation, and the Research Foundation for Microbial Diseases of Osaka University (for MY). This study was also funded by grants-in-aid for Scientific Research (B:17H03913) and Challenging Exploratory Research (19K22940) from the Ministry of Education, Culture, Science, Sports, and Technology (MEXT) of Japan, and by a Livestock Promotional Subsidy from the Japan Racing Association.

References

Adachi, O., T. Kawai, K. Takeda, M. Matsumoto, H. Tsutsui, M. Sakagami, K. Nakanishi and S. Akira (1998) Targeted disruption of the MyD88 gene results in loss of IL-1- and IL-18-mediated function. Immunity, 9: 143-150.

Bando, H., Y. Lee, N. Sakaguchi, A. Pradipta, J. S. Ma, S. Tanaka, Y. Cai, J. Liu, J. Shen, Y. Nishikawa, M. Sasai and M. Yamamoto (2018a) Inducible Nitric Oxide Synthase Is a Key Host Factor for *Toxoplasma* GRA15-Dependent Disruption of the Gamma

Interferon-Induced Antiparasitic Human Response. MBio, 9: e01738-18.
Bando, H., Y. Lee, N. Sakaguchi, A. Pradipta, R. Sakamoto, S. Tanaka, J. S. Ma, M. Sasai and M. Yamamoto (2019) *Toxoplasma* Effector GRA15-Dependent Suppression of IFN-gamma-Induced Antiparasitic Response in Human Neurons. Frontiers in Cellular and Infection Microbiology, 9: 140.
Bando, H., N. Sakaguchi, Y. Lee, A. Pradipta, J. S. Ma, S. Tanaka, D. H. Lai, J. Liu, Z. R. Lun, Y. Nishikawa, M. Sasai and M. Yamamoto (2018b) *Toxoplasma* Effector TgIST Targets Host IDO1 to Antagonize the IFN-gamma-Induced Anti-parasitic Response in Human Cells. Frontiers in Immunology, 9: 2073.
Bando, H., A. Yoshimura, M. Koketsu, A. Soga, Y. Taniguchi, M. Ozaki, M. Suzuki, H. Kanuka and S. Fukumoto (2015) Serological Survey of *Toxoplasma gondii* in Wild Sika Deer in Eastern Hokkaido, Japan. The Journal of Protozoology Research, 25: 1-2.
Batz, M. B., S. Hoffmann and J. G. J. Morris (2012) Ranking the disease burden of 14 pathogens in food sources in the United States using attribution data from outbreak investigations and expert elicitation. Journal of Food Protection, 75: 1278-1291.
Behnke, M. S., A. Khan, J. C. Wootton, J. P. Dubey, K. Tang and L. D. Sibley (2011) Virulence differences in *Toxoplasma* mediated by amplification of a family of polymorphic pseudokinases. Proceedings of the National Academy of Sciences of the United States of America, 108: 9631-9636.
Boothroyd, J. C. (2009) *Toxoplasma gondii*: 25 years and 25 major advances for the field. International journal for parasitology, 39: 935-946.
Courret, N., S. Darche, P. Sonigo, G. Milon, D. Buzoni-Gatel and I. Tardieux (2006) CD11c- and CD11b-expressing mouse leukocytes transport single *Toxoplasma gondii* tachyzoites to the brain. Blood, 107: 309-316.
Dubey, J. P. (2010) Toxoplasmosis of Animals and Humans. CRC Press.
Etheridge, R. D., A. Alaganan, K. Tang, H. J. Lou, B. E. Turk and L. D. Sibley (2014) The *Toxoplasma* pseudokinase ROP5 forms complexes with ROP18 and ROP17 kinases that synergize to control acute virulence in mice. Cell Host and Microbe, 15: 537-550.
Fentress, S. J., M. S. Behnke, I. R. Dunay, M. Mashayekhi, L. M. Rommereim, B. A. Fox, D. J. Bzik, G. A. Taylor, B. E. Turk, C. F. Lichti, R. R. Townsend, W. Qiu, R. Hui, W. L. Beatty and L. D. Sibley (2010) Phosphorylation of immunity-related GTPases by a *Toxoplasma gondii*-secreted kinase promotes macrophage survival and virulence. Cell Host and Microbe, 8: 484-495.
Fisch, D., H. Bando, B. Clough, V. Hornung, M. Yamamoto, A. R. Shenoy and E. M. Frickel (2019) Human GBP1 is a microbe-specific gatekeeper of macrophage apoptosis and pyroptosis. The EMBO Journal, 38: e100926.
Frenkel, J. K. and J. S. Remington (1980) Hepatitis in toxoplasmosis. The New England journal of medicine, 302: 178-179.
Gay, G., L. Braun, M. P. Brenier-Pinchart, J. Vollaire, V. Josserand, R. L. Bertini, A. Varesano, B. Touquet, P. J. De Bock, Y. Coute, I. Tardieux, A. Bougdour and M. A. Hakimi (2016) *Toxoplasma gondii* TgIST co-opts host chromatin repressors dampening STAT1-dependent gene regulation and IFN-gamma-mediated host defenses. Journal of Experimental Medicine, 213: 1779-1798.
Gov, L., A. Karimzadeh, N. Ueno and M. B. Lodoen (2013) Human innate immunity to *Toxoplasma gondii* is mediated by host caspase-1 and ASC and parasite GRA15. MBio, 4: e00255-13.
Gov, L., C. A. Schneider, T. S. Lima, W. Pandori, M. B. Lodoen (2017) NLRP3 and Potassium Efflux Drive Rapid IL-1beta Release from Primary Human Monocytes during *Toxoplasma gondii* Infection. The Journal of Immunology, 199: 2855-2864.
Guzman, E. and M. Montoya (2018) Contributions of Farm Animals to Immunology. Frontiers in Veterinary Science, 5: 307.
Hakimi, M. A., P. Olias and L. D. Sibley (2017) *Toxoplasma* Effectors Targeting Host Signaling and Transcription. Clinical microbiology reviews, 30: 615-645.
Hunter, C. A. and L. D. Sibley (2012) Modulation of innate immunity by *Toxoplasma gondii* virulence effectors. Nature Reviews Microbiology, 10: 766-778.
Jensen, K. D., K. Hu, R. J. Whitmarsh, M. A. Hassan, L. Julien, D. Lu, L. Chen, C. A. Hunter and J. P. Saeij (2013) *Toxoplasma gondii* rhoptry 16 kinase promotes host resistance to oral infection and intestinal inflammation only in the context of the dense granule protein GRA15. Infection and Immunity, 81: 2156-2167.
Jensen, K. D., Y. Wang, E. D. Wojno, A. J. Shastri, K. Hu, L. Cornel, E. Boedec, Y. C. Ong, Y. H. Chien, C. A. Hunter, J. C. Boothroyd and J. P. Saeij (2011) *Toxoplasma* polymorphic effectors determine macrophage polarization and intestinal inflammation. Cell Host and Microbe, 9: 472-483.
Lee, Y., M. Sasai, J. S. Ma, N. Sakaguchi, J. Ohshima, H. Bando, T. Saitoh, S. Akira and M. Yamamoto (2015) p62 Plays a Specific Role in Interferon-gamma-Induced Presentation of a *Toxoplasma* Vacuolar Antigen. Cell reports, 13: 223-233.
Ma, J. S., M. Sasai, J. Ohshima, Y. Lee, H. Bando, K. Takeda and M. Yamamoto (2014) Selective and strain-specific NFAT4 activation by the *Toxoplasma gondii* polymorphic dense granule protein GRA6. The Journal of experimental medicine, 211: 2013-2032.
MacMicking, J. D. (2012) Interferon-inducible effector mechanisms in cell-autonomous immunity. Nature reviews Immunology, 12: 367-382.
Montazeri, M., T. M. Galeh, M. Moosazadeh, S. Sarvi, S. Dodangeh, J. Javidnia, M. Sharif and A. Daryani (2020) The global serological prevalence of *Toxoplasma gondii* in felids during the last five decades (1967-2017): a systematic review and meta-analysis. Parasites and Vectors, 13(1): 82.
Nathan, C. and Q. W. Xie (1994) Nitric oxide synthases: roles, tolls, and controls. Cell, 78: 915-918.
Ohshima, J., M. Sasai, J. Liu, K. Yamashita, J. S. Ma, Y. Lee, H. Bando, J. C. Howard, S. Ebisu, M. Hayashi, K. Takeda, D. M. Standley, E. M. Frickel and M. Yamamoto (2015) RabGDIalpha is a negative regulator of interferon-gamma-inducible GTPase-dependent cell-autonomous immunity to *Toxoplasma gondii*. Proceedings of the National Academy of Sciences of the United States of America, 112: E4581-4590.
Ohshima, J., Y. Lee, M. Sasai, T. Saitoh, J. Su Ma, N. Kamiyama, Y. Matsuura, S. Pann-Ghill, M. Hayashi, S. Ebisu, K. Takeda, S. Akira and M. Yamamoto (2014) Role of mouse and human autophagy proteins in IFN-gamma-induced cell-autonomous responses against *Toxoplasma gondii*. Journal of Immunology, 192: 3328-3335.
Olias, P., R. D. Etheridge, Y. Zhang, M. J. Holtzman and L. D. Sibley (2016) *Toxoplasma* Effector Recruits the Mi-2/NuRD Complex to Repress STAT1 Transcription and Block IFN-gamma-Dependent Gene Expression. Cell Host and Microbe, 20: 72-82.
Platanias, L. C. (2005) Mechanisms of type-I- and type-II-interferon-mediated signaling. Nature Reviews Immunology, 5: 375-386.
Reese, M. L., G. M. Zeiner, J. P. Saeij, J. C. Boothroyd and J. P. Boyle (2011) Polymorphic family of injected pseudokinases is paramount in *Toxoplasma* virulence. Proceedings of the National Academy of Sciences of the United States of America, 108: 9625-9630.
Rosowski, E. E., D. Lu, L. Julien, L. Rodda, R. A. Gaiser, K. D. Jensen and J. P. Saeij (2011) Strain-specific activation of the NF-kappaB pathway by GRA15, a novel *Toxoplasma gondii* dense granule protein. Journal of Experimental Medicine, 208: 195-212.
Rosowski, E. E., Q. P. Nguyen, A. Camejo, E. Spooner and J. P. Saeij (2014) *Toxoplasma gondii* Inhibits gamma interferon (IFN-gamma)- and IFN-beta-induced host cell STAT1 transcriptional activity by increasing the association of STAT1 with DNA. Infection and Immunity, 82: 706-719.
Rosowski, E. E. and J. P. Saeij (2012) *Toxoplasma gondii* clonal strains all inhibit STAT1 transcriptional activity but polymorphic effectors differentially modulate IFN gamma induced gene expression and STAT1 phosphorylation. PLoS One, 7: e51448.
Steinfeldt, T., S. Konen-Waisman, L. Tong, N. Pawlowski, T. Lamkemeyer, L. D. Sibley, J. P. Hunn and J. C. Howard (2010) Phosphorylation of mouse immunity-related GTPase (IRG) resistance proteins is an evasion strategy for virulent *Toxoplasma gondii*. PLoS biology, 8: e1000576.
Stelzer, S., W. Basso, J. Benavides Silván, L. M. Ortega-Mora, P. Maksimov, J. Gethmann, F. J. Conraths and G. Schares (2019) *Toxoplasma gondii* infection and toxoplasmosis in farm animals: Risk factors and economic impact. Food and Waterborne Parasitology, 15: e00037.
Suzuki, Y., M. A. Orellana, R. D. Schreiber and J. S. Remington

(1988) Interferon-gamma: the major mediator of resistance against *Toxoplasma gondii*. Science, 240: 516-518.

Taylor, G. A., C. G. Feng and A. Sher (2007) Control of IFN-gamma-mediated host resistance to intracellular pathogens by immunity-related GTPases (p47 GTPases). Microbes and Infection, 9: 1644-1651.

Thomas, S. R., D. Mohr and R. Stocker (1994) Nitric oxide inhibits indoleamine 2,3-dioxygenase activity in interferon-gamma primed mononuclear phagocytes. The Journal of biological chemistry, 269: 14457-14464.

Yamamoto, M., M. Okuyama, J. Ma, T. Kimura, N. Kamiyama, H. Saiga, J. Ohshima, M. Sasai, H. Kayama, T. Okamoto, D. C. S. Huang, D. Soldati-Favre, K. Horie, J. Takeda and K. Takeda (2012) A cluster of interferon-γ-inducible p65 GTPases plays a critical role in host defense against *Toxoplasma gondii*. Immunity, 37: 302-313.

Yao, K., J. Fang, Y. L. Yin, Z. M. Feng, Z. R. Tang and G. Wu (2011) Tryptophan metabolism in animals: important roles in nutrition and health. Frontiers in Bioscience, 3: 286-297.

Zhao, Y., D. J. Ferguson, D. C. Wilson, J. C. Howard, L. D. Sibley and G. S. Yap (2009) Virulent *Toxoplasma gondii* evade immunity-related GTPase-mediated parasite vacuole disruption within primed macrophages. Journal of Immunology, 182: 3775-3781.

JOURNAL OF
INTEGRATED FIELD SCIENCE
Field Science Center, Graduate School of Agricultural Science, Tohoku University

Symposium mini review

Morphological Variation of Bermudagrass along Longitudinal and Latitudinal Gradients under in-situ and ex-situ conditions

Jing-Xue ZHANG[1], Yu SHEN[1], Miao-Li WANG[2], Zhi-Peng GUO[2], Ming-Hui CHEN[1], Jin-Qian[1] and Xue-Bing YAN[1]

[1]College of Animal Science and Technology, Yangzhou University, Yangzhou 225000, China
[2]College of Animal and Veterinary Science, Henan Agricultural University, Zhengzhou 450002, China

Keywords
Cynodon dactylon, Morphology, Variation, Longitude, Latitude

Corresponding Author
Xuebing YAN,
yxbbjzz@163.com

Abstract

Bermudagrass [*Cynodon dactylon* (L.) Pers.], a polymorphic and cosmopolitan warm-season grass, was widely used for turf, forage and soil stabilization. Estimation of genetic variation based on the morphological characteristics have been developed in an attempt to eliminate the environmental effects. Our work focuses on comparing the value of morphological characterization of in situ and ex situ to find whether there is a strong environmental influence on the morphological characteristics of *C. dactylon* along longitudinal and latitudinal gradients. No significant differences were found in the variation of plant height, leaf width, internode length, and stolon diameter between the same populations in situ and ex situ. Both morphological characterization of *C. dactylon* in situ and ex situ are equally valuable for the estimation of the germplasm collection and evolutionary studies of the species.

Morphological variation under in-situ condition

C. dactylon are also highly diversified in ecology and morphology, with growing in tropic, subtropic, and temperate zones, and from Africa to South America, Africa, Europe, and South Asia (Harlan and De Wet, 1968; Taliaferro, 1995; Dong and Shen, 2003). Larger morphological sizes of *C. dactylon* appeared at the low- and high-latitude regions, while leaves of the erect shoot and the internode length enlarged significantly with the collection sites moving from east to west (Zhang *et al*., 2018; Wang *et al*., 2020). This supports the conjecture that at low- and high-latitude sites, leaf length, leaf width and circumference are being selected in the opposite direction when compared to the mid-latitude sites. High morphological variations were significantly correlated with climate factors and soil nutrients. Environmental effects on plant morphological characters were obvious in this study, reflecting plasticity for morphological appearance against longitude and latitude. Morphological size was influenced by longitude and latitude-related environmental factors, suggesting a different breeding goals and morphological adaptation. The morphological variations could lead to physiological changes and improved ecological tolerances. Some results of other studies which suggest evolutionary adaptation to an expanded range might require modifications in vegetative development (size, growth) (Geber and Eckhart, 2005).

Morphological variation under ex-situ condition

The environment along longitudinal and latitudinal gradients have possibly high effect on the morphological, so the pattern of morphological variation under homogeneous growing conditions ex situ was compared with those already reported in situ to validate this methodology. Morphological characteristics including plant height, leaf width, internode length, stolon length and stolon diameter of Bermudagrass were examined on the materials that were grown in the common garden. The pattern of variation revealed by variation coefficients and cluster analysis were similar both in ex situ conditions and in situ conditions, because in both studies the populations formed the same morphotypes. Inconsistencies were found between the two conditions, the internode length and stolon diameter of high- latitude populations tended to have greater size than those from the low- and mid-latitudes under ex situ conditions, while under in situ conditions, these two characters were lower at mid-latitude. Internode length and stolon diameter at different latitudes are influenced by latitude-related soil characters and climate factors for adaptive phenotypic plasticity. However, we think that this inconsistency has little relevance. The morphological

characterization of fruit was used to estimate the evolution and diffusion (Harries, 1978). Morphological characterization in situ can be a useful methodology with low technological for genetic diversity studies, the design of strategies for the conservation of *C. dactylon*.

Relationship among the morphological characters and ploidy levels for bermudagrass breeding

Morphological traits varied with ploidy level across the 27 geographic regions along longitudinal and latitudinal gradients. Among ploidy levels, plant height and leaf width of triploids were smaller than those of the hexaploids, indicating that the Chinese wild *C. dactylon* contained much wider morphological diversity among different ploidy levels. Polyploids have been studied for their morphological, physiological and developmental differences from diploids to find the correlative evidence explaining observations that polyploids can adapt better to different environments. One sample of morphological differences between diploids and polyploids are the larger cell sizes in polyploids including those of the stomata (Speckman *et al*., 1965; Melaragno *et al*., 1993; Masterson, 1994; Hodgson *et al*., 2010). A hexaploid Bermudagrass such as *C.dactylon* cv. 'Tifton 10' tends to have thick stolons and coarse-textured long leaves (Wu *et al*., 2005, 2006). Morphological effects of polyploidization could include bigger flowers, delayed and prolonged flowering, an altered length/width ratio of leaves, a darker green coloration of the leaves, or thicker leaves and stems as in Buddleja (Rose *et al*., 2000), Salvia (Kobayashi *et al*., 2008). Inducing novel morphological characteristics by ploidy breeding is a powerful tool that could lead to commercial success in plants.

Acknowledgements

I would like to thank Professor Xue-Bing Yan and the Organizing Committee of the International Symposium on Integrated Field Science for the invitation to participate at this event.

References

Dong, K. H. and Y. X. Shen (2003) Forage Production Science. China Agricultural Press, Beijing.

Geber, M. A. and V. M. Eckhart (2005) Experimental studies of adaptation in Clarkia xantiana. II. Fitness variation across a subspecies border. Evolution, 59: 521-531.

Harlan, J. R. and J. M. J. De Wet (1968) Sources of variation in *Cynodon dactylon* (L.) Pers. Crop Science, 9: 774-778.

Harries, H. C. (1978) The evolution, dissemination and classification of *Cocos nucifera* L. Botanical Review, 44: 265-319.

Hodgson, J. G., M. Sharafi, A. Jalili, S. Díaz, G. Montserrat-Martí, C. Palmer and B. Cerabolini (2010) Stomatal vs. genome size in angiosperms: the somatic tail wagging the genomic dog? Annals of Botany, 105(4): 573-584.

Kobayashi, N., S. Yamashita, K. Ohta and T. Hosoki (2008) Morphological characteristics and their inheritance in colchicine-induced Salvia polyploids. Japan Journal of the Japanese Society for Horticultural Science, 77(2): 186-191.

Masterson, J. (1994) Stomatal size in fossil plants: evidence for polyploidy in majority of angiosperms. Science, 264: 421-424.

Melaragno, J. E., B. Mehrotra and A. W. Coleman (1993) Relationship between endopolyploidy and cell size in epidermal tissue of arabidopsis. The Plant Cell Online, 5: 1661-1668.

Rose, J., J. Kubba and K. Tobutt (2000) Induction of tetraploidy in Buddleia globosa. Plant Cell Tissue Organ Cult, 63: 121-125.

Speckman, G., J. Post and H. Dijkstra (1965) Length of stomata as an indicator for polyploidy in rye-grasses. Euphytica, 14: 225-228.

Taliaferro, C. M. (1995) Diversity and vulnerability of bermuda turfgrass species. Crop Science, 35: 327-332.

Wu, Y. Q., C. M. Taliaferro, G. H. Bai, D. L. Martin and M. P. Anderson (2005) Genetic diversity of *Cynodon* transvaalensis Burtt-Davy and its relatedness to hexaploid *C. dactylon* (L.) Pers. as indicated by AFLP markers. Crop Science, 45: 848-853.

Wu, Y. Q., C. M. Taliaferro, G. H. Bai, D. L. Martin and J. A. Anderson (2006) Genetic analyses of Chinese *Cynodon* accessions by flow cytometry and AFLP markers. Crop Science, 46: 917-926.

Wang, M. L., J. X. Zhang, Z. P. Guo, Y. Z. Guan, G. Qu, J. Y. Liu, Y. X. Guo and X. B. Yan (2020) Morphological variation in *Cynodon dactylon* (L.) Pers. and its relationship with the environment along a longitudinal gradient. Hereditas, 157(1).

Zhang, J. X., M. L. Wang, Z. P. Guo, Y. Z. Guan, Y. X. Guo and X. B. Yan (2018) Variations in morphological traits of bermudagrass and relationship with soil and climate along latitudinal gradients. Hereditas, 155(1).

JOURNAL OF
INTEGRATED FIELD SCIENCE
Field Science Center, Graduate School of Agricultural Science, Tohoku University

Symposium mini review

Genomic Prediction in Japanese Black Beef Cattle: Some Topics

Shinichiro OGAWA

Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi, Japan

Keywords
Japanese Black cattle,
single nucleotide polymorphism,
breeding value prediction,
the degree of marbling,
genome-wide high-density DNA marker

Corresponding Author
Shinichiro OGAWA,
shinichiro.ogawa.d5@tohoku.ac.jp

Abstract

The Japanese Black is a representative of the beef cattle breeds constructing Wagyu in Japan. The beef produced are high quality, especially highly marbled (shimofuri), and they are also famous overseas. Carcass traits including the degree of marbling have been remarkably improved through use of a low number of elite sires with high predicted genetic abilities (breeding values) for meat quality. Breeding values are predicted by using a deep pedigree information and a large amount of carcass performance data of fattened steers and heifers. Breeding values for growth performance and feed efficiency of young bulls and those for female reproductivity of cows have been also evaluated using pedigree information. Recently, prediction of breeding values by using genotype information on genome-wide high-density single nucleotide polymorphisms (SNPs) as DNA markers, also referred to as genomic prediction, have been studied in Japanese Black cattle. Genomic prediction is believed to have a potential to achieve more efficient breeding in livestock species. In this review, some relevant topics for genomic prediction in Japanese Black cattle, as well as the possibility of genomic prediction and the future issues in this breed, will be discussed.

Japanese Black cattle as the primary beef breed of Wagyu

Wagyu is a general term used for modern native Japanese beef cattle breeds in Japan. Wagyu cattle consist of four beef breeds: the Japanese Black, Japanese Brown, Japanese Shorthorn, and Japanese Polled. The Japanese Black cattle is the primary Wagyu breed and is well known to excel in meat quality, especially in marbling. For the detailed description about establishing the Japanese Black breed, see, for example, Hirooka (2014), Motoyama *et al*. (2016), and Gotoh *et al*. (2018). In short, native Japanese cattle in Japan were crossed with British and Continental breeds during an approximately 10-year period in the early 1900s. And then, under a completely closed breeding system, the four breeds of Wagyu were fixed through strict selection over many years (Namikawa, 1992).

In Japanese Black cattle, the genetic abilities (breeding values) have been evaluated for several economically important traits relating to carcass performance of fattened progeny, growth and feed efficiency of young bulls, and female reproductive efficiency of dams. For the detailed explanation about the progeny-testing for the representative carcass traits in this breed, see, for example, Inoue (2004) and Sasaki (2007). Beef quality traits including the degree of marbling have received more emphasis in domestic beef production of Japanese Black cattle since the relaxation of beef import restrictions in Japan in 1991. In the same year, genetic evaluation of carcass traits using a mixed model methodology (Henderson, 1973) based on a deep pedigree information was introduced using relevant field data collected at carcass markets (Wagyu Registry Association, 2007). Assuming the infinitesimal model to breeding value (Fisher, 1918; Bulmer, 1980), the whole genome is targeted in the genetic evaluation but is treated as an unobserved black box. Subsequently, while there has been steady genetic improvement attained in carcass traits, it is known that there is a sharp decline occurred in effective population size of this breed, because of intensive use of few sires with higher predicted breeding values for degree of marbling (e.g., Nomura *et al*., 2001; Nomura, 2002; Honda *et al*., 2004).

Numerous studies have reported the estimated heritabilities of, and genetic correlations among various kinds of traits in Japanese Black cattle. Oyama (2011) summarized the estimated values of direct and maternal heritabilities for body weight and daily gain traits of calves, those of direct

heritability for body weight, daily gain, feed intake, and feed conversion traits of young bulls, those of direct heritability for and genetic correlation among carcass traits of fattened steers and heifers, and those of direct and maternal heritabilities for female reproductive traits of dams. In general, carcass and growth traits were moderately to highly heritable but female reproductive traits had low heritabilities, and the genetic correlation between two fat deposition traits, marbling and subcutaneous fat thickness, was low (Oyama, 2011).

Genetic parameters for different traits have been also estimated. For example, several studies estimated the heritabilities of body measurement traits and their genetic correlation with carcass and female reproductive traits (e.g., Baco *et al*., 1997; Kuchida *et al*., 1994; Munim *et al*., 2012; Oyama *et al*., 1996). Heritabilities of traits relating to meat quality and their genetic correlations with carcass traits have been also reported (e.g., Inoue *et al*., 2008; Komatsu *et al*., 2014; Nogi *et al*., 2011; Onogi *et al*., 2017; Sakuma *et al*., 2016). Heritabilities of image analysis traits for the shape of ribeye and marbling in ribeye and other muscles have been estimated (e.g., Goto *et al*., 2020; Kuchida *et al*., 2006; Osawa *et al*., 2008). Moderate to high heritability has been estimated for growth curve parameters (e.g., Inoue *et al*., 2020; Onogi *et al*., 2019; Takeda *et al*., 2018; Wada & Nishida, 1987), residual feed intake, residual daily gain, and residual feed intake and daily gain (e.g., Hoque & Oikawa, 2004; Okanishi *et al*., 2008; Takeda *et al*., 2018), and predicted methane emission traits (Uemoto *et al*., 2020). For female reproductive efficiency for heifer, Inoue *et al*. (2020) estimated the heritability of first service conception rate to be 0.03 and Setiaji and Oikawa (2020) estimated the heritability of non-return rate, the number of inseminations, and interval from first to successful insemination to be 0.027, 0.019, and 0.011, respectively. Ogawa and Satoh (2021) reported that the estimated heritability of calving interval was low but the genetic correlation between different ages of dam was consistently high. Inoue *et al* (2017) estimated the direct and maternal heritabilities of calving difficulty to be 0.24 and 0.61, respectively. Nishida *et al*. (2006) estimated the heritability of the number of services per conception at different parity to be ~0.1 in most cases. Nishimura *et al*. (2010) estimated the heritability for semen characteristics traits in bulls to be around 0.1. Nishi *et al*. (2016) estimated the heritabilities of carcass defects including blood splash, intramuscular edema, muscle steatosis, bruising, trim loss, and other defects, and Oyama *et al*. (2020) estimated the heritabilities of defective appearances including white spotting, tongue defect, and nipple defect. Inoue *et al*. (2015) estimated the heritabilities of internal diseases of fattened steers and their genetic correlations with carcass traits. Takeda *et al*. (2017) estimated the heritability of temperament of calves and its genetic correlation with carcass traits. These estimates could provide valuable information for the development of appropriate, sound future breeding plans in Japanese Black cattle (Oyama, 2011).

Studies for genomic prediction for carcass traits in Japanese Black cattle

In the late twentieth-century, breakthroughs occurred in molecular biology and genetic engineering that established the technological basis for modern genomics and biotechnology. This facilitated quantitative trait locus (QTL) mapping and marker-assisted selection (MAS). The MAS assumes the use of a small number of DNA markers for major causative genes with large effects. However, there is now a general consensus that most complex and quantitative traits are usually affected by a large number of small-effect genes (de los Campos *et al*., 2013).

Meuwissen *et al*. (2001) proposed the idea of a new type of MAS that simultaneously treats all chromosome segments by using genome-wide DNA markers such as single nucleotide polymorphisms (SNPs). Genome-wide high-density SNPs are used with the expectation of tracing all underlying QTLs, or to explain all additive genetic variances of a trait by exploiting the status in linkage disequilibrium between QTLs and SNPs. Prediction of breeding values using genome-wide DNA markers is often referred to as genomic prediction (GP), and selection based on the result of GP is genomic selection (GS). For the detailed explanation for GP and GS written in Japanese, see, for example, Matsuda *et al*. (2013) and Nagamine (2012).

After developing the commercial SNP chip which can determine the genotypes of SNPs identified using samples other than Japanese Black cattle (Matukumalli *et al*., 2009), GS following GP was introduced into routine genetic evaluation and selection of dairy cattle, especially Holstein cattle (e.g., Hayes *et al*., 2009; VanRaden *et al*., 2009); this is partly because of the possibility of reducing the costs of progeny testing schemes (Schaeffer, 2006). Even though the potential for GS to improve genetic gain in beef cattle would be substantial (Pimentel *et al*., 2012; Van Eenennaam *et al*., 2011), there are no reports providing the information on GP accuracy for Japanese Black cattle. Ogawa *et al*. (2014) estimated the variance of carcass weight and marbling score explained by the genome-wide 38,502 SNP markers using 872 fattened steers (**Fig. 1**). This study showed that the genome-wide SNP markers genotyped by using the commercial chip can capture most additive genetic variances of carcass weight and marbling score in Japanese Black cattle. Ogawa *et al*. (2016a) assessed the accuracy of GP for carcass weight and marbling score, using 1,791 steers as the training population and 189 animals as the validation population (**Fig. 2**). The accuracy of GP was middle to high for both traits, implying

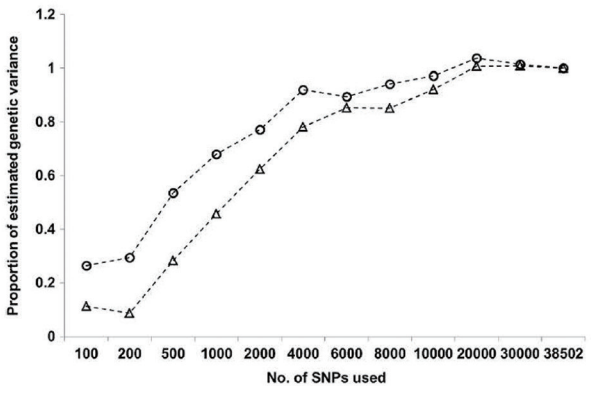

Fig. 1. Changes in proportions of estimated genetic variances with increasing SNP marker density. Circles: carcass weight; triangle: marbling score (Ogawa *et al.*, 2014).

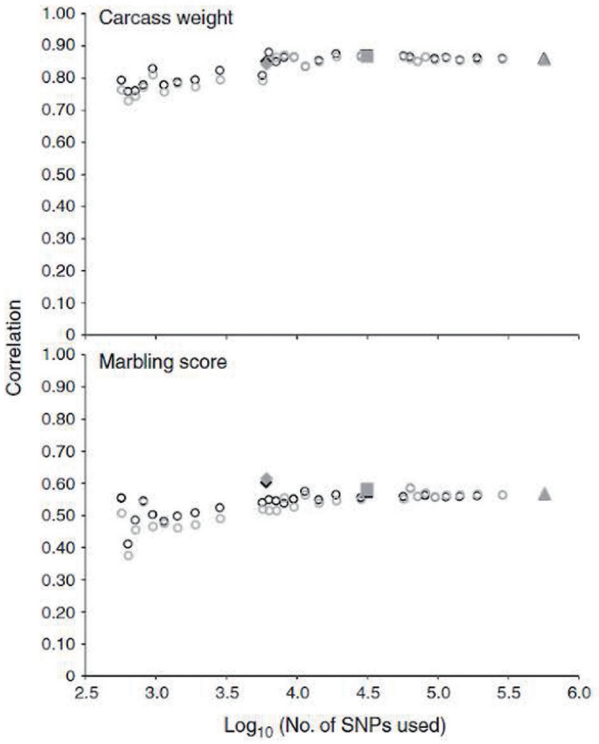

Fig. 2. Changes in the accuracy of genomic prediction. Triangles, squares and rhombuses show the results obtained using high-density, 50K and low-density single nucleotide polymorphism (SNP) sets, respectively (Ogawa *et al.*, 2016a).

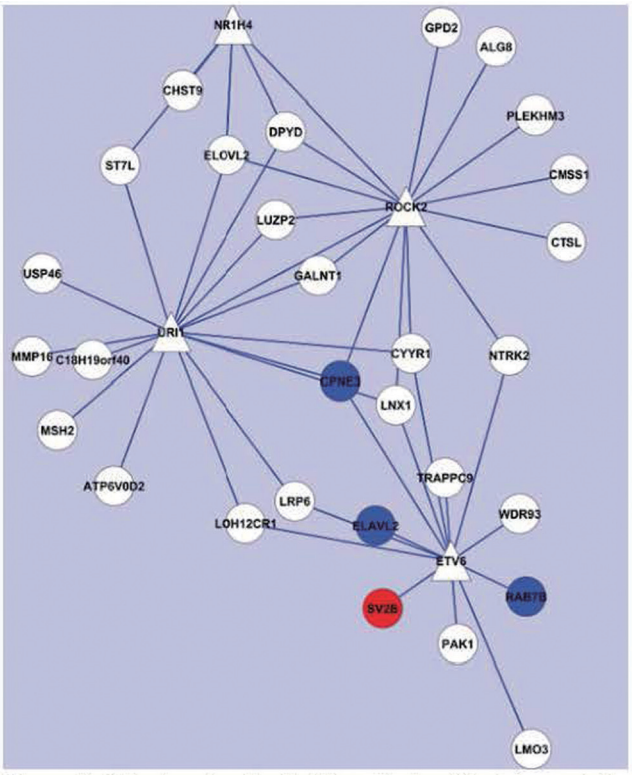

Fig. 3. Subnetwork extracted from the top 3 hub transcription factors (TFs) and their neighboring genes (Okada *et al.*, 2018). Node colors correspond to gene tissue specificity: gland (red), nervous system (blue), and nontissue-specific genes (white). Node shapes indicate gene classification: triangle (TF) and circle (other genes).

that GP for carcass traits could be available in pre-selecting young Japanese Black breeding animals. Watanabe (2016) compared the accuracies of GP for six carcass traits with those of parent average of breeding value, which is used as a classical indicator for pre-selection, and showed that the accuracy was higher for GP than parent average.

Some possible future challenges in this breed

In Japanese Black cattle, the study about GP for fatty acid composition in meat (Onogi *et al*., 2015), semen production traits (Atagi *et al*., 2017), and feed efficiency traits (Takeda *et al*., 2020) were also reported. In the future, GP for other traits including female reproductive efficiency, resistance to disease and heat stress, and traits related to environmental load should be performed.

Integrating multi-omics data is a hot topic (e.g., Snelling *et al*., 2013; Suravajhala *et al*., 2016; Takagi *et al*., 2014). Okada *et al*. (2018) estimated candidate gene-gene interaction network for feed efficiency in cattle combining the results of the genome-wide association study for seven feed utilization traits in Japanese black cattle and public RNA-expression data from different tissues of multiple foreign breeds (**Fig. 3**). The results could give meaningful insight into the trait, and GP incorporating such a biological information might increase the performance of GP in cattle (e.g., Gao *et al*., 2017; Melzer *et al*., 2013; Tiezzi *et al*., 2018).

The study about long-term implementation of GP and GS in Japanese Black cattle is essential (e.g., Maltecca *et al*., 2020; Neyhart *et al*., 2017; Ogawa *et al*., 2016b). For example, GS can boost the speed of genetic improvement, while this might also bring further decrease in genetic diversity of this breed, as already observed in dairy cattle (Doekes *et al*., 2018; Doublet *et al*., 2019). High-density SNP markers could be also available for assessing the genetic diversity of cattle populations (e.g., Decker *et al*., 2014; Eusebi *et al*., 2019; VanRaden *et al*., 2011). Therefore, genome-wide DNA markers might be a powerful tool to achieve the efficient genetic improvement while considering the genetic diversity of Japanese Black cattle (e.g., Gómez-Romano *et al*., 2016; Thomasen *et al*., 2013; Wang *et al*., 2017).

Acknowledgements

The works of Ogawa *et al*. (2014, 2016a,b) were partly supported by the Japanese Ministry of Agriculture, Forestry, and Fisheries, by the Japanese Racing and Livestock Promotion Foundation (H20-5), and by the Research Fellowship of the Japanese Society for the Promotion of Science for Young Scientists (No.15J02417). The author thanks the staff of the Shirakawa Institute of Animal Genetics for their technical assistance for collecting SNP genotype data. The work of Ogawa and Satoh (2021) was supported by the Sasakawa Scientific Research Grant from The Japan Science Society (2018–4019). The author thanks the Miyagi Prefectural Government Livestock Industry division for kindly providing the data.

References

Atagi, Y., A. Onogi, M. Kinukawa, A. Ogino, K. Kurogi, K. Uchiyama, T. Yasumori, K. Adachi, K. Togashi and H. Iwata (2017) Genetic analysis of semen production traits of Japanese Black and Holstein bulls: genome-wide marker-based estimation of genetic parameters and environmental effect trends. *Journal of Animal Science*, 95(5): 1900-1912. DOI: 10.2527/jas.2016.1186

Baco, S., H. Harada and R. Fukuhara (1997) Genetic relationships of body measurements at registration to a couple of reproductive traits in Japanese Black cows. *Animal Science Technology (Japan)*, 69(1): 1-7. DOI: 10.2508/chikusan.69.1

Bulmer, M. G. (1980) The mathematical theory of quantitative genetics. Oxford University Press, Oxford, UK.

Decker, J. E., S. D. McKay, M. M. Rolf, J. Kim, A. Molina Alcalá, T. S. Sonstegard, O. Hanotte, A. Götherström, C. M. Seabury, L. Praharani, M. E. Babar, L. C. A. Regitano, M. A. Yildiz, M. P. Heaton, W. -S. Liu, C. -Z. Lei, J. M. Reecy, M. Saif-Ur-Rehman, R. D. Schnabel and J. F. Taylor (2014) Worldwide patterns of ancestry, divergence, and admixture in domesticated cattle. *PLoS Genetics*, 10(3): e1004254. DOI: 10.1371/journal.pgen.1004254

de los Campos, G., J. M. Hickey, R. Pong-Wong, H. D. Daetwyler and M. P. L. Calus (2013) Whole-genome regression and prediction methods applied to plant and animal breeding. *Genetics*, 193(2), 327-345. DOI: 10.1534/genetics.112.143313

Doekes, H. P., R. F. Veerkamp, P. Bijma, S. J. Hiemstra and J. J. Windig (2018) Trends in genome-wide and region-specific genetic diversity in the Dutch-Flemish Holstein–Friesian breeding program from 1986 to 2015. *Genetics Selection Evolution*, 50: 15. DOI: 10.1186/s12711-018-0385-y

Doublet, A. -C., P. Croiseau, S. Fritz, A. Michenet, C. Hozé, C. Danchin-Burge, D. Laloë and G. Restoux (2019) The impact of genomic selection on genetic diversity and genetic gain in three French dairy cattle breeds. *Genetics Selection Evolution*, 51: 52. DOI: 10.1186/s12711-019-0495-1

Eusebi, P. G., A. Martinez and O. Cortes (2020) Genomic tools for effective conservation of livestock breed diversity. *Diversity*, 12(1): 8. DOI: 10.3390/d12010008

Fisher, R. A. (1918) The correlation between relatives on the supposition of mendelian inheritance. *Earth and Environmental Science Transactions of The Royal Society of Edinburgh*, 52(2): 399-433. DOI: 10.1017/S0080456800012163

Gao, N., J. W. R. Martini, Z. Zhang, X. Yuan, H. Zhang, H. Simianer and J. Li (2017) Incorporating gene annotation into genomic prediction of complex phenotypes. *Genetics*, 207: 489-501. DOI: 10.1534/genetics.117.300198

Gómez-Romano, F., B. Villanuva, J. Fernández and J. A. Woolliams (2016) The use of genomic coancestry matrices in the optimisation of contributions to maintain genetic diversity at specific regions of the genome. *Genetics Selection Evolution*, 48: 2. DOI: 10.1186/s12711-015-0172-y

Goto, Y., R. Asa, K. Hagiya and K. Kuchida (2020) A new evaluation method of marbling fineness in *M. longissimus thoratic* of Japanese Black Cattle. *Nihon chikusan Gakkaiho*, 91(2): 103-110. (in Japanese) DOI: 10.2508/chikusan.91.103

Gotoh, T., T. Nishimura, K. Kuchida and H. Mannen (2018) The Japanese Wagyu beef industry: current situation and future prospects – A review. *Asian-Australasian Journal of Animal Sciences*, 31(7): 933-950. DOI: 10.5713/ajas.18.0333

Hayes, B. J., P. J. Bowman, A. J. Chamberlain and M. E. Goddard (2009) Invited review: genomic selection in dairy cattle: progress and challenge. *Journal of Dairy Scienc*e, 92(2): 433-443. DOI: 10.3168/jds.2008-1646

Henderson, C. R. (1973) Sire evaluation and genetic trends. In: Proceedings of the Animal Breeding and Genetics Symposium in Honour of J. L. Lush. American Society for Animal Science, Blackburgh, Champaign, IL, pp. 10-41.

Hirooka, H. (2014) Marbled Japanese Black cattle. *Journal of Animal Breeding and Genetics*, 131(1): 1-2. DOI: 10.1111/jbg.12073

Honda, T., T. Nomura, Y. Yamaguchi and F. Mukai (2004) Monitoring of genetic diversity in the Japanese Black cattle population by the use of pedigree information. *Journal of Animal Breeding and Genetics*, 121(4): 242-252. DOI: 10.1111/j.1439-0388.2004.00452.x

Hoque, M. A. and T. Oikawa (2004) Comparison and relation among different estimates of residual feed intake for Japanese Black (Wagyu) bulls. *Animal Science Journal*, 75(3): 201-205. DOI: 10.1111/j.1740-0929.2004.00176.x

Inoue, K. (2004). A Challenge to a national genetic evaluation for Japanese Black Cattle in Japan (An inter-prefecture progeny testing scheme for beef cattle). *The Journal of Animal Genetics*, 32(1): 17-28. (In Japanese) DOI:10.5924/ABGRI2000.32.17

Inoue, K., T. Honda and K. Oyama (2015) Genetic relationships between internal diseases diagnosed at slaughter and carcass traits in Japanese Black cattle. *Journal of Animal Science*, 93(6): 2714-2721. DOI: 10.2527/jas.2014-8497

Inoue, K., M. Hosono, H. Oyama and H. Hirooka (2020) Genetic associations between reproductive traits for first calving and growth curve characteristics of Japanese Black cattle. *Animal Science Journal*, 91(1): e13467. DOI: 10.1111/asj.13467

Inoue, K., M. Hosono and Y. Tanimoto (2017) Inferring causal structures and comparing the causal effects among calving difficulty, gestation length and calf size in Japanese Black cattle. *Animal*, 11(12): 2120-2128. DOI: 10.1017/S1751731117000957

Inoue, K., N. Shoji and M. Kobayashi (2008) Genetic relations among fat melting point, fatty acid composition and carcass traits in Japanese Black Cattle. *Nihon Chikusan Gakkaiho*, 79(1): 1-8. (in Japanese) DOI: 10.2508/chikusan.79.1

Komatsu, T., N. Shoji, K. Saito and K. Suzuki (2014) Effects of genetic and environmental factors on muscle glycogen content in Japanese black cattle. *Animal Science Journal*, 85(8), 793-798. DOI: 10.1111/asj.12201

Kuchida, K., K. Chiba, T. Yamagishi, H. Uchida and A. Nishida (1994) The relationship between the body measurements of cows and the growth traits of their calves in Japanese Black cattle. *Animal Science Technology (Japan)*, 65(4): 334-339. (in Japanese) DOI: 10.2508/chikusan.65.334

Kuchida, K., T. Osawa, T. Hori, H. Kotaka and S. Mariyama (2006) Evaluation and genetics of carcass cross section of beef carcass by computer image analysis. *The Journal of Animal Genetics*, 34(2): 45-52. (in Japanese) DOI: 10.5924/abgri2000.34.2_45

Maltecca, C., F. Tiezzi, J. B. Cole and C. Baes (2020) Symposium review: Exploiting homozygosity in the era of genomics—Selection, inbreeding, and mating programs. *Journal of Dairy Science*, 103(6): 5302-5313. DOI: 10.3168/jds.2019-17846

Matsuda, H., Y. Taniguchi and H. Iwaisaki (2013) Representative methods for genomic prediction and current status of the approaches using biological knowledge. *The Journal of Animal Genetics*, 41(2): 93-99. (in Japanese) DOI: 10.5924/abgri.41.93

Matukumalli, L. K., C. T. Lawley, R. D. Schnabel, J. F. Taylor, M. F. Allan, M. P. Heaton, J. C'Connell, S. S. Moore, T. P. L. Smith, T. S. Sonstegard and C. P. Van Tassell (2009) Development and characterization of a high density SNP genotyping assay for cattle. *PLoS ONE*, 4(4): e5350. DOI: 10.1371/journal.pone.0005350

Melzer, N., D.Wittenburg and D. Repsilber (2013) Integrating milk metabolite profile information for the prediction of traditional milk traits based on SNP information for Holstein cows. *PLoS One*, 8(8): e70256. DOI: 10.1371/journal.pone.0070256

Meuwissen, T. H. E., B. J. Hayes and M. E. Goddard (2001) Prediction of total genetic value using genome-wide dense marker maps. *Genetics*, 157(4): 1819-1829.

Motoyama, M., K. Sasaki and A. Watanabe (2016) Wagyu and the factors contributing to its beef quality: A Japanese industry overview. *Meat Science*, 120: 10-18. DOI: 10.1016/j.meatsci.2016.04.026

Munim, T., T. Oikawa, T. Ibi and T. Kunieda (2013) Genetic relationship of body measurement traits at early age with carcass traits in Japanese black cattle. *Animal Science Journal*, 84(3): 206-212. DOI: 10.1111/asj.12005

Nagamine, Y. (2012). Genomic study using large number of SNP markers and future of livestock animal breeding. *Nihon Chikusan Gakkaiho*, 83(1): 1-8. (in Japanese) DOI: 10.2508/CHIKUSAN.83.1

Namikawa, K. (1992) Wagyu: Japanese Beef Cattle - Historical Breeding Processes of Japanese Beef Cattle and Preservation of Genetic Resources as Economic Farm Animal. Kyoto, Japan, Wagyu Registry Association.

Neyhart, J. L., T. Tiede, A. J. Lorenz and K. P. Smith (2017) Evaluating methods of updating training data in long-term genomewide selection. *G3 (Bethesda)*, 7(5): 1499-1510. DOI: 10.1534/g3.117.040550

Nishi, K., T. Shimogiri, A. Kusano, S. Sakamoto, K. Shiromoto, K. Kawabe, S. Okamoto, T. Honda and K. Oyama (2016) Estimation of genetic parameters for carcass defects of Japanese Black cattle in Kagoshima. *Animal Science Journal*, 87(5): 655-660. DOI: 10.1111/asj.12475

Nishida, A., M. A. Aziz, S. Nishida and K. Suzuki (2006) Modelling number of services per conception of Japanese Black cattle by random regression. *Journal of Animal Breeding and Genetics*, 123(1): 56-53. DOI: 10.1111/j.1439-0388.2006.00560.x

Nishimura, K, T. Honda and K. Oyama (2010) Genetic variability of semen characteristics in Japanese Black bulls. *The Journal of Animal Genetic*, 38(2): 73-76. DOI: 10.5924/abgri.38.73

Nogi, T., T. Honda, F. Mukai, T. Okagaki and K. Oyama (2011) Heritabilities and genetic correlations of fatty acid compositions in longissimus muscle lipid with carcass traits in Japanese black cattle. *Journal of Animal Science*, 89(3), 615-621. DOI: 10.2527/jas.2009-2300

Nomura, T. (2002) Effective size of populations with unequal sex ratio and variation in mating success. *Journal of Animal Breeding and Genetics*, 119(5): 297-310. DOI: 10.1046/j.1439-0388.2002.00347.x

Nomura, T., T. Honda and F. Mukai (2001) Inbreeding and effective population size of Japanese Black cattle. *Journal of Animal Science*, 79(2): 366-370. DOI: 10.2527/2001.792366x

Ogawa, S., H. Matsuda, Y. Taniguchi, T. Watanabe, S. Nishimura, Y. Sugimoto and H. Iwaisaki. (2014) Effects of single nucleotide polymorphism marker density on degree of genetic variance explained and genomic evaluation for carcass traits in Japanese Black beef cattle. *BMC Genetics*, 15: 15. DOI: 10.1186/1471-2156-15-15

Ogawa, S., H. Matsuda, Y. Taniguchi, T. Watanabe, Y. Kitagawa, I. Tabuchi, Y. Sugimoto and H. Iwaisaki (2016a) Genomic prediction for carcass traits in Japanese Black cattle using single nucleotide polymorphism markers of different densities. *Animal Production Science*, 57(8): 1631-1636. DOI: 10.1071/AN15696

Ogawa, S., H. Matsuda, Y. Taniguchi, T. Watanabe, A. Takasuga, Y. Sugimoto and H. Iwaisaki (2016b) Accuracy of imputation of single nucleotide polymorphism marker genotypes from low-density panels in Japanese Black cattle. *Animal Science Journal*, 87(1): 3-12. DOI: 10.1111/asj.12393

Ogawa, S. and M. Satoh (2021) Random regression analysis of calving interval of Japanese Black cows. *Animals*, 11(1): 202. DOI: 10.3390/ani11010202

Okada, D., S. Endo, H. Matsuda, S. Ogawa, Y. Taniguchi, T. Katsuta, T. Watanabe and H. Iwaisaki (2018) An intersection network based on combining SNP coassociation and RNA coexpression networks for feed utilization traits in Japanese Black cattle. *Journal of Animal Science*, 96(7): 2553-2566. DOI: 10.1093/jas/sky170

Okanishi, T., M. Shojo, T. Katsuta, K. Oyama and F. Mukai (2008) Genetic analysis of residual feed intakes and other performance test traits of Japanese Black cattle from revised protocol. *Animal Science Journal*, 79(3): 291-296. DOI: 10.1111/j.1740-0929.2008.00529.x

Onogi, A., A. Ogino, T. Komatsu, N. Shoji, K. Shimizu, K. Kurogi, T. Yasumori, K. Togashi and H. Iwata (2015) Whole-genome prediction of fatty acid composition in meat of Japanese Black cattle. *Animal Genetics*, 46(5):557-559. DOI: 10.1111/age.12300

Onogi, A., A. Ogino, A. Sato, K. Kurogi, T. Yasumori and K. Togashi (2019) Development of a structural growth curve model that considers the causal effect of initial phenotypes. *Genetics Selection Evolution*, 51: 19. DOI: 10.1186/s12711-019-0461-y

Onogi, A., S. Sasaki, M. Kobayashi, A. Ogino, T. Nozaki, K. Kurogi, T. Yasumori, K. Togashi and H. Iwata (2017) A genetic analysis of meat compositions in Japanese Black cattle: Genetic parameters and sex influence. *Journal of Animal Breeding and Genetics*, 134(5): 373-382. DOI: 10.1111/jbg.12280

Osawa, T., K. Kuchida, S. Hidaka and T. Kato (2008) Genetic parameters for image analysis traits on *M. longissimus thoracis* and *M. trapezius* of carcass cross section in Japanese Black steers. *Journal of Animal Science*, 86(1): 40-46. DOI: 10.2527/jas.2007-0359

Oyama, H., K. Imamura, S. Sakamoto, K. Nishi, K. Kawabe, S. Okamoto, T. Honda, K. Oyama and T. Shimogiri (2020) Estimation of genetic parameters of defective appearances in Japanese Black heifer calves in Kagoshima. *Animal Science Journal*, 91(1): e13338. DOI: 10.1111/asj.13338

Oyama, K. (2011) Genetic variability of Wagyu cattle estimated by statistical approaches. *Animal Science Journal*, 82(3): 367-373. DOI: 10.1111/j.1740-0929.2011.00895.x

Oyama, K., F. Mukai and T. Yoshimura (1996) Genetic relationships among traits recorded at registry judgment, reproductive traits of breeding females and carcass traits of fattening animals in Japanese Black cattle. *Animal Science Technology (Japan)*, 67(6): 511-518. DOI: 10.2508/CHIKUSAN.67.511

Pimentel, E. C. G. and S. König (2012) Genomic selection for the improvement of meat quality in beef. *Journal of Animal Science*, 90(10): 3418-3426. DOI: 10.2527/jas.2011-5005.

Sakuma, H., K. Saito, K. Kohira, F. Ohhashi, N. Shoji and Y. Uemoto (2016) Estimates of genetic parameters for chemical traits of meat quality in Japanese black cattle. *Animal Science Journal*, 88(2), 203-212. DOI: 10.1111/asj.12622

Sasaki, Y. (2007). Selection criterion for Wagyu cattle - visual judging to gene diagnosis -. *The Journal of Animal Genetics*, 35(1): 33-49. DOI: 10.5924/abgri2000.35.33

Schaeffer, L. R. (2006) Strategy for applying genome-wide selection in dairy cattle. *Journal of Animal Breeding and Genetics*, 123(4): 218-223. DOI: 10.1111/j.1439-0388.2006.00595.x

Setiaji, A. and T. Oikawa (2020) Genetics of heifer reproductive traits in Japanese Black cattle. *Asian-Australasian Journal of Animal Sciences*, 33(2): 197-202. DOI: 10.5713/ajas.19.0118

Snelling, W. M., R. A. Cushman, J. W. Keele, C. Maltecca, M. G. Thomas, M. R. Fortes and A. Reverter (2013) Breeding and genetics symposium: networks and pathways to guide genomic selection. *Journal of Animal Science*, 91(2): 537-552. DOI: 10.2527/jas.2012-5784

Suravajhala, P., L. J. A. Kogelman and H. N. Kadarmideen (2016) Multi-omic data integration and analysis using systems genomics approaches: methods and applications in animal production, health and welfare. *Genetics Selection Evolution*, 48: 38. DOI: 10.1186/s12711-016-0217-x

Takagi, Y., H. Matsuda, Y. Taniguchi and H. Iwaisaki (2014) Predicting the phenotypic values of physiological traits using SNP genotype and gene expression data in mice. *PLoS One*, 9(12): e115532. DOI: 10.1371/journal.pone.0115532

Takeda, K., H. Uchida and K. Inoue (2017) Genetic relationships between temperament of calves at auction and carcass traits in Japanese Black cattle. *Animal Science Journal*, 88(10): 1475-1481. DOI: 10.1111/asj.12787

Takeda, M., Y. Uemoto, K. Inoue, A. Ogino, T. Nozaki, K. Kurogi, T. Yasumori and M. Satoh (2017) Evaluation of feed efficiency traits for genetic improvement in Japanese Black cattle. *Journal of Animal Science*, 96(3): 797-805. DOI: 10.1093/jas/skx054

Takeda, M., Y. Uemoto, K. Inoue, A. Ogino, T. Nozaki, K. Kurogi, T. Yasumori and M. Satoh (2020) Genome-wide association study and genomic evaluation of feed efficiency traits in Japanese Black cattle using single-step genomic best linear unbiased prediction method. *Animal Science Journal*, 91(1): e13316. DOI: 10.1111/asj.13316

Thomasen, J. R., A. C. Sørensen, G. Su, P. Madsen, M. S. Lund and B. Guldbrandtsen (2013) The admixed population structure in Danish Jersey dairy cattle challenges accurate genomic predictions. *Journal Animal Science*, 91(7): 3105-3112. DOI: 10.2527/jas.2012-5490

Tiezzi, F., M. E. Arceo, J. B. Cole and C. Maltecca (2018) Including gene networks to predict calving difficulty in Holstein, Brown Swiss and Jersey cattle. *BMC Genetics*, 19: 20. DOI: 10.1186/s12863-018-0606-y

Uemoto, Y., M. Takeda, A. Ogino, K. Kurogi, S. Ogawa, M. Satoh and F. Terada (2020) Genetic and genomic analyses for predicted methane-related traits in Japanese Black steers. *Animal Science Journal*, 91(1): e13383. DOI: 10.1111/asj.13383

Van Eenennaam, A. L., J. H. J. van der Werf and M. E. Goddard

(2011) The value of using DNA markers for beef bull selection in the seedstock sector. *Journal of Animal Science*, 89(2): 307-320. DOI: 10.2527/jas.2010-3223

VanRaden, P. M., K. M. Olson, G. R. Wiggans, J. B. Cole and M. E. Tooker (2011) Genomic inbreeding and relationships among Holsteins, Jerseys, and Brown Swiss. *Journal of Dairy Science*, 94(11): 5673-5682. DOI: 10.3168/jds.2011-4500

VanRaden, P. M., C. P. Van Tassell, G. R. Wiggans, T. S. Sonstegard, R. D. Schnabel, J. F. Taylor and F. S. Schenkel (2009) Invited review: Reliability of genomic predictions for North American Holstein bulls. *Journal of Dairy Science*, 92(1): 16-24. DOI: 10.3168/jds.2008-1514

Wada, Y. and A. Nishida (1987) Genetic aspects of the growth curve characteristics in Japanese Black cows. *Japanese Journal of Zootechnological Science*, 58(12): 1078-1085.

Wagyu Registry Association (2007) Breeding and Improvement of Wagyu. 2nd edition. Wagyu Registry Association, Kyoto, Japan. (in Japanese)

Wang, Y., D. Segelke, R. Emmerling, J. Bennewitz and R. Wellmann (2017) Long-term impact of optimum contribution selection strategies on local livestock breeds with historical introgression using the example of German Angler cattle. *G3 (Bethesda)*, 7(12): 4009-4018. DOI: 10.1534/g3.117.300272

Watanabe, T. (2016) Genomic breeding value evaluation for economically important traits of Japanese Black cattle. *The Journal of Animal Genetics*, 44(1-2): 3-10. (in Japanese) DOI: 10.1534/g3.117.300272

Outline

Name

International virtual symposium “New Insights on Animal Science”

Dates

November 22nd, 2020

Venue

Virtual on-line symposium by ZOOM directed from Tohoku University

Organizers

- Center for Food and Agricultural Immunology (CFAI)
- Graduate School of Agricultural Science, Tohoku University
- Integrated Field Science Center, Graduate School of Agricultural Science, Tohoku University

Organizing Committee

Shin-ichiro Ogura	Haruki Kitazawa
Sanggun Roh	Tomonori Nochi
Kenshiro Hara	Yoshinobu Uemoto
Tao Zhuang	Kwonjung Yi

Sponsors

Japan Society for the Promotion of Science (JSPS) Core-to-Core Program “Establishment of international agricultural immunology research-core for a quantum improvement in food safety”

Introduction of CFAI (Director of CFAI: Dr. Haruki Kitazawa)

The Center for Food and Agricultural Immunology (CFAI) was established in the Graduate School of Agricultural Science, Tohoku University, on April 1, 2015, and has been launched the second five-year period from April 2020 taken over the first period. CFAI consists of the following 4 divisions: 'Agricultural Immunology', 'Safety and Function Evaluation', 'Cooperation with Society' and 'Research and Administration'. The mission of CFAI is to create an intellectual and technical basis for drug-independent health promotion and food safety systems in agriculture. We hope to do this based on interdisciplinary research on the agricultural immunology of plants, livestock as well as fish and shellfish. CFAI has international collaborations with UC Davis and Texas A&M University in the US, Wageningen University and Utrecht University in the Netherlands, Yangzhou University in China and CERELA-CONICET in Argentina. CFAI has also established links with the Food and Agricultural Immunology Network (FAIN) in the Japan Society for the Promotion of Science (JSPS) Core-to-Core Program, A Advanced Research Networks entitled Establishment of international agricultural immunology research-core for a quantum improvement in food safety, including above organizations. A key aim of CFAI is global professional developments in these areas. In order to achieve our mission, we are especially keen to promote research exchange of young researchers and graduate students in the leading international academias.

CFAI will now hold an international virtual symposium entitled "New Insights on Animal Science" in the Graduate School of Agricultural Science, Tohoku University on November 22nd, 2020 as an important event in the Core-to-Core Program. This symposium mainly consists of the topics from selected young scientists and PhD students in Yangzhou University and Tohoku University. Through this symposium, I hope that CFAI will be able to develop further the theoretical foundations of animal science by including participants whose specialties include all fields of sciences.

Haruki Kitazawa (CFAI, Tohoku University)

Haruki Kitazawa is Director of the International Education and Research Center for Food and Agricultural Immunology (CFAI), and Professor of the Laboratory of Animal Animal Food Function (LAFF), Graduate School of Agricultural Science, Tohoku University. He got his PhD on Immunomodulatory functions of phosphopolysaccharides produced by lactic acid bacteria in 1993. He has recently conducted international research works with CERELA-CONICET under JSPS several Joint Research Projects. He also contributes as an editorial board member in Frontiers in Nutrition, Microorganisms etc, and also as a guest editor in Frontiers in Immunology and other international journals. His present research activities focus, in particular, on immunoregulatory activities of immunobiotics and immunosymbiotics, which are a member of probiotics able to beneficially regulate the mucosal immune system, in the gut together with the development of immunobiotic evaluation system via pattern recognition receptor family in vitro by using a variety of livestock cell lines.

Shin-ichiro Ogura, Symposium Chair
Field Science Center, Graduate School of Agricultural Science, Tohoku University

Shin-ichiro Ogura is Director of Integrated Field Science Center (FSC) and Vice Director of Tohoku Agricultural Science Center for Reconstruction (TASCR), and Professor of Laboratory of Land Ecology, Graduate School of Agricultural Science, Tohoku University. He got his PhD in Graduate School of Agricultural Science, Tohoku University in 1997. Following this, he worked at Faculty of Agriculture, Miyazaki University as Assistant Professor, from 1998 to 2003. Since 2003, he has been working at Tohoku University. During his career, he had an opportunity of staying in Macaulay Land Use Research Institute, Scotland in 2002. He is focusing on the plant-animal interactions in grassland ecosystems such as pasture and forage crop production and utilization by ruminant animals, especially diet selection, foraging behavior and nutrient uptake of grazing ruminants. He is now conducting researches to evaluate nutritional characteristics of native plants and its role to animal production, to establish a grazing system in species-rich vegetation.

Evaluation, Conservation and Utilization for Chinese Indigeous Sheep and Goat

Wei SUN

Professor
Director of Institute of Agricultural Science and Technology Development (IASTD)
Joint International Research Laboratory of Agriculture & Agri-Product Safety of the Ministry of Education
Yangzhou University

E-mail: sunwei@yzu.edu.cn

Sheep and goat are two of the earliest animals to be domesticated for agricultural purposes. China is one of the countries that have the richest sheep and goat breed resources in the world.

Prof. Sun will talk about "the germplasm conservation and utilization of Chinese indigenous sheep & goat breeds". His representation will cover the following 5 aspects: Firstly, the origin and domestication of Chinese indigenous sheep & goat; Secondly, Sheep & goat germplasm resources in China; Thirdly, Conservation of sheep & goat germplasm resources in China; Fourthly, Utilization of sheep & goat germplasm resources in China; and, Finally, Problems and countermeasures of germplasm conservation and utilization in China.

Research Biography

Wei Sun received his PhD degree on animal genetic, breeding and reproduction from Yangzhou University. He is the director of Institute of Agricultural Science and Technology Development, and had experience in Commonwealth Science and Industries Research Organization (CSIRO), Brisbane, Australia as a visiting scholar. He is also a member of an expert group on National Meat Sheep Genetic Improvement Program; the Deputy secretary-general, Sheep-raising Council Branch of Chinese Animal Husbandry and Veterinary Society; Chief Expert, Huaihai Mutton Sheep Research and Development Center of Jiangsu province. His research interest is to study the Evaluation, Protection, Utilization and Development of Genetic Resources of Sheep(Goat) Breeds; Molecular Markers and Cultivation of New Strain (Breed) of Sheep (Goat); and Sheep (Goat) (Epi-) Genetics and Functional Genomics.

Immunosecurity: Strengthen the Impact of Immune Regulation in Domestic Animals

Cheol-Heui YUN

Full Professor
Department of Agricultural Biotechnology and Research Institute of Agriculture and Life Sciences,
Seoul National University

E-mail: cyun@snu.ac.kr

Animal farming has been affected by a number of factors including national and international restrictions based on their guidelines and regulations, disease outbreak, welfare issues, balance between demand and supply of the meats and animals together with their by-products or processed-products, and economic and cultural demand. The global population increases coincident with an enhancement of living standards in developing countries, which is likely to create a high demand for animal-derived proteins over time. With that in mind, we are under great pressure and constantly facing a challenge in relation with the issues including climate change (i.e., global warming), banning on the use of antibiotics as a feed additive concordant with disease outbreak for both domestic animals (African swine fever, avian influenza, foot-and-mouse disease) and human (corona pandemic for instance). Yet, high productivity together with high quality meat from the animal would be most desirable for producers and animal farming industry. Thus, it is fair to say that current animal farming is under various psychological and physical stressful conditions, which can be categorized into nutritional factors, environmental factors, biological factors and physical factors.

Basic, however essential, components of immune system include the recognition of self and non-self, and its remarkable specificity for subtle chemical differences that distinguish one foreign pathogen from another. Domestic animal immunology is seemingly important as we could find answers to the convoluted questions such as finding suitable antibiotic replacements, immunomodulants and vaccines with appropriate adjuvants. In this mini-review, I attempted to categorize aforementioned questions and to provide a direction towards our future of the animal science and biotechnology.

Research Biography

Professor Cheol-Heui Yun was educated at the Chonnam National University for BSc and the Seoul National University for his MSc in the area of Animal Nutrition. Professor Yun completed his PhD at the University of Saskatchewan, Canada in the area of immune modulation and mucosal immunology. Then, he pursued his professional career at leading research institutes in different region of the world including International Vaccine Institute (IVI, Seoul, Korea), United States Department of Agriculture (USDA, Greenbelt, MD, USA), National Institutes of Health (NIH, Bethesda, MD, USA) and Gothenburg University (Gothenburg, Sweden) where he undertook research related to stressors, vaccine, infection and host protective immunity. He published over 230 SCIE papers to date and has been the recipient of prestigious awards, including Seoul National University Excellence in Teaching Award (2018), Distinguished teaching award by Kukdam Foundation (2010), and official commendation for from the cabinet minister of the Ministry of Science and Technology, Korea (2007).

He was invited as a professor at Seoul National University since 2006, and currently serves as editors of a number of societies including World Journal of Immunology, Frontiers in Immunology (Molecular Innate Immunity Section, and Cytokines and Soluble Mediators in Immunity Section), Journal of Biomaterials and Tissue Engineering. He currently also serves as a co Editor-in-Chief of Asian-Australasian Journal of Animal Sciences. On the other hand, he is acting as an Ethics editor of the science editing, and Korean Journal of Women Health Nursing. Recently, he is actively involving in Ethical issues where he is acting as a Chair of Committee on Publication Ethics, Korean Council of Science Editors (KCSE) and Secretary General of Council of Asian Science Editors (CASE).

The Liver tissue: Another Endocrine Organ in Ruminant

Sanggun ROH

Associate Professor
Graduate School of Agricultural Science, Tohoku University

E-mail: sanggun.roh.a3@tohoku.ac.jp

The liver plays a crucial role in controlling the homeostasis as a secondary endocrine organ. In ruminant, the hepatic function is dramatically changed during weaning and parturition through the signaling of hormones and blood metabolites. The management systems, feed composition and feeding program affects much to increasing pathological processes of energy metabolism disorder in liver. The endocrine system and liver are codependent, and the either organs can lead to alter the quantity and quality of animal production. The presentation will give our data about the physiological roles of chemerin and ANGPTL8 as the hepatic hormones, and the regulatory factor of its production, to understand the hepato-endocrine interplay.

Research Biography

Sanggun Roh has worked in Seoul National University, Obihiro University, Monash University, Shinshu University, North Carolina State University from postgraduation. In 2009, he moved to Tohoku University Graduate School of Agricultural Science as an associate professor. He is now doing the international collaboration with overseas research groups in USA, Canada, German, South Korea, China, Thailand, French and German. He also served the adjunct associate professor in Seoul National University from 2017 to 2019. His research interest is to study the physiological and metabolic adaptations in three key tissues (adipose, mammary gland and liver) through the rumen in ruminant production.

Receptors in Spermatozoa – Their expressions and Functions

Yuki HIRADATE

Assistant professor
Graduate School of Agricultural Science, Tohoku University

E-mail: yuki.hiradate.d4@tohoku.ac.jp

It is known that various types of receptors that are thought to function during fertilization are expressed in spermatozoa. These receptors possibly crosstalk with ligands secreted from the female reproductive organs, but the mechanism remains unclear. Neurotensin (NTS), 13 amino acids of peptide hormone, is localized in uterine and oviduct epithelium. Interestingly, *Nts* mRNA expression is increased in ovulation, suggesting it possesses a specific role for fertilization. Further expressions of NTS receptors are confirmed in spermatozoa, suggesting it functions for fertilization. *In vitro* culture of spermatozoa with NTS facilitates acrosome exocytosis and protein tyrosine phosphorylation, both are involved in sperm capacitation. Receptors for NTS are also expressed in early embryos, and the addition of NTS results in an increase in blastocyst rate. These results indicate that NTS and its receptors contribute to fertilization and embryonic development.

Research Biography

Yuki Hiradate received his PhD degree on animal reproductive biology from Tohoku University Graduate School of Agricultural Science in 2012. After working as a post-doctoral fellow at the National Institute of Infectious Diseases from 2016, he has served Tohoku University Graduate School of Agricultural Science as an assistant professor since 2017. His research interest is mechanism for male gametogenesis and crosstalk between male and female gametes mediated by secretory factors during fertilization.

Genome-wide CRISPR/Cas9 Screen for Porcine Epidemic Diarrhea Virus Resistance in Pig Intestinal Epithelial Cells

Haifei WANG

Lecturer
College of Animal Science and Technology, Yangzhou University

E-mail: hyfiwang@yzu.edu.cn

Porcine epidemic diarrhea which is caused by porcine epidemic diarrhea virus (PEDV) resulted in large economic losses in the pig industry because of the high morbidity and mortality approaching 100% in neonatal piglets. Recently, PEDV is still identified as the main pathogen causing severe diarrhea in pig farms, highlighting the urgency to genetically improve the ability of pigs to resist PEDV infections. Genome-wide CRISPR screen has been proven to be an effective technology to identify functional genes resistant to viral infections. Here, we designed >90 thousand sgRNAs targeting porcine protein coding genes and cloned them into CRISPR knockout vectors. After transfection and puromycin resistance selection in IPEC-J2 cell, cell libraries with gene knockout were obtained. PEDV was added to the cell library and cultured for 14 days with consistent infection. Finally, survival cells were collected, amplified by PCR, and sequenced by high-throughput sequencing platform. The MAGeCK software was used to compare the enrichment differences of sgRNA between the survival cells and control cells. A subset of genes including ERN1, THEM19, KDM2B, and SULT2A1 with high potential resistance to PEDV infection was screened out. These findings will be helpful for identifying genes with resistance to PEDV infection and further contribute to genetic resistance breeding for porcine epidemic diarrhea.

Research Biography

Haifei Wang received his PhD degree on animal genetics and breeding from China Agriculture University in 2016. Since September of 2016, he has served College of Animal Science and Technology of Yangzhou University as a lecturer. His research interests focus on identifying genes and genetic markers involved in regulating porcine pathogen infections and clarifying the underlying molecular mechanisms.

Immune Evasion Mechanisms of Zoonotic Protozoan Parasite Toxoplasma Gondii in Mammalian Host

Hironori BANDO

Assistant Professor
Graduate School of Agricultural Science, Tohoku University
Department of Immunoparasitology, RIMD Osaka University

E-mail: hironori.bando.d4@tohoku.ac.jp

Toxoplasma gondii is a zoonotic pathogen that causes toxoplasmosis, a contagious disease affecting most mammals, including livestock, wild animals, and humans. Toxoplasmosis in livestock causes miscarriages or stillbirths, resulting in economic damage to farmers, and posing a challenge in animal husbandry. Moreover, cases of transmission to humans have recently shown an increasing trend annually. Thus T. gondii is now an important pathogen that requires immediate attention, both from animal and public health perspectives. However, there is still no vaccine or preventative medicine. Therefore, it is required to promote research on the development of novel treatment methods for toxoplasmosis and the elucidation of the host immune responses–parasite interactions in host cells. Interferon-γ (IFN-γ) is critical for anti-T. gondii responses in mammalian host, however, detailed mechanisms of host immune responses and parasite virulence mechanisms are still unclear. In this study, at first, we showed that IFN-γ induced tryptophan degradation by indole-2,3-dioxygenase (IDO1) plays an important role in the IFN-γ-induced anti-T. gondii response. Next, we focused on T. gondii virulence mechanisms and analyzed the role of TgGRA15 to suppress IFN-γ-dependent immunity. We generated GRA15 deficient T. gondii by Cas9/CRISPR system, and showed that IDO1-dependent anti-T. gondii response is inhibited TgGRA15-dependently. We also showed that T. gondii infection indirectly reduces IDO1 protein levels via iNOS expressed in hepatocytes stimulated with IL-1β that is produced from infected monocytes in a manner dependent on GRA15. Thus, we have demonstrated that iNOS in humans is a pro-Toxoplasma host factor that promotes the growth of the parasite.

Research Biography

Hironori Bando received his PhD degree on Animal and Food Hygiene from Obihiro University of Agriculture and Veterinary Medicine in 2013. He belonged to Research Institute for Microbial Diseases Osaka University from 2013 to 2019 as a specially appointed assistant professor. Since 2019, he has belonged to Tohoku University Graduate School of Agricultural Science as an assistant professor. His research interest is to study the immunology of host parasite relationships (Immunoparasitology).

Morphological Variation and Genetic Patterns of Bermudagrass along Longitudinal and Latitudinal Gradients

Xuebing YAN

Professor
College of Animal Science and Technology, Yangzhou University

E-mail: yxbbjzz@163.com

This complex environmental heterogeneity coupled with the long-standing history offers scenarios suitable for and favoring the evolution and existence of variation of morphological traits. Understanding the population genetic pattern and process of gene flow requires a detailed knowledge of how landscape characteristics structure populations. Geographic patterns in morphological variation and ploidy level of 570 *Cynodon dactylon* (L.) Pers. (common bermudagrass) individuals sampled from 28 geographic sites along a latitudinal and longitudinal gradient across China were observed. Genetic diversity and structure within these collections was estimated via expressed sequence tag-derived simple sequence repeat (EST-SSR). Considerable variations in morphological traits were observed at different longitudes and latitudes. Larger morphological sizes of *C. dactylon* appeared at the low and high-latitude regions, while the leaves of the erect shoot and the internode length enlarged significantly with the collection sites moving from east to west. Higher within-population genetic diversity appeared at low-latitude, as well as having positive correlation with temperature and precipitation. No isolation by distance and notable admixture structure existed among populations along latitudes, but low gene flow means a rich genetic differentiation among populations of *C. dactylon* along longitudinal gradient. The genetic diversity increased with the ploidy level of *C. dactylon* at different latitudes, suggesting polyploidy creates higher genetic diversity. Groups of individuals with the same ploidy at different longitudes were separated further away by genetic distance along with the increasing ploidy levels. The findings of this study are related to landscape population evolution, polyploidy speciation, preservation, and use of bermudagrass breeding.

Research Biography

Xuebing Yan received his PhD degree on grass science from China Agricultural University in 2005. He belonged to the Henan Agricultural University from 2007 to 2018 and had experience in the USDA Forage and Rangeland Research Lab, in the USA from 2013 to 2014. Since 2018, he has belonged to the College of Animal Science and Technology, Yangzhou University as a distinguished Professor. His research interest is to study the population genetic differentiation of grass and the forage processing and utilization.

Genomic Prediction in Japanese Black beef cattle: some Topics

Shinichiro OGAWA

Assistant Professor
Graduate School of Agricultural Science, Tohoku University

E-mail: shinichiro.ogawa.d5@tohoku.ac.jp

The Japanese Black is a representative of the beef cattle breeds constructing Wagyu in Japan. The beef produced are high quality, especially highly marbled (shimofuri), and they are also famous overseas. Carcass traits including the degree of marbling have been remarkably improved through use of a low number of elite sires with high predicted genetic abilities (breeding values) for meat quality. Breeding values are predicted by using a deep pedigree information and a large amount of carcass performance data of fattened progenies. Breeding values for growth performance and feed efficiency of young bulls and those for female reproductivity of cows have been also evaluated using pedigree information. Recently, prediction of breeding values by using genotype information on genome-wide high-density single nucleotide polymorphisms (SNPs) as DNA markers, also called genomic prediction, have been studied in Japanese Black cattle. Genomic prediction is believed to have a potential to achieve more efficient breeding in livestock species. In this presentation, some relevant topics for genomic prediction in Japanese Black cattle, as well as the possibility of genomic prediction and the future issues in this breed, will be discussed.

Research Biography

Shinichiro Ogawa received his Doctor of Agriculture from Kyoto University in 2017 (JSPS Research Fellowship for Young Scientists DC2, No.15J02417). Since 2017, he has served Graduate School of Agricultural Science, Tohoku University as an assistant professor. His current research interest is to explore an efficient breeding and selection scheme for Japanese Black cattle and pigs.

The Actions of Orphan Nuclear Receptor RORgamma on Hepatic Cholesterol Metabolism in Piglets

Demin CAI

Professor
College of Animal Science and Technology, Yangzhou University
Institute of Epigenetics and Epigenomics

E-mail: demincai@yzu.edu.cn

Time-restricted feeding (TRF) is a dieting strategy based on nutrients availability and diurnal rhythm, shown to improve lipid metabolism efficiency. Previous study reveals that retinoic acid-related (RAR) orphan receptor (ROR) γ is closely linked to animal lipid metabolism. However, the functional role of RORγ in liver physiology of pigs in response to TRF has not been determined, largely due to the lack of functional models and molecular tools. We established porcine liver organoids and subjected them to restricted nutrients supply for 10-h during the light portion of the day. Our results showed that TRF regimen did not alter hepatocyte physiology but downregulated the hepatic CHO biosynthesis program along with the reduced cellular CHO content in porcine liver organoids. Using unbiased bioinformatic analysis of a previous ChIP-seq data and ChIP-qPCR validation, we revealed RORγ as the predominant transcription factor that responded to TRF. This was likely through RORγ direct binding to the MVK gene (encoding mevalonate kinase) and recruiting the enrichment of co-factor p300, histone marks H3K27ac and H3K4me1/2, as well as RNA Polymerase II (Pol-II) at the locus of MVK. Our findings demonstrate that TRF triggers the RORγ-mediated chromatin remodeling at the locus of CHO biosynthesis genes in porcine liver organoids and further improves lipid metabolism.

Research Biography

Demin Cai received his PhD degree on animal physiology and biochemistry from Nanjing Agricultural University, College of Veterinary Medicine in 2015. He belonged to Department of Biochemistry and Molecular Medicine of University of California at Davis from 2014-2015 as a Joint-PhD student, from 2016-2019 as a post-doc fellow and from 2019-2020 as a faculty of assistant research scientist. Since 2020, he has served Yangzhou University College of Animal Science and Technology as a professor. His research interest is to study the actions of circadian rhythm, epigenetics, and orphan-nuclear-receptor-controlled molecular metabolism for improving pig growth and health.

Selection of Wakame Assimilative and Adhesive Lactobacilli and Their Genomic Characterization

Binghui ZHOU[1,2,3], Leonardo ALBARRACIN[4], Yuki MASUMIZU[1,2], Yuhki INDO[1,2], Mikado TOMOKIYO[1,2,3], Md Aminul ISLAM[1,2,3], Wakako IKEDA-OHTSUBO[1,2,3], Tomonori NOCHI[1,2,3], Hisashi ASO[1,2,3], Julio VILLENA[4] and Haruki KITAZAWA[1,2,3]

[1]Graduate School of Agricultural Science, Tohoku University
[2]CFAI
[3]C-to-C, [4]CERELA-CONICET

Synbiotics, the combination of prebiotics and probiotics, have been considered as potential candidates for antimicrobial substitutes in the livestock industry due to their positive effects on gastrointestinal disorders. Wakame, an edible seaweed, has prebiotic and immunomodulatory properties and therefore, wakame waste have been proposed to be used in feed formulation as a prebiotic in combination with immunomodulatory probiotics (immunobiotics). In order to select wakame assimilating immunobiotics for the development of symbiotic feeds, we isolated lactobacilli from the porcine intestine using a component adjusted wakame broth, and screened them in vitro for their immunomodulatory properties, adhesion capacities and wakame-assimilative abilities1). The immunomodulatory effect of lactobacilli was evaluated in porcine intestinal epithelial (PIE) cells after the activation of Toll-like receptor (TLR)-3 or TLR4. The adhesion of lactobacilli to porcine mucin and PIE cells were evaluated by Biacore and fluorescence assays, respectively. The pH, turbidity, viable bacterial count and sugar consumption were measured following incubation of Lactobacillus strains in the enzyme-treated wakame medium. A total of 116 lactobacilli strains were isolated, 8 of which were selected for further research because of their differential immunomodulatory abilities. The 8 strains showed different adhesion abilities to porcine mucin and PIE cells. No correlation between the immunomodulatory and adhesion capabilities were found. The sequencing of the complete genome of the 8 strains and the genomic analysis revealed that immunomodulation and adhesion depend on the combination of several cell-surface bacterial factors acting simultaneously on the intestinal cells of the porcine host2,3). All lactobacilli were able to utilize saccharides in enzyme-treated wakame. Wakame improved the survival of lactobacilli in simulated gastric conditions. The synergistic combination of the immunomodulatory effects of wakame and lactobacilli selected in this work could be used as a highly efficient functional feed to improve immune health status in pigs.

1) Masumizu, Zhou et al., Microorganisms (2019).
2) Zhou, Albarracin et al., Microbiol. Res. Announc. (2020).
3) Zhou, Albarracin et al., Microorganisms (2020).

Short-chain Fatty Acids Regulate the Immune Responses via G Protein Coupled Receptor 41 in Bovine Rumen Epithelial Cells

Tianyu YANG[1,2], Kang ZHAN[1,2], Xiaoxiao GONG[1,2], Yinyin CHEN[1,2], Maocheng JIANG[1,2] and Guoqi ZHAO[1,2]

[1]Institute of Animal Culture Collection and Application
[2]College of Animal Science and Technology, Yangzhou University

The rumen immune system often suffers when challenging antigens from lysis of dead microbiota cells in the rumen. However, the rumen epithelium innate immune system can actively respond to the infection. Previous studies have demonstrated G protein-coupled receptors 41 (GPR41) as receptors for short chain fatty acids (SCFAs) in human. We hypothesized that SCFAs, the most abundant microbial metabolites in rumen, may regulate the immune responses by GPR41 in bovine rumen epithelial cells (BRECs). Therefore, the objective of study was to firstly establish an immortal BRECs line and investigate the regulatory effects of SCFAs and GPR41 on innate immunity responses in BRECs. These results showed that long-term BRECs cultures were established by SV40T-induced immortalization. The concentrations of 20 mM SCFAs significantly enhanced the levels of GPR41, IL1β, TNFα, chemokines, and immune barrier genes by transcriptome analysis. Consistent with transcriptome results, the expression of GPR41, IL1β, TNFα, and chemokines were markedly upregulated in BRECs treated with 20 mM SCFAs by qRT-PCR compared with control BRECs. Remarkably, the GPR41 knockdown (GPR41KD) BRECs treated with 20 mM SCFAs significantly enhanced the proinflammatory cytokines IL1β and TNFα expression compared with wild type BRECs treated with 20 mM SCFAs, but reduced the expression of CCL20, CXCL2, CXCL3, CXCL5, CXCL8, CXCL14, Occludin, and ZO-1. Moreover, GPR41 mRNA expression is positively correlated with CCL20, CXCL2, CXCL3, CXCL8, CXCL14, and ZO-1. These findings revealed that SCFAs regulate GPR41-mediated levels of genes involved in immune cell recruitment and epithelial immune barrier and thereby mediate protective innate immunity in BRECs.

The Effects of Heat Stress on the Immune Function and Morphological Structure of Avian Gut-associated Lymphoid Tissues

Ryota HIRAKAWA[1,2], Motoi KIKUSATO[1,2], Siti NURJANAH[1,2], Kyohei FURUKAWA[1,2], Mutsumi FURUKAWA[1,2], Katsuki USAMI[1,2], Kan SATO[1,2], Masaaki TOYOMIZU[1,2] and Tomonori NOCHI[1,2]

[1]International Education and Research Center for Food and Agricultural Immunology
[2]Graduate School of Agricultural Science, Tohoku University

High ambient temperature increases susceptibility to infections caused by enteric pathogens, such as Salmonella spp. in broiler chickens. Among numerous factors involved in immune activation and regulation, secretory immunoglobulin (Ig), especially IgA, has been known to play a key role in the gastrointestinal tract in protecting the tissues from pathogen-medicated functional disorder. However, knowledge of the effect of heat stress (HS) on the gastrointestinal Ig production in chickens remains obscures. This study investigated the immune function and morphological structure of cecal tonsils and cecal patches, both of which are composed of lymphoid follicles, in broiler chickens reared under either thermoneutral (24.5°C) or HS conditions (34.5°C). Our results revealed that both cecal tonsils and cecal patches displayed severe depression of Bu1+ B cells and CD3+ T cells including CD4+ T cells and CD8+ T cells in the HS conditions. HS also caused a hypoplasia of germinal centers in cecal tonsils, wherein mature B cells differentiate into plasmablasts undergoing Ig class-switching. In contrast, such morphological structure necessary for B cell differentiation was maintained adequately in the cecal patches even under the HS conditions. Due to the resistance of cecal patches to HS, the production of IgM, IgA and IgY in the gastrointestinal tract of heat-stressed chickens was sufficiently sustained at same level as those of thermoneutral chickens. These findings suggested that the breeding and/or feeding strategy to preserve the immune function and morphological structure of cecal patches may be useful to maintain the health of chickens in the HS conditions.

Identification of Predictor Genes of Feed Efficiency in Beef Cattle by Applying Machine Learning (ML) Methods to Multi-tissue Transcriptome Data

Weihao CHEN[1,2], Antonio REVERTER[2], Yutao LI[2] and Wei SUN[1]

[1]College of Animal Science and Technology, Yangzhou University
[2]CSIRO Agriculture & Food, Queensland Bioscience Precinct

Machine learning (ML) methods have shown promising results in identifying candidate genes when applied to large transcriptome datasets. However, no attempt has been made to compare the performance of combining different ML methods together in the prediction of high and low feed efficiency (HFE and LFE) animals. In this study, using RNA-seq data of five tissues from 18 Nellore bulls, we evaluated the prediction accuracies of five analytical methods in classifying animals according to their feed efficiency potential. Of five methods, the two-step ML method combining RF and XGBoost (RX), identified the smallest subsets of potential predictor genes across all tissues with the highest classification accuracy for 9 HFE and 9 LFE animals. Besides, genes identified by the RX, there was a correlation between the gene's prediction ranking ("Gain" values) and its relevance to the networks ("Betweenness"), reflecting a key biological role to the phenotype. When comparing co-expression gene network differences between LFE and HFE groups from the RX, the number of connections between genes with maximum expression in skeletal muscle represented the biggest change between HFE and LFE networks. This indicates more FE related pathways activated in HFE. The results demonstrate a great potential for applying a combination of ML methods to large transcriptome datasets to identify biologically important genes for accurately classifying FE samples.

Identification of the Mechanism Responsible for Maternal IgA Secretion That Depends on the Gut Microbial Stimulation in Peyer's Patches

Katsuki USAMI[1,2], Kanae NIIMI[1,2], Mutsumi FURUKAWA[1,2], Saeka UCHINO[1,2], Kouichi WATANABE[1,2], Hisashi ASO[1,2] and Tomonori NOCHI[1,2]

[1]International Education and Research Center for Food and Agricultural Immunology
[2]Graduate School of Agriculture Science, Tohoku University

Improving breastfeeding quality increases mammalian health across generations. Although the interorgan network among distinct tissues has been implicated in maintaining essential behaviors, including breastfeeding, most details remain unknown. We discuss the essential role of Peyer's patches (PPs), a secondary lymphoid tissue in the small intestine, in breastfeeding. Specifically, PPs constitute an important source of plasma cells, recruited from the mammary glands, to produce maternal IgA, which is transferred from the mother to the offspring through breastfeeding. A more significant advance in this study was that limited intestinal microorganisms belonging to Bacteroidales were identified as essential bacteria in the gastrointestinal tract for stimulating the immune functions in PPs to produce maternal IgA in milk. Our results provide significant insights into the development of novel strategies for transferring sufficient amounts of maternal IgA to the next generation via breastfeeding.

Establishment of Evaluation System of Porcine Intestinal Barrier Integrity and Preliminary Screening of Candidate lncRNA Related to Intestinal Barrier

Weiyun QIN, Haifei WANG, Zhengchang WU, Shenglong WU and Wenbin BAO

College of Animal Science and Technology, Yangzhou University

Intestinal barrier damage is one of the important factors in leading to diarrhea and intestinal inflammation in piglets. In order to screen candidate lncRNAs involved in PEDV infection through intestinal barrier, a total of 43 Duroc×Landrace×Yorkshire 8-day-old ternary crossbred pigs were used in this study, which included 28 diarrhea piglets and 15 normal piglets. Firstly, the pathogeny was identified by RT-PCR, and then the intestinal barrier function was evaluated by D-lactic acid- and DAO ELISA, HE and AB-PAS stainning, scanning electron microscopy and transmission electron microscopy observation. Based on this, lncRNA-seq was performed using the confirmed phenotype of diarrhea piglets. 112 differentially expression lncRNAs were identified and we locked XR_002344446.1. lncRNA expression increased in intestinal mucosa after PEDV infection and mainly localized in the cytoplasm by FISH assays. Functionally, knowdown of lncRNA using siRNA in IPEC-J2 cells significantly increased their susceptibility to PEDV infection, Furthermore, we observed that lncRNA interference decreased the intestinal mucosal permeability and destroyed the connection of tight junction. Mechanistically, by using biotinylated-lncRNA probe to perform RNA pull-down assay in IPEC-J2 cells, we identified cytoskeletal proteins was abundantly pulled down by lncRNA in IPEC-J2 cells. In conclusion, we speculated lncRNA regulates tight jucntion through binding cytoskeletal proteins, the connection between lncRNA and cytoskeleton proteins was broken when PEDV enters the intestine, resulted in the disruption of the tight junctional distribution of ZO1 to the intracellular localization.

Evaluation of Testicular Toxicity By Sperm Epigenetic Status

Kazuya SAKAI, Kenshiro HARA and Kentaro TANEMURA

Graduate School of Agricultural Science, Tohoku University

Testicular toxicity is a frequent adverse effect of the surrounding environment such as temperature, radiation, and environmental chemicals. However, there is no effective biomarker to detect testicular toxicity noninvasively. To find new biomarkers, we focused on epigenetic factors in the male germline. In this study, we investigated changes to sperm DNA methylation and sperm RNA profiles in mouse models of testicular toxicity induced by doxorubicin (DXR). We established mouse models of early-stage testicular toxicity and testicular pre-toxicity by the administration of 0.2 mg/kg and 0.02 mg/kg DXR, respectively, twice weekly for 5 weeks. Histological analysis showed sparse abnormalities in testicular tissue; however, western blotting analysis revealed reduced testicular expression levels of DNA methyltransferases Dnmt3a and Dnmt3b in both DXR-treated groups. Interestingly, comprehensive sperm DNA methylation analysis using Methyl-CpG binding domain protein-enriched genome sequencing (MBD-seq) revealed that hypomethylation was the most frequent change induced by DXR. Moreover, in sperm RNA-seq analysis, we found that some differences in RNA contents between DXR-treated and untreated groups. These findings suggest that sperm epigenetic factors may be used as an early diagnostic marker for testicular changes not detected by conventional toxicity analysis.

Genetic Diversity and Population Structure of Bermudagrass [*Cynodon dactylon* (L.) Pers.] along Latitudinal Gradients and the Relationship with Polyploidy Level

Jingxue ZHANG[1,2], Miaoli WANG[1], Zhipeng GUO[1], Yongzhuo GUAN[1], Jianyu LIU[1], Xuebing YAN[2] and Yuxia GUO[1]

[1]College of Animal and Veterinary Science, Henan Agricultural University
[2]College of Animal Science and Technology, Yangzhou University

Understanding the population genetic pattern and process of gene flow requires a detailed knowledge of how landscape characteristics structure populations. Although *Cynodon dactylon* (L.) Pers. (common bermudagrass) is widely distributed in the world, information on its genetic pattern and population structure along latitudinal gradients is limited. Genetic diversity among different ploidy levels was also compared in the study. The material used consisted of 296 C. *dactylon* individuals sampled from 16 geographic sites from 22◦350 N to 36◦180 N. Genetic diversity was estimated using 153 expressed sequence tag-derived simple sequence repeat (EST-SSR) loci. Higher within-population genetic diversity appeared at low-latitude, as well as having positive correlation with temperature and precipitation. The genetic diversity increased with the ploidy level of C. *dactylon*, suggesting polyploidy creates higher genetic diversity. No isolation by distance and notable admixture structure existed among populations along latitudes. Both seed dispersal (or vegetative organs) and extrinsic pollen played important roles for gene flow in shaping the spatial admixture population structure of C. *dactylon* along latitudes. In addition, populations were separated into three clusters according to ploidy levels. C. *dactylon* has many such biological characters of perennial growth, wind-pollination, polyploidy, low genetic differentiation among populations, sexual and asexual reproduction leading to higher genetic diversity, which gives it strong adaptability with its genetic patterns being very complex across all the sampled latitudes. The findings of this study are related to landscape population evolution, polyploidy speciation, preservation, and use of bermudagrass breeding.

List of scientific papers in 2020 published by field science group in Graduate School of Agricultural Science, Tohoku University

The Forest-Andisols Group

Eguchi, K., E. Oguri, T. Sasaki, A. Matsuo, D. D. Nguyen, W. Jaitrong, B. E. Yahya, Z. Chen, R. Satria, W. Y. Wang and Y. Suyama (2020) Revisiting museum collections in the genomic era: potential of MIG-seq for retrieving phylogenetic information from aged minute dry specimens of ants (Hymenoptera: Formicidae) and other small organisms. Myrmecological News, 30: 151-159.

Fukasawa, Y. (2020) How does wood-inhabiting fungal community affect forest recovery after deforestation events in subalpine coniferous forest? Journal of Integrated Field Science, 17: 12-14.

Fukasawa, Y. and K. Kaga (2020) Effects of wood resource size and decomposition on hyphal outgrowth of a cord-forming basidiomycete, *Phanerochaete velutina*. Scientific Reports, 10: 21936.

Fukasawa, Y., E. C. Gilmartin, M. Savoury and L. Boddy (2020) Inoculum volume effects on competitive outcome and wood decay rate of brown- and white-rot basidiomycetes. Fungal Ecology, 45: 100938.

Fukasawa, Y., M. Savoury and L. Boddy (2020) Ecological memory and relocation decisions in fungal mycelial networks: responses to quantity and location of new resources. The ISME Journal, 14: 380-388.

Fukasawa, Y., Y. Ando, S. N. Suzuki, M. Aizawa and D. Sakuma (2020) Climate influences the effect of fungal decay type on regeneration of Picea jezoensis var. hondoensis seedlings on decaying logs. Canadian Journal of Forest Research, 50: 73-79.

Hamabata, T., A. Matsuo, M. P. Sato, S. Kondo, K. Kameda, I. Kawazu, T. Fukuoka, K. Sato, Y. Suyama and M. Kawata (2020) Natal origin identification of green turtles in the North Pacific by genome-wide population analysis with limited DNA samples. Frontiers in Marine Science, 7: 658.

Hirano, T., T. Saito, Y. Tsunamoto, J. Koseki, L. Prozorova, D. V. Tu, K. Matsuoka, K. Nakai, Y. Suyama and S. Chiba (2019) Role of ancient lakes in genetic and phenotypic diversification of freshwater snails. Molecular Ecology, 28(23): 5032-5051.

Kitomi, Y., E. Hanzawa, N. Kuya, H. Inoue, N. Hara, S. Kawai, N. Kanno, M. Endo, K. Sugimoto, T. Yamazaki, S. Sakamoto, N. Sentoku, J. Wu, H. Kanno, N. Mitsuda, K. Toriyama, T. Sato and Y. Uga (2020) Root angle modifications by theDRO1homolog improve rice yields in saline paddy fields. Proceedings of the National Academy of Sciences, 117(35): 21242-21250.

Nagasawa, K., H. Setoguchi, M. Maki, H. Goto, K. Fukushima, Y. Isagi, Y. Suyama, A. Matsuo, Y. Tsunamoto, K. Sawa and S. Sakaguchi (2020) Genetic consequences of plant edaphic specialization to solfatara fields; phylogenetic and population genetic analysis of *Carex angustisquama* (Cyperaceae). Molecular Ecology, 29(17): 3234-3247.

Nakajima, S., S. K. Hirota, A. Matsuo, Y. Suyama and F. Nakamura (2020) Genetic structure and population demography of white-spotted charr in the upstream watershed of a large dam. Water, 12: 2406.

Nanzyo, M., H. Hirai, K. Ito and H. Kanno (2020) Variations between weathered pumice particles of the Nantai-Shichihonzakura and Nantai-Imaichi tephra from polished sections. Soil Science and Plant Nutrition, 66(5): 693-701.

Nguyen, D. D., E. Oguri, A. Yamada, C. C. Lin, C. Zhilin, A. D. Nguyen, Y. Suyama and K. Eguchi (2020) Genome-wide MIG-seq and morphometric data reveals heterospecificity of the *Gnamptogenys taivanensis* group (Hymenoptera: Formicidae: Ectatomminae) in the northern mountainous region of Vietnam. Raffles Bulletin of Zoology, 68: 539-555.

Nochi, T., S. Suzuki, S. Ito, S. Morita, M. Furukawa, D. Fuchimoto, Y. Sasahara, K. Usami, K. Niimi, O. Itano, M. Kitago, S. Matsuda, A. Matsuo, Y. Suyama, Y. Sakai, G. Wu, F. W. Bazer, K. Watanabe, A. Onishi and H. Aso (2020) Elucidation of the effects of a current X-SCID therapy on intestinal lymphoid organogenesis using an in vivo animal model. Cellular and Molecular Gastroenterology and Hepatology, 10(1): 83-100.

Onosato, K., T. Shitara, A. Matsumoto, A. Matsuo, Y. Suyama and Y. Tsumura (2020) Contact zone of two different chloroplast lineages and genetic guidelines for seed transfer in *Quercus serrata* and *Q. crispula*. Plant Species Biology, 36(1): 72-83.

Prasetyo, E., Widiyatno, S. Indrioko, M. Na'iem, T. Matsui, A. Matsuo, Y. Suyama and Y. Tsumura (2020) Genetic diversity and the origin of commercial plantation of Indonesian teak on Java Island. Tree Genetics & Genomes, 16: 34.

Sakaguchi, S., K. Nagasawa, Y. Umetsu, J. Nagasawa, S. Ichikawa, S. Kinoshita, K. Hiratsuka, Y. Suyama, Y. Tsunamoto, Y. Isagi and H. Setoguchi (2020)

Phylogenetic origin of *Magnolia pseudokobus* (Magnoliaceae), a rare *Magnolia* extinct in the wild, revealed by chloroplast genome sequencing, genome-wide SNP genotyping and microsatellite analysis. Journal of Forest Research, 25(5): 322-328.

Seiwa, K., Y. Negishi, Y. Eto, M. Hishita, K. Masaka, Y. Fukasawa, K. Matsukura and M. Suzuki (2020) Successful seedling establishment of arbuscular mycorrhiza- compared to ectomycorrhizal-associated hardwoods in arbuscular cedar plantations. Forest Ecology and Management, 468: 118155.

Strijk, J. S., H. T. Binh, N. V. Ngoc, J. T. Pereira, J. W. Ferry Slik, R. S. Sukri, Y. Suyama, S. Tagane, J. J. Wieringa, T. Yahara and D. D. Hinsinger (2020) Museomics for reconstructing historical floristic exchanges: Divergence of Stone Oaks across Wallacea. PLoS ONE 15(5): e0232936.

Suddee, S., S. Tagane, P. Souladeth, D. Kongxaysavath, S. Rueangreua, Y. Suyama and E. Suzuki (2020) *Coleus bolavenensis* (Lamiaceae), a new species from Laos. Thai Forest Bulletin (Botany), 48(1): 82-85.

Tagane, S., P. Souladeth, A. Nagahama, Y. Suyama, N. Ishii, N. Tanaka and T. Yahara (2020) Twenty-five new species records in the flora of Laos. National History Bulletin of Siam Society, 64(1): 25-41.

Takano, A., S. Sakaguchi, P. Li, A. Matsuo, Y. Suyama, G. H. Xia, X. Liu and Y. Isagi (2020) A narrow endemic or a species showing disjunct distribution? Studies on *Meehania montis-koyae* Ohwi (Lamiaceae). Plants, 9: 1159.

Tomimatsu, H., A. Matsuo, Y. Kaneko, E. Kudo, R. Taniguchi, T. Saitoh, Y. Suyama and A. Makita (2020) Spatial genet dynamics of a dwarf bamboo: Clonal expansion into shaded forest understory contributes to regeneration after an episodic die-off. Plant Species Biology, 35(3): 185-196.

Wulantuya, K. Masaka, Bayandala, Y. Fukasawa, K. Matsukura and K. Seiwa (2020) Gap creation alters the mode of conspecific distance-dependent seedling establishment via changes in the relative influence of pathogens and mycorrhizae. Oecologia, 192: 449-462.

Yonemichi, T., T. Tsukagoshi, T. Karukome, Y. Hisamoto, Y. Ohmori, C. Lian, M. P. Sato, T. Sasaki, A. Matsuo, Y. Suyama and S. Goto (2020) Salt tolerance seedlings derived from putative hybrid pines (*Pinus* × *densi-thunbergii*): possibility of candidate materials for coastal forest restoration. Journal of Japanese Forestry Society, 102(2): 101-107 (in Japanese with English summary).

Zhang, M., T. Yahara, S. Tagane, S. Rueangruea, S. Suddee, E. Moritsuka and Y. Suyama (2020) *Cryptocarya kaengkrachanensis*, a new species of Lauraceae from Kaeng Krachan National Park, southwest Thailand. PhytoKeys, 140: 139-157.

The Ruminant Production Group

Adeyemi, O. S., A. O. Eseola, W. Plass, O. Atolani, T. Sugi, Y. Han, G. E. Batiha, K. Kato, O. J. Awakan, T. D. Olaolu, C. O. Nwonuma, O. Alejolowo, A. Owolabi, D. Rotimi and O. T. Kayode (2020) Imidazole derivatives as antiparasitic agents and use of molecular modeling to investigate the structure-activity relationship. Parasitology research, 119(6): 1925-1941.

Bando, H., Y. Fukuda, M. Yamamoto and K. Kato (2020) *Toxoplasma gondii* effectors TgIST and TgGRA15 differentially target host IDO1 to antagonize the IFN-γ-induced anti-*T. gondii* response in human cells. *Journal of Integrated Field Science*, 17: 37-37.

Fisch, D., B. Clough, M. C. Domart, H. Bando, A. P. Snijders, L. M. Collinson, M. Yamamoto, A. R. Shenoy and E. M. Frickel (2020) Human GBP1 differentially targets *Salmonella* and *Toxoplasma* to drive recognition of microbial ligands and caspase-mediated death. *Cell Reports,* 32(6): 108008.

Fukasawa, M. (2020) Establishing good relationships between human and cattle. Stockmanship for better management. Handout for chikusan kankei shin-gijutu happyoukai, 29-32 (in Japanese).

Fukasawa, M. (2020) Management based on good human-animal relationships. Animal Industry, 782: 11-15 (in Japanese).

Fukasawa, M. and T. Kosako (2020) Stockmanship for better management. Japanese Journal of Grassland Science, 65(4): 263-266 (in Japanese).

Fukasawa, M., T. Komatsu and Y. Higashiyama (2020) The influence of concentrate-rich diet on daily sleep and lying postures of Japanese black cows. Animal Science Journal, 91: e13356. DOI: 10.1111/asj.13356

Han, Y., O. S. Adeyemi, M. H. B. Kabir and K. Kato (2020) Screening of compound libraries for inhibitors of *Toxoplasma* growth and invasion. Parasitology research, 119(5): 1675-1681.

Kabir, M. H. B., M. Itoh, A. A. Shehata, H. Bando, Y. Fukuda, F. Murakoshi, A. Fujikura, H. Okawa, T. Endo, A. Goto, M. Kachi, T. Nakayama, Y. Kano, S. Oishi, K. Otomaru, M. I. Essa, K. Kazama, X. Xuan and K. Kato (2020) Distribution of *Cryptosporidium* species isolated from diarrhoeic calves in Japan. Parasitology International, 78: 102153.

Kabir, M. H. B., O. Ceylan, C. Ceylan, A. A. Shehata,

H. Bando, M. I. Essa, X. Xuan, F. Sevinc and K. Kato (2020) Molecular detection of genotypes and subtypes of *Cryptosporidium* infection in diarrheic calves, lambs, and goat kids from Turkey. Parasitology International, 79: 102163.

Kabir, M. H. B., Y. Han, S-H. Lee, A. B. Nugrahaa, F. C. Recuenco, F. Murakoshi, X. Xua and K. Kato (2020) Prevalence and molecular characterization of *Cryptosporidium* species in poultry in Bangladesh. One Health, 9: 100122.

Kakihara, H. and S. Ogura (2020) Effect of livestock dung on pasture soil acidification and grass growth. 2019 Annual Report of the Project of Integrated Compost Science (PICS), pp. 38-41 (in Japanese).

Komissarov, M. and S. Ogura (2020) Siltation and radiocesium pollution of small lakes in different catchment types far from the Fukushima Daiichi nuclear power plant accident site. International Soil and Water Conservation Research, 8(1): 56-65.

Kondoh, D., J. Tomiyasu, R. Itakura, M. Sugahara, M. Yanagawa, K.Watanabe, P. A. Alviola, S. A. Yap, E. A. Cosico, F. A. Cruz, A. R. Larona, A. J. F. Manalad, J. S. Masangkay, Y. Sugiura, S. Kyuwa, S. Watanabe, Y. Une, T. Omatsu, H. Bando and K. Kato (2020) Comparative histological studies on properties of polysaccharides secreted by vomeronasal glands of eight Laurasiatheria species. Acta Histochem, 122(3): 151515.

Miyata, M., R. C. Robinson, T. Q. P. Uyeda, Y. Fukumori, S. I. Fukushima, S. Haruta, M. Homma, K. Inaba, M. Ito, C. Kaito, K. Kato, T. Kenri, Y. Kinosita, S. Kojima, T. Minamino, H. Mori, S. Nakamura, D. Nakane, K. Nakayama, M. Nishiyama, S. Shibata, K. Shimabukuro, M. Tamakoshi, A. Taoka, Y. Tashiro, I. Tulum, H. Wada and K. I. Wakabayashi (2020) Tree of Motility – History of Motility Systems in the Tree of Life –. Genes to Cells, 25: 6-21.

Murakoshi, F., H. Bando, T. Sugi, O. S. Adeyemi, M. Nonaka, T. Nakaya and K. Kato (2020) Nullscript inhibits *Cryptosporidium* and *Toxoplasma* growth. International journal for parasitology. Drugs and drug resistance, 14: 159-166.

Murakoshi, F., T. Nakaya and K. Kato (2020) Detection and Epidemiological Analysis of Symbiotic Viruses from Protozoa. Journal of integrated field science, 17: 15-17.

Ogura, S. (2020) Preface – trend and challenge of weed control in pasture and forage crop fields. Japanese Journal of Grassland Science, 66(1): 21-22 (in Japanese).

Ogura, S., Y. Maekawa and S. Tanaka (2020) Effects of grazing experience in a wasted mulberry plantation on foraging manner of mulberry leaves by cow. Japanese Journal of Grassland Science, 65(4): 231-235 (in Japanese with English synopsis).

Saito, H., Y. Murata, M. Nonaka and K. Kato (2020) Screening of a library of traditional Chinese medicines to identify compounds and extracts which inhibit *Toxoplasma gondii* growth. Journal of Veterinary medical Science, 82: 184-187.

Sakaguchi, N., M. Sasai, H. Bando (Equal-contribution), Y. Lee, A. Pradipta, J. S. Ma and M. Yamamoto (2020) Role of Gate-16 and Gabarap in Prevention of Caspase-11-Dependent Excess Inflammation and Lethal Endotoxic Shock. *Frontiers in Immunology,* 11: 561948.

Shehata, A. A., H. Bando, Y. Fukuda, M. H. B. Kabir, F. Murakoshi, M. Itoh, A. Fujikura, H. Okawa, T. Endo, A. Goto, M. Kachi, T. Nakayama, Y. Kano, S. Oishi, K. Otomaru, K. Kazama, M. I. Essa and K. Kato (2020) Development of a highly sensitive method for the detection of Cryptosporidium parvum virus type 1 (CSpV1). Japanese Journal of Veterinary Research, 68(3): 159-170.

Takaki, Y., Y. Takami, T. Watanabe, T. Nakaya and F. Murakoshi (2020) Molecular identification of *Cryptosporidium* isolates from ill exotic pet animals in Japan including a new subtype in *Cryptosporidium fayeri*. Veterinary Parasitology: Regional Studies and Reports, 21: 100430.

The Rice Production Group

Asami, H., Y. Miura, T. Watanabe, T. Uno, R. Tajima, M. Saito and T. Ito (2020) Silicate fertilizer improves grain quality in brewers' rice (Oryza sativa L.) 'Toyonishiki'. Japanese Journal of soil science plant nutrition, 91(1): 11-20 (in Japanese).

Kaneda, Y., M. Nishida, F. Takakai and T. Sato (2020) New growth diagnosis standards of high-yielding rice and demonstration of high yielding using NDVI by GreenSeeker handheld crop sensor. Japanese Journal of soil science plant nutrition, 91(6): 417-425 (in Japanese).

Moritsuka, N., H. Saito, R. Tajima, Y. Takahashi and H. Hirai (2020) Farm-Scale Estimation of Total Nitrogen Content in Surface Paddy Soils by Extraction with Commercially Available Hydrogen Peroxide. Agronomy, 10: 40.

Obara, K., K. Homma, R. Tajima, M. Maki, Y. Saito, N. Hashimoto, S. Yamamoto and C. Hongo (2020) Analysis of RGB Images to Estimate SPAD Values in Rice for UAV Remote Sensing. Japanese Journal of Crop Science, 89(1): 50-51 (in Japanese).

Takahashi, T., K. Nakano, R. Nira, E. Kumagai, M. Nishida and M. Namikawa (2020) Conversion of soil particle size distribution and texture classification from ISSS system to FAO/USDA system in Japanese paddy soils. Soil Science and Plant Nutrition, 66(3): 407-414.

Takamoto, A., T. Takahashi, K. Togami, M. Nishida, K. Tsuchiya and M. Namikawa (2020) Estimation of nitrogen uptake at the mature stage of rice from the dry yield of rough rice. Japanese Journal of soil science plant nutrition, 91(1): 1-10 (in Japanese).

Marine Bio-Production Group

Fujii, T., D. J. Pondella, V. L. G. Todd and A. Guerin (2020) Editorial: Seafloor heterogeneity: artificial structures and marine ecosystem dynamics. Frontiers in Marine Science, 7: 378. DOI: 10.3389/fmars.2020.00378

Yamada, Y., Y. Ujibe, T. Shimizu, Y. Sato, Y. Tashiro, J. Sato, M. Ikeda and M. Takagi (2020) Genetic disturbance of the endangered Japanese aucha perch, *Coreoperca kawamebari,* in Tokushima Prefecture. Japanese Journal of Conservation Ecology, 25: 9-23 (in Japanese with English abstract).

Yamazaki, D., O. Miura, S. Uchida, M. Ikeda and S. Chiba (2020) Comparative seascape genetics of co-distributed Monodonta spp. intertidal snails in the Japanese and Ryukyu archipelagoes. Marine Ecology Progress Series, 657: 135-146 (in press).

Zhou, J., D. Kitazawa, T. Yoshida., T. Fujii, J. Zhang, S. Dong and Q. Li (2020) Numerical simulation of dissolved aquaculture waste transport based on water circulation around shellfish and salmon farm sites in Onagawa Bay, Northeast Japan. Journal of Marine Science and Technology. DOI: 10.1007/s00773-020-00773-y

Integrated Field Control Group

Ikeyama N. and T. Sumita (2020) Evaluation of Direct Seeding in Rice Cultivation in Shonai District of Yamagata Prefecture. Journal of rural society and economics, 38(2): 10-18 (in Japanese).

Inoue, S., A. Ito and C. Yonezawa (2020) Mapping Paddy Fields in Japan by Using a Sentinel-1 SAR Time Series Supplemented by Sentinel-2 Images on Google Earth Engine. Remote Sensing, 12(10): 1622-1622.

Inoue, S., A. Ito and C. Yonezawa (2020) PADDY FIELD MAPPING IN EASTERN PART OF ASIA USING SENTINEL-1 AND SENTINEL-2. Proceedings of 2020 IEEE International Geoscience and Remote Sensing Symposium, pp.5171-5174.

Kuwabara, Y., A. Miura, T. Sumita and Y. Fujii (2020) Commitment and Network Formation of Successors in Community-based Farming : A Case Study of Agricultural Newcomers in Yamagata Prefecture. Journal of rural planning, 39(Special issue): 232-237 (in Japanese).

Murata, H., M. Hara, Y. Yamazaki, M. Saito and C. Yonezawa (2020) Drone-based monitoring of the current situation of oyster aquaculture area in Nagatsura-ura Lagoon, Miyagi Prefecture for grasping environmental carrying capacity. Proceedings of the 69th Autumn meeting of the remote sensing society of Japan, pp.135-136 (in Japanese with English abstract).

Narisawa, T. and C. Yonezawa (2020) Temporal changes of a meadow at Noyuki area in Katsurao village, Fukushima prefecture after the Great East Japan Earthquake. Journal of the Japanese Agricultural Systems Society, 36(3): 39-48 (in Japanese with English abstract).

Saito, M., C. Yonezawa, T. Matsunami, T. Kanno and H. Uchino (2020) Evaluation of drainage methods on corn fields using remote sensing data. Proceedings of the 2020 Autumn conference of the Japanese Agricultural Systems Society, pp.11-12 (in Japanese).

Seleky, R. N., W. Ozawa and T. Sumita (2020) Characteristics of Farms with Successors: A Case Study of Margomuyo Village, Sleman District, Yogyakarta Province, Indonesia. Journal of rural Society and Economics, 38(2): 115-126.

Sugawara Y. and T. Sumita (2020) Tourist's Evaluation of Agriculture and a Rural Area, and its Related Issues : An Analysis of Attitude of Tourists, Journal of rural society and economics, 38(2): 19-27 (in Japanese).

Sugawara, Y. and T. Sumita (2020) Travel Agent's Evaluation of Agriculture and a Rural Area, and its Related Issues—An Analysis of Attitude of Travel Agent's Employees － Journal of rural society and economics, 37(2): 1-9.

Suzuki H., T. Sumita and Y. Kato (2020) Measures to Secure Human Resources for Management Succession in Farming Corporations : A Case analysis of securing successors to smooth generational change. Journal of rural society and economics, 38(2): 78-86 (in Japanese).

Yamamoto, K., K. Ueda, T. Sumita, Y. Fujii and S. Matsushita (2020) The Effects of Commuter Farming on Making Decision of Spouses at Separation Farmer : A Case Study in the Citrus Production Island Area.

Journal of rural society and economics, 38(2): 58-67 (in Japanese).
Yonezawa, C. and M. Watanabe (2020) DETECTION OF FLOODING AGRICULTURAL FIELD BY TYPHOON HAGIBIS ON 2019 USING SAR IMAGERY. Proceedings of 2020 IEEE International Geoscience and Remote Sensing Symposium, pp.6863-6866.

Guidelines for Authors

The Journal of Integrated Field Science (JIFS) publishes articles in all areas of field science in agricultural science. The JIFS is an English journal launched in 2003 when the Integrative Field Science Center, Graduate School of Agricultural Science, Tohoku University, was officially established. The JIFS places the edit committee under which original scientific articles, including short papers, proceedings, reviews, descriptions, and data are published. An original manuscript will be peer-reviewed by two referees. In principle, our journal publishes one volume per year, and it began publication as an online journal from volume 15 (JIFS: http://www.agri.tohoku.ac.jp/jp/about/field/jifs/index.html). We will also publish all the manuscripts on the website as an e-journal (Tohoku University Repository: https://tohoku.repo.nii.ac.jp/).

Ethical Issues

Authors' Responsibility

The manuscript will not be accepted if it has been published in other periodicals or is under consideration for publication elsewhere. All authors must have participated in the conception, design, implementation, analysis, or interpretation of the data.

The submitted manuscript is predicated on the assumption that all listed authors have concurred in the submission and that the final version of the manuscript has been reviewed and approved by all authors. All authors must agree to the terms stated in the **Authors' Responsibility Form** and sign the form. The responsible author(s) are required to submit a copy of the completed **Authors' Responsibility Form** to the JIFS Editorial Office by e-mail (jifs.grp@tohoku.ac.jp).

Permissions

Authors wishing to include figures, tables, or text passages that have already been published elsewhere are required to obtain permission from the copyright owner(s) and to include evidence that shows such permission has been granted when submitting their papers. Any material received without such evidence will be assumed to have originated from the authors.

Ethical Rules for Experiments and Researches

In case the research works involve issues that require obtaining consent and/or cooperation of the third party, consideration in handling of personal information, or actions related to bioethics and/or biosafety (including the laws and regulations and the guidelines in the country/region(s) where the joint international research is to be conducted), all the works should be approved by an internal or external ethical jury. Such works may include research involving questionnaire surveys, interviews and/or behavior surveys (containing personal histories, images, and/or personal information), handling of donated specimens, human genome analysis, recombinant DNA, and experimentation with animals.

Registration of Molecular Sequences

New molecular sequences in manuscripts must be deposited in an appropriate international data bank. The database accession numbers must be included in the manuscript. For taxonomical studies based on molecular sequences, the source specimen of molecular information should be deposited in an appropriate institution.

Conflict of Interest

Authors/reviewers should clearly declare any conflicts of interest.

Scientific Misconduct

The Editorial Board of the JIFS reserves the right to reject any manuscript that contains any fraudulent activity (falsification, fabrication, plagiarism, etc.). Any research that does not meet the acceptable standards of research behavior as determined by national/local regulations, institutional review boards, or their equivalents regarding human and animal experimentation will be rejected.

The Editorial Board of the JIFS also reserves the right to withdraw a published article without the consent of the author(s), in case of any misconduct of the author(s) related to the published article. The decision of withdrawal by the Editorial Board and the reasons for the decision will be notified to the author(s) by e-mail.

Preparing the Manuscripts

General Preparation

Times Roman or Times New Roman (+ Symbol) font with standard size (e.g., 12-point) should be used in the text and tables. Pages and lines in the text must be numbered consecutively throughout the manuscript. The file name must include the corresponding author's name. Manuscripts must be written in English and typed in double-line spacing (25–28 lines per page) with 20-mm margins. Short reports should not exceed four printed pages, including references, tables, and figures. Use the automatic page- and line-numbering function to number the pages throughout the manuscript.

• Do not use field functions.
• Use tab stops or other commands for indents, not the space bar.
• Use the table function, not spreadsheets, to make tables.
• Use the equation editor or MathType for equations.
• Footnotes to the text are not allowed, and any such material should be incorporated into the text as parenthetical matter.

Each abbreviation should be defined when appearing for the first time and used consistently thereafter. Avoid abbreviations that are used only in a specific field and are unfamiliar to general readers. Insert a space between a number and a symbol, for instance, mean ± S.D., =, <.

Parts of the Manuscript

The manuscript should be submitted in separate files: main text file (including title page, acknowledgments, and references), tables, and figures. Manuscripts should be divided into the following sections:

1) Title page
   - Title of the manuscript
   - The full names of all the authors
   - The author's institutional affiliations where the work was conducted, with a footnote for the author's present address if different from where the work was conducted.
2) Abstract and keywords
3) Main text
4) Acknowledgments (optional)
5) References
6) Tables (each table complete with title and footnotes)
7) Figure legends
8) Figures
9) Appendices (optional)
10) Supporting Information (optional)

Each figure, Appendices, and Supporting Information should be supplied as separate files.

Title Page

The title page must contain a main title, a running title, full name(s) of all the author(s), their affiliation(s) where the work was conducted, and information for the corresponding author, including complete corresponding address and e-mail address. Abbreviations should not be used in the main title, except for commonly used ones, such as DNA and RNA. A vague title must be avoided. The title should be written for readers outside the discipline. The running title must not exceed 50 characters, including spaces.

Abstract and Keyword

The abstract should state the backgrounds/rationale, objectives, findings, and main conclusions of the work in a single paragraph of 300 words or less. State the significance of your findings in the abstract. The abstract should be written for readers outside the discipline. Abbreviations should be avoided in the abstract as far as possible. A heading is not required. Tables, figures, or references are not allowed in the abstract. Provide five to seven keywords (not appearing in the title) in alphabetical order positioned beneath the abstract. Abbreviations should be avoided in the keywords as far as possible.

Main Text

Each type of papers should contain the following sections:

• Original articles: Introduction, Materials and Methods, Results, and Discussion.
• Review articles: Introduction, other optional sections, and Conclusion(s).

Authors should use headings to divide the sections of their manuscript, if appropriate: Introduction, Materials and Methods, Results, Discussion, and Conclusion(s). Regardless of the article type, the backgrounds and the aim of the study should be clearly written in the Introduction for a broad readership of the JIFS.

In cases where a study deals with human subjects or live animals, the Materials and Methods section of the manuscript must declare that all experiments were performed in compliance with the relevant laws and/or institutional guidelines, and the study was approved by the institutional review board, if necessary. A statement must also be included stating that informed consent was obtained from human subjects.

Acknowledgments (Optional)

Contributions from individuals who do not meet the criteria for authorship should be listed, with permission from the contributor, in the Acknowledgments section. All funding sources should also be mentioned. Thanks to anonymous reviewers are not appropriate.

References

References are cited in the main text by the first author's last name and year of publication in parentheses, as shown below.

• Single author: (Aragaki, 2020)
• Two authors: (Aragaki and Ayase, 2020)
• Three or more authors: (Momota *et al*., 2020)
• Two or more references can be cited in the order of year of publication, such as (Takagi *et al*., 1993; Tamai *et al*., 1995; Sasaki *et al*., 2000; Hayami *et al*., 2012)

References are listed after the main text in alphabetical order under the first author's name: Last name(s), followed by initials of first and middle names; year of publication (in parentheses); title of article; title of periodical (abbreviated according to Index Medicus and italicized); number of volume (bold); and the first and last pages. Publications by the same author(s) in the same year should be listed as 2007a, 2007b, etc. In a reference with more than fifteen authors, give the first fifteen authors and then use *et al*.

When a book is referred to, the reference should include the author's name; year of publication (in parentheses); title of book (capitalized and italic); edition (if any); publisher; place of publication; and page(s).

Note the following examples.

1) A journal article:
   Momota, K., Takagi, R., Sasaki, A., Tamai, S. and Ikeda, M. (2021) The effect of birds and cows on marine ecosystem in Miyagi Prefecture. *J. Integr. Field Sci*., **6**: 109-115.
2) A chapter in an edited book:
   Aragaki, Y. and Ikeda, M. (2021) The effect of courtship dance of fish on marine biodiversity in Onagawa Bay. In *Fish, Cattle and Biodiversity, 2nd ed*., edited by Ogura, S. and Fukasawa, M. Tohoku University Press, Sendai, pp. 108-150.
3) A book:
   Ogura, S. and Fukasawa, M. (2021) *Fish, Cattle and Biodiversity, 2nd ed*., Tohoku University Press, Sendai.

Tables

• Tables should be prepared in editable files, such as Word and Excel.
• Table(s) are placed after References.
• Number tables sequentially with Arabic numerals (e.g., Table 1) in the order in which they are cited in the manuscript.
• A large table is not welcome. The final printed size of the table, including its title, legends, and footnotes, should be less than one printed page.
• Abbreviations should not be used in the title as far as possible.
• Footnotes to the tables should be indicated by superscript lowercase letters (or asterisks for significance values and other statistical data) and included beneath the table body.
• All abbreviations used in the tables must be defined in the footnotes.

Figures

• Figures should be prepared in PowerPoint, J-PEG, TIFF, EPS, or PDF files.
• Number figures sequentially with Arabic numerals (e.g., Fig. 1) in the order in which they are cited in the manuscript.
• Figure legends should be placed after the References.
•Authors should emphasize the most essential points in each figure by providing an understandable title and legend to each figure.
• Abbreviations should not be used in the titles as far as possible.
• Indicate the notable findings with some symbols or marks in each figure.
• Provide explanations for symbols or marks in the figure legends.
• All abbreviations used in the figures must be defined in the legends.
• High-quality photographs may be required for printing morphological data.
• Provide a scale bar in a representative morphological photograph.

Manuscript Submission

All files should be sent to the editorial board as attached files of e-mail to the Editorial Office of JIFS (jifs@grp.tohoku.ac.jp).

The manuscript files should be provided in the Word version of the manuscript (the title page, abstract, main text, references, and legends of figures), the Word or Excel version of tables, and the PowerPoint, J-PEG, TIFF, EPS, or PDF version of the figures.

The JIFS requests no submission charges.

Peer Review

All submitted manuscripts, including the Invited Review articles, are subject to initial review by Editor(s) and iThenticate

plagiarism screening. The Editor(s) will determine whether a submitted manuscript should be returned without peer review or sent for peer review. If the manuscript is returned without peer review, the author is notified immediately.

If the manuscript warrants full peer review, the Editor assigns two independent reviewers, including the Editorial Board Members. All reviewers who review the manuscript remain anonymous to the authors. Every manuscript is treated as confidential information. The Editor-in-Chief makes the final decision based on the comments and recommendations provided by the reviewers.

Submission of the Revised Manuscript

The response letter should summarize the changes made and include a list of your point-to-point responses to the reviewers' critiques. The revised manuscript and the Word version of the response letter should be sent by e-mail to the Editorial Office of JIFS.

Post Acceptance

Once a manuscript is accepted, the corresponding author must sign the **Copyright Transfer Agreement** on behalf of all authors and return it to the editorial office. The Form will be sent to the corresponding author by e-mail from the editorial office together with a proof.

Your accepted paper may be substantially edited by the Editor(s), or requested to be partly rewritten after acceptance to ensure that your publication is accurate, clear, and understandable to the broad readership of the JIFS. Editors reserve the right to request authors to revise the manuscript when required.

A copyedited proof will be sent to the corresponding author within a few weeks. Authors should review the proof and return requested corrections within 48 hours. Tables are adapted to the JIFS style in a printing company we contract with. Authors should therefore carefully check the correctness of tables in the proof.

Copyright and Permissions

Copyright

As a condition of publication, all authors must transfer copyright to the JIFS Editorial Office. The articles published in the JIFS after volume 19 are marked by the Creative Commons (CC) License; the CC BY-NC-ND 4.0 (Attribution-NonCommercial-NoDerivatives 4.0 International) License. Anyone may download, reuse, copy, reprint, or distribute the article marked by the CC BY-NC-ND 4.0 without modifications or adaptations for non-profit purposes if they cite the original authors and source properly.

Permissions for Reuse and Reproduction

It is our policy that authors reserve the right to reuse or reproduce their own article, in whole or in part, for non-commercial use, provided that acknowledgment is given to the original source of publication. Non-commercial use includes uses in the author's own thesis, dissertation, textbook, review article, deposit in any repository, websites, lectures at the conference or other meetings, and so on. Authors are not required to obtain permission from the Editorial Board of JIFS in such cases.

Please refer to the supplement Permissions if you request other types of permissions for reuse and reproduction.

Publication Fees

There is no publication fee, regardless of color or black and white.

Reprints

Reprints in pdf are available for free. Printed reprints amount to 200 yen per copy.

Next Publication, volume 19 of the JIFS will be published in March 2022. Please send your manuscript to us by November 30, 2021, if you want your study to appear in the forthcoming volume.

Send to: jifs@grp.tohoku.ac.jp
Submission Deadline: November 30, 2021

Contact

If you have any questions about the submission process, you may contact the editorial office.

The JIFS Editorial Office
Graduate School of Agricultural Science, Tohoku University
468-1 Aramaki Aza Aoba, Aoba-ku, Sendai 980-0845, JAPAN
Tel/Fax: +81-022-757-4194
E-mail: jifs@grp.tohoku.ac.jp