学　位　論　文　内　容　の　要　旨
Abstract of Doctoral Dissertation

博士の専攻分野の名称 博士（生命科学）氏　名
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学　位　論　文　題　名
Title of Doctoral Dissertation

Glycomics Approach of Livestock Management Toward the Discovery of Novel Biomarkers Indicating an Environmental Stress
(糖鎖解析による家畜管理法の試み–環境ストレスを反映するバイオマーカーの探索)

Since stressful environmental stimuli are one of the main causes of health issues, deficient immune responses and production in livestock, more attention needs to be placed on the principles of The Welfare Quality® Assessment protocol in order to enhance the performance and productivity of the farm animals.

The temperature-humidity index (THI) has been used in assessments of heat stress. However, it is important to note that the THI value cannot be used as a feasible indicator to alert farmers to any alterations in an animal's metabolic/homeostatic immune balance perturbed by "complicated heat stress", while the THI may be an important parameter for simply showing the temperature and humidity of the feeding environment. Moreover, farmers have not been able to fully recognize the signs of cows being physically affected by slight heat stress. Additionally, traditional physiological approaches are also insufficient for elucidating a direct correlation between hormone secretions due to the stress adaptability of the animal; therefore, the best indicator for identifying animals under stress is a stress-related change in biological functions with suppression of the immune system. Posttranslational protein glycosylation is a basic principle for controlling the structures and functions of most glycoproteins in relation to cellular differentiation, adhesion, immunity, signal transduction, growth control, malignant alterations, and influenced by environmental stimuli. These structural alterations may provide highly sensitive biomarkers to indicate changes in general metabolism and the homeostatic immune balance directly/indirectly induced by various diseases without any direct biopsy of the tissues/organs affected. Based on some climatic data at Sapporo on 2012, heat stress commonly assessed by the THI was estimated, maximum in August. The results indicated that animal performance and milk production were significantly influenced by seasonal variations which may strongly depend on marked changes in the diet and metabolic effects on normal microbial protein synthesis. THI values may contribute to the development of a management system for short-term heat stress in order to improve animal comfort. However, the relationship between long-term damage caused by the integration of many environmental stresses and the performance/milk productivity of lactating Holstein dairy cows currently remains unclear due to the lack of sensitive biomarkers to indicate alterations in the general metabolism and homeostatic immune balance of Holstein dairy cows. Thus I performed, for the first time, large-scale glycomics of 336 dairy Holstein cow serum samples collected for 9 months in Hokkaido, Japan (between February and October) using glycoblotting combined with MALDI-TOF/MS and DMB/HPLC of whole serum glycoproteins to detect dynamic changes in the expression levels and profiles of 36 *N*-glycan structures. A multivariate analysis revealed a correlation between the serum expression levels of these season-specific glycoforms and productivity, there were an increase in glycans terminating with two/three Neu5Gc residues in summer and autumn, and *N*-glycans in winter dominantly displaying Neu5Ac, which caused by changes in the expression levels of *exo*-mannosidases/glycosyltransferases and intracellular concentrations of various sugar nucleotides in relation to their biosynthetic pathways. The dynamic interconversion between Neu5Ac and Neu5Gc provides highly sensitive biomarkers because of the sialylation of IgG and other serum glycoproteins was considered to putatively modulate immune cell functions in infectious diseases, inflammation, neurodegeneration, autoimmune diseases, and cancer through interactions with Fcγ/FcRn receptors and/or various pattern recognition receptors (PRRs)

including sialic acid-binding immunoglobulin-like receptors (Siglec) on dendritic cells and macrophages. The sialylation profile of serum IgG Fc *N*-glycans as well as those of major serum glycoproteins in fetal bovine serum may differ markedly from those observed in the serum samples of mature Holstein dairy cows. The bi-antennary *N*-glycan structures bearing two Neu5Ac residues have potential as specific serum biomarkers closely related to maternal immune defenses. Since the *N*-glycans of bovine colostrum IgG were modified with Neu5Gc and Neu5Ac residues, the immunity of newborn calves may be specifically controlled by the Nue5Gc residues of IgG *N*-glycans because, fetal bovine serum glycoproteins were not modified with the Neu5Gc residue, and may be required epigenetically in order to adapt to the dynamic changes associated with early environmental stresses in order to construct a beneficial intestinal microbiota and shape early immunity. The upregulation of serum total Neu5Gc expression levels may directly indicate an increase in damage caused by various environmental stresses in Holstein dairy cows. After quantitative analysis of dynamic sialylation in serum *N*-glycans glycoproteins, the Neu5Ac content was markedly higher in winter to indicate reproductive stress while in summer and autumn were dominantly terminated by the Neu5Gc residue to confirm the evidence of environmental stresses. The results demonstrated that the Neu5Gc/Neu5Ac ratio of whole serum glycoproteins was a highly sensitive and efficient biomarker for indicating the general performance and milk productivity of Holstein dairy cows.

In conclusion, a glycoblotting allowed for a large-scale serum *N*-glycome analysis and the quantitation of non-reducing terminal sialic acids was established in the present study, I discovered highly sensitive serum *N*-glycan biomarkers indicating an environmental/heat stress affecting the general health condition, especially homeostatic immune balance of Holstein dairy cows. Consequently, serum Neu5Gc levels are a promising alternative biomarker to the individual *N*-glycans. The serum ratio Neu5Gc/Neu5Ac correlated well with the performance and milk productivity of Holstein dairy cows, while the THI value was not capable of predicting such long-term damage by a heat/environmental stress. The significance of the interconversion between Neu5Ac and Neu5Gc of serum glycoproteins in the regulatory mechanism of immune responses may be determined in terms of the interactions between glycoform-engineered IgG and Fcγ/FcRn receptors or key animal lectins/PRRs on dendritic cells/macrophages. Moreover, Neu5Ac quantifications are a promising alternative embryonic biomarker to the individual *N*-glycans of serum in cows.

Finally, I strongly confirmed that glycomics profiling is one of the non-invasive approaches to investigate the immune balance and productivities in livestock management under integrated heat/environmental stresses.