学　位　論　文　審　査　の　要　旨

Doctoral Dissertation Evaluation Review

博士の専攻分野の名称　博士（生命科学）　氏　名　Ibrahim Farag Mohammed Rehan
Degree requested: Doctor of Life Science　Name: Ibrahim Farag Mohammed Rehan

審査担当者	主査 /Chief examiner	教授 西村紳一郎 <Professor Shin-Ichiro Nishimura >
Examiners	副査 /Associate examiner	教授 幸田敏明 <Professor Toshiaki Koda >
	副査 /Associate examiner	教授 門出健次 <Professor Kenji Monde >
	副査 /Associate examiner	准教授 比能洋 <Associate Professor Hiroshi Hinou>

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）

博士学位論文審査等の結果について（報告）
Results of Evaluation of the Doctoral Dissertation (Report)

Recently, studies on glycomics analysis of human model are actively being done. Many of them aim to diagnose the disease in order to find a drug discovery. However, the research is still limited in animal model.

In this study, based on animal's metabolic/homeostatic immune balance, the author performed a novel research, on 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, and there was an increase in high mannose *N*-glycan in spring, glycans terminating with two/three Neu5Gc residues in summer and autumn, and *N*-glycans in winter dominantly displaying Neu5Ac, which was 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 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 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. The bi-antennary *N*-glycan structures bearing two Neu5Ac residues have a 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, since fetal bovine serum glycoproteins were not modified with the Neu5Gc residue, and may be required epigenetically in order to be adapted to the dynamic changes associated with early environmental stresses in order to construct a beneficial intestinal microbiota and to shape early immunity. The upregulation of serum total Neu5Gc expression levels may directly indicate an increase in damages caused by various environmental stresses in Holstein dairy cows. After quantitative analysis of dynamic sialylation in serum *N*-glycans, the Neu5Ac

content was markedly higher in winter to indicate reproductive stress while in summer and autumn it was dominantly terminated by the Neu5Gc residue to confirm the evidence of environmental stresses.

In conclusion, the author has new findings by discovering 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 for the individual *N*-glycans. The serum ratio Neu5Gc/Neu5Ac was 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. These results will contribute to confirm 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. Therefore, we acknowledge that the author is qualified to be granted the Doctorate of (Life Science) from Hokkaido University.