



HOKKAIDO UNIVERSITY

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Effects of Maternal Undernutrition on DNA Methylation, Gene Expression,
and Metabolites of Fetal Thymus and Growth in Wagyu (Japanese Black) Cattle
(和牛 (黒毛和牛) における母牛の低栄養が胎仔胸腺のDNAメチル化、遺伝子発現、
代謝産物および成長に及ぼす影響に関する研究)

Livestock production has played an important role in ensuring food security and economic stability, which however, has recently become increasingly difficult due to factors such as rising production costs, declining profitability, and negative impacts on animal health and ecosystems. In beef production, the negative impact on productivity caused by infectious diseases is also becoming higher, and efficient production by healthy livestock with strong immunity is required. Additionally, maternal nutrition significantly impacts fetal development, influencing postnatal outcomes in accordance with the DOHaD theory. Recently, this concept of has garnered attention for its implications in livestock production, as maternal nutrition can induce epigenetic modifications and alter gene expression, thereby affecting fetal organogenesis and physiology. Health and subsequent productivity are important for livestock. The thymus, in particular, is essential for establishing and maintaining immune homeostasis both prenatally and postnatally. This study aimed to examine the effects of maternal nutrient restriction (MNR) on DNA methylation, gene expression, and metabolite profiles in the Wagyu fetal thymus and to assess the resulting physiological and growth changes in neonatal Wagyu calves.

To investigate the effects of maternal low nutrition on calf growth, eight pregnant cows were divided into two groups during late gestation. The control group (CON, n=4) was fed 100% of their nutritional requirements, while the maternal low nutrition group (LOW, n=4) was fed 60% until calving. Metabolic hormones and metabolites were analyzed from blood samples of cows and their newborn calves, and colostrum was examined for the same traits. The secretion of GH in pregnant cows was higher in the LOW group relative to the CON group, and this disparity persisted postpartum and was concurrently observed in the colostrum content ($p < 0.05$). In the LOW group, there was a tendency towards lower overall insulin secretion compared to the CON group ($p < 0.10$). The concentration of plasma glucose was lower, while NEFA levels were higher in the LOW group compared to the CON group throughout the nutrient treatment period ($p < 0.001$). Additionally, plasma protein concentration was lower in the LOW group compared to the CON group throughout the nutrient treatment period, and this disparity persisted postpartum and was concurrently observed in the colostrum content ($p < 0.05$). In the offspring, GH secretion was higher in LOW offspring compared to CON offspring ($p < 0.05$); however, the overall disparity in GH secretion between the two groups of offspring was not evident. The level of IGF-1 was greater in LOW offspring on calving day ($p < 0.05$). Nonetheless, the overall secretion of IGF-1 was lower in LOW offspring compared to CON offspring ($p < 0.01$). Calves in the study were classified by gender and group, with male calves in the CON group and females in the LOW group. At birth, LOW offspring had a lower birth weight compared to CON

offspring (29.0 ± 2.63 kg vs. 40.25 ± 1.70 kg; $p < 0.05$), and this difference in body weight persisted through 5 months of observation. At weaning (90 days of age) and 150 days of age, the weights were 130.3 ± 3.5 kg vs. 90.6 ± 5.0 kg and 197.63 ± 5.83 kg vs. 141.25 ± 8.38 kg ($p < 0.01$) for CON and LOW offspring, respectively. Nonetheless, no differences in growth rate and feed conversion ratio were observed.

Next, 11 pregnant Wagyu cows were divided into two groups: the CON group ($n=6$) was fed 120% of their nutritional requirements until 8.5 months of fetal age, and the LOW group ($n=5$) was fed 60%. Experiments were first focused on histological and immunohistochemical analysis of the thymus. Thymus weight was significantly reduced in the LOW group compared to the CON group ($p < 0.01$). Histological analysis showed a decrease in medullary area in the LOW group compared to the CON group ($p < 0.01$). Analysis of T cell subsets showed that the LOW group had a significantly greater number of CD4⁺ T cells and a higher CD4⁺ T cell to nucleus ratio compared to the CON group ($p < 0.001$). Whole Genome Bisulfite Sequencing (WGBS) revealed 8,869 differentially methylated genes (DMG, $q < 0.01$), with 4,566 genes exhibiting hypo-methylation and 4,303 genes showing hyper-methylation in the LOW group. Intergenic regions exhibited the highest frequency of DNA methylation, followed by intron; however, the promoter regions had the fewest changes in DNA methylation in both groups. KEGG pathway analysis indicated that hypo-DMGs were associated with pathways such as the Ras signaling pathway, cGMP-PKG signaling pathway, and relation of hypothalamic-pituitary-adrenal (HPA) axis synthesis and secretion, including GnRH, oxytocin, renin, aldosterone, relaxin, cortisol, and insulin ($q < 0.001$). Hyper-DMG enriched pathways included Hippo, Wnt signaling, parathyroid hormone, and Fc gamma R-mediated phagocytosis ($q < 0.001$). RNA sequencing identified 94 differentially expressed genes (DEGs, $q < 0.05$), with 66 upregulated and 28 downregulated genes in the LOW group. KEGG pathway analysis revealed upregulated genes linked with metabolic pathways, oxidative phosphorylation, chemical carcinogenesis-reactive oxygen species, and glutathione metabolism, while downregulated genes were associated with MAPK signaling, C-type lectin receptor signaling, and natural killer cell-mediated cytotoxicity ($q < 0.10$). Moreover, key overlapping genes such as GRIA1, CACNA1D, and SLC25A4 were enriched in pathways including the cAMP signaling pathway, cGMP-PKG signaling pathway ($q < 0.10$). Metabolomic analysis revealed elevated levels of histidine, alanine, serine, histamine, and phenylalanine in response to MNR. The results of Metabolite Set Enrichment Analysis (MSEA) highlighted alterations in specific metabolic pathways, particularly those related to amino acid metabolism ($p < 0.10$). Additionally, CD4 T cell expression in the fetal thymus was significantly higher in the LOW group compared to the CON group.

Maternal nutrition in Wagyu cattle induces epigenetic changes that regulate gene expression related to the dynamics of metabolism, which in turn may affect stress and immune responses, especially in helper T cells. Furthermore, maternal low nutrition alters physiological regulation, as evidenced by changes in GH and IGF-1 secretion. These findings are expected to influence epigenetic modifications that affect postnatal growth, physiological function, and immune system regulation in Wagyu cattle. The results of this study, which revealed that maternal nutrition in large ruminants significantly affects the fetal thymus gland, which influences calf immunity, are significant as basic scientific findings in the field of livestock research in the development of postnatal health and efficient feeding techniques and are expected to contribute to the establishment of new beef production systems.