Study on early nutritional factors associated with metabolism disorders in modern broilers
-Gluconeogenesis specificity and the relation between systemic metabolic control regulation and 25-hydroxychorecalciferol in newly hatched chicks

Summary of Doctoral Thesis

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The production of chicken meat, which is a source of animal protein that can respond quickly to the increase in the world population, is increasing. Modern broilers are rapidly renewed for breeding improvement due to increased demand for chicken meat and the increase in breast meat is particularly remarkable. On the other hand, new problems related to the myodegeneration have been reported. Myodegeneration including White Striping (WS), pectoral major muscle with white lines, and Wooden Breast (WB), a sclerotic muscle has been reported. However, the pathogenesis of the degeneration has not been elucidated yet. Broilers have promoted growth by suppressing muscle proteolysis, but the association with metabolic disorders was considered.

Therefore, in this study, the possibility of the metabolic disorder was investigated while the onsets of WS and WB were confirmed by using the commercial broiler. Although it has been reported that chickens increase gluconeogenesis before and after hatching, this is contrary to the muscle proteolysis inhibition of the broiler growth promoting

factor, and the metabolic contradiction seems to have some relation with the onset of the myodegeneration, and the glucose (GLU) amount of the embryo before hatching and the specificity at the time of inhibition of gluconeogenesis were examined. Furthermore, we investigated the influences of in ovo administration of vitamin D_3 (VD_3) on broiler growth, which has been suggested to be related to the metabolic induction of tissue formation from skeletal formation in recent years, and then investigated the effects of 25-hydroxycholecalciferol ($25(OH)D_3$), a metabolic form, on the metabolic control system of newly hatched chicks.

1. Examination of WS and WB symptoms

In Experiment 1, 12 Ross commercial broilers at 43 days of age were selected 6 WB probable broilers and 6 non-probable broilers by Kawasaki et al. (2016) in collaboration with the Research and Development Center of Nosan Corporation in order to confirm the onset of the WS and WB symptoms. After observing the macroscopic features of appearance, body weight measurement and blood sampling were carried out, and pectoral major muscle, suture muscle, liver, kidney, heart, testis, and diencephalon were collected after observing the macroscopic features of the onset site. Hematologic chemistry study was performed, then mRNA expression of decorin and TGF-β, associated with collagen formation in the pectoral major muscles, were measured. Though the WB onset area showed the appearance feature in which both wings could not contact due to the sclerosis of the

pectoral major muscle, the difference in body weight in this experiment was not recognized. In the WS onset site of pectoral major muscle, white line material which was reported to be adipocyte and connective tissue was confirmed along the muscle fiber. In the WB onset site, in addition to the muscle sclerotization, internal bleedings were scattered and the colloidal substance similar to the muscle decomposition material observed in hatching was confirmed over the whole of pectoral major muscle, and the sclerosis symptom seemed to be the prelude before the muscle decomposition or collapse, and the possibility of causing the physiological metabolic contradiction of catabolism before and after hatching and the anabolism related to the muscle proteolysis suppression of broiler seemed to be the necessity of examination. Since there was a strong correlation between decorin and TGF-β in pectoral major muscle and correlation between TGF-β and VDR, it was presumed that VD was related to muscle metabolism, and it seemed to be meaningful to focus on VD in this Experiment.

2. Examination of gluconeogenesis specificity

In order to investigate the possibility of causing the physiological metabolic discrepancy of anabolic catabolism before and after hatching in the experiment 1, the timing and site of GLU accumulation in embryos were clarified, and how the administration of metformin, a gluconeogenesis inhibitor, affected catabolism was confirmed. 24, 16, 8, 8, 20, and 10 Cobb broiler breeder eggs were used in experiment 2, 3, 4, 5, 6, and 7 respectively, and the hatching eggs were incubated at

37.8° C and >60% RH. 8 embryos used for GLU measurement were collected at Day 17, 18 and 19 of incubation in experiment 2. 8 embryos were collected at Day 17 and 19 of incubation in experiment 3, and then hatching muscle, femoral muscle, glandular stomach, muscular stomach, heart, and liver were collected. 8 embryos were collected at Day 19 of incubation in experiment 4, and then thymus, Fabricius capsule, spleen, and brain were collected. Blood was collected from 5 egg yolk arteries at Day 19 of incubation in experiment 5. 0, 100, 200, and 300 mg/mL metformin was in ovo administered at Day 17 of incubation to confirm the dose-response on embryonic viability and hatchability of newly hatched chick in experiment 6. 0 or 200 mg/mL metformin was in ovo administered at Day 17 of incubation same as experiment 6 in experiment 7. Incubation was continued until Day 19 of incubation, eggs were cleaved, and embryos and yolk sac weights were measured. The GLU content of the embryos increased linearly from day 17 to day 19 of incubation ($P<0.05$). The GLU concentration in the liver decreased significantly with growth ($P<0.05$), but no difference was observed in other tissues, suggesting that GLU in the liver was transported to the blood. Administration of metformin reduced embryonic mortality and hatchability of newly hatched chicks, but blood GLU concentrations were not affected, suggesting that the presence of strong feedback and glycemic maintenance and gluconeogenesis control are important in chicken life maintenance, that feedback is enhancing gluconeogenesis-associated muscle

proteolysis, and that broiler growth may be caused by breeding to suppress muscle proteolysis.

3. Examination on the effect of VD_3 on broiler development

Physiological effects of VD_3, the starting material for VD considered to be related to muscle metabolism in experiment 1, were confirmed and their function on growth was investigated. 60 Ross broiler breeder eggs were used and incubated at temperatures of 37.8° C and >60% RH in experiment 8. Distilled water (control group), soybean oil (soybean oil group), or VD_3(VD_3 group dissolved in soybean oil were 0.5 mL in ovo administered at 20 eggs respectively at Day 18 of incubation. Hatched chicks were discriminated between sexes and 12 birds (6 males and 6 females) were selected for the same average body weight after weighing. Thereafter, commercially available broiler starter diet (ME 3,100 kcal/kg, CP 21 %) was fed for 4 weeks, liver and pectoral major muscles were collected at 28 days of age, and mRNA expressions of IGF-1 and IGF-1 receptors were measured. In addition, the length of the tibia was measured. There were no differences in body weight at hatch and 28 days of age among groups, but the tibial length was longer in males than in females in the VD_3 group, with interaction between treatment and sexes, and longer in males in the VD_3 group than in the control group. Although the differences in tibial bone length may be attributed to the combination of VD_3 and soybean oil, it was not clear whether or not VD_3 alone was the effect, but the mRNA expression of muscle IGF-1 receptors was high only in females in the

VD_3 soybean oil administration group, and the expression levels of liver IGF-1 were high in the VD_3 soybean oil administration group, suggesting that growth was promoted only in males and bone elongation was affected. However, mRNA expression of the IGF-1 receptor in the pectoral major muscle, which is an indicator of development, was increased only in females, and mRNA expression of IGF-1 in the liver was increased in the presence of VD_3. The root cause of the sex difference seemed to be the involvement of sex steroid hormone, but WS and WB onsets were considered to be due to the health of the female for the development considering that the onset rate is low, or the onset rate is slow compared with the male in the industry. It was speculated that the lack of heathy promoted the suppression of muscle proteolysis in males. In addition, although VD_3 has been used in broiler diets, myodegeneration of WS and WB has been a concern. However, the association between muscle metabolism and VD function has been suggested in this experiment, suggesting that there may be an effect of VD morphology differences.

4. 25(OH)D_3 Relationship with systemic metabolic regulation system

In experiments 9 and 10, which investigated the relationship with systemic metabolic control systems to determine the effect of different VD forms on broilers, were divided into 4 groups with VD_3 of 3,000 or 5,000 IU/kg and 0 or 69 μg/kg 25(OH)D_3 based on basal diet (ME 3, 200 kcal/kg, CP 20%) using Ross broiler chicks . Feed was fed ad libitum, drinking water, and body weight was measured at 11 days of

age, blood was collected, and then euthanized in cervical dislocation in experiment 9. The collected blood was analyzed for 25(OH)D_3 content, GLU content, free fatty acid content, and calcium content, and the liver, kidneys, pectoral major muscle, and diencephalon were collected, and the expression of VDR mRNA in the respective organs was measured, and the expression of proopiomelanocortin (POMC), agouti-related peptide (AgRP), neuropeptide Y (NPY), and VDR mRNA in the diencephalon was measured. Analysis of mRNA expression in the diencephalon in the melanocortin system involved in energy-metabolic control in experiment 9 revealed that the NPY and AgRP involved in anabolism did not vary with VD, while the POMC involved in catabolism varied in the presence of 25(OH)D_3, similar to the response of VDR mRNA expression in the kidneys. The mRNA of POMC and VDRs in the diencephalon was also correlated, suggesting an effect on catabolism by 25(OH)D_3. Furthermore, in Experiment 10, GLU tolerance in each group was investigated by an oral GLU tolerance test. As a result, the addition of 3000IU VD_3 and 69 μg/kg 25(OH)D_3 tended to be lower. Differences with other groups were observed at 120 minutes after oral administration and insulin sensitivity increased. It was inferred that the GLU regulation function by VD balance might occur in the GLU tolerance state.

This study suggested that muscle breakdown may be occurring in the presence of WS and WB and VD is involved in muscle metabolism. The strong control of hyperglycemia and gluconeogenesis was observed

before and after hatch, and the existence of feedback by dynamic adaptation led to catabolism of muscle proteolysis, and it was considered that the degradation suppression was done by breeding. The VD effect on the growth was also considered to be degradation inhibition due to lack of health in males. The association of the melanocortin system and VDR with the regulation of energy metabolism and the possibility of glycemic regulation was suggested to be affected by the presence of $25(OH)D_3$. Thus, catabolic regulation by using gluconeogenesis and $25(OH)D_3$ could lead to the control of WS and WB onsets.