別紙４

学 位 論 文 の 要 旨

論文題目　Studies on the innate immune function and its response to probiotics treatment in the intestine of broiler chicks
(ブロイラーヒナ腸管における自然免疫機能とそれに及ぼすプロバイオティクス給与の影響に関する研究)

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The intestinal tract which contains the microbiota complex has risks of the infections by pathogenic bacteria. The innate immunity plays important roles in the immunodefense against the infection by pathogenic bacteria in younger chick before adaptive immune system develops. Antimicrobial peptides (AMPs) have a broad spectrum of antimicrobial activity. Thus, the enhancement of the innate immune system including AMPs synthesis in the chick intestine in important for the safe chick breeding. Antibiotics treatment had been used as growth promoter, but it has a risk of appearance of resistant bacteria which is the serious problem for the public health. Probiotic treatment is expected to improve growth-performance by modification of the luminal microbiota and metabolome in intestine. Since probiotics treatments are also expected to enhance the intestinal immunodefense functions in the intestine, probiotics may be useful for the safe chick production instead of antibiotics. Thus, the aim of this study was to determine the expression profiles of innate immune molecules in the chick intestine and whether intestinal microbe and probiotics enhanced their expressions.

1. The expression profiles of avian β-defensins (AvBDs) and pro-inflammatory cytokines in the chick intestine

Avian β-defensins (AvBDs) and proinflammatory cytokines play important roles for innate immune response. However, the expression profiles of these innate immune molecules in the intestine during the growth of chick are not well studied. This study examined the

changes in the expression of AvBDs and proinflammatory cytokines and the localization of AvBD2 in the intestine of broiler embryos and chicks during their growth. The ileum and cecum of embryonic day 19 (ED19), day-old (D0) and 7-day-old (D7) chicks were collected, and the gene expression levels of 10 AvBDs (AvBD1 to 8, 10, and 12) and proinflammatory cytokines (L-1β, -6, and -8) were analyzed by real-time PCR, and the localization of AvBD2 was examined by immunohistochemistry. The results showed that the expression levels of several *AvBDs* (*AvBD1, 2, 4, 6, 7, 8,* and *10*) in the ileum and cecum were highest at ED19 or D0, and their expressions levels were tendency to decrease at D7. The expression levels of *IL-1β*, *-6*, and *-8* in the ileum were higher at D7 than that of ED19. Whereas the expression levels of *IL-1β*, *-6*, and *-8* in the cecum were greater at D0 than ED19, and their expression levels were declined at D7. The AvBD2 positive cells were localized in the lamina propria beneath the surface epithelium of villi and crypts. The number of positive cells in the mucosa of cecum was greater at D0 than ED19 and D7. These results suggest that AvBDs and proinflammatory cytokines are expressed and synthesised in the intestine of embryos and newly-hatched chicks, and their expression may decrease with the growth of chicks. Those AvBDs and proinflammatory cytokines may play roles in protection of intestine tissues from infection.

2. The immune response of avian β-defensin (AvBDs) and pro-inflammatory cytokines in the chick intestine against TLR ligands

Toll-like receptors (TLRs) recognize the bacterial components (microbe-associated molecular patterns; MAMPs), then induce the expression of innate immune molecules including AMPs and cytokines. It was examined whether different TLR ligands (bacterial pattern) affect the expression of proinflammatory cytokines (*IL-1β* and *IL-6*) and AvBDs (*AvBD1*, *AvBD4*, and *AvBD7*) in the chick intestine. The ileum and cecum of 3-day-old chicks were cultured with or without TLR2, TLR4, and TLR21 ligands—namely Pam3CSK4, LPS, and CpG-ODN, respectively—for 1 or 3 h. Some tissues were examined histologically. The gene expression profiles of proinflammatory cytokines and AvBDs were examined in these tissues using real-time PCR. The mucosa of ileum and cecum contained leukocytes including eosinophilic heterophil-like cells, luminal and crypt epithelial cells, and other enterocytes. Pam3CSK4 tended to downregulate the expression of *IL-1β*, *AvBD1*, and *AvBD7* in the ileum but to upregulate their expression in the cecum. LPS downregulated the

expression of *IL-1β* and *IL-6* in both the ileum and the cecum, whereas it upregulated the expression of *AvBD1*, *AvBD4*, and *AvBD7* in the cecum. CpG-ODN upregulated the expression of *IL-6* and *AvBD7* in the ileum and *IL-1β* in the cecum, and tended to downregulate the expression of *IL-1β* and *AvBDs* in the ileum. It is suggested that the expression levels of proinflammatory cytokines and *AvBDs* in the chick intestine are affected by TLR2, TLR4, and TLR21 ligands, and thus the synthesis of innate immune factors in the intestine may be modulated by the luminal microbe molecules.

3. *Effect of antibiotics treatment on the microbial composition and expression of antimicrobial peptide and cytokines in the chick cecum*

The evidence showing that the microbiota complex directly affect the innate immune system in the intestine remains to be confirmed. It was examined whether antibiotics administration affected the intestinal microbiota in the cecum contents and the expression of innate immune molecules including TLRs, pro- and anti-inflammatory cytokines, and AMPs in the chick cecum and ceca-tonsils. The neonatal chicks were orally given with PBS with or without antibiotics (penicillin and streptomycin) till day 13, and the cecum and ceca-tonsil were collected at day 7 and day 14. The microbiota diversity in the cecum contents at day 7 and day 14 were modified by the administration of antibiotics. At day 7, the expression levels of *TLRs*, proinflammatory cytokines, and AMPs in the chick ileum and cecum and ceca-tonsil were downregulated by antibiotics treatment. whereas, the expression levels of *TLRs* and proinflammatory cytokines were upregulated in the cecum and ceca-tonsil at day 14. The AvBD2 and CATH1 immunoreactive cells were localized in leucocyte cells in the mucosa of cecum and ceca-tonsil at day 7 and 14 in chicks treated with or without antibiotics. These results suggest that TLRs, pro- and anti-inflammatory cytokines, and AMPs expressions are affected by the luminal microbiota in the cecum at early phase of life in chicks.

4. *Effect of probiotics treatment on the microbial composition and expression of antimicrobial peptides and pro- and anti-inflammatory cytokines in the chick ileum and cecum*

The live probiotics treatments are expected to improve microbiota complex and metabolome in the intestinal contents and the expressions of immune molecules in intestinal mucosa. The aim of this study was to determine the effect of different commercial probiotics

on the innate immune molecules (TLRs, pro- and anti-inflammatory cytokines, and AMPs) in the chick ileum and cecum. The day-old chicks were given 500 μl water with or without live Lactobacillus reuteri (LR) or Clostridium butyricum (CB) from day 1 to day 6 after hatching, and the ileum and cecum were collected on day 7. Both of LR and CB treatment increased the expression levels of several innate immune molecules in the ileum and cecum, whereas these effectives were different between LR and CB. These results suggest that probiotic LR and CB treatments modulate the expression of innate immune molecules. Since two different probiotics showed the different modification in the expression of innate immune molecules, mixture of different probiotics may be of better benefit as a probiotics treatment to enhance the innate immune system in the intestine.

5. Conclusion

Based on the results of this study, it was concluded that the expressions of innate immune molecules including AMPs in the chick intestine are affected by the bacterial components and luminal microbiota complex, and the probiotics treatments enhance these expressions. This finding may be useful to develop more effective probiotics to enhance the intestinal immunity in the future studies for production of healthy chicks under a sustainable and safe environment.