Effect of IL-8, IL-2 and IL-15 Gene Expression in Different Tissues of Jinghai Yellow Chicken Infected by *Eimeria Tenella*

Chengli Wang¹, Hailiang Yu², Xiaohui Wang², Wenbin Zou², Changhao Mi², Tingyan Hu², Guojun Dai²

¹Jiangsu Agri-animal Husbandry Vocational College, Jiangsu, China
²College of Animal Science and Technology, University of Yangzhou, Yangzhou, China

Corresponding author e-mail: Guojun Dai, daigji@yzu.edu.cn

Abstract. For research the role of IL-8, IL-2 and IL-15 genes in immune regulation after *E. tenella* infection, with Jinghai yellow chickens as material, the mRNA expression levels of IL-8, IL-2 and IL-15 genes in ten different tissues after infection with different doses of *E. tenella* oocysts were compared to determine the expression difference of the three genes in the same tissue with different infection doses. The results showed that IL-8, IL-2 and IL-15 genes were expressed in all tissues at the 7th day of infection, and the infection group was higher than the control group in most tissues. The mRNA expression of IL-8 gene in infection group II in the liver was significantly higher than that in the other two groups (P<0.05), and was extremely far higher the other two groups in the spleen and cecum (P<0.01). The mRNA expression of IL-8 gene in infection group II was also far higher in the bursa and thymus than that in the control group (P<0.05). The mRNA expression of IL-2 gene in infection group II in liver and lungs were significantly (P<0.05) and extremely significant (P<0.01) higher than that in the control group, respectively. The IL-15 gene mRNA expression. The infection group was obvious (P<0.05) and extremely significantly (P<0.01) higher in the lung and cecum than that in the control group, respectively. These results indicate that the three genes play a role in the infection of *E. tenella* in chickens, and provide a basis for the subsequent study of the relationship between these three genes and chicken coccidiosis resistance.

1. Introduction
Cytokines have a wide range of biological functions, which, in the host immune response, play important roles in the induction and activation of immunocompetent cells [1]. With the continuous discovery of the types and functions of cytokines, their role in resistance to coccidia has attracted the attention of many researchers. To study the expression of cytokines in the process of coccidial infection is helpful for us to understand the immune regulation mechanism of coccidial infection. Swaggerty et al [2] did research and found that the offspring of broiler individuals with high expression of IL-6 and IL-8 had significantly higher resistance to coccidia than those with low expression of IL-6 and IL-8. Lin Yuxin et al [3] sequenced the cecal transcriptome of chickens infected and uninfected with *E. tenella*. They found that IL-6, IL-8, IL-15, IL-12β and TGFβ2 were significantly enriched in the “Cytokine and Cytokine Receptor” Interaction Pathway during the infection. In this study, Real-time PCR technology was used for detecting the expression of IL-8, IL-2 and IL-15 genes in different tissues of infected groups and control groups, so as to explore the relationship between gene expression and coccidial infection, which enhances Our mastery of...
immunity mechanism of host resistance to coccidial infection and the interaction mechanism between host and coccidia, and provides some reference for further study of gene function.

2. Materials and methods

2.1. Experimental animals and Eimeria tenella sporulated oocysts
We randomly selected 18 1-day-old Jinghai yellow chickens from Jiangsu Jinghai Group, raised them to 30 days old, and then Natural clusters them into three groups, six in each group. 7500 E. tenella sporulated oocysts were fed to each chicken of Infection Group I, 15000 E. tenella sporulated oocysts were fed to each chicken of Infection Group II and the chickens of control group were fed with saline of the same amount. The experimental chickens were fed with feed without any coccidia or anticoccidial drugs, and were kept in flame-sterilized cages without pathogens. After fecal examination was carried in the pre-trial period, no coccidial infection was found. The sporulated E. tenella oocysts were donated by the parasite research office of Yangzhou University College of Veterinary Medicine, and they were collected from the offspring after one-generation breeding of healthy non-coccidian-immune chickens [4].

2.2. Collection of tissue samples
On the 7th day after infection, after slaughter, quickly collect the heart, liver, lung, kidney, spleen, small intestine, cecum, glandular stomach, bursa of Fabricius and thymus gland of the experimental chicken, totally 10 tissue samples. After place them in cryopreservation tubes, and then immediately store them with liquid nitrogen on site, and then transfer them to the laboratory, and then frozen in a -70 °C refrigerator for standby.

2.3. Extraction of RNA from chicken tissues (Trizol method) and synthesis of cDNA
RNA extraction kits for tissue samples were purchased from Nanjing Vazyme Co., Ltd, and the specific operation steps should be carried out according to the kit instructions. Nanodrop 1000 Concentration Analyzer is used for detecting the concentration and purity of RNA. When the OD260/OD280 ratio is between 1.8-2.0, it indicates that the RNA has high purity and can be used for cDNA synthesis. The name of the reverse transcription kit for cDNA synthesis is R123-HiScript Q RT SuperMix for qPCR (+gDNA wiper), which was purchased from Nanjing Vazyme Co., Ltd, and the specific operation is carried out according to the instructions of the kit.

2.4. Design and synthesis of fluorescent quantitative primers
According to the chicken IL-8, IL-2 and IL-15 gene sequences published in GenBank, the primer design software, Primer Premier 5.0, was used to design cross-intron primers. β-actin gene was used as the reference gene. Synthesis of all primers from Sangon Biotech (Shanghai) Co., Ltd, and the details of primer design can be seen in Table 1.

| Gene     | Primers sequence                | Annealing temperature | Length/bp |
|----------|---------------------------------|-----------------------|-----------|
| β-actin  | F: CAGCCATCTTTTTGGGTAT          | 60°C                  | 169       |
|          | R: CTGGATCTCCTGTCATCC           |                       |           |
| IL-2     | F: ATCTTTTGGCTGATTTCGTTAG       | 60°C                  | 165       |
|          | R: CTGGGTCTTCAGTTGGTTGTAG       |                       |           |
| IL-8     | F: AGTTCATCCACCCCTAATCC         | 60°C                  | 106       |
|          | R: CACACCTCTCTTCATCCCTT         |                       |           |
| IL-15    | F: AAAGAGATGCTGGGAGTG           | 60°C                  | 142       |
|          | R: AAAGGCAAAAGAAATGGGC          |                       |           |

2.5. Reaction conditions and reaction system of fluorescence quantitative PCR
The fluorescence quantitative PCR reaction kit (Q311-ChamQ SYBR qPCR Master Mix) was purchased from Nanjing Vazyme Co., Ltd, and the system composition is: 2×ChamQ SYBR qPCR
Master Mix 10 μL, forward primers and reverse primers are respectively 0.4 μL, 50× ROX Reference Dye 1 0.4 μL, cDNA10 μL, ddH2O 6.8 μL. The reaction procedure is: pre-denaturation, 95 ℃ 30 s; denaturation 95 ℃ 5 s, anneal 60 ℃ 34s, and 40 cycles; dissolution curve, 95 ℃, 15 s, 60 ℃, 1 min; 95 ℃, 15 s.

2.6. Calculation and statistical analysis of relative expression
The results of fluorescence quantification should be processed and analyzed by 2-ΔΔCt method. The 2-ΔΔCt specific calculation formula is: ΔΔCt = (mean Ct value of the target gene of the group to be tested - mean Ct value of the reference gene of the group to be tested) - (mean Ct value of the target gene of the control group - mean Ct value of the reference gene of the control group). Where, Ct (initial cycle number) is the number of cycles corresponding to the threshold value of fluorescence signal when fluorescence signal starts from background to exponential growth stage [5]. Use SPSS25.0 statistical software to analyze the expression of the target gene in different tissues by one-way ANOVA, and use Duncan method to carry out multiple comparisons.

3. Results and analysis

3.1. RNA extraction results
From Figure 1, we can see three bands, which are 28 s, 18 s and 5 s respectively, the 28 s and 18 s bands were clear and the RNA integrity is good, which can be used in the next step of the experiment.

Figure 1. Total RNA extraction results.

3.2. Fusion curve of target gene
Use ABI7500 fluorescence quantitative PCR system to carry out quantitative detection for the target gene. As shown in Figure 2, the four gene real-time fluorescence quantitative PCR products have only one specific peak, and the response specificity is good.
3.3. Expression of IL-8 gene in each tissue

From table 2, we can see that IL-8 gene expresses in all tissues. In the heart, lungs, kidney, spleen, cecum, glandular stomach and bursa of Fabricius, the expression of IL-8 gene in the infected group is higher than that in the control group, and the expression of IL-8 gene in the liver, spleen, cecum and bursa of Fabricius in the infected group II were far higher than that in the other two groups (P<0.05), and the expression in hymus gland is far higher than that in infection group I(P<0.01), and far higher than that the control group (P<0.05); the expression level of IL-8 gene in spleen of infected group I is significantly higher than that of control group (P<0.01).

Table 2. Expression of IL-8 gene in different tissues

| Sample          | Control group | Infection group I | Infection group II |
|-----------------|---------------|-------------------|-------------------|

Figure 2. Real-time PCR melt curve for the β-actin, IL-8, IL-2 and IL-15 gene in various tissues.
Heart 0.98±0.08 0.99±0.09 1.06±0.07
Liver 1.93±0.35b 1.84±0.61b 4.84±0.53a
Lungs 21.12±5.06 27.51±6.68 26.97±7.10
Kidney 1.33±0.43 2.17±0.22 1.92±0.26
Spleen 5.73±1.08c 8.89±1.33b 15.95±1.69A
Small intestine 7.66±2.21 5.49±2.04 4.84±2.62
Liver 1.93±0.35b 2.32±0.85ab 4.19±1.99a
Lungs 9.30±1.39b 17.41±1.77A 18.74±6.20A
Kidney 1.63±0.30b 4.32±1.72a 1.94±0.27b
Spleen 3.82±1.15 4.51±0.59 4.37±1.23
Small intestine 1.85±0.72b 4.39±1.28a 5.72±1.01a
Cecum 5.61±0.46c 13.69±2.80b 18.06±1.75A
Glandular stomach 5.93±1.34 6.04±2.36 7.80±2.87
Bursa of Fabricius 3.63±1.16 2.26±1.03 3.50±1.04
Thymus gland 2.53±0.61b 3.58±1.03b 6.37±1.25a

Note: the different capital letters in the same line indicated the difference at 0.01 and 0.05 lever, the same or no letters indicated there were no differences. The same as below.

3.4. IL-2 gene expression in each tissue
From table 3, we can see that IL-2 gene expresses in all tissues. For all tissues except bursa of Fabricius, the expression of IL-2 in infected group is higher than that in control group. For liver tissue, the expression of IL-2 gene in infected group II is significantly higher than that in control group (P<0.05); for lung tissue, the expression of IL-2 gene in infected group I is extremely significant higher than control group (P<0.01); for kidney tissue, the expression of IL-2 gene in infected group I is significantly higher than that in other two groups (P<0.05); for small intestine tissue, the expression of IL-2 gene in infected group is significantly higher than that in the control group (P<0.05); for the caecum, the expression of IL-2 gene in the infected group II is extremely significant higher than that in the infected group I(P<0.01), and the expression of IL-2 gene in the infected group I is extremely significant higher than control group (P<0.01); for the thymus gland, the expression of IL-2 gene in the infected group II is significantly higher than that in the other two groups (P<0.05).

Table 3. Expression of IL-2 gene in different tissues

| Sample                | Control group | Infection group I | Infection group II |
|-----------------------|---------------|------------------|-------------------|
| Heart                 | 1.00±0.07     | 1.02±0.09        | 1.05±0.09         |
| Liver                 | 1.20±0.24b    | 2.32±0.85ab      | 4.19±1.99a        |
| Lungs                 | 9.30±1.39b    | 17.41±1.77A      | 18.74±6.20A       |
| Kidney                | 1.63±0.30b    | 4.32±1.72a       | 1.94±0.27b        |
| Spleen                | 3.82±1.15     | 4.51±0.59        | 4.37±1.23         |
| Small intestine       | 1.85±0.72b    | 4.39±1.28a       | 5.72±1.01a        |
| Cecum                 | 5.61±0.46c    | 13.69±2.80b      | 18.06±1.75A       |
| Glandular stomach     | 5.93±1.34     | 6.04±2.36        | 7.80±2.87         |
| Bursa of Fabricius    | 3.63±1.16     | 2.26±1.03        | 3.50±1.04         |
| Thymus gland          | 2.53±0.61b    | 3.58±1.03b       | 6.37±1.25a        |

3.5. Expression of IL-15 gene in each tissue
From table 4, the IL-15 gene expresses in all tissues. For all tissues except small intestine, the expression of IL-15 in infected group is higher than that in control group. For lung tissue, the expression of IL-15 gene in two infected groups is significantly higher than that in control group (P<0.05); For Cecum tissue, the expression of IL-15 gene in two infected groups is extremely significant higher than that in control group (P<0.01); For glandular stomach tissue, the expression of IL-15 gene in infected group II is extremely significantly higher than that in control group (P<0.01), but not significantly different to that in infected group I.

Table 4. Comparison of IL-15 gene expression among different tissues.

| Sample                | Control group | Infection group I | Infection group II |
|-----------------------|---------------|------------------|-------------------|
| Heart                 | 0.99±0.08     | 0.96±0.06        | 1.05±0.09         |
| Liver                 | 8.03±1.56     | 8.53±1.36        | 10.93±1.43        |
| Lungs                 | 5.89±1.35b    | 9.04±0.79a       | 10.74±2.07a       |
| Kidney                | 2.52±0.77     | 3.15±0.08        | 3.04±0.88         |
| Spleen                | 3.61±0.91     | 4.21±1.26        | 5.78±0.85         |
4. Discussion

Cytokine is a kind of immune factor which has a wide range of biological activity function, it plays an important role in the induction and activation of immunocompetent cells [1]. The study on the expression pattern of cytokines in the course of coccidiosis infection is beneficial to the in-depth understanding of the immune regulation mechanism of anti-coccidiosis infection, and provides reference for the development of coccidiosis vaccine and the use of immune enhancers and adjuvants [6]. IL-8 is an important chemotactic cytokine in chicken body, and it binds to CXCR1 receptor on immune cells, and after it takes the immune cells to the affected area and make it activated, it participates in the immune response, so as to realize the resistance function of immune cell. At the same time, it can promote the survival, proliferation of endothelial cells and the expression of anti-apoptosis genes, regulate the generation of blood vessels, thus promoting wound healing [7,8]. After Min et al [9] inoculated pcDNA3-1E vaccine containing IL-8 and IL-15 genes to the experimental chickens, they found that CD3+ lymphocytes increased significantly, and the abscission number of oocysts in feaces decreased significantly. Swaggerty et al [10] did a research and found that the anti-coccidia ability of offspring of broiler individuals with high expression of IL-6 and IL-8 was far higher than that of individuals with low expression of IL-6 and IL-8. Xin Shijie et al [11] compared the chickens uninfected by E. tenella with chickens infected by E. tenella and found that the expression of IL-8 gene in the spleen and cecum of the infected group significantly increased. The results show that IL-8 gene expresses in all tissues, and for most tissues, the expression of IL-8 gene in the infected group is higher than that in the control group. The expression of IL-8 gene in the liver of toxic group II is significantly higher than that in the other two groups, the expression in the spleen and cecum is significantly higher than that in the other two groups, the expression in the bursa of Fabricius is significantly higher than that in the control group, and the expression in thymus gland is significantly higher than that in toxic group 1, and significantly higher than that in control group, which indicates that IL-8 gene plays some role in the response of chickens to E. tenella infection.

Interleukin-2 (IL-2) is a multifunctional cytokine, which is, under the induction of antigens and other factors, mainly produced by helper T cells. By binding to the IL-2R receptor on the target cell surface, IL-2 plays an important role in promoting B cell differentiation, NK cell activation and MHCII antigens expression stimulation [12-13]. At present, there are some studies showing that the recombinant IL-2 protein can inhibit the coccidia replication, reduce the cecum lesions, increase the IgA antibody secretion and increase the relative weight gain [14-15]. Cornelissen et al [16] did a research and found that the proliferation of macrophages and CD4+ in cecum tissue could be promoted after chicken was infected with E. tenella. Lillegaard et al [17] studied the effect of chicken IL-2 eukaryotic expression plasmid on E. tenella gene, and the result showed that IL-2 could, as an immune adjuvant, play an important role in inhibiting coccidia replication. Lowenthal et al [18] did a research and found that the chicken injected with cytokines such as IL-2 could induce the secretion of IgA antibody in immune cells, which enhanced the immunity to diseases.

As a cell growth factor recently discovered, IL-15 plays a function of biological activity similar to IL-2, which can enhance the cytotoxic activity of natural killer cells (NK) and stimulate its secretion of cytokines, which plays an important role in the early defense stage of host virus infection [19-20]. Lillegaard et al [21], through experiments, proved that IL-15 can stimulate the proliferation of T cell recipient cells and memory phenotype CD8 T cells induced by Salmonella in body, and can induce protective immunity to Toxoplasma gondii. When Ding et al [22] used IL-16, IL-15, IL-2 cytokines or Th1 regulation cytokines in combination with coccidia vaccine, they found that the efficacy of coccidia vaccine against coccidia was significantly enhanced. Min et al [8] did a research and it

|                | Small intestine | Cecum       | Glandular stomach | Bursa of Fabricius | Thymus gland |
|----------------|----------------|------------|-------------------|-------------------|-------------|
| IL-8 expression| 3.90±0.29      | 4.64±0.93  | 5.94±0.20AB       | 3.50±0.38B        |
|                | 0.96±0.13      | 1.36±0.71  | 2.32±0.42         | 1.93±0.11         |
|                | 1.43±0.30      |            |                   |                   |

Discussion

Cytokines are a kind of immune factor with a wide range of biological activity function, playing an important role in the induction and activation of immunocompetent cells [1]. The study on the expression pattern of cytokines in the course of coccidiosis infection is beneficial to a deep understanding of the immune regulation mechanism of anti-coccidiosis infection, providing reference for the development of coccidiosis vaccine and the use of immune enhancers and adjuvants [6]. IL-8 is an important chemotactic cytokine in chicken body, binding to CXCR1 receptor on immune cells, promoting immune cells to the affected area, and activating it, to realize the resistance function of immune cells. At the same time, it can promote the survival, proliferation of endothelial cells and the expression of anti-apoptosis genes, regulating blood vessel generation, promoting wound healing [7,8]. After Min et al [9] inoculated pcDNA3-1E vaccine containing IL-8 and IL-15 genes to experimental chickens, they found that CD3+ lymphocytes increased significantly, and the number of oocysts in feces decreased significantly. Swaggerty et al [10] conducted research and found that the anti-coccidia ability of offspring of broiler individuals with high expression of IL-6 and IL-8 was significantly higher than that of individuals with low expression of IL-6 and IL-8. Xin Shijie et al [11] compared chickens uninfected by E. tenella with chickens infected by E. tenella and found that the expression of IL-8 gene in the spleen and cecum of the infected group significantly increased. The results show that IL-8 gene is expressed in all tissues, and for most tissues, the expression of IL-8 gene in the infected group is higher than that in the control group. The expression of IL-8 gene in the liver of toxic group II is significantly higher than that in the other two groups, the expression in the spleen and cecum is significantly higher than that in the other two groups, the expression in the bursa of Fabricius is significantly higher than that in the control group, and the expression in thymus gland is significantly higher than that in toxic group I, and significantly higher than that in control group, indicating that IL-8 gene plays some role in the response of chickens to E. tenella infection.

Interleukin-2 (IL-2) is a multifunctional cytokine, under the induction of antigens and other factors, mainly produced by helper T cells. By binding to the IL-2R receptor on cell surface, IL-2 plays an important role in promoting B cell differentiation, NK cell activation and MHCII antigens expression stimulation [12-13]. At present, there are some studies showing that recombinant IL-2 protein can inhibit coccidia replication, reduce cecum lesions, increase IgA antibody secretion and increase relative weight gain [14-15]. Cornelissen et al [16] conducted research and found that macrophages and CD4+ in cecum tissue could be promoted after chicken was infected with E. tenella. Lillegaard et al [17] studied the effect of chicken IL-2 eukaryotic expression plasmid on E. tenella gene, and the result showed that IL-2 could, as an immune adjuvant, play an important role in inhibiting coccidia replication. Lowenthal et al [18] conducted research and found that chicken injected with cytokines such as IL-2 could induce IgA antibody secretion in immune cells, enhancing immunity to diseases.

As a cell growth factor recently discovered, IL-15 plays a function of biological activity similar to IL-2, which can enhance the cytotoxic activity of natural killer cells (NK) and stimulate cytokine secretion, playing an important role in the early defense stage of host virus infection [19-20]. Lillegaard et al [21], through experiments, proved that IL-15 can stimulate the proliferation of T cell recipient cells and memory phenotype CD8 T cells induced by Salmonella in body, and can induce protective immunity to Toxoplasma gondii. When Ding et al [22] used IL-16, IL-15, IL-2 cytokines or Th1 regulation cytokines in combination with coccidia vaccine, they found that coccidia vaccine efficacy against coccidia was significantly enhanced. Min et al [8] conducted research and it
showed that, compared with the number of CD3 lymphocytes in chickens only fed with pcDNA3-1E gene vaccine, the number of CD3 lymphocytes in chickens inoculated with both pcDNA3-1E vaccine and IL-15 gene plasmid at the same time significantly increased, which could enhance the effect of the vaccine against coccidia. In this study, IL-15 gene expresses in all tissues. In the lung tissue, the expression of IL-15 gene of the two infected groups was significantly higher than that in the control group; in the cecum tissue, the expression of IL-15 gene in the infected group was significantly higher than that in the control group; in the glandular stomach tissue, the expression of IL-15 gene in the infected group was significantly higher than that in the control group. In conclusion, we can see that IL-15 gene plays an important role in the process of chicken being infected with E. tenella.

5. Conclusions
The comparative results of IL-8, IL-2 and IL-15 gene expression in 10 different tissues infected with different doses of E. tenella shows that the three genes play a certain role in the process of E. tenella infection, especially in the E. tenella infected site, the cecum. The results provide a basis for the study of the relationship between the three genes and the chicken coccidiosis immunity and resistance.

Acknowledgement
This thesis is supported by the construction of Jiangsu modern agricultural industry technology system (JATS [2019] 449), Jiangsu Province University Discipline Construction Project (PAPD). Special Fund for National Modern Agricultural Industry Technology System (CARS-41-G23). Research project of the Jiangsu Agri-animal Husbandry Vocational College (S2020060)

Special thanks to Xie Kaizhou, Wang Jinyu, Zhang Tao and Zhang Suixi for their help in the research work

References
[1] Mucksová J, Chalupský K, Plchý J, et al. Simultaneous detection of chicken cytokines in plasma samples using the Bio-Plex assay [J]. Poultry Science, 2018, 97(4):57-63.
[2] Swaggerty CL, Pevzner IY, and Kogut MH. Selection for pro-inflammatory mediators produces chickens more resistant to Eimeria tenella [J], Poultry Science. 2015, 94(11):37–42.
[3] Lin Y.X. RNA Sequencing Analysis of Chicken Cecum Tissues Following E. tenella Infection and Coccidiosis Evaluation of Jinghai Yellow Chicken Cross-Breeding System Parents(D), Yangzhou University, 2015.
[4] YU H.L ZOU W.B. WANG X.H, et al. RNA Sequencing Analysis of Cecum Tissues of Jinghai Yellow Chickens Infected by E. tenella [J], Biotechnology Bulletin, 2019, 35(11):64-71.
[5] Zhang J.J. Studies on Polymorphisms of Chicken IL-6 Gene Sequence and Relationship with Coccidiosis-resistant parameters of Jinghai Yellow Chicken (D), Yangzhou University, 2017.
[6] Hou J, Li R. L, Pan H, et al. Research Advances of Artificial Induction and Detection Methods of Drug-resistance in Chicken Coccidia [J]. China Poultry, 2010, 32(19):49-51.
[7] WERB LMCZ. Inflammation and cancer [J]. Nature, 2002, 420(6917):860.
[8] Li A, Dubey S, Varney ML, et al. IL-8 directly enhanced endothelial cell survival, proliferation, and matrix metalloproteinases production and regulated angiogenesis [J]. The Journal of Immunology, 2003, 170(6):3369-3376.
[9] Min W, Lillehoj HS, Burnside J, et al. Adjuvant effects of IL-1β, IL-2, IL-8, IL-15, IFN-α, IFN-γ, TNF-β4 and lymphotactin, on DNA vaccination against eimeria acervulina. Vaccine. 2001, 20(2), 267-275.
[10] Swaggerty CL, Pevzner IY, and Kogut MH. Selection for pro-inflammatory mediators produces chickens more resistant to Eimeria tenella [J], Poultry Science. 2015, 94(11):37–42.
[11] Xin S J, Wang X H, Dai G J, et al. Effect and correlation analysis of Eimeria tenella infection on IL-6, IL-8 and CCLi2 genes expression in spleen and caecum of Jinghai Yellow Chicken (Gallus gallus) [J]. Acta Agriculturae Zhejiangensis, 2019, 31(01):44-51.
[12] Boyman O, Kovar M, Rubinstein M P, et al. Selective stimulation of T cell subsets with antibody-cytokine immune complexes[J]. Science, 2006, 311(5769): 1924-1927.

[13] Fehniger TA, Bluman EM, Porter MM, et al. Potential mechanisms of human natural killer cell expansion in vivo during low dose IL-2 therapy[J]. J Clin Invest, 2000, 106(1):117-24.

[14] Kotenko SV, Gallagher G, Baurin VV, et al. IFN-lambdas mediate antiviral protection through a distinct class II cytokine receptor complex [J]. Nat Immunol, 2003, 4(1):69-77.

[15] Sheppard P, Kindsvogel W, Xu W, et al. IL-28, IL-29 and their class II cytokine receptor IL-28R[J]. Nat Immunol, 2003, 4(1): 63-68.

[16] Cornelissen JBWJ, Swinkels WJC, Boersma WA, et al. Host response to simultaneous infections with Eimeria acervulina, maxima and tenella: A cumulation of single responses[J]. Veterinary Parasitology, 2009, 162(1):58-66.

[17] Lillehoj HS, Kang SY, Keller L, et al. Lymphokines secreted by an avian T cell lymphoma or by sporozoite-stimulated immune T lymphocytes protect chickens against avian coccidiosis[J]. Exp Parasitol, 1989, 69(1): 54-64.

[18] Lowanthal JW, Connick TE, Mewayers PG, et al. Development of T cell immune responsiveness in the chicken [J]. Immunol Cell Biol, 1994, 72(2): 115-122.

[19] Loza M J, Zamai L, Azzoni I, et al. Expression of type 1 (interferon gamma) and type: (interleukin-13, interleukin-5) cytokines at distinct stages of natural killer cell differentiation from progenitor cells [J]. Blood, 2002, 99: 1273-1281.

[20] Rodella L, Zamai L, Rezzani R, et al. Interleukin 2 and interleukin 15 differentially predispose natural killer cells to apoptosis mediated by endothelial and tumour cells [J]. Br J Haematol, 2001, 115: 442-450.

[21] Lillehoj H S, Min W, Kang D C, et al. Molecular, cellular, and functional characterization of chicken cytokines homologous to mammalian IL-15 and IL-2[J]. Veterinary Immunology & Immunopathology, 2001, 82(3):229-244.

[22] Ding X, Lillehoj HS, Quiroz MA, et al. Protective Immunity against Eimeria acervuline following In Ovo Immunization with a Recombinant Subunit Vaccine and Cytokine Genes [J]. Infection & Immunity, 2004, 72(12):6939-6944.