**Research Article**

**Research on the Effect of *Pediococcus pentosaceus* on *Salmonella enteritidis*-Infected Chicken**

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Received 2 July 2020; Accepted 28 September 2020; Published 12 October 2020

Academic Editor: Mansour El-Matbouli

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*Salmonella enteritidis* can cause significant morbidity and mortality in humans and economic loss in the animal industry. Improving the innate immunity is an effective method to prevent *S. enteritidis* infection. *Pediococcus pentosaceus* is a Gram-positive coccus which had probiotics properties. Numerous previously published studies reported that probiotics were beneficial to gut microbiota by changing the intestinal flora structure and inhibiting the harmful microbial growth to enhance the innate immunity. We investigated the immunological effects of *P. pentosaceus* on *Salmonella*-infected chickens by the following experiment. A total of 120 broilers from AA line were fed and divided into 2 groups (treated and control groups) for the experiment from day 1. The control group was fed with the basic diet, while the treated group was fed with the basic diet adding *P. pentosaceus* microcapsule with the bacterial concentration of 1 g/kg in the feed and bacterial counts $2.5 \times 10^9$ CFU/g. All the birds were given with 0.5 ml of *S. enteritidis* bacterial suspension ($10^9$ CFU/ml) through oral cavity at day 9. The number of dead birds was recorded and used in the analysis. The bacterial culture method and quantitative real-time PCR analysis were used to evaluate the effects of *P. pentosaceus* on chickens infected with *S. enteritidis* and to ascertain the mechanism of the effect. The results showed that the *P. pentosaceus* could restrain the pathogenicity of *S. enteritidis* and reduce the death rate from 44.4% to 23.3%. The flora in the caecum exhibited “rising-declining” trends, and the gene (*TLR4, MyD88, TRAF6, NF-κB, TNF-a, IL6, and IL8*) expression pattern was different between the experimental and control group. *P. pentosaceus* as a probiotic may competitively inhibit the growth of *S. enteritidis* and control the inflammatory response through regulating the gene expression which involved in the toll-like receptor pathway and inflammation pathway.

1. Introduction

*Salmonella enteritidis* is a common pathogenic bacterium for all species of mammals and fowls. *S. enteritidis* can cause serious economic loss in the animal industry, especially in the poultry production, and which also can influence human health [1]. Reports of worldwide human morbidity caused by infection with *S. enterica* started to appear as early as the mid-1970s [2], and the pathogenic factor most commonly associated with food like eggs [3]. Due to the pathogenicity and universality of *S. enteritidis*, the prevention is more important than treatment. Thus, improving the immunity of animals is an effective method to prevent infection.

*Pediococcus pentosaceus* (CGMCC No. 6566) is a Gram-positive coccus with probiotic properties [4]. An abundance of previous studies have reported that probiotics are beneficial to the gut microflora by changing the intestinal flora structure and inhibiting the harmful microbe growth in order to enhance the innate immunity [4–6]. Probiotics as an immunity activator can lead to production of antibodies and improve the phagocytic function of cells in order to stimulate the immune system, by inducing humoral immunity and cellular immunity, thus enhancing the resistance to diseases [7–11]. The *P. pentosaceus* bacterium used in this study was obtained from a Chinese indigenous Caoke chicken, as previous research had shown that *P. pentosaceus* can improve...
2.2. Birds

To further investigate, whether *P. pentosaceus* can improve the immunity of chicken needs to be further investigated.

Toll-like receptors (TLRs) are essential components of the innate immune system. To date, more than ten TLR genes have been found, including the toll-like receptor 4 (TLR4) gene which encodes an important factor for the innate immune system that senses bacterial lipopolysaccharide (LPS) and is a key player in the defense against pathogenic microorganisms [12]. Macrophages induce the innate immunity by recognizing pathogens through the TLRs that sense the pathogen-associated molecular patterns. The myeloid differentiation factor 88 (MyD88) encodes an essential adapter protein molecule for most TLRs that mediate the induction of inflammatory cytokines through nuclear factor κB (NF-κB) [13]. LPS can act through two different methods to activate the TLR4/NF-κB pathway: one is dependent on MyD88, whereas the other is not. Lomaga et al. [14] showed that TNF receptor-associated factor 6 (TRAF6) was crucial not only in interleukin 1 (IL-1) and CD40 signaling, but also, surprisingly, in LPS signaling and was also essential for prenatal and postnatal survival. The immune response is a complex process involving the innate immune system, whose activation is indicated by the release of inflammatory factors, such as IFN-β, TNF-α, IL6, and IL8, which play an important role in this process.

In this study, we aimed to investigate the effect of the *P. pentosaceus* on *S. enteritidis* infection. To this end, we evaluated the body condition change, caecum flora count, and the expression of TLR pathway genes and inflammatory factor genes in the spleen and caecum of *S. enteritidis*-infected chickens, which had been fed a diet with or without *P. pentosaceus*.

2. Materials and Methods

2.1. Birds and Management. A total of 120 arbor acre broilers (AA) were divided into 2 groups (treated and control groups) and fed for the experiment from day 1. The control group was fed the basic diet (composition and nutrient levels are shown in Table 1), while the treated group was fed the basic diet plus *P. pentosaceus* microcapsule (1 g/kg, bacterial counts 2.5 × 10⁹ CFU/g), which was formed according to the Ning’s method [15]. All birds were reared cage free under standard conditions of temperature, humidity, and ventilation. The chickens had free access to feed and water during the entire rearing period. Birds were managed with due consideration to bird welfare. All procedures involving animals were approved by the Institutional Animal Care and Use Committee of the Sichuan Agriculture University (DK20134457). The experimental animals were anesthetized by intravenous injection of pentobarbital sodium at a dose of 40 mg/kg and euthanized by high cervical dislocation. Alleviated the suffering of experimental animals as much as possible during the experiment and cremated them centrally after the experiment.

2.2. Birds’ Treatment and Sample Collection. All the birds were given with 0.5 ml of *S. enteritidis* bacterial suspension (10⁹ CFU/ml) through oral cavity at day 9. For each group, 4 birds per time (1-, 3-, 7-, and 14-day postinjection) were collected for evaluating and sample collection. Each birds’ spleen and one of caecum were collected immediately after slaughter and transferred to liquid nitrogen for RNA extraction. Another caecum was collected, and the contents were extracted for bacterial culturing. Body temperature was measured through the cloaca at 5 time points, 0- and 12-hour postinjection (hpi), and 1-, 3-, and 7-day postinjection (dpi).

2.3. Caecum Microbiota Culture and Count. The contents of the caecum (0.2 g) were added into 1.8 ml peptone and mixed. Then, the mixture was diluted to 10, 10², 10³, and 10⁴ and cultured in media (Brilliant Green Agar for *Salmonella* and MacConkey medium for *Escherichia coli*). *Escherichia coli* was cultivated in a 37°C incubator for 24 h and identified as Gram-negative, while the *Salmonella* was cultivated at 37°C in an incubator for 18-24 h and identified by triple sugar iron agar.

2.4. Total RNA Isolation and cDNA Synthesis. Total RNA was isolated from the spleens and caecum (about 100 mg from each tissue sample) using Trizol reagent (Invitrogen Corp., Carlsbad, CA, USA) following the manufacturer’s instructions. Total RNA concentration and purity were determined at A260, 280, and 230 nM using the NanoVue.

| Composition       | Content (%) |
|-------------------|-------------|
| Corn              | 55.18       |
| Soybean meal      | 24.90       |
| Fermentation protein | 5         |
| Compound oil      | 4           |
| Wheat bran        | 4           |
| Rapeseed meal     | 2.65        |
| Calcium bicarbonate | 1.79       |
| Calcium carbonate | 0.87        |
| Mineral premix¹   | 0.50        |
| DL-methionine     | 0.16        |
| Choline           | 0.10        |
| Mildew preventive | 0.10        |
| Vitamin premix²   | 0.03        |
| Salt              | 0.40        |
| Bentonite         | 0.30        |
| Nutrient levels   | Content (%) |
| Crude protein     | 19.24       |
| Crude cellulose   | 3.08        |
| ME (MCal/kg of DM)| 2.72        |
| Lysine            | 1.05        |
| Available phosphorous | 0.21      |
| Dicalcium phosphate| 1.88       |
| Calcium carbonate | 0.91        |
| Methionine        | 0.17        |
Plus spectrophotometer (GE Healthcare, Chicago, IL, USA), and RNA integrity was evaluated by agarose-formaldehyde electrophoresis.

The first strand cDNA was obtained using the ImProm-II Reverse Transcription System (TaKaRa Biotechnology Co. Ltd., Dalian, China). The reaction was performed in a volume of 40 μL containing 8 μL of 5× PrimerScript Buffer, 2 μL of PrimerScript RT Enzyme Mix I, 1 μL of 50 μM Oligo dT forward and reverse primer, 2 μL of 100 μM Random hexamers, 4 μL of total RNA (400 ng), and 22 μL RNase-free dH₂O. The reverse transcription (RT) reaction was performed at 37 °C for 15 min with a final step of 85 °C for 15 s and then stored at −20 °C.

2.5. Real-Time Quantitative PCR Analysis of mRNA Expression. The expression levels of 8 chicken gene mRNAs at different stages of development in different tissues were measured by real-time quantitative PCR (qPCR). Expression of the chicken β-actin gene (GenBank accession number NM_205518) was used as an internal control. Primers were designed and synthesized by TaKaRa Biotechnology Inc. (Table 2).

The cycling conditions consisted of an initial denaturation step of 2 min at 95 °C, followed by 39 cycles of 5 s at 95 °C, 30 s at 60 °C, 30 s at 72 °C, followed by a final extension period of 5 min at 72 °C. A melting curve analysis was performed at a temperature of 65 °C to 95 °C, increasing at a rate of 0.5 °C/s. The qPCR reaction was performed in a volume of 10 μL, which included 5 μL 2× SYBR Green SuperMix (Bio-Rad, Inc.), 1 μL of 10× diluted cDNA, 0.4 μL of forward and reverse primers (350 nM stocks), and 3.2 μL nuclease-free H₂O. Each assay was conducted in triplicate in 96-well plates (Bio-Rad, Inc.). A no template control (NTC) for each primer set was included in each run. The range of amplification efficiency of those genes and β-actin was from 95% to 105%.

2.6. Statistical Analyses. The 2^−ΔΔCt method of quantification [16] was used to calculate the gene expression values. The formula of ΔΔCt was followed.

\[
\Delta\Delta Ct = (Ct, target - Ct, action) \times \text{time} - (Ct, target - Ct, action) \times \text{time0.}
\] (1)

Using the GLM procedure of SAS 8.2 (SAS Institute Inc., Cary, NC, USA), we analyzed the differences in gene expression between groups and at different time points by ANOVA and define the significance level as \(P < 0.05\).

3. Results

3.1. Body Temperature. The results of the mean body temperature in the two groups were shown in Figure 1. Both groups had normal temperature at 0 hpi, whereas all animals had increased temperature after given with Salmonella. The control group had the highest temperature (42.2°C) at 1 dpi and then showed continuous decline, until it reached 41°C at 3 dpi. The experimental group experienced continuous rise in temperature during the first 3 dpi, when it reached up to 42.4°C. However, the temperature declined at 7 dpi, but it was still higher than it was before injection with Salmonella.

3.2. Health State and Death Rate. Most of the birds showed clinical symptoms at 12 hpi, which included depression, decreased ingestion, diarrheal disease, being afraid of the cold, and dyspnea. Those symptoms were more serious in the control group than in the experimental group. The infected chicken fed a diet without \(P. \ pentosaceus\) started to die at 1 dpi, and most of them died between 3 and 7 dpi, while...
3.4. Gene Expression. The relative expression of \textit{Escherichia coli} in the control group was significantly higher than that in the experimental group, and the difference was significant \((P < 0.05)\) at 1 and 3 dpi.

The relative expression of the \textit{MyD88} gene during the time it was evaluated exhibited a declining trend in both the control group and experimental group (Figure 2(b)). However, overall, the expression of this gene was higher in the control group than that in the experimental group, and there was a very significant difference \((P < 0.01)\) at 1 and 7 dpi and a significant difference \((P < 0.05)\) at 14 dpi.

The relative expression of the \textit{TRAf6} gene showed a declining trend in the control group, which was highest at 1 dpi (Figure 2(c)). The experimental group was different from the control group, which was lowest at 1 dpi, but then continuously rose to the highest level at 7 dpi. The experimental group was lower than the control group at 1 dpi, and the difference was highly significant \((P < 0.01)\). The experimental group showed higher expression than the control group at 7 and 14 dpi, and the difference was significant \((P < 0.05)\) and highly significant \((P < 0.01)\), respectively.

The relative expression of the \textit{NF-κB} gene exhibited a “declining-rising-declining” trend, which was highest at 7 dpi. Overall, the control group was higher than the experimental group at 1 dpi and 3 dpi, and the difference was very significant \((P < 0.01)\).

3.4.2. Toll Pathway Gene Expression in Caecum. The relative expression of the \textit{TLR4} gene exhibited a declining trend both in the control and experimental group (Figure 3(a)), and the expression in the experimental group was highly significantly \((P < 0.01)\) lower than that in the control group at the first three time points and significantly \((P < 0.05)\) lower at 14 dpi.

The relative expression of the \textit{MyD88} gene showed different trends between the control group and experimental group (Figure 3(b)). The control group exhibited a declining trend, which was highest at 1 dpi, and subsequently continued its decline. The trial group had a “rising-declining” trend and was highest at 7 dpi. A comparison of the two groups indicated that their difference was very significant \((P < 0.01)\) at 1 dpi and 3 dpi.

The relative expression of the \textit{TRAf6} gene showed different trends between the control and experimental groups (Figure 3(c)). The control group exhibited a declining trend, which was highest at 1 dpi, and continued to decline. The experimental group had a “rising-declining” trend, which was highest at 7 dpi, but the expression was not significantly different between 3 dpi and 7 dpi. A comparison of the two groups revealed that the control group had a lower expression than the experimental group, and the difference was significant \((P < 0.05)\) and very significant \((P < 0.01)\) at 3 dpi and 7 dpi, respectively.

The relative expression of the \textit{NF-κB} gene showed a different trend between the control and experimental groups (Figure 3(d)). The control group had a declining trend, and it was highest at 1 dpi and continued to decline. The experimental group exhibited a “rising-declining” trend and highest at 7 dpi. A comparison of the two groups revealed that

![Figure 1: Comparison of body temperature postinfection between the two groups.](image-url)
the experimental group, and the di-

The control group always showed a higher expression than both in the control and experimental groups (Figure 4(a)).

Table 3: Comparison of *Salmonella* and *Escherichia coli* load in the caecum of postinfection between the two groups of chicken (mean ± SD log 10).

| Strain            | Group        | Sample size | 1 d        | 3 d       | 7 d       | 14 d      |
|-------------------|--------------|-------------|------------|-----------|-----------|-----------|
| *Salmonella*      | Control group| 4           | 7.44 ± 0.42<sup>a,x</sup> | 8.00 ± 0.33<sup>y</sup> | 7.86 ± 0.16<sup>a,y</sup> | 7.77 ± 0.31<sup>a,y</sup> |
|                   | Trial group  | 4           | 7.20 ± 0.18<sup>a,x</sup> | 7.27 ± 0.69<sup>a,x</sup> | 7.39 ± 0.20<sup>a,x</sup> | 6.65 ± 0.42<sup>a,x</sup> |
| *Escherichia coli*| Control group| 4           | 8.89 ± 0.09<sup>y</sup> | 9.76 ± 0.40<sup>y</sup> | 9.77 ± 0.45<sup>y</sup> | 8.96 ± 0.34<sup>a,x</sup> |
|                   | Trial group  | 4           | 7.63 ± 0.35<sup>a,x</sup> | 8.42 ± 0.42<sup>a,x</sup> | 8.83 ± 0.39<sup>a,x</sup> | 8.37 ± 0.41<sup>a,x</sup> |

Note: **a** represent the comparison of the data in the group, and different superscripts mean significant difference (*P* < 0.05); **b** represent the comparison of the data between groups, and different superscripts mean significant difference (*P* < 0.05).

Figure 2: Expression of (a) TLR4, (b) MyD88, (c) TRAF6, and (d) NF-κB in the spleen samples of broiler chickens in the control and the treatment groups. Note: **** means the expression of the target gene between trial group and control group had highly significant (*P* < 0.01) difference; *** means the expression of the target gene between trial group and control group had significant (*P* < 0.05) differences. Error bars indicate the standard deviation.

The expression in the experimental group was significantly (*P* < 0.05) lower than that in the control group, which was very significantly (*P* < 0.01) higher than that in the control group at 7 dpi.

3.4.3. Inflammatory Factor Gene Expression in Spleen. The relative expression of the *IFN-β* gene showed a declining trend both in the control and experimental groups (Figure 4(a)). The control group always showed a higher expression than the experimental group, and the difference was highly significant (*P* < 0.01) and significant (*P* < 0.05) at 1 dpi and 14 dpi, respectively.

The relative expression of the *TNF-α* gene exhibited different trends in the control and the experimental groups (Figure 4(b)). The control group had a declining trend, which was highest at 1 dpi, and continued to decline. The experimental group had a "rising-declining" trend, which was highest at 3 dpi. Expression in the experimental group was higher than in the control group, and the difference was not significant (*P* > 0.05) at 3, 7, and 14 dpi.

The relative expression of the *IL6* gene was highest at 1 dpi in the control group (Figure 4(c)), and it was very significantly (*P* < 0.01) higher than that in the experimental group. The expression exhibited a rising trend in the experimental group, which was highest at 7 dpi. A comparison of the two groups revealed that the expression was always higher in the experimental group than that in the control group, and the difference reached significance (*P* < 0.05) at 14 dpi.
3.4.4. Inflammatory Factor Gene Express in Caecum. The relative expression of the IL8 gene showed the same trend in the control and experimental groups (Figure 4(d)). The expression in both groups reached the highest levels at 7 and 14 dpi and subsequently declined to a level lower than that at 1 dpi. A comparison of the two groups revealed that there was no difference in expression at 1 dpi, while the expression was higher in the control group than that in the experimental group at 3 and 7 dpi, and the difference was significant at 7 dpi. Remarkably, the expression was higher in the experimental group than in the control group at 14 dpi, and the difference was highly significant (P < 0.01).

3.5. Gene Expression in Different Tissues. To further determine whether the expression of the above genes was tissue specific, we analyzed the expression levels of those genes in two different tissues. The results showed that the expression of four TLR pathway genes was “decreased” in both the spleen and caecum tissues in the control group, and the highest expression was at 1 dpi. The expression was different in the experimental group. Among the four inflammatory factor genes, expression of IFN-β, TNF-a, and IL6 genes exhibited the same trends in those two tissues as over the time period at which they were evaluated, while the IL8 gene showed different trends in the tissues examined.
4. Discussions

4.1. Body Temperature and Flora Number. After injection with the Salmonella bacterium, the body temperature rose in both groups. As the time progressed, the health of the birds in the control group deteriorated, the body temperature declined, and the number of dead birds rose. The rise in the body temperature was beneficial for developing resistance to the disease, whereas the low temperature was not beneficial to the enhancement of the inflammatory reaction whereby germs are killed. As the chicken was infected by Salmonella, the organism mounted an inflammatory response to kill germs, which led to a rise in the body temperature.

The death of chickens in the control group mainly occurred between 3 and 7 dpi, and the body temperature was lower than that of animals in the experimental group. This result was consistent with those of previous research. For instance, Liping and Yujie [17] reported that the broiler chicken fed a diet supplemented with complex microorganisms can improve the resistance to S. pullorum and reduce the chicken death rate to 20%. Additional studies reported that lactobacillus can improve the immune function and enhance the natural immunity and intestinal mucosa resistance [18–20]. We can speculate that continuous supplementation of the diet with P. pentosaceus can reduce the harmful effects of pathogenic bacteria in chicken.

Several studies have reported that probiotics can compete with Salmonella and Escherichia Coli to bind with Caco-2 to inhibit the pathogenic bacterium growth [21–23], and the microbial community structure was altered as the E. coli number increased when infected by S. pullorum [18]. Our study results on flora number revealed that the flora number in caecum had increased but not significantly and quickly returned to normal levels in the experimental group. Meanwhile, in the control group, the flora number quickly increased and the difference was significant. This result is consistent with those of the other studies and indicates that the infection with the pathogenic bacterium can change the microbial community structure in caecum, and the effect was less intense in the experimental group than that in the control group. It is possible that this may be caused by physical and mechanical competitive inhibition of the probiotics to reduce the Salmonella seeding. Additional studies indicated that Lactobacillus, as one of the probiotics, can secrete organic acid to reduce the pH value, produce secondary metabolites like bacteriostatic toxin, and inhibit the pathogenic bacterium growth to protect the intestinal biological barrier [24, 25]. P. pentosaceus which belongs to the Lactobacillaceae family may be share the same resistance mechanism.

4.2. Gene Expression. The TLR pathway plays an important role in mediating Salmonella transmembrane signal transduction and stimulates the body immune system. TLR4, MyD88, TRAF6, and NF-κB are important genes in the TLR pathway, and their expression can indicate the activation intensity of the TLR pathway. After injection with
Salmonella, the gene expression changed along with time, which was consistent with previously reported results [26–29], indicating that the TRL4 pathway was connected with the S. enteritidis infection. A comparison of the experimental and control groups revealed that the gene expression patterns of the two groups were not consistent, which indicated that the added P. pentosaceus in the diet can affect the gene expression. The TLR4 and MyD88 gene expression in spleen and caecum were higher in the control group than that in the experimental group. These results suggested that the pathogenic bacterium activated the TLR pathway, which was higher in the control group than that in the experimental group. This activation may occur as a result of the P. pentosaceus’ competitive inhibition, physical, and mechanical that decreases the Salmonella seeding thus reducing the stimulation of intestinal cells by LPS to attenuate the immune response. Research has shown that TLR4 expression in enterocyte was blocked, which contributes to maintaining the steady state [30]. Higher expression of NF-κB can increase the inflammatory reaction through activation of the downstream inflammation factor genes in the TLR4 pathway. TNF-α as an important inflammatory factor that can act as an anti-infection pyrogenic factor that causes fever. IL6 can strengthen the effects of the other cytokines. The expression of inflammatory factor genes declined at 3 d, a result that was consistent with the body temperature change trend. According to our results, we can speculate that P. pentosaceus can restrain the activation of the TLR4 pathway by S. enteritidis, thus causing a reduction of TLR4 signaling, a decline of the expression of the MyD88 gene, and the decline of the expression of downstream inflammation factor genes. Whether the change in the expression of those genes caused the protein changes needs to be further investigated in the future. Why the expression of these genes declined between 3 dpi and 7 dpi in the control group, while they were increased in the experimental group? According to the results of the evaluation of the body temperature and flora number change and chicken death condition, we surmised that the decline in body temperature was not a contributing factor to the inflammatory response, which may have allowed the bacteria to grow and cause the rise in microbial load and the increased death rate.

In this study, we just comparatively analyzed the effect of added and not added Pediococcus pentosaceus on Salmonella enteritidis-infected chicken, so we do not design the healthy control group, and this is a bug. On this basis, it seems obvious that further study is needed to design the healthy control group.

5. Conclusions

Through this research, we can speculate that the P. pentosaceus can not completely eliminate the S. enteritidis infection and it just inhibited the bacterial growth. The mechanism of inhibition of S. enteritidis by P. pentosaceus may involve the inhibition of the expression of certain genes usually induced

![Figure 5](image_url)

**Figure 5:** Expression of (a) IFN-β, (b) TNF-α, (c) IL6, and (d) IL-8 genes in the caecum samples of broiler chickens in the control group and the treatment group. Note: “**” means the difference in the expression of the target gene between the experimental and control groups was highly significant (P < 0.01); “*” means the difference in expression of target gene between the experimental and control groups was significant (P < 0.05). Error bars represent standard deviation.
by *S. enteritidis* or alternatively may result from direct effects on the TLR4 gene and inhibition of its expression.

**Abbreviations**

*S. enteritidis*: Salmonella enteritidis  
*P. pentosaceus*: Pediococcus pentosaceus  
TLRs: Toll-like receptors  
TLR4: Toll-like receptor 4  
LPS: Lipo polysaccharide  
MyD88: Myeloid differentiation factor 88  
NF-κB: Nuclear factor κB  
TRAF6: TNF receptor-associated factor 6.

**Data Availability**

Data supporting the findings is contained within the manuscript.

**Ethical Approval**

All procedures involving animals were approved by the Institutional Animal Care and Use Committee of the Sichuan Agriculture University.

**Conflicts of Interest**

The authors report no conflict of interest. The authors alone are responsible for the content and writing of the manuscript.

**Authors’ Contributions**

D. Lan carried out the molecular genetic studies and drafted the manuscript. X.Y. Xun, Y.D. Hu, and N.Z. Li carried out the immunoassays. X.Y. Xun, C.W. Yang, and X.S. Jiang participated in the design of the study and performed the statistical analysis. Y.P. Liu conceived of the study, participated in its design and coordination, and helped to draft the manuscript. All authors read and approved the final manuscript. D. Lan, X.Y. Xun, and Y.D. Hu contributed equally to this work.

**Acknowledgments**

The authors acknowledge Dr. Wang Ye for providing purely technical help and thank the editor and reviewers. This study was supported by the project of the application basis project from the Science and Technology Department of Sichuan Province (No. 2019NZJ0009, No. 2020YFN0093, and 2016NYZ0043) and the project of Sichuan Science Research (No. 2018HH0083 and No. 2019JDKP0048).

**References**

[1] S. Suzuki, “Pathogenicity of *Salmonella enteritidis* in poultry,” *International Journal of Food Microbiology*, vol. 21, no. 1-2, pp. 89–105, 1994.

[2] A. J. Bäumler, B. M. Hargis, and R. M. Tsolis, “EPIDEMIOLOGY: Enhanced: Tracing the origins of *Salmonella* outbreaks,” *Science*, vol. 287, no. 5450, pp. 50–52, 2000.

[3] J. Guard-Petter, “The chicken, the egg and *Salmonella enteritidis*,” *Environmental Microbiology*, vol. 3, no. 7, pp. 421–430, 2001.

[4] V. A. Torok, R. J. Hughes, K. Ophel-Keller, M. Ali, and R. MacAlpine, “Influence of different litter materials onecal microbiota colonization in broiler chickens,” *Poultry Science*, vol. 88, no. 12, pp. 2474–2481, 2009.

[5] V. A. Torok, K. Ophel-Keller, M. Loo, and R. J. Hughes, “Application of methods for identifying broiler chicken gut bacterial species linked with increased energy metabolism,” *Applied and Environmental Microbiology*, vol. 74, no. 3, pp. 783–791, 2008.

[6] H. W. Kim, F. F. Yan, J. Y. Hu, H. W. Cheng, and Y. H. B. Kim, “Effects of probiotics feeding on meat quality of chicken breast during postmortem storage,” *Poultry Science*, vol. 95, no. 6, pp. 1457–1464, 2016.

[7] M. Kalliomäki, S. Salminen, H. Arvilommi, P. Kero, P. Koskinen, and E. Isolauri, “Probiotics in primary prevention of atopic disease: a randomised placebo-controlled trial,” *The Lancet*, vol. 357, no. 9262, pp. 1076–1079, 2001.

[8] J. Schrezenmeir and M. de Vrese, “Probiotics, prebiotics, and synbiotics—approaching a definition,” *The American Journal of Clinical Nutrition*, vol. 73, no. 2, pp. 361s–364s, 2001.

[9] P. A. Barrow, “Probiotics for Chickens,” in *Probiotics*, pp. 225–257, Springer, 1992.

[10] E. Jonsson and P. Conway, “Probiotics for pigs,” in *Probiotics*, pp. 259–316, Springer, 1992.

[11] G. Perdigón and S. Alvarez, “Probiotics and the immune state,” in *Probiotics*, pp. 145–180, Springer, 1992.

[12] M. A. Lamaga, W. C. Yeh, I. Sarosi et al., “NF-κB activation via MyD88-dependent toll-like receptor signaling is inhibited by trichotheccene mycotoxin deoxynivalenol,” *The Journal of Toxicological Sciences*, vol. 41, no. 2, pp. 273–279, 2016.

[13] K. Sugiyama, M. Muroi, M. Kinoshita et al., “NF-κB activation via MyD88-dependent toll-like receptor signaling is inhibited by trichotheccene mycotoxin deoxynivalenol,” *The Journal of Toxicological Sciences*, vol. 41, no. 2, pp. 273–279, 2016.

[14] M. A. Lamaga, W. C. Yeh, I. Sarosi et al., “TRAF6 deficiency results in osteopetrosis and defective interleukin-1, CD40, and LPS signaling,” *Genes & Development*, vol. 13, no. 8, pp. 1015–1024, 1999.

[15] Y. X. Ning Yuchang, “Preparation of microcapsules and characterisitic research of Lactobacillus from chicken,” *Animal Husbandry and Veterinary Medicine*, vol. 38, no. 2, pp. 220–224, 2011.

[16] K. J. Livak and T. D. Schmittgen, “Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCt method,” *Methods*, vol. 25, no. 4, pp. 402–408, 2001.

[17] X. Loping and L. Yujie, “The efeccion of complex microorganism on broiler immunity,” *Feed Reviews*, vol. 1, no. 1, pp. 27–28, 2003.

[18] H. S. Gill, K. J. Rutherford, J. Prasad, and P. K. Gopal, “Enhancement of natural and acquired immunity by *Lactobacillus rhamnosus* (HN001), *Lactobacillus acidophilus* (HN017) and *Bifidobacterium lactis* (HN019),” *British Journal of Nutrition*, vol. 83, no. 2, pp. 167–176, 2000.

[19] C. M. Galdeano and G. Perdigón, “The probiotic bacterium *Lactobacillus casei* induces activation of the gut mucosal immune system through innate immunity,” *Clinical and Vaccine Immunology*, vol. 13, no. 2, pp. 219–226, 2006.

[20] K. A. Baken, J. Ezendam, E. R. Gremmer et al., “Evaluation of immunomodulation by *Lactobacillus casei* Shirota: immune
function, autoimmunity and gene expression,” *International Journal of Food Microbiology*, vol. 112, no. 1, pp. 8–18, 2006.

[21] S. Delgado, E. O’sullivan, G. Fitzgerald, and B. Mayo, “Subtractive screening for probiotic properties of Lactobacillus species from the human gastrointestinal tract in the search for new probiotics,” *Journal of Food Science*, vol. 72, no. 8, pp. M310–M315, 2007.

[22] M. Candela, F. Perna, P. Carnevali et al., “Interaction of probiotic Lactobacillus and Bifidobacterium strains with human intestinal epithelial cells: adhesion properties, competition against enteropathogens and modulation of IL-8 production,” *International Journal of Food Microbiology*, vol. 125, no. 3, pp. 286–292, 2008.

[23] B. Ridwan, C. Koning, M. Besselink et al., “Antimicrobial activity of a multispecies probiotic (Ecologic 641) against pathogens isolated from infected pancreatic necrosis,” *Letters in Applied Microbiology*, vol. 46, no. 1, pp. 61–67, 2008.

[24] G. F. Bills, G. Platas, A. Fillola et al., “Enhancement of antibiotic and secondary metabolite detection from filamentous fungi by growth on nutritional arrays,” *Journal of Applied Microbiology*, vol. 104, no. 6, pp. 1644–1658, 2008.

[25] G. Reid and J. Burton, “Use of Lactobacillus to prevent infection by pathogenic bacteria,” *Microbes and Infection*, vol. 4, no. 3, pp. 319–324, 2002.

[26] S. C. Adams, Z. Xing, J. Li, and C. J. Cardona, “Immune-related gene expression in response to H1N1H9 low pathogenic avian influenza virus infection in chicken and Pekin duck peripheral blood mononuclear cells,” *Molecular Immunology*, vol. 46, no. 8-9, pp. 1744–1749, 2009.

[27] A. Rauf, M. Khatri, M. V. Murgia, K. Jung, and Y. M. Saif, “Differential modulation of cytokine, chemokine and toll like receptor expression in chickens infected with classical and variant infectious bursal disease virus,” *Veterinary Research*, vol. 42, no. 1, p. 85, 2011.

[28] G. D. Raj, T. M. C. Rajanathan, K. Kumanan, and S. Elankumaran, “Changes in the cytokine and toll-like receptor gene expression following infection of indigenous and commercial chickens with infectious bursal disease virus,” *Indian Journal of Virology*, vol. 22, no. 2, pp. 146–151, 2011.

[29] M. St. Paul, A. I. Mallick, L. R. Read et al., “Prophylactic treatment with toll-like receptor ligands enhances host immunity to avian influenza virus in chickens,” *Vaccine*, vol. 30, no. 30, pp. 4524–4531, 2012.

[30] K. Takahashi, Y. Sugi, A. Hosono, and S. Kaminogawa, “Epigenetic regulation of TLR4 gene expression in intestinal epithelial cells for the maintenance of intestinal homeostasis,” *The Journal of Immunology*, vol. 183, no. 10, pp. 6522–6529, 2009.