Ecotoxicology and Environmental Safety 210 (2021) 111825

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Synergistic use of florfenicol and *Salvia miltiorrhiza* polysaccharide can enhance immune responses in broilers

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ARTICLE INFO

Edited by: Dr Yong Liang

Keywords:
Florfenicol
Salvia Miltiorrhiza polysaccharide
Immune response
Broilers

ABSTRACT

To explore the effect of florfenicol (FFC) combined with *Salvia miltiorrhiza* polysaccharide (SMPs) on immune function of Broilers. One hundred and twenty-one-day-old chicks were chosen and divided into 6 groups. The group A received standard basal diet only, the group B received a basal diet with FFC (0.15 g/L diet), and the group C, D, E received a basal diet with FFC (0.15 g/L diet) and SMPs (1.25 g/L, 2.5 g/L, 5 g/L diet) , the group F received a basal diet with SMPs (5 g/L diet). FFC can significantly inhibit the growth performance of broilers, but has no significant damage to the immune function of broilers. The combination of FFC and SMPs can improve the growth performance of broilers, increase the number of leukocyte subtypes in blood ($P < 0.05$), increase the number of Newcastle disease (ND) and avian influenza (AI) antibodies in blood, the number of immunoglobulins, and the content of cytokines ($P < 0.05$). In addition, it significantly improve the lymphocyte conversion rate of broiler peripheral blood ($P < 0.05$). So that, synergistic use of FFC and SMPs can enhance immune responses in Broilers.

1. Introduction

With the wide application of antibiotics, people gradually realize that antibiotics can not only kill pathogens, but also affect the immune function of the body. So that, understanding the regulation mechanism of different kinds of antibiotics on the immune function of organism will be of positive significance in guiding the clinical selection of drugs (Yang et al., 2017). Florfenicol (FFC) is recognized as one of the most prominent broad-spectrum antibiotics in Livestock and poultry feeding industry (Zhang et al., 2015). FFC is often used to treat infections caused by various pathogens, including *Escherichia coli, Salmonella typhimurium, Staphylococcus aureus, Streptococcus, Pasteurella multocida* and *Mycoplasma pneumoniae* (HO et al., 2000; Shin et al., 2005; Wisselink et al., 2006). There are some reports about the suppressive effects of FFC on the humoral immune parameters in mammals (Guan et al., 2011; Lis et al., 2011; Shuang et al., 2011), birds (Hassanin et al., 2014; Khalifeh et al., 2009a), and fish (Er and Dik, 2014; Reda et al., 2013), and those studies pointed out that the cellular immunity could be altered in a dose-dependent manner following administration of FFC. In addition, FFC induced hypoplasia and atrophy of the spleen and thymus, induced cell cycle arrest, as well as splenocyte apoptosis, and decreased the proliferation and viability of lymphocytes and the humoral and cellular immunity of the treated mice (Hu et al., 2016). However, the effect of FFC on the poultry immune function was still controversial. In previous study, poultry administration for FFC has been reported before to adversely affect the immune system and B cell maturation (Chrząstek et al., 2011; Lis et al., 2019) oral FFC treatment probably through maintaining intact mucosal surfaces and changing the normal flora bacterial contents caused an up regulation in the threshold of cell mediated immunity as interferon response; on the other hand, FFC exerts a down regulation effect on the humoral immune responses (Khalifeh et al., 2009a). In other study, no significantly lower immune response against NDV vaccine was detected in chicks received FFC at a dosage of 50 mg/Kg feed (Cao et al., 2004). In conclusion, the effect of FFC on immune function of broilers is not clear, and further research is needed.

SMPs has the effect of enhancing immunity (Sun et al., 2011). It has been proved that SMPs has a significant promoting effect on lymphocyte

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https://doi.org/10.1016/j.ecoenv.2020.111825
Received 13 July 2020; Received in revised form 23 November 2020; Accepted 17 December 2020
Available online 4 January 2021

proliferation in mice; it can significantly improve the phagocytosis of peritoneal macrophages in mice, which shows that SMPs can significantly promote the proliferation of lymphocyte in mice, and the promoting effect is in accordance with the concentration, showing a strong role in immune promotion. In addition, in the absence of macrophages and B cells, SMPs can promote the proliferation of T cells, suggesting that this is the direct effect of SMPs, rather than the indirect effect of cytokines secreted by other cells, suggesting that T cells are also one of the target cells of SMPs and interact with mitogen Con A. Comparisons showed that SMPs could promote cellular immune function (Wang et al., 2014b). Although there are many reports on the immune regulation of SMPs on mice, there are few reports on its effect on the immune function of broilers. Therefore, in this experiment, we want to explored whether SMPs can alleviate the immune deficiency of broilers.

In this experiment, the development of immune organs, the changes of leukocyte subsets and the content of immunoglobulin were measured. Through this experiment, we want to reveal whether the therapeutic dose of FFC (0.15 g/L) can damage the immune function of broilers, and the effect of SMPs combined with FFC on immune function of broilers. This will have certain significance for the standard use of FFC and the development of SMPs as animal immunomodulatory drugs.

2. Materials and methods

2.1. Drugs and reagents

One-day-old SPF chickens (Arbor Acres broilers) were purchased from Hebei Dawu Agricultural Group Poultry Company Ltd (Baoding, China). FFC (purity≥95%) were purchased from Shenniu Biological Technical Co. Ltd (Shandong, China). SMPs (purity≥95%) were purchased from SHENGYAKAI Biological Technical Co. Ltd (Hunan, China), which consisted of five different monosaccharides, including mannose, rhamnose, arabinose, glucose and galactose, in the molar ratio of 2.24:2.35:1.27:0.98:1.11. Commercial kits for detecting chickens IgA, IgG, IgM, IFN-γ, IL-2 and IL-4 obtained from Shanghai Hengyuan Biotech CO.,Ltd (Shanghai China). Other chemicals used in these experiments of analytical grade were from commercial sources.

2.2. Experimental materials

A total of 240 broilers were randomly divided into six groups, 40 chicks in per group. Group A: chickens began to drink water and feed normally from 1-day-old; Group B: 0.15 g/L of FFC was added to drinking water at 1-day-old and fed freely; Group C: chickens were fed with 0.15 g/L FFC and 1.25 g/L SMPs in drinking water at 1-day-old, feeds were taken freely. group D: chickens were fed with 0.15 g/L FFC and 2.5 g/L SMPs in drinking water at 1-day-old, feeds were taken freely. group E, chickens were fed with 0.15 g/L FFC and 5 g/L SMPs in drinking water at 1-day-old, feeds were taken freely. Group F: chickens were fed with 5 g/L SMPs in drinking water at 1-day-old, feeds were taken freely. After 5 consecutive days, normal drinking water and feed were given to each group. Blood was collected from subwing veins of chickens at age of d21 and d42, respectively, and organs of chickens were executed. All broilers were vaccinated with Newcastle disease and avian influenza vaccine at 1-day old, 7-day old and 21-day old (Sarfati-Mizrahi et al., 2010). All the experimental protocols were approved by the Animal Care and Use Committee of Agricultural University of Hebei prior to the initiation of the study.

2.3. Weight gain and immune organ index

Each chickens recorded their initial body weight, weighed each chicken again after feeding to 21-day-old and 42-day-old in each group, the body weight difference between 21-day-old/42-day-old and 1-day-old was calculated respectively as the weight gain of each chickens, and randomly selected 10 broilers in each group to execute, and took their spleen, thymus and bursa to weigh and calculate organ index. organ index = organ weight (mg)/body weight(g).

2.4. Detection of leukocyte subsets in chicken blood

The whole blood of chickens was taken by anticoagulant tube, and then detected by Hematology analyzer (Mindray Medical International, Shenzhen, China). The number of white blood cells (WBC), lymphocytes (Lymph), mononuclear cells (Mon) and neutrophils (Gran) in the blood of chickens in each group were recorded (Hu et al., 2014).

2.5. Detection of ND and AI antibodies tilters in broiler serum

Before grouping, ten 1-day-old chickens were randomly selected to detect Newcastle disease (ND) and avian influenza (H9N2, AI) maternal antibodies, and then, At the age of d7, d14, d21, d28, d35 and d42. Ten chickens were randomly selected from each group. Blood was collected from each chicken (chickens were eliminated after blood collection) and serum was separated by centrifugation. The hemagglutination inhibition (HI) test was carried out with 96-hole V-shaped hemagglutination platelets. The titer of antibodies was determined by log2.

2.6. Measurement of lymphocyte transformation rate in peripheral blood

At the age of 42 days, MTT (sigma, St. Louis, MO, USA) was used to detect the dynamic changes of T lymphocyte transformation rate in peripheral blood. Three milliliter blood of each chicken was collected from the wing vein, and added EDTA was used for anticoagulation. Take 1 mL of blood containing anticoagulant, and slowly add it to the tube containing lymphocyte separation solution (the ratio of blood and separation solution is 1:1), centrifuge for 20 min at 3000 rpm/min, absorb the middle cloudy white blood cell layer, wash it three times with D′hanks solution (Gibco, Los Angeles, CA, USA) and centrifuge for 10 min at 2000 rpm /min. Then 1 mL erythrocyte lysate was added to tubes and centrifuged for 5 min at 2000 rpm/min after reaction 2 min. Remove the supernatant after centrifugation. Then adding RPMI1640 culture medium (Gibco, Los Angeles, CA, USA) to suspend cells, the cell survival rate was more than 95% by trypan blue staining.

RPMI 1640 medium was used to adjust the number of cells to 2×10^6/mL. PHA (sigma, St. Louis, MO, USA) (final concentration of 10 ug/mL) and cell suspension were added to 96-well cell culture plate, 100 μL /hole, and ten replicates were set in each group. After slight shaking, the cells were cultured at 37 ℃ for 72 h in 5% CO_2 incubator (Thermo, Waltham, MA,USA). Ten milliliter MTT was added to each hole and reaction for 4 h, 100 μL DMSO was added to each hole. After shaking and mixing for 30 min, the cells were decomposed for 12 h and the absorbance at 590 nm was measured by Automatic enzyme-linked immunosorbent assay systems (BioTek Instruments, Inc., USA).

2.7. Detection of immunoglobulin and cytokines in serum

Serum was separated by venous blood collection at 21 and 42 days old. The contents of IgG, IgM, IgA, IL-2, IL-4 and IFN-γ in chicken serum were determined by ELISA kit (Henyuan Biotechnology Co., Ltd., Shanghai, China) .

2.8. Statistical analysis

Using SPSS21.0 software (IBM Corporation, Armonk, NY) to test the normality and homogeneity of variance of the counting data. Then the mean differences among groups were compared by one-way ANOVA. If the variance is homogeneous, LSD test is used; if the variance is not homogeneous, Dunnett-13 test is used. The data in this experiment were mean ± standard deviation (SD) and significant differences among groups were set at a value of $P < 0.05$, $P < 0.01$.

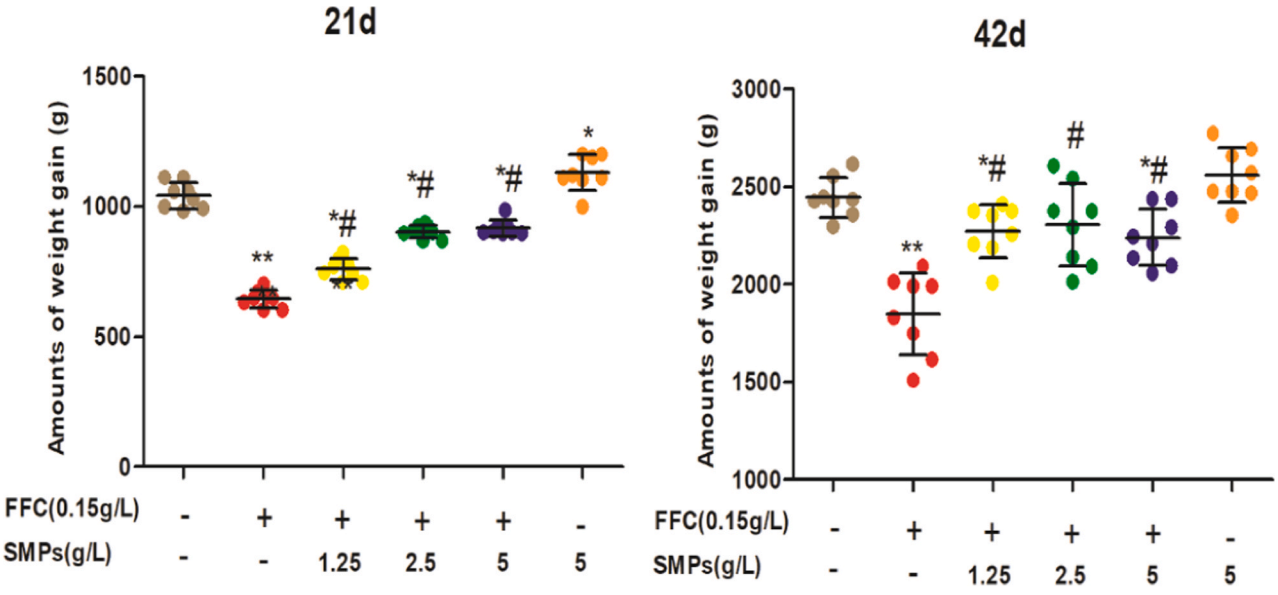

Fig. 1. Amounts of weight gain of each group in d21 and d42. A colored dot represents a biological repeat, and the same color represents the same group. ** compared with control group, $P < 0.01$, * compared with control group, $P < 0.05$; # compared with FFC group, $P < 0.01$, ## compared with FFC group. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

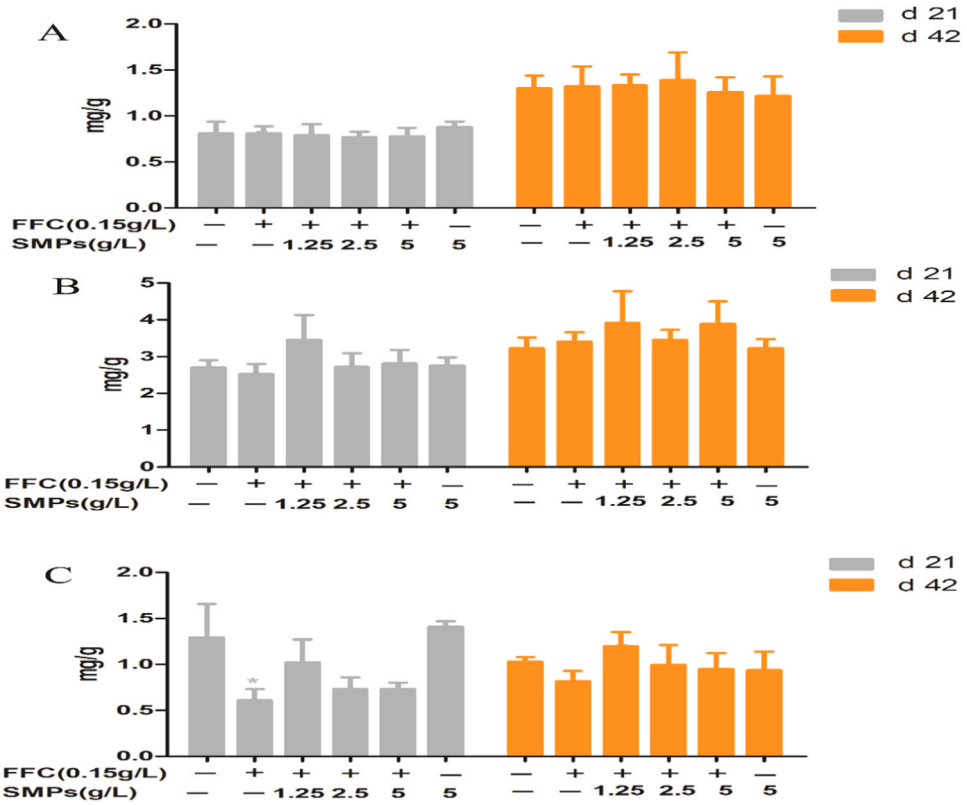

Fig. 2. Spleen index(A), thymus index(B) and bursa of Fabricius index(C) in each group. ** compared with control group, $P < 0.01$, * compared with control group, $P < 0.05$; # compared with FFC group, $P < 0.01$, ## compared with FFC group.

3. Results

3.1. Growth performance

As shown in Fig. 1, compared with the control group, 0.15 g/L of florfenicol significantly decreased the weight gain of Broilers ($P < 0.01$) at d21 and d42, while 1.25 g/L, 2.5 g/L and 5 g/L of SMPs significantly reduced the weight gain of broilers caused by FFC ($P < 0.05$).

3.2. Immune organ index

In our study we found that there was no significant difference in

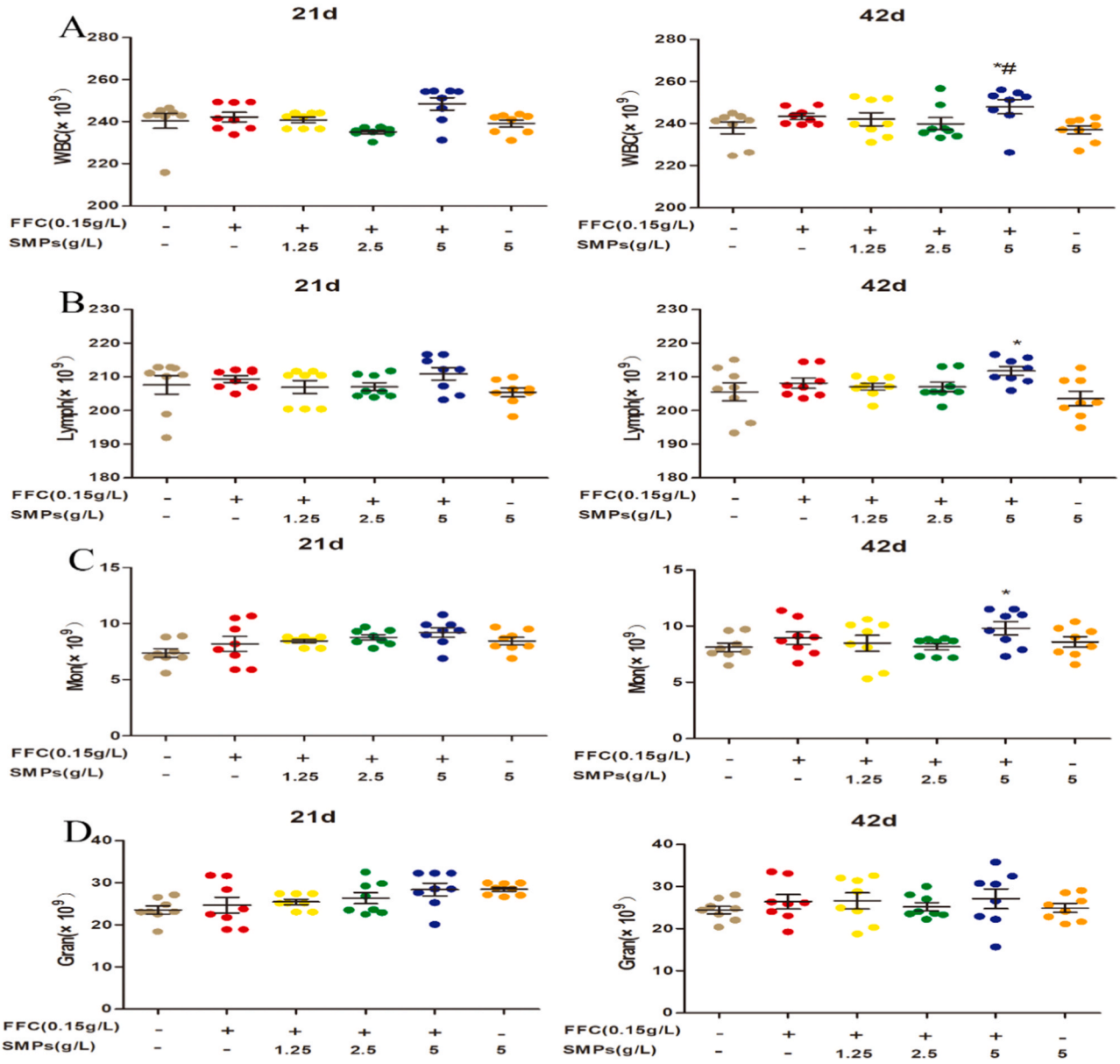

Fig. 3. Amounts of WBC(A), Lymph(B), Mon(C) and Gran(D) in the blood of broilers. ** compared with control group, $P < 0.01$, * compared with control group, $P < 0.05$; # Compared with FFC group, $P < 0.01$, ## compared with FFC group.

spleen index and thymus index between per group chinks at d21 and d42 ($P > 0.05$, Fig. 2A and Fig. 2B). As for bursa of Fabricius index, the bursa index of florfenicol group was significantly lower than that of other groups at d21($P < 0.05$, Fig. 2C), and there was no significant difference between groups at d42 ($P > 0.05$, Fig. 2C).

3.3. Leukocyte subtypes in blood of broilers

The main function of leukocytes is to phagocytize bacteria and prevent diseases, the change of leukocyte subsets reflects the disease resistance of broiler. As shown in Fig. 3, we detected WBC, Lymph, Mon and Gran in the blood of broilers. It was found that there was no significant difference in WBC, Lymph and Mon contents among the groups at age of 21 days ($P > 0.05$), but at age of 42 days, WBC, Lymph and Moon contents in blood, only 5 mg/L SMPs and FFC combined group had significantly higher than other groups ($P < 0.05$). For the content of Gran, there was no significant difference between the groups at age of d21 and d42($P > 0.05$).

3.4. ND and AI antibodies tilter in serum of broilers

We detected the antibody to Newcastle disease. The results showed as shown in Fig. 4A, for d7-d42, compared with the control group, FFC combined with three doses of SMPs (5 g /L, 2.5 g/L and 1.25 g/L) significantly increased the titer of ND antibody ($P < 0.05$). while at d14, d28 and d35, FFC significantly increased the titer of antibody to Newcastle disease in broilers ($P < 0.05$). In the same time, Compared with FFC group, 5 g/L SMPs combined with FFC could significantly increase the antibody titer of Newcastle disease in Broilers ($P < 0.05$). In the same time, we detected the titers of antibodies against avian influenza H9N2 in broilers. We found that compared with control group, 5 g/L SMPs combined with FFC could significantly increase the AI antibody titer in d14, d28, d35 and d42 (Fig. 4B).

3.5. Contents of IgA, IgG and IgM in serum of broilers

As shown in the Fig. 5A, the content of IgA in the FFC-2.5 g/L SMPs

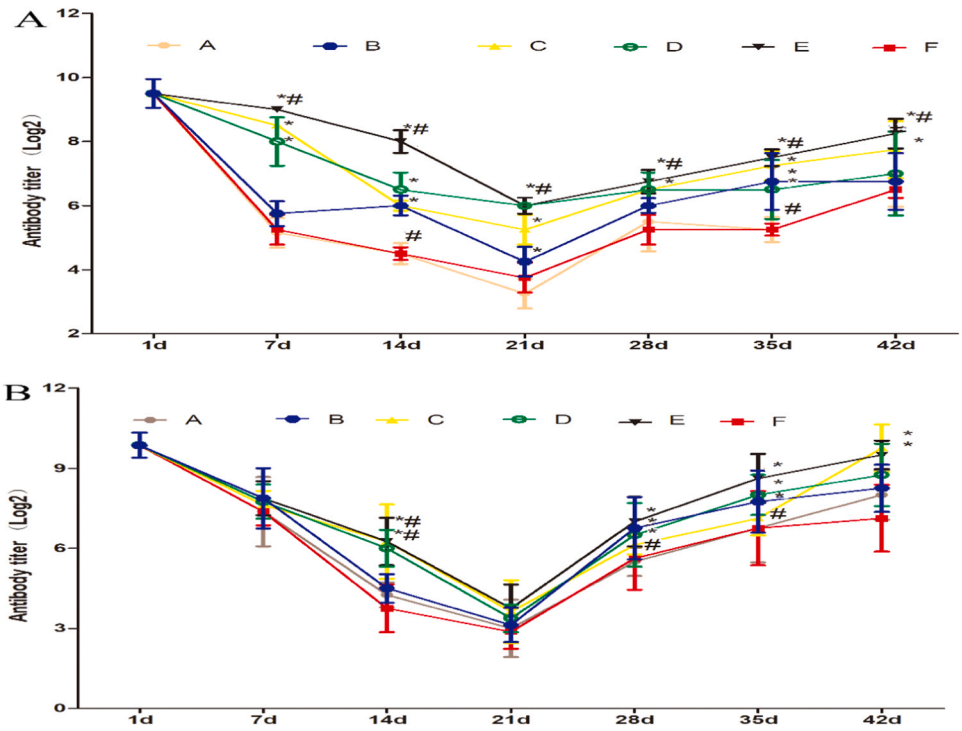

Fig. 4. Antibody titer of ND(A) and AI (B) in each group. Group A: chickens began to drink water and feed normally from 1-day-old; Group B: 0.15 g/L of FFC was added to drinking water at 1-day-old. Group C: chickens were fed with 0.15 g/L FFC and 1.25 g/L SMPs in drinking water at 1-day-old. group D: chickens were fed with 0.15 g/L FFC and 2.5 g/L SMPs in drinking water at 1-day-old. group E, chickens were fed with 0.15 g/L FFC and 5 g/L SMPs in drinking water. group F, chickens were fed 5 g/L SMPs in drinking water. ** compared with control group, $P < 0.01$, * compared with control group, $P < 0.05$; # compared with FFC group, $P < 0.01$, ## compared with FFC group.

group and the FFC-5 g/L SMPs combination group significantly higher than the control group and FFC group at the d21($P < 0.05$). However, at day42, only 5 g/L SMPs-FFC combination group was significantly higher than the control group and FFC group($P < 0.05$), while there was no significant difference between other groups($P < 0.05$). Compared with the control group, the contents of IgG in the SMPs-FFC combination group increased significantly at day21 and day42($P < 0.05$), contents of IgG in the FFC group was no significant difference at d21 and d42 ($P > 0.05$); Compared with the FFC group, the contents of IgG in the SMPs-FFC combintion group increased significantly at d21and d42 ($P < 0.05$), contents of IgG in the FFC-5 g/L combintion group increased significantly at day42($P < 0.05$) (Fig. 5B). However, the expression of IgM in each group were no significant difference at d21($P > 0.05$). Compared with the control group, both the FFC group and the FFC-5 g/L SMPs combination group could significantly increase the IgM content at d42($P < 0.05$) (Fig. 5C).

3.6. Contents of IFN-γ, IL-2 and IL-4 in serum of broilers

As shown in the Fig. 6A, the content of IFN-γ in the FFC group and the FFC-SMPs combination group significantly higher than the control group on the d21($P < 0.05$). However, at d42, the content of IFN-γ in only 5 g/L SMPs-FFC combination group was significantly higher than the control group($P < 0.05$), while there was no significant difference between other groups($P > 0.05$). The results of IL-2 contents showed that compared with the control group, the IL-2 contents in SMPs-FFC combination group and the FFC group increased significantly at d21 and d42 ($P < 0.05$); Compared with the FFC group, the contents of IL-2 in the 2.5 g /L SMPs-FFC combination group and 5 g/L SMPs-FFC combination group increased significantly at d42 ($P < 0.05$) (Fig. 6B). However, the expression of IL-4 in 5 g/L SMPs-FFC combination was significantly higher than the control group at d21, in the same time, the expression of IL-4 in 5 g/L SMPs-FFC combination was significantly higher than the FFC group at d21($P < 0.05$); but at d42, there was no significant difference in the expression of IL-4 between each group ($P > 0.05$) (Fig. 6C).

3.7. Lymphocyte transformation rate in peripheral blood

Lymphocyte transformation rate is an important indicator of cellular immunity. We detected the lymphocyte transformation rate in the blood of broilers in each group. We found that there was no significant difference in the lymphocyte transformation rate among each group at the age of d21($P > 0.05$) (Fig. 7A), but at the age of d 42, compared with the control group, both the FFC group and the SMPs-FFC combination group could significantly improve the lymphocyte transformation in the blood ($P < 0.05$) (Fig. 7B). Compared with the FFC group, 5 g/L SMPs-FFC combination group can significantly improve the blood lymphocyte conversion rate($P > 0.05$) (Fig. 7B).

4. Discussion

Florfenicol has been widely used in the treatment of bacterial diseases, but its function of immune regulation is still uncertain. In fact, the reported dose of florfenicol, which can cause animal immunosuppression, is far greater than its recommended therapeutic dose (Hu et al., 2016). While the immunotoxicity of the recommended therapeutic doses (0.15 g/L, drinking water) or lower doses of florfenicol was rarely reported. In this experiment, broilers were added the recommended therapeutic doses of florfenicol in drink water to explore the effect of florfenicol on the immune function of broilers. SMPs has the function of immune regulation, and can improve the immunity of immunocompromised animals (Liu et al., 2013; Wang et al., 2014a; Zhang et al., 2012b). We observed the effect of FFC combined with SMPs on immune function of broilers. The results showed that low dose of FFC had no significant damage to immune organ index, antibody titer and immunoglobulin content of broilers, besides significantly inhibiting weight gain. We also can see the weight gain is decreased in the groups of FFC+SMPs treatment, FFC combined with SMPs (5 g/L) also significantly increase blood antibody titer and immunoglobulin content in broilers, but the immune organ index was almost no difference. The results showed that the combination of FFC and SMPs (5 g/L) could improve the immunity of broilers.

The immune system is diverse and can respond accurately to

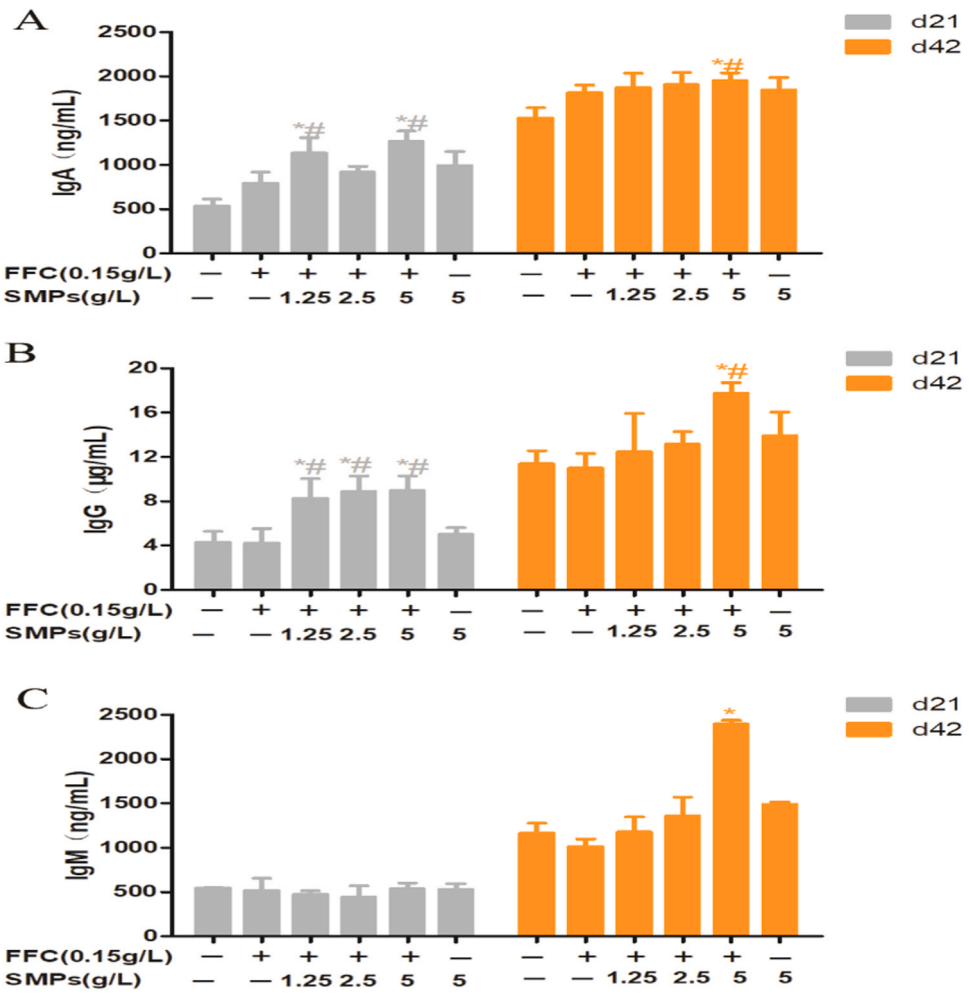

Fig. 5. Contents of IgA, IgG and IgM in Chicken Serum. ** compared with control group, $P < 0.01$, * compared with control group, $P < 0.05$; # compared with FFC group, $P < 0.01$, ## compared with control group.

different types of infectious organisms (Mckee et al., 2010). Cellular immunity is very important in the immune system. T lymphocyte plays an important role in preventing intracellular biological infection. In addition, in T lymphocytes, Th cells can induce antibody-producing lymphocytes and Tc cells to help phagocytes phagocytize microorganisms. Anti-biochemical oxygen demand (BOD) produced by B lymphocyte mediated fluid immunity by IES (Marciani et al., 2000). The spleen is an important lymphatic organ that links innate and adaptive immunity (Ciavarra et al., 2005). Splenic lymphocytes, including T cells and B cells, play an important role in the body's immune response. Splenic lymphocyte proliferation is the most direct indicator of the body's immunity. Producing effective T and B lymphocyte immunity depends on lymphocyte stimulating cell proliferation. As we all know, PHA stimulates T cells, while LPS stimulates B cell proliferation (Su et al., 2014). Our results showed that 5 g/L SMPs-FFC combination group can significantly improve the blood lymphocyte conversion rate, which may contribution to improve the immunity of broilers.

The titer of antibody in chickens reflects the strength of humoral immunity. Humoral immunity is an important specific immune response mediated by B cells (Khalifeh et al., 2009a), which is one of the main factors for the body to resist infectious diseases. Chicken B cells develop and mature in bursa of Fabricius medulla. Mature B cells are stationary, secrete antibodies and exert humoral immune effects (Peng et al., 2009). The higher the antibody level, the stronger the resistance of chickens to infectious diseases, and the less the chance of infectious diseases in chickens (Gheorghiu et al., 1985). Therefore, the combination of florfenicol and Salvia miltiorrhiza polysaccharide in each dosage group could increase the antibody level of Newcastle disease and avian influenza in peripheral blood of chickens, which indicated that florfenicol and Salvia miltiorrhiza polysaccharide could increase the antibody level of Newcastle disease in chickens and enhance the humoral immunity.

IgG, IgM and IgA are the main immunoglobulins produced by activated B lymphocytes, reflecting the humoral immune status (Lebacqverheyden et al., 1974). Among them, IgG is the main component of antibodies in serum and body fluids. It mainly participates in the body's re-humoral immune response. It has the functions of regulating phagocytosis, agglutination and precipitation of antigens, neutralizing pathogens and toxins, and activating complement transduction pathways (Venkatesh Prasanna et al., 2009). IgM is the earliest immunoglobulin produced by activated B cells stimulated by antigen (Dekruyff et al., 1985). IgM has a strong antigen binding ability and plays an important role in the early humoral immune defense (Gonzalez-Suarez et al., 2009). IgA is also an important immunoglobulin involved in humoral immunity. Their content can reflect the level of humoral immunity in chickens. Experiments have shown that Chinese herbal medicine can improve the immune organ index of chickens, and can improve the level of chicken immunoglobulin, which can improve the immune ability of chickens. Our results showed that the combination of florfenicol and Salvia miltiorrhiza polysaccharide could significantly increase the levels of IgG, IgM and IgA in serum of chickens. The results showed that the combination of florfenicol and Salvia miltiorrhiza polysaccharide had better effect, promoted the production of immunoglobulin and

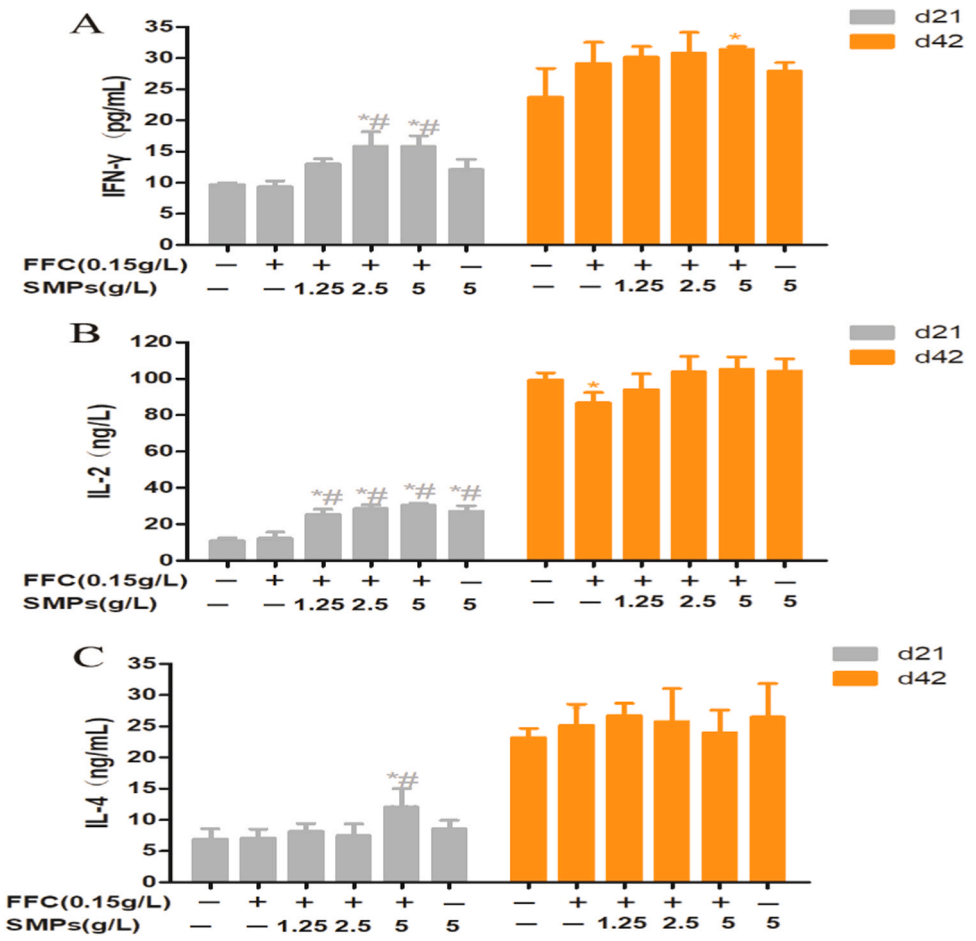

Fig. 6. Contents of IFN-γ (A), IL-2 (B) and IL-4 (C) in Chicken Serum at d21 and d42. ** compared with control group, $P < 0.01$, * compared with control group, $P < 0.05$; # compared with FFC group, $P < 0.01$, ## compared with FFC group.

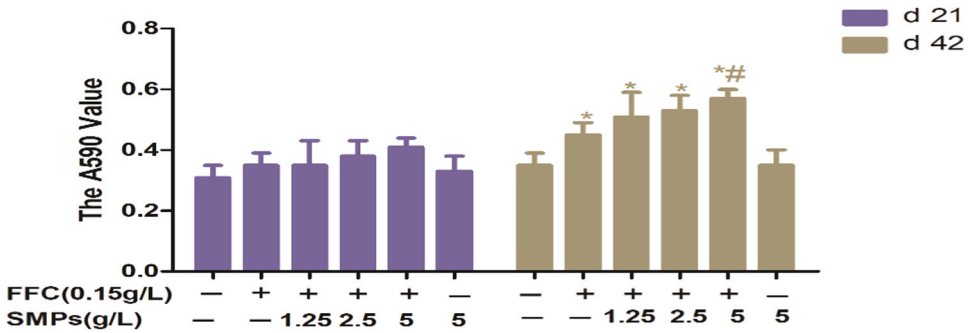

Fig. 7. Peripheral blood lymphocyte transformation rate of broilers in each group at d21 and d42. ** compared with control group, $P < 0.01$, * compared with control group, $P < 0.05$; # compared with FFC group, $P < 0.01$, ## compared with FFC group.

enhanced the immunity of broilers.

The cytokines secreted by helper T cells can be divided into Th1-related cytokines (IFN-γ and IL-2) and Th2-related cytokines (IL-4 and IL-6) (Pinto et al., 2006). IFN-γ mediates cellular immunity by promoting T cell proliferation and differentiation to activate phagocytosis and kill intracellular pathogens. IL-2 is the activation and differentiation of T lymphocytes. In addition, it can promote the secretion of immunoglobulin to induce B cell differentiation and proliferation of killer cells (Feria-Romero et al., 2011). IL-4 plays an important role in the differentiation of Th2 and strongly induces the secretion of IL-5, IL-9 and IL-10 (Yee et al., 2013). Our results showed that the combination of florfenicol and Salvia miltiorrhiza polysaccharides could significantly increase the contents of IFN-γand IL-2, but had no significant effect on the contents of IL-4.

5. Conclusion

FFC can significantly inhibit the growth performance of broilers, but has no significant damage to the immune function of broilers. The combination of florfenicol and SMPs can improve the growth performance of broilers, increase the number of leukocyte subtypes in blood, increase the antibodies tilters of Newcastle disease and avian influenza, the number of immunoglobulins, and the content of cytokines in serum of broilers. In addition, it can improve the lymphocyte conversion rate of

broiler peripheral blood. So that, synergistic use of FFC and SMPs can enhance immune responses in Broilers.

CRediT authorship contribution statement

WS and YB conceived and designed the study, and critically revised the manuscript. CH performed most of the experiments and analyzed the results, CH and XW drafted the manuscript. YW assisted in experimental design, data interpretation and manuscript preparation. CH, YG and YC contributed to samples collection, performed ELISA and helped with the data analysis. XW, YC and CH participated in Lymphocyte transformation test and fed animals. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This study was financially supported by Subsidized Project for Cultivating Innovative Ability of Postgraduates of Hebei Province (no. CXZZBS2019096), Hebei Province Innovation Capacity Promotion Project-Hebei Veterinary Biotechnology Innovation Center Open Project (No. 20567668H), Precision Animal Husbandry Discipline Group Construction Project of Hebei Agricultural University (1090064).

References

Cao, J., Li, K., Lu, X., Zhao, Y., 2004. Effects of florfenicol and chromium (III) on humoral immune response in chicks. Asian Australas. J. Anim. Sci. 17, 208–213.
Chrząstek, K., Madej, J.P., Mytnik, E., Wieliczko, A., 2011. The influence of antibiotics on B-cell number, percentage, and distribution in the bursa of Fabricius of newly hatched chicks. Poult. Sci. 90, 2723–2729.
Ciavarra, R.P., Taylor, L., Greene, A.R., Yousefieh, N., Horeth, D., van Rooijen, N., Steel, C., Gregory, B., Birkenbach, M., Sekellick, M., 2005. Impact of macrophage and dendritic cell subset elimination on antiviral immunity, viral clearance and production of type 1 interferon. Virology 342, 177–189.
Dekruyff, R.H., Clayberger, C., Cantor, H., 1985. Monoclonal helper T cells induce B cell responses to T-independent antigens: antigen-specific T cells are directly stimulated by activated B cells in the absence of antigen. J. Immunol. 134, 86–90.
Er, A., Dik, B., 2014. The effects of florfenicol on the values of serum tumor necrosis factor-α and other biochemical markers in lipopolysaccharide-induced endotoxemia in brown trout. Mediat. Inflamm. 2014, 1–5.
Feria-Romero, I.A., Chávez-Rueda, K., Orozco-Suárez, S., Blanco-Favela, F., Calzada-Bermejo, F., Chávez-Sánchez, L., Manuel-Apolinar, L., Hernández-González, R., Aguilar-Setién, Á., Tesoro-Cruz, E., 2011. Intranasal anti-rabies DNA immunization promotes a Th1-related cytokine stimulation associated with plasmid survival time. Arch. Med. Res. 42, 563–571.
Gheorghiu, M., Mouton, D., Lecoeur, H., et al., 1985. Resistance of high and low antibody responder lines of mice to the growth of avirulent (BCG) and virulent (H37Rv) strains of mycobacteria. Clin. Exp. Immunol. 59, 177–184.
Gonzalez-Suarez, M.L., Salinas-Carmona, M.C., Perez-Rivera, I., 2009. IgM but not IgG monoclonal anti-Nocardia brasiliensis antibodies confer protection against experimental actinomycetoma in BALB/c mice. FEMS Immunol. Med. Microbiol. 57, 17–24.
Guan, S., Lu, J., Shen, X., Qian, W., Liu, J., Deng, X., 2011. Florfenicol impairs the immune responses to vaccination against foot-and-mouth disease in mice. Immunopharmacol. Immunotoxicol. 33, 609–613.
Hassanin, O., Abdallah, F., Awad, A., 2014. Effects of florfenicol on the immune responses and the interferon-inducible genes in broiler chickens under the impact of E. coli infection. Vet. Res. Commun. 38, 51–58.
HO, S.-P., HSU, T.-Y., CHEN, M.-H., WANG, W.S., 2000. Antibacterial effect of chloramphenicol, thiamphenicol and florfenicol against aquatic animal bacteria. J. Vet. Med. Sci. 62, 479–485.
Hu, D., Zhang, T., Zhang, Z., Wang, G., Wang, F., Qu, Y., Niu, Y., Liu, S., 2014. Toxicity to the hematopoietic and lymphoid organs of piglets treated with a therapeutic dose of florfenicol. Vet. Immunol. Immunopathol. 162, 122–131.
Hu, D., Han, Z., Li, C., Lv, L., Cheng, Z., Liu, S., 2016. Florfenicol induces more severe hemotoxicity and immunotoxicity than equal doses of chloramphenicol and thiamphenicol in Kunming mice. J. Immunopharmacol. 38, 472–485.
Huang, Y., Jiang, C., Hu, Y., Zhao, X., Shi, C., Yu, Y., Liu, C., Tao, Y., Pan, H., Feng, Y., Liu, J., Wu, Y., Wang, D., 2013. Immunoenhancement effect of rehmannia glutinosa polysaccharide on lymphocyte proliferation and dendritic cell. Carbohydr. Polym. 96, 516–521.
Kashi, V.P., Jacob, R.A., Paul, S., Nayak, K., Satish, B., Swaminathan, S., Satish, K.S., Ranga, U., 2009. HIV-1 Tat-specific IgG antibodies in high-responders target a B-cell epitope in the cysteine-rich domain and block extracellular Tat efficiently. Vaccine 27, 6739–6747.
Khalifeh, M.S., Amawi, M.M., Abu-Basha, E.A., Yonis, I.B., 2009a. Assessment of humoral and cellular-mediated immune response in chickens treated with tilmicosin, florfenicol, or enrofloxacin at the time of Newcastle disease vaccination. Poult. Sci. 88, 2118–2124.
Lebacqverheyden, A.M., Vaerman, J.P., Heremans, J.F., 1974. Quantification and distribution of chicken immunoglobulins IgA, IgM and IgG in serum and secretions. Immunology 27, 683–692.
Lis, M., Szczypka, M., Suszko, A., Świtała, M., Obmińska-Mrukowicz, B., 2011. The effects of florfenicol on lymphocyte subsets and humoral immune response in mice. Pol. J. Vet. Sci. 14, 191–198.
Lis, M., Szczypka, M., Suszko-Pawłowska, A., et al., 2019. Modulation of lymphocyte subsets and humoral immune response by florfenicol administered to sheep red blood cell-immunized broiler chickens. Pol. J. Vet. Sci. 22, 481–487.
Liu, L., Jia, J., Zeng, G., Zhao, Y., Qi, X., He, C., Guo, W., Fan, D., Han, G., Li, Z., 2013. Studies on immunoregulatory and anti-tumor activities of a polysaccharide from Salvia miltiorrhiza Bunge. Carbohydr. Polym. 92, 479–483.
Marciani, D.J., Press, J.B., Reynolds, R.C., Pathak, A.K., Pathak, V., Gundy, L.E., Farmer, J.T., Koratich, M.S., May, R.D., 2000. Development of semisynthetic triterpenoid saponin derivatives with immune stimulating activity. Vaccine 18, 3141–3151.
Mckee, A.S., Macleod, M.K., Kappler, J.W., Marrack, P., 2010. Immune mechanisms of protection: can adjuvants rise to the challenge? BMC Biol. 8, 37–42.
Peng, J.P., Liu, J., Chen, N.H., 2009. Construction of yeast two hybrid cDNA library of chicken B cells bursa of fabricius. Acta Agric. Univ. Jiangxiensis.
Pinto, R.A., 2006. T helper 1/T helper 2 cytokine imbalance in respiratory syncytial virus infection is associated with increased endogenous plasma cortisol. Pediatrics 117, 878–886.
Reda, R.M., Ahmed, E.N.G., Ibrahim, R.E., et al., 2013. Effect of oxytetracycline and florfenicol as growth promoters on the health status of cultured Oreochromis niloticus. Egypt. J. Aquat. Res. 39, 241–248.
Sarfati-Mizrahi, D., Lozano-Dubernard, B., Soto-Priante, E., Castro-Peralta, F., Flores-Castro, R., Loza-Rubio, E., Gay-Gutiérrez, M., 2010. Protective dose of a recombinant Newcastle disease LaSota-avian influenza virus H5 vaccine against H5N2 highly pathogenic avian influenza virus and velogenic viscerotropic Newcastle disease virus in broilers with high maternal antibody levels. Avian Dis. 54, 239–241.
Shin, S.J., Kang, S.G., Nabin, R., Kang, M.L., Yoo, H.S., 2005. Evaluation of the antimicrobial activity of florfenicol against bacteria isolated from bovine and porcine respiratory disease. Vet. Microbiol. 106, 73–77.
Shuang, G., Yu, S., Weixiao, G., Dacheng, W., Zhichao, Z., Jing, L., Xuming, D., 2011. Immunosuppressive activity of florfenicol on the immune responses in mice. Immunol. Invest. 40, 356–366.
Su, X., Pei, Z., Hu, S., 2014. Ginsenoside Re as an adjuvant to enhance the immune response to the inactivated rabies virus vaccine in mice. Int. Immunopharmacol. 20, 283–289.
Sun, X.G., Fu, X.Q., Cai, H.B., Liu, Q., Li, C.H., Liu, Y.W., Li, Y.J., Liu, Z.F., Song, Y.H., Lv, Z.P., 2011. Proteomic analysis of protective effects of polysaccharides from Salvia miltiorrhiza against immunological liver injury in mice. Phytother. Res. 25, 1087–1094.
Wang, N., Yang, J., Lu, J., Qiao, Q., Wu, T., Du, X., Bao, G., He, X., 2014a. A polysaccharide from Salvia miltiorrhiza Bunge improves immune function in gastric cancer rats. Carbohydr. Polym. 111, 47–55.
Wang, N., Yang, J., Lu, J., Qiao, Q., Wu, T., Du, X., Bao, G., He, X., 2014b. A polysaccharide from Salvia miltiorrhiza Bunge improves immune function in gastric cancer rats. Carbohydr. Polym. 111, 47–55.
Wisselink, H.J., Veldman, K.T., Van den Eede, C., Salmon, S.A., Mevius, D.J., 2006. Quantitative susceptibility of Streptococcus suis strains isolated from diseased pigs in seven European countries to antimicrobial agents licenced in veterinary medicine. Vet. Microbiol. 113, 73–82.
Yang, J.H., Bhargava, P., McCloskey, D., Mao, N., Palsson, B.O., Collins, J.J., 2017. Antibiotic-induced changes to the host metabolic environment inhibit drug efficacy and alter immune function. Cell Host Microbe 22, 757–765.e3.
Zhang, Q.Q., Ying, G.G., Pan, C.G., Liu, Y.S., Zhao, J.L., 2015. Comprehensive evaluation of antibiotics emission and fate in the river basins of China: source analysis, multimedia modeling, and linkage to bacterial resistance. Environ. Sci. Technol. 49, 6772–6782.
Zhang, X.D., Xu, D.Z., Li, J.H., et al., 2012b. Study on the immunocompetence of polysaccharide extracted from root of Salvia miltiorrhiza. Zhong Yao Cai 35, 949–952.