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Research Article

Immunomodulatory Effects of *Robinia pseudoacacia* Polysaccharides on Live Vaccine against Infectious Bronchitis in Immunosuppressive Chickens

Qiuyan Sun, Fang Li, Caixia Wang, and Meiyan Shen

Department of Veterinary Medicine, Shandong Vocational Animal Science and Veterinary College, Weifang 261061, China

Correspondence should be addressed to Fang Li; sdmylifang@163.com

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In order to investigate the immunomodulatory effect of *Robinia pseudoacacia* Polysaccharides (RPPS) on vaccine against Infectious Bronchitis (IB) in immunosuppressive chickens, the artificial leukemia chicken model was established and then the IB live vaccine (H120 strain) was immunized. The immunomodulatory efficacy of RPPS was determined by the antibody titer, the lymphocyte transformation rate in peripheral blood, the $CD4^+$ and $CD8^+$ T lymphocyte levels in peripheral blood, and the cytokine levels in the serum. The results showed that RPPS could not only enhance the immune effect of IB live vaccine but also improve the immunity of immunosuppressive chickens. Thus, the function of RPPS immunopotentiator could be further developed.

1. Introduction

In China's large-scale chicken farms, reticuloendotheliosis virus (REV), Marek's disease virus (MDV), avian leukosis virus (ALV), chicken infectious bursal disease virus (IBDV), and other viruses could cause different levels of immunosuppression in chickens; therefore, diseases induced by the viruses are called immunosuppressive diseases. Immunosuppressive diseases have caused significant reduction in poultry production performance, immune system function, and the ability of poultry to respond to vaccine immunization, leading to huge damage and losses to the poultry industry [1, 2]. Studies have found that immunosuppressive diseases were very common in the poultry industry in China and had different levels of inhibitory effect on the antibody production after vaccine immunization in chicks. Avian leukosis (AL) is an avian infectious disease caused by avian leukosis virus (ALV). Infection of ALV could induce growth retardation, decreased production performance, increased mortality and feed consumption, increased development of multitissue tumors, and also immunosuppression in chicken flocks indirectly causing immune failure of other vaccines, which resulted in a huge economic loss to the poultry industry.

Infectious Bronchitis (IB) caused by infectious bronchitis virus (IBV) is characterized by cough, sneezing, tracheal vocalization, difficulty in breathing, asphyxia, kidney enlargement, paleness, urate deposition, "spotted kidney," and egg production and quality decline [3]. At present, it has seriously affected the development of China's poultry industry [4, 5]. Studies have shown that *Robinia pseudoacacia* Linn had various biological effects including antitumor, antioxidation, antibacterial, antiviral, and immune regulation function [6, 7] and had rare toxicity on the body. *Robinia pseudoacacia* Linn is a flower of the perennial deciduous tree eucalyptus of the genus Rhododendron. *Robinia pseudoacacia* Linn has a remarkable curative effect on blood vomiting, hematuria, hemorrhoid hemorrhage, wind-heat redness, hypertension, hyperlipidemia, cervical lymphatic tuberculosis, vascular sclerosis, diabetes, retinitis, psoriasis, etc. It can deworm and treat pharyngitis and has extremely high medicinal value [8, 9], so *Robinia pseudoacacia* Linn has high medical value and belongs to healthy products in people's life as a traditional Chinese medicine. *Robinia pseudoacacia* polysaccharides (RPPS) are a kind of plant polysaccharides. So far, there have been few reports on the immunomodulatory effects of *Robinia pseudoacacia*

polysaccharides (RPPS) on livestock and poultry, especially on RPPS for immunosuppressive chickens. The immune regulation of IB vaccine has not been reported at all. Therefore, we established a model of artificial leukemia chicken and then immunized them with live IB vaccine (H120 strain). The efficacy was determined by detecting the antibody titer, immune organ index, lymphocyte conversion rate in the peripheral blood, $CD4^+$ and $CD8^+$ T cell levels in the peripheral blood, and cytokine levels in the serum. The study will lay the foundation for further development of application value and market prospect of *Robinia pseudoacacia* polysaccharides and of green ecological animal husbandry in China.

2. Materials and Methods

2.1. Reagents. Concanavalin A (ConA), lymphocyte separation solution, IL-2 ELISA kit, and IFN-γ ELISA kit were purchased from Sigma, USA. RPMI-1640 (Gibco) and fetal bovine serum (Gibco) were purchased from Invitrogen, USA.

2.2. Instrument. PE-6800VET automatic animal blood analyzer was from Japan Sysmex company. Enzyme standard analyzer RT-6100 was from Rayto, Shenzhen, China.

2.3. Strains and Vaccines. The IBV H120 (infectious bronchitis H120) strain with $TCID_{50}$ of $10^{-7.32}$/0.1 mL was kindly provided by Professor Yuyan Wang from the Department of Pathogenic Biology of Fudan University, School of Medicine. ALV-J (Avian Leukosis virus-J) NX0102 strain $TCID_{50}$ of $10^{-5.96}$/0.1 mL was kindly provided by Professor Ruiliang Zhu of Shandong Agricultural University. IB H120 live vaccine was purchased from Qingdao Yibang Bioengineering Co. Ltd. (production batch number: 150132016).

2.4. Extraction and Determination of Robinia pseudoacacia Polysaccharides (RPPS) [10]. Fresh *Robinia pseudoacacia* flowers, collected from Shandong Animal Husbandry and Veterinary Vocational College in May, were cleaned and dried at 60°C, then pulverized by ultrafine pulverizer and extracted by boiled alcohol precipitation method. The flowers were deproteinized by sevage method and purified by C18 solid phase extraction column. The content of polysaccharides was measured by PMP (1-phenyl-3-methyl-5-pyrazolone) precolumn derivatization ultrahigh performance liquid chromatography tandem mass spectrometry.

2.5. Experimental Animals

2.5.1. Establishing the Model of Artificial Leukemia Chicken. 140 one-day-old SPF white-feathered chicks were intraperitoneally injected with ALV-J $10^{5.96}$ $TCID_{50}$/0.1 mL at a dose of 0.1 mL per chick; the artificial leukemia chicken model was established.

2.5.2. Grouping of Experimental Animals. The 1-day-old SPF white-feathered chicks were purchased from the SPAFAS chicken farm in Jinan, Shandong. The chickens used during the study were kept in strict accordance with the SPF chicken breeding management requirements in the SPF animal house of the Shandong Animal Husbandry and Veterinary Vocational College. 210 one-day-old SPF white-feathered chicks were randomly divided into 3 groups with 70 animals each; the three groups were group I, group IV, and group V. 140 artificial leukemia chicken model was divided into 2 groups, which were group II and group III. Three groups, groups I to II, were inoculated subcutaneously with 10 mg/mL of *Robinia pseudoacacia* polysaccharides daily at 2 days of age. The inoculation dose was 0.1 mL per chick for 3 days. In groups III and IV, the chicks were injected subcutaneously with saline, 0.1 mL/day for 3 days, and groups I to IV were inoculated intranasally with live IB vaccine at 7 days. Group V served as the blank control group. Ten chickens were randomly selected from each group at 7 d, 14 d, 21 d, 28 d, 35 d, 42 d, and 49 d after immunization for follow-up evaluation after being for 8 h before the test.

2.6. Determination of IB Antibody Titer in the Serum. 1.0 mL aseptically-collected heart blood from chickens was centrifuged with a speed of 3000 rpm/min for 25 min in a centrifuge tube, with the supernatant removed. The IB antibody titer was determined by a fixed virus dilution serum neutralization test.

2.7. Determination of Immune Organ Index. Each group of chickens was weighed and then the bursa, spleen, and thymus were removed. The organs, their surface water gently wiped with sterile filter paper, were weighed separately, and the bursa, spleen, and thymus indexes were calculated according to the formula: immune organ index (mg/g) = (immune organ mass/body weight).

2.8. Determination of Peripheral Blood Lymphocyte Transformation Rate. 2 mL heart blood, collected with EDTA-Na vacuum sterile blood collection tube, was shaken up and diluted with 2 mL RPMI-1640.4 mL human lymphocyte separation solution (density 1.085) which was added in the tube followed by centrifugation at 20°C with a speed of 2000 rpm/min for 20 min. Afterward, the supernatant was removed and the solution was centrifuged for 2 more times. After cell precipitation, RPMI-1640 containing 10% fetal bovine serum was added in for suspension cultivation, and a drop of the cell suspension was counted. The cell suspension was diluted to 2×10^6 cells/mL. Then the lymphocyte suspension was inoculated into a 96-well cell culture plate with a density of 0.1 mL/well. Each sample was replicated by 4 wells, in which 3 wells were added with Congo protein (ConA) at a final concentration of 50 μg/mL, and the last well was used as a negative control. All the samples were put into an incubator at 37°C with the volume of 5% CO_2 until the appearance of single-layer cells. Afterward, 5 μL of 5 mg/L MTT solution was added in each well for another 4 hours of incubation. Then the supernatant was gently discarded and 200 μL of DMSO solution was added into each well followed by a 10-minute shaking. The absorbance value was measured at 490 nm with a microplate reader for the calculation of the lymphocyte conversion rate.

2.9. Determination of $CD4^+$ and $CD8^+$ T Lymphocyte Levels in the Peripheral Blood. 2 mL heart blood was collected with EDTA-Na vacuum aseptic blood collection tube, and the

content of $CD4^+$ and $CD8^+$ T lymphocyte in the peripheral blood was measured and calculated by PE-6800VET automatic animal blood analyzer.

2.10. Determination of Cytokine Levels in the Serum. 2 mL aseptically-collected heart blood from chickens was centrifuged at 3000 rpm/min for 25 min in a centrifuge tube, with the supernatant removed and stored at −20°C. The content of cytokine IL-2 and IFN-γ in the serum was measured referring to the manufacturer instruction of IL-2 ELISA kit and IFN-γ ELISA kit.

2.11. Data Analysis. Data analysis was worked out based on SPSS Statistics 19.0 statistical analysis software. Multiple comparisons were performed using the LSD method, and the test data were expressed as mean ± standard deviation (Mean ± SD) and the significant difference was analyzed.

3. Results

3.1. Extraction and Content Determination of Polysaccharides from Robinia pseudoacacia (RPPS). Polysaccharide was extracted from the fresh locust flower, dried, and pulverized via water boiling and precipitation with ethanol, with the extract of 25.7%. The content of polysaccharide was 71.3% by PMP precolumn derivatization high-performance liquid chromatography tandem mass spectrometry.

3.2. Changes of IB Antibody Titer in the Serum. The IB antibody titer in each group of serum is shown in Figure 1. The titer of IB antibody in the serum of group I was significantly higher than that of the other groups ($P < 0.01$), and the retention time of the high-level antibody titer was longer. The difference of IB antibody titer in the serum between group II and group IV was not obvious ($P > 0.05$); however, the two groups were much higher than group III in antibody titer in the serum. The IB antibody titer in the serum of group V was 0. The IB antibody titer in the serum of groups I to IV peaked at 21 days after immunization and then began to decrease. The IB antibody titer in the serum of group III was extremely lower than that of group I ($P < 0.01$), group II, and group IV ($P < 0.05$).

3.3. Changes of the Immune Organ Index

3.3.1. The Change of the Bursal Index. The bursal index of group I was much higher than that of the other groups ($P < 0.01$). The difference of the bursal index between group II and group IV was not obvious ($P > 0.05$), but their indexes were significantly higher compared with that of group III. The bursal index of group III was obviously lower than that of group I ($P < 0.01$), group II, and group IV ($P < 0.05$), shown in Figure 2.

3.3.2. The Change of the Thymus Index. The thymus index of group I was significantly higher than that of the other groups ($P < 0.01$). The thymus index of group IV was much higher than that of groups II, III, and V ($P < 0.05$). The thymus index of group II was much higher than that of group III ($P < 0.05$). The thymus index of group III was obviously lower than that of group I ($P < 0.01$), group II, group IV, and group V ($P < 0.05$), shown in Figure 3.

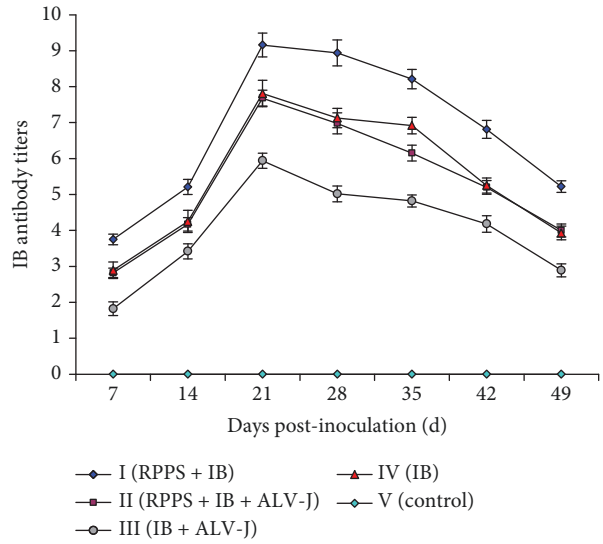

Figure 1: Changes of antibody titer in the serum.

3.3.3. The Change of the Spleen Index. The spleen index of group I was significantly higher than that of the other groups ($P < 0.01$). The spleen index of group IV was much higher than that of groups II, III, and V ($P < 0.05$). There was no remarkable difference in the spleen index between group II and group V ($P > 0.05$). The spleen index of group III was obviously lower than that of group I ($P < 0.01$), group II, group IV, and group V ($P < 0.05$), shown in Figure 4.

3.4. Changes of Peripheral Blood Lymphocyte Transformation Rate. The changes of transformation rate of peripheral blood lymphocytes of each group are shown in Figure 5. The results showed that the transformation rate of peripheral blood lymphocytes in group I was significantly higher than that of the other groups ($P < 0.05$). The transformation rate of peripheral blood lymphocytes in group II was obviously lower than that of group IV ($P < 0.05$). The transformation rate of peripheral blood lymphocytes of group III was much lower than that of the other groups ($P < 0.05$) and was decreased rapidly after 28 days.

3.5. Changes of $CD4^+$ T Lymphocytes in the Peripheral Blood. The changes of $CD4^+$ T lymphocytes in the peripheral blood are shown in Table 1. The content of $CD4^+$ T lymphocytes in the peripheral blood of group III was significantly lower than that of the other groups ($P < 0.01$). The content of $CD4^+$ T lymphocytes in the peripheral blood of group I was obviously higher than that of the other groups ($P < 0.05$). The content of $CD4^+$ T lymphocytes in the peripheral blood of group II and group IV showed no observable difference ($P > 0.05$). The content of $CD4^+$ lymphocytes in the peripheral blood of group II and IV was much higher than that of group V ($P < 0.05$). The content of $CD4^+$ lymphocytes in the peripheral blood of group I to group IV reached a peak after 21 days and then slowly decreased.

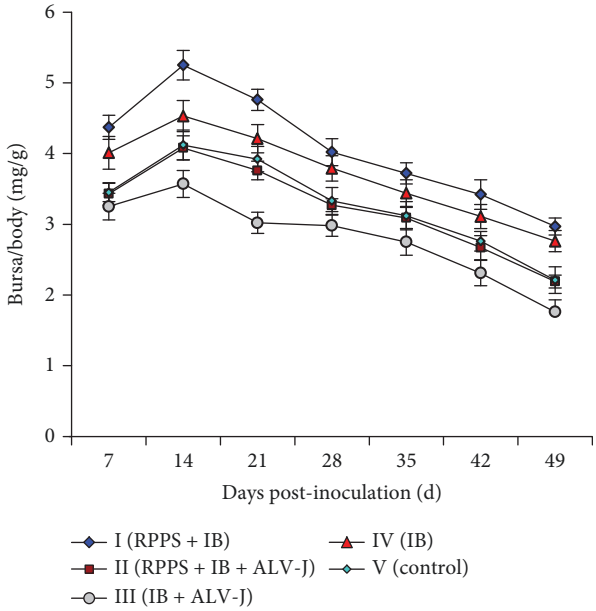

Figure 2: Changes of the bursa index.

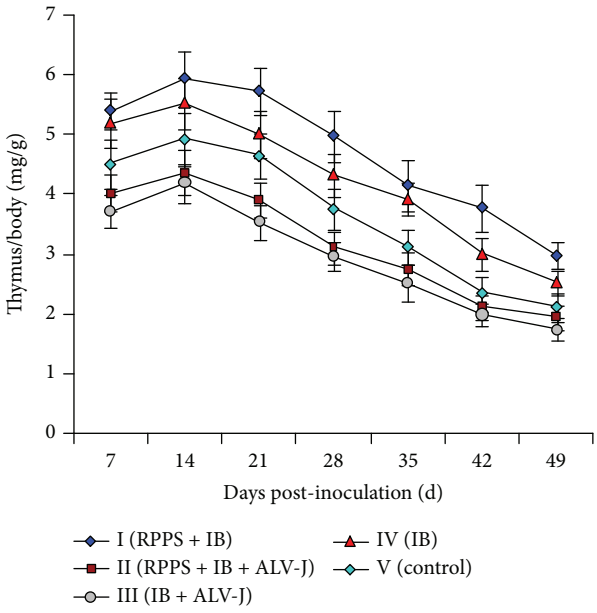

Figure 3: Changes of the thymus index.

3.6. Changes of $CD8^+$ T Lymphocytes in the Peripheral Blood. The changes of $CD8^+$ T lymphocytes in the peripheral blood are shown in Table 2. The content of $CD8^+$ lymphocytes in the peripheral blood of group I was significantly higher than that of the other groups ($P < 0.01$). The content of $CD8^+$ lymphocytes in the peripheral blood of group III was obviously lower than that of the other groups ($P < 0.01$). The content of $CD8^+$ lymphocytes in the peripheral blood of group II and group IV showed no observable difference ($P > 0.05$). The content of $CD4^+$ lymphocytes in the peripheral blood of group I to group IV reached a peak after 21 days and then decreased slowly.

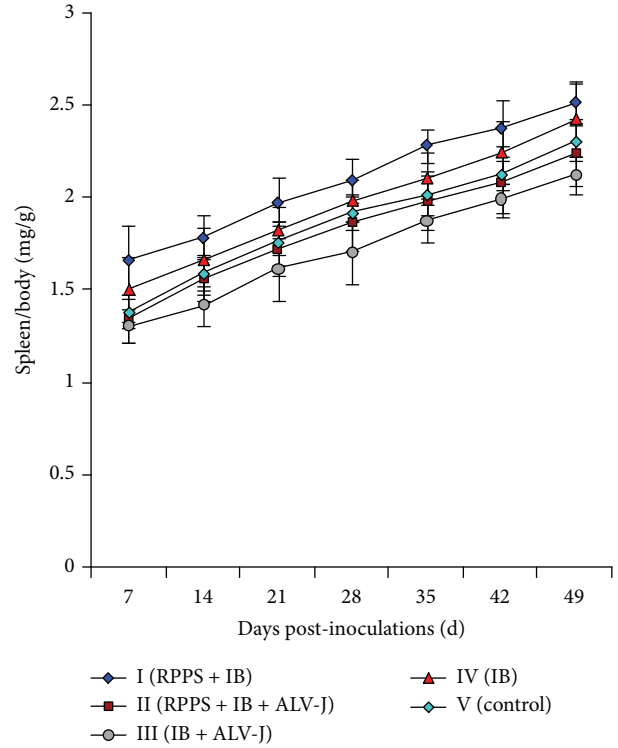

Figure 4: Changes of the spleen index.

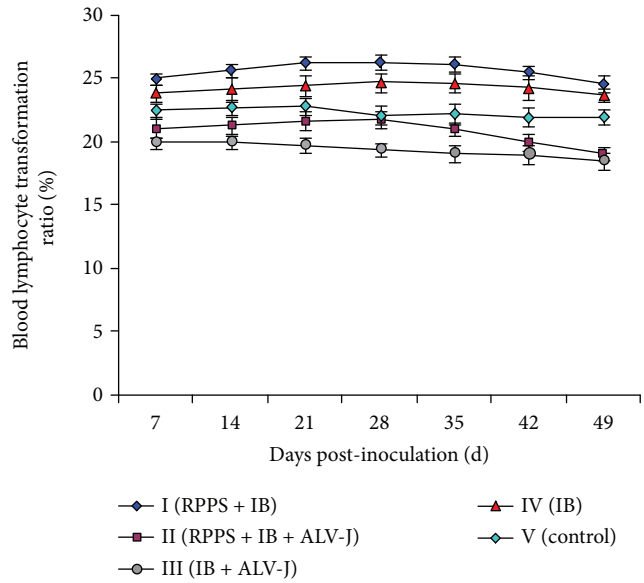

Figure 5: Effect of RPPS on blood lymphocyte transformation rate in chickens (%).

3.7. Changes of Cytokine IL-2 Level in the Serum. The results in Figure 6 showed that the level of cytokine IL-2 in the serum of group I was significantly higher than that of the other groups ($P < 0.01$). The level of cytokine IL-2 in the serum of group II was much higher than that of group III ($P < 0.05$). The level of cytokine IL-2 in the serum of group

Table 1: Effects of RPPS on $CD4^+$ T lymphocyte in the peripheral blood.

Groups	Days postinoculation and lymphocyte transformation rates						
	7	14	21	28	35	42	49
I (RPPS+IB)	34.53 ± 4.21^{a}	46.98 ± 5.11^{a}	49.28 ± 4.57^{a}	48.23 ± 4.22^{a}	45.69 ± 3.65^{a}	44.12 ± 3.52^{a}	40.78 ± 3.47^{a}
II (RPPS+IB+ALV-J)	31.98 ± 3.47^{b}	42.65 ± 3.78^{b}	45.02 ± 4.23^{b}	44.42 ± 3.57^{b}	41.88 ± 3.59^{b}	41.03 ± 3.43^{b}	37.67 ± 3.55^{b}
III (IB+ALV-J)	22.96 ± 3.76^{A}	24.02 ± 3.43^{A}	25.98 ± 4.42^{A}	25.55 ± 3.45^{A}	25.01 ± 3.54^{A}	24.9 ± 3.68^{A}	24.76 ± 3.71^{A}
IV (IB)	32.09 ± 3.41^{b}	42.77 ± 4.85^{b}	45.68 ± 3.98^{b}	44.79 ± 3.62^{b}	42.52 ± 3.73^{b}	41.78 ± 3.67^{b}	38.23 ± 4.02^{b}
V (control)	29.95 ± 2.97^{c}	35.27 ± 3.78^{c}	39.76 ± 4.52^{c}	39.02 ± 4.07^{c}	37.99 ± 3.82^{c}	35.03 ± 3.57^{c}	34.79 ± 3.24^{c}

Note: groups with different superscript lowercase letters are statistically different at $P < 0.05$; groups with superscript capital letters are statistically extremely different ($P < 0.01$).

Table 2: Effects of RPPS on $CD8^+$ T lymphocyte in the peripheral blood.

Groups	Days postinoculation and lymphocyte transformation rates						
	7	14	21	28	35	42	49
I (RPPS+IB)	18.32 ± 4.71^{A}	24.55 ± 5.43^{A}	27.87 ± 5.46^{A}	26.97 ± 5.22^{A}	25.12 ± 3.65^{A}	24.37 ± 3.52^{A}	23.05 ± 3.47^{A}
II (RPPS+IB+ALV-J)	15.32 ± 3.12^{a}	19.21 ± 3.41^{a}	22.94 ± 3.65^{a}	22.65 ± 2.89^{a}	21.19 ± 2.78^{a}	20.05 ± 3.21^{a}	19.53 ± 2.96^{a}
III (IB+ALV-J)	12.47 ± 2.76^{B}	13.53 ± 3.25^{B}	15.05 ± 3.76^{B}	14.76 ± 2.98^{B}	13.51 ± 2.74^{B}	11.65 ± 2.77^{B}	10.56 ± 2.12^{B}
IV (IB)	15.71 ± 2.92^{a}	19.65 ± 4.52^{a}	23.32 ± 4.78^{a}	22.77 ± 4.23^{a}	21.23 ± 3.42^{a}	20.45 ± 3.67^{a}	19.72 ± 3.02^{a}
V (control)	13.98 ± 2.21^{b}	18.21 ± 4.02^{b}	21.45 ± 4.33^{b}	20.98 ± 3.67^{b}	19.88 ± 3.82^{b}	18.21 ± 3.57^{b}	17.92 ± 2.96^{b}

Note: groups with different superscript lowercase letters are statistically different at $P < 0.05$; groups with superscript capital letters are statistically extremely different ($P < 0.01$).

II was obviously lower than that of group IV ($P < 0.05$). The levels of cytokine IL-2 within the first 7 days of group III and group V showed no observable difference ($P > 0.05$). However, the difference became significant from the 7^{th} day to the 28^{th} day and returned the value within the first 7 days after 28 days ($P > 0.05$).

3.8. Changes of Cytokine IFN-γ Level in the Serum. The results in Figure 7 showed that the level of cytokine IFN-γ in the serum of group I was significantly higher than that of the other groups ($P < 0.01$). There was no obvious difference in the IFN-γ level between group III and group V ($P > 0.05$). The level of cytokine IFN-γ of groups II and IV showed no observable difference within the first 14 days ($P > 0.05$), while the difference became significant after 14 days ($P < 0.05$). The cytokine IFN-γ content in the serum of group II was much higher than that of group III ($P < 0.05$).

4. Discussion

According to grouping of experimental animals, I marks the group of vaccine and RPPS (group I), II the group of chicken leukemia, vaccine, and RPPS (group II), III the group of chicken leukemia and vaccine (group III), IV the group of vaccine strain (group IV), and V the control group (group V).

Changes in the serum antibody titer of the body can accurately and intuitively reflect the state of humoral immunity [11]. The titer of IB antibody in the serum of group I was obviously higher than that of the other groups ($P < 0.01$), indicating that RPPS can significantly improve the serum antibody titer of IB live vaccine. IB antibody titer in the serum of both groups II and IV was not notably different ($P > 0.05$) but was much higher than that of group III, indicating that RPPS can significantly increase the serum antibody level in immunosuppressive chickens.

The brusa fabricius, thymus, and spleen are the main immune organs of birds. These organs are the main sites for immune cell formation, differentiation, and antibody formation. The developmental status of immune organs directly influences to resist pathogenic microorganisms. So the immune organ index can be used to reflect the immune status of birds; the increase of immune organ index is an enhancement of immune function. The detection of avian bursal, thymus, and spleen index changes can directly determine the immune status of animals [12]. The study observed the bursal index, thymus index, and spleen index of chickens. The results showed that the bursal index, thymus index, and spleen index of chickens in group I were all remarkably higher than those of the rest of the groups ($P < 0.01$). The bursal index, thymus index, and spleen index of chickens in group III were remarkably lower than those of both group I ($P < 0.01$) and group II ($P < 0.05$). This study found that RPPS could significantly increase the bursal index, thymus index, and spleen index of chickens and also the bursal index, thymus index, and spleen index of immunosuppressive chickens, suggesting that RPPS could enhance immune function in poultry.

Lymphocyte transformation rate is the most direct indicator of cellular immunity [13]. The conversion rate of lymphocytes in the peripheral blood of RPPS and IB vaccine group was significantly higher than the other groups; the infected ALV-J and IB vaccine groups decreased the

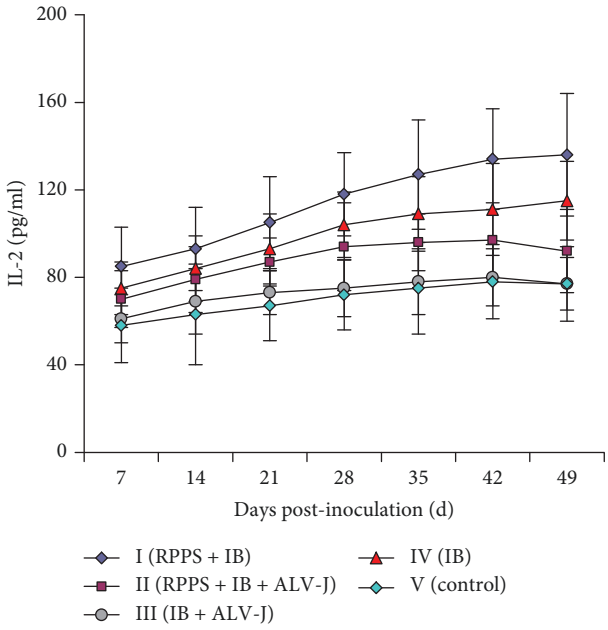

Figure 6: Changes of cytokine IL-2 level in the serum.

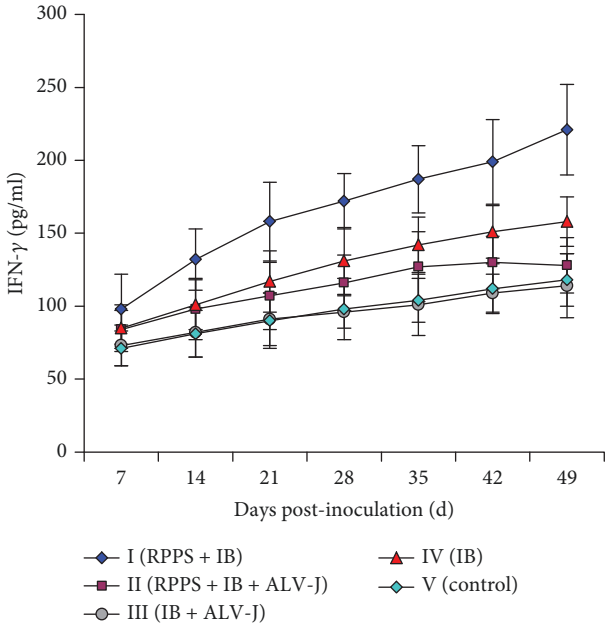

Figure 7: Changes of cytokine IFN-γ level in the serum.

conversion rate of lymphocytes in the peripheral blood compared with the other groups. These results imply that RPPS could increase the conversion rate of lymphocytes in the peripheral blood. And the immunosuppressive disease could reduce the lymphocyte transformation rate of chicken.

The mature $CD4^+$ and $CD8^+$ T cells in the peripheral blood are mutually exclusive subsets. $CD4^+$ T cells belong to T helper (Th) cells, which are divided functionally into Th1 and Th2 cells based on the distinct patterns of cytokine production. $CD8^+$ T cells are immune effector cells that belong to cytotoxic T lymphocytes and are responsible for the removal of target cells through the direct killing effect; the increased counts of $CD4^+$ and $CD8^+$ lymphocytes reflect the enhanced T cell-mediated immunity. So the content of $CD4^+$ and $CD8^+$ T in the peripheral blood is the basis for evaluation of immune status [14]. The content of $CD4^+$ lymphocytes in the peripheral blood of RPPS and IB vaccine group was significantly higher than that of the other groups ($P < 0.05$); the content of $CD4^+$ lymphocytes in the peripheral blood of infected ALV-J and IB vaccine group was extremely lower than the other groups ($P < 0.01$). The results indicate that RPPS could remarkably increase the content of $CD4^+$ lymphocytes in the peripheral blood of chickens and RPPS can increase the content of $CD4^+$ lymphocytes in the peripheral blood of chickens with immunosuppressive disease. The content of $CD8^+$ lymphocytes in the peripheral blood of group RPPS and IB vaccine group was remarkably higher than that of the other groups ($P < 0.01$); the content of $CD8^+$ lymphocytes in the peripheral blood of infected ALV-J and IB vaccine group was significantly lower than the other groups ($P < 0.01$); the difference of $CD8^+$ lymphocytes in the peripheral blood between groups II and IV is not significant ($P > 0.05$). The results indicate that RPPS could significantly increase the content of $CD8^+$ lymphocytes in the peripheral blood of chickens and the content of $CD8^+$ lymphocytes in the peripheral blood of immunosuppressive chickens.

IL-2 mainly produced by activated T lymphocytes has important immunoregulatory effects in the immune system and can enhance multiple functions of immune cells, stimulating helper T cells and natural killer cells to produce cytokines and facilitate their proliferation. Therefore, IL-2 level can indirectly reflect the level of immune response [15]. The content of cytokine IL-2 in the serum of group I was significantly higher than that of the other groups ($P < 0.01$), indicating that RPPS could remarkably increase the content of cytokine IL-2 in chicken serum. The content of cytokine IL-2 in the serum of group II was obviously higher than that of group III ($P < 0.05$), suggesting that RPPS could increase the cytokine IL-2 content in the serum of immunosuppressive chickens. The content of cytokine IL-2 in the serum of group II was much lower than that of group IV ($P < 0.05$), indicating that the immunosuppression disease could inhibit the production of cytokine IL-2 in chicken serum.

IFN-γ can stimulate natural killer cells and enhance their killing function. It can also promote macrophage and cytotoxic T lymphocyte maturation and stimulate B lymphocytes to secrete antibodies, thereby enhancing the body's immune function [16]. The cytokine IFN-γ level in the serum of group I was significantly higher than that of the other groups ($P < 0.01$), indicating that RPPS can remarkably increase cytokine IFN-γ production in chicken serum. The difference of cytokine IFN-γ in the serum between group III and group V was not obvious ($P > 0.05$), indicating that immunosuppressive disease can reduce the function of vaccine to stimulate IFN-γ production. The content of cytokine IFN-γ in the serum of group II was much higher than that of group III ($P < 0.05$), indicating that RPPS can dramatically increase the content of cytokine IFN-γ in the serum of immunosuppressive chickens.

5. Conclusion

RPPS can significantly enhance the vaccine immune effect and improve the immune function of immunosuppressive chickens. Thus, the efficacy of RPPS in immunity enhancement can be further studied.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

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