Effects of Lactic Acid Bacteria and Yeast Composite Preparation and Its Combination with Virginiamycin on Serum Biochemical Indices, Antioxidant Capacity, and Immune Function in Broiler Chickens: Postprint

Authors: Zhang Caifeng, Qi Guanghai, Wang Xiaocui, Wu Shugeng, Zhang Haijun, Wang Jing, Shi Zhaoguo, Wang Liang, Wang Hui

Date: 2018-12-24T00:00:00+00:00

Abstract

This experiment aimed to investigate the effects of a lactic acid bacteria and yeast compound preparation (LS) and its combined use with virginiamycin (VM) on serum biochemical indices, antioxidant capacity, and immune function in broiler chickens. A total of 400 healthy 1-day-old Arbor Acres broiler male chicks were selected and divided into 4 groups according to the principle of similar body weight, with 5 replicates per group and 20 chickens per replicate. The control group was fed a basal diet, while the experimental groups were fed experimental diets supplemented with 30 mg/kg VM (VM group), 15 mg/kg VM + 0.1% LS (VM+LS group), and 0.1% LS (LS group) in the basal diet, respectively. The experimental period lasted 42 days. The results showed that: 1) Compared with the control group, serum total protein content at 42 days of age was significantly increased ($P<0.05$) in the VM+LS and LS groups. 2) At 21 days of age, compared with the control group, serum malondialdehyde content was significantly decreased ($P<0.05$) in the VM, VM+LS, and LS groups; serum glutathione peroxidase activity was significantly increased ($P<0.05$) in the VM+LS and LS groups. At 42 days of age, compared with the control group, serum total superoxide dismutase and glutathione peroxidase activities were significantly increased ($P<0.05$), while serum malondialdehyde content was significantly decreased ($P<0.05$) in the VM+LS and LS groups. 3) There were no significant differences ($P>0.05$) in immune organ indices among groups at 21 and 42 days of age, but numerically, the VM+LS and LS groups were higher than the control and VM groups. Serum immunoglobulin G (IgG) and immunoglobulin A (IgA) contents in 21-day-old broilers were significantly higher

(P<0.05) in the LS group than in the control group; serum IgG, IgA, and immunoglobulin M (IgM) contents in 42-day-old broilers were significantly higher (P<0.05) in the LS group than in the control group; serum IgG content in 42-day-old broilers was significantly higher (P<0.05) in the VM+LS group than in the control group. It can be concluded that dietary supplementation with 0.1% LS and its combination with 15 mg/kg VM can both improve the antioxidant capacity and immune function of broiler chickens, with the effect of 0.1% LS alone being more pronounced.

Full Text

Effects of Lactobacillus and Saccharomycetes Compound Preparation and Its Combination with Virginiamycin on Serum Biochemical Indexes, Antioxidant Capacity and Immune Function of Broilers

Zhang Caifeng[1],[2], Qi Guanghai[1], Wang Xiaocui[1], Wu Shugeng[1], *Zhang Haijun[1]*, Wang Jing[1], Shi Zhaoguo[2], Wang Liang[3], Wang Hui

[1]Key Laboratory of Feed Biotechnology of Ministry of Agriculture, National Engineering Research Center of Biological Feed, Feed Research Institute, Chinese Academy of Agricultural Sciences, Beijing 100081, China
[2]College of Animal Science and Technology, Gansu Agricultural University, Lanzhou 730070, China
[3]Beijing General Station of Animal Husbandry, Beijing 100107, China
Xiamen Honghegu Biotechnology Co., Ltd., Xiamen 361000, China

Corresponding authors: Wu Shugeng, professor, E-mail: wushugeng@caas.cn; Zhang Haijun, associate professor, E-mail: zhanghaijun@caas.cn

---

Abstract

This experiment investigated the effects of a Lactobacillus and Saccharomycetes compound preparation (LS) and its combination with virginiamycin (VM) on serum biochemical indexes, antioxidant capacity, and immune function in broilers. Four hundred one-day-old healthy Arbor Acres (AA) male broilers were randomly allocated into four groups with five replicates each (20 broilers per replicate). The control group received a basal diet, while experimental groups received basal diets supplemented with 30 mg/kg VM (VM group), 15 mg/kg VM + 0.1% LS (VM+LS group), or 0.1% LS (LS group). The 42-day trial revealed several key findings. First, serum total protein content at 42 days of age was significantly higher in the VM+LS and LS groups compared to the control (P<0.05). Second, at 21 days of age, serum malondialdehyde (MDA) content was significantly reduced in the VM, VM+LS, and LS groups (P<0.05), while serum glutathione peroxidase (GSH-Px) activity was significantly elevated in

the VM+LS and LS groups (P<0.05). At 42 days of age, the VM+LS and LS groups showed significantly increased serum total superoxide dismutase (T-SOD) and GSH-Px activities (P<0.05) and significantly decreased serum MDA content (P<0.05) compared to the control. Third, although no significant differences were observed in immune organ indexes among groups at 21 and 42 days of age (P>0.05), the VM+LS and LS groups exhibited numerically higher values than the control and VM groups. The LS group demonstrated significantly higher serum immunoglobulin G (IgG) and immunoglobulin A (IgA) contents at 21 days of age (P<0.05), and significantly higher serum IgG, IgA, and immunoglobulin M (IgM) contents at 42 days of age (P<0.05) compared to the control. The VM+LS group also showed significantly higher serum IgG content at 42 days of age (P<0.05) relative to the control. These results indicate that dietary supplementation with 0.1% LS alone or in combination with VM can enhance the antioxidant capacity and immune function of broilers, with the individual addition of 0.1% LS showing more pronounced effects.

Keywords: probiotics; Lactobacillus; Saccharomycetes; virginiamycin; broiler; antioxidant capacity; immune function

Introduction

The broiler industry represents the fastest-growing and most highly intensified sector of China' s animal agriculture [1]. Feed antibiotics have been widely and extensively used in livestock production [2] to prevent disease and promote growth, but this practice has led to increased bacterial resistance [3-4]. The global movement to ban antibiotics in feed began in 1986, and an increasing number of countries have since implemented their own restrictions. Consequently, nutritional modulation of broiler health has become a major research focus.

Lactobacillus and Saccharomycetes can promote the development of immune organs such as the spleen, thymus, and bursa of Fabricius in broilers [5-9], and significantly increase the contents of immunoglobulin M (IgM), immunoglobulin A (IgA), and immunoglobulin G (IgG) in tears, organ fluids, intestinal fluid, and bile [10], thereby enhancing immunity. These microorganisms mutually promote growth through complementary metabolites and quorum sensing. Our previous research has confirmed that dietary supplementation with 0.1% Lactobacillus and Saccharomycetes compound preparation can improve broiler growth performance by regulating intestinal health [11]. However, research on the effects of this compound preparation as an antibiotic alternative or in combination with antibiotics on broiler antioxidant capacity and immune function remains insufficient to fully explain the mechanisms underlying its growth-promoting effects [11]. Therefore, this study examined the impacts of dietary Lactobacillus and Saccharomycetes compound preparation alone and in combination with virginiamycin on serum biochemical indexes, antioxidant capacity, and immune function in broilers, aiming to provide a theoretical basis for the rational appli-

cation of this probiotic preparation in broiler production.

1.1 Experimental Materials

The Lactobacillus and Saccharomycetes compound preparation was provided by Xiamen Honghegu Biotechnology Co., Ltd., containing *Lactobacillus* (BCRC 16092) at 2.5×10 CFU/g and *Saccharomycetes* (BCRC 20262) at 1.3×10 CFU/g. Virginiamycin was purchased from Phibro Animal Health Corporation (USA) with an active ingredient content of 50%.

1.2 Experimental Design and Management

Four hundred one-day-old healthy Arbor Acres (AA) male broilers were randomly divided into four groups according to similar body weight principles. The groups included: a control group fed the basal diet, a VM group receiving basal diet + 30 mg/kg VM, a VM+LS group receiving basal diet + 15 mg/kg VM + 0.1% LS (with measured values of 2.3×10 CFU/g Lactobacillus and 1.3×10 CFU/g Saccharomycetes in the final diet), and an LS group receiving basal diet + 0.1% LS (same measured values). Each group consisted of five replicates with 20 broilers per replicate. Birds were raised in four-tier cage systems for 42 days, divided into a starter phase (1-21 days) and a grower phase (22-42 days). The basal diets were formulated according to NRC (1994), Chinese Feeding Standard of Chickens (NY/T 33-2004), and the AA Broiler Management Guide. Diet composition and nutrient levels are presented in Table 1 . All diets were cold-pelleted (maximum temperature 65 °C during pelleting) and rapidly cooled.

During the trial, birds were exposed to natural light supplemented with artificial lighting (24 h/d for the first 7 days, then 23 h/d thereafter) and provided ad libitum access to feed and water. Room temperature was maintained at 33 °C for the first 3 days, then reduced by 2 °C weekly until reaching 24 °C, which was maintained for the remainder of the experiment. Routine immunization and disinfection procedures followed the AA Broiler Management Guide. Temperature and humidity were recorded 24 h daily, with regular cleaning and mortality recording throughout the experimental period.

1.3 Sample Collection and Analysis

1.3.1 Immune Organ Index On days 21 and 42 of the experiment, two broilers with body weight close to the replicate average were selected from each replicate, euthanized by jugular venesection, and the spleen, bursa of Fabricius, and thymus were carefully dissected and weighed to calculate organ indexes using the formula: Immune organ index (%) = [immune organ weight (g) / live body weight (kg)] × 100.

1.3.2 Serum Sample Preparation On days 21 and 42, two broilers per replicate with body weight near the replicate average were selected for blood collection (5 mL from the wing vein using anticoagulant tubes). Samples were centrifuged at 3,000 r/min for 10 min, and the supernatant was aliquoted into 1.5 mL Eppendorf tubes and stored at -20 °C for subsequent analysis of serum biochemical indexes, antioxidant indexes, and immunoglobulin contents.

Serum Biochemical Indexes: Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities, along with total protein (TP), albumin (ALB), uric acid (UA), creatinine (CRE), total bilirubin (TBILI), and glucose (GLU) contents were determined using commercial kits with a semi-automatic biochemical analyzer (CHEM-5 model). All kits were purchased from Shanghai Kehua Biotechnology Co., Ltd.

Serum Antioxidant Indexes: Serum total antioxidant capacity (T-AOC) and GSH-Px activity were measured by colorimetric methods, malondialdehyde (MDA) content by thiobarbituric acid method, and total superoxide dismutase (T-SOD) activity by xanthine oxidase method. All assay kits were obtained from Nanjing Jiancheng Bioengineering Institute.

Serum Immunoglobulin Contents: Serum IgG, IgA, and IgM contents were determined by enzyme-linked immunosorbent assay (ELISA) using kits from Nanjing Jiancheng Bioengineering Institute.

1.4 Statistical Analysis

Data were analyzed using one-way ANOVA in SPSS 16.0 software. Duncan' s multiple comparison tests were performed when significant differences were detected. Significance was declared at $P<0.05$. Results are expressed as "mean ± standard deviation."

Results

2.1 Effects on Serum Antioxidant Indexes

The effects of LS and its combination with VM on serum antioxidant indexes are shown in Table 2 . At 21 days of age, dietary supplementation significantly affected serum MDA content ($P<0.001$) and GSH-Px activity ($P=0.049$). Compared with the control group, serum MDA content was significantly lower in the VM, VM+LS, and LS groups ($P<0.05$). The VM+LS and LS groups exhibited significantly higher serum GSH-Px activity than the control and VM groups ($P<0.05$), with no significant difference between the VM+LS and LS groups ($P>0.05$).

At 42 days of age, dietary treatments significantly influenced serum T-AOC ($P=0.035$), MDA content ($P=0.004$), and T-SOD ($P<0.001$) and GSH-Px activities ($P=0.004$). The VM+LS group showed significantly higher serum T-AOC

compared to the control (P<0.05), while the LS group did not differ significantly from the VM+LS group (P>0.05). Both VM+LS and LS groups had significantly lower serum MDA content than the control (P<0.05), with no significant differences among the VM group and the other three groups (P>0.05). The VM+LS and LS groups demonstrated significantly higher serum T-SOD and GSH-Px activities than the control and VM groups (P<0.05), with the LS group showing significantly higher T-SOD activity than the VM+LS group (P<0.05).

These results indicate that both 0.1% LS alone and in combination with 15 mg/kg VM can enhance serum T-SOD and GSH-Px activities while reducing serum MDA content, thereby improving the redox status of broilers.

2.2 Effects on Immune Organ Indexes

The effects of LS and its combination with VM on immune organ indexes are presented in Table 3 . Dietary supplementation did not significantly affect immune organ indexes at either 21 or 42 days of age (P>0.05). However, numerically, the VM+LS and LS groups showed higher values compared to the control and VM groups. These findings suggest that 0.1% LS alone or combined with 15 mg/kg VM may have a tendency to increase the spleen index.

2.3 Effects on Serum Immunoglobulin Contents

The effects of LS and its combination with VM on serum immunoglobulin contents are shown in Table 4 . Dietary supplementation significantly affected serum IgG (P<0.001) and IgA contents (P=0.001) at 21 days of age. The LS group exhibited significantly higher serum IgG content compared to the control and VM groups (P<0.05), and also significantly higher than the VM+LS group (P<0.05). Additionally, the LS group showed significantly higher serum IgA content than all other groups at 21 days of age (P<0.05).

At 42 days of age, dietary treatments significantly influenced serum IgG (P<0.001), IgA (P=0.038), and IgM (P=0.011) contents. Both VM+LS and LS groups had significantly higher serum IgG content than the control and VM groups (P<0.05), with the LS group showing significantly higher values than the VM+LS group (P<0.05). The LS group also demonstrated significantly higher serum IgA and IgM contents compared to the control and VM groups (P<0.05), while no significant differences were observed between the control and VM groups (P>0.05).

These results demonstrate that both 0.1% LS alone and in combination with 15 mg/kg VM can increase serum immunoglobulin contents in broilers, with the individual LS supplementation showing more pronounced effects.

2.4 Effects on Serum Biochemical Indexes

The effects of LS and its combination with VM on serum biochemical indexes are presented in Table 5 . At 21 days of age, dietary supplementation did not significantly affect serum AST, ALT, or ALP activities, nor TBILI, ALB, UA, GLU, TP, or CRE contents ($P>0.05$). At 42 days of age, no significant differences were observed among groups in serum AST, ALT, or ALP activities, or in TBILI, UA, GLU, ALB, or CRE contents ($P>0.05$). However, compared with the control group, serum TP content was significantly increased by 11.07% in the VM+LS group and by 5.91% in the LS group ($P<0.05$).

These findings indicate that both 0.1% LS alone and in combination with 15 mg/kg VM can increase serum TP content in broilers, suggesting that LS supplementation promotes protein synthesis in the body.

Discussion

3.1 Effects on Serum Biochemical Indexes

Serum enzyme activities such as AST, ALT, and ALP reflect the degree of liver cell damage [12]. Serum TP, which includes ALB and globulin, along with ALB content, reflects protein absorption and metabolism status in the body. Elevated TP content not only indicates improved absorption and utilization of amino acids and proteins but also forms an important foundation for maintaining humoral immunity [13]. Previous studies have shown that dietary supplementation with 0.1%-0.2% Lactobacillus did not significantly affect ALP activity in broiler serum [14], while a compound probiotic preparation containing Lactobacillus, Saccharomycetes, and Bacillus significantly increased serum TP content in broilers [15]. Additionally, dietary supplementation with 2.0‰ *Lactobacillus plantarum* significantly elevated serum TP content in broilers [16]. Our results align with these reports, showing that 0.1% LS increased serum TP content at 42 days of age compared to the control and 30 mg/kg VM groups, though serum ALB content remained unchanged. The increased TP content may be associated with elevated immunoglobulin levels, which is consistent with our finding that 0.1% LS increased serum immunoglobulin contents. Furthermore, limited research exists on the combined use of Lactobacillus or Saccharomycetes with antibiotics. In our study, partial replacement of VM with LS resulted in significantly higher serum TP content at 42 days of age in the VM+LS group compared to the 30 mg/kg VM group, with no significant difference from the 0.1% LS group. This aligns with our previous finding that the combination of 0.1% LS with 15 mg/kg VM produced better growth-promoting effects [11], suggesting that this combination more effectively improves protein metabolism, possibly through synergistic interactions.

3.2 Effects on Antioxidant Capacity

Under normal conditions, free radical generation and elimination maintain dynamic equilibrium in the body. However, stress can lead to excessive free radical production and oxidative damage. T-AOC is a crucial indicator for measuring antioxidant capacity, reflecting both non-enzymatic and enzymatic systems' handling of free radical metabolism and their compensatory response to external stimuli. MDA is the end product of lipid peroxidation, reflecting the degree of oxygen radical-mediated lipid peroxidation, while T-SOD and GSH-Px are important antioxidant enzymes that influence mitochondrial enzyme activity and respiratory chain complex activity.

Previous studies have demonstrated that administering Lactobacillus and *Enterococcus faecium* in drinking water significantly improved antioxidant levels in broilers [17], and dietary supplementation with 0.2% *Lactobacillus plantarum* significantly increased serum T-AOC, protecting broilers from oxidative damage and maintaining health status [8]. Additionally, dietary supplementation with 0.20% yeast culture significantly reduced serum MDA content and maintained health in broilers [18]. Our results show that 0.1% LS significantly increased serum T-AOC, enhancing the antioxidant capacity of the defense system. The preparation also significantly elevated serum T-SOD activity, improving the ability to scavenge superoxide anion radicals and protect cells from damage. Moreover, 0.1% LS significantly increased serum GSH-Px activity, protecting cell membrane structural integrity and effectively resisting peroxide interference and damage. The significant reduction in serum MDA content indicates decreased lipid peroxidation in the body. With increasing broiler age, LS demonstrated superior effects compared to antibiotics, suggesting that LS improves growth performance [11] by enhancing redox status and maintaining better health conditions.

Virginiamycin irreversibly inhibits bacterial protein synthesis, leading to bacterial death, and is commonly used to prevent and treat Gram-positive bacterial infections. Dietary antibiotics have been shown to increase serum T-AOC in 24-day-old broilers [8], indicating that antibiotics may improve animal health by enhancing antioxidant capacity, which aligns with our findings. In our study, the 30 mg/kg VM group exhibited higher serum T-SOD activity at 21 days of age and significantly lower serum MDA content compared to the control, demonstrating that antibiotics can improve superoxide radical scavenging capacity and antioxidant capability. Furthermore, both LS and VM+LS groups showed significantly increased serum T-AOC, T-SOD, and GSH-Px activities, along with significantly reduced serum MDA content and lipid peroxidation. The effects were more pronounced in the LS group during the starter phase, while similar effects were observed between LS and VM+LS groups during the grower phase. These results indicate that LS alone or combined with VM can enhance the antioxidant defense system, improve superoxide anion radical scavenging capacity, increase GSH-Px activity to protect cell membrane integrity, and ultimately improve antioxidant capacity and health status in broilers.

3.3 Effects on Immune Function

The thymus and bursa of Fabricius are central immune organs in poultry and primary sites for humoral immunity, while the spleen is a peripheral immune organ involved in systemic immunity. Increased relative organ weight indicates enhanced immune status [19]. Previous research showed that compared to antibiotics, 0.2% *Lactobacillus plantarum* increased spleen and bursal indexes by 40.6% and 47.7%, respectively [8], demonstrating that Lactobacillus enhances peripheral and humoral immunity by increasing immune organ indexes. In our study, dietary supplementation with 0.1% LS alone or combined with 15 mg/kg VM tended to increase the spleen index, indicating a potential role in promoting immune organ development. Although no significant differences were observed in immune organ indexes among groups, the VM+LS group exhibited the highest spleen and bursal indexes, suggesting possible synergistic effects between 0.1% LS and 15 mg/kg VM that warrant further investigation.

Immunoglobulins are antibody-active animal proteins with antibacterial, antiviral, and antitoxin functions. Poultry primarily produce three types: IgA, IgG, and IgM. IgG activates complement and neutralizes toxins, while IgA enters mucosal surfaces to neutralize infectious agents [6], with their contents directly reflecting immune status. Studies have shown that probiotic preparations can promote production of secretory IgA (sIgA) for non-specific immunity [6]. Dietary supplementation with 0.2% *Lactobacillus plantarum* increased serum IgG content by 24.3% and 16.2% compared to antibiotic and control groups, respectively [8]. Additionally, 1% composite preparation of *Lactobacillus graminis* and *Lactobacillus gasseri* significantly increased serum IgA and IgG contents [20], and oral administration of chicken-derived Lactobacillus significantly elevated serum IgG and IgA contents [21]. These findings demonstrate that Lactobacillus, whether used alone or in combination with other bacteria, can significantly enhance broiler immune function. Our results show that 0.1% LS significantly increased serum IgG, IgA, and IgM contents at 42 days of age compared to the control, confirming that LS can substantially improve immune function in broilers.

Previous research indicated that dietary supplementation with 50 mg/kg VM significantly increased serum IgG content at 21 days of age in broilers [22], which differs from our findings. This discrepancy may be attributed to different VM dosage levels. Furthermore, the combination of 0.1% LS with 15 mg/kg VM significantly increased serum IgG content at 42 days of age compared to the control, suggesting synergistic immune enhancement. However, the LS group alone showed significantly higher serum IgG and IgM contents at 42 days of age than the VM+LS group, indicating that individual supplementation with 0.1% LS is more effective for improving immunity than its combination with 15 mg/kg VM. This aligns with our previous growth performance results [11], demonstrating that LS alone improves health status and growth performance more effectively.

Conclusion

Dietary supplementation with 0.1% Lactobacillus and Saccharomycetes compound preparation alone or in combination with 15 mg/kg virginiamycin can improve the antioxidant capacity and immune function of broilers, thereby enhancing health status. The individual addition of 0.1% LS demonstrates more pronounced effects.

References

[1] Chen Q. Study on cost-benefit and efficiency of broiler production in China [D]. PhD thesis. Beijing: Chinese Academy of Agricultural Sciences, 2013.
[2] Zhu Y G, Ouyang W Y, Wu N, et al. Sources and control strategies of antibiotic resistance [J]. Bulletin of Chinese Academy of Sciences, 2015, 30(4): 509–516.
[3] Knapp C W, Dolfing J, Ehlert P A, et al. Evidence of increasing antibiotic resistance abundances archived soils since 1940 [J]. Environmental Science & Technology, 2009, 44(2): 580–587.
[4] D' Costa V M, King E, Kalan L, et al. Antibiotic resistance is ancient [J]. Nature, 2011, 477(7365): 457–461.
[5] Zhang C F, Wang X C, Zhang H J, et al. Effects of Lactobacillus and Saccharomycetes compound preparation on growth performance, slaughter performance and intestinal health of broilers [J]. Chinese Journal of Animal Nutrition, 2017, 29(4): 1248–1256.
[6] Zhang X Y. Study on application effect of compound probiotic preparation of Lactobacillus and Bacillus in broiler production [D]. Master' s thesis. Nanchang: Jiangxi Agricultural University, 2013.
[7] Li J N, Sun P, Qin C F, et al. Research progress on effects of Lactobacillus on local and systemic immune regulation in animals [J]. Acta Veterinaria et Zootechnica Sinica, 2013, 44(11): 1700–1705.
[8] Galdeano C M, Perdigón G. The probiotic bacterium *Lactobacillus casei* induces activation of the gut mucosal immune system through innate immunity [J]. Clinical and Vaccine Immunology, 2006, 13(2): 219–226.
[9] Cao X L. Effects of *Bacillus subtilis* and *Lactobacillus johnsonii* on T-cell immunity and intestinal mucosal immunity in piglets [D]. Master' s thesis. Ya' an: Sichuan Agricultural University, 2007.
[10] Guo X Y, Zhang M, Han F, et al. Effects of compound probiotic preparation and Astragalus polysaccharides on immune organ indexes and immune efficacy against Newcastle disease and avian influenza in chickens [J]. Chinese Agricultural Science Bulletin, 2016, 32(32): 20–24.
[11] Ma C Q. Development of probiotic preparation and its effects and mechanisms on immune function in chicks [D]. PhD thesis. Beijing: China Agricultural

University, 2004.
[12] Mohan B, Kadirvel R, Natarajan A, et al. Effect of probiotic supplementation on growth, nitrogen utilisation and serum cholesterol in broilers [J]. British Poultry Science, 1996, 37(2): 395–401.
[13] Yuan L H. Effects of low temperature and vitamin A on performance and biochemical indexes of growing laying ducks [D]. Master's thesis. Harbin: Northeast Agricultural University, 2008.
[14] Toghyani M, Tohidi M, Gheisari A A, et al. Performance, immunity, serum biochemical and hematological parameters in broiler chicks fed dietary thyme as alternative for an antibiotic growth promoter [J]. African Journal of Biotechnology, 2010, 9(40): 6819–6825.
[15] Zhao J W. Effects of probiotic preparation on ammonia in chicken houses and immune system of broilers [D]. Master' s thesis. Tai' an: Shandong Agricultural University, 2011.
[16] Liu N Z, Chen J, Cui S F, et al. Effects of different concentrations of *Lactobacillus plantarum* on immune function in broilers [J]. Jilin Agriculture, 2012(4): 59–60.
[17] Capcarova M, Weiss J, Hrncar C, et al. Effect of *Lactobacillus fermentum* and *Enterococcus faecium* strains on internal milieu, antioxidant status and body weight of broiler chickens [J]. Journal of Animal Physiology and Animal Nutrition, 2010, 94(5): e215–e224.
[18] Xiao M, Gao Z H, Zhang S C, et al. Effects of yeast culture on immune function, antioxidant capacity and serum biochemical indexes in broilers [J]. Guangdong Agricultural Sciences, 2013, 40(5): 103–106.
[19] Sun B, Chen J, Liu J. Effects of dietary Astragalus polysaccharides on intestinal flora and immune organ indexes in broilers [J]. Heilongjiang Animal Science and Veterinary Medicine, 2014(13): 86–88.
[20] Tan M, Watarai S, Isogai E, et al. Induction of intestinal IgA and IgG antibodies preventing adhesion of verotoxin-producing *Escherichia coli* to Caco-2 cells by oral immunization with liposomes [J]. Letters in Applied Microbiology, 2003, 36(3): 135–139.
[21] Wang J, Xu L, Sun W, et al. Effects of chicken-derived Lactobacillus on growth performance and immune function in chicks [C]//Proceedings of the 14th National Poultry Science Symposium. Harbin: Chinese Association of Animal Science and Veterinary Medicine, 2009.
[22] Yan D F, Liu J X, Qi W T, et al. Effects of microencapsulated *Lactobacillus salivarius* on performance, serum antioxidant capacity and immune function in broilers [J]. Journal of the Chinese Cereals and Oils Association, 2013, 28(11): 52–57.

Note: Figure translations are in progress. See original paper for figures.