# Effects of Astragalus Polysaccharide and Clostridium butyricum on Immune Performance, Antioxidant Capacity, and Intestinal Morphology in Layer Ducklings (Postprint)

**Authors:** Zhao Wenwen, Yuan Wenhua, Junda Shen, Yuan Huikun, Wang Yuan, Li Guoqin, Lu Lizhi, Diao Xinping

**Date:** 2018-12-25T00:00:00+00:00

## Abstract

This experiment aimed to investigate the effects of dietary supplementation with Astragalus polysaccharide, Clostridium butyricum, and their combination on immune performance, antioxidant capacity, and intestinal morphology in egg-type ducklings. A total of 600 healthy 1-day-old Shaoxing male ducklings were randomly divided into 5 groups with 6 replicates per group and 20 ducks per replicate. Group I (control) was fed a basal diet, while groups II–V were fed the basal diet supplemented with 40 mg/kg bacitracin zinc (antibiotic group), 800 mg/kg Astragalus polysaccharide, 250 mg/kg Clostridium butyricum, and a combination of 800 mg/kg Astragalus polysaccharide + 250 mg/kg Clostridium butyricum, respectively. The experimental period lasted 28 days. The results showed: 1) Serum immunoglobulin A (IgA) content in group V was significantly higher than that in groups I, II, and IV ($P<0.05$), while serum immunoglobulin G (IgG) and complement 3 (C3) and complement 4 (C4) contents in group V were higher than those in the other groups ($P>0.05$). 2) The thymus index and spleen index in group V were significantly higher than those in groups I and II ($P<0.05$), while the bursa of Fabricius index in group V showed no significant difference from the other groups ($P>0.05$). 3) The total antioxidant capacity (T-AOC) in serum and liver of groups III, IV, and V was significantly higher than that in group I ($P<0.05$), and the activities of total superoxide dismutase (T-SOD) and glutathione peroxidase (GSH-Px) in serum and liver were significantly higher than those in groups I and II ($P<0.05$), while the malondialdehyde (MDA) content in serum and liver of group IV was significantly lower than that in group I ($P<0.05$). 4) The crypt depth in the duodenum, jejunum, and ileum of group V was significantly lower than that in groups I and II ($P<0.05$), and the villus height/crypt depth ratio in the jejunum and ileum of group V was

significantly higher than that in groups I and II (P<0.05). In conclusion, dietary supplementation with the combination of Astragalus polysaccharide and Clostridium butyricum was more effective than individual supplementation of either Astragalus polysaccharide or Clostridium butyricum in improving immune performance, antioxidant capacity, and intestinal villus morphology in egg-type ducklings.

## Full Text

# Effects of Astragalus Polysaccharides and *Clostridium butyricum* on Immune Function, Antioxidant Capacity, and Intestinal Morphology of Egg Ducklings

**ZHAO Wenwen[1,2], YUAN Wenhua[1,2], SHEN Junda[2], YUAN Huikun[1], WANG Yuan[1], LI Guoqin[2,3], LU Lizhi[2,3*], DIAO Xinping[1*]**

[1]College of Animal Science and Technology, Northeast Agricultural University, Harbin 150030, China
[2]Institute of Animal Husbandry and Veterinary Science, Zhejiang Academy of Agricultural Sciences, Hangzhou 310000, China
[3]Key Laboratory of Information Traceability for Agricultural Products, Ministry of Agriculture and Rural Affairs, Hangzhou 310021, China

### Abstract

This experiment investigated the effects of dietary supplementation with Astragalus polysaccharides (APS), *Clostridium butyricum*, and their combination on immune function, antioxidant capacity, and intestinal morphology in egg ducklings. A total of 600 one-day-old healthy Shaoxing male ducklings were randomly allocated to five groups with six replicates per group and 20 ducklings per replicate. Group I (control) received a basal diet, while groups II–V received the basal diet supplemented with 40 mg/kg bacitracin zinc (antibiotic group), 800 mg/kg APS, 250 mg/kg *C. butyricum*, and 800 mg/kg APS + 250 mg/kg *C. butyricum*, respectively. The 28-day trial revealed: (1) Serum immunoglobulin A (IgA) content in group V was significantly higher than in groups I, II, and IV (P<0.05), while IgG, complement 3 (C3), and complement 4 (C4) levels were elevated compared to other groups (P>0.05). (2) Thymus and spleen indices in group V were significantly higher than in groups I and II (P<0.05), though the bursa of Fabricius index showed no significant differences (P>0.05). (3) Serum and hepatic total antioxidant capacity (T-AOC) in groups III, IV, and V was significantly higher than in group I (P<0.05), with total superoxide dismutase (T-SOD) and glutathione peroxidase (GSH-Px) activities also significantly elevated compared to groups I and II (P<0.05). Malondialdehyde (MDA) content in group IV was significantly lower than in group I (P<0.05). (4) Crypt depth in the duodenum, jejunum, and ileum of group V was significantly reduced compared to groups I and II (P<0.05), while villus height-to-crypt depth ratios in

the jejunum and ileum were significantly increased (P<0.05). These findings demonstrate that combined APS and *C. butyricum* supplementation more effectively enhances immune function, antioxidant capacity, and intestinal villus morphology than either additive alone.

**Keywords:** Astragalus polysaccharides; *Clostridium butyricum*; immunity; antioxidant capacity; intestinal morphology

*Corresponding authors: LU Lizhi, Professor, E-mail: lulizhibox@163.com; DIAO Xinping, Associate Professor, E-mail: diaoxp63@163.com

## Introduction

Antibiotics and chemotherapeutic agents have been widely used as feed additives in livestock production to prevent disease and promote growth. However, extensive antibiotic use enhances bacterial resistance, increases antibiotic residues in animal products, disrupts intestinal microbial balance, and contributes to the proliferation of resistant bacteria and emergence of superbugs, thereby posing direct and indirect threats to human health. Astragalus polysaccharides (APS), a primary component of the traditional Chinese herb Astragalus membranaceus, have been shown to regulate immune function, improve growth performance, and enhance immunity in animals. Microecological preparations represent a novel class of green, pollution-free additives that maintain intestinal flora balance and strengthen immunity. Previous research has demonstrated that appropriate concentrations of Astragalus extract promote probiotic growth, and that synergistic effects exist between probiotics and APS. This study examined the individual and combined effects of APS and *Clostridium butyricum* on immune function, antioxidant capacity, and intestinal morphology in egg ducklings to provide a reference for their application in duck production.

### 1.1 Experimental Materials

Astragalus polysaccharides (60% purity) and bacitracin zinc (10% purity) were provided by Xi' an Zebang Biotechnology Co., Ltd. *Clostridium butyricum* ( 2×10  CFU/g) was supplied by Hubei Lvxue Biotechnology Co., Ltd.

### 1.2 Experimental Design and Basal Diet

Six hundred one-day-old healthy Shaoxing male ducklings of similar body weight were randomly divided into five groups with five replicates per group and 20 ducklings per replicate. The basal diet was formulated according to NRC (1998) standards and Taiwan Livestock Society (1993) requirements for laying ducks. Diet composition and nutrient levels are presented in Table 1 . Group I served as the control (basal diet only), group II (antibiotic group) received the basal diet + 40 mg/kg bacitracin zinc, group III received the basal diet + 800 mg/kg

APS, group IV received the basal diet + 250 mg/kg *C. butyricum*, and group V received the basal diet + 800 mg/kg APS + 250 mg/kg *C. butyricum*.

### 1.3 Management Practices

The trial was conducted at the Guowei Poultry Industry Co., Ltd. breeding base in Shaoxing, Zhejiang Province, for 28 days. Ducklings were raised on net floors with ad libitum access to feed and water. Warm water was provided during the first week, followed by room-temperature water thereafter. Lighting combined natural and artificial illumination: 24 h during week 1, gradually decreasing to 16 h by week 3, and natural light from week 4 onward. Temperature was controlled automatically. Water and feed troughs were cleaned daily, and strict sanitation protocols were maintained. Ducklings were monitored daily for health status, feed intake, water consumption, and fecal abnormalities. Routine immunization and management procedures were followed.

### 1.4 Sample Collection and Processing

On day 28, two ducklings approaching average body weight were selected from each replicate, weighed, and blood samples were collected via jugular venipuncture. Serum was separated by centrifugation at 3,500 rpm for 15 minutes and stored at -20°C. Following blood collection, ducklings were euthanized. Livers (excluding gallbladders) were harvested and stored at -20°C. Immune organs (spleen, thymus, and bursa of Fabricius) were dissected, trimmed of connective tissue and fat, and weighed. Approximately 2 cm segments from the mid-duodenum, jejunum, and ileum were rinsed in physiological saline and fixed in neutral buffered formalin (pH 7.4) for hematoxylin-eosin (HE) staining. All tools were autoclaved, and work surfaces were disinfected with 75% ethanol to prevent microbial contamination.

### 1.5 Analytical Methods

**1.5.1 Serum Immune Indices** Serum immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), complement 3 (C3), and complement 4 (C4) concentrations were determined using colorimetric methods with an A6 semi-automatic biochemical analyzer. Assay kits were provided by Beijing Huaying Biotechnology Co., Ltd.

**1.5.2 Immune Organ Indices** Immune organ index (g/kg) = fresh organ weight / pre-slaughter fasting body weight.

**1.5.3 Serum and Hepatic Antioxidant Indices** Total antioxidant capacity (T-AOC), malondialdehyde (MDA) content, glutathione peroxidase (GSH-Px) activity, and total superoxide dismutase (T-SOD) activity in serum and liver were measured using commercial assay kits with an A6 semi-automatic biochemical analyzer according to manufacturer instructions. Kits were supplied by Beijing Huaying Biotechnology Co., Ltd.

**1.5.4 Intestinal Morphology** Fixed duodenal, jejunal, and ileal tissues were dehydrated, cleared, paraffin-embedded, sectioned, and stained with HE. For each group, 40× magnification fields were photographed from each slide to measure villus height (VH) and crypt depth (CD), from which VH/CD ratios were calculated.

### 1.6 Statistical Analysis

Data were pre-processed using Excel 2007 and analyzed via one-way ANOVA with SPSS 22.0 software. Duncan' s multiple range test was used for post-hoc comparisons. Results are expressed as means ± standard error, with $P<0.05$ considered statistically significant.

---

## Results

### 2.1 Effects on Serum Immune Indices

As shown in Table 2 , serum IgA concentrations in groups III and V were significantly higher than in groups I, II, and IV ($P<0.05$), representing increases of 9.55%, 7.92%, and 6.86% (group III) and 13.07%, 11.39%, and 10.29% (group V), respectively, compared to groups I, II, and IV. Serum IgM concentration in group V was 32.14% higher than in group II ($P<0.05$). Although not statistically significant ($P>0.05$), serum IgG, C3, and C4 concentrations were highest in group V.

### 2.2 Effects on Immune Organ Indices

Table 3 shows that the thymus index in group V was significantly higher than in groups I, II, III, and IV ($P<0.05$), with increases of 45.32%, 27.59%, 25.59%, and 22.70%, respectively. Group IV also exhibited an 18.43% increase compared to group I ($P<0.05$), while groups I, II, and III did not differ significantly ($P>0.05$). Spleen indices in groups IV and V were significantly elevated compared to groups I and II ($P<0.05$). The bursa of Fabricius index was highest in group V, though differences among groups were not significant ($P>0.05$).

### 2.3 Effects on Serum and Hepatic Antioxidant Indices

Serum antioxidant parameters on day 28 (Table 4 ) revealed that T-AOC in groups III, IV, and V increased by 50.17%, 47.67%, and 90.03% compared to group I ($P<0.05$), with group V showing a 68.48% increase over group II ($P<0.05$). Serum MDA concentration in group IV was 43.71% lower than in group I ($P<0.05$). GSH-Px activity in group V exceeded that of groups I, II, III, and IV by 80.53%, 60.08%, 25.97%, and 29.70%, respectively ($P<0.05$). T-SOD activity in group V was significantly higher than in all other groups ($P<0.05$).

Hepatic antioxidant indices demonstrated that T-AOC in group V increased by 90.00%, 68.89%, 26.67%, and 27.73% compared to groups I, II, III, and IV, respectively ($P<0.05$ for groups I and II). Hepatic MDA content in group IV decreased by 43.66% relative to group I ($P<0.05$). GSH-Px activities in groups III, IV, and V were significantly elevated compared to groups I and II ($P<0.05$). Similarly, T-SOD activities in these groups were significantly higher than in groups I and II ($P<0.05$).

### 2.4 Effects on Intestinal Morphology

Table 5 presents intestinal morphological data. Villus height in the duodenum, jejunum, and ileum did not differ significantly among groups ($P>0.05$). Crypt depth in the duodenum of group V was significantly reduced compared to groups I, II, and IV ($P<0.05$). Jejunal crypt depth in groups IV and V was significantly lower than in group I ($P<0.05$). Ileal crypt depth in groups III, IV, and V decreased significantly compared to groups I and II ($P<0.05$). Although duodenal VH/CD ratios were similar across groups ($P>0.05$), jejunal VH/CD ratio in group V was significantly higher than in groups I and II ($P<0.05$). Ileal VH/CD ratios in groups III, IV, and V were also significantly elevated compared to groups I and II ($P<0.05$).

---

## Discussion

### 3.1 Serum Immune Indices

Livestock immune function depends on immune factors (immunoglobulins, cytokines, complement) to recognize and eliminate antigenic threats, maintaining physiological homeostasis. IgA, IgG, and IgM are three critical immunoglobulins in poultry, with their concentrations serving as important indicators of immune status. Complement C3 and C4 levels reflect immune competence and disease progression. Previous studies have shown that APS promotes dendritic cell maturation and increases IgA, IgG, and IgM concentrations, thereby enhancing immunity. *Clostridium butyricum* has been reported to elevate serum IgA, IgG, and IgM levels in broilers and weaned piglets. Synergistic interactions between probiotics and APS have been shown to promote intestinal IgA secretion. Our results demonstrate that the APS-*C. butyricum* combination (group V) significantly increased serum IgA concentration and elevated IgM, IgG, C3, and C4 levels compared to other treatments. This synergy likely enhances APS absorption in the intestine, collectively improving immune performance.

### 3.2 Immune Organ Indices

Immune organ development and proliferation of immune cells increase organ weight and enhance immune function. The immune organ index serves as a crucial indicator of immune competence in young poultry. Numerous studies have

demonstrated that APS promotes immune organ development and significantly increases organ indices. Dietary *C. butyricum* supplementation has been shown to significantly increase thymus and spleen indices in 21-day-old meat ducks. Combined probiotic and APS administration improves immune organ indices in laying hens and indigenous chickens. Our findings indicate that the APS-*C. butyricum* combination significantly increased thymus and spleen indices compared to the control and antibiotic groups, while the bursa of Fabricius index was also elevated, though not significantly. This suggests that the compound additive promotes immune organ growth, possibly by modulating the intestinal microbial environment to favor beneficial bacteria, whose metabolites (amino acids, organic acids, vitamins) subsequently nourish immune organ development.

### 3.3 Antioxidant Indices

GSH-Px and SOD collaboratively protect cellular health by reducing organic hydroperoxide damage and eliminating lipid peroxides. Elevated MDA content indicates intensified peroxidation reactions and greater cellular damage. APS has been demonstrated to possess immunomodulatory and antioxidant properties, increasing SOD activity while decreasing MDA content in egg yolk. Dietary *C. butyricum* supplementation enhances antioxidant capacity in Cherry Valley ducks. Our results show that serum and hepatic T-AOC, T-SOD, and GSH-Px activities were highest in the combination group (V), while MDA content was lowest in *C. butyricum*-supplemented groups. This indicates that combined supplementation more effectively enhances antioxidant capacity than individual additives, possibly because *C. butyricum* colonization disrupts substrates required for oxidative reactions in the intestine.

### 3.4 Intestinal Morphology

The small intestine is the primary site for nutrient absorption. Increased villus height and decreased crypt depth enhance digestive and absorptive capacity, while higher VH/CD ratios indicate superior intestinal function. APS supplementation has been shown to increase duodenal villus height and reduce crypt depth in broilers. *Clostridium butyricum* promotes proliferation of beneficial intestinal bacteria while inhibiting pathogens. Combined APS-probiotic administration increases villus length and width. Our results demonstrate that the APS-*C. butyricum* combination more effectively reduced crypt depth in all intestinal segments and increased VH/CD ratios in the jejunum and ileum compared to individual supplements. This synergistic effect likely increases beneficial bacterial populations, reduces pathogens, and leverages butyric acid—a primary *C. butyricum* metabolite that promotes intestinal epithelial cell organization and regeneration—to improve intestinal morphology.

---

## Conclusion

Dietary supplementation with the Astragalus polysaccharides and *Clostridium butyricum* combination enhances immune function, antioxidant capacity, and intestinal morphology in egg ducklings, demonstrating superior efficacy compared to individual supplementation, antibiotic treatment, or control diets.

---

## References

[1] XIN N, DIAO QY, ZHANG NF. Application of Bacillus in animal nutrition and feed[J]. China Feed, 2010(14): 26-29.

[2] WANG RZ, HUANG YQ, CHEN W, et al. Application of *Clostridium butyricum* in poultry production[J]. Feed Research, 2010(6): 22-23.

[3] ZHENG X, ZHENG YH. Research progress and control measures of superbugs at home and abroad[J]. Chinese Animal Husbandry and Veterinary Abstracts, 2012(1): 69-75.

[4] LIU ZY. Effects of Astragalus polysaccharides on growth performance, immune function and blood indices of guinea fowl[J]. Heilongjiang Animal Science and Veterinary Medicine, 2013(7): 130-132.

[5] CHEN HL, LI DF, CHANG BY, et al. Effects of Chinese herbal polysaccharides on the immunity and growth performance of young broilers[J]. Poultry Science, 2003, 82(3): 364-370.

[6] XIAO YN. Astragalus polysaccharides and their application in broiler production[J]. Shandong Journal of Animal Science and Veterinary Medicine, 2013(4): 81-82.

[7] NEWBOLD CJ, WALLACE RJ, MCLNTOSH FM. Mode of action of the yeast *Saccharomyces cerevisiae* feed additive ruminants[J]. British Journal of Nutrition, 1996, 76(2): 249-261.

[8] LIU Y, QI H, YAN XM. Promoting effect of active components of Astragalus on probiotics[J]. Journal of Dalian Institute of Light Industry, 2005, 24(3): 192-194.

[9] CEN L, CHEN L, ZHANG XL, et al. Study on probiotics and Astragalus polysaccharides to improve growth performance of piglets[J]. China Animal Husbandry and Veterinary Medicine, 2009, 36(12): 17-19.

[10] YI L, NI XQ, PAN KC, et al. Effects of microecological preparations on production performance and immune function of chicks[J]. China Poultry, 2004, 26(23): 10-13.

[11] LIN J, KANG H, LIANG J, et al. CpG oligonucleotides and Astragalus polysaccharides are effective adjuvants in cultures of avian bone-marrow-derived dendritic cells[J]. British Poultry Science, 2015, 56(1): 30-38.

[12] WANG HF, GUO B. Immunomodulatory effect of Astragalus polysaccharides on duodenal mucosa of broilers[J]. Chinese Journal of Animal Nutrition, 2015, 27(5): 1534-1539.

[13] YANG CM, CAO GT, FERKET PR, et al. Effects of probiotic, *Clostridium butyricum*, on growth performance, immune function, and cecal microflora in broiler chickens[J]. Poultry Science, 2012, 91(9): 2121-2129.

[14] ZHENG YX, WANG C, ZOU XT, et al. Effects of *Clostridium butyricum* on growth performance, intestinal structure and immune function of weaned piglets[J]. Chinese Journal of Animal Nutrition, 2018, 30(7): 2683-2689.

[15] ZOU ZM, LIANG LH, CUI H, et al. Effects of functional amino acids interaction with Astragalus polysaccharides and probiotics on intestinal microecological environment and intestinal health of piglets[J]. Feed Industry, 2016, 37(21): 35-39.

[16] HUANG KH, YOU CB, ZHOU XG, et al. Study on the effect and mechanism of threonine interaction with Astragalus polysaccharides and probiotics on immune function of piglets[J]. Heilongjiang Animal Science and Veterinary Medicine, 2015(23): 122-125.

[17] WANG HL, LIU DD, JIANG SW, et al. Effects of compound microecological preparation and Astragalus polysaccharides on growth performance, intestinal flora and immune function of broilers[J]. Feed Industry, 2014, 35(6): 10-14.

[18] LIN XH, SUN HM, GU W, et al. Effects of *Bacillus subtilis* B7 on growth performance and immune performance of broilers[J]. China Animal Husbandry and Veterinary Medicine, 2013, 40(2): 91-95.

[19] SU LL, WANG SM, JIANG YH, et al. Effects of Astragalus polysaccharides soaking eggs on antioxidant performance of immune organs in chicks[J]. Heilongjiang Animal Science and Veterinary Medicine, 2014(11): 157-159.

[20] WANG JL, ZHANG SY, XU CY, et al. Effects of Astragalus polysaccharides on production performance and some immune indices of broilers[J]. China Feed, 2010(7): 18-21.

[21] JIA ZX. Effects of *Clostridium butyricum* on growth performance, immune and antioxidant function and intestinal chyme VFA content in Cherry Valley ducks[D]. Master' s thesis. Nanjing: Nanjing Agricultural University, 2014.

[22] LYU X. Effects of Astragalus polysaccharides and probiotics on production performance and immune function of Hy-Line brown layers[J]. Feed Review, 2014(12): 24-28.

[23] ZHAO YH, LI YX, JIAO FC, et al. Effects of Astragalus polysaccharides and probiotics on production performance and immune effect of Gushi chickens[J]. Jiangsu Agricultural Sciences, 2012, 40(9): 202-203.

[24] YANG WJ, WANG H, YANG J. Efficacy of probiotics and their application in food[J]. Sichuan Food and Fermentation, 2005, 41(1): 27-30.

[25] MA M. Research progress on glutathione peroxidase and glutathione S-transferase[J]. Progress in Veterinary Medicine, 2008, 29(10): 53-56.

[26] WANG YM. Free radicals and glutathione peroxidase[J]. Pharmaceutical Journal of Chinese People' s Liberation Army, 2005, 21(5): 369-371.

[27] SUN B, CHEN J, LIU J, et al. Effects of Astragalus polysaccharides supplementation on intestinal flora and immune organ indices of broilers[J]. Heilongjiang Animal Science and Veterinary Medicine, 2014(13): 86-88.

[28] TAO H. Effects of Astragalus polysaccharides on intestinal development and antioxidant capacity of broilers[D]. Master' s thesis. Changchun: Jilin Agricultural University, 2012.

[29] ZUO ZY, YANG WR, YANG ZB, et al. Effects of dietary Astragalus polysaccharides on antioxidant capacity and egg quality of laying hens[J]. Chinese Journal of Veterinary Science, 2012, 32(1): 130-134.

[30] ZHENG NZ, LI L, XIN QW, et al. Effects of selenium yeast, Astragalus polysaccharides and their combination on growth performance, meat quality and antioxidant capacity of mule ducks[J]. Chinese Journal of Animal Nutrition, 2016, 28(12): 3856-3866.

[31] ZHAO X, RAN L, YANG BL, et al. Study on the effect of *Clostridium butyricum* live preparation on intestinal flora[J]. Chinese Journal of Microecology, 1999, 11(6): 332-333.

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