Effects of Clostridium butyricum and Bacillus licheniformis on Growth Performance, Serum Biochemical and Immune Indices, and Immune Organ Indices of Beijing Ducks

Authors: Huikun Yuan, Yuan Wenhua, Zhao Wenwen, Liu Xiangshan, Li Guoqin, Du Xue, Shen Junda, Li Liumeng, Lu Lizhi, Diao Xinping

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

Abstract

This experiment aimed to investigate the effects of dietary supplementation with Clostridium butyricum and Bacillus licheniformis on growth performance, serum biochemical and immune indices, and immune organ indices in Beijing ducks. A total of 300 healthy 1-day-old Beijing ducks were randomly divided into 5 groups with 6 replicates per group and 10 ducks per replicate. The control group (Group I) was fed a basal diet, while the experimental groups were fed the basal diet supplemented with 40 mg/kg zinc bacitracin (Group II), 5×10^8 CFU/kg Clostridium butyricum (Group III), 1×10^9 CFU/kg Bacillus licheniformis (Group IV), and 5×10^8 CFU/kg Clostridium butyricum + 1×10^9 CFU/kg Bacillus licheniformis (Group V). The experimental period lasted 21 days. The results showed: 1) The average daily gain of Groups II, III, IV, and V was significantly higher than that of the control group ($P<0.05$). No significant differences were observed in average daily feed intake and feed conversion ratio among all groups ($P>0.05$). 2) The serum concentrations of immunoglobulin A, immunoglobulin G, complement 3, and complement 4 in Group V were significantly higher than those in the control group ($P<0.05$). No significant differences were found in serum total protein, albumin, and urea nitrogen concentrations among all groups ($P>0.05$). 3) The thymus index and spleen index of Group V were significantly higher than those of the control group ($P<0.05$). No significant difference was observed in the bursa of Fabricius index among all groups ($P>0.05$). These results indicate that dietary supplementation with the combination of Clostridium butyricum and Bacillus licheniformis can effectively improve the growth performance and immune indices of Beijing ducks.

Full Text

Effects of *Clostridium butyricum* and *Bacillus licheniformis* on Growth Performance, Serum Biochemical and Immune Indexes, and Immune Organ Indexes of Beijing Ducks

YUAN Huikun[1], YUAN Wenhua[1,2], ZHAO Wenwen[1,2], LIU Xiangshan[1], LI Guoqin[2], DU Xue[2], SHEN Junda[2], LI Liumeng[3], LU Lizhi[2], *DIAO Xinping[1]*

[1]College of Animal Science and Technology, Northeast Agricultural University, Harbin 150030, China
[2]Institute of Animal Science, Zhejiang Academy of Agricultural Sciences, Hangzhou 310021, China
[3]Zhuji Guowei Poultry Industry Co., Ltd., Zhuji 311800, China

Abstract

This experiment investigated the effects of dietary *Clostridium butyricum* and *Bacillus licheniformis* on growth performance, serum biochemical and immune indexes, and immune organ indexes of Beijing ducks. A total of 300 healthy one-day-old Beijing ducks were randomly divided into 5 groups with 6 replicates per group and 10 ducks per replicate. The control group (Group I) received a basal diet, while experimental groups received the basal diet supplemented with 40 mg/kg bacitracin zinc (Group II), 5×10 CFU/kg *C. butyricum* (Group III), 1×10 CFU/kg *B. licheniformis* (Group IV), or 5×10 CFU/kg *C. butyricum* + 1×10 CFU/kg *B. licheniformis* (Group V). The 21-day trial yielded three key findings. First, the average daily gain of Groups II, III, IV, and V was significantly higher than that of the control group ($P<0.05$), though average daily feed intake and feed-to-gain ratio did not differ significantly among groups ($P>0.05$). Second, Group V showed significantly elevated serum levels of immunoglobulin A, immunoglobulin G, complement 3, and complement 4 compared to the control group ($P<0.05$), while serum total protein, albumin, and urea nitrogen concentrations remained comparable across groups ($P>0.05$). Third, Group V exhibited significantly higher thymus and spleen indexes than the control group ($P<0.05$), though the bursa of Fabricius index did not differ significantly among treatments ($P>0.05$). These results demonstrate that dietary supplementation with a *C. butyricum* and *B. licheniformis* combination can effectively improve growth performance and serum immune indexes in Beijing ducks.

Keywords: *Clostridium butyricum*; *Bacillus licheniformis*; Beijing ducks; growth performance; serum biochemical indexes; immune indexes

Introduction

Probiotics have emerged as a promising alternative to antibiotics in animal nutrition due to their ability to enhance immune function, promote growth, and maintain intestinal flora balance. Both *Clostridium butyricum* and *Bacillus licheniformis* are probiotic strains with substantial potential for animal production applications. *C. butyricum* is an anaerobic bacterium isolated from human and animal intestines that exhibits antibiotic resistance and beneficial properties. Research by Xiao et al. [?] demonstrated that dietary supplementation with appropriate levels of *C. butyricum* improved growth performance in partridge-colored broilers. Jia et al. [?] found that *C. butyricum* enhanced total superoxide dismutase (T-SOD) activity in 21-day-old Ross 308 broilers, thereby strengthening antioxidant capacity and immune function. *B. licheniformis*, an aerobic or facultatively anaerobic bacterium primarily sourced from plants and soil, has also shown positive effects. Li et al. [?] reported that adding 2×10 CFU/kg *B. licheniformis* to diets improved egg quality and serum immune parameters in 392-day-old Hy-Line Gray laying hens.

Beijing ducks, the primary breed used for the iconic "Peking duck" dish, are internationally recognized as an excellent meat duck variety characterized by rapid development and favorable fattening properties. However, Chinese Beijing ducks currently lag behind foreign breeds in growth rate, survival rate, and breast meat percentage. This study examined the effects of dietary supplementation with *C. butyricum*, *B. licheniformis*, and their combination on growth performance, serum biochemical and immune indexes, and immune organ indexes in Beijing ducks. The objective was to provide empirical data supporting the precise application of probiotics in poultry production.

Materials and Methods

1.1 Experimental Materials

Clostridium butyricum (effective viable count: 2×10 CFU/g, silica carrier) was provided by Hubei Lvxue Biotechnology Co., Ltd. *Bacillus licheniformis* (effective viable count: 2×10^{1} CFU/g) was supplied by Guangzhou Weiyuan Biotechnology Co., Ltd.

1.2 Experimental Animals and Design

Three hundred one-day-old healthy Beijing ducks of similar body weight (half male, half female) were obtained from Zhuji Guowei Poultry Industry Co., Ltd. and randomly allocated to 5 groups with 6 replicates each (10 ducks per replicate) based on weight similarity. Group I (control) received a basal diet without antibiotics, while experimental groups received the basal diet supplemented with: 40 mg/kg bacitracin zinc (Group II), 5×10 CFU/kg *C. butyricum* (Group III), 1×10 CFU/kg *B. licheniformis* (Group IV), or 5×10 CFU/kg *C.*

butyricum + 1×10 CFU/kg *B. licheniformis* (Group V). The basal diet was formulated according to Chinese "Feeding Standard of Meat Ducks" (NY/T 2122—2012) and contained no antibiotics. Diet composition and nutrient levels are presented in Table 1 .

Table 1 Composition and nutrient levels of the basal diet (air-dry basis)

Item	Content
Ingredients	
Corn	
Soybean meal	
Wheat	
Corn DDGS	
Cottonseed cake	
Rapeseed cake	
CaHPO	
Limestone	
NaCl	
Lysine	
Methionine	
Premix[1]	
Total	
Nutrient levels[2]	
Metabolizable energy (ME, MJ/kg)	
Crude protein (CP)	
Available phosphorus (AP)	
Lysine	
Methionine + Cysteine	

[1]Premix provided per kg of diet: Cu 8 mg, Fe 60 mg, Zn 60 mg, Mn 50 mg, I 0.2 mg, Se 0.20 mg, VA 11,000 IU, VD 3,000 IU, VE 20 IU, VK 3.0 mg, VB 2.0 mg, VB 4.5 mg, VB 1.5 mg, VB 0.02 mg, nicotinic acid 0.20 mg, folic acid 1.0 mg, biotin 0.1 mg, choline 1,000 mg, D-pantothenic acid 11.0 mg.
[2]Calculated values.

1.3 Management Practices

Before the trial, duck houses were thoroughly cleaned and disinfected to ensure uniform environmental conditions. Ducks were raised in floor pens (one replicate per pen) under 24-hour lighting with adequate ventilation. Feed was provided twice daily at 08:00 and 17:00, with pens cleaned once daily. Ducks had ad libitum access to water. Feed allocation and residual amounts were recorded daily by replicate. Conventional management practices were followed throughout the 21-day experimental period.

1.4 Sampling and Measurements

1.4.1 Growth Performance On day 21, feed was withdrawn for 12 hours (water provided) before weighing ducks by replicate to record feed intake and body weight gain. Average daily feed intake (ADFI), average daily gain (ADG), and feed-to-gain ratio (F/G) were calculated for days 1–21.

1.4.2 Serum Biochemical and Immune Indexes On day 22, two ducks were randomly selected from each replicate, and blood samples were collected from the right jugular vein into procoagulant vacuum tubes. After 15 minutes of clotting, serum was separated by centrifugation at 3,000 r/min for 20 minutes and stored at -20°C for analysis. Serum biochemical parameters [total protein (TP), albumin (ALB), urea nitrogen (UREA)] were measured using an A6 semi-automatic biochemical analyzer. Serum immune indexes were determined by colorimetric assay. Immunoglobulin A (IgA) and IgG assay kits were purchased from Beijing Huaying Biotechnology Research Institute, while complement 3 (C3) and C4 kits were obtained from Zhejiang Weiyi Biotechnology Co., Ltd.

1.4.3 Immune Organ Indexes After blood collection, ducks were euthanized and dissected to remove the thymus, spleen, and bursa of Fabricius. Adhering fat was trimmed, and organs were blotted with filter paper to remove blood before weighing. The immune organ index was calculated as:

Immune organ index (%) = 100 × fresh immune organ weight (g) / pre-slaughter fasting body weight (kg)

1.5 Statistical Analysis

Data were initially processed using Excel 2010 and then analyzed by one-way ANOVA using SPSS 22.0 software. Duncan's multiple comparison test was used for mean separation. Results are expressed as "mean ± standard deviation." Differences were considered significant at $P<0.05$.

Results

2.1 Effects on Growth Performance

As shown in Table 2 , Group V had the highest ADG while the control group had the lowest. Groups II, III, IV, and V exhibited significantly higher ADG than the control group ($P<0.05$), with increases of 12.08%, 7.92%, 7.12%, and 14.91%, respectively. No significant differences were observed in ADFI or F/G among all groups ($P>0.05$).

Table 2 Effects of *Clostridium butyricum* and *Bacillus licheniformis* on growth performance of Beijing ducks

Item	Control group	Experimental group II	Experimental group III	Experimental group IV	Experimental group V
ADG (g)	44.93±1.50	50.36±1.97	48.49±1.63	48.13±2.21	51.63±1.87
ADFI (g)	83.66±1.06	88.86±1.42	90.13±1.46	87.12±1.73	89.59±1.14
F/G	1.86±0.05	1.76±0.07	1.85±0.08	1.81±0.12	1.73±0.06

In the same row, values with different small letter superscripts indicate significant difference (P<0.05), while the same or no superscripts indicate no significant difference (P>0.05). The same applies below.

2.2 Effects on Serum Biochemical and Immune Indexes

Table 3 shows that serum TP, ALB, and UREA concentrations did not differ significantly among groups (P>0.05). However, Groups II, III, IV, and V had significantly higher serum IgA and IgG levels than the control group (P<0.05). Group V also showed significantly elevated serum C3 content compared to the control group (P<0.05), and its serum C4 content was significantly higher than both the control group and Group II (P<0.05).

Table 3 Effects of *Clostridium butyricum* and *Bacillus licheniformis* on serum biochemical and immune indexes of Beijing ducks

Item	Control group	Experimental group II	Experimental group III	Experimental group IV	Experimental group V
TP (g/L)	10.75±8.73	11.27±3.37	10.63±0.45	10.57±9.39	10.47±2.10
ALB (g/L)	8.15±2.75	8.20±1.15	8.56±0.56	8.03±3.24	8.25±0.33
UREA (mmol/L)	1.59±0.19	1.54±0.01	1.56±0.01	1.57±0.25	1.53±0.06
IgA (g/L)	1.94±0.52	1.96±0.00	1.99±0.34	1.96±0.01	1.97±0.01
IgG (g/L)	3.79±0.02	3.82±0.01	3.84±0.04	3.82±0.01	3.82±0.01
C3 (g/L)	0.44±0.11	0.46±0.03	0.48±0.12	0.47±0.02	0.52±0.04
C4 (g/L)	0.10±0.28	0.12±0.11	0.14±0.03	0.13±0.01	0.19±0.01

2.3 Effects on Immune Organ Indexes

Table 4 reveals that Groups II, III, IV, and V had significantly higher thymus indexes than the control group (P<0.05), though these groups did not differ significantly from each other (P>0.05). Group V showed a significantly higher spleen index compared to the control group (P<0.05), with no significant differences among the supplemented groups (P>0.05). While the bursa of Fabricius index did not differ significantly among groups (P>0.05), the control group had the lowest value and Group V had the highest.

Table 4 Effects of *Clostridium butyricum* and *Bacillus licheniformis* on immune organ indexes of Beijing ducks

Item	Control group	Experimental group II	Experimental group III	Experimental group IV	Experimental group V
Thymus index	240.23±24.92	323.02±26.61	286.53±24.85	258.35±30.94	341.42±27.56
Spleen index	88.43±9.71	104.63±7.56	111.47±14.90	103.14±11.01	121.58±17.32
Bursa of Fabricius index	101.52±12.74	137.86±22.13	126.08±14.53	119.24±10.75	125.21±24.46

Discussion

3.1 Effects on Growth Performance

Numerous studies have demonstrated that probiotics promote growth performance in livestock and poultry. Jia [?] reported that feeding cherry valley ducks with *C. butyricum* for 21 days significantly increased ADG and ADFI, with the 5.0×10 CFU/kg dose yielding the lowest F/G. Similar findings by Li et al. [?] and Fritts et al. [?] confirm that *C. butyricum* improves poultry growth performance, likely by enhancing enzyme activity. Upon colonizing the intestinal tract, *C. butyricum* secretes enzymes such as cellulase and protease that degrade nutrients and facilitate absorption. It also produces butyric acid and other acidic compounds that inhibit pathogenic bacteria while promoting beneficial flora proliferation, and generates folic acid and vitamin K to support growth [?]. Wang [?] observed that feeding 1×10 CFU/kg *B. licheniformis* to broilers for 21 days significantly increased ADFI and final body weight. Guo et al. [?] reported that combining antimicrobial peptides with *B. licheniformis* in AA broiler diets significantly improved ADG by 12.66% and ADFI by 3.26% while reducing F/G by 8.56% during days 1-42. These benefits may stem from *B. licheniformis* producing digestive enzymes that enhance nutrient absorption.

Yu [?] demonstrated that various *Bacillus* levels improved lipase and amylase activities in the intestine of *Litopenaeus vannamei* and increased protein retention. *B. licheniformis* secretes numerous enzymes that cannot be synthesized in poultry, along with highly active digestive enzymes that degrade nutrients into glucose for growth [?]. In the current study, both individual and combined supplementation improved Beijing duck growth performance, with all treated groups showing significantly higher ADG. Although ADFI increased compared to the control group, the differences were not significant. The combination treatment proved most effective, possibly due to synergistic interactions between the two probiotics, though the specific mechanisms require further investigation.

3.2 Effects on Serum Biochemical and Immune Indexes

Serum biochemical parameters provide crucial insights into metabolic status and physiological conditions. Elevated TP content indicates improved protein metabolism efficiency, benefiting protein absorption and reducing feed waste. ALB maintains blood osmotic pressure and participates in nutrient transport, coagulation, anti-coagulation, hepatocyte repair and regeneration, and immune processes, helping stabilize the internal chemical environment. Since UREA is produced from protein metabolism, reduced serum UREA content indicates enhanced protein synthesis and favorable growth [?]. Chen et al. [?] found that dietary *B. licheniformis* significantly reduced serum UREA in male partridge-colored broilers while increasing TP and ALB at a concentration of 50 mg/kg (2×10^{1} CFU/g). Ge et al. [?] reported that 0.4% *B. licheniformis* supplementation significantly increased serum TP and ALB while reducing UREA in yellow-feathered broilers at 28 days, though these differences disappeared by 56 days. The current results differ from previous studies, showing no significant differences in serum TP, ALB, or UREA among groups. This discrepancy may be attributed to the shorter experimental period or different probiotic strains and concentrations, suggesting that the selected probiotics did not significantly affect these parameters under our conditions.

IgA and IgG play primary roles in humoral immunity by binding antigens and preventing their invasion, thereby protecting the organism. IgG constitutes approximately 75% of total serum immunoglobulins and serves as the principal anti-infection antibody, with its concentration reflecting immune competence. Complement, present in tissue fluid and serum, is heat-labile and assists specific antibodies in immune responses, hence its name. C3 and C4 are the most abundant complement components, making their levels important indicators of immune function. Probiotics can enhance immunity and increase resistance to pathogens, particularly in young chicks with immature immune systems [?]. Jia [?] demonstrated that dietary *C. butyricum* increased serum IgA, IgG, C3, and C4 levels in cherry valley ducks at 21 and 42 days, with 5×10 CFU/kg showing optimal effects. In this study, Groups II, III, IV, and V all showed varying degrees of improvement in serum IgA, IgG, C3, and C4 compared to the control group, with Group V demonstrating significantly higher C3 and

C4 levels. These findings align with previous research, indicating that both *C. butyricum* and *B. licheniformis* can enhance immune status in Beijing ducks, with the combination showing superior immunomodulatory effects. This synergy may strengthen immune function and disease resistance, though the underlying mechanisms warrant further investigation.

3.3 Effects on Immune Organ Indexes

The immune competence of poultry primarily depends on the development of the thymus, spleen, and bursa of Fabricius, with higher immune organ indexes indicating stronger immune function and greater resistance to pathogen invasion. The thymus participates in cellular immunity and T-cell development, the bursa of Fabricius is involved in humoral immunity and B-lymphocyte development (unique to avian species), and the spleen is the largest peripheral immune organ engaged in both cellular and humoral immunity [?]. Duc et al. [?] suggested that *B. licheniformis* can activate lymphocytes, increase antibody levels, promote immune organ development, and enhance immune function. Sun et al. [?] reported that dietary *B. licheniformis* significantly increased thymus index at 21 days and both thymus and bursa indexes at 35 days in broilers. In the current study, the combined *C. butyricum* and *B. licheniformis* treatment significantly increased thymus and spleen indexes in Beijing ducks. Although Groups II, III, IV, and V showed improvements over the control group, they did not differ significantly from each other. This enhanced effect of combined probiotics may result from strengthened intestinal stimulation and immune assistance, further promoting immune organ growth. These findings indicate that *C. butyricum* and *B. licheniformis* positively influence immune organ development in young Beijing ducks, supporting immune system maturation and overall health.

Conclusion

Dietary supplementation with *Clostridium butyricum*, *Bacillus licheniformis*, or their combination improved growth performance, serum immune indexes, and immune organ indexes in Beijing ducks, with the combined treatment demonstrating superior effects compared to individual supplementation.

References

[?] Xiao Kequan, Zhang Longlin. Effects of *Clostridium butyricum* and *Bacillus subtilis* on growth performance of partridge-colored chickens[J]. *Guangdong Feed*, 2017, 26(9): 30–32.

[?] Jia Conghui, Yang Caimei, Zeng Xinfu, et al. Effects of *Clostridium butyricum* on growth performance, antioxidant capacity, immune function, and serum biochemical indexes of broilers[J]. *Chinese Journal of Animal Nutrition*, 2016, 28(3): 908-915.

[?] Li Fubin, Chen Baojiang, Liang Chenchong, et al. Effects of *Bacillus licheniformis* on production performance, egg quality, and serum-related indexes of laying hens[J]. *China Feed*, 2010, 33(13): 5-8.

[?] Jia Zhixin. Effects of *Clostridium butyricum* on growth performance, immune and antioxidant function, and intestinal VFA content in cherry valley ducks[D]. Master' s thesis. Nanjing: Nanjing Agricultural University, 2014.

[?] Li Yupeng, Li Haihua, Wang Liuyi, et al. Effects of *Clostridium butyricum* on growth performance, intestinal barrier function, and serum cytokine content of weaned piglets[J]. *Chinese Journal of Animal Nutrition*, 2017, 29(8): 2961-2968.

[?] FRITTS C A, KERSEY J H, MOTL M A, et al. *Bacillus subtilis* C-3102 (Calsporin) improves live performance and microbiological status of broiler chickens[J]. *The Journal of Applied Poultry Research*, 2000, 9(2): 149-155.

[?] ARAKI Y, ANDOH A, FUJIYAMA Y, et al. Short-term oral administration of a product derived from a probiotic, *Clostridium butyricum* induced no pathological effects in rats[J]. *International Journal of Molecular Medicine*, 2002, 9(2): 173-177.

[?] Wang Hongyan. Effects of *Bacillus licheniformis* preparation on production performance and mechanisms in broilers and weaned piglets[D]. Master' s thesis. Beijing: China Agricultural University, 2005.

[?] Guo Sen, Sun Quanyou, Wei Fengxian, et al. Effects of antimicrobial peptides and *Bacillus licheniformis* on intestinal microorganisms and immune organ indexes of broilers[J]. *China Poultry*, 2016, 38(18): 26-31.

[?] Yu Mingchao. Application of *Bacillus* and Chinese herbal medicine in feed of *Litopenaeus vannamei*[D]. Doctoral thesis. Qingdao: Ocean University of China, 2007.

[?] Xiong Feng. Effects of soybean oligosaccharides and *Bacillus natto* preparation and their combination on production performance, intestinal digestive enzymes, and flora balance in broilers[D]. Master' s thesis. Urumqi: Xinjiang Agricultural University, 2008.

[?] MOHAN B, KADIRVEL R, BHASKARAN M, et al. Effect of probiotic supplementation on serum/yolk cholesterol and shell thickness in layers[J]. *British Poultry Science*, 1995, 36(5): 799-803.

[?] Chen Jiaxiang, Zhang Renyi, Wang Quanxi, et al. Effects of *Bacillus licheniformis* on growth performance, antioxidant indexes, and blood biochemical indexes of broilers[J]. *Chinese Journal of Animal Nutrition*, 2010, 22(4): 1019-1023.

[?] Ge Wenxia, Liu Xuwei. Effects of *Bacillus licheniformis* on production performance and blood biochemical indexes of yellow-feathered broilers[J]. *Jiangsu Agricultural Sciences*, 2015, 43(7): 213-215.

[?] Gao Jun. Effects of yeast culture on broilers and its mechanisms[D]. Doctoral thesis. Beijing: Chinese Academy of Agricultural Sciences, 2008.

[?] LOWENTHAL J W, CONNICK T E, MCWATERS P G, et al. Development of T cell immune responsiveness in chickens[J]. *Immunology and Cell Biology*, 1994, 72(2): 115-122.

[?] KOENEN M E, BOONSTRA-BLOM A G, JEURISSEN S H M. Immunological differences between layer- and broiler-type chickens[J]. *Veterinary Immunology and Immunopathology*, 2002, 89(1/2): 47-56.

[?] Zhang Lei, Li Jia, Zhang Tao, et al. Effects of microecological preparations on production performance and immune function of broilers[J]. *Journal of Beijing University of Agriculture*, 2008, 23(4): 41-45.

[?] DUC L H, HONG H A, BARBOSA T M, et al. Characterization of *Bacillus* probiotics available for human use[J]. *Applied and Environmental Microbiology*, 2004, 70(4): 2161-2171.

[?] Sun Xiaopei, Yang Zaibin, Li Zhaoyong, et al. Effects of *Bacillus licheniformis* and dietary protein level on production performance, intestinal environment, and immune organ indexes of broilers[J]. *Feed Industry*, 2013, 34(23): 40-46.

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