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Original Article

Evaluation of the Effect of Bacillus Subtilis and Pediococcus Acidilactici Mix on Serum Biochemistry, Growth Promotation of Body and Visceral Organs in Lohmann Brown Chicks

■Author(s)

Yu W[I*] https://orcid.org/0000-0002-5807-004X
Hao X[I*] https://orcid.org/0000-0003-0034-2812
Zhiyue W[I,II] https://orcid.org/0000-0001-5608-538X
Haiming Y[I] https://orcid.org/0000-0003-0171-4649
Lei X[I] https://orcid.org/0000-0001-5518-0510

[I] College of Animal Science and Technology, Yangzhou University, Jiangsu Province, PR. China, 225009.
[II] Joint International Research Laboratory of Agriculture and Agri-Product Safe Ministry of Education of China, Yangzhou University, Jiangsu Province, PR. China, 225009.

*These authors contributed equally to the paper

■Mail Address

Corresponding author e-mail address
Dr. Haiming Yang
College of Animal Science and Technology, Yangzhou University; address: East Wen hui Road 48, Yangzhou City, China.
Phone: +86 514 87979045
Email: yhmdlp@qq.com

■Keywords

Probiotic, Bacillus subtilis, Pediococcus acidilactici, chick, growth performance, serum biochemical variables.

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ABSTRACT

Recently the use of antibiotic growth promoter (AGPs) in poultry feed is banned in China, leading to the increasing demand for AGPs substitutes. Probiotics have been considered as a potential substitute. The purpose of this study was to evaluate the effects of probiotic on serum biochemistry, and growth promotion of body weight and visceral organs and in *Lohmann* Brown chicks aged 0 to 9 weeks.

Five hundred and forty 1-day-old male chicks were randomly divided into three groups, with six replicates and 30 chicks in each replicate. The experiment was carried out over 70 days. Dietary treatments were: (1) basal diet alone (control group); (2) basal diet containing 0.1% probiotics (probiotic group); and (3) basal diet containing 0.01% zinc bacitracin in the feed (antibiotic group).

The body weight (BW) of probiotic group was significantly higher than that of the control group during the whole trial period (p<0.05). ADG (0~70 d) in the probiotic group and the antibiotic group was higher than that in the control group (p<0.05). The Oliver weight and serum total protein (TP) in the probiotic group was higher than that in the control group on the 63rd day (p<0.05). The length of the duodenum, the weight of the spleen, duodenum and Jejunum in both the probiotic and antibiotic groups were higher than those in the control group (p<0.05). The ileum weight in the probiotic group was significantly higher than those in the control group (p<0.05). The ileum length in the probiotic group was significantly longer than that in both the antibiotic and control groups (p<0.05).

Probiotic mix with Bacillus subtilis and Pediococcus acidilactici play a similar role in promoting growth of body and visceral organs, and it is a promising growth promoter for *Lohmann* Brown chicks (0 to 9 weeks).

INTRODUCTION

The application of antibiotics has made a great contribution to the development of animal husbandry because of its remarkable improvement in animal production performance and animal health. However, the disadvantages hidden behind the huge economic benefits brought by feed antibiotic additives have been gradually recognized. In order to eliminate the threat of antibiotic abuse to human health and at the same time to ensure the efficiency and efficiency of animal husbandry production, it is necessary to find a non-toxic, non-residual antibiotic alternative in feed additive. Under this background, feed additives that can promote growth are constantly being developed, among which probiotics are considered to be one of the best substitutes for antibiotics (Markovic, *et al.*, 2009; Yang, *et al.*, 2009). Probiotics are defined in the literature as "living microbial products that contribute to intestinal micro ecological balance and

have beneficial effects on hosts by improving the properties of human or animal inherent flora" (Fuller, 1989; Isolauri *et al.*, 1998).

Many previous studies have explored the application of *Bacillus subtilis*, *acid-producing Streptococcus* and *Bacillus natto* in poultry production. For example, it can improve poultry performance (Yang *et al.*, 2012; Ribeiro *et al.*, 2014), product quality (Mikulski *et al.*, 2012; Zhang *et al.*, 2012; Lei *et al.*, 2013; Zhou *et al.*, 2015; Youssef *et al.*, 2013), nutrient digestibility (Li *et al.*, 2014; Hossain, *et al.*, 2015), and immune function (Liao *et al.*, 2015). In addition, it can improve the environment or reduce environmental pollution (Zhang & Kim, 2013; 2014). However, most of the studies on the beneficial effects of probiotics on birds focused on broilers (Mookiah *et al.*, 2014; Mountzouris *et al.*, 2010) and most of the studies in laying hens were at the age of 20 to 50 weeks (Abdelqader *et al.*, 2013; Youssef *et al.*, 2013; Nahashon *et al.*, 1992; Mohebbifar *et al.*, 2013; Panda *et al.*, 2008). This is proof of the need to study the growth promotion effects of probiotics as an alternative of antibiotics in the early growing stage of chickens (0 to 9 weeks).

In this study, we hypothesized that under normal (not challenged with pathogens) feeding conditions, the combination of the *Bacillus subtilis* and *Pediococcus acidilactici* can replace the antibiotic to achieve the growth promoting effect of both body weight and visceral organs in the early growing stage of the layer-type chicks. The purpose of this study was to evaluate the effects of probiotic (*Bacillus subtilis* and *Pediococcus acidilactici* mix) on serum biochemistry, and growth promotation of body weight and visceral organs and in *Lohmann* Brown chicks aged 0 to 9 weeks.

MATERIAL AND METHODS

Probiotics and antibiotic

The commercial probiotics used in this experiment (Baolai-Leelai Bioengineering Co. Ltd., Shandong, China) contain $1x10^8$ cfu Bacillus subtilis and $1x10^8$ cfu Pediococcus acidilactici per gram of product. Bacitracin zinc premix (10% of bacitracin zinc in premix) was a representative of the antibiotic in the present study (Xinxing Veterinary Pharmaceutical Factory, Tianjin, China). There was no probiotic or antibiotic in the vitamin-mineral premix.

Birds and Experimental Treatment

All the procedures used in the experiment were approved by the Committee of Animal Protection and Utilization institutions of Yangzhou University (Yangzhou, China). Five hundred and forty 1-day-old male *Lohmann* Brown chicks were purchased from a commercial hatchery (Xing Da Company, Jinhu, China) and initial BWs were obtained. The birds were randomly divided into three groups with six replicates and 30 birds in each replicate; the experimental period was 70 days. The treatments were as follows: basal diet (control group), basal diet containing 0.1% probiotics mix (probiotic group) and basal diet containing 0.01% zinc bacitracin in feed (antibiotic group). The birds were fed the starter diets from d 1 to 42 (Starter phase) and finisher diets from d 43 to 70 (Grower phase). The nutrient levels of the diets were based on Chinese nutrient requirements for layer chickens (ZB B43005-86) as shown in Table 1.

Table 1 – Ingredients and nutrient composition of diets[1]

Ingredient	Starter (Days 1 to 42)	Grower (Days 43 to 70)
Corn (%)	64.27	66.47
Soybean meal (%)	27.63	25.32
Corn protein powder[2] (%)	3.00	0.00
Bran[3] (%)	1.60	5.00
Dicalcium phosphate (%)	1.50	0.99
Limestone (%)	0.70	0.87
DL –Methionine (%)	0.00	0.05
NaCl (%)	0.30	0.30
Vitamin –mineral premix	1.00	1.00
Calculated nutrition		
composition[4,5]	11.97	11.72
ME, (MJ / kg)	19.00	16.25
CP (%)	0.93	0.80
Lysine (%)	0.31	0.29
Methionine (%)	0.80	0.70
Ca (%)	0.71	0.62
Total P (%)	0.46	0.35
Available P (%)		

[1]Nutrient level of the diets was based on Chinese nutrient requirements for layer chickens (ZB B43005 –86).

[2]Crude protein (CP) content was 51.3%, and metabolizable energy (ME) was 3.41 Mcal/kg.

[3]Crude protein content was 14.3%, and ME was 1.35 Mcal/kg.

[4]Provided per kilogram of diet (Days 1 to 42): vitamin A, 1500 IU; vitamin D_3, 200 IU; vitamin E, 10 IU; riboflavin, 3.6 mg; pantothenic acid, 10 mg; niacin, 27 mg; vitamin B_{12}, 9 µg; choline chloride, 1300 mg; biotin, 0.15 mg; folic acid, 0.55 mg; thiamine, 1.8 mg; pyridoxine, 3 mg; Fe, 80 mg; Zn, 40 mg; Mn, 60 mg; I, 0.35 mg; Cu, 8 mg; Se, 0.15 mg.

[5]Provided per kilogram of diet (Days 43 to 70): vitamin A, 1500 IU; vitamin D_3, 200 IU; vitamin E, 5 IU; riboflavin, 1.8 mg; pantothenic acid, 10 mg; niacin, 11 mg; vitamin B_{12}, 3 µg; choline chloride, 500 mg; biotin, 0.10 mg; folic acid, 0.25 mg; thiamine, 1.3 mg; pyridoxine, 3 mg; Fe, 60 mg; Zn, 35 mg; Mn, 30 mg; I, 0.35 mg; Cu, 6 mg; Se, 0.10 mg.

–control group: basal diet without probiotics and antibiotic

–probiotic group: basal diet containing 0.1% probiotics

–antibiotic group: basal diet containing 0.01% zinc bacitracin mix (10%).

All birds were raised in stainless steel pens of identical size. The light schedules were based on

the *Lohmann* Commercial Management Guide. A standard *Lohmann* management procedure was used throughout the experiment. All birds drank and ate freely, during the experiment.

Sampling and Analytical Methods

Birds were weighed by electronic scales on days 1, 14, 28, 42, 56, and 70 to determine BW and ADG. Because feed wastage could not be controlled for consumption and feed-to-gain ratio, they were not measured in the experiment.

On days 21, 42, and 63, twelve birds per treatment were randomly selected, individually weighed, and sampled for blood from the wing vein. On day 63, the birds were euthanized by cervical dislocation, and the heart, liver, spleen, bursa, and thymus were removed and weighed. The length of intestinal segments was measured when it was taken out and then it was weighed after the chyme was removed.

Before harvesting the serum, blood samples were centrifuged at 1800 ×*g* for 10 min at 4°C after clotting. Serum samples were stored at –20°C until their variables were measured.

The serum growth hormone (GH) was determined using Iodine [^{125}I] Growth Hormone Radioimmunoassay kits (North Biological Technology Research Institute, Beijing, China) according to the manufacturer's instructions.

Serum biochemical variables, total protein (TP), albumin (ALB), glucose (GLU), and alkaline phosphatase (ALP), were measured using the Unicel Dxc 800 Synchron Automatic biochemistry analyzer system (Beckman Coulter, Brea, CA, USA).

Statistical Analysis

Data were input into an Excel spreadsheet to establish a database and analyzed using a one-way ANOVA (SPSS 17.0, SPSS Inc. and Chicago, IL, USA) to determine differences between treatment groups. Differences were significant when $p<0.05$.

RESULTS

Growth Performance

The results of growth performance of birds are presented in Table 2. Birds in the probiotic group had greater BW than in the control group during the overall experimental period ($p<0.05$). On the 14th day, the weight of poultry in probiotic group was higher than that in antibiotic group ($p<0.05$), but there was no significant difference between probiotic group and antibiotic group on the 28th and 70th day ($p>0.05$). On the 56th and 70th day, compared with the control group, the addition of antibiotic led to the improvement of BW ($p<0.05$).The probiotic group had higher ADG from day 1 to 14 and day 56 to 70 compared with the control group ($p<0.05$). The antibiotic group had higher ADG from days 42 to 56 compared with the control group ($p<0.05$). Probiotic treatment or antibiotic treatment led to increased ADG during the overall experimental period compared with the control group ($p<0.05$). On the 14th day of the experiment, BW and ADG in probiotic group were higher than those in antibiotic group ($p<0.05$).

Table 2 – Effects of probiotics/antibiotics on growth performance in *Lohmann* Brown chicks.

Parameter	Age (d)	Control group1	Probiotic group	Antibiotic group
BW (g)	1	36.6 ± 2.3	36.2 ± 2.0	36.0 ± 2.1
BW (g)	1	36.6 ± 2.3	36.2 ± 2.0	36.0 ± 2.1
	14	142 ± 2^{a}	146 ± 1.0^{b}	142 ± 2.0^{a}
	28	308 ± 14^{a}	320 ± 10^{b}	315 ± 8.0^{ab}
	42	560 ± 20^{a}	578 ± 21^{b}	573 ± 15^{ab}
	56	715 ± 20^{a}	765 ± 34^{b}	757 ± 37^{b}
	70	943 ± 68^{a}	1016 ± 36^{b}	1005 ± 48^{b}
ADG (g)	1 to 14	7.52 ± 0.26^{a}	7.87 ± 0.14^{b}	7.58 ± 0.16^{a}
	14 to 28	11.9 ± 0.90	12.4 ± 0.70	12.3 ± 0.60
	28 to 42	18.0 ± 0.50	18.4 ± 1.00	18.5 ± 0.60
	42 to 56	11.1 ± 0.90^{a}	13.4 ± 1.10^{b}	13.1 ± 2.4^{b}
	56 to 70	16.2 ± 3.8	17.9 ± 1.16	17.7 ± 1.7
	1 to 70	12.9 ± 1.0^{a}	14.0	

Note: $^{a-b}$Different superscript letters within a row indicate a statistically significant difference (p<0.05).

–control group: basal diet without probiotics and antibiotic

–probiotic group: basal diet containing 0.1% probiotics

–antibiotic group: basal diet containing 0.01% zinc bacitracin mix (10%).

Serum Biochemical Variables

The results of serum biochemical variables of birds are show in Table 3. Serum ALB of the probiotic group was higher than the control group and the antibiotic group on day 21 (p<0.05). Furthermore, the inclusion of probiotics resulted in increased TP compared with the control group and antibiotic group on day 63 (p<0.05). There was no significant difference between the treatments group birds with respect to ALP, GLU, and GH (p>0.05).

Table 3 – Effects of probiotics /antibiotics on serum biochemical variables in *Lohmann* Brown chicks.

Parameter	Age (d)	Control group	Probiotic group	Antibiotic group
ALB	21	11.1 ± 0.40[a]	11.8 ± 0.70[b]	11.4 ± 0.90[ab]
(g/L)	42	11.3 ± 0.40	11.6 ± 0.80	11.6 ± 0.70
	63	12.0 ± 1.0	11.8 ± 0.60	11.5 ± 0.90
TP	21	28.6 ± 1.0	29.9 ± 1.6	29.4 ± 2.3
(g/L)	42	32.3 ± 1.1[ab]	33.2 ± 1.1[a]	31.2 ± 1.7[b]
	63	32.3 ± 1.2[a]	33.6 ± 1.6[b]	31.4 ± 1.6[a]
ALP	21	1940 ± 718	2044 ± 642	2087 ± 942
(U/L)	42	1578 ± 591	1995 ± 774	2105 ± 459
	63	828 ± 186	778 ± 266	766 ± 194
GLU	21	15.8 ± 1.3	15.2 ± 0.80	15.4 ± 1.2
(mmol/L)	42	14.1 ± 0.60	13.7 ± 0.90	13.8 ± 0.90
	63	11.9 ± 0.60	12.5 ± 0.70	11.6 ± 1.8
GH	21	855 ± 131	871 ± 172	891 ± 90.0
(ng/ml)*10^3	42	892 ± 191	873 ± 385	867 ± 92.0
	63	863 ± 84.0	839 ± 63.0	805 ± 129

Note: [a-b]Different superscript letters within a row indicate a statistically significant difference (p<0.05).–control group: basal diet without probiotics and antibiotic

–probiotic group: basal diet containing 0.1% probiotics

–antibiotic group: basal diet containing 0.01% zinc bacitracin mix (10%).

Visceral Organs

The results of the growth of the small intestine are show in Table 4. The duodenum was significantly longer in the probiotic and antibiotic groups compared to the control group (p<0.05). The probiotic group had significantly longer ileums compared with the control and antibiotic groups (p<0.05). In addition, the weight of duodenum and ileum in birds fed the probiotic feed was significantly higher than that in the control group (p<0.05). The weight of the duodenum in the probiotic group and antibiotic group was significantly higher than that in the control group (p<0.05). There was no significant difference between the treatment group birds with respect to the Length and weight of the Jejunum. There was significant difference between the probiotic group and antibiotic group, only in the ileum length (p<0.05), but there was no significant difference in other variables.

Table 4 – Effects of probiotics/antibiotics on small intestine growth in *Lohmann* Brown chicks on day 63.

Variables	Control group	Probiotic group	Antibiotic group
Duodenum length (cm)	22.4 ± 1.4[a]	24.6 ± 2.4[b]	24.3 ± 2.4[b]
Jejunum length (cm)	49.7 ± 6.4	47.5 ± 5.2	47.0 ± 3.4
Ileum length (cm)	42.4 ± 4.2[a]	47.3 ± 4.8[b]	43.8 ± 2.8[a]
Duodenum weight (g)	5.01 ± 0.67[a]	5.61 ± 0.82[b]	5.66 ± 0.57[b]
Jejunum weight (g)	9.41 ± 0.87	10.03 ± 0.85	9.68 ± 1.43
Ileum weight (g)	5.43 ± 0.95[a]	6.38 ± 0.73[b]	6.01 ± 0.87[ab]

Note: [a-b]Different superscript letters within a row indicate a statistically significant difference (p<0.05).–control group: basal diet without probiotics and antibiotic

–probiotic group: basal diet containing 0.1% probiotics

–antibiotic group: basal diet containing 0.01% zinc bacitracin mix (10%).

The results of visceral organ growth are show in Table 5. The addition of probiotics resulted in significantly increased (p<0.05) liver weights compared with the control. Birds fed either the probiotic or antibiotic diet had higher spleen weights than the control birds (p<0.05). However, there was no significant difference in heart, thymus, and bursa weights between treatment group birds (p>0.05).

Table 5 – Effects of probiotics/antibiotics on other visceral organs growth in *Lohmann* Brown chicks on day 63.

Organ weight (g)	Control group	Probiotic group	Antibiotic group
Heart	3.88 ± 0.50	4.02 ± 0.33	4.05 ± 0.25
Liver	18.6 ± 2.4[a]	21.4 ± 2.8[b]	20.7 ± 3.3[ab]
Spleen	2.12 ± 0.45[a]	2.66 ± 0.59[b]	2.69 ± 0.82[b]
Thymus	3.45 ± 0.92	3.53 ± 0.66	3.56 ± 0.99
Bursa	0.34 ± 0.07	0.40 ± 0.12	0.34 ± 0.07

Note: [a-b]Different superscript letters within a row indicate a statistically significant difference ($p<0.05$).

–control group: basal diet without probiotics and antibiotic

–probiotic group: basal diet containing 0.1% probiotics

–antibiotic group: basal diet containing 0.01% zinc bacitracin mix (10%).

DISCUSSION

It is well known that growth and development in chickens are influenced by the different numbers and types of normal flora colonization in the intestinal mucosa and intestinal contents (Chichlowski *et al.*, 2007; Hernández *et al.*, 2008; Choct, 2009). Probiotics is a new feed additive as a potential substitute for antibiotic. The organic acids produced by probiotics can directly kill pathogenic bacteria or form a microenvironment that is not conducive to the growth of harmful bacteria, thus improves immune function (Zulkifli *et al.*, 2000; Panda *et al.*, 2008) and nutrient retention (Nahashon *et al.*, 1994; Mountzouris *et al.*, 2010). The organic acids such as lactic acid and acetic acid produced by probiotics can reduce the pH value of the intestinal tract and create the microecological environment which is beneficial to the growth and reproduction of the probiotic bacteria, increase the number of beneficial bacteria and inhibit the growth of pathogenic microorganisms (Koenen *et al.*, 2004; An *et al.*, 2008; Mountzouris *et al.*, 2010). The body weight (BW) and ADG of the probiotic group were higher than those of the control group during the whole experiment period, which indicated that probiotics promoted the growth of *Lornman* chicken. This was in agreement with previous studies in broilers (Zulkifli *et al.*, 2000; Timmerman *et al.*, 2006; Wang & Gu , 2010). On the contrary, other studies (Lee *et al.*, 2010; Zhang *et al.*, 2011) reported no beneficial effects on chicken growth performance. Different probiotics have favorable or unfavorable effects on the body through a variety of complex and interactive mechanisms. The difference of experimental results may be due to the different types of probiotics and the level of feeding and management. In the current experiment, the addition of a mix of *Bacillus subtilis* (1×10^5 cfu/kg of diet) and *Pediococcus acidilactici* (1×10^5 cfu/kg of diet) significantly increased the BW and ADG in *Lohmann* Brown chicks. Similarly Bacillus subtilis was reported to improve body weight gain in broilers (Fritts *et al.*, 2000; Hooge *et al.*, 2004). The reason may be that Bacillus subtilis increases the height of villi, which can provide more surface areas to absorb nutrients (Sen *et al.*, 2012) and reduce pathogenic bacteria in the intestine, thus improving nutrient absorption (Wu *et al.*, 2011; Park & Kim, 2015). The addition of lactic acid bacteria can also improve the intestinal structure and promote the absorption and utilization of more nutrients (Chichlowsk *et al.*, 2007; Awad *et al.*, 2009). Furthermore, Pediococcus acidilactici-based probiotics can effectively enhance the resistance of birds to coccidiosis and partially protected against the negative growth effects associated with infection (Lee *et al.*, 2007) therefore leading to an improved growth performance. In this study, the addition of the probiotic group improved the length of the duodenum and the jejunum of Lohmann Brown chicks on day 63. This is similar to the results reported by Awad *et al.* (2009). This indicated that the probiotics mix might have improved growth performance by increasing intestinal length and greater nutrient absorption. The results showed that the probiotics could improve the growth performance of chickens, which was similar to that of antibiotic. Probiotic supplementation did not affect the serum levels of ALP, GLU, or GH. However, in the probiotic group, ALB on day 21 and TP on day 63 were significantly higher than those in the control group. The good nutritional status can maintain the serum total protein and the albumin content at a high level, and the increase of the both contents of the two shows that the metabolic activity of the body is vigorous (Dhanalakshmi *et al.*, 2007).

In the current study, we found that liver weights were significantly higher in the probiotic group compared with the control group. This was in agreement with the study of Wang *et al.* (2014), whereas, not in line with the result of Awad *et al.* (2009) who reported no effect on liver weights. The spleen is an important lymphoid organ, which produces immunoglobulins,

complement, and other immune substances that play an important role in immunity. Chen *et al.* (2013) suggested measuring immune organ weight as a method for evaluating immune status in chickens. Spleen weight could be increased with spleen swelling under pathological conditions (Syed *et al.*, 2012). Whereas, under the normal situation of the present study, the higher spleen weight in the probiotic group and antibiotic group suggested that the probiotic diet can improve the immune function of the body, which is consistent with the observation of Li *et al.* (2009). In addition, under pathological conditions, there was no interaction between coccidiosis vaccine and feed additives on growth performance and carcass yield of broilers, and probiotics could be safely used in broilers feed (Xi *et al.*, 2019). This is also demonstrated by the data of the present study, where the body weight of the probiotic group was higher than the antibiotic group.

There was no significant difference between the probiotic group and the antibiotic group in the organs (heart, liver, spleen, thymus, and bursa) weights, which indicated that dietary supplementation with probiotics mix or antibiotic had similar influence on the growth of the heart, liver, and main immune organs of chickens.

CONCLUSION

In conclusion, adding Bacillus subtilis and Pediococcus acidilactici mix to chicken feed resulted in similar effect as zinc bacitracin, an alternative to antibiotics, where the growth of body weight and visceral organs were promoted in *Lohmann* Brown chicks (0 to 9 weeks). Therefore, Bacillus subtilis and Pediococcus acidilactici mix is a promising growth promoter for *Lohmann* Brown chicks (0 to 9 weeks) under non-pathological state.

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