

Effects of probiotics on the growth performance and intestinal micro flora of broiler chickens

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Abstract: Antibiotics have been used in poultry industry for decades to promote growth and protect animals from diseases, followed by various side effects. In efforts of searching for a better alternative, probiotic is of extensive attention. We investigated the effects of *Bacillus subtilis*, *Rhodopseudomonas palustris*, *Candida utilis* and *Lactobacillus acidophilus* as 0.1% (W/W) feed additives on broiler growth performance and intestinal microflora. The results showed the probiotics treatments significantly improved growth of broilers. Broilers supplemented with *B. subtilis* and *L. acidophilus* weighed 18.4% and 10.1% more than birds in control group at 42 days of age. Furthermore the feed conversion ratios of the birds in the two groups were also improved, decreasing 9.1% and 12.9%, respectively. Further study indicated a significant increase of cecal *Lactobacilli* concentration in broilers supplemented with probiotics, especially in *L. acidophilus* treatment group. Meanwhile, the count of cecal *Actinomyces* in birds treated with probiotics was significantly lower compared with the control group. In conclusion, probiotics such as *B. subtilis* and *L. acidophilus* are good alternatives to antibiotics in promoting growth resulting from a beneficial modulation of the intestinal micro flora, which leads to increased efficiency of intestinal digestion in the host animal.

Keywords: Probiotics, broilers, growth performance, feed conversion ratio, intestinal micro flora.

INTRODUCTION

Probiotics have been defined as “Live microorganisms (bacteria or yeasts), which when ingested or locally applied in sufficient numbers confer one or more specified demonstrated health benefits for the host” (Anil *et al.*, 2007). In fact, probiotics have been used for quite a long time throughout human history. In the early 20th century, Russian immunologist Elie Metchnikoff first observed the beneficial effect of bacteria (Metchnikoff 1907). He suggested that *Lactobacilli* in yogurt could have a positive effect on the intestinal micro flora, which meant a lot to human health and longevity.

The poultry industry during the past two decades has been one of the most dynamic and ever expanding sectors in the world (Alkhalif *et al.*, 2010). For about fifty years, antibiotics have been used as feed additives in poultry industry to promote animal growth and protect animals against infection of pathogenic microorganisms (Ferket *et al.*, 2002). However, with increasing problems brought by abuse of antibiotic, such as antibiotic resistance, there are bans on subtherapeutic antibiotic usage in the poultry industry in Europe (Edens 2003). And probiotics have been regarded as good replacement feed additives (Tomasik *et al.*, 2003). Probiotics frequently used in the poultry are *Bifidobacteria*, *Bacillus*, *Saccharomyces* and *Lactobacilli*, which are able to benefit the host animals basing on different mechanisms (Liu *et al.*, 2012 and Zhang *et al.*, 2011 and Li *et al.*, 2009). *Lactobacilli* can

produce lactic acids and make an acidic intestinal environment, which is bad for the growth of putrefactive and potentially pathogenic bacteria (Servin 2004). *Bacillus* species have potential attributes of colonization, immunostimulation, and anti-microbial activity (Le *et al.*, 2004) and can secrete protease, amylase, and lipase (Santoso *et al.*, 1995). *Saccharomyces* play an important role in vitamin and amino acid production. Photosynthetic bacteria have effects on improving water quality, promoting growth and preventing the host from diseases (Qi *et al.*, 2009).

Here the effects of supplementing the feed with 0.1% (W/W) *Bacillus subtilis*, *Candida utilis*, *Lactobacillus acidophilus* and *Rhodopseudomonas palustris* on the production performance and intestinal microflora of broilers were studied, respectively. The results indicated that broilers supplemented with *B. subtilis* and *L. acidophilus* weighed 18.4% and 10.1% more than those in control group and the feed conversion ratios of the birds in this two groups were also improved, decreasing 9.1% and 12.9%, respectively. It also showed a significant increase of cecal *Lactobacilli* concentration in broilers supplemented with probiotics, especially in *L. acidophilus* treatment group. Meanwhile, the count of cecal *Actinomyces* in birds treated with probiotics was significantly lower compared with the control group. Probiotics such as *B. subtilis* and *L. acidophilus* are good alternatives to antibiotics in promoting growth resulting from a beneficial modulation of the intestinal microflora, which leads to increased efficiency of intestinal digestion in the host animal.

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MATERIALS AND METHODS

Probiotic Strains

Four commercial probiotic products obtained from research cooperation base of University of Science & Technology, Beijing, and Shijiazhuang USTB-Sheng'an Biotech Co., LTD were used in this study. The composition of the four products was *B. subtilis*, *R. palustris*, *C. utilis* and *L. acidophilus* in a concentration of 3×10^{10} , 5.8×10^9 , 7×10^8 and 8×10^9 CFU/g, respectively.

Table 1: Composition of the basal diet

Ingredients	Starter 0-21 days	Grower 22-42 days
Composition g/kg		
Maize	535.3	588.6
Soybean meal	355.2	315.3
Fish meal	39.9	36.3
Vegetable oil	35.2	30.2
Limestone	15.2	12.7
Salt	3.0	3.0
Monocalcium phosphate	9.2	7.8
Vitamin premix	2.0	2.0
Mineral premix	2.0	2.0
DL-methionine	1.5	1.0
L-lysine	1.0	0.6
Choline chloride	0.5	0.5
Calculated chemical composition (g/kg wet weight)		
ME MJ/kg	12.9	12.8
Dry matter	88.9	88.7
Crude protein	221.6	206.3
Lysine	11.2	9.5
Methionine + cystine	8.5	7.6
Calcium	10.2	8.7
Total phosphorus	6.9	6.3

^aThe vitamin premix supplied the following per kilogram of diet: vitamin A, 9800 IU; vitamin D₃, 2200 IU; vitamin E, 40 IU; vitamin K₃, 2 mg; thiamine, 1 mg; riboflavin, 7 mg; pyridoxine, 6 mg; vitamin B₁₂, 0.035 mg; nicotinic acid, 50 mg; pantothenic acid, 15 mg; folic acid, 1.5 mg; biotin, 0.15 mg; ^bThe mineral premix supplied the following per kilogram of diet: Fe, 70 mg; Cu, 9 mg; Mn, 90 mg; Zn, 60 mg; Se, 0.16 mg; and I, 0.5 mg.

Experimental design and husbandry

All practice in the process of this study concerning animal care followed principles required by the university. Twenty 11-day-old male broiler chicks with original average body weight of 327 g for each with no significant differences were assigned at random to 5 experimental groups with each group consisted of 4 birds and raised floor pens (0.90 m²/bird) for 31 days. Chicks were vaccinated at hatch for Marek's, Newcastle and Infectious Bronchitis Disease. Broilers were fed with a commercial maize-soybean diet (Lv *et al.*, 2012) without any antibiotics or growth promoters. The feed was formulated for starter (0-21 days) and grower (22-42 days) growth

periods (table 1). Broilers in group T1, T2, T3 and T4 were supplied with basal diet containing 0.1% (W/W) *B. subtilis*, *R. palustris*, *C. utilis* and *L. acidophilus*, respectively. table 2 shows the viable count of probiotic containing in one gram feed. Feed and water were supplied for consumption *ad lib*. Temperature was controlled at range of 20 to 25°C. At night, incandescent lamps were used for lighting. There was not any antibiotic fed during the whole experiment of 31 days.

Growth performance

All birds in each group were weighed individually at 11, 14, 21, 28, 35 and 42 days of age. Daily weight gain (DWG) in overall period was calculated. The amounts of added feed to each pen were recorded daily, and feed residues in each pen were weighed. Feed consumption was therefore calculated. The feed conversion ratios (FCR) for the overall period of 31 days could be calculated: feed consumption/weight gain. The number and body weight of dead birds were recorded daily, then the mortality was calculated.

Intestinal microflora

On the 42nd day, one bird from each group was randomly selected and sacrificed. The cecum was ligated with light twine and collected in sterile bags. The cecal digesta was collected in a sterilized tube, stored at -20°C. Approximately 100mg of cecal digesta was mixed with 900µL of sterile saline solution (0.9% NaCl) and homogenized 3 min using a homogenizer. Each cecal digesta homogenate was serially diluted from initial 10⁻¹ to 10⁻⁹, and subsequently plated on selective agar media for enumeration of target bacterial groups. The following bacterial species were identified: total number of bacteria on Plate Count agar and aerobic incubation at 37°C for 24 h; *Lactobacilli* on MRS agar medium and anaerobic incubation at 37°C for 48h; *Saccharomyces* on Rose Bengal Medium with 0.01% chloramphenicol and aerobic incubation at 28°C for 4d; *Actinomyces* on Gause's Synthetic Agar with 50µg/mL K₂Cr₂O₇ and aerobic incubation at 28°C for 6d. Each determination was conducted in triplicate. The results are presented as colony forming units (CFU) per gram of wet cecal digesta (Biernasiak *et al.*, 2009).

STATISTICAL ANALYSIS

Each individual broiler in each group was handled as one experimental unit. Data were subjected to one-way analysis of variance (ANOVA) using the SPSS 19.0 (2010, SPSS Inc., USA).

RESULTS

Effects of probiotics on growth performance

To accurately assess the growth performance, initial and weekly body weights were examined during the whole

period (fig. 1). The final body weight, DWG and FCR were shown in table 3. No death occurred during the entire feeding period in all the groups. table 3 clearly demonstrated that the probiotic treatments improved the final body weight, DWG and FCR of the broilers. After the starter period of 11-21 days, the differences of the groups in the body weight became greater (fig. 1). The body weight of broiler in group T1 with *B. Subtilis* treatment was the highest in 21, 28, 35 and 42 days, respectively. *B. Subtilis* treatment group significantly increased the DWG and improved the FCR for overall period ($P<0.05$), compared with other groups in table 3.

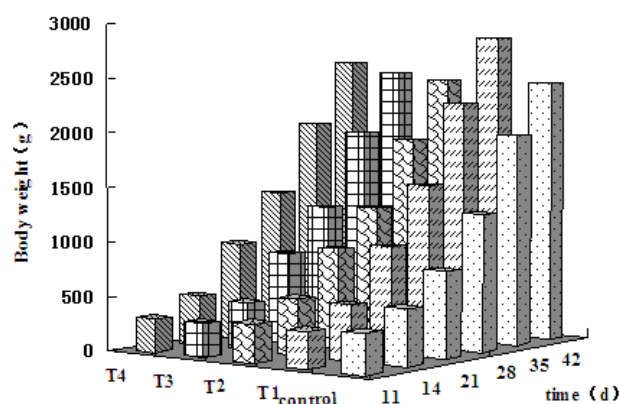


Fig. 1: Growth performance of broilers

T1: *B. subtilis* treatment with 3×10^7 *B. subtilis* cells per g feed; T2: *R. palustris* treatment with 5.8×10^6 *R. palustris* cells per g feed; T3: *C. utilis* treatment with 7×10^5 *C. Utilis* cells per g feed; T4: *L. acidophilus* treatment with 8×10^6 *L. acidophilus* cells per g feed.

Effects of probiotics on intestinal microflora

The cecal microflora composition of broilers at 42 d of age is shown (table 4, fig. 2). The total number of bacteria in cecum of broilers supplemented with *B. subtilis*, *R. palustris*, *C. utilis*, and *L. acidophilus* was 4, 1, 191 and 6 times more than control group, respectively. *C. utilis* suppylment significantly increased the total number of bacteria in cecum, which was two orders of magnitude higher than the other groups and reached 1.3×10^8 CFU/g wet cecal digesta. In comparison with the control group, the count of *Lactobacilli* in cecum of broilers treated with *B. subtilis*, *R. palustris*, *C. utilis*, and *L. acidophilus* group was 4, 6, 7 and 10 times higher than those of control, respectively. The number of *Saccharomycetes* in the cecum of birds treated with *B. subtilis* and *R. palustris* was 89% and 92% less while that of *C. utilis* and *L. acidophilus* group was 19 and 16 times more than control group, greatly different depending on the probiotics used. The population of *Actinomycetes* in cecum of birds supplemented with *B. subtilis*, *R. palustris*, *C. utilis*, and *L. acidophilus* decreased by 91%, 100%, 91% and 87% respectively, demonstrating inhibition of probiotics on growth of *Actinomycetes*.

DISCUSSION

This finding is in agreement with several reports demonstrating that probiotic supplemented to the birds improve the body weight and DWG. Khaksefidi *et al* showed that body weight gain of birds fed diet treated with 50mg/kg of probiotic containing *Bacillus subtilis* were significantly higher than birds of the control group and the FCR were better (Khaksefidi *et al.*, 2006). Liu *et al* reported that treatment of the wheat-based diet with *Lactobacillus reuteri* Pg4 increased body weight gains of the broiler chickens (Liu *et al.*, 2007). Mountzouris *et al* found that broilers treated with probiotic containing *Pediococcus* strain, *Enterococcus* strain, *Lactobacillus* strains and *Bifidobacterium* strain in feed and water displayed a growth-promoting effect and better FCR (Mountzouris *et al.*, 2007). Probiotic preparation is effective in promoting broilers growth and improving FCR. This result may be due to probiotic's immunomodulatory activity and ability to fortify beneficial members of the intestinal microflora, improving efficiency of digestion and nutrient absorption processes of the host (Dalloul *et al.*, 2003 and Mountzouris *et al.*, 2010 and Alkhalif *et al.*, 2010). Particularly, broilers in T1 group treated with *B. subtilis* weighed 18.4% more than those of control group and FCR decreased 9.1%. It was also worth noting that birds in T4 group treated with *L. acidophilus* got an increase of 10.1% in body weight and a significant decrease of 12.9% in FCR ($P<0.05$) compared with birds in control group.

Further study showed that the probiotics significantly modified the composition of intestinal microflora in broilers. Similar results were also acquired by other researchers. Chen *et al* found that feed supplemented with *Bacillus subtilis* and *Saccharomyces cerevisiae* Y10 increased the *Lactobacillus* population and depressed *Escherichia coli* population in small and large intestine (Chen *et al.*, 2013). Particularly, there's considerable increase in the number of *Lactobacilli* in cecum of broilers fed with probiotics, especially in *L. acidophilus* treatment group. In this study, *L. acidophilus* treatment decreased the cecal concentrations of *Saccharomycetes* and increased the concentration of *Lactobacilli*. As a kind of normal gut flora, *L. acidophilus* created an intestinal environment with low pH and low Eh that inhibited the intestinal growth of pathogenic bacteria, yeast and fungi. In addition, *L. acidophilus*'s strong ability to attach to the chicken intestine also caused the development of *Lactobacilli* in the cecum of broilers supplied with *L. acidophilus* (Jin *et al.*, 1996). *L. acidophilus* is good for the balance of intestinal microflora (Zhang *et al.*, 2004). This result coincide with the growth performances in our study that T4 group treated with *L. acidophilus* displayed a great increase of 10.1% in body weight and the most significant improvement of 12.9% in FCR. These results indicated that *L. acidophilus* made profound changes in

Table 2: The viable count of probiotic containing in one g feed (CFU/g)

Probiotic supplementation	—	<i>B. Subtilis</i>	<i>R. palustris</i>	<i>C. utilis</i>	<i>L. acidophilus</i>
Groups	Control	T1	T2	T3	T4
Viable count (CFU/ g)	—	3×10^7	5.8×10^6	7×10^5	8×10^6

Table 3: Growth performance

Growth performance	Treatments				
	Control	T1	T2	T3	T4
Final body weight (g) *	2480±208 ^a	2937±128 ^b	2527±253 ^a	2610±165 ^a	2730±359 ^c
Daily weight gain (g) *	56±5 ^a	82±3 ^b	66±6 ^a	71±4 ^a	77±10 ^c
Feed conversion ratio	2.19	1.99	2.20	2.00	1.91

Table 4: viable count of bacteria, *Lactobacilli*, *Actinomyces* and *Saccharomyces* in cecal digesta of broilers 42 days (Mean±SD)

Bacteria	Treatments				
	Control	T1	T2	T3	T4
total bacteria ($\times 10^5$ CFU/g)	7.0±0.6	34.9±1.7	14.0±0.8	1339.3±0.6	45.5±0.6
<i>Lactobacilli</i> ($\times 10^5$ CFU/g)	2.4±0.3	12.8±1.4	17.8±0.7	19.7±3.0	27.3±0.7
<i>Saccharomyces</i> ($\times 10^3$ FU/g)	1.23±0.20	0.13±0.11	0.10±0.06	24.83±3.15	21.43±0.76
<i>Actinomyces</i> ($\times 10^3$ CFU/g)	370.0±40.00	31.67±6.81	0.07±0.06	35.08±4.86	48.58±4.25

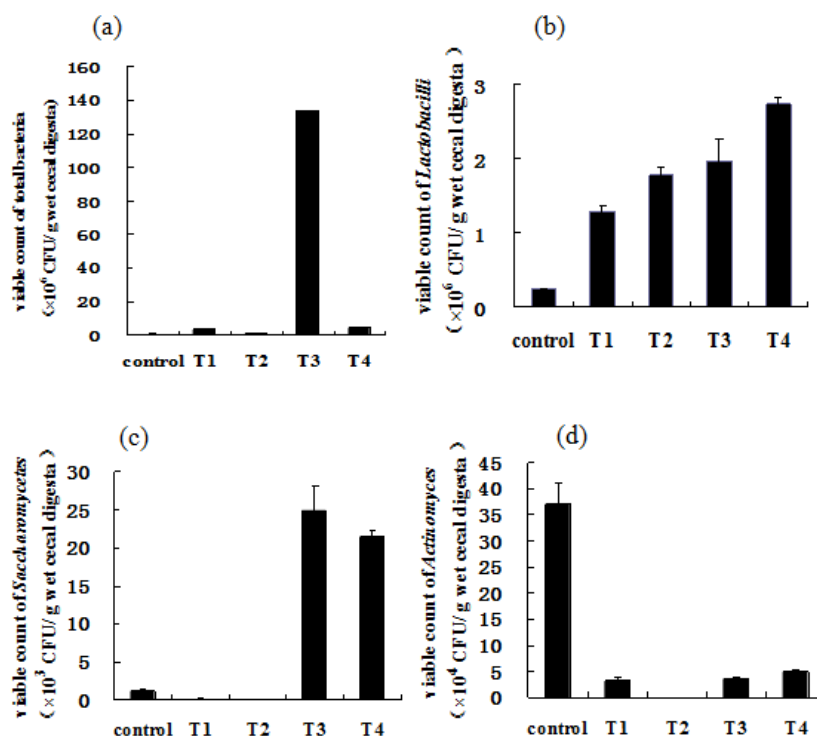


Fig. 2: Effects of probiotics on intestinal micro flora

T1: *B. subtilis* treatment with 3×10^7 *B. subtilis* cells per g feed; T2: *R. palustris* treatment with 5.8×10^6 *R. palustris* cells per g feed; T3: *C. utilis* treatment with 7×10^5 *C. utilis* cells per g feed; T4: *L. acidophilus* treatment with 8×10^6 *L. acidophilus* cells per g feed.

- (a) viable count of total bacteria in cecal digesta of broilers.
- (b) viable count of *Lactobacilli* in cecal digesta of broilers.
- (c) viable count of *Saccharomyces* in cecal digesta of broilers.
- (d) viable count of *Actinomyces* in cecal digesta of broilers

the intestinal flora through promoting development of performance of broilers. beneficial bacteria, thus significantly improved the growth

CONCLUSION

Probiotics administration in feed of broilers displayed a growth-promoting effect. Significantly improvement in DWG and FCR of the broilers were shown in *B. subtilis* and *L. acidophilus* treatments, compared with control and other probiotic treatment groups. Further study indicated a significant increase of cecal *Lactobacilli* concentration in broilers supplemented with probiotics, especially in *L. acidophilus* treatment group. Meanwhile, the count of cecal *Actinomyces* in birds treated with probiotics was significantly lower compared with the control group. In conclusion, probiotics especially *B. subtilis* and *L. acidophilus* used here are potential alternatives to antibiotics in promoting growth resulting from a beneficial modulation of the intestinal micro flora, which leads to increased efficiency of intestinal digestion in the host animal.

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