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Original Research Article

Study of *Bacillus subtilis* on growth performance, nutrition metabolism and intestinal microflora of 1 to 42 d broiler chickens

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ABSTRACT

To study the influence of different levels of *Bacillus subtilis* on growth performance, nutrition metabolism and intestinal microflora of 1 to 42 d Arbor Acres (AA) broilers, a total of 800 one-day-old healthy broilers were randomly divided into 5 groups with 4 replicates per group and 40 broilers per replicate. Broilers were fed a basic diet (group 1) which acted as the control group, and 4 other groups (2 to 5) were fed the basal diet with *B. subtilis* added at concentrations of 100, 150, 200 and 250 mg/kg, respectively for 42 days. The results showed as follow: the average daily gain (ADG) of group 4 was significantly higher than ($P < 0.05$) that of group 1, and the average daily feed intake (ADFI) of group 5 was the highest but the differences between groups were not significant ($P > 0.05$). The feed to gain ratio (F/G) of all the experimental groups was lower than that of the control and the difference was significant in group 4 ($P < 0.05$). In addition, supplementation of *B. subtilis* increased the apparent metabolism of crude protein ($P > 0.05$), crude fat ($P > 0.05$), dry matter ($P > 0.05$) and organic matter ($P < 0.05$). *B. subtilis* decreased the *Escherichia coli* and *Salmonella* populations in the cecum. This shows that adding *B. subtilis* to the broiler diet can improve the growth performance, increase feed efficiency, regulate serum index and reduce harmful bacteria in the intestinal tract. Based on our study, it could be recommended that addition of *B. subtilis* at 200 mg/kg could improve the growth performance of broilers.

1. Introduction

As a kind of green feed additive, probiotics has many advantages such as they improve livestock production, keep animals' intestinal healthy and enhance the animals' immunity without toxic side-effect or drug residues (Abdur-Rahman et al., 2014; Dragana et al., 2014). But most probiotics preparations are vulnerable to environment changes. As the holding time extends, viable bacterium will gradually die. Therefore, the amount of viable bacterium in the feed microorganism additives getting access to the intestinal tracts of animals is small, which significantly reduces the effect of additive (Leser et al., 2008; Qin, 2009; Xiang et al., 2009.). *Bacillus subtilis* can form spores in adverse environment that has some unique biological characters such as resistance to acid, alkali, and heat. They also grow fast. Thus, the spores can still plant in intestinal tracts to grow and breed on arrival after the extrusion process for granulating in feed processing and the expose to strong acidic environment in animals' stomach. Moreover, *B. subtilis* is aerobic bacteria, it takes a large amount of free oxygen while reproducing in the intestinal tract thus it can strongly restrain the growth of the majority of aerobic pathogen bacteria, enhance the growth of anaerobic probiotics such as *Lactobacillus*, yeast and *Bifidobacterium* (Wang et al., 2006). Therefore, it is useful to restore and maintain the intestinal flora balance of animal, improve immune function, enhance animals' resistance to disease, and promote their growth (Gao et al., 2012; Tannock et al., 2000; Zhou et al., 2012). Now *B. subtilis* has become an advanced research hotspot on animal

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probiotics study and it was one of bacteria approved by the Ministry of Agriculture that can be applied in animal husbandry (Lei et al., 2008; Tom et al., 2004; Wei et al., 2009).

Based on previous researches, we studied the effects of different levels of *B. subtilis* additive on growth performance, nutrition metabolism and intestinal microflora in AA chickens so that to provide theoretical basis for safe and healthy AA broiler production.

2. Materials and methods

2.1. Test materials

B. subtilis preparation: the total content of viable bacterium $\geq 2 \times 10^{10}$ cfu/g (provided by Guangzhou Ayhke Biotechnology Co., Ltd); one-day-old AA broiler.

2.2. Experimental design and basal diets

A total of 800 one-day-old healthy AA broilers with approximate weight were randomly divided into 5 treatments with 4 replicates per group and 40 broilers per replicate. The broilers in group 1 as the control were fed basal diet; those in groups 2 to 5 as treated were fed the basal ration supplemented with *B. Subtilis* at 100, 150, 200 and 250 mg/kg, respectively. The trial period was 42 days.

2.3. Experimental diet

With reference of the NRC (1994) and China's Standard of Broiler Diet (GB43005-86), the basal diet was made of corn and soybean meal at 2 stages. The composition and nutrients levels of the basal diets are showed on Table 1.

2.4. Feeding management

One week before the test, preparations including sterilizing hen-houses and experimental utensils, making animals adapt to cage-rearing, controlling temperature and humidity were made. The relative humidity of 60% to 65% was remained through natural ventilation, and the lighting was kept 24 h per day through supplementing with artificial light during the whole experiment. In the first week of the experiment, the temperature of the hen-house was controlled between 32 and 33 °C, and then gradually lowered to 25 °C by 2 °C per week until the end of the experiment.

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

Ingredients	1 to 21 d	22 to 42 d	Nutrition levels[1]	1 to 21 d	22 to 42 d
Corn	58.20	60.18	ME, MJ/kg	12.45	12.94
Soybean meal	30.20	28.20	Crude protein	21.02	20.01
Wheat bran	2.15	2.60	Calcium	1.02	0.96
Fish meal	3.25	3.10	Total phosphorus	0.70	0.65
Stone powder	2.48	2.36	Available phosphorus	0.46	0.38
$CaHPO_4$	1.26	1.17	Methionine	0.52	0.50
Soybean oil	1.18	1.23	Lysine	1.15	1.10
NaCl	0.20	0.20	Methionine + Cystine	0.90	0.86
Lysine	0.26	0.12			
Methionine	0.20	0.10			
Premix[2]	0.50	0.50			
Total	100.00	100.00			

[1] The metabolizable energy was calculated value, while the others were measured value.

[2] One kilogram of premixed feed could provide: VA 8,000 IU, VD_3 1,000 IU, VE 20 IU, VK 0.5 mg, VB_1 3 mg, VB_2 9 mg, VB_6 7 mg, VB_{12} 0.03 μg, niacin 35 mg, D-pantothenic acid 10 mg, folic acid 0.55 mg, biotin 0.18 mg, Fe 100 mg, Cu 8 mg, Zn 100 mg, Mn 120 mg, I 0.7 mg, Se 0.3 mg.

During the experiment, diet and water was provided *ad libitum*, the hen-houses were cleaned and disinfected regularly. The flocks of broilers were observed, and their behavior, incidence and mortality were recorded. The experimental broilers were immunized according to the conventional procedure.

2.5. Nutrition metabolism

From days 30 to 42, 4 broilers were selected randomly in each replicate and put into metabolic cages individually. Total feces collection method was used to determine the metabolism of dry matter (DM), organic matter (OM), crude protein (CP), crude fat, calcium (Ca), and phosphorus (P). The feed intake and feces were recorded every day, and 1/3 feces were frozen at −20 °C. When the metabolism experiment ended, all the frozen feces were mixed. Before chemical analysis, feces samples were dried at 120 °C for 2 h and then at 65 °C for 72 h, then finely ground to a size that could pass through 1-mm sifter.

2.6. Measuring items and methods

2.6.1. Growth performance

The broilers of each replicate were weighed at the beginning, day 42 and every week of the experiment. Feed intake and the number of living chickens were recorded to calculate the average daily gain (ADG), average daily feed intake (ADFI) and feed to gain ratio (F/G) of every replicate.

ADG = (Final weight - Initial weight)/(Days of test × Number of broilers);

ADFI = Feed consumption/(Days of test × Number of broilers);

F/G = Feed consumption/Weight gain.

2.6.2. Nutrition apparent metabolism rate

We analyzed the feed and feces sample for DM, OM, CP, and crude fat by the standard of P.R.C (GB/T6435-86 for water, GB/T 6432-1994 for CP, GB/T 6433-1994 for crude fat, GB/T 6438-2007 for crude ash). The apparent metabolism rate was calculated by the formula.

Nutrition apparent metabolism rate (%) = 100 × (Nutrition in feed intake − Nutrition in feces)/Nutrition intake.

2.7. Test of intestinal flora

Four broilers were selected randomly in each replicate to collect the duodenal contents at the end of the experiment. About 1 g of the samples was put into sterilized tubes and diluted with sterilized normal water to 1:10. Using a micropipette, 0.1 mL dilution was pipetted and plated on agar plates using L shape glass rod, and then incubated for 24 h in 37 °C before counting colonies on plates. Every sample was repeated 3 times. Eosin methylene blue agar medium was used for culturing *Escherichia coli* and Salmonella-Shigella agar (SS) medium was used for *Salmonella*. All the media were bought from Guangdong Huankai Biology Co., Ltd.

2.8. Statistical analysis

ANOVA model of SPSS17.0 statistical program was applied to data analysis on single-factor variance. Multiple comparisons were conducted with the application of LSD method. The significant level

is set to be $P < 0.05$. The results of the data were expressed as means ± standard deviation.

3. Results

3.1. Effects of B. subtilis on growth performance of AA broiler

The difference of ADFI between experimental groups was not significant ($P > 0.05$), and that of group 5 was the highest. Feed/gain ratios of experimental groups were lower than that of the control group, and the F/G value in group 4 was significantly lower than that in the control ($P < 0.05$). As shown of ADG, different levels of *B. Subtilis* could improve the growth performance of AA broiler. Among all the test groups, group 4 had the best effect with a 9.52% increase of growth performance compared with the control group ($P < 0.05$) (Table 2).

3.2. Effects of B. Subtilis on nutrients metabolism of AA broilers

In this study, apparent metabolism rate of DM were dramatically increased compared with the control group ($P < 0.05$), but there was no significant difference in apparent metabolism rates of CP, crude fat, crude ash and OM between the treatments and the control ($P < 0.05$) (Table 3).

3.3. Effects of B. subtilis on microflora of duodenum in AA broiler

As can be seen in Table 4, *B. subtilis* could significantly decrease the amount of total aerobe, *Salmonella* and *E. coli* ($P < 0.05$) and the effect of group 5 was the best. However, *B. subtilis* could increase the amount of *Lactobacillus*. Thus, *B. subtilis* had considerable inhibitory action on the amounts of total aerobe, *Salmonella* and *E. coli* in the cecum of AA broilers.

4. Discussion

4.1. Effects of B. subtilis on growth performance and nutrition metabolism of AA broilers chickens

Some studies have shown that *B. subtilis* can improve the growth performance of broilers (Sogaard et al., 1990; Zhang et al., 2014). *B. subtilis* can secret highly-active protease, lipase and amylase to decompose plant complex carbohydrates, increase the digestibility of nutrients and provide more nutrition to animals (Li et al., 2014). In this experiment, we observed that *B. subtilis* could significantly increase growth performance, decrease F/G but had no obvious effects on feed intake. The similar results have been published (Huang, 2012; Hu et al., 2008; Tan et al., 2012), but differed to Afsharmanesh and Lee's (Afsharmanesh et al., 2013; Lee et al., 2014). The possible reason was that Afsharmanesh's diet was wet

Table 2
Effects of *B. subtilis* on growth performance of AA chicken.

Item	Groups				
	1	2	3	4	5
Initial weight, g	32.74 ± 0.83	32.70 ± 0.65	32.57 ± 0.74	32.65 ± 1.60	32.63 ± 0.72
Final weight, kg	1.89 ± 0.15^{a}	1.93 ± 0.28^{ab}	2.04 ± 0.34^{ab}	2.07 ± 0.16^{b}	2.05 ± 0.23^{b}
ADFI, g	89.53 ± 2.44	88.99 ± 8.54	91.75 ± 1.62	86.47 ± 3.40	93.13 ± 8.93
ADG, g	44.29 ± 1.60^{a}	45.14 ± 2.57^{ab}	47.81 ± 3.71^{ab}	48.57 ± 1.81^{b}	48.05 ± 4.52^{ab}
F/G	1.91 ± 0.09^{b}	1.86 ± 0.22^{ab}	1.81 ± 0.11^{ab}	1.78 ± 0.13^{a}	1.82 ± 0.20^{ab}

ADFI = average daily feed intake; ADG = average daily gain; F/G = feed to gain ratio.
^{a,b} In the same row, values with the same or no letter superscripts mean no significant difference ($P > 0.05$), while values with different letter superscripts mean significant difference ($P < 0.05$).

Table 3
Effects of *B. subtilis* on nutrient metabolism (%) of AA broiler.

Item	Groups				
	1	2	3	4	5
Crude protein	68.34 ± 4.46	71.76 ± 5.24	70.59 ± 3.97	71.96 ± 4.81	72.16 ± 2.73
Crude fat	62.37 ± 2.47	64.72 ± 5.12	65.62 ± 3.46	65.12 ± 4.07	66.82 ± 5.24
Crude ash	34.62 ± 1.38	34.95 ± 1.46	36.04 ± 2.36	36.78 ± 3.48	35.39 ± 3.02
Dry matte	70.85 ± 3.35^{a}	74.74 ± 4.12^{ab}	75.93 ± 5.13^{ab}	76.16 ± 3.84^{b}	76.19 ± 3.846^{b}
Oganic matter	70.43 ± 5.32	70.82 ± 4.82	71.37 ± 3.32	72.25 ± 4.22	72.79 ± 4.52

^{a,b} In the same row, values with the same or no letter superscripts mean no significant difference ($P > 0.05$), while values with different letter superscripts mean significant difference ($P < 0.05$).

Table 4
Effects of *B. subtilis* on microflora [flora numbers, lg (cfu/g)] in the cecum of AA broilers.

Item	Groups				
	1	2	3	4	5
Total aerobe	8.79 ± 0.61^{a}	8.21 ± 0.43^{ab}	8.12 ± 0.66^{ab}	7.85 ± 064^{b}	7.86 ± 041^{b}
Lactobacillus	8.35 ± 0.28^{a}	8.79 ± 0.35^{ab}	8.86 ± 0.42^{bc}	9.35 ± 0.45^{c}	9.37 ± 0.39^{c}
Salmonella	6.63 ± 0.14^{b}	6.35 ± 0.07^{ab}	6.37 ± 0.32^{ab}	5.75 ± 0.14^{a}	6.28 ± 0.26^{ab}
Escherichia coli	8.54 ± 0.61^{b}	7.48 ± 0.37^{a}	7.47 ± 0.29^{a}	7.31 ± 0.36^{a}	7.01 ± 0.28^{a}

^{a,b,c} In the same row, values with the same or no letter superscripts mean no significant difference ($P > 0.05$), while values with different letter superscripts mean significant difference ($P < 0.05$).

wheat-based diets but our diet was a corn-soybean meal dry powdery feed. Lee's experiment bird was male Ross 708.

According to the studies, adding *B. subtilis* into the basal diet of broilers had no significant effect on the ADG of broilers aged from 0 to 21 days, while the increase of broilers aged from 22 to 49 days and 0 to 49 days were 11.3% and 8.4%, respectively (Zhu et al., 2008). Lv et al. (1998) fed broilers with *B. subtilis* additives and found the ADG of broilers aged from 0 to 3 weeks was increased by 4.9% compared with the control group. Another study showed that adding *B. subtilis* had no significant effect on growth performance in the first 2 weeks but in the third week the growth performance was improved (Hao et al., 2008). The effect of *B. subtilis* on improving the growth performance of broilers is not instantly but accumulated as time goes (Hu et al., 2008). But other studies showed that the effect of *B. subtilis* on improving the growth performance were stronger at the beginning than later (Alexopoulos et al., 2004; Liu et al., 2011). Our experiment received similar results, which was the growth performance increased by 9.52%.

Some results have shown that *B. subtilis* can significantly increase metabolism rates of CP, crude fat and crude ash and utilization rate. Our study showed that *B. subtilis* could increase the apparent metabolic rate of CP, crude fat, crude ash and OM, which were similar to others' results (Gao et al., 2012; Li et al., 2014; Zhang et al., 2014), and similar to Sen (2012) in DM apparent metabolism rate. Feng (2015) fed yellow broilers with fermented cottonseed meal diet supplemented with *B. subtilis* and noticed that apparent metabolism rates of CP, Ca and P were increased. But Sadeghi's (2014) result showed that supplement probiotic (*B. subtilis*) had no significant effect on crude ash and Ca contents of tibia. *B. subtilis* played an important role on broilers' health. Supplement of *B. subtilis* preparation in diet, water and other methods could significantly decrease the morbidity and mortality, as well as increase the survival rate (Liu et al., 2011; Yi et al., 2005).

4.2. Effects of B. subtilis on cecum microflora in AA broilers

Cecum is an important site of fermentation and influences animal health and production. *B. subtilis* can maintain the balance of intestinal microenvironment and increase feed conversion ratio by maintaining the advantage of intestinal beneficial bacteria and compete with pathogen for nutrients (Leser et al., 2008). Some studies showed that *B. subtilis* can secret pathogen-suppressive substances that have bacteriostatic action on common pathogens such as *Staphylococcus aureus*, *E. coli* which were highly sensitive to sample concentrate and the bacteriostatic effect is equivalent to normal antibiotics (Anas et al., 2013; Leng et al., 2006; Quan, 2002; Ushakova et al., 2013). *B. subtilis* could suppress *E. coli* while promoting anaerobic intestinal probiotics growth or live in symbiosis with them (Dong et al., 2004; Dragana et al., 2014).

The results of this study showed that *B. subtilis* could decrease the amount of *Salmonella* and *E. coli* significantly in cecum and this antibacterial effect was because its “microbial oxygen consumption”. *B. subtilis* are aerobes that need large amount of oxygen in the growth and reproduction. It could promote the growth of anaerobic probiotics by consuming lots of free oxygen in animal's digestive tract, and maintain the balance of intestinal microenvironment to suppress the growth of aerobes like *E. coli*.

5. Conclusions

1) *B. subtilis* can improve the growth performance and decrease the F/G of AA broiler significantly, and there is an increasing tendency of the apparent metabolism of CP, crude fat, DM and OM.
2) *B. subtilis* has a good inhibitory effect on intestinal *E. coli* and *Salmonella*. Concentration of 250 mg/kg has the best inhibitory effect.
3) Valued by growth performance, the optimal supplemented of *B. subtilis* is 200 mg/kg.

Conflict of interest

The authors declare that they have no conflict of interest.

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