Livestock Science 155 (2013) 343–347

Contents lists available at SciVerse ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Short communication

Effects of *Bacillus subtilis* UBT-MO_2 on growth performance, relative immune organ weight, gas concentration in excreta, and intestinal microbial shedding in broiler chickens

Z.F. Zhang, J.H. Cho, I.H. Kim *

Department of Animal Resource and Science, Dankook University, Cheonan, Choongnam 330-714, South Korea

ARTICLE INFO

Article history:
Received 18 December 2012
Received in revised form
18 May 2013
Accepted 20 May 2013

Keywords:
Bacillus subtilis UBT-MO_2
Broilers
Excreta noxious gas
Immune organ weight

ABSTRACT

This study was conducted to investigate the effects of *Bacillus subtilis* UBT-MO_2 on growth performance, relative immune organ weight, gas concentration in excreta, and intestinal microbial shedding in broiler chickens. A total of 480 1-d-old mixed sex broilers were randomly allotted to a 2×2 factorial arrangement of treatments with 2 levels of enramycin (0 or 5 ppm) and *B. subtilis* (0 or 10^5 cfu/kg) for 35 d. Each treatment had 6 replicate pens with 20 broilers in each pen. Diets were fed in 2 phases: starter phase (from *d* 0 to 21) and grower phase (from *d* 22 to 35). Overall, broilers fed diets supplemented with *B. subtilis* had 4.4% greater ($P=0.01$) body weight gain than those fed non-probiotic diets. The feed conversion ratio in broilers fed diets containing enramycin was decreased by 2.8% and 4.2% during *d* 0–21 ($P=0.05$) and throughout the experimental period ($P=0.02$), respectively, than those fed diets without antibiotic. Broilers fed *B. subtilis* diets had 30.9% greater ($P=0.02$) relative weight of thymus than those fed diets without probiotic. Dietary supplementation with *B. subtilis* resulted in 26.9% and 37.9% lower ($P=0.03$) NH_3 and H_2S concentrations, respectively, in excreta compared with no supplementation. However, no differences were observed in intestinal bacterial concentrations among treatments. In conclusion, dietary supplementation with 10^5 cfu/kg of *B. subtilis* could improve the growth performance of broiler chickens, and reduced NH_3 and H_2S emissions.

1. Introduction

Several European countries have banned the use of dietary antibiotics, which led to the investigation of antibiotic growth promoter alternatives in animal industry (Hooge et al., 2004; Simon et al., 2003). *Bacillus subtilis* are not normal intestinal microorganisms but rather facultative anaerobes that are able to grow in the gut (Clements et al., 2001). Many previous studies have reported that 10^6–10^9 cfu *B. subtilis*/kg could have some beneficial effects on intestinal microbes, thus, improving the growth performance of broilers (Teo and Tan, 2007; Zhang et al., 2012). However, Lee et al. (2010) reported that feeding 1.5×10^5 cfu/g of *B. subtilis* did not affect the body weight gain in broilers (*d* 1–22). Moreover, Huang et al. (2004) reported that the optimal concentration for administering probiotics was strain-dependent and a greater inclusion rate did not always result in better performance.

Additionally, poultry manure is one of the major sources of N pollution (Song et al., 2012), in which NH_3 is a major aerial pollutant (Kristensen and Wathes, 2000) with adverse effects on the production of broilers (Miles et al., 2004). Dietary supplementation of *B. subtilis* has

* Corresponding author. Tel.: +82 41 550 3652; fax: +82 41 565 2949.
E-mail address: inhokim@dankook.ac.kr (I.H. Kim).

1871-1413
http://dx.doi.org/10.1016/j.livsci.2013.05.021

been found to reduce NH_3 emission in poultry by improving the activity of enzymes and the utilization of N (Santoso et al., 1999; Tanaka and Santoso, 2000). However, the efficacy of probiotic could be influenced by many factors, such as age of animals, strain of microorganism, and inclusion level (Chen et al., 2006). Therefore, this experiment was conducted to evaluate the effects of *B. subtilis* UBT-MO_2 (10^5 cfu/kg) and antibiotic (enramycin) on growth performance, relative immune organ weight, gas concentration in excreta, and intestinal microbial shedding in broiler chickens.

2. Material and methods

2.1. *B. subtilis UBT-MO_2*

The *B. subtilis* UBT-MO_2 final product was provided by a commercial company (DMJ Biotech Co. Ltd., Yeongi-gun, Chungcheongnam-do, South Korea), which is composed of spray-dried spore forming *B. subtilis* UBT-MO_2 endospores. The product was determined to contain at least 10^8 cfu *B, subtilis*/kg.

2.2. Experimental animals and husbandry

A total of 480 1-d-old mixed sex Arbor Acres broiler chickens (BW of 45.8 ± 0.5 g) were purchased from a commercial hatchery (Yang Ji Company, Cheonan, Choongnam, South Korea). All birds were randomly placed in stainless steel battery brooders (1.75×1.55 m^2) with concrete floors covered with clean rice bran. The temperature of the room was maintained at 33 ± 1 °C for the 1st 3 d, after which the temperature was gradually reduced by 3 °C a week until reaching 24 °C. The temperature of the room was maintained at 24 °C until the end of the experiment. Artificial light was provided 24 h/d by the use of fluorescent lights. The Animal Welfare Committee of Dankook University approved the animal care protocol used for this experiment.

2.3. Experimental design and diets

The broilers were randomly allotted to a 2×2 factorial arrangement of treatments with 2 levels each of enramycin (0 or 5 ppm) and *B. subtilis* (0 or 10^5 cfu/kg) for 35 d. There were 6 replications pens per treatment with 20 birds per pen. The diets were fed during the experiment in 2 phases, consisting of a starter phase from *d* 0 to 21 and a grower phase from *d* 22 to 35. All diets were formulated to meet or exceed the NRC (1994) recommendations for Arbor Acres broilers, and offered in mash form (Table 1).

2.4. Sampling and measurements

The broilers were weighed on *d* 0, 21, and 35, and the feed intake was recorded throughout the experimental period, after which the body weight gain, feed intake, and feed conversion ratio were calculated. At the end of experiment, 30 chicks per treatment (5 chicks per pen) were randomly chosen, weighed individually, and then sacrificed by cervical dislocation. The liver, spleen, thymus, and bursa of Fabricius were removed and weighed, and the organ weight was expressed as a percentage of body weight.

Table 1
Ingredient composition and nutrient content of diets[a].

Item	Starter	Grower
Ingredients, %		
Corn	43.90	49.13
Wheat	20.00	20.00
Soybean meal, 44% CP	21.75	18.33
Corn gluten meal	5.00	3.72
Meal and bone meal	2.83	2.40
Salt	0.16	0.15
Limestone	1.10	1.11
Tallow	4.44	4.42
Vitamin-mineral premix[b]	0.22	0.24
Antioxidant	0.05	0.05
DL-Met, 88%	0.20	0.18
Lys, 78.4%	0.30	0.24
Thr, 98.5%	0.05	0.03
Total	100.00	100.00
Calculated composition		
ME, Mcal/kg	3.19	3.21
Analyzed composition, %		
CP	21.12	19.79
Lys	1.19	1.02
Met+Cys	0.93	0.82
Ca	0.90	0.85
Available P	0.52	0.49

[a] Starter diet was provided during *d* 0–21, whereas grower diet was provided during *d* 22–35.

[b] Supplied per kilogram of diet: vitamin A, 12,500 IU; vitamin D_3 3250 IU; vitamin E (DL-a-tocophery acetate), 25 IU; vitamin K_3 2.25 mg; riboflavin, 8.0 mg; niacin, 50 mg; pantothenic acid, 15 mg; 50% cholinechloride, 1000 mg; cobalamin, 15 μg; cholecalciferol, 82.5 μg; biotin, 0.1 mg; folicacid, 0.75 mg; Fe (as $FeSO_4 \cdot 7H_2O$), 37.5 mg; Mn (as MnO_2), 37.5 mg; Zn (as $ZnSO_4 \cdot H_2O$), 37.5 mg; Cu (as $CuSO_4 \cdot 5H_2O$), 3.75 mg; Se (as $NaSeO_3 \cdot 5H_2O$), 0.23 mg; and I (as KI), 0.83 mg.

At the end of the experiment, NH_3 and H_2S contents in excreta were determined by the methods described by Yan et al. (2012). In brief, 300 g of fresh excreta samples were collected from each pen, and stored in 2.6 L plastic boxes in duplicates. The samples were fermented for 48 h at a temperature of 32 °C. After the fermentation period, 100 mL of the headspace air was sampled from approximately 2.0 cm above the excreta sample. Concentrations of NH_3 and H_2S were measured within the scope of 5.0–100.0 ppm (No. 3La, Detector Tube; Gastec Corp., Ayase, Kanagawa, Japan) and 2.0–20.0 ppm (4LK, Detector Tube; Gastec Corp.).

On *d* 35, 3 chicks from each replicate pen (18 broilers per treatment) were killed by cervical dislocation. One gram of small intestine and cecal contents were serially diluted from 10^{-3} to 10^{-7} with 9 mL of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ, US) and then mixed with stomacher (Seward Stomacher 400 circulator; Seward Limited, West Sussex, UK). Specifically, the samples were plated on MacConkey agar (Difco Laboraories, Detroit, MI, US) and lactobacilli medium III agar (Medium 638, DSMZ, Braunschweig, Germany) plates to enumerate *Escherichia coli* and *Lactobacillus*, respectively.

The MacConkey agar plates were then incubated for 24 h at 37 °C. The lactobacilli medium III agar plates were incubated for 48 h at 39 °C. The *E. coli* and *Lactobacillus* were counted immediately upon removal from the incubator.

2.5. Statistical analyses

In this experiment, all data were analyzed by ANOVA (SAS Inst. Inc., Cary, NC, US) using a 2×2 factorial arrangement of treatments with the pen being considered as the experimental unit. The model utilized included the effects of *B. subtilis* and enramycin, as well as the interactive effects. When a significant interaction was observed, the means of each treatment were compared using Fisher's protected least significant difference. Variability in the data is expressed as the standard error means (SEM) and a probability level of $P < 0.05$ was considered to be statistically significant.

3. Results

Broiler chickens fed diets with enramycin had 2.8% and 4.2% lower feed conversion ratio than chicks fed the non-enramycin diets during *d* 0–21 ($P=0.05$) and the overall experimental period ($P=0.02$), respectively (Table 2). Broilers fed the diets with *B. subtilis* had 4.4% greater ($P=0.01$) overall body weight gain. There is no difference in feed intake among dietary treatments throughout the experimental period.

The relative weights of bursa of Fabricius, liver, and spleen were not affected by dietary supplementation with *B. subtilis* or enramycin (Table 3). However, the relative weight of thymus was increased ($P=0.02$) by 30.9% in response to *B. subtilis* supplementation. The NH_3 and H_2S contents in excreta were decreased ($P=0.03$) by 26.9% and 37.9%, respectively, in broilers fed diets supplemented with *B. subtilis* compared with broilers fed non-*B. subtilis* diets. The concentrations of *E. coli* and *Lactobacillus* in small intestine and cecum were not influenced by enramycin or *B. subtilis*.

4. Discussion

No interactive effects were observed between *B. subtilis* and enramycin in the current study, and no comparisons of our results with others could be made because there seem to be no other studies conducted with *B. subtilis* in broilers. In our study, we found that the feed conversion ratio was improved by dietary supplementation of enramycin. In agreement with our results, Hassan et al. (2010) reported that enramycin improved feed conversion ratio of broilers, and similar results were found by other researchers (El-Husseiny et al., 2008; Pedroso et al., 2006).

As far as the growth promoting effects of *B. subtilis* is concerned, Hooge et al. (2004) found that the addition of 0.003% *B. subtilis* C-3102 spores increased the body weight of broiler chickens. Additionally, Zhang et al. (2012) found that body weight gain was improved in broilers fed with the diets supplemented with 10^8 cfu *B. subtilis*/kg. Beneficial effects of *B. subtilis* on broiler performance were also consistent with some studies conducted in broilers (Mountzouris et al., 2010; Zhou et al., 2010), whereas, a few studies did not report positive effects (Lee et al., 2010; Willis and Reid, 2008). From the current study, we could induce that 10^5 cfu *B. subtilis*/kg improved the body weight gain in broilers from *d* 0 to 35.

In the current study, no differences in the relative weight of livers, spleens, and bursa of Fabricius among dietary treatments were observed. Zhang et al. (2012) reported that the relative weights of liver and bursa of Fabricius were unaffected by dietary inclusion of 10^8 kg^{-1} *B. subtilis*. However, in that study, the relative weight of spleen was increased by 3.8% by adding *B. subtilis* in broiler diets, and similar results were reported by Awad et al. (2009). This inconsistency may be due to different concentrations and species of the direct-fed microorganisms. Interestingly, we found that the relative weight of thymus

Table 2
Effects of *Bacillus subtilis* probiotic on growth performance in broiler chickens[a,b].

Item	−*Bacillus subtilis*		+*Bacillus subtilis*		SEM[c]	*P*-value		
	−Ant[b]	+Ant	−Ant	+Ant		*Bacillus subtilis*	Ant	*Bacillus subtilis* × Ant
d 0–21								
BWG, g	896	918	930	946	11	0.27	0.11	0.78
FI, g	1276	1274	1303	1274	21	0.54	0.51	0.57
FCR	1.42	1.39	1.40	1.35	0.03	0.11	0.05	0.77
d 22–35								
BWG, g	802	821	848	864	21	0.06	0.43	0.99
FI, g	1649	1555	1634	1629	38	0.09	0.85	0.95
FCR	2.06	1.89	1.93	1.89	0.04	0.66	0.24	0.89
d 0–35								
BWG, g	1698	1739	1778	1810	12	0.01	0.24	0.87
FI, g	2925	2829	2937	2903	53	0.08	0.57	0.38
FCR	1.72	1.63	1.65	1.60	0.02	0.62	0.02	0.10

[a] BWG—body weight gain, FI—feed intake, and FCR—feed conversion ratio.
[b] *Bacillus subtilis* and antibiotic (Ant, enramycin) were supplemented at 10^5 cfu/kg and 5 ppm, respectively, or combined at 10^5 cfu *Bacillus subtilis*/kg and 5 ppm enramycin.
[c] Standard error of the means; 6 replicate pens of 20 chicks/pen per treatment.

Table 3
Effects of *Bacillus subtilis* probiotic on immune organ relative weight (% of body weight), gas concentration in excreta, and intestinal microbial shedding in broiler chickens[a].

Items	−*Bacillus subtilis*		+*Bacillus subtilis*		SEM[b]	*P*-value		
	−Ant	+Ant	−Ant	+Ant		*Bacillus subtilis*	Ant	*Bacillus subtilis* × Ant
Immune organ relative weight								
Bursa of Fabricius	0.25	0.30	0.29	0.34	0.03	0.31	0.18	0.93
Liver	2.55	2.25	2.41	2.48	0.12	0.74	0.35	0.16
Spleen	0.11	0.11	0.14	0.14	0.02	0.13	0.96	0.09
Thymus	0.26	0.29	0.34	0.38	0.03	0.02	0.07	0.85
Gas concentration in excreta, ppm								
NH_3	78.3	60.9	42.9	58.9	6.2	0.03	0.42	0.69
H_2S	14.0	6.3	5.6	7.0	2.0	0.03	0.31	0.78
Microbial shedding, log_{10} cfu/g								
Small intestine								
Lactobacillus	7.43	7.53	7.51	7.62	0.15	0.14	0.13	0.11
E. coli	6.45	6.28	6.37	6.22	0.21	0.28	0.19	0.14
Cecum								
Lactobacillus	7.95	8.06	8.04	8.16	0.15	0.14	0.13	0.11
E. coli	6.97	6.86	6.79	6.82	0.21	0.28	0.19	0.14

[a] *Bacillus subtilis* and antibiotic (Ant, enramycin) were supplemented at 10^5 cfu/kg and 5 ppm, respectively, or combined at 10^5 cfu *Bacillus subtilis*/kg and 5 ppm enramycin.
[b] Standard error of the means; 6 replicate pens of 5 chicks/pen per treatment for relative immune organ weight, 6 replicate pens of 20 chicks/pen per treatment for gas concentration in excreta, and 6 replicate pens of 3 chicks/pen per treatment for intestinal microbial shedding.

was increased in response to feeding broilers with the diets containing *B. subtilis*, which was consistent with Li et al. (2009) who reported that thymus relative weight was increased by 4×10^{10} cfu/kg of *B.* based probiotics in Hy-Line chicks from *d* 0 to 42. Measurement of the immune organ weight is a common method of evaluating the immune status in chickens (Heckert et al., 2002). The enhanced immune ability may also, in turn, explain the increased body weight gain in the current study. In other words, 10^5 cfu *B. subtilis* UBT-MO_2/kg had some beneficial immune effects on broiler chickens.

The most interesting finding of our study is that NH_3 and H_2S contents in excreta were decreased in broilers fed the diets supplemented with *B. subtilis* (10^5 cfu/kg). In agreement with our results, Chen et al. (2006) reported that fecal NH_3 content was decreased after finishing pigs were fed diets supplemented with *Bacillus*-based probiotics in a 42-d study. Ferket et al. (2002) suggested that the fecal noxious gas emission by animals is ultimately related to nutrient utilization and the intestinal microflora ecosystem. Yan et al. (2011) also demonstrated that the fecal noxious gas content is related to the nutrient digestibility because the increased digestibility may allow less substrate for the microbial fermentation in the large intestine, which consequently decrease the fecal noxious gas content (Yan et al., 2012). Moreover, Apata (2008) and Li et al. (2008) found that probiotics improved the nutrient digestibility in broiler chickens. In our study, the intestinal microbial shedding was unaffected by dietary treatments, but we found that the body weight gain was improved by inclusion of *B. subtilis*. Therefore, the beneficial effects of *B. subtilis* on body weight gain and excreta gas emission were probably due to the improved nutrient utilization.

Probiotic intake could result in the creation of gut microecology conditions that suppress harmful microorganisms (Rada and Rychly, 1995) and favor beneficial microorganisms, thereby, enhancing gut health. Li et al. (2009) reported that 4×10^{10} cfu *Bacillu scereus*/kg increased the concentrations of beneficial bacterial numbers (lactobacilli and bifidobacteria) and decreased the concentration of harmful bacterial numbers (*E. coli*). Similar results were reported by other reporters (Hassan and Ryu, 2012; Mountzouris et al., 2010; Teo and Tan, 2007). In contrast, other studies using multi-strain, single-species (Jin et al., 1998) and multi-strain, multi-species (Priyankarage et al., 2003) probiotics have shown no changes in the gut microflora profile of broilers, which is in agreement with the results of the present study. However, it is difficult to directly compare different studies conducted to evaluate the efficacy of probiotics because their efficacy depends on species viability and composition, application method, administration level, overall diet, farm hygiene, bird age, and environmental stress factors (Ghadban, 2002; Patterson and Burkholder, 2003).

5. Conclusions

In conclusion, the results of this study demonstrated that the administration of 10^5 cfu *B. subtilis* UBT-MO_2/kg to broilers could improve the growth performance, and increase the relative weight of thymus. In addition, treatments with *B. subtilis* UBT-MO_2 reduced the NH_3 and H_2S concentrations in excreate, which may reduce the release of odor emissions from broiler houses.

Conflict of interest

There was no conflict of interest.

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