JPSA
Japan Poultry Science Association

http://www.jstage.jst.go.jp/browse/jpsa
doi:10.2141/jpsa.0160069

Administration of *Bacillus Amyloliquefaciens* and *Saccharomyces Cerevisiae* as Direct-Fed Microbials Improves Intestinal Microflora and Morphology in Broiler Chickens

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This study was conducted to investigate the effects of *Bacillus amyloliquefaciens* (*BA*) and *Saccharomyces cerevisiae* (*SC*) as directed-fed microbials on performance, intestinal microflora, and intestinal morphology in broiler chickens. A total of four hundred one-day-old broiler chickens were randomly divided into 16 pens of 25 chickens each, and every treatment had 4 replicated pens with two pens of males and females respectively. A formulated corn-soybean meal based control diets and experimental diets, including 0.1% *BA* (1×10^7 colony-forming units (CFU)/kg), the mixture of 0.05% *BA* (5×10^6 CFU/kg) and 0.05% *SC* (5×10^6 CFU/kg), and 10 ppm antibiotic (avilamycin), were fed for 5 weeks. The results showed no significant difference in the growth performance among all treatments. Supplementation of the mixture of *BA* and *SC* increased acetate and propionate and decreased the *E. coli* in ceca compared to control and antibiotic treatment. The treatments with antibiotic, *BA*, and the mixture of *BA* and *SC* compared to control treatment increased villus height / crypt depth ratio and decreased ammonia in excreta. In addition, supplementation of *BA* and the mixture of *BA* and *SC* compared to antibiotic treatment increased serum high-density lipoprotein, and decreased serum glutamic-oxaloacetic transaminase, respectively. In conclusion, supplementation of the mixture of *BA* and *SC* was better than added *BA* only, and the mixed probiotics product could potentially alter the use of avilamycin in broiler diets.

Key words: antibiotic, *Bacillus amyloliquefaciens*, broilers, *Saccharomyces cerevisiae*

J. Poult. Sci., 54: 134−141, 2017

Introduction

Antibiotics are used to promote animal growth and improve immunity to stressors, including heat and the intensive farming system. However, the misuse and overuse of antibiotics may cause antibiotic resistance in chickens. This could pose a threat to public health if dangerous infections of drug-resistant bacteria were spread via the food chain (Smith *et al*., 2003). As a result, the use of antibiotics as growth promoters was banned from the EU on January 1, 2006 (Burch, 2006).

Direct-fed microbials (DFM), including *Lactobacillus*, *Bacillus* and *Saccharomyces cerevisiae* (*SC*), have been studied as potential alternatives to feed additives (Salim *et al*., 2013). *Bacillus* is gram-positive bacteria that can produce endospores to protect itself from a stressful environment. Dietary supplementation with 2% *Bacillus amyloliquefaciens* (*BA*) was reported to decrease NH_3 emissions in broiler excreta (Ahmed *et al*., 2014). In addition, *BA* could also stabilize the cecal microbial population by increasing *Lactobacillus* counts and decreasing *E. coli* (An *et al*., 2008; Ahmed *et al*., 2014). While Mountzouris *et al*. (2015) reported that *SC* diet supplements had no effect on growth performance. Haldar *et al*. (2011) showed that *SC* supplements could increase ileal villus height and decrease *E. coli* counts in the digestive systems of broilers.

BA and *SC* demonstrated outstanding production of extracellular enzymes including protease, amylase and lipase (Shirazi *et al*., 1998). These enzymes may improve the

Received: April 19, 2016, Accepted: September 13, 2016
Released Online Advance Publication: November 25, 2016
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nutritional digestibility of protein and starch in broilers (Diaz, 2007). The combination of *Lactobacillus*, *Bacillus* and *SC* as DFM resulted in benefits to growth performance, immune response and improved intestinal morphology in early stage broilers (Salim *et al*., 2013).

It is hypothesized, therefore, that DFM supplementation has potential benefits for the poultry industry. Few reports, however, have studied the mixture of *BA* and *SC* as a feed additive to the broiler diet. Therefore, this study was conducted to assess the effects of mixed *BA* and *SC* supplementation on growth performance, intestinal microbiota and intestinal morphology in broiler chickens, compared to the use of antibiotics (avilamycin).

Materials and Methods

Growth Curve, Acid and Basic Tolerance, and Enzyme Activity of BA and SC

The *BA* was inoculated in Lysogeny broth (LB) and the *SC* was inoculated in Yeast-Mald (YM) at 37℃ for 24 hours. After inoculation, 1 mL of these broth culture was added to 9 mL LB and YM broth and colony-forming units (CFU) of *BA* and *SC* were measured in 0, 6, 12, 18 and 24 hours. The assay of acid and basic solution tolerance was modified from the method of Hairul Islam *et al*. (2011). The pH of acid and basic solution was adjusted to 2.0, 3.0 and 12.0 with HCl and NaOH. 1 mL of *BA* or *SC* broth culture was added to stimulated solution and visible colony counts were calculated after 3 hours. The enzyme activities of *BA* were assayed by radio enzyme diffusion which according to method of Walsh *et al*. (2005) and APIZYM system (bioMerieux, Marcy-l'Étoile, France). The APIZYM test was performed according to the instructions. The reaction was terminated by addition of one drop each of A and B APIZYM reagents and the following results were examined in the end. The assay of mannanase and β-glucosidase were according to the methods of Lai *et al*. (2015) and Hernandez *et al*. (2003).

Experimental Birds and Housing

This study was conducted at National Chung Hsing University, Taiwan; the experimental protocol for animal use was approved by the Animal Care and Use Committee. A total of four hundred one-day-old broiler chickens (*Ross* 308) were evenly divided by gender and randomly allocated to one of 4 treatments, each of which had 4 replicate pens per treatment and 25 birds per pen (total of 100 birds per treatment). Initially, the average BW of the birds was similar among the different pens (average 46.0 to 46.5 g/bird approximately). All of the birds were placed in a temperature controlled house. The temperature was maintained at 33±1℃ until the birds reached 7 d of age, before gradually being decreased to 27±1℃ until the birds reached 21 d of age; after this point, the broilers were maintained at 27±1℃.

Feeding Schedule and Dietary Composition

The experiment lasted for 35 d and there were 2 phases: starter (1 to 21 d) and finisher (22 to 35 d). Diets (in mash form) and water were provided *ad libitum*. The birds in the control group were fed diets based on corn-soybean meal, and the other 3 groups were provided experimental diets. These were based on the basal diet but also contained an additional 10 ppm antibiotic (avilamycin), 0.1% *BA* to reach 1×10^7 CFU/g (Determined CFU, starter diet: 4×10^7 CFU/g, finisher diet: 4×10^7 CFU/g) as DFM-1 or a mixture of 0.05% *BA* to reach 5×10^6 CFU/g (Determined CFU, starter diet: 7×10^6 CFU/g, finisher diet: 8×10^6 CFU/g) and 0.05% *SC* to reach 5×10^6 CFU/g (Determined CFU, starter diet: 8×10^6 CFU/g, finisher diet: 8×10^6 CFU/g) as DFM-2 (Table 1). Starter and grower diets were offered to the birds from 1-21 d and from 22−35 d of age, respectively. During the entire experimental period (35 d), the diets were formulated to meet the requirements suggested by the *Ross* Broiler Management Manual (2009).

Table 1. **Ingredients and chemical composition of the experimental diets for broilers**

Ingredients	Starter diet (1−21 days)	Finisher diet (22−35 days)
	g/kg	
Corn, yellow	472.6	518.0
Soybean meal	345.2	295.9
Full fat soybean meal	100.0	100.0
Soybean oil	35.1	45.0
Monocalcium phosphate	18.6	16.6
Calcium carbonate	16.1	13.4
L-Lysine-HCl	3.8	3.2
DL-Methionine	2.0	1.3
NaCl	3.8	3.8
Choline-Cl	0.8	0.8
Vitamin premix[1]	1.0	1.0
Mineral premix[2]	1.0	1.0
Total	1000.0	1000.0
Calculated nutrient value		
ME, kcal/ kg	3050.10	3175.26
Crude protein, %	23.00	21.01
Calcium, %	1.05	0.90
Total Phosphorus, %	0.76	0.70
Available Phosphorus, %	0.50	0.45
Lysine, %	1.43	1.25
Methionine + Cystine, %	1.07	0.96

[1] Supplied per kg of diet: Vit A 15000 IU; Vit. D3 3000 IU; Vit. E 30 mg; Vit. K3 4 mg; Riboflavin 8 mg; Pyridoxine 5 mg; Vit. B12 25 μg; Ca-pantothenate 19 mg; Niacin 50 mg; Folic acid 1.5 mg; Biotin 60 μg.

[2] Supplied per kg of diet: Co ($CoCO_3$) 0.255 mg; Cu ($CuSO_4\cdot5H_2O$) 10.8 mg; Fe ($FeSO_4\cdot H_2O$) 90 mg; Zn (ZnO) 68.4 mg; Mn ($MnSO_4\cdot H_2O$) 90 mg; Se (Na_2SeO_3) 0.18 mg.

Performance, Serum and Intestinal Content Collection

Body weight (BW) of chickens per pen and feed intake (FI) were recorded at 1, 21 and 35 d of age. Body weight gain (BWG) and feed conversion ratio (FCR) were recorded and calculated. On day 35, 16 chickens per treatment (four birds from each pen) close to average weight were selected for sampling. Fifty grams of excreta per treatment, excreted over 2 h, were collected randomly in 3 plastic plates from the cages of selected birds to determine ammonia content. Blood from the brachial vein was collected (5 mL) via car-

diac puncture using a vacutainer tube. The blood was centrifuged at 3000×g for 30 min to obtain the serum. It was then stored at −20℃ until it was analyzed for cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL), serum glutamic-oxaloacetic transaminase (SGOT) and serum glutamic-pyruvic transaminase (SGPT) levels. After the blood was collected, the birds were euthanized by exsanguination and the gastrointestinal tract was removed from the carcass. About 15-cm segments of ceca and ileum were dissected and approximately 10 g content of each bird was collected in sterile plastic plates for subsequent study. The ileal and cecal contents of four chickens in same replicates were well mixed in same plastic plate for subsequent study.

Microbial Populations in Ileal and Cecal Content

To determine microbial populations, strains of *E. coli, C. perfringens* and lactic acid bacteria were cultured with chromogenic medium agar (CHROMagar™ ECC), TSC agar (BD Difco TM) with D-cyclosrine and MRS medium (de Man Rogosa and Sharpe agar, Difco 288130), respectively. After anaerobic incubation at 37℃ for 48 h, the microfloral counts were calculated. Bacterial populations were expressed as $\log_{10}$ CFU per gram of intestinal content.

Volatile Fatty Acid (VFA) Analysis of Cecal Contents

To determine VFA levels, including acetate, propionate, butyrate, isobutyrate, isovalate and n-valerate, 1 g of cecal content was mixed with 4 mL 25% metaphosphoric acid. Samples were centrifuged at 10,000×g for 20 min and the supernatant was transferred into a 2 mL tube stored at −20℃. The concentration of VFA was determined by gas chromatography with a flame ionization detector, fuse silica capillary column and nitrogen was used as the carrier gas. Volatile acid standard mixes (SUPELCO) were used as standard solutions.

Morphometric Analysis of the Small Intestine

At the end of the experiment (day 35), one bird per replicate cage from each treatment group (4 birds/treatment in total) was randomly selected and sacrificed. During necropsy, the gastrointestinal tract was removed and the small intestine was divided into two parts: the jejunum (from the pancreatic loop to Meckel's diverticulum) and the ileum (from Meckel's diverticulum to the ileo-caeco-colic junction). 3 cm long segments from the center of each tissue were fixed in 10% formalin for later morphometrical assays. The formalin-fixed gut wall was washed in PBS and embedded in paraffin wax. 3 μm sectioned tissue was stained using hematoxylin and eosin methods. The samples were analyzed by light microscopy and software (Motic image plus 2.0, Shimadu, Kyoto, Japan) was used for measuring the villus height and crypt depth in fifteen favorably-oriented and representative samples per treatment. The ratio of villus height to crypt depth was also calculated.

Ammonia Analysis of Ileal and Excreta Content

The assay of ammonia analysis was modified from the methods of Weatherburn (1967). 1 g of ileal and excreta sample was mixed with 4 mL 25% metaphosphoric acid and centrifuged at 15000×g for 10 min. 1.5 mL of supernatant was transferred into a 2 mL Eppendolf tube and stored at −20℃. 25 μL of supernatant was mixed with 1 mL reagent A (5 g of phenol with 250 mg of sodium nitroprusside per liter of solution) and 1 mL reagent B (25 g of sodium hydroxide with 16.8 mL sodium hypochlorite per liter of solution). After waiting 15 min for color development at 37℃, absorbance was measured at 630 nm. NH_4Cl was used as the standard solution.

Serum Characteristics Determination

Serum biochemical values, including serum glutamic-oxalocetic transaminase, serum glutamic-pyruvic transaminase, cholesterol, high-density lipoprotein and low-density lipoprotein, were measured using an automatic biochemical analyzer (7150 auto-analyzer, Hitachi, Tokyo, Japan).

Statistical Analysis

Data was subjected to ANOVAs as a completely randomized design using GLM function of the SAS software (SAS, 2004). Determination of significant statistical differences among the mean values of the four treatment groups used Tukey's honestly significant difference test with a significance level of $P<0.05$.

Results

Growth Curve of BA and SC, Acid and Basic Solution Tolerance, and Enzyme Activity

The growth curve, acid and basic solution on the viability of *BA* and *SC* are data not shown (Figure S1 and Table S1). Decreasing number of *BA* and *SC* after the acid and basic solution tolerance test are less than 2 log CFU/mL. The results of enzyme activities of *BA* were indicated that *BA* had enzyme activities including protease, xylanase, cellulase, amylase, cysteine aminopeptidase, acid phosphatase, phosphohydrolase, and α-glucosidase (data not shown, in Table S2 and Figure S2). The β-glucosidase activity of *SC* is 1.59 mU, and the Mannanase activity of *SC* is 13.28 mU.

Growth Performance

The effects of dietary antibiotic, DFM-1 and DFM-2 supplementation on the growth performance of broilers at difference phases are shown in Table 2. From 1−21 d, 22−35 d, and 1−35 d of age, there were no significant differences in BW, BWG, FI or FCR among all treatment groups ($P>0.05$).

Microbial Population in Ileum and Cecum

The effects of dietary antibiotic, DFM-1 and DFM-2 supplementation on intestinal microflora are shown in Table 3. Treatment had no significant effect on *Lactobacillus* or *Clostridium perfringens* ($P>0.05$). Supplementation with DFM-2 and antibiotics resulted in significantly lower amounts of *E. coli* in ileal content compared to the control group ($P<0.01$). DFM-2 also significantly reduced *E. coli* concentration in cecal content when compared to the antibiotic treatment and control groups ($P<0.05$).

Volatile Fatty Acids in Cecum

The effects of dietary antibiotic, DFM-1 and DFM-2 supplementation on cecal VFA are shown in Table 4. There were no significant differences in isobutyric acid, isovaleric acid or n-valeric acid levels among all treatment groups. However, treatment with DFM-1 and DFM-2 significantly

Table 2. **Effect of *Bacillus amyloliquefaceins* and *Saccharomyces cerevisiae* supplementation on growth performance of broilers[1]**

Item	Experimental diets				SEM	*P* value
	Control	Antibiotic	DFM-1	DFM-2		
1−21 d						
Body weight (g)	811	801	831	823	29.02	0.900
Feed consumption (g)	923	921	999	968	27.10	0.172
Weight gain (g)	764	755	785	777	28.81	0.887
FCR	1.21	1.22	1.25	1.28	0.03	0.377
21−35 d						
Body weight (g)	2139	2168	2179	2162	110.28	0.995
Feed consumption (g)	2064	2061	2061	2067	94.89	1.000
Weight gain (g)	1327	1367	1348	1338	88.17	0.990
FCR	1.57	1.51	1.54	1.55	0.03	0.703
1−35 d						
Feed consumption (g)	2986	2981	3061	3035	105.57	0.940
Weight gain (g)	2092	2121	2132	2115	110.00	0.995
FCR	1.44	1.41	1.44	1.44	0.03	0.904

[1] Each value represents the mean of 4 replicates with 4 birds in each replicate.
DFM-1=*B. amyloliquefaceins*; DFM-2=the mixture of *B. amyloliquefaceins* and *S. cerevisiae*.

Table 3. **Effect of *Bacillus amyloliquefaceins* and *Saccharomyces cerevisiae* supplementation on intestinal microflora concentration of broilers (35 d)[1]**

Item	Experimental diets				SEM	*P* value
	Control	Antibiotic	DFM-1	DFM-2		
Lactic acid bacteria	---------- Log_{10} CFU/g ----------					
Ileum	8.64	8.81	8.84	8.80	0.09	0.573
Ceca	11.27	11.37	11.28	11.36	0.13	0.944
E. coli	---------- Log_{10} CFU/g ----------					
Ileum	7.33^{a}	6.07^{c}	6.92^{ab}	6.49^{bc}	0.15	0.001
Ceca	8.23^{a}	8.14^{ab}	7.45^{bc}	7.28^{c}	0.19	0.027
Clostridium perfringens	---------- Log_{10} CFU/g ----------					
Ileum	7.40	7.41	7.36	7.19	0.21	0.905
Ceca	8.49	8.48	8.47	8.41	0.25	0.996

[1] Each value represents the mean of 4 replicates with 4 birds in each replicate.
[a, b] Means with in the same row with different letters are significantly different ($P<0.05$).
DFM-1=*B. amyloliquefaceins*; DFM-2=the mixture of *B. amyloliquefaceins* and *S. cerevisiae*.

increased in total VFA and acetic acid compared to the antibiotic and control groups ($P<0.05$), while treatment with DFM-2 resulted in significantly greater propionic acid concentrations ($P<0.01$). In addition, supplementation with DFM-1 significantly increased butyric acid levels ($P<0.05$).

Intestinal Morphology

The effects of dietary antibiotic, DFM-1 and DFM-2 supplementation on the intestinal morphology of broilers are shown in Table 5 and Fig. 1. There were no significant differences in villus height, crypt depth or villus height / crypt depth ratio in the jejunum among all treatment groups ($P>0.05$). However, supplementation with antibiotics, DFM-1 and DFM-2 resulted in higher ileal villus height and villus height / crypt depth ratio compared to the control group ($P<0.01$).

Ammonia in Ileum and Excreta

The effects of dietary antibiotic, DFM-1 and DFM-2 supplementation on excreta and ileal ammonia concentrations are shown in Table 6. None of the treatments had a significant effect on ileal ammonia levels ($P>0.05$), however supplementation with antibiotics, DFM-1 or DFM-2 significantly decreased NH_3 in excreta ($P<0.01$).

Serum Characteristics

The effects of dietary antibiotic, DFM-1 and DFM-2 supplementation on serum characteristics are shown in Table 7. Supplementation with DFM-1 and DFM-2 significantly reduced serum SGOT and increased HDL compared to the antibiotic treatment group ($P<0.05$). However, none of the treatments had a significant effect on SGPT, serum cholesterol or LDL ($P>0.05$).

Table 4. **Effect of *Bacillus amyloliquefaceins* and *Saccharomyces cerevisiae* supplementation on cecal VFA concentration of broilers (35 d)[1]**

Item	Experimental diets				SEM	*P* value
	Control	Antibiotic	DFM-1	DFM-2		
	---------- μmole/g ----------					
Acetic acid	11.8[b]	11.84[b]	16.02[a]	18.73[a]	0.93	0.011
Propionic acid	3.98[b]	3.94[b]	4.66[b]	8.84[a]	0.66	0.002
Isobutyric acid	0.55	0.52	0.76	0.90	0.09	0.068
Butyric acid	5.46[b]	6.33[b]	8.83[a]	6.06[b]	0.66	0.041
Isovaleric acid	0.90	0.88	1.21	1.34	0.12	0.079
n-Valeric acid	0.93	0.94	1.17	1.32	0.13	0.195
Total VFA	23.62[b]	24.44[b]	32.64[a]	37.70[a]	1.82	0.002

[1] Each value represents the mean of 4 replicates with 4 birds in each replicate.
[a, b] Means with in the same row with different letters are significantly different ($P<0.05$).
VFA=Volatile fatty acid; DFM-1=*B. amyloliquefaceins*; DFM-2=the mixture of *B. amyloliquefaceins* and *S. cerevisiae*.

Table 5. **Effect of *Bacillus amyloliquefaceins* and *Saccharomyces cerevisiae* supplementation on intestinal morphology of broilers (35 d)[1]**

Item	Experimental diets				SEM	*P* value
	Control	Antibiotic	DFM-1	DFM-2		
Jejunum						
Villus height, μm	1407	1414	1425	1425	28.40	0.974
Crypt depth, μm	199	199	188	198	6.21	0.550
Villus height/Crypt depth	7.17	7.18	7.60	7.26	0.22	0.451
Ileum						
Villus height, μm	782[c]	993[a]	992[a]	929[b]	14.94	<0.001
Crypt depth, μm	166	177	171	174	5.13	0.571
Villus height/Crypt depth	4.81[b]	5.51[a]	5.86[a]	5.50[a]	0.19	0.008

[1] Each value represents the mean of 16 replicates (1 bird per replicate×4 replicates per treatment×4 measurements per section).
[a, b, c] Means with in the same row with different letters are significantly different ($P<0.05$).
DFM-1=*B. amyloliquefaceins*; DFM-2=the mixture of *B. amyloliquefaceins* and *S. cerevisiae*.

Discussion

The use of mixed probiotics as a feed additive is currently popular in the livestock industry. The *Bacillus* specie used in this study, *BA*, has demonstrated tolerable *in vitro* ability in both gastric juice (pH 3.0) and 0.3% bile salts; enzyme production, including protease (107.5 U/g), cellulase (0.531 U/g) and xylanase (0.075 U/g); and adhesion to the epithelial cells of the chicken crop (Teng *et al*., 2015). *SC* is well known for its production of extracellular lipase that may improve fat digestion in animals (Shirazi *et al*., 1998). Moreover, the cell walls of *SC,* making up 15 to 25% of its dry weight, is mainly composed of glucan and mannoproteins, including mannan-oligosaccharide and β-glucan (Yalçinkaya *et al*., 2008). It was reported to improve immune response and modulate intestinal microflora in animals (Corrigan *et al*., 2011; Vervicka and Oliveira, 2013). As mentioned above, a mixture of *BA* and *SC* was used to assess the benefits of DFM supplementation in broiler diets.

In this study, supplementation with *BA* and *SC* had no impact on growth performance across all treatment groups. This is in keeping with the findings of Chen *et al*. (2009), who reported that supplementation with *Bacillus subtilis* alone (10^6 CFU/g) or a mixture of *Bacillus subtilis* (10^6 CFU/g) and *SC* (10^8 CFU/g) had no effect on growth performance in broilers. Moreover, Ahmed *et al*. (2014) also showed that adding 0.1% and 0.5% *BA* (*Bacillus amyloliquefaciens* KB3) or supplementation with 10^9 CFU/g *BA* did not affect growth performance in broilers. However, *Bacillus* could play an important role in modulating the cecal microbial population. Lei *et al*. (2014) pointed out that adding *BA* to broiler diets could decrease *E. coli* (from 6.99 to 6.11 log CFU/g) and increase *Lactobacillus* (from 7.88 to 8.47 log CFU/g) in the cecum. In this study, DFM-1 and DFM-2 decreased the cecal counts of *E. coli* compared to the antibiotic and control groups. Therefore, dietary supplementation with antibiotics or a mixture of *BA* and *SC* could decrease ileal *E. coli*. Similarly, Kim *et al*. (2011) reported that diets with avilamycin decreased the population of *E.coli* in intestine of broilers and Salim *et al*. (2013) found that supplementation with a probiotic mixture that included *Lactobacillus reuteri, Bacillus subtilis* and *SC*, decreased *E. coli* amounts in

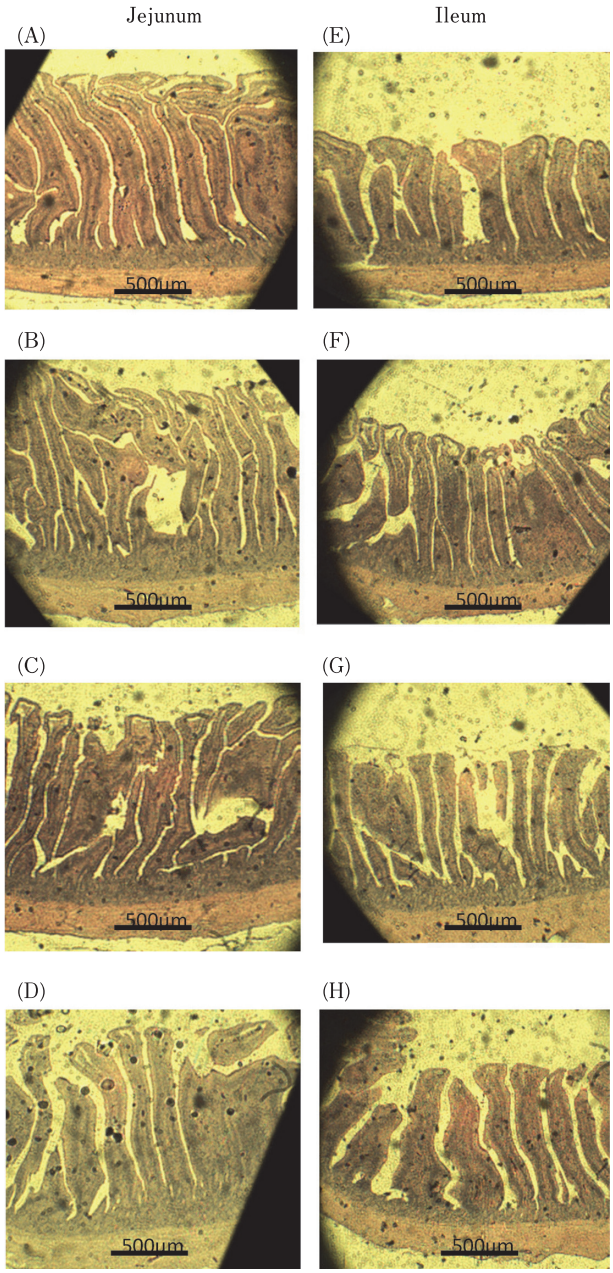

Fig. 1. **Photomicrography of jejunum and ileum of 35 d broiler in treatments with control, antibiotic, DFM-1 (*Bacillus amyloliquefaciens*), and DFM-2 (the mixture of *Bacillus amyloliquefaciens* and *Saccharomyces cerevisiae*).** (A) Control, jejunum (B) Antibiotic, jejunum (C) DFM-1, jejunum (D) DFM-2, jejunum (E) Control, ileum (F) Antibiotic, ilium (G) DFM-1, ileum (H) DFM-2, ileum. Hematoxylin and eosin stain (40×) (method according to Huang *et al*., 2012).

broilers' ceca. Wiyada (2012) reported that the antimicrobial products produced by *Bacillus*, such as bacilysin, macrolactin and bacillaene from nonribosomal peptide synthetases and polyketide synthetases, could inhibit intestinal pathogenic bacteria. Furthermore, both *Bacillus* and *SC* consume oxygen rapidly, encouraging anaerobic probiotics in the intestines to produce lactate in order to impair the growth of opportunistic pathogens (Wu *et al*., 2011; Song *et al*., 2013). VFA production (acetate, propionate, and butyrate) is also responsible for the reduced numbers of *Enterobacteriaceae* and *Salmonella* (Van der Wielen *et al*., 2000, 2001). In the current study, DFM-1 and DFM-2 groups had higher acetate and total VFA than the control or antibiotic groups; moreover, the DFM-2 group had greater propionate levels than other treatments which results in reducing number of cecal *E. coli*. Similarly, broiler fed with a mixture of 11 probiotic *Lactobacillus* stains had decreased population of *E. coli* in cecal digesta through production of more acetic acid, propionic acid and total VFA (Mookiah *et al*., 2014).

The inhibition of pathogenic bacteria in *BA* supplemented diets could increase villus height and villus height to crypt depth ratio in broilers (Lei *et al*., 2014). Increasing villus height means greater nutrient absorption because of the increase in intestinal surface area (Xu *et al*., 2002). The crypt is known as the villus factory; deeper crypts indicate faster cell turnover to meet the demand for new tissue in response to inflammation or sloughing due to bacterial toxins or pathogen (Yason *et al*., 1987). Additional tissue turnover leads to nutrient waste in order to maintain intestinal morphology and lowers feed efficiency (Xu *et al*., 2002). In the current study, treatment with antibiotics, DFM-1 and DFM-2 resulted in increased villus height and villus height / crypt ratio in the ileum. Diaz (2007) showed that supplementation with 0.1% *BA* could improve intestinal morphology, resulting in higher nutritional digestibility of protein (8.1%), fat (7.3%) and starch (3.8%) in broilers.

Clearly, better digestibility will decrease the excretion of non-utilized nutrients, resulting in less ammonia in the excreta of broilers. Microorganisms in manure degrade nitrogenous compounds, with ammonia as the major end product (Ferket *et al*., 2002). High concentration of ammonia in poultry houses suppresses growth performance, immune response and increases disease susceptibility (Wei *et al*., 2015). In this study, supplementation with *BA* or a mixture of *BA* and *SC* significantly decreased fecal ammonia compared to the control and antibiotic groups. Ahmed *et al*. (2014) also reported that adding various concentration of *BA* (0.1%, 0.5%, 1% and 2%) to broiler diets decreased fecal ammonia emissions, which correlates with our own findings.

Serum enzymes, including SGOT and SGPT, are mainly monitored to evaluate liver damage. Antibiotic supplementation increased hepatic work load due to the metabolic demand of incoming antibiotics and resultant increase in SGOT (Murwani and Bayuardhi, 2007), while the mix of probiotics (*Lactobacillus plantarum*, *L. bulgaris*, *L. acidophilus*, *L. rhamnosus*, *Bifadobacterum binfadum*, *Streptococus thermophilus*, *Enterococus faecium*, *Aspergillus oryzae* and *Candida pintolopesi*) was studied to decrease the SGOT of broilers (Khan *et al*., 2013). In the current study, supplementation with *BA* or a mixture of *BA* and *SC* decreased SGOT when compared to the antibiotic group, either.

Table 6. **Effect of *Bacillus amyloliquefaceins* and *Saccharomyces cerevisiae* supplementation on ileal and fecal ammonia concentration of broilers (35 d)[1]**

Item	Experimental diets				SEM	*P* value
	Control	Antibiotic	DFM-1	DFM-2		
	---------- μmole/g ----------					
Ileum[1]	59.70	54.69	47.82	52.85	3.28	0.354
Excreta[2]	56.61[a]	46.16[b]	46.41[b]	42.34[b]	1.72	0.008

[1] Each value represents the mean of 4 replicates with 4 birds in each replicate.
[2] Each value represents the mean of 3 replicates.
[a, b] Means with in the same row with different letters are significantly different ($P<0.05$).
DFM-1=*B. amyloliquefaceins*; DFM-2=the mixture of *B. amyloliquefaceins* and *S. cerevisiae*.

Table 7. **Effect of *Bacillus amyloliquefaceins* and *Saccharomyces cerevisiae* supplementation on serum characteristics of broilers (35 d)[1]**

Item	Experimental diets				SEM	*P* value
	Control	Antibiotic	DFM-1	DFM-2		
	---------- U/L ----------					
SGOT	177.7[ab]	210.7[a]	140.3[b]	154.0[b]	9.69	0.010
SGPT	4.5	5.3	4.3	4.4	0.42	0.393
	---------- mg/dL ----------					
CHO	70.7	64.3	61.0	69.3	2.84	0.213
HDL	47.5[ab]	44.0[b]	48.0[a]	49.7[a]	0.87	0.028
LDL	8.7	7.7	7.7	8.0	0.90	0.890

[1] Each value represents the mean of 4 replicates.
[a, b] Means with in the same row with different letters are significantly different ($P<0.05$).
DFM-1=*B. amyloliquefaceins*; DFM-2=the mixture of *B. amyloliquefaceins* and *S. cerevisiae*;
SGOT=Serum glutamic-oxaloacetic transaminase; SGPT=Serum glutamic-pyruvic transaminase; CHO=Cholesterol; HDL=High-density lipoprotein; LDL=Low-density lipoprotein.

In addition, treatments with DFM-1 and DFM-2 not only decreased SGOT, but also increased serum HDL compared to the antibiotic treatment. The DFM-2 treatment group had higher VFA levels in the cecum that it may suggest that the suppressed hepatic cholesterol synthesis and increased HDL to redistribute cholesterol from the plasma to the liver (Chen and Anderson, 1984).

In conclusion, supplementation with DFM-2 increased acetate and propionate in the cecum, reduced *E. coli* counts in the ileum and cecum, increased ileal villus height and villus height / crypt ratio, increased serum HDL and decreased SGOT. Moreover, supplementation with a mixture of *BA* and *SC* is better than *BA* alone, suggesting that a mixed probiotics product could be a potential alternative to antibiotic growth promoters (e.g. avilamycin) in broiler diets.

Acknowledgments

The authors thank the Ministry of Science and Technology (MOST 104-2815-C-005-038-B and MOST 103-2628-B-005-001-MY3) for financially supporting this study.

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