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Effects of *Bacillus subtilis*, Kefir and β-Glucan Supplementation on Growth Performance, Blood Characteristics, Meat Quality and Intestine Microbiota in Broilers

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ABSTRACT A total of 528 broilers (47±0.1 g; 1 day of age) were used in a 35-day feeding trial to evaluate probiotics, prebiotics and their interactive effects on growth performance, blood characteristics, relative organ weight and meat quality. Broilers were randomly distributed into 1 of 6 treatments on the basis of body weight (BW) (4 replicate pens per treatment, 22 broilers per pen). The dietary treatments were CON, basal diet; B, CON + 0.1 g kg-1 *Bacillus subtilis*; K, CON + 1 g kg-1 Kefir; G, CON + 1 g kg-1 β-glucan; GB, G + 1 g kg-1 *Bacillus subtilis*; and GK, G + 1 g kg-1 Kefir. The overall effects indicated that broilers fed the K, G and GK diets had greater body weight gain (BWG) than those fed the CON diet (P<0.05). The number of white blood cells increased (P<0.05) in the GB group compared with the CON, B and G treatments; however, the lymphocyte percentage in the B group was higher than in the G group. The weight of bursa fabricii was lower in the B and G groups compared to the K group (P<0.05), whereas a higher spleen weight was observed in chickens that were fed the GB and GK diets compared to the B group (P<0.05). The treatments did not affect the meat quality parameters, except for meat redness, which improved with all of the supplementation groups (P<0.05). The population of *Lactobacillus* spp. in gizzard was significantly higher in the K treatment compared with CON, B, G and GB. In conclusion, supplementation with kefir and β-glucans improved growth performance.

(Key words: broiler, *Bacillus subtilis*, β-glucan, kefir, growth performance, meat quality)

INTRODUCTION

Previously, antibiotics were usually included in diets as growth promoters. However, due to the regular use, antibiotic resistance emerged as another problem and thus a ban was enforced on the use of in-feed antibiotics for livestock growth promotion (Simon, 2005). In search for the better alternatives, probiotics and prebiotics have gained a great attention to replace in-feed antibiotics. This study focuses first on the definitions of probiotics, prebiotics, and the possible synbiotics effects of β-glucans as a prebiotic with either *Lactobacillus*-based or *Bacillus subtilis*-based probiotics. β-Glucans are indigestible polysaccharides that can be extracted from the cell walls of yeasts, fungi, and various grains as an immune modulator to improve the immune system and act as a mechanism against pathogenic activities (Cheng et al., 2004). Several studies have shown that dietary supplementation of β-glucans improved the growth performance (An et al., 2008) or immune system's capability (Cheng et al., 2004; Wang et al., 2008; Zhang et al., 2008). The relative weight of the spleen as an organ, which is related to immune system, was increased by 3.9% by β-glucan supplementation (Zhang et al., 2012). Previous work in the author's laboratory showed an interaction between β-glucans and kefir (Cho et al., 2013). The interaction between prebiotics and probiotics has been already confirmed several times (Cho et al., 2013; Zhang et al., 2012), however, it seems crucial to know which kinds of probiotics (kefir as a *Lactobacillus*-based probiotic or *B. subtilis*) can make a better combination with β-glucans. Different species of *B. subtilis* are considered to be probiotic microorganisms that can improve intestinal health. Dietary *Bacillus*-based direct-fed microbials (DFM) are reported to have beneficial effects on animal and poultry growth and feed conversion efficiency (An et al., 2008; Park and Kim, 2015; Zhang et al., 2012). Use of compounds that have *B. subtilis* in them could possibly improve intestinal health (Aliakbarpour et al., 2012; Lei et al., 2014) and manipulation of intestinal microflora (Lei et al., 2014). Kefir is a popular traditional Middle Eastern beverage created from the fermentation of milk with kefir grains and other cultures prepared from grains. Kefir grains contain

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a complex mixture of lactobacilli, bacteria, and yeasts, as well as the probiotic bacteria that is found in yogurt (Fuller, 1989; Zhang et al., 2012). Thus, kefir can be defined as a probiotic food ingredient and feed additive. The current experiment was undertaken to demonstrate the effects of β-glucans, kefir and *B. subtilis* as different sorts of probiotics on growth performance, blood characteristics, relative organ weight, and meat quality.

MATERIALS AND METHODS

All broilers used in this trial were handled in accordance with the guidelines of the Animal Care and Use Committee of Dankook University.

1. Preparation of Kefir, β-Glucan and *B. subtilis*

A concentrated β-glucan was derived from *Agrobacterium* sp. R259 KCTC 10197B, which was derived by an aerobic fermentation method (Genebio), and was guaranteed to contain 86.1 g kg^{-1} β-1,3/1,6-glucan, 42 g kg^{-1} protein, and 13 g kg^{-1} lipids. A fermenter with volume of 34 tonnes was used in this method. The fermentation temperature was controlled at a bout 30℃ with an aeration rate of 0.75 air volume per culture volume perminute. The solids in the broth were isolated by a decanter. Thereafter, the solids were put into a high-speed bead mill to disrupt the cells, and then protein was sieved. The final product with purity of 9.5% was obtained after drying the screened slurry usin g a spray dryer. The *Bacillus subtilis* UBT-MO2 probiotic isolated from the soil around poultry farms (DMJ Biotech Co. Ltd., Youngi-gun, Chungcheongnam-do, South Korea) is composed of spray-dried spore-forming *B. subtilis* endospores, which is determined to contain at least 10^8 cfu *B. subtilis*/kg. The test Kefir was provided by a commercial company, Genebio (Seoul, South Korea) from sterilize ultra-high temperature milk inoculated with 5% active kefir grains under aseptic conditions and incubating at 22℃ for 20 hours (Marshal and Cole, 1985). Viable counts of lactic acid bacteria in the kefir samples were then determined by plating serial 10-fold dilutions (in 10 g/L peptone solution) onto Lactobacilli MRS agar plates to verify the number of *Lactobacillus*. The Lactobacilli MRS agar plates were incubated for 48 h at 39℃ and the number of *Lactobacillus* bacteria in the kefir samples was $5.3±4.2×10^8$.

2. Birds and Diets

A total of 528 day-of-hatch mixed sex ROSS 308 broiler chicks (Yang Ji Company, Cheonan, South Korea) with initial BW of 47±0.1 g were randomly placed in battery brooders with 4 replicate pens of 22 birds/pen for each treatment. There were 6 treatments in this experiment: CON, basal diet; B, CON + 1 g kg^{-1} *Bacillus* (10^8 cfu *Bacillus subtilis*/kg); K, CON + 1 g kg^{-1} kefir; G, CON + 1 g kg^{-1} β-glucan; GB, G + 1 g kg^{-1} *Bacillus* (10^8 cfu *Bacillus subtilis*/kg); GK, G + 1 g kg^{-1} kefir. Birds were given access to water and a maizesoybean meal-based feed as starter including 3,100 kcal/kg metabolism energy (ME), 220 g kg^{-1} crude protein (CP) and finisher diets (ME 3,050 kcal/kg, CP 190 g kg^{-1}) (Table 1) *ad libitum*. Chicks were kept under daylight (electric) lamp lighting on a light regime consisting of 23 h light and 1 h darkness. The temperature of the room was maintained at 33±1℃ for the first 3 d and decreased to 23℃ until the end of the experiment with the relative humidity was around 60%. The diets were provided during the experiment in 2 phases consisting of a starter phase from d 0～21 and a finisher phase from d 21～35.

3. Growth Performance Parameters

Birds were weighed on d 0, d 21 and d 35, with birds in each pen being weighed as a group. Mean BW of the birds in each treatment was calculated from the pen replicates for each weighing day. Body weight gain (BWG) was calculated individually for each period and cumulatively based on pen weights. For the same period, feed intake (FI) of each pen as a group was measured as BW (d 21 and d 35) with cumulative averages calculated. Feed conversion ratio (FCR) was calculated as (feed intake)/(body weight gain).

4. Sampling and Measurements

On d 21 and d 35 of the experiment, 8 broilers were randomly selected from each treatment (2 birds/pen) and blood samples were collected from the wing vein using a sterile syringe. Half of the sample was transferred into either a vacuum (clot activator with gel) or a 5 mL K3 EDTA vacuum tube (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ) and stored at −4℃. The white blood cell (WBC), red blood

Table 1. Diet composition (as-fed basis)

Ingredient (g/kg)		Starter[1]	Grower[1]
Corn		556.7	632.1
Soybean meal (CP 48%)		282.5	246.1
Corn gluten meal (CP 60%)		65.0	35.0
Soybean oil		55.0	48.9
Tricalcium phosphate		24.6	22.9
Limestone		8.9	7.5
Salt		2.0	2.0
DL-Methionine		0.7	0.7
L-Lysine-HCl		0.6	0.8
Vitamin premix[2]		2.0	2.0
Trace mineral premix[3]		2.0	2.0
Chemical composition[4] (g/kg)	ME (Mcal/kg)	3,100.0	3,050.0
	Crude protein	220.0	190.0
	Lysine	11.0	10.0
	Ca	10.0	9.0
	P	8.0	7.5

[1] Starter diets, provided during weeks 0～3; grower diets, provided during weeks 4～5.
[2] Provided per kg of diet: 15,000 IU of vitamin A, 3,750 IU of vitamin D_3, 37.5 mg of vitamin E, 2.55 mg of vitamin K_3, 3 mg of vitamin B_1, 7.5 mg of vitamin B_2, 4.5 mg of vitamin B_6, 24 mg of vitamin B_{12}, 51 mg of niacin, 1.5 mg of folic acid, 126 mg of biotin and 13.5 mg of pantothenic acid.
[3] Provided per kg of diet: 37.5 mg of Zn, 37.5 mg of Mn, 37.5 mg of Fe, 3.75 mg of Cu, 0.83 mg of I, 62.5 mg of S and 0.23 mg of Se.
[4] Calculated values.

cell (RBC) and lymphocyte counts in the whole blood were determined using an automatic blood analyser (ADVIA, Bayer, Tarrytown, NY).

Plasma was separated from whole blood by centrifuging at 3,000 g for 15 min. Immunoglobin G (IgG) was analysed using nephelometry (Dade Behring, Marburg, Germany). On d 35, 20 broilers (5 chickens/pen) were randomly selected from each treatment and then killed by cervical dislocation. The liver, spleen, bursa Fabricii, breast meat and abdominal fat and were then removed and cleaned. The breast muscle was stored at 4℃ before analysis. Relative organ weight was expressed as a percentage of the live body weight. The breast meat Hunter lightness (L*), redness (a*) and yellowness (b*) values were measured using a Minolta CR410 chromameter (Konica Minolta Sensing Inc., Osaka, Japan). Drip loss was measured using approximately 2 × 2 × 2 cm of meat sample according to the plastic bag method described by Honikel (1986). The water holding capacity (WHC) was measured according to the methods of Kauffman et al. (1986). Cooking loss was determined using 5 g of breast meat, which was heat-treated in plastic bags separately in a water bath (100℃) for 5 min. Samples were cooled at room temperature. Cooking loss was calculatedas (sample weight before cooking－sample weight after cooking)/sample weight before cooking × 100. At one hour postmortem, duplicate pH values for each breast muscle sample were measure dusing a pH meter by inserting a glass electrode directly in the thickest part of the pectoralis major (Fisher Scientific, Pittsburgh, PA, US).

The same slaughtered broiler chickens were used for microbial counts. Gizzard, Ileal and caecal contents were collected into Qorpak glass containers (118 mL) under CO_2, sealed and placed on ice until transported to the laboratory for enumeration of microbial populations. Gizzard, caecal and ileal samples were assessed for populations of *Lactobacillus* and *Escherichia coli*. One gram of the composite excreta sample from each cage was diluted with 9 mL of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ) and then homogenised. Viable counts of bacteria in the gizzard, caecal and ileal samples were then conducted by plating serial 10-fold dilutions (in 1% peptone solution) onto lactobacilli medium III agar plates (Medium 638; DSMZ, Braunschweig, Germany), MacConkey agar plates (Difco Laboratories, Detroit, MI) supplemented with glacial acetic acid (1 mL/L) and mupirocin (100 mg/L) extracted from antimicrobial discs to isolate the *Lactobacillus* and *Escherichia coli*, respectively. The lactobacilli medium III agar plates were then incubated for 48 h at 37℃ under anaerobic conditions. The MacConkey plates were incubated for 24 h at 37℃ and the Wilkins-Chalgren agar plates were incubated for 72 h at 37℃. The microflora colonies were counted immediately after removal from the incubator. The concentration of microflora was expressed as log10 colony-forming units per gram of intestinal content.

5. Statistical Analysis

All data were statistically analysed by one-way ANOVA using the GLM procedure of SAS system for windows (SAS Institute Inc., 2001, Cary, NC), with pen as the experimental unit. The mean differences among all treatments were separated by Tukey multiple range tests, with a $P<0.05$ indicating significance.

RESULTS

1. Growth Performance

Live-weight gain, feed intake and feed conversion ratio for the experimental period are shown in Table 2. From 0 to 3 weeks period, birds fed diets with either *B. subtilis*, kefir or β-glucans grew faster ($P<0.05$) than those of the CON and GK groups, but feed conversion ratio was significantly ($P<0.05$) lower in the CON, K and GB groups compared to GK group. From 4~5 weeks period, the body weight gain of broilers in the GK group was better ($P<0.05$) than those of the CON and B group, but the feed conversion and feed intake did not change. Body weight gain significantly increased by kefir, β-glucans and mix of kefir and β-glucans during the overall 35-d period, however, feed intake and feed conversion were not affected from the treatments.

2. Blood Profile

For the blood characteristic, the group receiving feed supplemented with *B. subtilis* had the highest RBC in the 3rd week but there was not any difference among all treatments in the end of experiment (Table 3). For lymphocyte, all groups (K, G, GB and GK) except *B. subtilis* group had a higher lymphocyte percentage in the 3rd week compared to control diet, but percentage of lymphocyte was significantly ($P<0.05$) higher only in the group receiving *B. subtilis* than that of the β-glucan diet at the end of experiment. There were no differences in total protein and IgG between birds fed the supplemented diets in both 3rd and 5th week. At 35 d of age, total WBC were significantly ($P<0.05$) greater in GB than those in CON, B and G groups (Table 3).

3. Relative Organ Weight

Table 4 presents relative organ weight in broiler chickens fed the CON, B, K, G and mixture of G with GB or GK. Relative weights of the liver, abdominal fat and breast meat did not affect from the treatments ($P>0.05$), but kefir was able to increase the weight of bursa Fabricii compare to both *B. subtilis* and β-glucan groups at the end of experiment. Results also showed that the weight of spleen was significantly increased in the GB and GK groups compared to B group.

Table 2. Effects of *Bacillus subtilis*, kefir and beta-glucan on growth performance in broilers[1]

	Item	CON	B	K	G	GB	GK	SE[2]
0~3 (weeks)	Weight gain (g)	788^{b}	830^{a}	833^{a}	833^{a}	807^{ab}	785^{b}	6.1
	Feed intake (g)	986^{c}	1,067^{abc}	1,020^{abc}	1,090^{ab}	1,012^{bc}	1,097^{a}	24.1
	Feed conversion	1.251^{b}	1.285^{ab}	1.224^{b}	1.308^{ab}	1.254^{b}	1.397^{a}	0.041
3~5 (weeks)	Weight gain (g)	991^{c}	1,011^{bc}	1,047^{abc}	1,056^{ab}	1,032^{abc}	1,093^{a}	19.9
	Feed intake (g)	2,113	2,102	2,129	2,093	1,999	2,150	88.4
	Feed conversion	2.132	2.079	2.033	1.982	1.937	1.967	0.113
0~5 (weeks)	Weight gain (g)	1.779^{b}	1,841^{ab}	1,880^{a}	1,889^{a}	1,839^{ab}	1,878^{a}	23.1
	Feed intake (g)	3,099	3,169	3,129	3,183	3,011	3,246	86.3
	Feed conversion	1.741	1.721	1.664	1.685	1.637	1.728	0.069

[1] Abbreviations: (CON), basal diet; (B), CON + 1 g kg^{-1} *Bacillus subtilis*; (K), CON + 1 g kg^{-1} kefir; (G), CON + 1 g kg^{-1} betaglucan; (GB), G + 1 g kg^{-1} *Bacillus subtilis*; (GK), G + 1 g kg^{-1} kefir.

[2] Standard error of the means.

[a~c] Means in the same row with different superscripts differ ($P<0.05$).

Table 3. Effects of *Bacillus subtilis*, kefir and beta-glucan on blood profile in broilers[1]

	Item	CON	B	K	G	GB	GK	SE[2]
3 (week)	RBC (×10^6/μL)	1.85^b	2.25^a	2.02^b	2.01^b	1.95^b	2.01^b	0.07
	WBC (×10^6/μL)	244	277	244	287	258	267	28.6
	Lymphocyte (%)	74.7^b	80.5^{ab}	81.5^a	83.0^a	84.5^a	85.5^a	1.92
	Total protein (g/dL)	3.1	3.2	2.9	3.1	2.85	3.18	0.11
	IgG (g/dL)	1.13	1.31	1.55	1.44	1.31	1.26	0.42
5 (week)	RBC (×10^6/μL)	1.84	1.94	1.89	1.99	2.02	2.03	0.06
	WBC (×10^6/μL)	468^b	482^b	530^{ab}	484^b	572^a	521^{ab}	18.91
	Lymphocyte (%)	79.2^{ab}	83.2^a	75.7^{ab}	71.7^b	76.2^{ab}	80.0^{ab}	2.99
	Total protein (g/dL)	3.85	3.72	3.81	3.91	3.83	4.13	0.28
	IgG (g/dL)	1.06	1.4	1.4	1.12	1.18	1.19	0.34

[1] Abbreviations: (CON), basal diet; (B), CON + 1 g kg^{-1} *Bacillus subtilis*; (K), CON + 1 g kg^{-1} kefir; (G), CON + 1 g kg^{-1} betaglucan; (GB), G + 1 g kg^{-1} *Bacillus subtilis*; (GK), G + 1 g kg^{-1} kefir.
[2] Standard error of the means.
[a,b] Means in the same row with different superscripts differ (p<0.05).

Table 4. Effects of *Bacillus subtilis*, kefir and beta-glucan on relative organ weight and meat quality in broilers[1]

	Item	CON	B	K	G	GB	GK	SE[2]
% of body weight	Liver	3.213	3.424	2.904	3.018	3.215	2.968	0.29
	Spleen	0.172^{ab}	0.116^b	0.150^{ab}	0.158^{ab}	0.208^a	0.195^a	0.02
	Fat	1.235	1.221	1.485	1.298	1.566	1.679	0.17
	Bursa Fabricii	0.111^{ab}	0.075^b	0.144^a	0.076^b	0.120^{ab}	0.103^{ab}	0.02
	Breast meat	16.8	17.1	16.33	16.29	15.1	17.03	0.93
Meat quality	L* (lightness)	52.35	51.9	53.12	52.92	55.39	50.87	1.64
	a* (redness)	11.91^b	14.82^a	14.29^a	14.87^a	15.98^a	14.13^a	0.69
	b* (yellowness)	11.87	10.98	11.16	8.15	11.87	12.21	1.28
	Cook loss (%)	20.89	21.02	20.65	21.26	22.34	21.35	0.21
	Drop loss (%)	1.23	1.15	1.02	1.18	1.23	1.22	0.12
	WHC[3] (%)	65.15	64.25	68.25	65.25	65.64	68.35	3.65
	pH	5.95	5.94	5.86	5.89	5.92	5.98	0.21

[1] Abbreviations: (CON), basal diet; (B), CON + 1 g kg^{-1} *Bacillus subtilis*; (K), CON + 1 g kg^{-1} kefir; (G), CON + 1 g kg^{-1} betaglucan; (GB), G + 1 g kg^{-1} *Bacillus subtilis*; (GK), G + 1 g kg^{-1} kefir.
[2] Standard error of the means.
[3] Water holding capacity.
[a,b] means in the same row with different superscripts differ (P<0.05).

4. Meat Quality

Meat quality measurements of chicks are presented in Table 4. All *B. subtilis*, kefir and β-glucan supplemented diets increased redness of meat, but they did not affect lightness, yellowness, cook loss, drip loss, WHC and pH.

5. Intestinal Microbiota

Results of the bacterial counts in different segments of the digestive tract are shown in Table 5. The population of *Lactobacillus* spp. in gizzard was significantly higher in K treatment compared with CON, B, G and GB. No differences were observed in ileum and cecum. There was no significant difference among treatments in populations of coliforms.

DISCUSSION

In a previous study, we observed that *B. subtilis* improved BWG in chicken (Zhang et al., 2012). In the current study, an improvement occurred in weight gain and feed efficiency when *B. subtilis* (1 g/kg) was supplemented to broiler chickens diet from 1 to 21 d; however, this early improvement was not carried through to 35 d of age, which was in agreement with Park and Kim IH (2015) who reported that growth performance of broilers did not improve by *Bacillus*-based DFM. On the contrary, others have found significant improvement for BWG (Zhang et al., 2012; An et al., 2008). The results of many studies, in which the effects of probiotics on growth performance in poultry were investigated, were not consistent because of the various microorganisms with different characteristics, considered as probiotics, application methods, ages, diets and environmental conditions (Zhang et al., 2012). It is well known that *B. subtilis* has a positive effect on the bird's performance since they can produce enzymes to improve the digestibility of nutrients and produce peptides to kill pathogens, but the positive effects of *B. subtilis* are mostly observed when the birds were under bacterial challenges. *B. subtilis* spores reduced intestinal colonization of *Salmonella* and *E. coli* in birds with salmonella challenge (Park and Kim, 2015), which can modulate the gut microflora and improve the immune system of the birds. It can be the reason that the result of this experiment did not show a significant difference compared with the control group. As has been previously reported by our laboratory (Cho et al., 2013) and also observed in this current study, kefir increased body weight gain in broilers. Cho et al. (2013) reported equivalent efficiencies when using kefir or avilamycin, an antibiotic. Both kefir and avilamycin improved body weight of broilers when 1 g kg^{-1} kefir was added to the broiler diet. Kefir has been relatively less studied as a component in broiler diets but several studies have been conducted on the effects of kefir in humans (Fuller, 1989) as well as in broiler chickens (Cho et al., 2013). Toghyani et al. (2015) reported that inclusion of 2% milk kefir in drinking water improves growth performance. Kefir is considered a probiotic-fermented product (Fuller, 1989) obtained by the interaction of lactic acid bacteria, yeasts and acetic acid bacteria, trapped in a complex matrix of polysaccharides (Zhang et al., 2012). The mechanism by which kefir

Table 5. Effects of *Bacillus subtilis*, kefir and beta-glucan on microbiota of intestine in broilers[1]

Item (log_{10}g/CFU)		CON	B	K	G	GB	GK	SE[2]
Lactobacillus spp.	Gizzard	6.46^{bc}	6.43^{bc}	6.91^{a}	6.41^{bc}	6.37^{c}	6.79^{ab}	0.17
	Ileum	7.79	7.82	8.01	8.09	8.14	8.06	0.15
	Caeca	8.11	8.06	8.19	8.20	8.26	8.35	0.11
Coliforms	Gizzard	4.11	3.89	3.96	3.79	4.18	3.88	0.19
	Ileum	6.11	6.14	5.97	6.16	6.01	6.02	0.14
	Caeca	6.70	6.48	6.39	6.42	6.46	6.37	0.16

[1] Abbreviations: (CON), basal diet; (B), CON + 1 g kg^{-1} *Bacillus subtilis*; (K), CON + 1 g kg^{-1} kefir; (G), CON + 1 g kg^{-1} betaglucan; (GB), G + 1 g kg^{-1} *Bacillus subtilis*; (GK), G + 1 g kg^{-1} kefir.

[2] Standard error of the means.

[a,b] means in the same row with different superscripts differ (P<0.05).

affects poultry performance is largely unknown, where as, it is welles tablished that probiotics which contain *Lactobacillus* culture can control the pathogens population and alter gastrointestinal flora (Chen et al., 2016). These effects can increase the activity of intestinal enzymes and digestibility of nutrients (Dierck, 1989). It has been previously reported that adding *Lactobacillus* culture into broiler feed result in an increase in feed conversion ratio (Yu et al., 2007; Chen et al., 2016). It is noteworthy that the different effects of probiotics, including relation of intestinal microflora (Yu et al., 2007; Chen et al., 2016) and immune system (An et al., 2008). In the current experiment, the results showed that the kefir makes suitable environment for microbial and it would be helped on the immune system. These results combined with earlier published studies show the positive effects of kefir with respect to growth performance of broilers. In the current study, the β-glucan supplemented group has a higher BWG compared with the CON group. However, the absence of an antibiotic treatment in this study did not assure our lab about the capability of competing β-glucan with antibiotics. Moon et al. (2016) showed that the broilers receiving β-glucan-supplemented diets had similar weight gain to those raised on antibiotics which means β-glucan can improve the performance comparable to antibiotics. β-glucan increase BW gain with selectively improving immune system (An et al., 2008; Zhang et al., 2008). In recent years, there has been increasing generated by β-glucan because of its immune modulating capabilities, which may also benefit growth performance. However, our laboratory's previous studies have shown that feeding 0.1 kg/100 kg β-glucan to broiler chickens resulted in no significant response in weight gain (Cho et al., 2013; Zhang et al., 2012). This result is in contrast to Wang et al. (2008) who observed a significant change in the growth rate when young broilers with β-glucan in their diets were treated with *E. coli*. It has become common practice to either feed beneficial bacteria directly (probiotic), dietary components that favour these beneficial microbial populations (prebiotic), or a combination of both (symbiotic) in poultry diets (Cho et al., 2013; Zhang et al., 2012). In the current study, there has been a significant correlation between the kefir and β-glucans supplements and positive weight gain.

There was no effect of increasing the level of RBC, WBC, lymphocyte, or IgG when diets were supplemented with *B. subtilis*. However, in another study the positive effects of *Bacillus*-based probiotics on immune system were confirmed (An et al., 2008). These different results may be due to the age of animals, environmental stress factors, species composition, viability, and others. In this study, the addition of kefir and β-glucan increased the number of WBC in the third week. WBC is known for its function as a defence system (Grasman, 2002). β-glucan did not affect the blood characteristics in the current study but there was a positive effect between *B. subtilis* with β-glucan for the number of WBC at the end of the experiment. Increasing interest has been generated on β-glucan in recent years because of its immune modulating capabilities which may benefit growth performance. However, the absence of a challenge in this study has resulted in no deleterious effects on immune system, lymphocyte counts, or IgG.

After the addition of *B. subtilis* to the feed at 1 g/kg in the current study, the overall effects of *B. subtilis* on relative organ weight examined in this study were in general unaffected and similar to previous reports of our lab (Zhang et al., 2012). However, Park and Kim (2015) showed that *B. subtilis* B2A significantly increased the weight of bursa of Fabricii as an organ which is in related to immune system. The weight of bursa Fabricii was higher for the kefir supplemented group compared with *B. subtilis* and β-glucans at the end of experiment. bursa Fabricii is an important organ for T and B lymphocytes maturation as parts of WBC (Grasman, 2002) which contribute to the immune system. These results agree with those of Sato et al. (2009), who reported that the T cell immune system was increased in neonatal chicks fed several strains of a *Lactolacillus*-suplemented diet. The bigger bursa Fabricii may explain the stimulation of the blood lymphocyte in this study, which is contributed to immune system. In this experiment, the final relative weight of bursa Fabricii and spleen were unaffected by the dietary β-glucans supplementation. Several other authors have shown similar results of bursa Fabricii and spleen weights in broilers (Cho et al., 2013; Zhang et al., 2012). There was an interaction for the weight of spleen as an immune-based organ when *B. subtilis* and β- glucans were used in the diet, however, this combine additives treatment did not change the size of the bursa Fabricii and the percentage of lymphocytes.

All supplemented groups showed an increase in the redness value of breast meat. In other previous study the meat colour was affected in broilers which were given *Bacillus*-based probiotic (Liu et al., 2012; Cho et al., 2013). However, other studies in which probiotics were given, did not show any change in meat colour, drip loss (Park and Kim, 2015) and cooking loss (Kabir, 2009) in broilers. Moon et al. (2016) reported that β-glucan did not show any effects on meat colours. Little is known about the mechanism by which kefir and β-glucan supplementation change the colour of meat since different factors can affect the meat colour. Many factors such as bird sex, age, exogenous chemicals, processing procedure, and freezing may affect poultry meat colour. It can be suggested that the composition, viability and application method of the probiotics may affect these results.

The concentration of *Lactobacillus* spp. in gizzard was significantly increased in the groups fed diets containing kefir than that of control, while the colonization of total *E. coli* were not changed by the supplemented groups. It is interesting to note that, in this study, either kefir or *B. subtilis* did not change both the concentrations of ileal and cecal *Lactobacillus* and *E. coli*, whereas probiotics are known to modify intestinal microbiota in favour of useful bacteria and reduce the colonization of pathogens, consequently gut health and improving the growth performance.

CONCLUSION

In summary, our results have demonstrated that the addition of kefir as a probiotic and β-glucan as a prebiotic to the chicken diets have caused a positive effect on BWG and meat colour in broiler. There were positive effect of kefir and β-glucan supplementation on BWG and meat colour. *B. subtilis* and kefir also increased the weight of spleen. Further experiments with improved immune systems, are required to test whether this probiotic and prebiotic addition is as effective as antibiotics for growth performance and protecting broilers from harmful microorganisms.

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