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The Physiological Responses to Dietary Administration of Zinc Bacitracin and *Bacillus* Mixture on Low-Weight Day-Old Chicks

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Abstract

This study investigated the responses of low-weight day-old chicks to zinc bacitracin and *Bacillus* mixture on growth performance, hematology, intestinal selected microbiota populations, and carcass characteristics. A total of 192 unsexed Lohman MB-202 day-old broiler chicks were randomly allotted to four dietary treatment groups of 48 chicks each (6 replicates of 8 chicks) and fed for 35 days. The four treatments were CONT (basal diet without supplementation), AGP (basal diet with 0.04% zinc bacitracin), PROB (basal diet supplemented with 0.5% *Bacillus* mixture), and PROB+AGP (basal diet supplemented with 0.5% *Bacillus* mixture and 0.04% zinc bacitracin). There were no significant differences in final body weight, feed intake, and feed conversion ratio of broilers. The relative weight of thymus was higher (P= 0.01) in AGP than that in PROB and PROB+AGP birds. Birds in PROB+AGP had lower (P= 0.04) values of alanine aminotransferase (ALT) enzyme compared to those in CONT and AGP groups, but the difference was not significant when compared with birds in PROB group. Serum creatinine was lower (P < 0.01) in PROB+AGP than in CONT and AGP birds. Birds in AGP group tended (P = 0.09) to have higher globulin concentration in the serum compared to other birds. There was also a tendency (P = 0.07) for PROB+AGP birds to have higher albumin to globulin ratio compared with other birds. There was more lactic acid bacteria in ileal digesta (P = 0.05) in PROB+AGP than in AGP birds, but the difference was not significant when compared to CONT and PROB birds. Birds in AGP group had higher (P = 0.05) relative breast weight compared to other birds. In conclusion, *Bacillus* mixture did not affect performance and hematological parameters, but increased the intestinal population of lactic acid bacteria in broiler chicks.

Introduction

It is well documented that the weight of one-day-old broiler chicks determines their future weight at market age (Tona *et al*., 2004; Mendes *et al*., 2011; Toghyani *et al*., 2011). For example, chicks with low initial weights (34.40–35.22 g) had lower final body weights (BW) than those with heavier hatching weights (39.29–41.30 g) (Mendes *et al*., 2011). In another study, Michalczuk *et al*. (2011) showed that hatching chicks with BW higher than 42 g presented higher BW at day 36 compared to those with BW lower than or equal to 39 g at hatch. Similar findings have been observed in Pekin duck

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(Sözcü and Ipek, 2017). Birds with a heavier initial BW may have more developed digestive organs (such as gizzard and intestine), which may improve digestive capacity and thus growth performance of the birds (Sözcü and Ipek, 2017). In these respects, it is discouraged to use chicks with low initial BW in commercial broiler farms as their rearing will reduce economical benefits. Chicks with low initial BW are usually produced from the junior breeder flock (25–30-week-old) (Tona *et al*., 2004; Ulmer-Franco *et al*., 2010).

Due to the regular egg production cycle, broiler breeders would produce chicks from junior breeder flocks at specific times. Instead of culling chicks with low hatching weights, improving the management and rearing conditions can be implemented (Butcher and Nilipour, 2002; Ulmer-Franco *et al*., 2010). Another concern related to chicks from young breeder flock is a high mortality rate (Wyatt *et al*., 1985). The reasons behind this are unclear but may be associated with poor immune function. In order to be as productive as the heavier chicks, these chicks need distinct management and rearing conditions (Butcher and Nilipour, 2002; Ulmer-Franco *et al*., 2010). For example, providing extra vitamins and minerals to these chicks during the rearing period may improve their performance (Butcher and Nilipour, 2002). Some vitamins and minerals are essential for optimizing metabolism, and thus growth performance (Islam *et al*., 2004; Ajuwon *et al*., 2011) and immune functions in broiler chickens (Sanda, 2015; Sugiharto, 2016).

In-feed antibiotics have long been used as growth promoters, antimicrobial agents, and immune stimulants in broiler production (Lee *et al*., 2012; Sugiharto, 2016). In the case of low day-old weight chicks, in-feed antibiotics are essential for improving the growth and health of broilers. However, long-term application of such antibiotics may result in antibiotic residues in broiler meat which can pose risk to human health (Sugiharto, 2016). Hence, alternatives to in-feed antibiotics are important, especially for day-old low-weight chicks. Among the in-feed antibiotic substitutes, probiotics – which contains a wide spectrum of *Bacillus* strains – has attracted considerable interest from poultry nutritionists. Compared to *Lactobacillus*-based probiotic cultures, which is commonly used as a feed additive in poultry nutrition, probiotics containing a wide spectrum of *Bacillus* strains show better viability and stability, especially during feed processing and storage (Simon, 2005). The ability to form spores allows *Bacillus*-based probiotics to survive not only in the extreme conditions of the gastrointestinal tract, but to also be more stable during processing and feed storage (Elshaghabee *et al*., 2017). Therefore, probiotics that contain a wide spectrum of *Bacillus* strains seem to be more effective and practical in broiler production. In our previous study, we prepared a select mixture of *Bacillus* probiotics with vitamins and minerals (which we termed called "*Bacillus* mixture"), and found an improvement in the organ development and immune competence of broiler chicks (Isroli *et al*., 2017).

The objective of the present study was to investigate the response of low day-old weight chicks to the administration of zinc bacitracin and *Bacillus* mixture in terms of growth performance, blood parameters, intestinal microbiota populations, and carcass characteristics. In this study, the effects associated with *Bacillus* mixture was compared with zinc bacitracin without vitamins and minerals supplementation in low-weight chicks. To date, zinc bacitracin is still commonly used in Indonesian commercial poultry producers.

Materials and Methods

A total of 192 one-day-old chicks (unsexed Lohmann meat broilers; BW= 35.20 ± 0.25 g; mean ± standard deviation) were used in the present trial. Upon arrival, the chicks were weighed individually and randomly allotted to one of four groups of 48 chicks each (6 replicates of 8 chicks). The chicks were raised in an open-sided broiler house with wire floor pens for the entire trial period. Light was provided 24 h per day. To control the temperature and humidity, the broiler house was equipped with a plastic curtain, light bulb as heater, and blower fan. The dietary treatment groups were CONT (basal diet without any supplementation), AGP (basal diet with 0.04% zinc bacitracin), PROB (basal diet supplemented with 0.5% *Bacillus* mixture), and PROB+AGP (basal diet supplemented with 0.5% *Bacillus* mixture and 0.04% zinc bacitracin).

The study lasted for 35 days, and for the entire study period, the feed (in mash form) and water were provided *ad libitum*. The coccidiostat, enzymes, and other feed additives/supplements were not included in the basal diet. The basal

diet was formulated (Table, 1) to meet or exceed the Indonesian National Standards for Broiler Feed (SNI, 2006). To test the efficacy of the supplements in preventing the overgrowth of *Clostridium perfringens*, the diet was formulated to increase the *C. perfringens* proliferation in the intestine of birds by including bone meal, chicken feather meal, meat bone meal, and viscous cereals (M'Sadeq *et al*., 2015). The *Bacillus* mixture contained 12.10 log cfu/g multi-strains *Bacillus*(i.e., *Bacillus cereus* strain SIIA_Pb_E3, *Bacillus licheniformis* strain FJAT-29133, *Bacillus megaterium* strain F4-2-27 and *Bacillus* spp. 11CM31Y12), 0.100 mg vitamin A, 0.018 mg vitamin D_3, 0.100 mg vitamin E, 1200 mg Ca, 750 mg P, 0.08 mg Mg, 0.006 mg Co, 0.045 mg Cu, 0.015 mg Se, 0.180 mg S, 0.010 mg Zn, 0.060 mg KCl, 0.030 mg I, 0.060 mg Fe and 0.100 mg Mn. The *Bacillus* mixture was prepared in our laboratory, and zeolite was used as a carrier for the bacteria. The novel *Bacillus* strains have recently been isolated from the rumen content of cow, and exhibit antibacterial activity (*in vitro*) against *Escherichia coli* ATCC 25922 (unpublished data). These bacterial cultures also showed probiotic activities in broiler chicks during the brooding period *in vivo* (Isroli *et al*., 2017). The experiment was performed under the standard procedures of rearing and treating of farm animals stated in the law of the Republic of Indonesia number 18, 2009 regarding animal husbandry and health.

Table 1. Ingredients and chemical composition of basal diet

Items (%, unless otherwise noted)	Composition
Maize (CP 8.5%)	45.5
Soybean meal (CP 46%)	17.0
Wheat flour	10.0
Bread flour	5.00
Rice bran	4.45
Crude palm oil	3.50
Corn gluten meal	3.60
Distiller dried grains[1]	3.00
Meat bone meal	2.80
Hydrolyzed chicken feather meal	2.00
Bone meal	1.50
Lysine	0.55
Methionine	0.37
L-threonine	0.08
Salt	0.15
Premix[2]	0.50
Analyzed composition	
Metabolizable energy (Kcal/kg)[3]	3290
Crude protein	21.7
Crude fat	5.90
Crude fiber	6.79
Ash	10.9

[1]The dried residue remaining after the starch extraction from maize.
[2]Mineral-vitamin premix contained (per kg of diet); Ca 2.250 g, P 0.625 g, Fe 3.570 mg, Cu 0.640 mg, Mn 5.285 mg, Zn 0.003 mg, Co 0.001 mg, Se 0.013 mg, I 0.016 mg, vitamin A 375 IU, vitamin D 150 IU, vitamin E 0.080 mg.
[3]Value was calculated according to formula (Bolton, 1967) as follow: 40.81 {0.87 [crude protein + 2.25 crude fat + nitrogen free extract] + 2.5}.

Throughout the study period, birds were only vaccinated with commercial Newcastle disease virus (NDV) vaccine through eye drops and drinking water at day 4 and 18 of the experiment, respectively. Live BW and accumulative feed intake (FI) of broiler chicks were recorded at the end of experiment. Blood was collected from the bird's wing veins (6 birds per treatment) and placed in vacutainers containing ethylene diamine tetra acetic acid (EDTA) for the determination of complete blood counts. For the serum biochemical analysis, the rest of the blood was collected in the vacutainers without anticoagulant, permitted to clot at room temperature, and centrifuged at 448 *g* for 15 min. The serum was frozen until analyses (Sugiharto *et al*., 2017). After blood sampling, the birds were slaughtered, de-feathered, and eviscerated. The

internal organs were immediately obtained and weighed (Sugiharto *et al*., 2017). For the microbiological and short chain fatty acids (SCFAs) analyses, digesta were expelled by gently squeezing from the ileum (from Meckel's diverticulum to a point 4 cm proximal to the ileocecal junction) and cecum into the sterile sample bottles. The samples of breast muscle were collected for the determination of crude protein and fat content.

Complete blood counts were determined using a hematology analyzer (Prima Fully-auto Hematology Analyzer, PT. Prima Alkesindo Nusantara, Jakarta, Indonesia). The serum NDV antibody titers were measured according to hem agglutination inhibition (HI) assay (Villegas, 1987). The titers were presented as geometric mean titers (Log_2). To determine the levels of total triglyceride, total cholesterol, high-density lipoprotein-cholesterol (HDL-c), and low-density lipoprotein-cholesterol (LDL-c) cholesterol, uric acid, and creatinine in serum, we used enzymatic colorimetric/color methods. The spectrophotometric/photometric tests were used to determine total protein, albumin, alanine aminotransferase (ALT), and aspartate aminotransferase (AST) in the serum of broilers. Globulin was calculated from the difference between total protein and albumin in serum. These biochemical analyses in serum were conducted using kits (DiaSys Diagnostic System GmbH, Holzheim, Germany) according to the manufacturer's instructions.The bacterial populations in the ileal and cecal digesta of birds were determined (6 birds per treatment) according to Sugiharto *et al*. (2017) with few modifications. Coliform bacteria were enumerated on MacConkey agar (Merck KGaA, Darmstadt, Germany) after aerobic incubation at 38°C for 24 h as red colonies. The number of lactic acid bacteria (LAB) was determined on de Man, Rogosa and Sharpe (MRS; Merck KGaA, Darmstadt, Germany) agar following an aerobic incubation at 38°C for 48 h. The number of *C. perfringens* was determined on tryptose sulfite cycloserine (TSC; Merck KGaA, Darmstadt, Germany) agar plates following anaerobic incubation at 38°C for 48 h. Analysis of SCFAs concentrations in the cecal digesta was conducted by gas chromatography according to the conditions described by Sugiharto *et al*. (2015). Proximate analysis was conducted to analyze the crude protein and fat contents of meat according to the standard methods (AOAC, 1995). Crude protein content was determined according to the Kjeldahl method, while crude fat was determined by the Soxhlet extraction method using petroleum ether.

The data were analyzed according to a completely randomized design by ANOVA using the General Linear Models Procedure in SAS (1985). The pen was considered as the experimental unit. The differences ($P < 0.05$) among groups were further analyzed using Duncan's multiple-range test.

Results

Performance of broiler chicks

Data on the performance of broiler chicks are presented in Table 2. There were no significant differences in final BW, and feed conversion ratio (FCR) of broiler chicks between the treatment groups. However, birds in AGP and PROB groups tended ($P = 0.06$) to have higher feed intake than birds in CONT and PROB+AGP groups.

Table 2. Effects of zinc bacitracin and *Bacillus* mixture on performance of broiler chicks at 35 days of age

Items	Dietary treatments†				*SE*‡	*P*- value
	CONT	AGP	PROB	PROB+AGP		
Live BW (g)	909	972	955	917	40.8	0.66
Accumulative FI (g)	1,899	2,057	2,057	1,957	46.8	0.06
FCR (g/g)	2.19	2.22	2.22	2.24	0.04	0.98

†CONT: basal diet without any supplementation, AGP: basal diet with 0.04% zinc bacitracin, PROB: basal diet supplemented with 0.5% *Bacillus* mixture, PROB+AGP: basal diets supplemented with 0.5% *Bacillus* mixture and 0.04% zinc bacitracin; ‡*SE*: standard error.

Internal organ weights of broiler chicks

The relative weights of internal organs of broiler chicks are shown in Table 3. The relative weight of thymus was higher ($P = 0.01$) in AGP than in PROB and PROB+AGP birds. All other internal organs assessed had similar relative weights across the treatment groups.

Table 3. Effects of zinc bacitracin and *Bacillus* mixture on relative weights of internal organs in broiler chicks

Items (% live BW)	Dietary treatments†				*SE*‡	*P*-value
	CONT	AGP	PROB	PROB+AGP		
Heart	0.83	0.85	0.91	0.81	0.07	0.77
Liver	3.17	3.14	3.63	3.26	0.29	0.62
Proventriculus	0.43	0.47	0.46	0.50	0.04	0.66
Gizzard	1.20	1.15	1.16	1.18	0.10	0.98
Spleen	0.22	0.14	0.26	0.13	0.05	0.23
Thymus	0.24[ab]	0.30[a]	0.15[b]	0.20[b]	0.03	0.01
Bursa of Fabricius	0.18	0.16	0.22	0.32	0.07	0.37
Duodenum	0.64	0.70	0.68	0.65	0.04	0.73
Jejunum	1.12	1.14	1.13	1.16	0.09	0.99
Ileum	1.04	1.01	0.94	0.84	0.07	0.16
Caecum	0.49	0.50	0.56	0.66	0.10	0.63
Pancreas	0.32	0.35	0.27	0.32	0.03	0.24

[a,b] Means in the row with different letters show significant differences ($P < 0.05$).
†CONT: basal diet without any supplementation, AGP: basal diet with 0.04% zinc bacitracin, PROB: basal diets supplemented with 0.5% *Bacillus* mixture, PROB+AGP: basal diets supplemented with 0.5% *Bacillus* mixture and 0.04% zinc bacitracin; ‡*SE*: standard error.

Hematological parameters of broiler chicks

Complete blood counts and serum biochemical parameters of broilers are shown in Tables 4 and 5, respectively. In general, there were no substantial differences incomplete blood counts of broiler chicks. Regarding serum biochemical parameters, birds in PROB+AGP had lower (P = 0.04) levels of ALT enzyme compared to CONT and AGP groups, but the difference was not significant when compared to birds in PROB groups. Serum creatinine was lower ($P < 0.01$) in PROB+AGP birds than in CONT and AGP birds, but the difference was not significant when compared to PROB birds. Birds in AGP group tended (P = 0.09) to have a higher globulin concentration in the serum compared to birds in other treatments. PROB+AGP birds also tended to have higher albumin to globulin ratio when compared to other birds (P = 0.07).

Table 4. Effects of zinc bacitracin and *Bacillus* mixture on complete blood counts in broiler chicks

Items	Dietary treatments†				*SE*‡	*P*-value
	CONT	AGP	PROB	PROB+AGP		
Hemoglobin (g/dL)	8.76	9.74	9.38	9.07	0.79	0.84
Erythrocytes (10^6/µL)	2.05	2.26	2.11	2.06	0.16	0.80
Hematocrit (%)	29.1	31.3	30.3	30.0	2.76	0.95
MCV (fL)	140	138	144	145	2.80	0.31
MCH (pg)	42.5	42.9	44.3	43.8	1.03	0.56
MCHC (g/dL)	30.4	31.1	30.9	30.3	0.52	0.58
Leukocytes (10^3/µL)	19.1	21.8	25.6	22.1	2.36	0.36
Heterophils (10^3/µL)	0.46	0.66	0.68	0.63	0.10	0.40
Eosinophils (10^3/µL)	0.84	1.10	1.15	1.00	0.10	0.16
Lymphocytes (10^3/µL)	17.8	20.0	23.7	20.5	2.43	0.37
Thrombocytes (10^3/µL)	24.4	17.0	19.7	17.5	3.88	0.52

†CONT: basal diet without any supplementation, AGP: basal diet with 0.04% zinc bacitracin, PROB: basal diets supplemented with 0.5% *Bacillus* mixture, PROB+AGP: basal diets supplemented with 0.5% *Bacillus* mixture and 0.04% zinc bacitracin; ‡*SE*: standard error.

Intestinal bacterial populations of broiler chicks

The numbers of LAB in ileal digesta were higher (P = 0.05) in PROB+AGP than in AGP birds, but the difference was not significant when compared to CONT and PROB birds (Table, 6). There were no significant differences in the counts of coliform and *C. perfringens* in both the ileal and cecal digesta of broilers.

Table 5. Effects of zinc bacitracin and *Bacillus* mixture on serum biochemical parameters and antibody titer in broiler chicks

Items#	Dietary treatments†				*SE*‡	*P*-value
	CONT	AGP	PROB	PROB+AGP		
Total cholesterol (mg/dL)	133	140	142	153	14.5	0.81
HDL-c (mg/dL)	69.0	73.0	71.7	69.8	6.89	0.97
LDL-c (mg/dL)	54.8	72.6	53.2	74.2	12.4	0.51
Total triglyceride (mg/dL)	45.1	54.1	47.7	49.2	6.38	0.78
AST (U/L)	237	262	298	232	30.2	0.42
ALT (U/L)	4.27^{a}	6.28^{a}	2.15^{ab}	0.60^{b}	1.35	0.04
Total protein (g/dL)	2.67	3.15	2.63	2.63	0.20	0.21
Albumin (g/dL)	1.28	1.54	1.40	1.48	0.11	0.37
Globulin (g/dL)	1.39	1.62	1.24	1.15	0.13	0.09
A/G ratio	0.95	0.98	1.16	1.36	0.11	0.07
Uric acid (mg/dL)	4.09	4.26	7.44	4.69	1.31	0.26
Creatinine (mg/dL)	0.25^{a}	0.30^{a}	0.16^{ab}	0.07^{b}	0.04	<0.01
Antibody titer against NDV (Log_2 GMT)	2.60	2.60	3.60	2.20	0.64	0.47

^{a,b}Means in the row with different letters show significant differences ($P < 0.05$).
†CONT: basal diet without any supplementation, AGP: basal diet with 0.04% zinc bacitracin, PROB: basal diets supplemented with 0.5% *Bacillus* mixture, PROB+AGP: basal diets supplemented with 0.5% *Bacillus* mixture and 0.04% zinc bacitracin; ‡SE: standard error.
#HDL-c: high-density lipoprotein cholesterol, LDL-c: low-density lipoprotein cholesterol, AST: aspartate aminotransferase, ALT: alanine aminotransferase, A/G ratio: albumin to globulin ratio, NDV: Newcastle disease virus; GMT: geometric mean titer.

Table 6. Effects of zinc bacitracin and *Bacillus* mixture on selected bacterial populations in the intestine of broiler chicks

Items (log cfu/g)	Dietary treatments†				*SE*‡	*P*-value
	CONT	AGP	PROB	PROB+AGP		
Ileum						
Coliform	7.25	7.14	7.00	7.05	0.12	0.46
C. perfringens	5.42	6.29	4.71	5.48	0.61	0.37
LAB#	9.23^{ab}	9.10^{b}	9.42^{ab}	9.65^{a}	0.13	0.05
Caecum						
Coliform	7.21	7.13	7.45	7.51	0.15	0.24
C. perfringens	6.35	5.83	6.12	7.36	0.51	0.19
LAB#	9.42	9.56	9.54	9.78	0.15	0.41

^{a,b}Means in the row with different letters show significant differences ($P < 0.05$).
†CONT: basal diet without any supplementation, AGP: basal diet with 0.04% zinc bacitracin, PROB: basal diet supplemented with 0.5% *Bacillus* mixture, PROB+AGP: basal diet supplemented with 0.5% *Bacillus* mixture and 0.04% zinc bacitracin; ‡*SE*: standard error; #LAB: lactic acid bacteria.

Concentrations of short chain fatty acids in cecal digesta of broiler chicks

The concentrations of SCFAs in cecal digesta of broilers did not differ across treatment groups (Table, 7).

Carcass traits of broiler chicks

Table 8 shows the carcass characteristics of broiler chicks. Birds in AGP group had higher ($P = 0.05$) relative weight of breast compared to other birds. No significant differences were observed in the relative weight of eviscerated carcass, breast, thigh, drumstick, wing, and abdominal fat of broilers. Crude protein and fat contents of breast meat were also similar across treatments.

Table 7. Effects of zinc bacitracin and *Bacillus* mixture on concentrations of short chain fatty acids in cecal digesta of broiler chicks

Items (mmol/kg)	Dietary treatments†				*SE*‡	*P*-value
	CONT	AGP	PROB	PROB+AGP		
Acetic acid	76.1	65.0	62.8	66.3	9.70	0.77
Propionic acid	36.0	34.0	34.0	34.6	4.92	0.99
Butiric acid	21.2	22.7	23.7	21.9	3.81	0.97

†CONT: basal diet without any supplementation, AGP: basal diet with 0.04% zinc bacitracin, PROB: basal diet supplemented with 0.5% *Bacillus* mixture, PROB+AGP: basal diet supplemented with 0.5% *Bacillus* mixture and 0.04% zinc bacitracin; ‡*SE*: standard error.

Table 8. Effects of zinc bacitracin and *Bacillus* mixture on carcass characteristics of broiler chicks

Items	Dietary treatments†				*SE*‡	*P*-value
	CONT	AGP	PROB	PROB+AGP		
	% live weight					
Eviscerated carcass	60.2	63.4	61.5	61.3	1.58	0.56
	% Eviscerated carcass					
Breast	32.0[b]	33.8[a]	31.9[b]	31.7[b]	0.54	0.05
Thigh	16.4	15.6	16.8	16.8	0.46	0.26
Drumstick	15.4	15.4	15.7	15.1	0.76	0.98
Wing	14.2	13.6	14.3	14.8	0.44	0.31
Abdominal fat	0.69	0.99	0.81	0.61	0.25	0.73
	% proximate analysis					
Crude protein	23.2	23.7	22.7	23.1	0.38	0.34
Crude fat	0.53	0.60	0.57	0.57	0.04	0.65

[a,b] Means in the row with different letters show significant differences ($P< 0.05$).
†CONT: basal diet without any supplementation, AGP: basal diet with 0.04% zinc bacitracin, PROB: basal diet supplemented with 0.5% *Bacillus* mixture, PROB+AGP: basal diet supplemented with 0.5% *Bacillus* mixture and 0.04% zinc bacitracin; ‡*SE*: standard error.

Discussion

A number of studies have reported inferior growth performance in broilers with low hatching weight compared to those with heavier hatching weight (Butcher and Nilipour, 2002; Tona *et al*., 2004; Ulmer-Franco *et al*., 2010; Mendes *et al*., 2011; Michalczuk *et al*., 2011; Toghyani *et al*., 2011). Internal organs in broilers with low initial BW are also smaller (Sözcü and Ipek, 2017). As such, this study did not include a group of chicks with heavier hatching weight as negative control. As expected, the BW of broilers at 35 days of age in our study did not reach the target weight of Lohmann meat broilers, which have hatching weights of 42 g and weights of 2 kg on day 35 (Aviagen, 2007). The slower growth rate of birds with low hatching weights has been attributed to the slower differentiation of satellite cells due to the lower levels of myogenin and cyclin-dependent kinase inhibitor (p21) (both of which play an important role in muscle differentiation)(Andrés and Walsh, 1996). Satellite cells are the primary contributors to DNA synthesis for post-hatch muscle growth (Daughtry *et al*., 2017), so their slower differentiation may imply slower muscle hypertrophy or skeletal muscle growth of broiler chicks in later life. Supplementing of probiotic and antibiotic had no significant impact on the growth performance of birds in the present study, and the reasons for their ineffectiveness on growth performance in chicks with low initial BW remain largely unknown. Satellite cells are maximally active shortly after hatching and are very responsive to nutrition (Powell *et al*., 2013). In commercial hatcheries, broiler chicks often rely on nutrients from the remaining yolk sac during handling and transport. Indeed, chicks produced from young breeder flocks had smaller proportion of yolk sac (Vieira and Moran Jr., 1998; Ulmer-Franco *et al*., 2010). It could be possible that chicks with low initial BW may have lower availability of nutrients during the post-hatch period compared to those with heavier initial BW, which could slow proliferation and differentiation of satellite cells in broiler chicks (Li *et al*., 2012). Therefore, the growth-promoting effects of probiotics and antibiotic zinc bacitracin may be hindered by the delay in post-hatch muscle hypertrophy during the first week of broiler life, which may also slow down the growth rate of broilers at subsequent ages. Further, a number of other factors may also attenuate the growth-promoting effects of probiotics and in-feed antibiotics in the present study. For example, the quality of feed provided to the chicks contained 6.79% crude fiber, which is higher than the standard fiber content in broiler feed. In this study, there was a tendency for birds in AGP and PROB groups to have higher feed intake than birds in CONT and PROB+AGP groups. However, treatment with in-feed antibiotics or probiotics did not affect the FCR of broiler chicks. Similar results were reported by Olnood *et al*. (2015) and Mahmoud *et al*. (2017), who showed no substantial effects of antibiotic zinc bacitracin and probiotic *Bacillus subtilis*, respectively, on the FCR of broiler chicks.

Lymphoid organ weight is a common marker of immune status in broiler chickens, as the

development of immune organs is crucial for the optimal production of immunoglobulins (Heckert *et al*., 2002). In this study, the relative weight of thymus was lower in birds supplemented with *Bacillus* mixture or combination of *Bacillus* mixture and zinc bacitracin, compared to birds fed zinc bacitracin. Previous studies revealed an increase in thymus weight in birds following infections. For example, thymus size increased in birds after infections with *Chlamydia psittaci* HJ strain virus (Chu *et al*., 2016), H7N3 avian influenza (HPAI) virus (Kapczynsk *et al*., 2013), and infectious bursal disease virus (IBDV) (Mutinda *et al*., 2015). Therefore, the lower relative weight of thymus in birds treated with *Bacillus* mixture may be attributed to a lower degree of infections in the birds though this should be interpreted with caution as Rimondi *et al*. (2014) reported a significant reduction in thymus-to-body weight ratios in white leghorn chicks inoculated with Argentinean chicken anemia virus. Reasons for these differences across studies may be related to differences in the types or strains of birds tested, their holding conditions, the microorganisms used for infection (types, pathogenicity and doses), and the duration of infection. Nevertheless, birds in PROB and PROB+AGP tended to have lower globulin levels and higher A/G ratio when compared with those in AGP birds. This finding may support a lower infection incidence in birds supplemented with *Bacillus* mixture. Indeed, higher serum globulin and lower A/G ratio have been shown to be associated with infections in birds (e.g. with *E. coli* in broiler chicks and Pekin ducks, Sharma *et al*., 2015; Ogunbanwo *et al*., 2004; Facon *et al*., 2014). In our study, ALT enzyme levels were lower in birds in PROB+AGP and PROB compared to those in CONT and AGP groups. A decrease in serum ALT has been reported in broiler chicks in association with yeast supplementation (Aluwong *et al*., 2013) and commercial probiotics containing *Bacillus subtilis*, *Bacillus coagulans* and *Saccharomyces boulardii* (Haque *et al*., 2017). With regard to the role of vitamins and minerals, Huff *et al*. (1992) showed decreased ALT levels with supplementation of vitamin-mineral premixes in broiler chickens. Low ALT levels may be associated with a healthy liver or the absence of a toxicant-response in broiler chicks (Sugiharto *et al*., 2016). Sharma *et al*. (2015) reported that an *E. coli* infection was associated with increased ALT levels in bird serum. Hence, the lower serum ALT levels that we observed in probiotic-treated birds may be attributed to low levels of infections. In the present study, serum creatinine levels were lower in PROB+AGP and PROB birds compared to CONT and AGP birds. Previous studies by Mahmoud (2015) and Ismail (2017) showed an increase in serum creatinine levels in ducks and chickens infected by avian influenza viruses and NDV, respectively. Salim *et al*. (2011) showed a decrease in creatinine levels in broilers that were infected with *Salmonella typhimurium* but were supplemented with *Bacillus subtilis*. Probiotics seem to protect the chicks from infections or reduce the adverse effects of infections.

Results in the present study showed that treatment with *Bacillus* mixture was capable of increasing the population of LAB in the ilea of broiler chicks. Gao *et al*. (2017) also found a greater population of *Lactobacillus* in the gut of broilers fed *Bacillus subtilis*. Our data therefore suggest that *Bacillus* mixture treatment may favor the growth of good bacteria in the intestine of chicks, possibly because *Bacillus* spp. Can produce antibacterial substances/antimicrobial peptides (AMPs) (Teixeira *et al*., 2013), increase total SCFAs concentration in the ceca (Fujiwara *et al*., 2009), and lower the intestinal pH (Knap *et al*., 2011). Such conditions may inhibit the growth of harmful bacteria and support the growth of beneficial bacteria such as LAB in the intestine of broilers. In regards to the antibacterial activity of *Bacillus*-based probiotic, our study did not show a significant effect of probiotic treatment on the numbers of coliform bacteria and *C. perfringens* in the intestine of broilers. There were also no effects on the concentrations of SCFAs in the cecal contents of broiler chicks, which is similar to findings from Olnood *et al*. (2015). However, Milián *et al*. (2013) showed an increase in acetic, propionic as well as total SCFAs in the cecum of broiler chicks treated with *Bacillus subtilis*. These differences across studies may be due to differences in probiotic strains and fibre content in diets.

In-feed antibiotic treatment resulted in higher relative weights of breast muscle in broiler chicks in our study. Similarly, Izat *et al*. (1990) reported that feeding bambermycins (2.2 ppm) or bacitracin methylene disalicylate (27.5 ppm) increased the weight of carcass and breast meat in broiler chicks. Denli *et al*. (2003) showed that feeding broiler chicks the antibiotic flavomycin

resulted in higher carcass weight. However, Landy *et al*. (2011) showed no effect of flavophospholipol on the carcass weight of broiler chicks. The higher breast weight may be related to the higher carcass and live weights of broilers (Sözcü and Ipek, 2017).

Conclusion

Treatment with *Bacillus* mixture increases the intestinal population of LAB in low weight day-old broiler chicks. However, such treatment does not affect hematological parameters, growth performance, and carcass characteristics of broiler chicks with low initial BW.

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