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Effects of Dietary Fermented Garlic on the Growth Performance, Relative Organ Weights, Intestinal Morphology, Cecal Microflora and Serum Characteristics of Broiler Chickens

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ABSTRACT

The present study was conducted to evaluate the effects of feeding broilers with garlic fermented by *Leuconostoc citreum* SK2556. A total of 250 male broiler chicks was randomly housed into 25 floor pens. Five dietary treatments with five replicates of 10 chicks each (n=50 chicks/treatment). A corn and soybean meal based diet was used as the control diet (NC). The experimental diets were formulated by mixing the basal diet either with antibiotics (10 ppm; PC) or fermented garlic (FG) at the concentrations of 0.1% (FG1), 0.3% (FG3) or 0.5% (FG5) in diets. Daily weight gain, feed intake, and feed:gain ratio were not affected by any of the dietary treatments. Average daily gain on day 21 linearly increased (p=0.024) with increasing FG levels. The relative weight of the bursa of Fabricius showed a progressive decline with increasing the FG levels. Jejunal villus height was not influenced by dietary treatments. Villus width linearly decreased as FG levels increased (p=0.17). Jejunal crypt depth was significantly lower (p<0.05) in the FG1 and FG3 groups compared with the NC group. Villus height:crypt depth ratio linearly increased (p=0.018) with increasing FG levels. The population of cecal microflora was not altered by dietary treatments. Broiler chickens fed the FG5 diet exhibited (p<0.05) higher blood levels of total protein and cholesterol compared with those fed the NC diet. Collectively, the results show that dietary FG marginally affected growth performance, especially during the first days rearing, improved intestinal morphology, and altered blood characteristics of broiler chickens.

INTRODUCTION

Garlic (*allium sativum*) has long history of use as culinary or medicinal supplement. As garlic contains a sulfur volatile active component that has antibacterial, anti-inflammatory and antioxidant biological properties (Wilson and Demming-Adams, 2007), it has been explored as a potential alternative to antibiotics in poultry production. Nonetheless, inconsistent effects of garlic on the growth performance, egg production, gut physiology, nutrient digestibility, and lipid metabolism have been reported (Khan *et al.*, 2012). Garlic inclusion levels widely varied in those studies, being added at levels as low as 0.125% to as high as 5% to broiler diets.

Several attempts have been recently made to enhance garlic functionality and stability using fermentation with specific bacteria strains which are resistant to garlic, and are able to ferment it. The bacterial strains commonly used for garlic fermentation are *Lactobacillus plantarum*, *Bacillus subtilis*, *Weissella koreensis*, and *Leukonostoc mesenteroides*. Garlic fermented with either *W. koreensis* or *L. mesenteroides* equally produced higher sulfur-related metabolites, such as 3-vinyl-[4H]-1,2,-dithiin, all ylmethyl trisulfide, diallyl disulfide, diallyl trisulfide, but lower alliin compared with fresh garlic (Yan *et al.*, 2012; Hussain *et al.*, 2014).

Recent feeding trials have been tested the effect of fermented garlic on pig and broiler performance (Ao *et al.*, 2010, 2011; Kang *et al.*, 2010; Wang *et al.*, 2011; Yang *et al.*, 2012; Yan & Kim, 2013; Hossain *et al.*, 2014, 2015; Chun *et al.*, 2015). The addition of graded levels (e.g., dietary inclusion levels of 0.05, 0.1, 0.2%) of *L. mesenterodes*-fermented garlic in the diets of broiler chickens increased body weight and feed intake, but did not affect feed efficiency, when compared with the control-diet fed chickens (Hossain *et al.*, 2014, 2015). These results corroborated with the study of Kang *et al.* (2011), who used *L. plantarum* for garlic fermentation and added higher levels (1%) to a broiler diet. In contrast with the studies of Kang *et al.* (2011) and Hossain *et al.* (2014, 2015), dietary *W. koreensis*-fermented garlic failed to affect the growth performance of broiler chickens, albeit it enhanced host immunity, retarded meat lipid oxidation, and lowered serum total cholesterol and triglyceride concentrations compared with the control chickens (Ao *et al.*, 2011).

In order to further increase our understanding of the efficacy of fermented garlic, we tested whether garlic fermented by *L. citreum* SK2556, a novel patented strain isolated from an aged garlic product, could affect the growth performance of broiler chickens, and if indeed so, whether the FG-induced improvement in production performance was related to changes in intestinal morphology. To our knowledge, no studies on the effect of FG on the intestinal morphology of broiler chickens have been conducted to date. It was reported that dietary non-fermented garlic exhibited antibacterial activity and improved the intestinal morphology of broiler chickens (Abdullah *et al.*, 2010; Ham *et al.*, 2010). Thus, the present study aimed at investigating the effects of the dietary supplementation of FG on the growth performance, intestinal morphology and microbiota, and serum characteristics of broiler chickens.

MATERIALS AND METHODS

Preparation of the fermented garlic powder

The peeled garlic bulbs used in this study were purchased from a local market and ground in a mill (Philips HR 2860, Netherlands) to obtain a homogenized garlic juice. The garlic juice was then mixed with a buffer (K_2HPO_4 0.1 g/L, $MgCl_2$ 0.01 g/L, NaCl 1 g/L, CH_3COONa 1g/L) at the ratio of 30:70. The garlic and buffer mixture was sterilized by autoclaving at 121ºC for 15 min and cooled to room temperature. The mixture was then inoculated with a starter culture of 1% *Leuconostoc citreum* SK2556 in a shaking incubator at 37ºC for 24 h. The pH of the fermented garlic was 4.47. The *L. citreum* SK2556 culture contained a 1×10^8 cfu/mL. The *L. citreum* SK2556 strain used in this study was originally isolated from pickled garlic food, characterized and patented due to its ability to grow and ferment in medium containing garlic (Kim *et al.*, 2012).

Experimental design, animals and diets

A total of 250 feather-sexed male broiler chicks (Ross 308) were purchased from a local hatchery. Upon arrival, birds were individually weighed and randomly distributed into 25 floor pens with rice husks as a bedding material.

Five dietary treatments with five replicates (10 chicks per replicate, n=50 chicks/treatment) were applied. Starter and finisher diets based on corn and soybean meal were formulated (Tables 1 and 2) and used as

Table 1 – Ingredients and composition of basal diets

Item	Starter (0-21 days)	Grower (22-35 days)
Ingredient, %		
Corn	52.80	56.65
Wheat Powder	5.00	5.00
Fish meal	2.00	2.00
Giblet powder	2.00	3.00
Soybean meal	23.60	21.00
Full-fat soybean (extruded)	5.00	3.00
Limestone	1.33	1.27
Salt	0.24	0.24
Glucose	1.00	1.00
Dicalcium phosphate	1.00	1.00
Tallow	4.90	4.90
Vitamin and mineral premix[1]	0.15	0.15
L-Lysine-HCl	0.45	0.39
DL-Methionine	0.31	0.25
L-Threonine	0.07	0.05
Choline-HCl	0.05	0.05
Maduramicin	0.05	0.05
Total	100.0	100.0
Calculated nutrient composition		
Metabolizable energy, MJ/kg	13.7	13.1
Crude protein, %	21.04	20.70
Crude fat, %	8.59	8.43
Calcium, %	0.92	0.93
Available phosphorus, %	0.32	0.31

[1]Vitamin and mineral premix contains followings per kg of diet: vitamin A, 10,000,000IU; vitamin D_3, 5,000,000IU; vitamin E, 20,000IU; vitamin K_3, 3000mg; vitamin B_1, 2000mg; vitamin B_2, 6000mg; vitamin B_6, 3000mg; vitamin B_{12}, 16mg; niacin, 50,000mg; Ca-pantothenate, 13,000mg; folic acid, 13,000mg; Cu, 5000mg; I, 1250mg; Mn, 110,000mg; Zn, 100,000mg; Se, 300mg; Fe, 40,000mg; Co, 5000mg.

non-medicated control diets (negative control, NC). The experimental diets were formulated by mixing the basal diet with either 10 ppm avilamycin (positive control, PC) or three levels of fermented garlic at the concentrations of 0.1% (FG1), 0.3% (FG3), or 0.5% (FG5). All diets contained maduramicin (5 mg/kg of diet) as a coccidiostat. Diet and water were provided on an *ad-libitum* basis. The initial room temperature was set at 33ºC, gradually decreased to reach 25ºC at 15 days of age and kept constant thereafter (Kang and Kim, 2016). All animal care procedures were approved by Institutional Animal Care and Use Committee in Konkuk University.

Measurements

Body weight and feed intake were measured by pen on a weekly basis and used to calculate feed:gain ratio. On day 35, three birds per pen were randomly selected for blood sampling after euthanasia. Immediately after blood sampling, the liver, spleen, and bursa of Fabricius were excised, weighed and expressed as relative weight in grams per 100 g of body weight. For the morphological determination of the villi, 1-cm-long sections from mid-jejunum and mid-ileum were sampled. Cecal content was sampled and kept on ice until measurement of microflora on a same day of the sampling.

Intestinal morphology

Intestinal samples were fixed in 10% phosphate-buffered formalin for a minimum of 48 h, and 4.0μm sections were prepared as described elsewhere (Kim *et al.*, 2015). The sections were stained with standard hematoxylin-eosin solution and villus height, villus width (mid-point), and crypt depth were measured at 100× magnification under light microscopy. Villus surface area was calculated using the formula = (2π) × (VW/2) × (VL), where VW = villus width and VL=villus length (Sakamoto *et al.*, 2000). Twenty well-oriented villi per bird were measured and averaged for each bird.

Enumeration of the cecal microflora

Approximately 1 g of cecal contents were mixed with 9 volumes of sterilized ice-cold saline solution (w/v), vortexed, and serially diluted. A volume of 100 μL of cecal suspension was plated on the respective agar plates and incubated at 37ºC for 48 h. Colony was read and expressed as colony forming unit (cfu) per gram of cecal contents. The media used were MRS (Difco, USA), EMB (BBL™ Eosin Methylene Blue Agar, USA), and SS-agar (BBL™ Salmonella Shigella Agar, USA). No colonies were detected in SS-agar. Colonies in MRS and EMB agars were selected and confirmed by 16S rRNA by PCR analysis using commercial primers (forward primer 5'-AGA GTT TGA TCC TGG CTC AG-3' and reverse primer 5'-AAG GAG GTG ATC CAN CCR CA-3'; Lane, 1991). Nucleotide sequence from colonies in MRS exhibited 100% homology with *Bacillus licheniformis* and those in EMB with *Escherichia coli*.

Blood analysis

Plasma was used to measure albumin, total protein, GPT, GOT, GGT, total bilirubin, direct bilirubin, glucose, total cholesterol, HDL cholesterol, BUN, creatinine and uric acid. All analyses were performed at Optifarm laboratory (Cheongwong, Chungbuk, Korea).

Statistical analysis

Pen was considered an experimental unit. All data obtained was subjected to one-way analysis of variance (SPSS Ver. 21, IBM Corp., USA). If a significant effect was observed, the difference between treatments was determined by Tukey's test. Results were expressed as treatment means with their pooled SEM. In addition, linear contrasts were used to assess the effect of FG levels. A $p<0.05$ was considered statistically significant.

RESULTS

During the experimental period, none of the production-related parameters, i.e., daily body weight gain, feed intake and feed:gain ratio, were affected by the dietary treatments (Table 2). However, average daily gain on day 21 linearly increased ($p=0.024$) with increasing FG levels.

On day 35, organ weights, when expressed as relative to body weight, were not affected by the dietary treatments (Table 3); however, relative bursa weight progressively declined with increasing FG levels. Jejunal villus height did not differ among dietary treatments (Table 4). Villus width linearly decreased as FG levels increased ($p=0.17$). Jejunal crypt depth was significantly lower ($p<0.05$) in the FG1 and FG3 groups compared with the NC group. Jejunal villus area was not affected by the FG treatments. Villus height:crypt depth ratio ($p=0.056$) in the FG-fed groups tended to be higher ($p=0.0558$) compared with the NC group. Villus height:crypt depth ratio linearly increased ($p=0.0181$) with increasing FG levels. In contrast, villus height and width, villus surface area, crypt depth and villus height:crypt depth ratio in the ileum were not

Table 2 – Effect of fermented garlic on the growth performanceof broiler chickens[1,2]

Item	NC	PC	FG1	FG3	FG5	SEM	P	L
1-21 days								
Average daily gain, g/d/bird	34.57	34.63	35.86	36.70	36.88	0.715	0.104	0.024
Feed intake, g/d/bird	55.73	54.57	55.41	52.63	56.40	1.597	>0.50	>0.50
Feed:gain ratio, g/g	1.615	1.574	1.548	1.435	1.530	0.042	0.088	0.078
22-35 days								
Average daily gain, g/d/bird	70.69	69.22	68.52	69.43	66.26	2.577	>0.50	0.443
Feed intake, g/d/bird	100.28	96.14	95.30	95.65	97.42	2.167	>0.50	>0.50
Feed:gain ratio, g/g	1.430	1.389	1.392	1.388	1.476	0.054	>0.50	>0.50
1-35 days								
Average daily gain, g/d/bird	49.05	48.95	49.04	46.93	48.58	1.566	>0.50	>0.50
Feed intake, g/d/bird	76.63	70.77	70.56	66.46	71.63	2.371	0.465	>0.50
Feed:gain ratio, g/g	1.481	1.446	1.439	1.424	1.475	0.040	>0.50	>0.50

[1]NC, negative control; PC, positive control (antibiotic at 10 ppm); FG1, fermented garlic at 0.1%; FG3, fermented garlic at 0.3%; FG5, fermented garlic at 0.5%; SEM, pooled standard error of the mean; P, *p*-value analyzed by ANOVA ($p<0.05$); L, linear effect of dietary FG.

[2]Mean values are presented (n=5, each group).

Table 3 – Effect of fermented garlic on the relative organ weights of broiler chickens[1,2]

Item	NC	PC	FG1	FG3	FG5	SEM	P	L
Liver, g/100g BW	2.478	2.522	2.491	2.700	2.600	0.193	>0.50	0.319
Spleen, g/100g BW	0.127	0.122	0.126	0.116	0.148	0.020	0.287	0.308
Bursa of Fabricius, g/100g BW	0.244	0.219	0.220	0.212	0.193	0.024	0.158	0.018
Abdominal fat, g/100g BW	1.515	1.690	1.546	1.797	1.569	0.187	0.426	0.440

[1]NC, negative control; PC, positive control (antibiotic at 10 ppm); FG1, fermented garlic at 0.1%; FG3, fermented garlic at 0.3%; FG5, fermented garlic at 0.5%; SEM, pooled standard error of the mean; P, *p*-value analyzed by ANOVA ($p<0.05$); L, linear effect of dietary FG.

[2]Mean values are presented (n=5, each group).

Table 4 – Effect of fermented garlic on the intestinal morphology of broiler chickens[1,2]

Item	NC	PC	FG1	FG3	FG5	SEM	P	L
Jejunum								
Villus height, µm	896.1	827.7	753.7	734.5	1005.4	76.391	0.120	0.087
Villus width, µm	130.0	120.7	116.5	104.2	92.1	9.217	0.074	0.001
Villus area, mm^2	0.36	0.32	0.28	0.24	0.29	0.04	0.270	0.189
Crypt depth, µm	178.6^a	148.8^{ab}	108.6^b	108.3^b	133.5^{ab}	16.279	0.033	0.074
Villus height:crypt depth ratio, µm:µm	5.14	5.78	7.19	7.18	7.89	0.668	0.055	0.018
Ileum								
Villus height, µm	644.4	606.1	519.4	531.1	622.0	37.277	0.102	0.075
Villus width, µm	147.4	159.1	153.2	170.5	152.8	13.725	>0.50	>0.50
Villus area, mm^2	0.29	0.30	0.28	0.29	0.30	0.032	>0.50	>0.50
Crypt depth, µm	189.5	177.3	137.7	143.8	173.8	20.620	0.356	0.244
Villus height:crypt depth ratio, µm:µm	3.55	3.74	3.97	3.90	3.77	0.506	>0.50	>0.50

[1]NC, negative control; PC, positive control (antibiotic at 10 ppm); FG1, fermented garlic at 0.1%; FG3, fermented garlic at 0.3%; FG5, fermented garlic at 0.5%; SEM, pooled standard error of the mean; P, *p*-value analyzed by ANOVA ($^{a,b}p<0.05$); L, linear effect of dietary FG.

[2]Mean values are presented (n=5, each group).

affected by the dietary treatments. Neither PC or FG vs. NC affected the population of cecal microflora (Table 5). It was confirmed that the nucleotide sequence from colonies in MRS exhibited 100% homology with *Bacillus licheniformis* and those in EMB with *Escherichia coli*. None of dietary treatments affected blood parameters, such as albumin, total or direct bilirubin, GGT, uric acid, or glucose (Table 6). Creatinine, BUN, GPT, and GOT were not influenced by dietary FG compared with the NC group. Broiler chickens fed the FG5-added diet exhibited significantly higher total protein and cholesterol concentration compared with those fed the NC diet.

DISCUSSION

The present study aimed at investigating if garlic fermented by *L. citreum* SK2556, a novel patent strain isolated from prickled garlic product, could affect the growth performance and the intestinal morphology of

Table 5 – Effect of fermented garlic on the cecal microflora of broiler chickens[1,2]

Item	NC	PC	FG1	FG3	FG5	SEM	P	L
Lactic acid bacteria, cfu/g	10.99	10.53	10.82	10.53	10.95	0.139	0.112	>0.50
Escherichia coli, cfu/g	10.82	10.44	10.79	10.64	10.53	0.195	>0.50	0.350

[1]NC, negative control; PC, positive control (antibiotic at 10 ppm); FG1, fermented garlic at 0.1%; FG3, fermented garlic at 0.3%; FG5, fermented garlic at 0.5%; SEM, pooled standard error of the mean; P, *p*-value analyzed by ANOVA ($p<0.05$); L, linear effect of dietary FG.

[2]Mean values are presented (n=5, each group).

Table 6 – Effect of fermented garlic on the blood parameters of broiler chickens[1,2]

Item	NC	PC	FG1	FG3	FG5	SEM	P	L
Albumin, g/dl	0.69	0.72	0.55	0.79	0.67	0.070	>0.50	0.184
Total protein, g/dL	4.18	3.89	4.03	4.13	6.41	0.439	0.398	0.007
Total bilirubin, mg/dL	0.89	1.05	0.91	1.02	0.93	0.053	>0.50	0.341
Direct bilirubin, mg/dL	0.35	0.43	0.35	0.39	0.36	0.022	>0.50	>0.50
Uric acid, mg/dL	6.36	6.44	7.06	6.61	6.31	0.250	>0.50	0.603
Creatinine, mg/dL	0.51[ab]	0.49[ab]	0.47[b]	0.53[a]	0.53[a]	0.015	0.021	0.051
BUN, mg/dL	1.94	1.96	1.61	2.10	2.26	0.076	0.093	0.001
GPT, U/L	35.2[ab]	31.7[b]	26.3[b]	37.2[ab]	58.9[a]	5.983	0.006	<0.001
GOT, U/L	238.2	195.1	151.7	267.4	241.3	19.688	0.342	0.002
GGT, U/L	43.22	49.73	39.99	44.15	45.91	3.882	>0.50	>0.50
Glucose, mg/dL	326.9	316.3	304.4	318.0	310.7	5.300	>0.50	0.181
Total cholesterol, mg/dL	137.5	143.6	145.9	139.2	155.5	2.364	0.118	0.001
HDL cholesterol, mg/dL	75.0	70.7	70.3	80.6	80.2	3.045	0.158	0.045

[1]NC, negative control; PC, positive control (antibiotic at 10 ppm); FG1, fermented garlic at 0.1%; FG3, fermented garlic at 0.3%; FG5, fermented garlic at 0.5%; SEM, pooled standard error of the mean; BUN, blood urea nitrogen; GPT, glutamic pyruvic transaminase; GOT, glutamic oxaloacetic transaminase; GGT, gamma glutamyl transferase; HDL, high-density lipoprotein; P, *p*-value analyzed by ANOVA ([a,b]$p<0.05$); L, linear effect of dietary FG.

[2]Mean values are presented (n=5, each group).

broiler chickens. Earlier, we isolated *L. citreum* SK2556 strain from pickled garlic food, characterized and patented due to its ability to grow and ferment in a medium containing garlic (Kim *et al.*, 2012). In contrast with the strain used in this study, previously reported strains (i.e., *L. plantarum*, *B. subtilis*, *W. koreensis*, and *L. mesenteroides*) were not isolated from garlic, nor detailed information on their garlic-fermenting capacity was informed. However, earlier studies (Wang *et al.*, 2011; Hossain & Kim, 2014) reported that both *W. koreensis*- and *L. mesenteroides*-fermented garlic produced equal amounts of active metabolites, such as alliin, allylmethyltrisulfide, diallyltrisulfide, diallyldisulfide, and 3-vinyl-[4H]-1,2-dithiin.

The addition of FG to the broiler diets linearly increased average weight gain between 1-21 days, which partially explains the FG-induced decrease in feed:gain ratio. The results of the present study confirm the findings of previous studies (Choi *et al.*, 2010; Kang *et al.*, 2010; Ao *et al.*, 2011; Onyimonyi *et al.*, 2012; Hossain *et al.*, 2014, 2015) showing that neither FG or non-fermented garlic adversely affected the feed intake in broiler chickens. Thus, the underlying mechanism of the FG-induced increase in daily weight gain is likely the consequence of improvements in nutrient digestibility, especially at early ages. The latter assumption is supported by Issa & Abo Omar (2012), who reported that dietary garlic powder at the levels of 0.2 or 0.4% increased CP and EE digestibility in broiler chickens, although they did not find any garlic-mediated increase in growth performance. It was previously reported that dietary FG significantly increased body weight gain and feed:gain ratio, without any effect on feed intake, when added at the levels of 0.2% (Hossain *et al.*, 2014, 2015) or 1% (Kang *et al.*, 2010)in broiler diets.

In this study, we found that dietary FG significantly reduced jejunal crypt depth compared with the NC group. The ratio of villus height:crypt depth in jejunum linearly increased with increasing FG levels. The decrease in crypt depth observed in this study may indicate that FG may reduce villus turnover rate, and therefore, that relatively less energy is used for new tissue synthesis (Abdullah *et al.*, 2010), ultimately reducing energy requirements to maintain villus integrity. Given that villus:crypt ratio is an indicator of the digestive capacity of the small intestine (i.e., jejunum), the increased ratio observed in this study may provide the evidence that FG may play a role in digestion and absorption, as reported elsewhere (Adibmoradi *et al.*, 2006).Therefore, the question is how dietary FG affects intestinal morphology? One plausible explanation would be the effect of FG on gut microflora. Unfortunately, neither dietary FG or in-feed antibiotic (i.e., PC) affected the cecal microflora in this study. If this holds true for the jejunum, then the next plausible explanation is the direct impact of effectors present in the FG preparation on gut function, as also proposed by Abdullah *et al.* (2010).

To our surprise, we found that relative weight of bursa of Fabricius was linearly declined with increasing FG levels. This is in sharp contrast to the findings of Rahimi *et al.* (2011), who reported that dietary garlic significantly increased the relative weight of bursa of Fabricius compared with the non-medicated NC diet-fed chickens. In addition, Hossain *et al.* (2014, 2015) found no effect of *L. mesenteroides*-fermented garlic on the relative bursa weight of broiler chickens. At this stage, we do not know whether FG-mediated decrease in bursa weight impaired host immunity as we did not measure any immune-related parameters. Enhanced immune modulation by dietary garlic has been well established in broiler chickens (Ao *et al.*, 2011; Kim *et al.*, 2013) and it is considered the putative underlying mechanism in the garlic-mediated improvement of *Eimeria*-induced growth depression (Arczewska-Wlosek and Swiatkiewicz, 2013; Kim *et al.*, 2013). Similarly, the enhanced host innate and/or acquired immunity by plant-derived phytochemicals is known to prevent *Eimeria* infection in broiler chickens (Lee *et al.*, 2011; Kim *et al.*, 2013). Further studies are warranted to see whether dietary FG can alter host immune response in broiler chickens or render chickens resistant or susceptible to enteric pathogens, such as *Eimeria* spp. or *Clostridium perfringens*.

Blood parameters were not different between the NC and PC groups. This was expected, as antibiotics are not absorbed in the intestine, therefore are less likely to exhibit direct systematic effect. On the other hand, the addition of FG to the diet altered various blood characteristics of the evaluated broilers. First, higher serum total cholesterol levels were determined in the FG5 *vs*. NC groups, which is contrary to the well-established hypocholesterolemic effect of garlic (Chi *et al.*, 1982; Konjufca *et al.*, 1997; Ao *et al.*, 2010, 2011; Khan *et al.*, 2012). This contradictory result may be related to the added levels of garlic or to the fat source used. Chi *et al.* (1982) concluded that the effective garlic level to lower cholesterol in rats is approximately 2% when diets contain either exogenous cholesterol or 15% lard as a fat sources. Ao *et al.* (2010) also observed the hypocholesterolemic effect of fresh garlic added at the concentration of 2% to layer diets. In another report by Ao *et al.* (2011), the addition of *W. koreensis*-fermented garlic at 0.2% to a broiler diet, containing soybean oil as the single fat source, significantly lowered serum cholesterol compared with the NC group. On the other hand, adding garlic at 0.3% or 0.6% to broiler diets containing approximately 2% of soybean oil did not affect serum cholesterol levels in broiler chickens (Amouzmehr *et al.*, 2012). Whether FG at low levels in diets containing either animal fat or plant oil as a single fat ingredient would exhibit identical effect on lipid metabolism needs to be clarified. The higher total serum protein levels in FG5 compared with NC seems secondary to the increased cholesterol levels.

In conclusion, dietary FG linearly increased average daily gain during the starter phase (1-21 days) and decreased the relative weight of the bursa of Fabricius of 35-d-old broiler chickens. Jejunal, but not ileal, crypt depth was significantly lower both in the FG1 and FG2 groups compared with the NC group. Consequently, villus height:crypt depth ratio in the jejunum linearly increased as FG levels increased. Finally, dietary FG significantly increased serum total protein and cholesterol concentrations compared with the NC group. Further studies are warranted to determine of dietary FG can modulate local or systemic immune responses in naïve chicken or, preferentially, in enteric disease chicken models, and affect lipid metabolism in the presence or absence of exogenous cholesterol in the diet.

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