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Effects of dietary supplementation of 4-O-methyl-glucuronoarabinoxylan on growth performance, thigh meat quality and development of small intestine in female Partridge-Shank broilers

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Abstract

Recently, *Echinacea purpurea* and its extracts have gained much interest due to their improvement on meat quality, but little information is available on the application of the purified *Echinacea purpurea* polysaccharide (4-O-methyl-glucuronoarabinoxylan, 4OMG). Thus, this trial aimed at assessing the effects of dietary supplementation of 4OMG on growth performance, thigh meat quality and small intestine development of broilers. A total of 240 1-day-old female broiler chicks were randomly distributed to four groups with three replicates of 20 within each group. Each group received either 0, 15, 20 or 25 g 4OMG/kg DM of diet. During the entire experiment, broilers had ad libitum access to water and feed, and the feed intake was recorded daily. All broilers were weighed before and end of the experiment. For each group, three pens with a total of 20 broilers were randomly selected to slaughter after 30 days. Increasing dietary supplementation of 4OMG linearly increased final live weight and daily body weight gain ($P = 0.013$) of broilers, Gain-to-Feed ratio ($P < 0.001$), muscle pH ($P = 0.024$) and redness ($P = 0.001$), but decreased drip loss ($P = 0.033$), shear force value ($P = 0.004$) and hardness ($P = 0.022$) of the thigh meat. Broilers fed diet with higher 4OMG had greater weight index, villus height and ratio of villus height to crypt depth in both duodenum and jejunum. These results indicated that increasing dietary supplementation of 4OMG was beneficial for growth performance, meat quality and development of the small intestine of broilers.

Introduction

Chicken, a favourite meat for consumers, contains around 20 g protein and 5 g fat per 100 g raw meat without skin (Naji *et al.*, 2013). In addition, per 100 g raw meat of chicken may also deliver around 48 µg Vitamin A, 50 µg Vitamin B_1, 90 µg Vitamin B_2, 670 µg Vitamin E and around 9 mg calcium and 156 mg phosphorus (Zhang and Sun, 2008). Alternatively, chicken contains various other essential minerals and vitamins (Jinap *et al.*, 2013). Due to these nutritional characteristics, chicken has become one of the most affordable meat sources and its consumption more than doubled between 1970 and 2004, from 27.4 pounds per person to 59.2 pounds (boneless, edible weight) (Buzby and Farah, 2006).

Part of the rise in chicken consumption results from the chicken industry's response to demands by consumers in the nutritional quality of chicken. Oxidative rancidity is one of the major causes of deterioration in chicken for consumption, and it usually causes loss in texture and nutritional value of chicken (Colindres and Brewer, 2011). Therefore, any measures that enhance antioxidative function in broilers to improve the nutritional quality of chicken is essential. Phytogenic substances and extracts have a wide range of activities in animal (e.g. on digestive, immune and endocrine systems) and show physio-pathological (anti-inflammatory, anti-oxidative) and anti-microbial activities (static and cidal) (Nasir and Grashorn, 2010), which has been suggested as good antioxidants and has been used as feed additives in broilers production (Lee *et al.*, 2013). *Echinacea purpurea* L. (EP), a herb often used in animal feed, is well known for its immunological activity (Goel *et al.*, 2005). The most relevant compounds so far identified in the standardized derivatives of EP are alkamides, polyacetylenes, caffeic acid derivatives (echinacoside and cichoric acid), glycoproteins and polysaccharides (Bauer, 1998). Polysaccharides are polar constituents of EP, and purified polysaccharides with immunostimulatory properties have been isolated with systematic fractionation of the aerial parts of EP and subsequent pharmacological testing of the aqueous extracts (Wagner and Proksch, 1981). Despite the fact that EP polysaccharides have been shown to stimulate phagocytosis both *in vitro* and *in vivo* (Stimpel *et al.*, 1984; Lohmann-Matthes and Wagner, 1989), there is not

enough data available on the application of EP polysaccharides in livestock species, especially in poultry.

Small intestine is one of the important sites of nutrient absorption of poultry, and its better development may be beneficial for nutrient absorption (Mekbungwan *et al.*, 2002) as well as better performance of poultry (Swiatkiewicz and Hanczakowska, 2006). Wang *et al.* (2014) reported that EP polysaccharides may stimulate proliferation of intestinal epithelial cells in rat. Therefore, we hypothesized that dietary supplementation of EP polysaccharides in broilers may have beneficial effects on the development of small intestine, thereby resulting in improvement of performance and meat quality of broilers. Thus, this study aimed at assessing the effects of dietary supplementation of purified EP polysaccharide (4-O-methyl-glucuronoarabinoxylan, 4OMG) on growth performance, thigh meat quality and small intestine development of broilers.

Materials and methods

Broilers and management

Before the onset of the trial, all birds were individually wing tagged, and 20 broilers were randomly distributed to an experimental cage as a pen level with about 3.6 m^2 of area. The experimental cages were floored with stretch mesh and were provided with nipple drinkers and trough feeders. The lighting program was set at natural light. The temperature was decreased from 34°C at the beginning to 25°C at the end of the experiment. Relative humidity ranged from 55% to 60%, and other experimental parameters were approved by the Animal Ethics Committee of Anhui Science and Technology University (Fengyang, China). The broilers were vaccinated routinely against infectious bronchitis and Newcastle disease, but no type of medication was administered during the entire experimental period.

Experimental design

In the current experiment, a total of 240 1-day-old female broiler chicks (Partridge-Shank) were randomly distributed to four groups of 60 birds each. Partridge-Shank is a broiler with relatively smaller live weight, which has a unique taste and is popular among consumers, for detailed information see https://baike.so.com/doc/6305673-6519201.html. For each group, three replicates were conducted and 20 broilers were assigned to an experimental pen. Broilers had *ad libitum* access to the maize-soybean meal diet and water, and the diet was offered three times daily at 06:00, 12:00 and 18:00 h, respectively. The nutritional values of the maize-soybean meal diet corresponded to the NRC (1994) recommended requirements for broilers (Table 1). Based on dry matter (DM) of the formulated diet, each group received 4OMG (Dalian Macro Long International Trade Co., Dalian, China) dosages of 0, 15, 20 and 25 g/kg on DM of the diet. In the present trial, the used polysaccharide power (with 4OMG mass fraction higher than 90%) was extracted from above-ground parts of EP plant through systematic fractionation, ion-exchange and isolation. Due to lack of data for 4OMG, the present dosage of 4OMG power was selected according to Lee *et al.* (2013), in which broilers were supplemented with EP power ranged from 0 to 20 g/kg on DM basis of diet. During the experiment, feed intake was recorded daily by weighing feed offered and refused at the pen level. Before and end of the experiment, all broilers were weighed and the live weight was recorded to calculate the daily body weight gain and Gain-to-Feed ratio. The experiment lasted 30 days and at the end, three pens with a total of 20 broilers (each pen 6 or 7 broilers) of each group were randomly selected and slaughtered to determine the thigh meat quality and the development of small intestine.

Table 1. Ingredient and chemical composition of the basal diet fed to broilers

Item	g/kg dry matter (DM) of feed
Ingredient	
Maize meal	598.80
Soybean meal	340.00
Fish meal	30.00
Dicalcium phosphate	14.00
Limestone	12.00
Salt	2.80
DL-Methionine	1.10
Multi-vitamins[a]	0.30
Micronutrients[b]	1.00
Nutritional value of the diet fed to broilers	
Metabolizable energy[c] (MJ/kg DM)	12.12
Crude protein (g/kg DM)	217.04
Calcium (g/kg DM)	9.62
Available phosphorus (g/kg DM)	7.03
Lysine (g/kg DM)	11.01
Methionine (g/kg DM)	4.54

[a]Per kilogram multivitamins provided Vitamin A, 54 000 IU; Vitamin B_1, 2.0 mg; Vitamin B_2, 15.0 mg; Vitamin B_{12}, 30 mg; Vitamin D_3, 10 800 IU; Vitamin E, 1.5 mg; Vitamin K_3, 5.0 mg; nicotinic acid, 500 mg; and calcium pantothenate, 25 mg.
[b]Per kilogram micronutrients provided Cu, 1.0 g; Fe, 3.0 g; Se, 0.152 g; Zn, 3.4 g; I, 0.132 g; and Mn, 13.40 g.
[c]Metabolizable energy was estimated according to NRC (1994).

Chemical analysis

The compositions and chemical analyses of the diet fed to broilers are presented in Table 1. According to AOAC (2012), diet samples were analysed for DM (method 930.15), crude protein (method 990. 03), calcium and phosphorus (method 985.01) after ash sample preparation (method 975.03). Metabolizable energy was estimated according to NRC (1994), while dietary amino acids were analysed according to the method of Llames and Fontaine (1994).

Determination of thigh muscle pH value and colour

Thigh muscles (consisting of the biceps femoris, semitendinosus, semimembranosus and tensor facia latae) for analysis of meat quality were excised immediately after slaughter and stored at 4°C. The pH values of the thigh meat were measured 45 min after slaughter by inserting the electrodes of a portable meter (HI8424, Beijing Hanna Instruments Science & Technology Co. Ltd, Beijing, China) into the multiple thigh muscles. A colorimeter (Spectrophotometer, CR-10, Minolta, Tokyo, Japan) was used to measure the meat colour, in terms L* (lightness), a* (redness), and b* (yellowness), perpendicular to the muscles surface.

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Determination of water-holding capacity

The water-holding capacity of the thigh muscles was estimated using drip loss, and the drip loss was determined as described by Ren *et al.* (2019). Thigh muscles were trimmed to 5 cm × 3 cm × 1 cm and blotted to remove the surface water. They were placed in air-filled plastic bags, which were fastened to avoid evaporation and hung vertically in a refrigerator at 4°C for 24 h. The thigh samples were weighed before and after this procedure, and the drip loss was calculated as follows:

$$\text{Drip loss (g/100 g)} = 100 \times \frac{\text{initial thigh muscle weight (g)} - \text{final muscle fillet weight (g)}}{\text{initial thigh muscle weight (g)}} \quad (1)$$

Texture profile analysis

The texture of the cooked thigh muscle was determined according to both the recommended Texture Profile Analysis tests guidelines (Overview of Texture Profile Analysis, http://texturetechnologies.com/resources/texture-profile-analysis) and the method described by Naji *et al.* (2013). The meat samples were homogenized through 6 mm plates, then chicken thigh meat patties (5 cm diameter, 2 cm thickness and 30 g weight) were prepared. Half of each thigh patty was cooked to an internal temperature of 75°C to prepare the cooked meat samples. The centres of the meat samples were compressed twice to 90% of their original height with a texture analyser (Model A-XT2, Stable Micro Systems, Surrey, UK) attached to a needle (15 mm diameter) at a test speed of 1.00 mm/s and a trigger force of 0.001 kg and the holding time between compressions was 5 s.

In the present trial, hardness was expressed as the maximum force of the first compression, cohesiveness was expressed as the area of work during the second compression divided by the area of work during the first compression, and springiness was measured by the distance of the detected height during the second compression divided by the original compression distance. Gumminess and chewiness were calculated as follows:

$$\text{Gumminess } (g) = \text{Hardness } (g) \times \text{Cohesiveness} \quad (2)$$

$$\text{Chewiness } (g) = \text{Gumminess } (g) \times \text{Springiness} \quad (3)$$

Estimation of tenderness

In this trial, the tenderness of the uncooked thigh muscles was estimated using the shear force value as described by Ren *et al.* (2019). Before measurement, the thigh muscles were split into strips with size of 3.0 cm × 1.0 cm × 0.5 cm parallel to the muscle fibre. Then the thigh muscles were sheared perpendicular to the muscle fibre at a head speed of 200 mm/min with a digital muscle tenderness metre (C-LM3B, Northeast Agricultural University, Haerbin, China), and the max of force required to shear the cores was recorded in Newton (N).

Intestine histological analysis

After determination of weight and length, the small intestine samples were immediately spread as follows from: (1) the apex of the duodenum, (2) midway between the point of entry of the bile ducts and Meckel's diverticulum (jejunum) and (3) 10 cm proximal to the cecal junction (ileum). The removed tissues were fixed with 10% buffered formalin, then stained with haematoxylin and eosin and embedded in paraffin. The villus height (from the tip of the villi to the villus crypt junction) and crypt depth (defined as the depth of the invagination between adjacent villi) were evaluated under a light microscope. Data were acquired with a Carl Zeiss Axioskop microscope (Carl Zeiss GmbH, Jena, Germany) and a ZVS-47DE CDD camera (Optronics Inc., Goleta, USA) connected by an RGB line to a GraBIT PCI graphic card (Soft Imaging System GmbH, Munster, Germany) installed in a standard PC computer.

Statistical analysis

In the present trial, performance of broilers including live weights, daily body weight gain, feed intake and Gain-to-Feed ratio were calculated based on pen, while thigh meat quality and development of small intestine were calculated based on the individual bird.

The data were analysed with the PROC MIXED procedure of SAS 9.4 (Statistical Analysis for Windows, SAS Institute Inc., Cary, NC). Linear and quadratic effects of treatments indicated by orthogonal contrasts were used to evaluate effects of 4OMG supplementation. Duncan's multiple range test was conducted to determine the significance level of the particular comparison between treatment means, and the difference was considered significant at $P \leqslant 0.05$. The model including random and fixed effects was as follows:

$$Y_{ij} = \mu + B_i + P_j + \varepsilon_{ij} \quad (4)$$

where Y_{ij} is the dependent variable, μ is the overall mean, B_i is the random effect of broilers, i is the experimental unit, which will be pen ($n = 3$) for performance variables and individual bird ($n = 20$) for quality measurements. P_j is the fixed effect of 4OMG ($j = 0$, 15, 20 or 25 g/kg) and ϵ_{ij} is the error term.

Results

Increasing dietary supplementation of 4OMG linearly increased final live weight ($P = 0.013$) and daily body weight gain ($P = 0.013$) of broilers as well as Gain-to-Feed ratio ($P < 0.001$), but did not significantly influence the daily feed intake of broilers (Table 2).

Increasing dietary supplementation of 4OMG in broilers linearly increased muscle pH value ($P = 0.024$) and redness ($P = 0.001$), accompanied with lower drip loss ($P = 0.033$) and shear force value ($P = 0.004$) (Table 3). Increasing supplementation of 4OMG in broilers diet linearly decreased hardness of cooked thigh meat ($P = 0.022$), but did not significantly influence cohesiveness, springiness, gumminess and chewiness (Table 4).

Increasing dietary 4OMG supplementation in broilers quadratically increased duodenal weight index ($P = 0.046$) and villus height ($P = 0.004$) as well as ratio of villus height to crypt depth ($P = 0.028$) (Table 5). As shown in Table 6, increasing supplementation of 4OMG in broilers diet quadratically increased jejunal weight index ($P = 0.030$) and villus height ($P = 0.005$). Supplementation of 4OMG tended to increase the ratio of jejunal

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Table 2. Effects of dietary supplementation of 4-O-methyl-glucuronoarabinoxylan (4OMG) on the performance of 30-day-old broilers

	Dietary supplementation of 4OMG (g/kg DM)				SEM	*P* value[a]		
Item	0	15	20	25		Contrast	Linear	Quadratic
Final live weight (kg/pen)	10.2	10.6	11.7	11.3	0.35	0.026	0.013	0.268
Daily body gain (kg/pen)	0.34	0.35	0.39	0.38	0.011	0.026	0.013	0.268
Feed intake (kg DM/pen)	0.74	0.77	0.82	0.80	0.026	0.114	0.083	0.413
Gain-to-Feed ratio (kg/kg)	0.45	0.46	0.48	0.47	0.001	< 0.001	< 0.001	0.009

Results are given as means of three pens corresponding to 60 broilers in each treatment.
[a]Contrast, supplemental effect of 4OMG; Linear, linear effect of 4OMG; Quadratic, quadratic effect of 4OMG.

Table 3. Effects of dietary supplementation of 4-O-methyl-glucuronoarabinoxylan (4OMG) on thigh muscle pH, water-holding capacity and meat colour of 30-day-old broilers

	Dietary supplementation of 4OMG (g/kg DM)				SEM	*P* value[a]		
Item	0	15	20	25		Contrast	Linear	Quadratic
Muscle pH_{45min}	5.92	6.03	6.01	6.12	0.031	0.118	0.024	0.484
L* (lightness)	40.6	40.6	39.8	39.1	0.77	0.419	0.154	0.626
a* (redness)	10	13	17	18	1.4	0.004	0.001	0.500
b* (yellowness)	18	18	20	20	1.3	0.446	0.235	0.906
Drip loss (g/100 g)	4.1	3.7	3.2	3.6	0.22	0.033	0.094	0.045
Shear force value (Newton)	16	16	11	12	1.1	0.027	0.004	0.693

Results are given as means of three pens corresponding to 20 broilers in each treatment.
[a]Contrast, supplemental effect of 4OMG; Linear, linear effect of 4OMG; Quadratic, quadratic effect of 4OMG.

Table 4. Effects of dietary supplementation of 4-O-methyl-glucuronoarabinoxylan (4OMG) on cooked thigh meat texture of 30-day-old broilers

	Dietary supplementation of 4OMG (g/kg DM)					*P* value[a]		
Item	0	15	20	25	SEM	Contrast	Linear	Quadratic
Hardness (g)	331	324	255	275	20.4	0.070	0.022	0.517
Cohesiveness	0.21	0.20	0.23	0.22	0.032	0.931	0.945	0.761
Springiness	0.12	0.10	0.13	0.11	0.014	0.368	0.588	0.736
Gumminess (g)	66	68	57	55	10.3	0.599	0.354	0.883
Chewiness (g)	5.4	4.8	4.5	4.0	0.95	0.376	0.291	0.957

Results are given as means of three pens corresponding to 20 broilers in each treatment.
[a]Contrast, supplemental effect of 4OMG; Linear, linear effect of 4OMG; Quadratic, quadratic effect of 4OMG.

Table 5. Effects of dietary supplementation of 4-O-methyl-glucuronoarabinoxylan (4OMG) on duodenum development of 30-day-old broilers

	Dietary supplementation of 4OMG (g/kg DM)					*P* value[a]		
Item	0	15	20	25	SEM	Contrast	Linear	Quadratic
Weight index[b] (g/kg)	9.1	11.3	11.4	9.2	0.70	0.492	0.470	0.046
Length (cm)	19	21	21	19	1.1	0.281	0.890	0.133
Villus height (μM)	609	711	739	661	30.3	0.009	0.181	0.004
Crypt depth (μM)	238	216	208	203	17.2	0.155	0.154	0.625
Ratio of villus to crypt	2.6	3.3	3.5	3.3	0.26	0.021	0.168	0.028

Results are given as means of three pens corresponding to 20 broilers in each treatment.
[a]Contrast, supplemental effect of 4OMG; Linear, linear effect of 4OMG; Quadratic, quadratic effect of 4OMG.
[b]Weight index was calculated as: weight index (g/kg) = duodenal weight (g)/live weight (kg).

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Table 6. Effects of dietary supplementation of 4-O-methyl-glucuronoarabinoxylan (4OMG) on jejunum development of 30-day-old broilers

	Dietary supplementation of 4OMG (g/kg DM)					*P* value[a]		
Item	0	15	20	25	SEM	Contrast	Linear	Quadratic
Weight index[b] (g/kg)	14	16	18	15	1.1	0.069	0.347	0.030
Length (cm)	23	28	26	24	2.3	0.223	0.808	0.129
Villus height (μM)	667.1	750.4	758.4	685.2	26.6	0.044	0.617	0.005
Crypt depth (μM)	209	191	203	175	12.8	0.188	0.130	0.722
Ratio of villus to crypt	3.2	3.9	3.7	3.8	0.23	0.043	0.097	0.478

Results are given as means of three pens corresponding to 20 broilers in each treatment.
aContrast, supplemental effect of 4OMG; Linear, linear effect of 4OMG; Quadratic, quadratic effect of 4OMG.
[b]Weight index was calculated as: weight index (g/kg) = jejunal weight (g)/live weight (kg).

Table 7. Effects of dietary supplementation of 4-O-methyl-glucuronoarabinoxylan (4OMG) on ileum development of 30-day-old broilers

	Dietary supplementation of 4OMG (g/kg DM)					*P* value[a]		
Item	0	15	20	25	SEM	Contrast	Linear	Quadratic
Weight index[b] (g/kg)	9.2	11.1	9.5	9.3	0.82	0.812	0.325	0.408
Length (cm)	23	25	25	25	1.4	0.164	0.273	0.383
Villus height (μM)	538	577	570	554	44.3	0.582	0.845	0.552
Crypt depth (μM)	193	187	177	178	10.4	0.285	0.248	0.721
Ratio of villus to crypt	2.8	3.1	3.2	3.1	0.25	0.162	0.291	0.319

Results are given as means of three pens corresponding to 20 broilers in each treatment.
aContrast, supplemental effect of 4OMG; Linear, linear effect of 4OMG; Quadratic, quadratic effect of 4OMG.
[b]Weight index was calculated as: weight index (g/kg) = ileal weight (g)/live weight (kg).

villus height to crypt depth ($P = 0.097$), but no significant effects on the development of ileum (Table 7).

Discussion

So far, two purified polysaccharides (PS I and PS II) with immunostimulatory properties have been isolated with systematic fractionation of the aerial parts of EP and subsequent pharmacological testing of the aqueous extracts (Wagner and Proksch, 1981). Structural analysis showed that PS I to be a 4OMG with an average MW of 35 000, whereas PS II is an acidic arabinorhamnogalactan of MW 45 000 (Proksch and Wagner, 1987). Since the types and levels of the bioactive substances in EP play key roles in its nonspecific immunomodulatory properties (Randolph *et al*., 2003), thus the present in-depth investigation on the application of 4OMG in poultry should extend our understanding of the biological nature of EP.

Roth-Maier *et al*. (2005) noted that animals' performance is mainly influenced by the health and immune status, and a weak immune system may cause low daily gains while an enhanced immune system may maximum performance. In the present trial, the greater performance of broilers receiving 4OMG may result from an enhanced immune system. Bruneton (1995) reported that EP polysaccharides could enhance the immune system. Laboratory studies also have shown that purified polysaccharides from EP cell cultures possessed immunostimulatory activity to murine and human macrophages and mononuclear cells (Stimpel *et al*., 1984; Wagner *et al*., 1988). Roesler *et al*. (1991) reported that mice treated with EP polysaccharides could be protected from a lethal dose of either *Listeria monocytogenes* or *Candida albicans*. In addition, EP polysaccharides could also protect mice from H1N1 strain infection (Fusco *et al*., 2010). These reports indicated that EP polysaccharides may enhance the animal immune system, thus dietary supplementation of 4OMG was beneficial for growth of broilers. On the other hand, stimulating effects of 4OMG on better development of small intestine, especially intestinal villi, may also contribute to the growth of broilers. It is well known that small intestine is one of the important sites of nutrient absorption, and its better development may be beneficial for nutrient absorption (Mekbungwan *et al*., 2002) as well as better performance in animals (Swiatkiewicz and Hanczakowska, 2006). In the present trial, broilers fed diet with higher 4OMG had better development of small intestine, and this would be beneficial for nutrient absorption, thus resulting in the greater performance of broilers. The current results were in agreement with the report by Dehkordi and Fallah (2011) that feeding EP may improve feed conversion of broilers.

The present trial showed that increasing dietary supplementation of 4OMG in broilers may improve the development of small intestine, especially intestinal villi, and this may be related to the function of the 4OMG to promote cell differentiation. Wu *et al*. (2010) reported that EP extracts could significantly stimulate the proliferation of peripheral blood mononuclear cell. Wang

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et al. (2014) also reported that EP polysaccharides may well enhance the proliferation of rat intestinal epithelial cell. However, some researchers suggested that the improving effect of EP on intestinal cell growth may result from changes in the gut microflora, because piglets receiving a basal diet with EP had greater production of volatile fatty acids, especially butyrate in the duodenum, jejunum and ileum (Hanczakowska and Switkiewicz, 2012). It is well known that butyric acid may act as a primary source of energy for intestinal cells, thus it could stimulate cell proliferation (Bourassa *et al.*, 2016). So far, little information is yet available for the mechanism of 4OMG to enhance the development of small intestine. Therefore, further studies with 4OMG are still required.

Meat quality is affected by a number of factors including genetic, environment, feed, slaughtering conditions, processing and storage of meat, and feed additives have been reported as an important factor to influence physical and chemical characteristics as well as the sensory and microbial quality of meat (Nasir and Grashorn, 2010). In the current study, broilers fed diets with higher 4OMG had greater thigh muscle pH value, and this may be due to the inhibition effects of 4OMG on lactate dehydrogenase activity. Liu *et al.* (2008) reported that broilers fed diet with 10 g/kg supplementation of EP power had lower blood lactate dehydrogenase activity. Lactate dehydrogenase is a tetrameric enzyme, which converts pyruvate to lactate (Le *et al.*, 2010). As muscle is converted to meat, a shift occurs from aerobic to anaerobic metabolism, which favours the production of lactic acid, resulting in the pH of the tissue declining (Huff-Lonergan and Lonergan, 2005).

Broilers fed diets with higher 4OMG had lower drip loss of thigh muscle, this means that water-holding capacity of thigh muscles was enhanced, and it may be associated with 4OMG–induced enhancement of muscle pH. The ability of fresh meat to retain moisture is arguably one of the most important quality characteristics of raw products, but this water-holding capacity of muscle is usually affected by alteration of cell structure and lowering of pH (Offer and Knight, 1988). Huff-Lonergan and Lonergan (2005) noted that once the pH has reached the isoelectric point of the major proteins, especially myosin, the net charge of the protein is zero, meaning the numbers of negative and positive charges on the proteins are essentially equal. These negative and positive groups within the protein are attracted to each other, resulting in a reduction in the amount of water that can be attracted and held by that protein. Additionally, pH-induced lateral shrinkage of the myofibril may also lead to expulsion of water from the myofibrillar structure into the extramyofibrillar spaces and ultimately out of the muscle (Bendall and Swatland, 1988). The present enhanced muscle pH values were accompanied by increased water-holding capacity, which is consistent with the results of Toldra (2003), who reported a positive relationship between the meat pH value and its water-binding capacity.

Meat colour is an important parameter of meat quality characteristics, and it influences meat purchasing decisions more than any other quality factors because consumers use discolouration as an indicator of freshness and wholesomeness. In the present trial, broilers fed diets with higher 4OMG had greater redness of thigh muscle, and this may be due to the antioxidant capacity of 4OMG. It is well known that myoglobin is the principle protein responsible for meat colour, and oxygenation occurs when myoglobin is exposed to oxygen and is characterized by the development of a bright cherry-red colour (Mancini and Hunt, 2005). Discolouration results from oxidation of both ferrous myoglobin derivatives to ferric iron, but EP polysaccharides have a good capability for oxidation prevention of ferrous iron by slowing its conversion rate to ferric iron (Livingston and Brown, 1982; Lee *et al.*, 2009). Thus, dietary supplementation of 4OMG may protect meat colour, which was beneficial for the store and sale of chicken meat. The present result was in agreement with the report of Lee *et al.* (2013) that dietary supplementation of EP powder ranging from 5 to 20 g/kg could increase the redness of thigh muscle of broilers.

Meat tenderness also has a large impact on consumers' satisfaction with chicken meat. The development of meat tenderness is due to degradation of key muscle proteins, and the endogenous calpain system plays a major role in this process (Taylor *et al.*, 1995; Maddock *et al.*, 2005). During the conversion of muscle to meat, dramatic changes occur within the microenvironment of the muscle cell that can affect calpain activity (Winger and Pope, 1981; Maddock *et al.*, 2005). Rowe *et al.* (2004) reported that oxidative conditions in postmortem tissue decrease calpain activity and proteolysis, subsequently minimizing the extent of tenderization. Lee *et al.* (2009) reported that EP polysaccharides had antioxidant activity similar to that of ascorbic acid. Thus, dietary supplementation of 4OMG in broilers may contribute to improving oxidative conditions, resulting in improved tenderness of thigh muscles.

Texture profile analysis uses a double compression cycle to simulate the first and second bites, similar to a human subject, thus it may provide insight into how the meat behave when chewed. Faridi and Ahmadi (2015) reported that textural quality parameters of chicken meat could be changed by adding different complements such as *Virginiamycin* and *Echinacea* antibiotics, and diet with 20 g/kg *Echinacea* may lead to texture quality components decline in the leg. In the current study, dietary supplementation of 4OMG may lead to hardness decline in cooked thigh meat of broilers, and this may be associated with 4OMG–induced enhancement of water-holding capacity in thigh muscles. Battula *et al.* (2008) reported that higher muscle water-holding capacity could reduce liquid outflow, loss of soluble nutrients and flavour, therefore the meat could not become hard as well as tasteless.

Conclusions

It is clear that increasing dietary supplementation of 4OMG may better the development of small intestine, especially intestinal villi, which is beneficial for nutrition absorption of broilers. In addition, increasing supplementation of 4OMG in broilers diets was also beneficial for growth performance and meat quality of broilers.

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