Effects of Steam-Exploded Wood as an Insoluble Dietary Fiber Source on the Performance Characteristics of Broilers

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Effects of modified insoluble fiber originating from steam-exploded *Quercus mongolica* were studied relative to growth performance, blood parameters, intestinal morphology, and other intestinal characteristics in poultry broilers. First, the effect of steam-explosion on physicochemical properties of insoluble fiber from *Q. mongolica* was investigated. Steam-explosion (severity factor Log (*R*o) = 3.94) was found to increase the physical properties (water-holding capacity, oil-holding capacity, and swelling capacity) of *Q. mongolica* chip to different extents. Effects of feeding different concentrations of steam-exploded *Q. mongolica* on performance characteristics of broilers were investigated. Experimental diets of broilers consisted of a control diet (free of steam-exploded *Q. mongolica*), and four diets containing 0.5% to 2.0% steam-exploded *Q. mongolica* (severity factor Log (*R*o) = 3.94). A diet containing 1.0% steam-exploded *Q. mongolica* promoted broiler growth performance (body weight (858.9 g) and improved blood characteristics (130.0 mg/dL), intestinal morphology (V:C ratio 7.50), and organ weights (length of intestine 17.6 cm/100 g body weight).

Keywords: Quercus mongolica; Insoluble fiber; Steam-explosion; Performance characteristics; Broilers

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INTRODUCTION

Dietary fiber is considered a diluent of poultry diet (Rougière and Carré 2010), with negative effects on voluntary energy intake and nutrient digestibility (Mateos *et al*. 2002). However, dietary fiber improves gizzard function, digestibility of non-fiber nutrients, gastrointestinal tract health (Perez *et al*. 2011), and growth performance of broilers (González-Alvarado *et al*. 2010). In particular, the respective type and concentration of insoluble dietary fiber seems to be directly correlated with gastrointestinal tract development and growth performance (Jiménez-Moreno *et al*. 2009). A moderate amount of certain insoluble fiber sources, such as oat hulls (Sacranie *et al*. 2012), sunflower hulls (Kalmendal *et al*. 2011), and wood shavings (Hetland *et al*. 2010) can stimulate the development and health of the gastrointestinal tract in broilers.

In recent years, research on dietary fiber and its modification has increasingly gained attention. The physicochemical properties of fiber can be manipulated by various treatments, such as chemical, mechanical, thermal, or thermochemical methods, in order to improve functionality (Caprez *et al*. 1986; Bertin *et al*. 1988; Choi *et al*. 2008). Thermochemical treatments can change the physicochemical properties of dietary fiber by altering the ratio of soluble to insoluble fiber, with respect to the total dietary fiber content.

As one method of thermal treatment, steam explosion treatments are used to heat biomass using saturated steam, which is followed by an explosive decompression of the pressured system. During the explosion phase, steam and hot liquid water expand rapidly and are released from solid structures (Ibrahim *et al*. 2010). This process is known to substantially fractionate lignocellulosic biomass structure. Furthermore, the advantages of steam-explosion include significantly lower energy expenditure, lower financial effort, and less hazardous processing chemicals, compared to other methods of fiber modification.

To date, there are no results available on the effect of modified dietary fiber on growth performance, traits of the gastrointestinal tract, and nutrient digestibility in broilers. In this study, the effect of steam-explosion on physicochemical properties of *Quercus mongolica* biomass was evaluated. Moreover, the effect of insoluble fiber originating from steam-exploded *Quercus mongolica* on growth performance, blood characteristics, intestinal morphology, and organ weights of broilers was investigated.

EXPERIMENTAL

Materials

Quercus mongolica was collected from the forest around the city of Hongcheon in South Korea. The samples were chipped to a particle size of approximately 2 × 2 × 0.5 cm^3 for steam-explosion and stored at 20 °C, with moisture levels below 15%.

Steam Explosion

The chipped *Q. mongolica* material was treated according to the conditions shown in Table 1. Each experimental condition was expressed in terms of a severity factor log (R_o) (Eq.1), which combines reaction temperature and retention time (Fernandez- Bolaños *et al*. 1999).

$$\text{Severity factor } \log(R_o) = \{t \times \exp[(T - 100)/14.75]\} \quad (1)$$

Table 1. Steam-explosion Conditions

Temperature, *T* (°C)	Time, *t* (min)	Severity factor Log (R_o)
160	5	2.47
180	5	3.05
180	10	3.36
180	15	3.53
200	5	3.64
200	10	3.94
200	15	4.12
220	5	4.23
220	10	4.53
220	15	4.71

Physicochemical Properties

Carbohydrate and lignin content

The carbohydrate content was determined based on the total monomer content which was measured after a two-step acid hydrolysis procedure to fractionate fiber. The first step involved exposure to 72% H_2SO_4 at 30 °C for 60 min. In the second step, the reaction mixture was diluted to a final H_2SO_4 concentration of 4%, and subsequently autoclaved at 121 °C for 1 h. The solid residue remaining after this acid hydrolysis was considered to be the total lignin content. The carbohydrate (arabinose, xylose, mannose, galactose, glucose) content of this hydrolysis liquid was then analyzed by gas chromatography (GC) in a YL6100 device (Young Lin Ins. Co., Ltd., Anyang, South Korea), after hydrolysis with sulfuric acid and conversion into alditol acetates (ASTM method E1821-96 (1996)).

Dietary fiber content

Dietary fiber content was determined using an established method (AOAC 2000). Briefly, the samples were treated with thermo-stable α-amylase, and subsequently digested with a protease, followed by incubation with amyloglucosidase to remove starch and protein components. The insoluble dietary fiber was separated by centrifugation (at 1,000 g for 15 min) after enzymatic digestion of starch and protein, and soluble dietary fiber was precipitated with 95% ethanol. Dietary fiber was calculated as the sum of insoluble dietary fiber and soluble dietary fiber.

Water-holding and oil-holding capacity

Water-holding and oil-holding capacities were determined by mixing the fiber fraction with either distilled water (1:10, w/v) for 24 h, or with vegetable oil (1:5, w/v) for 30 min. After centrifugation at 1,000 g for 30 min, the respective holding capacities were measured as grams of either water or oil held by 1 g of fiber (Chau and Huang 2003).

Swelling capacity

Swelling capacity was defined as the volume of a sample upon immersion and soaking in water. Therefore, a dry sample (accurately weighed to 0.2 g) was placed in a test tube, to which 10 mL of water was added, followed by a hydration period of 18 h. Subsequently, the final volume of the sample was measured. The swelling capacity was expressed as mL/g of fiber (Ralet *et al*. 1993).

Animals, Diets, and Experimental Design

Experimental design

One-day-old male broiler chicks (Ross 308; N = 210) were randomly allocated to 30 groups of 7 chicks each. Each group was housed in a cage (3 m × 3 m) with a raised wire floor, a self-feeder, and a water source to provide ad libitum access to feed and water. The groups were assigned to five treatments (six cages per treatment), and fed one of the five diets. Environmental temperature in the first week of life was 35 °C, and decreased to 25 °C over the course of the experiment.

The diets were

1) basal diet (control),
2) basal diet + 0.5% steam-exploded *Q. mongolica*,
3) basal diet + 1.0% steam-exploded *Q. mongolica*,
4) basal diet + 1.5% steam-exploded *Q. mongolica*,
5) basal diet + 2.0% steam-exploded *Q. mongolica*.

The basal diet used in the study was a typical corn-wheat-soybean diet, formulated to meet nutrient requirements for nestling (0 to 21 d) and growing (22 to 35 d) periods (NRC 1994; Table 2).

Table 2. Composition and Nutrient Concentrations of the Basal Diet (air-dry basis)

Ingredient and Composition	Nestling Period (1 to 21 d)	Growing Period (22 to 35 d)
Ingredient (%)		
Corn	29.64	32.26
Wheat	30.30	30.00
Soybean meal	28.67	26.71
Canola meal	4.00	4.00
Vegetable oil	4.05	4.30
Salt	0.26	0.29
Limestone	1.02	1.51
Choline	0.12	0.10
Dicalcium phosphate	1.04	0.88
Vitamin/Mineral premix [1]	0.26	0.26
L-Lysine	0.66	0.38
DL-Methionine	0.29	0.30
Calculated value [2]		
Metabolizable energy, kcal/kg	3100.00	3200.00
Crude protein, %	23.00	20.00
Calcium, %	1.10	1.10
Available phosphorus, %	0.45	0.41
Lysine, %	1.30	1.10
Methionine, %	0.50	0.50
Cysteine, %	0.30	0.30

[1] Provided the following nutrients (per kg of air-dry diet): Vitamins: A 12,000 IU, D 33,000 IU, E 15 mg, K 2 mg, thiamine 2 mg, riboflavin 6 mg, pyridoxine 2 mg, calcium pantothenate 0.03 mg, folic acid 0.2 mg, niacin 45 mg, biotin 0.15 µg. Minerals: Ca 0.5%, Co 0.5 mg (as cobalt sulfate), Cu 10 mg (as copper sulfate), iodine 0.9 mg (as potassium iodine), Fe 80 mg (as ferrous sulfate), Mn 80 mg (as manganous oxide), Se 0.2 mg (as sodium selenite), Zn 80 mg (as zinc oxide).

[2] Calculated from NRC (1994)

Growth performance

The body weight of each individual was measured weekly. The feed input was weighed daily for each cage, and the leftover feed was weighed and discarded. Daily feed intake was calculated by dividing the amount of feed consumed by the number of days and animals.

Blood characteristics

Approximately 7 mL of blood was collected from the left wing vein of each individual using a 10 mL gauge syringe and a scalp vein needle. Blood samples were analyzed using commercial enzymatic kits (Merck, Germany) to determine blood urea nitrogen and cholesterol concentrations. After the experiment (following weighing and blood collection), three chicks per treatment were randomly selected and killed by cervical dislocation to investigate intestinal morphology and organ weights.

Intestinal morphology

Selected intestinal segments of approximately 2 cm were obtained from the midpoint of the duodenum, and from the midpoints between the bile duct and Meckel's diverticulum, and between Meckel's diverticulum and the ileo-cecal junction. Sections of 5-μm thickness were cut and stained with hematoxylin-eosin for examination under a light microscope. Ten villi with a lamina propria were randomly selected on each slide. Villus height (V) was defined as the length from the tip to the base, excluding the intestinal crypt. Villus thickness was measured at the half height of the villus, and the crypt depth (C) was defined as the distance from the villus base to the muscularis layer (not including the intestinal muscularis). The V:C ratio was calculated.

Organ weights

The gizzard and cecum were excised and weighed. The respective organ weights were recorded, and their weight relative to the total body weight was calculated and expressed as a percentage. The length of the intestine was measured after removal of its content.

Statistical Analysis

The effects of additives on growth performance, intestinal morphology, and organ and blood characteristics were analyzed statistically by an ANOVA, using SPSS software (SPSS Inc., Chicago, IL, USA). When significant differences were found, a least significant difference test was performed. Statistical significance is reported at $P < 0.05$.

RESULTS AND DISCUSSION

Effect of Steam-explosion Condition on Physicochemical Properties of *Q. mongolica*

Carbohydrate and lignin content

Steam-explosion can solubilize hemicelluloses, disorder the vegetative structure, and increase the accessible surface of lignocellulosic materials (Singh *et al*. 2015). In this study, the raw material was subjected to steam-explosion treatment at a severity factor of Log (R_o) = 2.47 (160 °C, 5 min), and severity factor Log (R_o) = 4.71 (220 °C, 15 min), respectively. The carbohydrate recovery ranged between 37.4% and 47.7% (Fig. 1). This is mainly attributable to the decrease in cellulose (expressed as glucose, in g) and hemicellulose fractions (expressed as arabinose, xylose, mannose, galactose, in g). More severe treatment conditions are expected to result in higher cellulose and hemicellulose degradation (Ballesteros *et al*. 2000). During the steam explosion, glycosidic bonds in hemicellulose and cellulose were hydrolyzed to some extent and the hemicellulose-lignin bonds were cleaved due to autohydrolysis pressure. This allowed the hemicellulose to be solubilized (Chen and Liu 2007).

The maximum cellulose content in the solid fraction was obtained from the severity factor Log (R_o) = 3.94. In the present study, arabinose, xylose, and galactose were completely removed by the treatment with the lowest severity factor Log (Ro) 3.36, while an increase in the severity factor value to 4.12 resulted in an increased solubilization of mannose. The lignin content of the solid fraction showed a substantial increase, compared to the raw material (Fig. 1). Lignin is not a polysaccharide but is chemically bound to hemicellulose in the plant cell wall, and therefore it is tightly associated with plant cell wall polysaccharides. Lignin also affects gastrointestinal physiology, as its phosphate groups bind strongly with positively charged ions (*e.g*. iron, zinc, calcium, and magnesium ions), and might thus influence mineral absorption in the gastrointestinal tract (Torre *et al*. 1991).

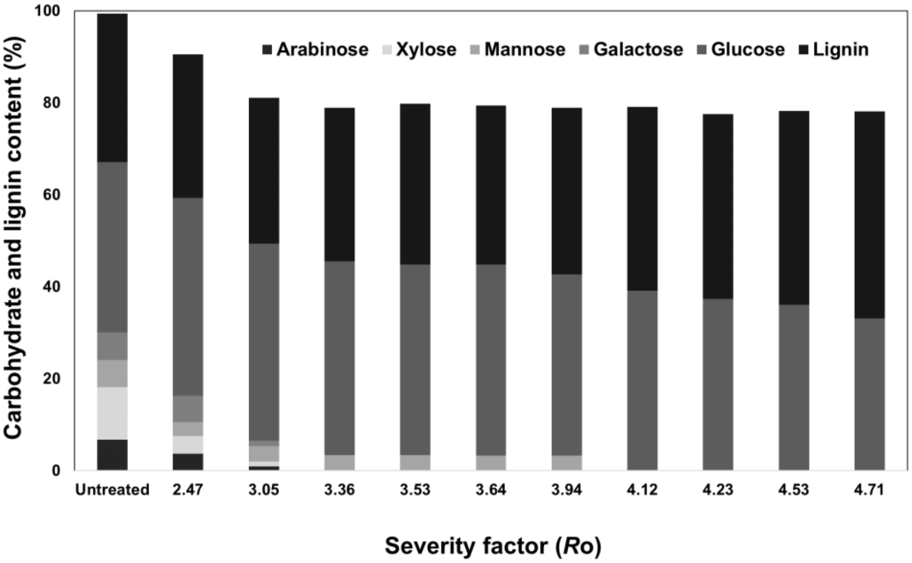

Fig. 1. Effect of steam-explosion condition on carbohydrate and lignin content of *Q. mongolica*

Physical properties

The physical properties of insoluble fiber investigated in this study include water-holding capacity, oil-holding capacity, and swelling capacity. Water-holding and oil-holding capacities were defined by the quantity of water and oil bound to the fiber without the application of any external force (apart from gravity and atmospheric pressure).

Steam-explosion treatment led to increases in water-holding capacity, oil-holding capacity, and swelling capacity of insoluble fiber (from 6.0 to 6.8 g/g, from 4.6 to 5.9 g/g, and from 1.5 to 4.1 mL/g, respectively; Figs. 2 to 4). Steam-explosion treatment significantly increased the water-holding capacity (at severity factor log (R_o) from 2.47 to 3.94), oil-holding capacity (at severity factor log (R_o) from 2.47 to 4.23), and swelling capacity (at severity factor log (R_o) from 2.47 to 3.94) of the insoluble fiber. In general, all physical characteristics changed at severity factor log (R_o) = 3.94.

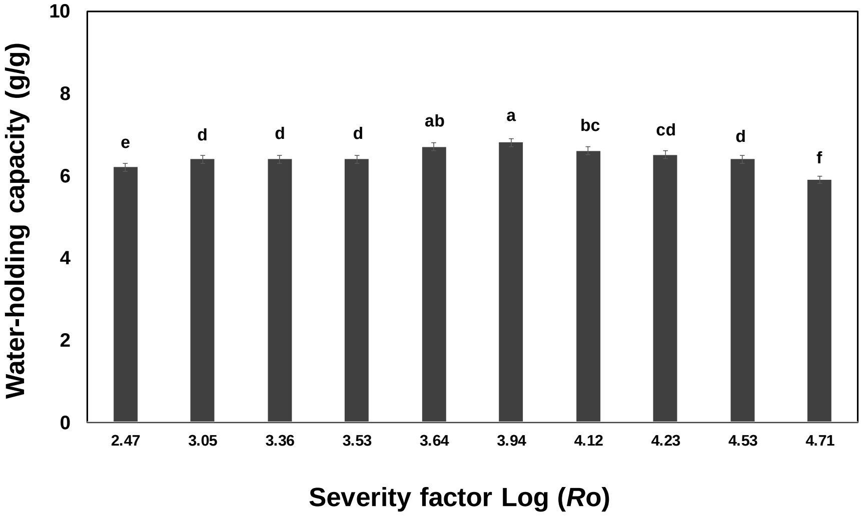

Fig. 2. Effect of steam-explosion condition on water-holding capacity of *Q. mongolica*

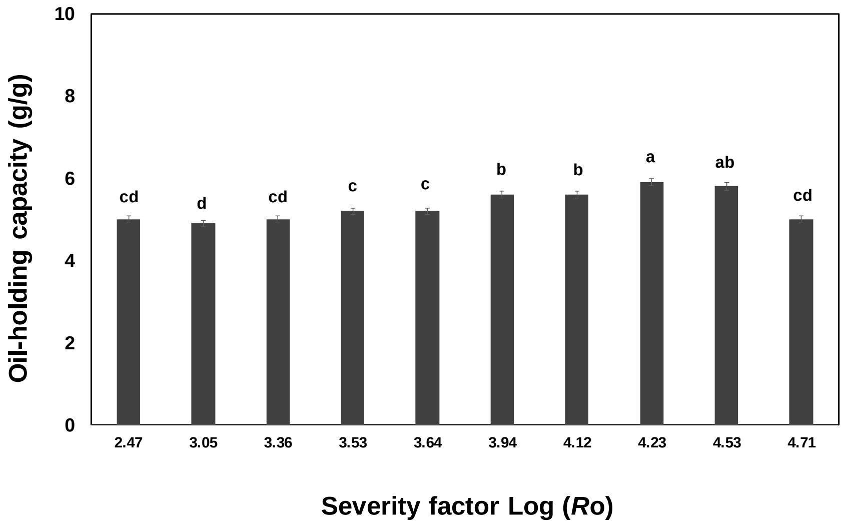

Fig. 3. Effect of steam-explosion condition on oil-holding capacity of *Q. mongolica*

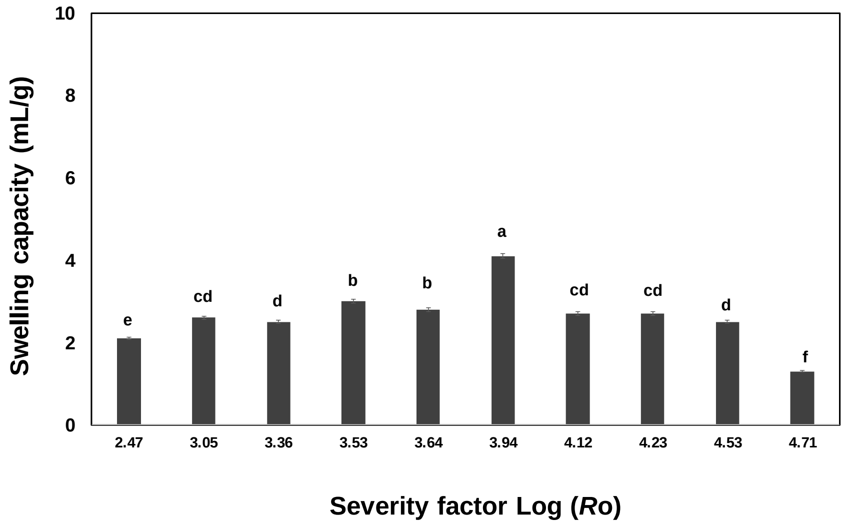

Fig. 4. Effect of steam-explosion condition on swelling capacity of *Q. mongolica.*

The ability to bind water is a function of the amount and characteristics of water-binding sites, as well as of the fiber structure (Robertson and Eastwood 1981). The increased water-holding capacity of steam-exploded insoluble fiber might be due to an increase in the amount of water, which can be bound by the structure after water has been released during the process of steam-explosion. The water-holding capacity of processed insoluble fiber from various fruit and vegetable sources generally varies from 2.8 to 42.5 g/g (Elleuch *et al*. 2011). Also, the increase of oil holding capacity was related to the increase of lignin content (Fig. 1).

Physicochemical Properties of Steam-exploded *Q. mongolica* Used in the Feeding of Broiler

The physicochemical properties of untreated and steam-exploded *Q. mongolica* are shown in Table 3. Total dietary fiber, insoluble dietary fiber, and soluble dietary fiber contents of untreated *Q. mongolica* were 90.5%, 89.9%, and 0.6%, respectively. Steam-explosion treatment of *Q. mongolica* caused a significant increase in total dietary fiber and insoluble dietary fiber content. Total dietary fiber content reached 93.3%, due to increase of insoluble dietary fiber content (93.0%) upon the steam-explosion treatment. There was a small but significant decrease in soluble dietary fiber content of steam-exploded *Q. mongolica* (0.3%), compared with untreated *Q. mongolica* (0.6%). Thermal treatments change the physicochemical properties of dietary fiber by altering the ratio of soluble and insoluble fiber (Caprez *et al.* 1986).

Hydration properties include water-holding capacity and swelling capacity. As shown in Table 3, the water-holding capacity increased from 6.0 g/g to 6.8 g/g after steam-explosion, and swelling capacity increased from 1.5 mL/g to 4.0 mL/g. Comparable findings have been reported previously (Martin-Sampedro *et al*. 2014). Swelling capacity depends on the physical structure (porosity and crystallinity) of the fiber matrix; an increase in swelling capacity might be attributed to a rise in the amount of short chains or the increased surface area of dietary fiber after steam explosion (Wang *et al*. 2015). The oil-holding capacity of steam-exploded *Q. mongolica* increased from 4.6 g/g to 5.7 g/g, which might be due to the higher lignin content (Fig. 1; Ma and Mu 2016; Luo *et al*. 2017). Caprez *et al*. (1986) similarly reported that thermal treatments of wheat bran affected the surface properties of dietary fibers and could lead to a significant increase in oil-holding capacity.

Steam-exploded *Q. mongolica* contains high concentrations of insoluble dietary fiber, which in combination with other improved physical properties suggests that this treatment method produces a promising alternative source of insoluble dietary fiber.

Table 3. Effect of Steam-explosion on Physicochemical Properties of *Q. mongolica*

	Untreated *Q. mongolica*	Steam-exploded *Q. mongolica**
Total dietary fiber, %	90.5	93.3
Insoluble dietary fiber, %	89.9	93.0
Soluble dietary fiber, %	0.6	0.3
Water holding capacity, g/g	6.0	6.8
Swelling capacity, mL/g	1.5	4.0
Oil holding capacity, g/g	4.6	5.7
***** Steam-explosion condition: severity factor Log (*R*o) = 3.94		

Effect of Feeding Graded Level of Steam-exploded *Q. mongolica* on Performance Characteristics of Broilers

Growth performance

Throughout the feeding period, body weight, daily weight gain, daily feed intake, and feed conversion ratio were influenced by steam-exploded *Q. mongolica* inclusion (Table 4). Broilers supplemented with the 1.0% steam-exploded *Q. mongolica* had a significantly higher body weight (858.9 g) than the control group (841.4 g; $P < 0.05$). Jiménez-Moreno *et al.* (2011) reported that the inclusion of dietary fiber significantly increased the relative body weight. This is because dietary fiber is retained in the gizzard for a long time. Supplementation with 1.0% steam-exploded *Q. mongolica* increased the body weight of broilers, but this effect was not found in broilers fed with diets containing 1.5 to 2.0% of steam-exploded *Q. mongolica*. Cellulose may have little effect on gizzard development or growth performance (Mateos *et al*. 2012). The increase of steam-exploded *Q. mongolica* concentrations led to a linear decrease in daily weight gain (43.2 to 47.0 g/d) and daily feed intake (82.3 to 76.2 g/d). The feed conversion ratio was lowest in broilers fed with a 1.0% steam-exploded *Q. mongolica* diet (1.71), which did not differ significantly from the control group.

Table 4. Effect of Steam-exploded *Q. mongolica* on Growth Performance, Blood Characteristics, Intestinal Morphology, and Organ Weights of Broiler Chicks

Item	Steam-exploded *Q. mongolica*, %[1]					SEM	*P*-value
	0.0[2]	0.5	1.0	1.5	2.0		
Growth performance							
Body weight (g)	841.41^{ab}[3]	828.46^{ab}	858.92^{a}	787.05^{b}	783.33^{b}	8.82	0.011
Daily weight gain (g/d)	48.26^{a}	46.67^{ab}	47.05^{ab}	43.24^{b}	43.26^{b}	0.56	0.002
Daily feed intake (g/d)	82.40^{a}	82.30^{a}	80.40^{ab}	77.80^{ab}	76.20^{b}	0.72	0.008
Feed conversion ratio (g/g)	1.71^{a}	1.76^{a}	1.71^{a}	1.80^{a}	1.76^{a}	0.01	0.164
Blood characteristics							
Urea nitrogen (mg/dL)	0.72	0.71	0.64	0.72	0.72	0.02	0.552
Cholesterol (mg/dL)	136.5	131.6	130.0	125.5	121.9	2.52	0.164
Intestinal morphology							
Villus height (µm)	283.63	308.85	312.91	305.44	275.13	15.85	0.949
Crypt depth (µm)	50.11^{ab}	49.35^{ab}	42.95^{b}	68.34^{ab}	78.34^{a}	4.50	0.033
V: C ratio	5.66^{ab}	6.19^{ab}	7.50^{a}	5.03^{ab}	3.42^{b}	0.48	0.072
Organ weights							
Gizzard (%)	1.99	1.79	2.05	2.02	2.05	0.04	0.198
Cecum (%)	0.51	0.51	0.57	0.56	0.60	0.02	0.211
Length of intestine (cm/100g body weight)	16.52	16.82	17.65	17.88	18.90	0.32	0.128

[1] The basal diet was diluted (wt/wt) with 0.5, 1.0, 1.5 or 2.0% of steam-exploded *Q. mongolica*, according to treatment.
[2] The control diet contained 0.0% of insoluble fiber of steam-exploded *Q. mongolica*.
[3] Different superscripts indicate a statistically significant difference ($P < 0.05$).

Blood characteristics

Diet supplementation with steam-exploded *Q. mongolica* did not affect blood urea nitrogen. However, broilers fed with 0.5% to 2.0% steam-exploded *Q. mongolica* had lower levels of blood cholesterol (131.6 to 121.9 mg/dL) than the control group (136.5 mg/dL). In general, the increase of steam-exploded *Q. mongolica* concentrations led to a linear decrease of blood cholesterol. The 0.5 to 2.0 % steam-exploded *Q. mongolica* diets produced a decrease of blood cholesterol from 131.6 to 121.9 (mg/dL). This finding is in line with the results of a previous study (El-kheir *et al*. 2009), probably because of the interference of cholesterol absorption by dietary fiber.

Intestinal morphology

The ratio of crypt depth to villi height is an indicator of digestion potential in the small intestine. A smaller ratio thus suggests a functional improvement of the intestinal mucosa. The villus height was lower in individuals fed with the 2.0% steam-exploded *Q. mongolica* diet (275.1 µm), than in the control group (283.6 µm). However, the crypt depth was larger in broilers fed with 1.5 to 2.0% steam-exploded *Q. mongolica* (68.34 to 78.34 µm), compared to the control diet (50.11 µm). Broilers fed with 1.0% steam-exploded *Q. mongolica* showed the highest V:C ratio (7.50). Changes in intestinal morphology, such as shorter villi and deeper crypts have been associated with the presence of toxins (Yason *et* *al*. 1987), or higher tissue turnover (Miles *et al*. 2006).

Organ weights

In general, the increase of steam-exploded *Q. mongolica* concentrations resulted in a linear increase of gizzard weight, cecum weight, and length of the intestine. Changes in the intestine weight indicated retention of insoluble fiber. However, throughout the feeding period, organ weights were not significantly affected by steam-exploded *Q. mongolica* supplementation.

CONCLUSIONS

1. Steam-explosion effectively increased the total dietary fiber content (particularly of insoluble dietary fiber) and improved the physicochemical properties (water-holding capacity, swelling capacity, and oil-holding capacity) of insoluble fiber of *Q. mongolica*.
2. The results indicated that broiler diet supplementation with up to 1.0% steam-exploded *Q. mongolica* resulted in greater body weight (858.9 g; $P < 0.05$), and a greater feed conversion ratio (1.71 g/g), compared to the control group (basal diet). Furthermore, broilers fed with 0.5% to 2.0% steam-exploded *Q. mongolica* had lower levels of blood cholesterol (131.6 to 121.9 mg/dL) than the control group. Urea nitrogen, however, was not affected by steam-exploded *Q. mongolica* supplementation, throughout the feeding period. Broilers fed 1.0% steam-exploded *Q. mongolica* had the highest V:C ratio (7.50), and the increase of steam-exploded *Q. mongolica* concentrations linearly increased gizzard weight, cecum weight, and length of the intestine.
3. In conclusion, the steam-explosion treatment (severity factor Log (R_o) = 3.94) could effectively improve the digestive functionality of insoluble fiber. Thus, steam-exploded *Q. mongolica* seems to be a promising supplement for improvement of poultry health and productivity.

ACKNOWLEDGMENTS

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (2018R1D1A1A02045243).

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Article submitted: June 4, 2018; Peer review completed: November 25, 2018; Revised version received and accepted: December 14, 2018; Published: January 8, 2019.
DOI: 10.15376/biores.14.1.1512-1524