



Determination of Growth Performance, Viscera Organ Weights and Ileal Intestinal Architecture of Broilers in Response to Drinking Water Added Extractions from Wooden Chips for the Starter Period

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ABSTRACT A total of 90 one-day-old male broilers (Ross 308) were randomly assigned to one of the three dietary treatments, each consisting of six replicates (5 broilers/cage). The dietary treatments were 1) control (CON: fresh clean water with no supplement); 2) low dose [LD: CON + 1.56% extractions from the wooden chips (EWC)] and 3) high dose (HD: CON + 12.5% EWC). Drinking water supplemented with EWC was provided using specifically designed individual nipple drinker units. Average daily water intake (ADWI), average daily gain (ADG), average daily feed intake (ADFI), and feed conversion ratio (FCR) were measured weekly for 21 days. One broiler from each cage was euthanized for measuring the visceral organ weights and collecting ileal tissue samples for ileal architecture analysis on day 21. Broilers assigned to the LD and HD watery groups showed higher ADWI than that in broilers consumed CON on day 7 ($P < 0.05$). The broilers subjected to HD treatment showed a deeper crypt depth ($P < 0.05$) than that in broilers subjected to LD and CON on day 21. Therefore, broilers consumed HD showed a lower ($P < 0.05$) villus height:crypt depth ratio than that broilers consumed CON on day 21. Broilers provided drinking water containing any of the tested concentrations of EWC showed no effect ($P > 0.05$) on growth performance, ileal villus height, and visceral organ weights as compared with those in the CON from hatch to 21 days. In conclusion, broilers fed HD showed reduction in villus height:crypt depth ratio without impairing growth performance and visceral organ weights for the experimental period.

(Key words: extractions from the wooden chips, growth performance, viscera organ weights, water intake)

INTRODUCTION

Many studies with respect to therapeutic or sub therapeutic use of antibiotic improved growth performance but decreased mortality and morbidity in production animals (Doyle, 2001). However, critical anxiety raised about transmitting bacterial antibiotic resistance in humans and discharge of antimicrobial compounds (i.e., zinc, copper) to the environment (Ogawara, 1981; Russell, 1991; Han, 2007). In this regard, much interests in alternatives/replacements for dietary antimicrobial products are increasing in poultry production (Patterson and Burkholder, 2003; Niewold, 2007).

There are many different kind of plant extract (barley, whole grains, oak, etc.) which affects antioxidant capacity depending on the amounts of polychemicals (Duh et al., 2001; Liu, 2007; Mateo et al., 2009; Num et al., 2017). However, many antioxidative phenolic compounds in plants are predominantly present in a covalently bound form with an insoluble polymer (Gong et al., 2012). Therefore, it is necessary to find an effective processing method to release these compounds. Several methods including heat treatment (steam explosion and torrefaction), far-infrared (FIR) radiation, and enzymatic treatment, have been studied in order to liberate and activate low-molecular-weight antioxidants from various plants (Ahajji

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et al., 2009). Heat treatment of wood at moderate temperatures (200~250°C) under an inert atmosphere (to avoid oxidative degradation) leads to formation of torrefied or heat-treated wood (Ahajji et al., 2009). Antioxidative phenolic compounds can be present in raw materials or produced during the heat-treatment process (Avat, 1993; Ahajji et al., 2009).

The purpose of the present study was to determine the beneficial effect of organic acids and phenolic compounds present in the extracts from Oak wood chips on broiler (Ross 308) growth performance, ileal intestinal architecture and viscera organ weights from hatch to 21 days.

MATERIAL AND METHODS

Experiment procedures were investigated and approved by the Animal Ethics Committee of the Chungnam National University (CNU-00903).

1. Housing and Managements

This study was conducted in the research facility of the Chungnam National University of South Korea. Eighteen wire floor cages ($0.85 \times 0.55 \times 0.35 \text{ m}^3$) were used as experiment units. Each cage consisted with specifically design individual nipple drinker unit with two nipple drinkers and a metal through feeder. All the broilers were allowed to *ad-libitum* access of corn and soybean-meal diet (Table 1) formulated to meet the nutrition requirement of poultry (NRC, 1994) without incorporation of any antibiotic growth promoters. The ambient temperature was maintained at $30 \pm 1^\circ\text{C}$ from days 1 to 3 and then gradually decreased to $25 \pm 1^\circ\text{C}$ and it was maintained until the end of the experiment.

2. Preparation of Extractions from Wooden Chips

Torrefaction of the oak wooden chips was achieved using a laboratory scale reactor as described by Nam et al. (2018). A prescribed amount of oak wooden chips was weighed and placed in the center of the reactor. An external dryer was used to dry the chips to <15% moisture prior to torrefaction. The wooden chips were torrefied at 240°C temperatures for 24 h. After torrefaction, the material was ground and sieved to a maximum particle size of 60 mesh. The torrefied material

Table 1. Composition (% as-fed basis) of the experimental diet

Ingredients	Amount
Corn	45.08
Wheat	5.90
Wheat bran	6.22
Soybean meal 48%	36.00
Vegetable oil	2.80
Limestone	1.50
Monocalcium phosphorus	1.70
Salt	0.30
Vit-min premix ¹	0.30
DL-methionine	0.20
ME (kcal/kg)	3,200
CP (%)	23
NDF (%)	10.9
Ca (%)	1.1
Avail. P (%)	0.4
Calculated values ²	
Lys (%)	1.3
Met (%)	0.5
Met+Cys (%)	0.9
Thr (%)	0.9
Trp (%)	0.3
Val (%)	1.1

¹ Supplied per kilogram of total diets: Fe ($\text{FeSO}_4 \cdot \text{H}_2\text{O}$), 80 mg; Zn ($\text{ZnSO}_4 \cdot \text{H}_2\text{O}$), 80 mg; Mn ($\text{MnSO}_4 \cdot \text{H}_2\text{O}$) 80 mg; Co ($\text{CoSO}_4 \cdot \text{H}_2\text{O}$) 0.5 mg; Cu ($\text{CuSO}_4 \cdot \text{H}_2\text{O}$) 10 mg; Se (Na_2SeO_3) 0.2 mg; I, ($\text{Ca}(\text{IO}_3)_2 \cdot 2\text{H}_2\text{O}$) 0.9 mg; vitamin A, 24,000 IU; vitamin D₃, 6,000 IU; vitamin E, 30 IU; vitamin K, 4 mg; thiamin, 4 mg; riboflavin, 12 mg; pyridoxine, 4 mg; folacine, 2 mg; biotin, 0.03 mg; vitamin B₈, 0.06 mg; niacin, 90 mg; pantothenic acid, 30 mg.

² The values were calculated according to the values of feedstuffs in NRC (1994).

was stored in a desiccator at room temperature until chemical analyses. Extractions of torrefied wood were performed in bottles using water. Upon completion of extraction, the extract was filtered through a filter paper. The concentration of polyphenol and flavonoids in oak wooden chip extracts were determined as 1.07 mg/g and 192.12 mg/g respectively

(Nam et al., 2018).

3. Experiment Design and Management

Ninety, day-old (Ross 308) male broilers with the initial body of 44.9 ± 0.2 (mean \pm SEM) were allotted to one of three treatments having 6 replicate pens (five broilers per pen) in a completely randomized design. Similar initial body weight and weight distribution were maintained across the replicate cages. The treatments were 1) control (CON: fresh clean water without any supplementations), 2) low dose (LD: CON+1.56% EWC), and 3) high dose (HD: CON+12.5% EWC). The study lasted for 21 days.

Pen basis daily water intake was measured using the calibrated water dispenser in the individual nipple drinker units. Body weights and feed intake were measured on each week for 3 weeks. Daily weight gain, daily feed intake and feed conversion ratio were calculated based on weekly body weights and feed intake on each pen.

4. Collection of the Samples

At the end of experiment (day 21), sample collection was conducted to collect viscera organs and ileal tissue samples. One broiler that closer to the mean body weight was selected from each replicate pen and weighted. Broiler was euthanized via cervical dislocation and sacrificed prior to the collection of samples. Afterward, ileal from the gastrointestinal tract was separated at the points of Meckel's diverticulum and ileocecal junction. A 3 cm segment of distal ileal was removed and cleaned with phosphate-buffered saline (PBS) solution. Collected samples were immediately stored in the plastic containers that contained 10% of neutral buffered formaldehyde (Sigma Chemical Co., St. Louis, MO) for the fixation prior to the mucosa architecture analysis. Thereafter, liver, spleen and pancreas were removed and trimmed the excess fat depositions on each organ. Organ weights were recorded and calculated the percentage of organ weights relative to live body weight of the respective broiler.

5. Ileal Intestinal Architecture

Ileal tissue samples were processed following dehydration, embedding, staining and mounting on the glass according to the Pelicano et al. (2005). Thereafter, well positioned 10 villi

and associated 10 crypts were selected for take villus height, villus width, crypt depth and mucosa thickness measurements using NIS-Elements viewer software (Version: 4.20; NIS Elements, Nikon, USA) with the aid of calibrated eyepiece of inverted microscope (eclipse TE2000, Nikon Instrument Inc., Melville, NY 11747-3064, USA).

6. Statistical Analyses

Completely randomized design was used for analyzed the data in general linear model (GLM) procedure of one-way ANOVA of SPSS software (version 24, Armonk, NY: IBM Corp.). Pen was used as the experiment unit for growth performance and water intake data while individual selected broiler was used as the experiment units for the intestinal ileal architecture and viscera organ weights analysis. Statistical significance was accepted at $P<0.05$. Turkey multiple range test between means were made when a significant treatment effect was observed.

RESULTS

Growth performance and water intake of broilers fed with three different EWC are shown in the Table 2. Broilers in the LD and HD groups showed higher ($P<0.05$) ADWI compared to those in the CON group on day 7. Given broilers LD and HD showed similar ($P>0.05$) ADG, ADFI and FCR compared to those in the CON from hatch to 21 days.

Ileal intestinal architecture of broilers fed with different watery treatments are shown in the Table 3. Broilers consumed HD showed higher ($P<0.05$) crypt depth presenting those drunk LD and CON on day 21. Therefore, broilers in HD had lower ($P<0.05$) villus height:crypt depth ratio compared to broilers received CON on day 21. No difference ($P>0.05$) was observed in ileal villus height, villus width and total mucosa thickness of broilers fed 3 different watery treatments on day 21.

Relative weights of the liver, spleen and pancreas did not affect ($P>0.05$) by the EWC treatments on day 21 (Table 4).

DISCUSSION

Previous studies demonstrated that broilers fed a diet su-

Table 2. Effect of extractions from wooden chips on growth performance of broiler for day 21

Item	CON ¹	LD ²	HD ³	SEM	P-value
ADG ⁴ (g/day)	Day 7	19.67	19.46	0.30	0.962
	Day 14	54.94	53.02	0.62	0.268
	Day 21	74.98	70.15	1.38	0.280
	Day 0~21	49.86	47.55	0.65	0.359
ADFI ⁵ (g/day)	Day 7	27.68	28.61	0.31	0.498
	Day 14	65.55	63.60	2.66	0.450
	Day 21	106.39	100.42	1.57	0.263
	Day 0~21	66.54	64.21	0.76	0.480
FCR ⁶ (g/g)	Day 7	1.41	1.47	0.02	0.624
	Day 14	1.20	1.20	0.01	0.823
	Day 21	1.42	1.45	0.02	0.673
	Day 0~21	1.34	1.37	0.01	0.662
ADWI ⁷ (mL/day)	Day 7	72.95 ^a	101.57 ^b	4.17	0.007
	Day 14	80.24	85.81	2.58	0.602
	Day 21	131.43	143.20	3.82	0.469
	Day 0~21	94.87	110.19	2.77	0.069

¹ Fresh clean water with no supplementation.² 1.56% extractions of wooden chips incorporated water.³ 12.5% extractions of wooden chips incorporated water.⁴ Average daily gain.⁵ Average daily feed intake.⁶ Feed conversion ratio.⁷ Average daily water intake.**Table 3.** Effect of extractions from wooden chips on ileal intestinal architecture of broiler on day 21

Item	CON ¹	LD ²	HD ³	SEM	P-value
Villus height (V)	668.62	646.75	626.02	18.05	0.656
Crypt depth (C)	54.74 ^a	54.05 ^a	77.30 ^b	3.96	0.013
V:C ratio	14.09 ^b	12.68 ^{ab}	8.50 ^a	0.89	0.018
Villus width	60.52	73.51	63.60	4.53	0.501
Total mucosa thickness	906.23	948.47	793.33	31.93	0.119

¹ Fresh clean water with no supplementation.² 1.56% extractions of wooden chips incorporated water.³ 12.5% extractions of wooden chips incorporated water.

plemented with EWC improved their growth and relevant intestinal functions including its morphology, digestibility and absorption in starter and grower phases (Zhu, 2013; Rattanawut

and Yamauchi, 2015). Abovementioned studies regarding EWC had mainly used it as an acidifying and antioxidant agents by reasons of present of organic acids and phenolic

Table 4. Effect of extractions from wooden chips on viscera organ weights of broiler on day 21

Item (%)	CON ¹	LD ²	HD ³	SEM	P-value
Liver	2.16	2.24	2.06	0.05	0.358
Spleen	0.08	0.07	0.08	0.01	0.686
Pancreas	0.26	0.24	0.26	0.01	0.280

¹ Fresh clean water with no supplementation.

² 1.56% extractions of wooden chips incorporated water.

³ 12.5% extractions of wooden chips incorporated water.

compounds, respectively (Watarai and Tana, 2005; Rattanawut and Yamauchi, 2015). Organic acids maintain the low pH of gastric contents that helps to suppress pathogenic microorganism and to increase protein digestion in broilers (Abdo, 2004; Wolfenden et al., 2007). Wang et al. (2008) mentioned that presence of phenolic compounds helps to reduce substrates that increase oxidative stress in Hubbard and Shiqizha broilers on day 21. However, there were few studies investigated that drinking water added EWC on growth performance and intestinal ileal architecture of broilers in starter phase (Yamauchi et al., 2014). Interestingly, drinking water acidification using organic acid is another implementation in broiler industry used for improving growth performance (Aclkgoz et al., 2011). Therefore, current study was designed to investigate the effect of organic acids and phenolic compounds in EWC on broilers growth performance, ileal intestinal architecture and viscera organ weights.

Current study observed that broilers in LD and HD increased water intake on day 7. Couple of studies of James and Wheeler (1949) and Smith et al. (2000) mentioned that the potassium in feed or drinking water increased the water intake in broilers. Although we were not in the position to analyze potassium content in EWC, the studies of Zulkarami et al. (2011) and Pari (2016) reported that EWC contained high concentration of potassium. Therefore, the results of our current study expressed that higher water intake could be due to reasonable amount of potassium in EWC.

In the current study, watery treatments did not significantly alter growth performance from hatch to day 21. Recent research outcome from Yamauchi et al. (2014) demonstrated that Sanuki Cochin fed 500 and 1,000 times diluted EWC had similar growth rate compared to those had clean fresh water. In addition, study of Aclkgoz et al. (2011) found no

difference between broilers had water added formic acid (pH 4.5) and those had water without formic acid (pH 7.4). In agreement to the findings, Watkins et al. (2004) concluded that water acidification using organic acids did not affect the growth performance of broilers in any stage. This could be due to high concentration of wooden chip extracts components (organic acids and phenols) may cause toxicity to broilers rather than acidifying water (Philipsen, 2006; Yamauchi et al., 2014).

In the aspect of ileal intestinal architecture of current study, there were higher crypt depth and low villus height to crypt depth ratio obtained by broilers accessed HD compared to those had CON. This result could be due to high turnover in enterocytes of broilers had HD (Savage et al., 1997; Demir et al., 2003). However, presumably broilers had HD in the current study was able to maintain their villus height without any adverse consequences compared to broilers had LD and CON. Similarly, Samanya and Yamauchi (2001) obtained that broilers (White Leghorn) fed a diet with EWC up to 5% did not alter the ileal villus heights on day 28. Further, Ross broilers fed a corn-soybean based diet supplemented with 0.1% of organic acid did not provide a significant difference on ileal architecture on day 21 (Gunal et al., 2006). In the current study, water supplementation with HD showed deeper crypt depth suggesting the high demand for new tissue in gut mucosa. Interestingly, Yason et al. (1987) mentioned that the deeper crypt have been associated with the availability of toxic compound in the intestine of broiler and turkey. The investigation would be suggesting that the presence of toxic compounds in EWC rather than organic acids and phenolic compounds. Many studies (Shamoto et al., 1999; Shamoto and Yamauchi, 2000) demonstrated that the incorporation of EWC in to broiler diets improved the gut morphology by

activation cell mitosis in the enterocytes. In this regard, Yamauchi et al. (2014) explained that the effect of organic components in EWC reduced the activity of the pathogenic microorganisms in the intestine that would be allowed improved gut health. It might be a one possible reason broilers had HD did not show impaired growth performance, although occurrence of many negative outcomes likely reduced ileal intestinal architecture indices (i.e., crypt depth and villous height/crypt depth ratio).

Broilers (Hubbard) fed organic acids compounds (i.e., 3% of citric acid and acetic acid) in EWC also did not show the significant effect on proportional weight of liver and spleen compared to broilers fed CON on day 42 (Sa et al., 2008). In opposing, Fushimi et al. (2001) suggested that dietary acidification might have stimulated the glycogenesis while inhibition of glycolysis in the liver that caused enlargement of hepatic tissues in rats. In contrast with current study, broilers fed 3% of citric acid and acetic acid diets showed higher pancreas weight compared to broilers fed control diet on day 42 (Sa et al., 2008). Gauthier (2002) and Jang et al. (2004) explained that organic acids could be a cause of hyper activation of pancreatic enzyme secretion that in turn enlargement of the pancreas in broilers.

In conclusion, broilers fed HD had in reduction of villus height:crypt depth ratio without impairing growth performance and viscera organs weights for the experimental period. However, we found that no significant difference of all measured factors between LD and CON in the current study.

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