Evaluation of *Achyranthes japonica* Nakai extract on growth performance, nutrient utilization, cecal microbiota, excreta noxious gas emission, and meat quality in broilers fed corn–wheat–soybean meal diet

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ABSTRACT This study was conducted to investigate the effects of dietary supplementation of *Achyranthes japonica* Nakai (**AJN**) extract as a natural feed additive on growth performance, nutrient utilization, cecal microbiota, excreta noxious gas emission, and meat quality in broilers fed corn–wheat–soybean meal diet. In total, seven hundred twenty 1-day-old male Ross 308 broilers with an average body weight (**BW**) of 43.36 ± 1.42 g were used in a 35-d feeding trial. Broilers were randomly assigned to 1 of the 4 treatments. Each treatment had 10 replication pens with 18 birds per replication. Dietary treatments composed of corn–wheat–soybean meal–based diets along with the addition of 0, 0.025, 0.05, and 0.1% of AJN extract. The BW gain and feed conversion rate were linearly influenced ($P < 0.05$) by the supplementation of AJN extract during days 8 to 21, 22 to 35, and the overall experiment. At the end of the experiment, the digestibility of dry matter and nitrogen and the population of cecal lactic acid bacteria were linearly improved ($P < 0.05$) in response to increasing AJN extract supplementation. Excreta emission of ammonia showed a linear decrease ($P < 0.05$) with the increasing levels of AJN extract. The breast muscle percentage linearly increased ($P < 0.05$) in birds fed AJN extract contained diets. In summary, the inclusion of AJN extract in corn–wheat–soybean meal diet improved growth performance, nutrient utilization, intestinal microbiota balance, and breast meat production and decreased excreta ammonia emission, which confirmed the applicability of AJN extract as a natural feed additive in broilers.

Key words: *Achyranthes japonica* Nakai, corn–wheat–soybean meal diet, broiler performance, cecal microbiota, nutrient utilization

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INTRODUCTION

In modern broiler production systems, feed costs are substantial and account for up to 80% of total costs (Saeed et al., 2017). Soybean meal (**SBM**) is the major source of dietary protein and plays an important role in the feedstuff for broilers' feed (Ukachukwu and Szabo, 2003). However, owing to location, seasonal differences, and insufficient production, supply of the conventional sources of protein such as SBM is unable to meet the requirements of the fast-growing poultry industry (Khan et al., 2016). Thus, low-SBM diets have been suggested as alternative low-cost feed and become more and more popular in broiler production. Additionally, the demand for quality and safe poultry products is still rising, which leads to an strong interest in natural feed additives such as medicinal herb products (Park et al., 2013, 2016). It was suggested that many physiologically active substances isolated from medicinal herbs such as flavonoids, isoprene, saponins, and terpenoids are beneficial in the prevention and treatment of diseases, and they also have antimicrobial and antioxidant activities (Cho et al., 2003; Park et al., 2014; Lan et al., 2017; Liu et al., 2017). In recent years, many studies have shown that supplementation of medicinal herbs has positive effects on performance, productivity, health immunity, and stabilization of gut microbiome in poultry (Guo et al., 2004; Gong et al., 2014; Zeng et al., 2015; Liu and Kim, 2017).

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Achyranthes japonica Nakai (**AJN**) is a perennial herb from the Amaranthaceae family, which is widely distributed in East Asia including Korea, China, and Japan (Jung et al., 2007). Traditionally, it was classified as a medicinal herb that activates blood flow and clears the stagnated blood (Park et al., 2004). In Korea, AJN was used to control pain and improve dysfunction in osteoarthritis patients (Han et al., 2005). The root of AJN contains various active components such as saponins, triterpenoids, phytoecdysteroids, 20-hydroxyecdysone, and inokosterone (He et al., 2017). Furthermore, the extract of AJN has been reported to have multiple physiological effects, including antiallergic, anti-inflammatory, antioxidant, hepatoprotective, and anticancer properties (Lee et al., 2012). However, to the best of our knowledge, little is known about the effects of AJN extract supplementation as a feed additive in broiler diets. Thus, the purpose of the present study was to investigate the effects of AJN extract supplementation on growth performance, nutrient utilization, cecal microbiota, excreta noxious gas emission, and meat quality in broilers fed corn–wheat–soybean meal diet.

MATERIALS AND METHODS

The experimental protocol (Ethics Approval Number: DK-1-1910) used in this study was approved by the Animal Care and Use Committee of Dankook University, South Korea.

Experimental Design, Animals, Diets, and Housing

A total of seven hundred twenty 1-day-old male Ross 308 broiler chickens with an average initial body weight of 43.36 ± 1.42 g were randomly assigned to 1 of the 4 treatments with 10 replications and 18 broilers per replications in a 35-d growth assay. The experiment was conducted in 3 phases: phase 1 (days 1–7), phase 2 (days 8–21), and phase 3 (days 22–35). The 4 dietary treatments were corn–wheat–soybean meal diet supplemented with 0, 0.025, 0.05, and 0.1% of AJN extract (Park and Kim, 2020). All diets were formulated to meet or exceed the NRC (1994) requirement for broiler chickens and provided in mashed form. All the feed were fed daily and mixed with AJN extracts daily. To ensure the products could mixed well into the diets, the extract was first mixed with 1 kg feed by hand mix, and then, this premix feed was mixed properly with the remaining feed by using a mixer according to the manufacturer's protocol. The composition of the basal diet is shown in Table 1, and all diets were presented in mash form. Broilers were housed in a temperature-controlled room with 3 floors of stainless steel battery cages (1.75 × 1.55 m^2). The temperature in the room was 33°C ± 1°C for the first 3 d and was then gradually decreased by 3°C per week to 20°C, which was maintained until the end of the experiment. The humidity was kept around 60% throughout the experiment. The broilers had free access to feed and water during the experiment.

AJN Extract Preparation

The AJN extract used in this study was provided by a commercial company (Synergen Inc., Bucheon, South Korea). The AJN plants were cultivated in South Korea. The manufacturing process of the AJN extracts is described briefly. After cleaning, the roots of *A. japonica* were powdered by using a mill (IKA M20; IKA, Staufen, Germany). The samples were extracted with distilled water at 80°C and then refluxed for 6 h to obtain the initial extract. The residues were extracted with distilled water (1:5) at 80°C for 2 h, and the extract solution was filtered under low temperature by a high-velocity centrifugal machine. The useful parts were collected by column and eluted with ethanol. After cooling and filtering, the samples were vacuum-dried with a temperature under 40°C. The extracts were completely dried in a freeze-drier and presented in mash form. The AJN extract contains active constituents of 1.15 mg/g total flavonoid, 4.26 mg/g total polyphenol, and 0.47 mg/g saponin.

Sampling and Measurements

Growth Performance and Nutrition Digestibility On days 0, 7, 21, and 35, chickens were weighed by pen, and feed intake was recorded to calculate body weight gain (**BWG**), average daily feed intake, and feed conversion ratio (**FCR**). From days 28 to 35 of experiment, broilers were fed diets mixed with 0.2% chromic oxide (Chromium (III) oxide, 98.5%, Samchun Pure Chemical Co., Ltd., Gyeonggi, Korea) as an indigestible marker to determine apparent total tract nutrient utilization of dry matter (**DM**), nitrogen (**N**), and gross energy (**GE**). Fresh excreta samples were collected from each pen on days 33, 34, and 35. Samples from same pen were mixed and pooled and were stored at −20°C until analysis. Before chemical analysis, the feed and excreta samples were thawed and dried at 70°C for 72 h in a forced-air oven (model FC-610, Advantec, Toyo Seisakusho Co. Ltd., Tokyo, Japan), after which they were finely ground by hand grounding through mortars to a size that could pass through a 1-mm screen. Then, feed and excreta samples were analyzed for DM and N (methods 943.01 and method 968.06; AOAC International, 2000). Gross energy was determined by measuring the heat of combustion in the samples, using a bomb calorimeter (Parr 6100; Parr108 instrument Co., Moline, IL). Chromium concentration was determined by atomic absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan), according to the method described by Williams et al. (1962). The equation for calculating utilization was as follows: utilization (%) = (1 − ((Ne × Cd)/(Nd × Ce))) × 100, where Ne = nutrient concentration in excreta (% DM), Nd = nutrient concentration in diet (% DM), Ce = chromium concentration in excreta (% DM), and Cd = chromium concentration in diet (% DM) (Liu et al., 2018a).

Cecal Microbiota Population and Excreta Noxious Gas Emission On day 35, the cecal contents of 2 birds from each pen (10 replications per treatment) were aseptically collected in individual sterile culture tubes, and then

Table 1. Ingredient composition of experimental diets as-fed basis.

Ingredients, %	Phase 1 (day 1–7)	Phase 2 (day 8–21)	Phase 3 (day 22–35)
Corn	43.41	55.09	58.59
Soybean meal	25.70	22.60	19.61
Wheat bran	10.30	0.30	0.30
Wheat flour	5.00	5.00	5.00
Rapeseed meal	-	2.00	-
Canola	-	2.00	-
Corn gluten	2.90	-	-
Sesame meal	2.00	2.00	2.00
Distillers dried grains with soluble	3.00	3.00	5.00
Meat meal	2.00	3.00	3.00
Tallow	1.00	1.80	3.10
Soy oil	0.50	-	-
Limestone	1.33	1.25	1.29
Monodicalcium phosphate	0.77	0.19	0.35
Salt	0.33	0.26	0.24
Methionine	0.36	0.33	0.34
Lysine	0.83	0.63	0.67
Threonine	0.19	0.18	0.14
Choline	0.13	0.10	0.10
Vitamin premix[1]	0.10	0.10	0.10
Mineral premix[2]	0.10	0.10	0.10
Phytase 1000G[3]	0.05	0.07	0.07
Chromium (III) oxide[4]	0.20	0.20	0.20
Total	100.00	100.00	100.00
Calculated nutrient composition			
Crude protein, %	21.99	20.49	18.49
Crude fat, %	4.08	4.95	6.08
Crude fiber, %	2.44	2.66	2.40
Crude ash, %	5.85	5.27	5.06
Metabolizable energy, kcal/kg	3,045	3,135	3,251
Calcium, %	0.96	0.90	0.89
Nonphytase phosphorus, %	0.45	0.43	0.42
Analyzed nutrient composition			
Total phosphorus, %	0.74	0.72	0.70
Lysine	2.20	1.68	1.52
Methionine	0.69	0.63	0.60
Threonine	0.97	0.91	0.76
Tryptophan	0.26	0.23	0.21
Methionine + cysteine	1.05	0.99	0.93

[1]Provided per kg of complete diet: 11,025 IU vitamin A; 1,103 IU vitamin D3; 44 IU vitamin E; 4.4 mg vitamin K; 8.3 mg riboflavin; 50 mg niacin; 4 mg thiamine; 29 mg d-pantothenic; 166 mg choline; 33 μg vitamin B12.

[2]Provided per kg of complete diet: 12 mg Cu (as $CuSO_4 \bullet 5H_2O$); 85 mg Zn (as $ZnSO_4$); 8 mg Mn (as MnO_2); 0.28 mg I (as KI); 0.15 mg Se (as $Na_2SeO_3 \bullet 5H_2O$).

[3]Phytase premix prepared by serial dilution with corn to contain 1,000 phytase units/g, provided by Easybiosystem, Seoul Feed Ltd., Seoul, Korea.

[4]The chromium oxide was added into diets as an indigestible marker which is exclusive of the total feed ingredients. The product was provided by Samchun Pure Chemical Co., Ltd., Gyeonggi, Korea.

placed on ice for transportation to the laboratory where analysis was immediately carried out. One gram of cecal sample was blended with 9 mL of sterile peptone water and vortexed for 1 min. Counts of viable bacteria in the cecal samples were determined by plating 10-fold serial dilutions (10^{-1} to 10^{-8}) onto Lactobacilli MRS agar (Difco Laboratories, Detroit, MI), MacConkey agar (Difco Laboratories), and Salmonella–Shigella agar (Difco Laboratories) plates to isolate lactic acid bacteria, coliform bacteria, and *Salmonella*, respectively. The lactobacilli agar plates were then incubated for 24 h at 37°C under anaerobic conditions. The MacConkey and Salmonella–Shigella agar plates were incubated for 24 h at 37°C under aerobic conditions. After the incubation periods, colonies of the respective bacteria were counted and expressed as the logarithm of colony-forming units per gram ($\log_{10}$ CFU/g).

At the end of the experiment, fresh excreta samples were collected from each pen for noxious gas emissions. A total of 30 g of samples were placed in 2.6-L sealed plastic boxes. The samples were allowed to ferment for 3 d at room temperature (25°C). After the fermentation period, the concentrations of ammonia ($\mathbf{NH_3}$), hydrogen sulfide ($\mathbf{H_2S}$), and total mercaptan were analyzed by using a gas sampling pump with different detection tubes (GV-100; Gastec Corp., Kanagawa, Japan; Gastec detector tube No. 3L, No. 4LT, and No. 70L). To collect the gas sample, adhesive plaster on the box was punctured, and 100 mL of the headspace air was sampled approximately 5 cm above the excreta (Park and Kim, 2020).

Breast Meat Quality and Relative Organ Weight On day 35 of the experiment, 2 chickens per pen (20 birds per treatment) were weighed and slaughtered in a local commercial slaughter house. The breast meat, abdominal

Table 2. Effect of dietary *Achyranthes japonica* Nakai (AJN) extract supplementation on growth performance in 35-day-old broilers.

	AJN extract (%)					*P*-value	
Items	0	0.025	0.05	0.1	SEM	Linear	Quadratic
IBW, g	43.21	43.31	43.42	43.44	0.64		
Days 1 to 7							
BWG, g/d/bird	105	106	108	109	3.06	0.374	0.907
ADFI, g/d/bird	130	130	131	133	4.48	0.598	0.775
FCR	1.242	1.232	1.226	1.234	0.06	0.916	0.895
Days 8 to 21							
BWG, g/d/bird	640	649	657	667	8.96	0.038	0.998
ADFI, g/d/bird	1,065	1,067	1,063	1,070	13.24	0.850	0.829
FCR	1.666	1.643	1.617	1.605	0.02	0.013	0.771
Days 22 to 35							
BWG, g/d/bird	937	944	962	976	11.92	0.020	0.781
ADFI, g/d/bird	1,758	1,748	1,758	1,749	23.75	0.868	0.992
FCR	1.877	1.852	1.828	1.793	0.03	0.029	0.864
Day 0 to 35							
BWG, g/d/bird	1,682	1,700	1,727	1752	15.18	0.002	0.810
ADFI, g/d/bird	2,953	2,945	2,952	2,952	29.20	0.975	0.881
FCR	1.756	1.733	1.709	1.686	0.02	0.004	0.997

The body weight gain expressed the average gain per bird per day.

Abbreviations: ADFI, average daily feed intake; BWG, body weight gain; FCR, feed conversion ratio; IBW, initial body weight; SEM, pooled standard error of the mean.

fat, gizzard, liver, spleen, and bursa of Fabricius were removed by trained personnel and weighed so that organ weight could be expressed as a percentage of body weight. Color values (L^* = lightness, a^* = redness, and b^* = yellowness) of breast muscle were determined using a Minolta CR-410 Chroma Meter (Konica Minolta Sensing Inc., Osaka, Japan). The pH values of each breast meat sample were measured via a glass-electrode pH meter (WTW pH 340-A, WTH Measurement Systems Inc., Ft. Myers, FL). To estimate the cooking loss, raw meat samples were packed into Cryovac Cook-In Bags after weighing and cooked in a water bath at 100°C for 30 min. Samples were cooled at room temperature for 1 h and weighed again. Cooking loss was calculated as the weight difference between the initial raw and final cooked samples. Drip loss was measured using approximately 4 g of meat sample hung in a zipper bag and stored at 4°C. After storage, moisture on the surface of the meat slice was carefully removed and weighed at days 1, 3, 5, and 7 after the sample was taken. The initial and final weight of each sample was used to calculate drip loss. To determine the water-holding capacity (WHC), 5 g of meat sample was heated to 90°C in a water bath for 30 min. The samples were cooled with ice and centrifuged at 1,000 × g for 10 min. Water-holding capacity (%) was calculated as the ratio of weight loss of the sample during centrifugation to that the original liquid.

Table 3. Effect of dietary *Achyranthes japonica* Nakai (AJN) extract supplementation on ileal nutrient utilization in 35-day-old broilers.

	AJN extract (%)					*P*-value	
Items, %	0	0.025	0.05	0.1	SEM	Linear	Quadratic
DM	71.16	71.71	71.86	72.71	0.33	0.003	0.654
N	69.40	70.29	70.96	71.74	0.57	0.006	0.927
GE	71.89	72.40	72.74	73.20	0.59	0.117	0.966

Abbreviations: DM, dry matter; GE, gross energy; N, nitrogen; SEM, pooled standard error of the mean.

Statistical Analysis

All data were subjected to statistical analysis in a randomized complete design using the General Linear Models procedures (SAS Institute, Cary, NC). The cage was used as the experimental unit for growth performance, nutrient utilization, and excreta gas emissions. For the cecal microbiota and meat quality measurements, the individual bird was used as the experimental unit. Orthogonal polynomials were used to assess the linear and quadratic effects of increasing concentration of supplemental AJN extract. Variability in the data was expressed as the pooled standard error of mean. $P < 0.05$ was considered statistically significant.

RESULTS

Growth Performance and Nutrition Utilization

The effects of dietary AJN extract supplementation on growth performance and nutrient utilization are described in Table 2 and Table 3, respectively. As shown in Table 2, FI was unaffected ($P > 0.05$) by AJN extract supplementation throughout the experimental period. As AJN extract supplementation increased, BWG was linearly increased ($P < 0.05$), and FCR linearly decreased ($P < 0.05$) during days 8 to 21, 22 to 35, and the overall experiment. There were significant ($P < 0.05$; Table 3) linear increases in the digestibility of DM and N in response to increasing AJN extract supplementation at the end of the experiment. No significant effects of AJN extract supplementation were

Table 4. Effect of dietary *Achyranthes japonica* Nakai (AJN) extract supplementation on cecal microbiota in 35-day-old broilers.

	AJN extract (%)					*P*-value	
Items (log_{10} cfu/g)	0	0.025	0.05	0.1	SEM	Linear	Quadratic
Lactic acid bacteria	7.01	7.09	7.14	7.20	0.05	0.012	0.836
Coliform bacteria	6.00	6.04	6.07	6.07	0.08	0.495	0.796
Salmonella	2.71	2.64	2.51	2.39	0.15	0.108	0.876

Abbreviation: SEM, pooled standard error of the mean.

found for GE digestibility at the end of the experiment ($P < 0.05$).

Cecal Microbiota Population and Excreta Noxious Gas Emission

As shown in Table 4, a significant linear increase ($P < 0.05$) because of increasing AJN extract supplementation was observed for cecal lactic acid bacteria counts, but the populations of cecal coliform bacteria and *Salmonella* were not affected ($P > 0.05$). A significant linear decrease ($P < 0.05$; Table 5) because of increasing AJN extract supplementation was observed for NH_3 concentration, but H_2S and total mercaptan concentrations were not affected ($P > 0.05$).

Breast Meat Quality and Relative Organ Weight

As shown in Table 6, dietary AJN extract supplementation did not influence ($P > 0.05$) the meat quality parameters such as pH, color, cooking loss, drip loss, and WHC. With regard to relative organ weight, breast muscle weight was linearly increased ($P < 0.05$) with increasing supplemental levels of AJN extract; whereas, the weight of liver, abdominal fat, bursa of Fabricius, spleen, and gizzard were not affected ($P > 0.05$) by AJN extract supplementation.

DISCUSSION

The aim of the current study was to evaluate the influence of dietary supplementation with 3 levels of AJN extract on performance characteristics in broilers fed corn–wheat–soybean meal diet. However, owing to a lack of available literature, direct comparisons of response to AJN supplementation of corn–wheat–soybean meal diet in broilers is impossible. Furthermore, owing to the synergistic effects among the active molecules in the extracts (Frankič et al., 2009), this study attempts to investigate the roles of AJN extract as a natural growth promoter for broilers. Similarly, Hernandez et al. (2004) reported that the dietary addition of extracts from sage, thyme, and rosemary improved broiler BW on day 35 and BWG from 14 to 21 d. Guo et al. (2004) indicated that dietary inclusion of the Chinese herbal medicine mixture positively improved BWG and FCR in broilers. Recently, Park et al. (2014) demonstrated that final weight and BWG were increased by the supplementation of herbal extract mixture of *Saposhnikovia divaricata*, *Lonicera japonica*, and *Chelidonium majus* in broiler diets. It was suggested that the main mechanisms of the positive effects of herb extract on growth performance of animals may be because of the increase in digestibility, improvement in the gut microbiome and modification of the digestive secretion morphology (Hashemi and Davoodi, 2010). However, the exact mechanism of AJN action in improving the growth performance of broilers has not been fully understood. The improved growth performance of broilers in this experiment may be because of the enhanced nutrient utilization. Furthermore, some research based on other animals investigated that AJN extracts exhibited antioxidative properties, antimicrobial activity, and immunostimulation effects, which may positively influence nutrient absorption to improve growth performance (Tahiliani and Kar, 2000; Chen et al., 2009). Thus, improved growth performance with the supplementation of AJN extracts in the current study may be associated with the stimulation in both digestion and absorption of the nutrients.

In response to increasing AJN extract supplementation, the nutrient utilization of DM and N were linearly increased at the end of the experiment. It is well known that herbs and their extracts exert beneficial actions within the digestive tract because of laxative and spasmolytic properties, and they also prevent flatulence

Table 5. Effect of dietary *Achyranthes japonica* Nakai (AJN) extract supplementation on excreta gas emission in 35-day-old broilers.

	AJN extract (%)					*P*-value	
Items, ppm	0	0.025	0.05	0.1	SEM	Linear	Quadratic
NH_3	13.58	13.18	12.80	12.23	0.38	0.023	0.822
H_2S	2.93	2.53	2.55	2.90	0.47	0.981	0.441
R.SH	2.00	1.75	2.00	2.00	0.75	0.942	0.870

Abbreviations: H_2S, hydrogen sulfide; NH_3, ammonia; R.SH, total mercaptan; SEM, pooled standard error of the mean.

Table 6. Effect of dietary *Achyranthes japonica* Nakai (AJN) extract supplementation on meat quality and organ weight in 35-day-old broilers.

	AJN extract (%)					*P*-value	
Items	0	0.025	0.05	0.1	SEM	Linear	Quadratic
pH value	7.67	7.69	7.63	7.69	0.05	0.996	0.632
Breast muscle color							
Lightness (L^*)	34.88	34.99	34.91	34.89	0.65	0.986	0.923
Redness (a^*)	37.52	37.71	37.81	37.51	1.09	0.991	0.823
Yellowness (b^*)	15.33	15.32	15.37	15.24	0.67	0.943	0.927
Cooking loss	19.23	20.42	20.97	20.95	2.46	0.612	0.807
WHC, %	44.97	45.19	43.27	42.75	5.72	0.742	0.950
Drip loss, %							
Day 1	4.76	4.75	4.67	4.62	0.10	0.311	0.881
Day 3	7.55	7.64	7.61	7.53	0.18	0.893	0.624
Day 5	10.83	10.79	10.81	10.67	0.20	0.591	0.810
Day 7	12.58	12.45	12.44	12.39	0.40	0.748	0.919
Relative organ weight, %							
Breast muscle	17.12	17.68	17.99	18.12	0.25	0.010	0.405
Liver	3.08	3.15	2.74	2.84	0.24	0.337	0.997
Abdominal fat	1.23	0.94	1.28	1.23	0.24	0.756	0.625
Bursa of Fabricius	0.14	0.14	0.16	0.16	0.02	0.390	0.997
Spleen	0.16	0.14	0.11	0.15	0.03	0.604	0.293
Gizzard	1.81	1.68	1.71	1.80	0.09	0.991	0.224

Abbreviations: SEM, pooled standard error of the mean; WHC, water-holding capacity.

(Chrubasik et al., 2005). Moreover, it has been suggested that the core mode of nutritional action of herbs and their extracts is the stimulation of digestive secretions, bile, and mucus, and enzyme activity enhancement (Windisch et al., 2008). For instance, Jang et al. (2007) reported that the inclusion of a plant extract mixture containing carvacrol, cinnamaldehyde, and capsaicin in the diet improved the activities of pancreatic trypsin and α-amylase, as well as jejunal chime content in 24-day-old broilers. Furthermore, Khalaji et al. (2011) proved that the inclusion of 0.3 g/kg and 0.5 g/kg of *Camellis L.* extract altered the villus height and ileal crypt depth in broilers. These could be part of the reasons which may explain the increased nutrient digestibility. Additionally, nonstarch polysaccharides (**NSP**) such as galactomannans and galactosides are the main antinutritional compounds in the SBM, which may negatively affect the nutrient utilization in broilers (Choct et al., 1996). It was reported that NSPs may influence digestion by increasing digesta viscosity, reducing lipid solution and absorption or increasing fermentation with subsequent flatulence (Roberts and Choct, 2006; Urbano et al., 2007). However, in the current experiment, we reduced the SBM levels in the based diets which may reduce the inclusion of the NSP antinutritional compounds in the diets. It could be another likely reason for the increased utilization in this study.

The gastrointestinal tract of broilers is the major position of feed digestion and nutrient absorption, which comprised over 900 species of bacteria (Wei et al., 2013). The cecum is the main site of microbial fermentation in the distal intestine and plays important roles in preventing pathogen colonization, removing harmful substances, circulating nitrogen, and absorbing additional nutrients (Yan et al., 2017). It was reported that the individual intestinal compartment owns the unique physical and chemical properties and was occupied with specialized microbiome composition (Dethlefsen et al., 2007). Therefore, stimulating beneficial bacteria such as lactic acid bacteria could be helpful to the gut microbiota balance, and this consequently affects the host growth, immunity, and well-being positively. In an *in vitro* study conducted by Jung et al. (2008), it was indicated that AJN extract received high antimicrobial effects against *Clostridium difficile*; furthermore, the efficiency of antimicrobial activity increased with the combination of lactic acid bacteria. Recently, Liu et al. (2018b) indicated that in challenged broilers, the supplementation of *Achyranthes bidentata* extract decreased the cecum *Escherichia coli* (*E. coli*) population. Moreover, Xie et al. (2018) reported that dietary inclusion of *A. bidentata* extract increased the counts of intestinal *Lactobacillus* and *Bifidobacterium* and decreased the number of *E. coli* in weaned piglets. The results of the present study indicated that the addition of AJN extract improved the cecal lactic acid bacteria population, which may improve the microbiome balance and leading to a healthy gastrointestinal tract. Furthermore, the improved microbiota balance also could be an explanation for the increased growth performance and nutrient utilization in this experiment.

Ammonia, H_2S, carbon dioxide, and other odorous gaseous compounds constituted the main noxious gas emissions from livestock farms, which is considered as severe environmental pollution posing serious health problems both to animals and workers (Sun et al., 2008). Among them, NH_3 emission is closely relevant to soil and water acidification and N deposition in ecosystems. Previous researchers hypothesized that the fecal noxious gas emission may be related to nutrient retention and the intestinal microbiome. Ferket et al. (2002) suggested that the gas emissions from farm animals are associated with harmful intestinal bacteria population. Ammonia is a by-product of the microbial decomposition of nutrient

compounds in the excreta (Li et al., 2012). But, in the present study, the counts of harmful bacteria such as coliform bacteria and *Salmonella* were not affected; whereas, lactic acid bacteria population was improved. In combination with the results of this experiment, it was suggested that excreta noxious gas emission may also be related to the beneficial bacteria population in the broiler intestine. On the other hand, decreased noxious gas content has also been suggested to be associated with the improvement of nutrient utilization (Yan et al., 2010, 2012). The increased nutrient utilization might result in less substrate for microbial fermentation to reduce the noxious gas emission. The possible explanation for the decreased NH_3 emission in the current study may be because of a two-way action.

Dietary supplementation of AJN extract had no significant effects on meat quality and relative organ weight, except for the breast meat percentage, the weight of which increased with AJN extract supplementation. The reports about the effects of herbs and their extracts on the carcass characteristics parameters in broilers are few, and the results are not univocal. In agreement with this study, Erener et al. (2011) reported that the addition of green tea extract improved the carcass weight of broilers. Similarly, Sang-Oh et al. (2013) indicated that broilers fed cinnamon powder had higher breast muscle percentage than those chickens fed normal diets. However, some other researchers demonstrated that there was no influence on carcass parameters with the supplementation of herbal extracts (Botsoglou et al., 2004; Garcia et al., 2007; Koreleski and Swiatkiewicz, 2007). For instance, Hong et al. (2012) reported that dietary supplementation of essential oils derived from oregano, anise, and citrus peel had no effects on breast muscles DM, WHC, fat content, and color in broilers. The different findings may be associated with differences in the herb types used in the studies. However, the exact mechanism by which AJN extracts exerts effects on the meat quality parameters is unclear, and the improvement in the breast meat percentage observed in this may be because of the antioxidative properties of AJN extracts (Frankič et al., 2009). Additionally, it was also suggested that the herbal extracts used in animal nutrition could stimulant the digestion of animals; thus, the improved nutrient utilization and growth performance may be another reason for the increased breast meat percentage. Furthermore, appearance is the major criterion for selection and evaluation of meat quality for consumers, which is not affected in this experiment. It could be concluded that the supplementation of AJN extract had no significant deleterious effect of meat quality parameters relevant to consumer acceptability.

In conclusion, supplementation with increasing levels of AJN extract improved growth performance during grower (day 8–21) and finisher (day 22–35) period, enhanced the nutrient utilization of DM and N, modified the cecal lactic acid bacteria population, decreased the concentration of excreta NH_3 emission, and increased breast meat production in broilers fed corn–wheat–soybean meal diet. Among the 3 levels of AJN extracts, addition of 0.1% had the best performing in broilers. This study provided a basis for future research on AJN as a feed additive in broilers.

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