Title Page

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Running Title	*Achyranthes japonica* extract in broiler diet
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Abstract:

Achyranthes japonica extract (AJE) is derived from a medicinal plant *Achyranthes japonica*, known for its anti-inflammatory, antioxidant, and antimicrobial properties. AJE contains multiple bioactive compounds, including saponins, triterpenoids, phytoecdysteroids, 20-hydroxyecdysone, and inokosterone. Aim of this investigation was to examine the impact of AJE as a phytogenic feed additive on growth performance, nutrient digestibility, excreta microbial count, noxious gas emissions, breast meat quality in broilers. About three hundred and sixty, day-old broilers (Ross 308) were assigned into four treatments (five replication cages/treatment, and 18 birds/case). Dietary treatments: CON, basal diet; 0.02% AJE, basal diet with 0.02%; 0.04% AJE, basal diet with 0.04% AJE, and 0.02% AJE, basal diet with 0.06% of AJE. Body weight gain increased linearly ($p < 0.05$) through the inclusion of AJE during days 7 to 21, 21 to 35, as well as the entire experimental period. Besides, feed intake increased ($p < 0.05$) linearly during days 21 to 35 and the entire experiment with the increased AJE doses in broiler diet. Dry matter digestibility was increased ($p < 0.05$) linearly along with increasing amounts of AJE. With increasing AJE supplementation, nitrogen and energy utilization tended to improve ($p < 0.10$). In summary, the addition of AJE in the corn-soybean meal diet led to higher body weight gain and increased feed intake as well as enhanced nutrient digestibility, among them the highest improvement was found in 0.06%-AJE indicating the acceptance of AJE as a phytogenic feed additive.

Keywords: *Achyranthes japonica*, broiler, body weight gain, nutrient utilization, phytogenic feed additive.

INTRODUCTION

For many years, therapeutic doses of antibiotics were used in livestock production to optimize the intestinal environment, growth, and avoid disease [1]. Recently, increased public awareness has been observed due to the antibiotic drug residues and resistance caused by long-term usage of antibiotic growth promoter in animal diets. As a result of the ban on antibiotic growth promoter in livestock, there has been a boom in research towards finding antibiotic growth promoter substitutes. The modern chicken industry has experienced a number of feed additives designed to increase the birds' resistance to disease and shift the population of gut microbiota. Because of their phytochemical properties, including phenolics, tannins, and flavonoids, which have a significant role in modifying nutrient digestibility and intestinal health, the use of medicinal plants in livestock diets has been extensively investigated in recent years [2].

Achyranthes japonica is a perennial plant with thickened roots. This plant belongs to the Amaranthaceae family, and can be found in east Asia, i.e. Korea, China, and Japan [3]. The root of *Achyranthes japonica* is often used as traditional medicine because it contains multiple bioactive compounds, including saponins, triterpenoids, phytoecdysteroids, 20-hydroxyecdysone, and inokosterone. A variety of physiological benefits of *Achyranthes japonica* have been identified in previous research, such as anti-allergic, hepatoprotective, anti-inflammatory, antioxidant, and cancer prevention properties [5]. Previous studies showed that the incorporation of *Achyranthes japonica* extract (AJE) enhanced the growth performance of broilers [6, 7], growing pigs [2], and finishing pigs [8]. Other plant extracts, like *Achyranthes bidentata* polysaccharides improved growth performance and ileal microbial count in pekin duck [9]. Dang et al. [8] showed that the incorporaton of *Achyranthes japonica Nakai* extract alleviated the adverse impact of a low-CP-diet on the growth of broilers. As the main component of AJE, flavonoids have the capacity to improve growth performance and nutrient digestibility in pigs [10]. Supplementation of AJE up to 0.10% has been shown to enhance growth, feed consumption, feed efficiency, and cecal *Lactobacillus* count in broiler chickens [6].

Considering the previous studies, we assumed that a broiler's diet supplemented with AJE would improve the growth, noxious gas emissions, and meat quality parameters of broilers. However, the optimum dose of AJE in broiler diets is not known yet. Therefore, the aim of this experiment was to assess the impact of dietary AJE at 0%, 0.02%, 0.04%, and 0.06% on broiler growth performance, nutritional digestibility, excreta bacteria count, noxious gas emissions, and meat quality characteristics.

MATERIALS AND METHODS

The Animal Care and Use Committee at Dankook University in South Korea reviewed the protocols for the experiments, and they gave their approval (DK-1-1956).

Animals, experimental design, diets, and housing

A total of 360, day-old-broilers (Ross 308) weighing 42.16 ± 0.56 g were alloted into four groups (five replicates/treatment and 18 birds/replicate). The whole of the feeding trial period was broken up into three distinct phases (days 1 to 7; days 7 to 21; and days 21 to 35). The treatment diets consisted of basal diet with varying amounts of AJE added (0.0%, 0.02%, 0.04%, and 0.06%, respectively). Table 1 shows the components that were used to prepare the basal diet. By exchanging the same amount of maize for AJE, the diet was adjusted. The preparation of diets was carried out with the instructions provided by Rostagno et al. [11]. The first five days, the birds were kept at 33℃, then from days 6 through 35, the temperature was dropped to 22℃, while the relative humidity hovered about 60%. During the feeding trial, the birds had free access to food and water.

Preparation of AJE

A commercial animal feed company was the supplier of the AJE (Synergen Inc., Bucheon, South Korea). At first, the roots of *Achyranthes japonica* were thoroughly washed, and then they were ground into a powder in a mill (IKAM20; IKA, Staufen, Germany). The powder was then added to a container of distilled water (at 80 ℃), and the mixture was allowed to reflux for four hours to get the extract. Once again, the residual residues were extracted using 1:5 distilled water (at 80℃ for 2 hours). After filtering the extract solution, the viable components were separated on a column and washed with ethanol. After being filtered, the extracted liquids were freeze-dried into a mush and distributed. As an end result, 0.47 mg/g of saponin, 1.15 mg/g of total flavonoid, and 4.26 mg/g of total polyphenol were present in the final AJE product.

Sampling and measurements

Body weight and feed intake of broilers were written down by cage on days 1, 7, 21, and 35 of the feeding trial to determine feed conversion ratio and body weight gain. Samples of fresh excreta were taken on the last three days of the feeding trial, which included a 0.2% chromic oxide (Cr_2O_3, 98.5%, Samchun Pure Chemical Co., Ltd., Gyeonggi, South Korea) addition to the diet. All samples were dried at 60 ℃ for 72 hours, and then subjected to AOAC-standard techniques for determining dry matter (methods 934.01), and nitrogen (methods 968.06) [12]. Gross energy was measured using bomb caloriemeter (Parr Instrument, Moline, IL, USA). An atomic absorption spectrophotometry was used to determine the amount of chromium (UV-1201, Shimadzu, Kyoto, Japan). Apparent total tract digestibility (%) = (1 - (Nf×Cd)/(Nd×Cf) × 100), where Nf represents nutrient concentration in feces (percent dry matter), Nd represents nutrient concentration in diet (percent dry matter), Cf represents chromium concentration in feces (percent dry matter), and Cd represents chromium concentration in diet (percent dry matter).

After the feeding trial was complete, feces samples were taken from 10 broilers across all treatments, pooled on a cage-by-cage basis, and frozen till analysis. The excreta samples were mixed with sterile peptone water using a 1:9 ratio, and then stirred in a vortex mixer for one minute. Using a serial dilution technique (from 101 to 106), samples were mixed and injected at a volume of 50 L into three different selective agar media: MacConkey agar for *coliform* bacteria (Difco Laboratories, Detroit, USA); Lactobacilli MRS agar for *Lactobacillus spp*. (Difco Laboratories, Detroit, USA); Salmonella-Shigella (SS) agar for *Salmonella* and *Shigella* bacteria (Difco Laboratories, Detroit, USA). For 24 hours, Petridishes were kept in an aerobic incubator at 37 degrees Celsius (MacConkey agar and SS agar). After a period of 24 hours, the number of live bacterial colonies was determined.

To determine noxious gas emissions about 300g of fresh excreta collected from each cage for four consequetive days. Excreta samples were collected and kept in two 2-liter sealed plastic containers at room temperature (20-24 ℃) for five days. During the fermentation process, gas concentrations were estimated using a gas sample pump kit (model GV100S, Gastec Corp., Japan). Total methyl mercaptans, hydrogen sulfide, and ammonia were all measured using complex gas meter (MultiRAE Lite model PGM-6208, RAE, USA). About 100 cc of air was collected from a sample taken roughly two inches below the surface of the excrement.

Once the experiment was complete, one broiler per cage was sent to a professional slaughterhouse to be weighed and slaughtered (5 birds per treatment). Trained staff members cut off the chicken's breast flesh, abdomen fat, liver, spleen, gizzard, and bursa of Fabricius before weighing it. We used a Minolta colorimeter (CR300, Tokyo, Japan) calibrated against a white plate to determine the exact shade of the chicken breast flesh (L*, a*, and b*). The pH of raw breast meat was determined 24 hours after a postmortem by homogenizing 10 g of flesh with 90 mL of double-distilled water and then using a digital pH meter (Testo 205, Lenzkirch, Germany). Samples of raw meat were weighed before being cooked in Cryovac Cook-In Bags for 30 minutes at 100℃. The samples were reweighed after resting at room temperature for one hour. The difference in mass between raw and cooked meat is known as cooking loss. Drip loss was measured using a sample of meat weighing about 4 grams into a zip-top bag and storing it in the fridge at 4 degrees Celsius. After being put into storage, the sample was weighed on days 1, 3, 5, and 7. Prior to weighing, the surface moisture was wiped from the meat. Droplet size was determined by comparing the starting and ending weights. Finally, 5 g of the meat was cooked to 90 ℃ in a water bath for 30 minutes to determine its water-holding capacity.

After that, we chilled the samples and centrifuged them at 1,000 g for 10 minutes. After centrifuging the samples, we calculated water-holding capacity by dividing the total weight loss by the initial liquid weight loss.

Statistical analysis

All of the data acquired was analyzed using the general linear models techniques (SAS Institute, Cary, NC). In this study, the cage was considered the experimental unit for growth performance, nutrient metabolizability, and noxious gas emissions. For meat quality parameters, the individual bird was considered as the experimental unit. Here $p<0.05$ was considered as significance and $p<0.10$ was considered as a trend.

RESULTS

The data presented in Table 2 show the effect of dietary AJE on growth performance. There was a linear increase ($p<0.05$) in body weight gain during d 7 to 21, d 21 to 35, and the overall feeding trial. Linearly increased ($p<0.05$) feed intake was found d 21 to 35, and overall feeding trial period. During d 1 to 7 dietary AJE supplementation showed no significant difference ($p>0.05$) in body weight gain, feed intake, and feed conversion ratio. No significant change was found in feed conversion ratio throughout all the stages of feeding trial.

At the end of the feeding trial, dry matter digestibility was increased linearly ($p<0.05$) with the increasing AJE supplementation in broiler diet (Table 3). Nitrogen and energy digestibility tended to improve ($p<0.10$) with the inclusion of AJE supplementation up to 0.06% in the broiler diet.

The data presented in Table 4 show that the excreta microbial count was not significantly influenced ($p > 0.05$) by dietary AJE supplementation. *Lactobacillus, E. coli,* and *Salmonella* count were not altered through the AJE supplementation up to 0.06%.

The addition of AJE in the diet did not have a significant effect ($p > 0.05$) on noxious gas emissions (Table 5). Emission of ammonia, hydrogen sulfide, methyl mercaptans were not changed at 35th day of the experiment through the supplementation of AJE.

Relative organ weight and meat quality parameters are shown in Table 6. No significant differences ($p > 0.05$) were found in breast muscle and abdominal fat relative organ weights of bursa of Fabricius, spleen, liver, and gizzard through dietary AJE supplementation. In this experiment, no significant differences ($p > 0.05$) were found in pH value, breast muscle color (L*, a*, b*), water-holding capacity, cooking loss, or drip loss of breast meat with increasing the AJE levels in the broiler diets among the four treatments.

DISCUSSION

Achyranthes japonica contains different antioxidant phytochemicals and bioactive compounds, that are responsible for different biochemical activities in the animal body [13]. Considering the beneficial bioactive compounds found in AJE, this study was done to investigate the viability of AJE as a phytogenic growth promoter in broiler chickens. We found that addition of AJE up to 0.06% improved body weight gain and feed intake in broiler chickens. Our findings are supported by some of the previous work on broilers [14]. Both Sun et al. [7] and Park and Kim [6] demonstrated increased body weight gain and feed conversion ratio when broilers were fed up to 0.10% *Achyranthes japonica* root extract. Furthermore, some other studies conducted on pigs [15], and broilers [16], demonstrated the capability of *Achyranthes* plants to boost body growth. On the other hand, no effect on growth performance was seen when 0.02 % *Achyranthes* plant extract was given in broilers [17] and 0.08% *Achyranthes* plant extract were given to piglets [18]. These varying outcomes might be the consequence of a variety of factors, including dosages, sources, feed formulas, stages, and animals. Hashemi and Davoodi [19] explained the bioactive components present in the plant extract improved growth performance by improving nutrient digestibility, healthy gut microflora count, and morphology of intestinal wall. Additionally, optimizing intestinal integrity, minimizing intestinal damage, balancing nutritional requirements for immune response, and limiting substrate for the microbiota are all ways to increase feed intake in poultry. Liu et al. [16] also noted plant extract helps to improve the gut microbial community, which may indirectly increase feed intake and the growth performance of the broilers. In addition, to improve palatability and flavor, some of the phytogenic additions have been shown to boost feed intake in broilers [20]. The higher feed intake in this study may be connected with the improved flavor by AJE; however, this assumption has to be confirmed by more research. Therefore, the enhanced body weight gain observed in the AJE supplemented groups in our research may have been the consequence of higher feed intake.

The digestibility of dry matter, nitrogen in broilers improved through the incorporation of AJE up to 0.06% in diets. Previously, Sun et al. [7], and Liu et al. [21] observed positive results when AJE was supplied in broilers up to 0.10%. Khalaji et al. [22] explained that, plant extracts have significant improved crypt depth and villus height of gut, potentially leading to enhanced nutrient absorption and overall digestive efficiency in broilers. Moreover, Jang et al. [23] showed that broilers fed plant extract mixtures increase the pancreatic trypsin, α-amylase, and intestinal maltase for better nutrient digestibility. A variety of bioactive components in phytogenic feed additives, including antioxidant and antibacterial properties, show stimulatory effects on the digestive tract of broilers [24]. Some previous studies proved that efficient nutrient utilization is usually related to higher villus height as well as decreased crypt depth in broilers [16]. Moreover, the improved digestibility of dry matter, ammonia, and nitrogen found in AJE-supplemented diets might be attributed to the digestive enzymes, as well as the improvement in intestinal morphology, which resulted in increased nutrient digestibility in broilers. We expect that inclusion of 0.6% AJE could improve a villus height and, plan to carry out the evaluation in the future study. Additionally, because of the antioxidant, antibacterial, and immune-stimulating properties of AJE, it may indirectly help in nutritional absorption [9].

The digestive system of broilers is the primary habitat for different types of bacteria, and the cecum is the primary location for the fermentation of microbes. It helps to balance pathogens, circulate nitrogen, absorb nutrients and improve growth [25, 26]. In addition, having a lot of beneficial bacteria in the animal body helps in the utilization of the nutrients needed and keeps gut microbiota in balance. The addition of phytogenic extract was shown to lower the intestinal pH level, raise the lactic acid bacteria count in the ileum, and at the same time, cecal contents have been shown to markedly lower the counts of *E. coli* and *C. perfringens* in the ileum and cecal contents [27]. Different health benefit microbial communities like *Lactobacilli*, *bifidobacteria* enhances the host's immune response to fight against harmful microorganism. Jung et al. [28] noted that 0.5% AJE had high antibacterial effects *in vitro* by preventing the proliferation of harmful *Clostridium difficile*. Moreover, some of the previous research found improved gut microbiota with the supplementation of AJE in broiler diets [29, 7]. Sun et al. [7] found increased *Lactobacillus* with decreased *E. coli* and *Salmonella* when 0.10% AJE was supplemented to the broiler diet. We could not find any significant changes in the excretal *Lactobacillus*, *E.coli*, and *Salmonella* count. In previous experiment, the microbial population improved linearly with increasing AJE supplementation up to 0.10% [6]. Compared to previous studies, lower dose of AJE (0.06% AJE) used in this experiment may be the probable reason for the lack of a significant effect.

Noxious gases from livestock farms are mostly comprised of ammonia, hydrogen sulfide, and carbon dioxide, which are responsible for significant environmental pollution as well as health concerns in both animals and farm personnel [30, 31]. The noxious gas emissions from their excreta are inversely proportional to their nutritional digestibility [32]. It has been shown that increasing digestibility and the complete oxidative breakdown of organic substrate in the intestine reduces odor and levels of noxious gases in the excreta. Moreover, the microbial population and noxious gas emissions are related to nitrogen digestibility and *Lactobacillus* population [33, 6]. Several previous studies found that addition of phytogenic feed additives comprising saponin and polyphenol lowered the volatile components in broiler excreta [4]. No statistically significant differences were found in noxious gas emissions between the treatment groups in this experiment. This result may be because of the similar microbial populations in our study as the noxious gas emissions are linked with fecal microbial count [4]. Yet another possible explanation might be the lower dose of AJE administered in this feeding trial as compared to some of the earlier research, which has used AJE concentrations of up to 0.10 percent [6].

We found that dietary AJE inclusion had no direct influence on meat quality or relative organ weights in broilers. In past studies, plant extracts were used to investigate the effects on a variety of meats, including chicken [6, 7]; pork [15]; lamb [34]; and duck [34]. Plant extract feed addition has been shown to improve physicochemical qualities [35], whereas some studies found no beneficial effects [36]. The contradictory results are attributable to the different diets, animals, and dosages of plant extracts used in the studies. Furthermore, the meat quality measures (breast muscle color, water holding capacity, cooking loss, drip loss) that were unaffected by AJE supplementation reveal that there are no negative impacts on customer acceptance.

CONCLUSION

In conclusion, supplementation with increasing levels of AJE improved body weight gain during day 7–21, day 21–35, and the overall period; feed intake during days 21–35 and the overall period; enhanced the nutrient digestibility of dry matter in broilers fed a corn–wheat–soybean meal diet. AJE may be suitable as a feed additive to improve growth performance, and feed utilization in broilers without affecting meat quality parameters. The result indicated that AJE could be used as a phytogenic feed additive in broiler diets to improve the growth performance and nutrient digestibity.

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CONFLICT OF INTEREST

No potential conflicts of interest were found, as confirmed by the authors.

ANIMAL WELFARE STATEMENT

Experiment on animals was accepted by Dankook University's Animal Care and Use Committee in Cheonan, Republic of Korea (DK-1-1956).

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Table 1. Feed composition of broiler (as fed-basis)

	Day 1-7	Day 7-21	Day 21-35
Ingredients (%)			
Corn	43.63	47.45	53.78
Soybean meal	35.08	31.28	28.18
Corn gluten meal	13.00	13.00	10.00
Wheat bran	3.00	3.00	3.00
Soybean oil	1.76	1.74	1.51
Tricalcium phosphate	1.81	1.81	1.81
Limestone	0.94	0.94	0.94
Salt	0.36	0.36	0.36
Methionine (99%)	0.19	0.19	0.19
Lysine	0.03	0.03	0.03
Mineral mix[1]	0.10	0.10	0.10
Vitamin mix[2]	0.10	0.10	0.10
Total	100.00	100.00	100.00
Calculated value			
ME, kcal/kg	3200	3200	3200
Crude protein, %	23.00	21.50	20.00
Calcium, %	1.10	1.08	1.07
Potassium, %	0.93	0.86	0.82
Sodium, %	1.08	0.97	0.89
Chlorine, %	0.57	0.58	0.59
Available P, %	0.54	0.53	0.52
Lys, %	1.26	1.15	1.06
Met, %	0.54	0.52	0.50
Methionine + Cystine, %	1.01	1.03	0.91

Fat,%	4.45	4.51	4.32
Fiber,%	3.55	3.48	3.30
Ash,%	6.76	6.57	6.30

[1] Provided per kg of complete diet: 37.5 mg Zn (as $ZnSO_4$); 37.5 mg Mn (as MnO_2); 37.5 mg Fe (as $FeSO_4 \cdot 7H_2O$); 3.75 mg Cu (as $CuSO_4 \cdot 5H_2O$); 0.83 mg I (as KI); and 0.23 mg Se (as $Na_2SeO_3 \cdot 5H_2O$).

[2] Provided per kg of complete diet: 15,000 IU of vitamin A, 37.5 IU of vitamin E, 3,750 IU of vitamin D_3, 2.55 mg of vitamin K_3, 24 ug of vitamin B_{12}, 51 mg of Niacin, 1.5 mg of Folic acid, 3 mg of Thiamin, 7.5 mg of Rivoflavin, 4.5 mg of vitamin B_6, 0.2 mg of Biotin and 13.5 mg of Ca-Pantothenate.

Table 2. The effect of dietary AJE supplementation on growth performance in broilers

Items	CON	0.02% AJE	0.04% AJE	0.06% AJE	SEM[2]	*p* value	
						Linear	Quadratic
d 1 to 7							
BWG, g	114.60	110.20	109.60	115.80	1.63	0.840	0.126
FI, g	143.20	143.40	142.00	142.40	2.54	0.880	0.985
FCR	1.259	1.309	1.300	1.233	0.03	0.773	0.400
d 7 to 21							
BWG, g	648.00	651.20	678.60	671.80	5.20	0.029	0.596
FI, g	988.60	981.40	992.20	990.00	7.45	0.837	0.878
FCR	1.527	1.508	1.464	1.474	0.02	0.182	0.660
d 21 to 35							
BWG, g	963.60	979.60	1004.00	1037.60	11.68	0.019	0.684
FI, g	1773.40	1793.20	1825.40	1859.00	13.52	0.018	0.781
FCR	1.841	1.833	1.821	1.798	0.021	0.496	0.874
Overall							
BWG, g	1726.40	1740.80	1792.60	1825.00	14.86	0.007	0.729
FI, g	2905.20	2917.80	2960.00	2991.80	16.06	0.042	0.757
FCR	1.683	1.677	1.653	1.642	0.02	0.324	0.928

[1]Abbreviations: CON, basal diet; AJE, *Achyranthes japonica* extract; FI, feed intake; BWG, body weight gain; FCR, feed conversion ratio;

[2]Standard error of the mean

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332 Table 3. The effect of dietary AJE supplementation on nutrient digestibility in broilers

Item, %	CON	0.02% AJE	0.04% AJE	0.06% AJE	SEM[2]	*p* value	
						Linear	Quadratic
D 35							
Dry matter	72.95	74.11	76.07	77.62	0.61	0.009	0.880
Nitrogen	73.20	73.55	75.64	76.14	0.67	0.096	0.960
Energy	72.87	73.41	75.48	75.82	0.66	0.094	0.946

333 [1]Abbreviations: CON, basal diet; AJE, *Achyranthes japonica* extract;

334 [2]Standard error of the mean

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Table 4. The effect of dietary AJE supplementation on excreta microbial count in broilers

Items, $\log_{10}$cfu	CON	0.02% AJE	0.04% AJE	0.06% AJE	SEM[2]	*p* value	
						Linear	Quadratic
D 35							
Lactobacillus	9.49	9.44	9.39	9.50	0.027	0.929	0.170
E.coli	5.97	6.20	6.11	5.97	0.041	0.805	0.121
Salmonella	3.62	3.61	3.48	3.69	0.070	0.902	0.443

[1]Abbreviations: CON, basal diet; AJE, *Achyranthes japonica* extract

[2]Standard error of the mean

Table 5. The effect of dietary AJE supplementation on noxious gas emissions in broilers

Items, ppm	CON	0.02% AJE	0.04% AJE	0.06% AJE	SEM[2]	*p* value	
						Linear	Quadratic
D 35							
Ammonia	14.50	16.25	17.00	14.25	1.187	0.988	0.398
Hydrogen Sulfide	6.75	8.00	7.75	5.75	0.667	0.613	0.268
Methyl mercaptans	8.00	6.25	8.00	7.00	0.656	0.845	0.794

[1]Abbreviations: CON, basal diet; AJE, *Achyranthes japonica* extract;

[2]Standard error of the mean

Table 6. The effect of dietary AJE supplementation on meat quality in broilers

	CON	0.02% AJE	0.04% AJE	0.06% AJE	SEM[2]	*p* value	
						Linear	Quadratic
pH value	7.42	7.45	7.34	7.27	1.32	0.140	0.561
Breast muscle color							
Lightness (L*)	55.88	55.54	54.74	57.47	0.68	0.535	0.288
Redness (a*)	12.23	11.61	12.53	11.44	0.42	0.718	0.795
Yellowness (b*)	12.55	13.66	12.89	13.85	0.37	0.369	0.925
WHC, %	33.85	38.87	37.75	33.80	1.32	0.915	0.108
Cooking loss, %	11.26	15.55	14.96	15.33	0.90	0.159	0.280
Drip loss, %							
d 1	1.95	2.07	1.83	2.03	0.17	0.997	0.924
d 3	5.33	5.75	5.30	5.34	0.36	0.901	0.809
d 5	11.17	11.06	11.28	10.72	0.30	0.707	0.740
d 7	15.12	14.40	14.43	14.21	0.31	0.366	0.704
Relative organ weight, %							
Breast muscle	15.99	16.17	16.82	16.97	0.35	0.290	0.990
Abdominal fat	1.31	1.25	1.34	1.30	0.08	0.914	0.939
Liver	2.21	2.39	2.45	2.45	0.07	0.246	0.536
Bursa of Fabricius	0.156	0.130	0.124	0.144	0.01	0.662	0.291
Spleen	0.108	0.118	0.104	0.098	0.01	0.513	0.594
Gizzard	1.41	1.47	1.32	1.26	0.07	0.405	0.722

[1]Abbreviations: CON, basal diet; AJE, *Achyranthes japonica* extract; WHC, water holding capacity;

[2]Standard error of the mean