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# Effect of *Achyranthes japonica* extract on growth, digestibility, microbiota, gas emission, and meat quality in broilers fed different protein diets

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## Abstract

*Achyranthes japonica* extract (AJE) is a multifuctional products that express anti-inflammatory, antioxidant and anti-microbial properties. This study was aimed to evaluate the effects of AJE addition to standard and low crude protein (LCP) diet on growth performance, nutrient digestibility, excreta bacterial count, excreta noxious gas emissions, breast meat quality, and organ weight of broiler chicken. A total of 340 one-day-old Ross 308 broilers [initial body weight (BW) of 43.10 ± 1.46 g, 5 replicate cages per treatment, and 17 birds per cage] were randomly distributed into 1 of 4 dietary treatment groups for a 35 day trial. The diets were provided based on three age stage of the broiler. In the starter stage broiler were fed basal diet. Experimental diet were fed to broiler from day 8 to 35. In growing (days 8–21) and finishing (days 22–35) stage broiler were fed: Standard crude protein (SCP) diet and LCP diet with 0.025% and 0.05% of AJE supplementation respectively. Here, the SCP and LCP diets were 21.50% and 20.86% CP during days 8–21 and 20.00% and 19.40% CP during days 22–35, respectively. The SCP diets with 0.025% AJE supplementation resulted in higher ($p < 0.5$) BW gain (BWG) at finishing stage and a tendency to lower feed conversion ratio and BWG in the overall period compared to LCP diets with or without AJE supplemenation. Moreover, dry matter and nitrogen digestibility were increased with SCP diet along with 0.025% of AJE. No significant difference was found in meat quality parameters except for pH. Interestingly, the $NH_3$ gas emission to the environment was found to be less with different levels of CP and AJE supplementation. Therefore, we concluded that the addition of 0.025% AJE to the SCP diet improved broiler growth performance and nutrient digestibility with low fecal $NH_3$ emissions.

**Keywords:** *Achyranthes japonica*, Broiler, Growth performance, Low and high protein diet

## INTRODUCTION

The population of the world is rapidly increasing, especially in developing countries. For their better growth and development, proteins are very essential elements. The broiler is a good source of high-quality protein. A significant portion of global protein demand is met by the broiler industry [1].

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**Availability of data and material**
Upon reasonable request, the datasets of this study can be available from the corresponding author.

**Authors' contributions**
Conceptualization: Khan SU, Cho SB, Kim IH.
Data curation: Khan SU.
Formal analysis: Khan SU.
Methodology: Khan SU, Cho SB.
Software: Khan SU.
Validation: Kim IH.
Investigation: Khan SU, Cho SB, Kim IH.
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Nutritionally balanced feed is more efficient for better broiler production. Feed cost accounts for 70% of the total production expenditure in the poultry industry [2]. Protein is the second-most vital and expensive nutrient component in ration formulation whose price are increasing day by day. Soybean meals are widely used in poultry's diet [3] because they are a preferred source of protein, which contains well-balanced amino acids that are highly digestible. Reducing dietary protein while maintaining essential amino acid balance can be an effective technique to minimize feed price and nitrogen release into the environment [4].

Since 2006, the use of antibiotics in livestock industry was banned by europian union due to residual effect on food [5]. After that, researchers are paying attention to alternative growth promoters like probiotics, prebiotics, herbal products, natural marine products, and organic acids [6]. Phytogenic feed additives are one of them. *Achyranthes japonica* extract (AJE) is a phytogenic feed additive belongs to the family *Amaranthaceae*. AJE is mostly available in Asian countries, especially in Japan, China, and South Korea [7]. AJE contains a wide range of bioactive compounds, including polyphenols, flavonoids, and saponins. These compounds have a variety of health benefits, such as anti-inflammatory, antioxidant, and anti-microbial effects [8,9]. In broilers, supplementation of AJE with low crude protein (LCP) diet showed higher growth performance by improving nitrogen digestibility and controlling the bacterial count in the gut [2,10]. Previous research on AJE addition with different crude protein (CP) levels is quite limited. Therefore, we hypothesized that the synergistic effect of AJE on nutrient digestibility and microbial population in broilers could help recover their production performance when supplemented with LCP diet. Our objective was to evaluate the impact of AJE supplementation on growth performance, nutrient digestibility, excreta bacterial count, and gas emission in broilers fed different protein diets.

# MATERIALS AND METHODS

## Experimental site and ethical considerations

Experimental research was conducted at the experimental farm of Dankook University, Jeonui (Sejong, Korea). All the research procedures and animal care activities were followed in accordance with the care and use of experimental animals. The experiment was approved by the Animal Care Committee of Dankook University in Korea (No. DK-1-1939).

## Additives information with processing method

For this study, we commercially purchased AJE from Synergen. In brief, the roots of *Achyranthes Japonica* were washed more than three times in clean water and dried at 80°C. The dried roots were then milled using an IKA M20 machine (Gemini BV, Apeldoorn, Netherlands). Milled specimens were extracted with distilled water for approximately 4 h to obtain an initial extract, and then it was filtered by a high-velocity centrifugal machine. Then the useful parts were taken and eluted with an ethanol solution, and the extracts were let cool completely before being dried in a spray dryer. Finally, 1.15 mg/g of flavonoids, 4.26 mg/g of total polyphenols, and 0.47 mg/g of saponin was obtained from the AJE.

## Animals, diets, and experimental design

A total of 340 one-day-old Ross 308 broiler chicks (43.10 ± 1.46 g) were randomly assigned to one of four experimental treatment groups. There were five replicate cages per treatment, including 17 birds per cage. The diets were provided based on three age stage of the broiler. In starter stage (days 1–8), broiler were fed basal (Control) diet. Experimental diet were provided to broliler from day 8 to 35 of age. In growing (days 8–21) and finishing (days 22–35) stage broiler were fed: Standard crude protein (SCP) diet and LCP diet with/without 0.025% and 0.050% AJE respectively. During the LCP diet formulation, 3% of the CP level was reduced based on the CP level of the SCP diet. CP levels in SCP and LCP were 21.5% and 20.86, respectively, during the growing stage. In the finishing stage, SCP and LCP diets contained 20% and 19.4%, respectively. A double amount of AJE was added to the LCP diet compared to the SCP diet as a recovery source for reduced CP levels. AJE was incorporated into the diet by replacing the same amount of corn. All the experimental diets (Table 1) were formulated following Aviagen's 2019 nutrient requirements for poultry [11]. The broiler were housed in an environmentally controlled shed. The battery cage system (1.75 × 1.55 $m^2$) is followed by three stages of a stainless-steel cage. Approximately 33℃ temperature was maintained during the brooding period, and 1℃ was reduced each week until 22℃ was reached. The cage was well arranged with attached feeders and a nipple drinker for ad libitum feed and water consumption. Fluorescent lights were used as a source of artificial light for 24 h per a day.

**Table 1. Ingredients and chemical composition of basal diets (as-fed basis)**

| Ingredients (%) | Starter | Grower | | Finisher | |
|---|---|---|---|---|---|
| | SCP | SCP | LCP (–3% CP) | SCP | LCP (–3% CP) |
| Corn | 43.63 | 47.45 | 49.07 | 53.78 | 55.31 |
| Soybean meal | 35.08 | 31.28 | 29.65 | 28.18 | 26.63 |
| Corn gluten meal | 13.00 | 13.00 | 13.00 | 10.00 | 10.00 |
| Wheat bran | 3.00 | 3.00 | 3.00 | 3.00 | 3.00 |
| Soybean oil | 1.76 | 1.74 | 1.74 | 1.51 | 1.51 |
| TCP | 1.81 | 1.81 | 1.85 | 1.81 | 1.86 |
| Limestone | 0.94 | 0.94 | 0.91 | 0.94 | 0.91 |
| Salt | 0.36 | 0.36 | 0.36 | 0.36 | 0.36 |
| Methionine (99%) | 0.19 | 0.19 | 0.17 | 0.19 | 0.17 |
| Lysine | 0.03 | 0.03 | 0.05 | 0.03 | 0.05 |
| Mineral mix[1)] | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Vitamin mix[2)] | 0.10 | 0.10 | 0.10 | 0.10 | 0.10 |
| Total | 100.00 | 100.00 | 100.00 | 100.00 | 100.00 |
| Analyzed value | | | | | |
| Crude protein (%) | 23.00 | 21.50 | 20.86 | 20.00 | 19.40 |
| Ca (%) | 1.10 | 1.08 | 1.08 | 1.07 | 1.07 |
| P (%) | 0.83 | 0.82 | 0.82 | 0.79 | 0.79 |
| Available P (%) | 0.54 | 0.53 | 0.53 | 0.52 | 0.52 |
| Lys (%) | 1.26 | 1.15 | 1.12 | 1.06 | 1.03 |
| Met (%) | 0.54 | 0.52 | 0.50 | 0.50 | 0.48 |
| ME (kcal/kg) | 3200 | 3200 | 3200 | 3200 | 3200 |
| Fat (%) | 4.45 | 4.51 | 4.54 | 4.32 | 4.35 |
| Fiber (%) | 3.55 | 3.48 | 3.46 | 3.30 | 3.27 |
| Ash (%) | 6.76 | 6.57 | 6.49 | 6.30 | 6.24 |

[1)]Provided per kg of complete diet: 37.5 mg Zinc (as $ZnSO_4$); 37.5 mg Mn (as $MnO_2$); 37.5 mg Fe (as $FeSO_4 \cdot 7H_2O$); 3.75 mg Copper (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, 3750 IU of vitamin D3, 2.55 mg of vitamin K3, 24 μg of vitamin B12, 51 mg of niacin, 1.5 mg of folic acid, 3 mg of thiamin, 7.5 mg of rivoflavin, 4.5 mg of vitamin B6, 0.2 mg of biotin, and 13.5 mg of calcium pantothenate.

SCP, standard crude protein; LCP, low crude protein; CP, crude protein; TCP, tricalcium phosphate; ME, metabolizable energy.


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### Growth performance

Body weight (BW) was recorded (340 birds) on days 0, 8, 22, and 35 in each pen, as along with feed intake (FI). Body weight gain (BWG) and Feed conversion ratio (FCR) was calculated by the using of recorded data during the experiment.

### Apparent nutrient digestibility

In total, 0.2% indigestible marker chromium oxide ($Cr_2O_3$) was added to the broilers' diets at the 28th day of age to measure the apparent total tract digestibility (ATTD) of dry matter, nitrogen, and metabolic energy. After 5 days of the supplemented diet, an excreta sample (2 sample/cage, 10 samples/treatment) was collected at days 35. The plastic plates were placed on the pen's floors for two continuous days, and 400 grams of excreta were taken from each pen. Until further chemical analysis was performed, samples were stored at –20℃. The excreta samples were cooled to 0℃ and de-moisturized at 70℃ for three days to perform a chemical analysis. The samples were then finely crushed and put through a 1-mm screen to filter. Following the guidelines established by the Association of Official Analytical Chemists (AOAC), samples of both feed and excreta were examined. Dry matter and nitrogen were analyzed using the 934.01 (AOAC) and 968.0 (AOAC) methods, respectively. During nitrogen analysis, a Kjeltec 2300 nitrogen Analyzer (Foss Tecator AB, Hoeganaes, Sweden) was used. Through the use of an automated adiabatic oxygen bomb calorimeter, the gross energy has been determined (Parr 6300 Calorimeter, Parr Instrument, Moline, IL, USA). To measure the concentration of chromium, an UV absorption spectrophotometer (UV-1201, Shimadzu, Kyoto, Japan) was used. The ATTD of a nutrient was calculated using the following equation:

ATTD (%) = $[1 - \{(Nf \times Cd) / (Nd \times Cf)\}] \times 100$, where Nf = concentration of nutrient in feces (% DM), Nd = concentration of nutrient in diet (% DM), Cf = concentration of chromium in feces (% DM), and Cd = concentration of chromium in diet (% DM).

### Excreta bacterial count

After taking the final BW at day 35 of age, fresh excreta samples were collected from each pen (2 samples/cage). For feces collection, randomly selected five birds from each cage were placed in an empty separate cage with clean plastic plates for 2 h. The 10 g of fresh mixed samples were collected from each plate. Immediately, the collected samples were sent to the laboratory for analysis. Five samples from treatment were taken, each of which contained 1 g of mixed excreta with 9 mL of 1% peptone broth (Becton Dickinson, Franklin Lakes, NJ, USA) and was vortexed. These "homogenized specimens" were mixed to a tenfold serial dilution with a 1% peptone broth solution and put on MacConkey agar plates (Difco Laboratories, Detroit, MI, USA), Lactobacilli medium agar plates (Medium 638; DSMZ, Braunschweig, Germany), and *Salmonella Shigella* agar plates (Becton Dickinson) to incubate *E. coli*, *Salmonella*, and *Lactobacillus*, respectively. The *Lactobacillus* bacterial count was incubated for 48 h at 39℃ under anaerobic conditions. *Salmonella Shigella* and MacConkey agar plates were incubated for 24 h at 37℃. Immediately, bacterial colonies were counted after being taken out of the incubator.

### Excreta gas emission

Finally, on day 35, grinded excreta samples (2 samples/cage) were collected. The collected samples were kept in 2.6-L plastic boxes, where there was a hole in the center of the box side wall that was airtight with tape. The samples were fermented at 28℃ for five days. A multi-gas detector meter (Model PGM-6208, RAE System, San Jose, CA, USA) was used to measure $NH_3$, $H_2S$, and methyl mercaptans on the first, third, and fifth days of fermentation. The average values of three days of measurements were recorded.

### Statistical analysis

All data were statistically analyzed as a randomized complete block design using GLM procedure of SAS software [12]. Preplanned contrast were performed to test the effects of different level of CP and AJE. Data was presented as the mean and standard errors of means. The Cage was considered as an experimental unit. Statistical significance was taken consideration when the *p*-value was remained under 0.05, and when it was less than 0.10, trends were taken into account.

## RESULTS

### Growth performance

The effect of AJE supplementation with different protein diets on broiler growth performance is summarized in Table 2. The SCP diet with (0.025%) AJE supplementation resulted in significantly higher BWG ($p \leq 0.05$) at the finishing stage and a tendency (0.0911, 0.0980) to lower FCR and higher BWG at the overall period compared to the LCP diet with or without (0.050%) AJE supplementation. In addition, throughout the experimental period, FI remained unaffected among all treatment groups.

### Apparent nutrient digestibility

The effects of different levels of protein diet with/without AJE supplementation on broiler apparent nutrient digestibility are shown in Table 3. The ATTD of dry matter and nitrogen shows significantly improved results with the SCP diet along with 0.025% AJE supplementation compared to the LCP diet with/without AJE supplementation. Interestingly, metabolizable energy digestibility remained unaffected ($p > 0.05$) throughout the experimental period in all the treatment groups.

### Excreta bacterial count

The effect of different protein diets with/without AJE supplementation on the cecal bacterial count

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

| Items | SCP | LCP | TRT1[1)] | TRT2 | SEM | SCP vs LCP | SCP vs TRT1 | SCP vs TRT2 | LCP vs TRT2 |
|---|---|---|---|---|---|---|---|---|---|
| Day 8–21 | | | | | | | | | |
| BWG (g) | 581 | 568 | 584 | 570 | 9 | 0.5176 | 0.8547 | 0.4553 | 0.9189 |
| FI (g) | 926 | 914 | 918 | 923 | 24 | 0.6701 | 0.7709 | 0.4437 | 0.7299 |
| FCR | 1.595 | 1.614 | 1.571 | 1.62 | 0.048 | 0.7713 | 0.7137 | 0.9292 | 0.8399 |
| Day 22–35 | | | | | | | | | |
| BWG (g) | 938 | 902 | 972 | 920 | 14 | 0.1693 | 0.0338 | 0.1309 | 0.8805 |
| FI (g) | 1,681 | 1,663 | 1,668 | 1,639 | 16 | 0.3455 | 0.1834 | 0.4816 | 0.1960 |
| FCR | 1.792 | 1.844 | 1.721 | 1.782 | 0.033 | 0.3049 | 0.1425 | 0.4429 | 0.7882 |
| Overall | | | | | | | | | |
| BWG (g) | 1,622 | 1,574 | 1,661 | 1,599 | 12 | 0.1248 | 0.0911 | 0.2189 | 0.9060 |
| FI (g) | 2,734 | 2,701 | 2,713 | 2,694 | 25 | 0.3950 | 0.5764 | 0.1147 | 0.4387 |
| FCR | 1.685 | 1.717 | 1.635 | 1.686 | 0.017 | 0.2036 | 0.0980 | 0.4760 | 0.5597 |

[1)]TRT1, SCP + 0.025% AJE; TRT2, LCP + 0.050% AJE.

AJE, *Achyranthes japonica* extract; SCP, standard crude protein; LCP, low crude protein; BWG, body weight gain; FI, feed intake; FCR, feed conversion ratio.

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

| Items | SCP | LCP | TRT1[1)] | TRT2 | SEM | SCP vs LCP | SCP vs TRT1 | SCP vs TRT2 | LCP vs TRT2 |
|---|---|---|---|---|---|---|---|---|---|
| Day 35 | | | | | | | | | |
| Dry matter | 70.71 | 69.52 | 75.16 | 71.30 | 1.40 | 0.2423 | 0.0283 | 0.2492 | 0.3964 |
| Nitrogen | 65.11 | 64.28 | 70.44 | 67.60 | 2.17 | 0.7088 | 0.0478 | 0.4982 | 0.4438 |
| Energy | 71.44 | 70.33 | 72.81 | 70.90 | 1.09 | 0.5238 | 0.4340 | 0.7536 | 0.7449 |

[1)]TRT1, SCP + 0.025% AJE; TRT2, LCP + 0.050% AJE.

AJE, *Achyranthes japonica* extract; SCP, standard crude protein; LCP, low crude protein.

in broiler feces is shown in Table 4. The bacterial populations of *Lactobacillus* increased and *E. coli* decreased significantly ($p$ < 0.05), respectively, as a result of 0.025% AJE supplementation along with SCP diet compared to LCP diet with/without AJE supplementation. Dietary CP and AJE supplementation levels both failed to have an effect on the *salmonella* population in broiler excreta samples throughout the experimental period.

## Excreta gas emission

Table 5 shows the effect of different protein diets with/without AJE supplementation on broiler excreta gas emissions. However, all the mentioned gases were not influenced by different levels of protein in the diets with AJE supplementation, except $NH_3$, throughout the experimental period.

## Meat quality and visceral percentage

The meat quality and visceral percentage of broilers are summarized in Table 6. The dietary supplementation of AJE along with different levels of protein had no significant improvement ($p$ > 0.05) on meat quality and visceral percentage. Surprisingly, the pH parameter shows a significant ($p$ < 0.05) effect with different level of AJE compared to SCP diet without supplementation.

**Table 4.** The effect of AJE supplementation on microbial in broilers

| Items | SCP | LCP | TRT1[1)] | TRT2 | SEM | SCP vs LCP | SCP vs TRT1 | SCP vs TRT2 | LCP vs TRT2 |
|---|---|---|---|---|---|---|---|---|---|
| Day 35 | | | | | | | | | |
| *Lactobacillus* | 7.82 | 7.67 | 8.02 | 7.69 | 0.08 | 0.1023 | 0.0274 | 0.1878 | 0.4513 |
| *Escherichia coli* | 5.82 | 5.92 | 5.56 | 5.85 | 0.09 | 0.2029 | 0.0457 | 0.2977 | 0.5440 |
| *Salmonella* | 3.73 | 3.87 | 3.68 | 3.71 | 0.11 | 0.5534 | 0.7614 | 0.9301 | 0.4967 |

[1)]TRT1, SCP + 0.025% AJE; TRT2, LCP + 0.050% AJE.

AJE, *Achyranthes japonica* extract; SCP, standard crude protein; LCP, low crude protein.

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

| Items | SCP | LCP | TRT1[1)] | TRT2 | SEM | SCP vs LCP | SCP vs TRT1 | SCP vs TRT2 | LCP vs TRT2 |
|---|---|---|---|---|---|---|---|---|---|
| Day 35 | | | | | | | | | |
| $NH_3$ | 16.75 | 13.50 | 12.00 | 9.00 | 1.14 | 0.1124 | 0.0589 | 0.0614 | 0.4845 |
| $H_2S$ | 1.08 | 1.23 | 0.95 | 0.90 | 0.41 | 0.8713 | 0.8396 | 0.7771 | 0.6572 |
| Methyl mercaptans | 6.50 | 7.00 | 5.25 | 5.25 | 1.94 | 0.4902 | 0.6643 | 0.6643 | 0.2699 |
| $CO_2$ | 1450 | 1450 | 1425 | 1375 | 280 | 0.5878 | 0.9456 | 0.8381 | 0.4586 |
| Acetic acid | 2.75 | 3.25 | 2.75 | 2.50 | 0.77 | 0.6690 | 1.0000 | 0.8303 | 0.5234 |

[1)]TRT1, SCP + 0.025% AJE; TRT2, LCP + 0.050% AJE.

AJE, *Achyranthes japonica* extract; SCP, standard crude protein; LCP, low crude protein.

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

| Items | SCP | LCP | TRT1[1)] | TRT2 | SEM | SCP vs LCP | SCP vs TRT1 | SCP vs TRT2 | LCP vs TRT2 |
|---|---|---|---|---|---|---|---|---|---|
| pH value | 7.76 | 7.59 | 7.58 | 7.68 | 0.04 | 0.0010 | 0.0008 | 0.0035 | 0.5567 |
| Breast muscle color | | | | | | | | | |
| CIE L* | 56.00 | 57.00 | 57.86 | 57.64 | 1.18 | 0.5496 | 0.2731 | 0.4570 | 0.8819 |
| CIE a* | 12.18 | 12.58 | 11.55 | 12.61 | 0.42 | 0.5828 | 0.3948 | 0.2940 | 0.6072 |
| CIE b* | 12.12 | 10.78 | 13.28 | 12.24 | 1.11 | 0.3609 | 0.4320 | 0.6516 | 0.1804 |
| WHC (%) | 52.03 | 53.88 | 54.56 | 53.75 | 3.44 | 0.7154 | 0.6177 | 0.8959 | 0.8148 |
| Cooking loss (%) | 20.03 | 18.56 | 20.58 | 20.50 | 1.55 | 0.5840 | 0.8386 | 0.8732 | 0.6968 |
| Drip loss (%) | | | | | | | | | |
| Day 1 | 3.03 | 3.70 | 3.16 | 3.54 | 0.71 | 0.4169 | 0.5375 | 0.5660 | 0.1750 |
| Day 3 | 6.94 | 6.52 | 6.21 | 6.42 | 0.37 | 0.5496 | 0.2731 | 0.4570 | 0.8819 |
| Day 5 | 13.40 | 13.13 | 13.71 | 12.78 | 0.66 | 0.5828 | 0.3948 | 0.2940 | 0.6072 |
| Day 7 | 17.05 | 17.94 | 16.67 | 16.81 | 0.45 | 0.3609 | 0.4320 | 0.6516 | 0.1804 |
| Relative organ weight (%) | | | | | | | | | |
| Breast muscle | 17.79 | 18.56 | 19.11 | 18.18 | 0.52 | 0.1990 | 0.1920 | 0.6871 | 0.0992 |
| Liver | 2.66 | 2.64 | 2.60 | 2.61 | 0.18 | 0.6474 | 0.7758 | 0.8002 | 0.8373 |
| Bursa of Fabricius | 0.16 | 0.15 | 0.14 | 0.16 | 0.02 | 0.9990 | 0.4821 | 0.8598 | 0.8608 |
| Abdominal fat | 0.86 | 0.93 | 1.18 | 1.1 | 0.24 | 0.3234 | 0.2798 | 0.4067 | 0.0797 |
| Spleen | 0.17 | 0.18 | 0.16 | 0.18 | 0.02 | 0.7269 | 0.8725 | 0.7220 | 0.4833 |
| Gizzard | 1.99 | 1.92 | 1.68 | 1.96 | 0.14 | 0.8825 | 0.1148 | 0.8489 | 0.7355 |

[1)]TRT1, SCP + 0.025% AJE; TRT2, LCP + 0.050% AJE.

AJE, *Achyranthes japonica* extract; SCP, standard crude protein; LCP, low crude protein; WHC, water holding capacity.

## DISCUSSION

AJE has several physiological and biological properties, like anti-oxidant, anti-inflammatory, and bioactive components, which have attracted the attention of researchers who are working on it as an alternative source of antibiotic growth promoters. There are limited studies performed in broilers with AJE supplementation, as it was recently introduced to the feed industry. Therefore, our main focus was to evaluate the effects of AJE with different levels of protein in diets as a natural growth promoter. Hoque and Kim, [2] reported that the LCP and HCPdiets with 0.025% and 0.05% of AJE significantly increased BWG compared to the LCP diet without AJE. Similarly, Park and Kim [13], Sun et al. [14] in broilers, and Ao and Kim [15] in ducks observed a linear increase in BWG and reduced FCR through the supplementation of *Achyranthes bidentata* extract. This improvement in BWG can be caused by several mechanisms since AJE supplementation has beneficial effects on the gut health of animals [16]. Hashemi and Davoodi [17] proposed that significant BWG was caused by increased digestibility, an improved intestinal microbial population, and enhanced digestive enzyme activities. This proposed mechanism was supported by our study as supplementation of AJE increased nitrogen and dry matter digestibility and created a favorable environment for the microorganism. As a result, BWG significantly increased at the finishing stage, though BWG and FCR tend to increase and decrease with (0.025%) AJE supplementation, respectively, at the overall period. On the other hand, reducing the CP level in diet had negative effects on broiler BWG and FCR in our study. Similarly, Liu and Kim, [18] in pig and [2] found the similar results in broiler. The negative effect of reducing the level of CP in the broiler diet can be minimized by the addition of essential amino acids [19]. Previous researchers explained that the LCP diet impairs the growth performance and gastrointestinal conditions in broilers [20]. However, in this experiment, supplementation of AJE in broiler diet failed to improve growth performance in LCP diet compared to SCP diet, although AJE showed improved growth performance with high protein levels. Regarding the negative effect of the LCP diet, [19] showed that reduced growth performance in broilers can be minimized by the addition of essential amino acids to the broiler diet. However, we request further study to find out the optimum AJE level in the LCP diet to show similar performance as a high protein diet.

In our study, the ATTD of DM and nitrogen digestibility were significantly improved with 0.025% of AJE supplementation. Similar findings were stated by [2,13,14]. Previous study showed that, plant extract supplementation has been shown to increase villi height and thus may improve nutrient absorption ability in broilers and quail [18]. Furthermore, the results from the other studies suggest that feeding plant extract can increase digestibility by stimulating bile secretion and enhancing the digestive enzyme of the small intestine [13].

The intestinal tract has vital roles in digestion and the absorption of nutrients. It has some specific functions and acts as a harbor for a wide range of microorganisms [21]. As a result, the increasing population of beneficial bacteria can help in the utilization of unprocessed nutrients and maintain the gut microbiota [2]. In the present study, AJE supplementation (0.025%) with a SCP diet increased *lactobacillus* and reduced *E. coli* populations in the broiler's excreta sample. Similarly, Lan et al., Sun et al. [22,14], and [2] found similar results. AJE supplementation creates a favorable condition in the digestive system of broilers that increases *lactobacillus* counts, although the mechanism is not yet understood. A large number of such beneficial bacteria inhibit the harmful bacteria in the animal gut [23] due to the competition for nutrients. A small amount of nutrients is left by the *lactobacillus* for the utilization and multiplication of *E. coli* bacteria. The anti-inflammatory and antioxidant properties of AJE could develop the normal gut microbiota balance by suppressing the pathogenic bacteria and lead to healthy intestinal development [13]. It is expected that a lower level of dietary protein will reduce the bacterial population in feces [24], but in this study, we got the reverse result. Similarly, Liu and Kim, [18] in pigs and [2] in broilers did not find an effect of protein levels on the microbial population. The mechanism of the effect of protein level on microbial count is yet not well known. The different sources of protein may be the possible reason for this result. Further study is required to determine the effect of protein in the microbial population.

Noxious gas emissions from animals in the environment are alarming issues due to climate change and negative effect on animal performance. Excreta noxious gas emissions of animals are related to the intestinal microflora, particularly the harmful bacterial population [25]. In this study, supplementation with 0.025% and 0.05% of AJE reduced the $NH_3$ emission in the excreta sample compare to SCP diet without supplementations. Similarly, Park and Kim [13] and Hoque and Kim [2] stated similar results in the broiler. Additionally, [26] found a lower amount of $H_2S$ and $NH_3$ gas in the environment through pig fecal. Mainly, ammonia gas is produced from the indigestible dietary protein released in feces and urine [2]. The AJE supplemented diet led the way to better microbiota balance and higher nutrient digestibility compared to the control diets in this study. Due to the increase in nitrogen digestibility and lactobacillus count, we conjectured that ammonia gas emissions may be reduced.

Good quality of meat takes the attention of consumers due to its texture, color, flavor, and long shelf life. The pH level of meat is measured by the amount of lactic acid present in the muscle tissue. The effects of plant extract on carcass quality are ambiguous. Our results suggested that AJE (0.025% and 0.050%) supplementation reduces the pH level compared to the control diet along with SCP and LCP, which has an overall effect on meat quality. Dang et al. [27] reported that supplementation of AJE to the diet had no positive effect on carcass quality. Interestingly, in our study, we found a lower pH level with AJE supplementation. We found lower pH may be due to a reduction in bacterial population caused by AJE supplementation. In addition, by using AJE supplementation, broiler meat texture, color, flavor, and shelf life increased compared to the control diet.

## CONCLUSION

Supplementation of 0.025% AJE with SCP diet improved the growth performance at finishing and overall experimental period and increased the DM and nitrogen digestibility in broilers. Reduced ammonia gas emissions and improved meat quality by keeping pH at a moderate level. However, further study is required to determine the optimum dose of AJE to recover the negative effect of LCP diet.

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