

Effect of *Achyranthes japonica* Nakai extract on growth performance, apparent nutrient digestibility, excreta microbial count and gas emission in broilers fed different protein diets

M.R. Hoque and I.H. Kim*

Dankook University, Department of Animal Resource & Science, Anseodong, Cheonan, Chungnam, 330-714, South Korea

KEY WORDS: *Achyranthes japonica*, bacterial population, broiler, digestibility, gas emission

Received: 29 June 2022
Revised: 19 September 2022
Accepted: 3 October 2022

* Corresponding author:
e-mail: inhokim@dankook.ac.kr

ABSTRACT. This aim of the study was to evaluate the effect of *Achyranthes japonica* Nakai extract (AJE) supplementation to a low-protein diet on growth performance, nutrient digestibility, excreta bacterial count and excreta gas emission in broilers. In total, 600 one-day-old male Ross 308 broilers, with an average body weight of 42.90 ± 1.43 g, were randomly divided into 20 birds/pen. In a 2×3 factorial design, pens were assigned to one of six dietary groups: high-crude protein (HCP) vs low-crude protein (LCP) supplemented with 2 doses (0.025%, 0.050%) of AJE or without supplementation (0%). The experimental diets were fed to broilers from day 8 to 35. The HCP diet resulted in higher body weight gain (BWG) and lower feed conversion ratio (FCR) compared to the LCP diet in all phases of the experiment. AJE (0.05%) supplementation increased BWG from day 22 to 35 and in the entire period of LCP diet administration. AJE supplementation to the LCP diet caused lower FCR between days 22 and 35, and an overall higher feed intake (FI). AJE showed a linear increase in BWG, FI and nitrogen digestibility over the entire trial period. Increasing doses of AJE linearly reduced the *Escherichia coli* count in excreta, and 0.05% AJE supplementation in the LCP group resulted in the lowest *E. coli* count. NH_3 emission was reduced in the group supplemented with 0.05% AJE. No interaction effect of CP and AJE was observed. When added to a low-protein diet, AJE supplementation presented a promising ability to sustain similar growth performance by improving nitrogen digestibility and modulating the intestinal bacterial population in broilers.

Introduction

In recent years, the broiler industry has shown rapid growth as broiler meat has become the main source of protein in the human diet (Forte et al., 2018). Over the past few decades, antibiotics have played an important role as growth-promoting agents and have improved broiler performance and health status. However, growing concerns about antibiotic resistance and residual effects on food materials has led to a ban on sub-therapeutic usage of antibiotics in livestock (Babaei et al., 2016). As an alternative,

phytogenic feed additives containing flavonoids, essential oils and phenols are gaining increasing attention, as they have antimicrobial, anti-inflammatory, and digestion-stimulating properties (Oso et al., 2019). Phytogenic additives caused improvements in growth, nutrient utilisation, and immune response, while decreasing faecal gas emissions in animals (Huang et al., 2011). *Achyranthes japonica* Nakai (AJN) belongs to the family *Amaranthaceae*. AJN is mostly available in Asian countries like Korea, Japan and China. AJN root contains different types of saponins, terpenoids, phytoecdisterols,

20-hydroxyecdysone, and inokosterone. In Korean traditional medicine, AJN has been used in hypertension, rheumatism, osteoarthritis, as well as an analgesic, and diuretic agent. In addition, AJN has been reported to exhibit antioxidant, anti-allergic, anti-inflammatory, and anti-carcinogenic properties (Bang et al., 2012; Jang et al., 2012). AJN supplementation resulted in improved average daily gain (ADG), nutrient digestibility, blood profile and reduced gas emissions in pigs (Liu and Kim, 2021). In broilers, AJN supplementation caused increased ADG, nutrient digestibility, excreta *Lactobacillus* counts and reduced NH₃ emissions (Park and Kim, 2020; Sun et al., 2020).

In the poultry industry, feed costs account for 70% of total production expenditure. Reducing dietary protein, while maintaining essential amino acid levels, can be an effective way of limiting feed expenses. Along with cost reductions, lower dietary protein levels may decrease nitrogen (N) release into the environment (Saleh et al., 2021). However, a reduced-protein diet has been reported to impair growth performance, and gastrointestinal conditions in broilers (Dozier et al., 2000). Yan et al. (2011), and Zeng et al. (2015) reported that plant extracts were more effective in diets with higher nutrient density. Thus, there may be an effect of protein level on the outcome of AJE supplementation. Previous studies have not reported any results of AJNE supplementation in combination with different crude protein (CP) levels. Hence, this study was conducted to explore the effect of AJN extract supplementation in combination with a low-protein diet, where feed cost and nitrogen excretion into the environment can be reduced, while preserving satisfactory growth performance.

Therefore, we hypothesised that the synergistic effect of AJE on nutrient digestibility and microbial population in broilers could help recover their production performance when supplemented with a low-protein 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.

Material and methods

The procedures adopted for this study were approved (DK-2-2009) by the Animal Care Committee of the Dankook University, Korea.

Animals and diets

In total, 600 one-day-old male Ross 308 broilers, with an average body weight of 42.90 ± 1.43 g,

were randomly divided into 30 pens, 20 chickens each. Thirty pens were randomly allocated to one of the six dietary treatments in a 2×3 factorial arrangement with two different dietary protein levels: high-crude protein (HCP) vs low-crude protein (LCP) supplemented with 2 doses (0.025%, 0.050%) of AJE or without supplementation (0%). For the LCP diet formulation, dietary protein was reduced by 2.5% compared to the HCP diet. Each treatment included five replication pens. The experimental diets were administered to broilers from day 8 to 35. All the experimental diets (Table 1) were formulated according to the guidance described by Rostagno et al. (2011). The birds were kept in floor pens with wood shavings as bedding. The experimental room was equipped with a temperature and light control system. Free access of broilers to feed and water was ensured by feeders and nipple drinkers attached to each pen. AJE (EXMUS, Synergen Inc, Bucheon, South Korea) was purchased in a mash form and supplied to broilers by mixing with feed. First, the roots of *A. japonica* were ground and refluxed for 6 h. The extracts were distilled with water for 2 h at 80 °C and then washed with ethanol. Subsequently, the filtered extracts were lyophilized in a freeze drier, resulting in a mash. According to the AJE supplier, the final product contained the following total flavonoid, total polyphenol and saponin levels (mg/g AJE): 1.15, 4.26, and 0.47, respectively.

Growth performance and apparent nutrient digestibility

Body weight was recorded (600 birds) on days 0, 8, 22, and 35 in each pen, as along with feed intake. Body weight gain (BWG), feed intake (FI) and feed conversion ratio (FCR) were calculated from data recorded during the experiment. Chromium oxide (Cr₂O₃) was added to diets on day 28 to determine the apparent total tract digestibility (ATTD) of dry matter (DM), N, and energy (ME). After 5 days of supplying chromium added feed, mixed (from different birds of the same pen) excreta samples (1 sample/pen, 5 samples/treatment) were collected on day 33 and 34. Plastic plates were placed on the pen floor for 2 consecutive days, and 400 gm of excreta samples were collected per pen. The samples were stored at -20 °C until chemical analysis. To perform the analysis, excreta samples were thawed to 0 °C and de-moisturized at 70 °C for 3 days. Subsequently, the samples were finely ground, and filtered through a 1-mm screen. Both feed and excreta samples were analysed according to the procedures of the Association of Official Analytical Chemists

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

Item	Starter	Grower		Finisher	
		PC	NC(-2.5% CP)	PC	NC(-2.5% CP)
Ingredients, %					
corn	43.63	47.45	48.83	53.78	55.09
soybean meal	35.08	31.28	29.83	28.18	26.80
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	1.75	1.51	1.51
tri calcium phosphate	1.81	1.81	1.85	1.81	1.85
limestone	0.94	0.94	0.91	0.94	0.92
salt	0.36	0.36	0.36	0.36	0.36
DL-Met (99%)	0.19	0.19	0.19	0.19	0.19
L-Lys	0.03	0.03	0.08	0.03	0.08
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
Calculated nutrient composition					
ME, kcal/kg	3200	3200	3200	3200	3200
crude protein, %	23.00	21.50	20.96	20.00	19.50
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	6.51	6.30	6.25
calcium, %	1.10	1.08	1.08	1.07	1.07
chlorine, %	0.57	0.58	0.59	0.59	0.59
sodium, %	1.08	0.97	0.97	0.89	0.90
potassium, %	0.93	0.86	0.87	0.82	0.82
phosphorus, %	0.83	0.82	0.82	0.79	0.79
available P, %	0.54	0.53	0.53	0.52	0.52
lysine, %	1.26	1.23	1.22	1.20	1.20
methionine, %	0.57	0.55	0.55	0.60	0.60
methionine + cystine, %	1.01	1.03	1.03	0.91	0.90
threonine, %	0.97	1.07	1.05	0.96	0.94
tryptophan, %	0.47	0.50	0.51	0.50	0.50
isoleucine, %	1.10	1.11	1.08	0.99	0.97
valine, %	1.30	1.32	1.30	1.17	1.15
leucine, %	1.92	1.86	1.85	1.88	1.84
phenylalanine + tyrosine, %	1.45	1.37	1.34	1.23	1.20
arginine, %	1.78	1.72	1.67	1.56	1.54
histidine, %	0.62	0.52	0.52	0.62	0.61

PC – positive control, NC – negative control, CP – crude protein, ME – metabolisable energy; ¹ provided per kg of complete diet: mg: Zn (as ZnSO₄) 37.5, Mn (as MnO₂) 37.5, Fe (as FeSO₄·7H₂O) 37.5, Cu (as CuSO₄·5H₂O) 3.75, I (as KI) 0.83, Se (as Na₂SeO₃·5H₂O) 0.23; ² provided per kg of complete diet: IU: vit. A 15 000, vit. D₃ 3 750, vit. E 37.5, vit. K₃; mg: thiamine 3, riboflavin 7.5, vit B₆ 4.5, niacin 51, folic acid 1.5, biotin 0.2, Ca-pantothenate 13.5; µg: vit. B₁₂

(AOAC International, 2000). DM was determined using method 934.01 (AOAC International, 2000); N was determined using a Kjeltac 2300 Nitrogen Analyzer (Foss Tecator AB, Hoeganaes, Sweden) according to method 968.0 (AOAC International, 2000). The heat of chemical reactions in the samples was measured using an oxygen bomb calorimeter (Parr 6100 Instrument Co., Moline, IL, USA) and gross energy was calculated according to the following equation:

$$GE \text{ (cal/g)} = \frac{\{(\text{bomb equivalent} \times T) \times A\}}{\text{sample dry weight (g)}}$$

where: T – temperature rise (°C), A – correction factors for wire, thread, N and sulphur.

Energy digestibility was calculated from the gross energy present in the diet and in the excreta samples. Chromic oxide concentration in the feed and excreta samples was determined by ashing and digesting ground samples with phosphoric acid-manganese sulphate, and potassium bromate

(Williams et al., 1962). The washed digest was incubated overnight in a mixture of calcium chloride solution and subsequently filtered. Chromium was detected by UV absorption spectrometry (Shimadzu UV-1201; Shimadzu, Kyoto, Japan). The apparent total tract digestibility (ATTD) of nutrients was estimated using the following formula: $ATTD (\%) = \{1 - [(Nf \times Cd)/(Nd \times Cf)]\} \times 100$, where Nf – nutrient concentration in faeces (% DM), Cd – chromium concentration in the diet (% DM), Nd – nutrient concentration in the diet (% DM), and Cf – chromium concentration in faeces (% DM).

Microbiological analysis of excreta

On day 35, fresh excreta samples (2 samples/pen) from each pen (10 samples/treatment) were collected after recording the final BW. From each pen, random 5 birds were kept in each pen with a clean plastic plate for 2 h. Fresh mixed (from different birds of the same pen) faeces samples (10 g) were collected from the plastic plates of each pen. The samples were transferred to the laboratory to directly conduct the analyses. Five samples were collected from each treatment and one gram of excreta was taken from each sample and mixed with 9 ml of 1% peptone broth (Becton, Dickinson and Co., Franklin Lakes, NJ, USA) and vortexed (VM-10, DAIHAN Scientific Company, Seoul, South Korea). These homogenised samples were subjected to a 10-fold serial dilution with 1% peptone broth solution and spread on MacConkey agar plates (Difco Laboratories, Detroit, MI, USA), *Lactobacillus* medium agar plates (Medium 638; DSMZ, Braunschweig, Germany), and *Salmonella-Shigella* (SS) agar plates (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) to culture *Escherichia coli*, *Lactobacillus*, and *Salmonella*, respectively. To count lactobacilli, the incubation was carried out for 48 h at 39 °C under anaerobic conditions. MacConkey agar plates and SS agar plates were incubated at 37 °C for 24 h. Bacterial colonies were counted immediately after removal from the incubator.

Excreta gas emission

Finally, on day 35, mixed excreta samples (2 samples/pen) from each pen (10 samples/treatment) were collected. The samples were stored in 2.6-l plastic boxes with a central hole in the box wall sealed with tape. The samples were fermented by incubating them at 28 °C for five days. NH₃, H₂S, and methyl mercaptans were measured on days 1, 3, and 5 of fermentation, with a multi-gas meter (MultiRae Lite model PGM-6208, RAE Systems, San Jose,

CA, USA). The average values of the three days of measurements were recorded.

Statistical analysis

The experiment was subjected to a 2 × 3 factorial design where data were analysed using the general linear model implemented in SAS software (SAS Institute, Inc., Cary, NC, USA). The pen served as an experimental unit. The values were log-transformed for microbial data analysis. Statistical analysis was performed to determine the effects of AJE levels, crude protein levels, and their interactions. Tukey's multiple range test was conducted to differentiate group mean values. Orthogonal polynomial contrasts were used to measure the effect of increasing AJE doses. The probability level of $P \leq 0.05$ was considered to be statistically significant.

Results

Growth performance

The results of different levels of AJE supplementation to protein diets on broiler growth performance are shown in Table 2. The HCP resulted in higher BWG ($P \leq 0.05$) and lower FCR ($P \leq 0.05$) compared to the LCP during all phases of the experiment. AJE supplementation (0.025% and 0.050%) in both the HCP and LCP diets resulted in higher ($P \leq 0.05$) BWG throughout the experiment compared to the LCP diet without AJE supplementation (0% AJE). In the period from day 8 to 21, AJE supplementation did not cause any changes in FCR ($P > 0.05$). In the period between day 8 and 21 and 22–35, FI was not affected by dietary protein level and/or AJE supplementation. However, in the overall measurement, 0.050% AJE supplementation to the HCP diet significantly increased FI compared to the LCP diet without AJE supplementation. Increasing levels of AJE supplementation resulted in a linear increase in BWG ($P \leq 0.05$) on days 22 to 35, and in the entire experiment. In the whole experimental period, AJE caused a gradual increase in FI ($P \leq 0.05$) and a linear decrease in FCR ($P \leq 0.05$). No interaction effect of protein and AJE levels on growth performance was observed.

Apparent nutrient digestibility

The effects of different protein diets with/without AJE supplementation on broiler apparent nutrient digestibility are presented in Table 3. The apparent total tract digestibility of dry matter and energy was not affected ($P > 0.05$) by protein levels

Table 2. Effect of increasing levels of *Achyranthes japonica* Nakai extract (AJE) supplementation to high- and low-protein diets on growth performance of broilers

Items	HCP			LCP			SEM	P-value				
	0	0.025%	0.050%	0	0.025%	0.050%		CP	AJE	CP*AJE	Linear	Quadratic
Day 8 to 21												
BWG, g	593 ^a	604 ^a	619 ^a	545 ^b	574 ^{ab}	576 ^{ab}	15	0.003	0.163	0.821	0.177	0.394
FI	949	972	979	927	966	972	18	0.468	0.119	0.874	0.662	0.915
FCR	1.604 ^{ab}	1.611 ^{ab}	1.585 ^b	1.702 ^a	1.683 ^{ab}	1.690 ^{ab}	0.036	0.005	0.911	0.898	0.554	0.193
Day 22 to 35												
BWG, g	1079 ^a	1085 ^a	1101 ^a	988 ^b	1058 ^a	1072 ^a	18	0.003	0.024	0.167	<0.001	0.526
FI	1919	1936	1968	1897	1932.4	1950	27	0.520	0.204	0.945	0.214	0.229
FCR	1.781 ^b	1.745 ^b	1.731 ^b	1.922 ^a	1.828 ^{ab}	1.798 ^{ab}	0.039	0.002	0.050	0.559	0.349	0.788
Overall												
Day 8 to 35												
BWG, g	1672 ^{ab}	1689 ^{ab}	1720 ^a	1533 ^c	1632 ^b	1648 ^{ab}	18	<0.001	<0.001	0.096	<0.001	0.154
FI	2868 ^{ab}	2908 ^{ab}	2947 ^a	2824 ^b	2899 ^{ab}	2922 ^{ab}	24	0.272	0.013	0.798	0.036	0.618
FCR	1.715 ^b	1.722 ^b	1.713 ^b	1.842 ^a	1.776 ^{ab}	1.773 ^{ab}	0.020	<0.001	0.178	0.187	0.004	0.091

HCP – high-protein diet supplemented with 0, 0.025% and 0.050% AJE, LCP – low-protein diet supplemented with 0, 0.025%, 0.050% AJE, CP – crude protein, BWG – body weight gain, FI – feed intake, FCR – feed conversion ratio, SEM – standard error of the mean; data represents 5 pens per treatment, 20 chickens/pen; ^{ab} – means within a row with different superscripts are significantly different at $P < 0.05$

Table 3. Effect of increasing levels of *Achyranthes japonica* Nakai extract (AJE) supplementation to high- and low-protein diets on nutrient digestibility in broilers

Items	HCP			LCP			SEM	P-value				
	0	0.025%	0.050%	0	0.025%	0.050%		CP	AJE	CP*AJE	Linear	Quadratic
Finishing												
Dry matter	76.7	77.1	78.2	74.1	77.7	76.8	1.23	0.248	0.160	0.418	0.692	0.381
Nitrogen	74.4 ^{ab}	74.7 ^{ab}	77.2 ^a	71.5 ^b	74.9 ^{ab}	75.1 ^a	1.34	0.282	0.034	0.437	0.024	0.288
Energy	75.2	75.7	76.4	74.7	74.7	75.4	1.44	0.466	0.794	0.970	0.194	0.460

HCP – high-protein diet supplemented with 0, 0.025% and 0.050% AJE, LCP – low-protein diet supplemented with 0, 0.025%, 0.050% AJE; CP – crude protein, SEM – standard error of the mean; data represents 10 samples per treatment; ^{ab} – means within a row with different superscripts are significantly different at $P < 0.05$

and/or AJE supplementation levels. Interestingly, 0.050% AJE added to the HCP and LCP diets improved nitrogen digestibility ($P \leq 0.05$) in broilers. In addition, a linear increase in nitrogen ATTD was observed with incrementing doses of AJE supplementation. No interaction effects of CP and AJE on nutrient digestibility were observed.

Excreta bacterial count

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

faeces is shown in Table 4. *Lactobacillus* population increased ($P \leq 0.05$) as a result of 0.025% AJE supplementation. At the finishing stage, 0.050% AJE supplementation to the HCP and LCP diets reduced *E. coli* count ($P \leq 0.05$) in the excreta samples. Incrementing doses of AJE supplementation caused a linear decrease in the *E. coli* count ($P \leq 0.05$) in excreta samples. Neither dietary protein levels nor AJE levels exerted an effect ($P > 0.05$) on *Salmonella* population in broiler excreta samples.

Table 4. Effect of increasing levels of *Achyranthes japonica* Nakai extract (AJE) supplementation to high- and low-protein level diets on excreta bacterial counts in broilers

Items, log ₁₀ CFU	HCP			LCP			SEM	P-value				
	0	0.025%	0.050%	0	0.025%	0.050%		CP	AJE	CP*AJE	Linear	Quadratic
Day 35												
<i>Lactobacillus</i>	9.43 ^{ab}	9.44 ^a	9.41 ^{ab}	9.10 ^b	9.42 ^{ab}	9.42 ^{ab}	0.0710	0.130	0.039	0.094	0.164	0.176
<i>Escherichia coli</i>	5.34 ^{ab}	5.22 ^{ab}	4.83 ^b	5.79 ^a	5.23 ^{ab}	4.92 ^b	0.193	0.248	0.006	0.485	0.032	0.825
<i>Salmonella</i>	3.17	3.06	3.07	3.32	3.10	3.15	0.361	0.762	0.762	0.991	0.518	0.377

HCP – high-protein diet supplemented with 0, 0.025% and 0.050% AJE, LCP – low-protein diet supplemented with 0, 0.025%, 0.050% AJE; CP – crude protein, SEM – standard error of the mean; data represents 10 samples per treatment; ^{ab} – means within a row with different superscripts are significantly different at $P < 0.05$

Gas emissions

Table 5 presents the results of feeding different CP and AJE levels on gas emissions of broiler excreta. The high protein diet without AJE supplementation (HCP) generated higher ($P \leq 0.05$) NH_3 gas emissions. The addition of AJE supplementation (0.050%) to both HCP and LCP diets reduced NH_3 gas emissions in broiler excreta samples. However, the concentration of H_2S and methyl mercaptans were not influenced either by CP and/or AJE levels. There was no interaction effect of CP and AJE levels on excreta gas emissions.

AJE supplementation did not reduce FCR, as FI increased in numerical values and became significantly higher in the overall period. Similarly, Dang et al. (2020) recorded a linear increase in ADFI in finishing pigs using AJE supplementation. Phyto-genic additives have been reported to increase feed intake in broilers by improving palatability and flavour (Naeemasa et al., 2015; van der Aar et al., 2017). Nevertheless, different phyto-genic additives may exert different effects as strong flavours and odours are also present in various plant extracts. In our study, the increased BWG in AJE-supplemented

Table 5. Effect of increasing levels of *Achyranthes japonica* Nakai extract (AJE) supplementation to high- and low-protein diets on excreta gas emissions in broilers

Items	HCP			LCP			SEM	P-value					
	0	0.025%	0.050%	0	0.025%	0.050%		CP	AJE	CP*AJE	Linear	Quadratic	
Day 35*													
NH_3	12.3 ^a	10 ^{ab}	8.75 ^b	11 ^{ab}	11.3 ^{ab}	9.25 ^b	0.87	0.818	0.024	0.361	0.247	0.409	
H_2S	6.25	5.75	5	4.75	4	6	0.92	0.336	0.743	0.287	0.288	0.519	
Methyl mercaptans	6.75	6.75	6	8.25	7.50	8.50	1	0.141	0.955	0.955	0.811	0.826	

HCP – high-protein diet supplemented with 0, 0.025% and 0.050% AJE, LCP – low-protein diet supplemented with 0, 0.025%, 0.050% AJE; CP – crude protein, NH_3 – ammonia, H_2S – hydrogen sulphide, SEM – standard error of the mean; data represents 10 samples per treatment; ^{ab} – means within a row with different superscripts are significantly different at $P < 0.05$

Discussion

AJE effect. AJE has been introduced to the feed industry very recently, thus few studies in this area are available for a comparison. Hence, we compared it to feed additives of similar composition in different species of the sample population. AJE supplementation proved to induce better growth performance in broilers, whereas FCR improved linearly with its supplementation. Similar results for AJE supplementation were reported by Liu and Kim (2021) in pigs, and Sun et al. (2020) and Park and Kim (2020) in broilers. Ao and Kim (2020) in turn observed a linear increase in BWG and a linear decrease in FCR in ducks administered *Achyranthes bidentata* extracts. AJE supplementation resulted in similar improvements in growth performance in growing and finishing pigs (Dang et al., 2020; Liu et al., 2021). This improvement in BWG could be caused by various mechanisms. Hashemi and Davoodi (2010) suggested that increased digestibility, improved intestinal microbial population and enhanced digestive enzyme activities were responsible for better BWG. This hypothesis was supported by our study, as AJE supplementation improved nitrogen digestibility and created favourable conditions for microorganisms. As a result, FCR tended to decrease in AJE-supplemented groups from day 22 to 35; however,

groups was due to higher FI, improved digestion, and healthy microbial community.

Only 0.050% AJE supplementation resulted in a clear improvement in nitrogen digestibility in our study. Similar findings were reported by Sun et al. (2020), Park and Kim (2020) and Liu et al. (2021). *Achyranthes* extracts exert antioxidant, antimicrobial, and immune-stimulating effects, thus they may indirectly facilitate nutrient absorption (Ao and Kim, 2020). Furthermore, plant extract supplementation has been shown to increase villus height, and thus improve nutrient absorption in quails and broilers (Liu et al., 2021). However, the most promising mechanism for increasing nutrient digestibility by plant extracts is the stimulation of digestive enzyme secretion, and a positive correlation between plant extracts and secretion of digestive enzymes, bile, and mucus was suggested by Windisch et al. (2008). The intestinal tract plays an important role in broiler nutrient digestion and utilisation. Its various parts perform specific functions and harbour a wide range of microorganisms (Yan et al., 2011). Thus, increasing the population of beneficial bacteria can help in the utilisation of unprocessed nutrients and maintain a healthy balance of the gut microbiota. In our study, AJE supplementation (0.050%) increased *Lactobacillus* and decreased *E. coli* population in excreta samples; similar results were reported by Lan et al.

(2016), Sun et al. (2020), Park and Kim (2020), and Liu et al. (2021). Although the mechanism is not yet clear, AJE supplementation facilitated the increase of *Lactobacillus* count in the digestive system of broilers. Higher abundance of these beneficial bacteria restrict the number of harmful bacteria in the gut (Jang et al., 2012), possibly due to competition for available nutrients. A large number of *Lactobacillus* would leave a small amount of nutrients for *E. coli* to utilise and multiply. The anti-inflammatory and anti-oxidant properties of AJE may help control this microbial balance in the gut (Liu and Kim 2021).

Noxious gas emissions from livestock farms is a hotly debated topic in environmental societies. Bad odours, nasal irritation and depression caused by harmful farm gases are similarly detrimental to animal growth, health, and human well-being (Liu and Kim, 2021). AJE supplementation (0.050%) reduced NH_3 emission in excreta samples in our study, similarly to studies where it lowered H_2S and NH_3 excretion in pigs (Dang et al., 2020; Liu et al., 2021). Harmful NH_3 is mainly caused by indigestible dietary proteins excreted with faeces and urine. According to Yan et al. (2011) and Hoque et al. (2021), nitrogen content in excreta depended on nutrient digestibility and microbial population. NH_3 is a by-product of nitrogen utilisation by harmful bacteria, and thus reduced *E. coli* population due to AJE supplementation in our study produced less harmful gases. In addition, nitrogen digestibility was also increased by AJE supplementation to the diet, which was consistent with the suggestion of Yan et al. (2011) and Hoque et al. (2021).

Effect of protein level

Reducing CP levels in broiler diets negatively affected ADG and FCR in our study. Although in different species, Galassi et al. (2010) and Liu and Kim (2021) found similar negative responses in pigs. In addition, this reduced protein level was not sufficient to increase feed intake in the LCP group. On the other hand, if essential amino acids (AA) were supplied in a diet reduced in protein, this negative effect may not have occurred in broilers (Peng et al., 2016). In our study, lysine and methionine were kept at the same level in the HCP and LCP diets, but other AA were not analysed. This reduction in growth performance could have occurred due to a lack of some amino acids.

Dietary protein levels had no effect on nutrient digestibility. In the absence of dietary protein in broilers, total nutrient retention could be increased

by nutrient absorption (Kidd et al., 2021); however, total tract digestibility did not differ as it was calculated as a percentage of each diets protein level. As the release of harmful gases depends on nutrient digestibility (Yan et al., 2011), we did not find any differences in their emissions, since no changes occurred in nutrient digestibility due to dietary protein reduction. On the other hand, Attia et al. (2017) observed a lower ammonia gas excretion due to reducing dietary protein by 3% and supplementing lysine and methionine. The difference could be due to reduced protein levels.

Indigestible nutrients are a food source for the intestinal bacterial population. Thus, lowering the protein level in broiler diets is expected to reduce to some extent the abundance of bacteria in faeces (Zhou et al., 2016). Liu and Kim (2021) found no effect of protein levels on the microbial population in finishing pigs. As the literature regarding the effect of protein levels on the microbial population in broilers is not extensive, the mechanism is difficult to describe unambiguously. Due to the type and source of proteins in the diet, the LCP diet could contain sufficient amounts of indigestible nutrients available for the gut microorganisms to maintain their population. Further research in broilers is needed to determine the impact of dietary protein levels on microbial populations.

Interaction of CP and AJE levels

There were no interactions found between CP and AJE levels in terms of broiler growth performance, digestibility, microbial count, and/or gas emissions; Liu and Kim (2021) presented similar results in growing-finishing pigs. Therefore, enhanced growth performance of broilers could be due to improved nutrient digestibility. This study demonstrated that there was no synergistic effect of CP level and AJE supplementation.

Conclusions

AJE supplementation at 0.050% helped broilers from the LCP group to obtain similar growth performance as the HCP group on days 22 to 35, and throughout the experimental period. This was due to the ability of AJE to improve nutrient digestibility and intestinal conditions for microbiota. In addition, 0.050% AJE supplementation has proven its value in reducing noxious NH_3 emissions. Improved protein utilisation through AJE supplementation contributed to better growth performance and reduced nitrogen excretion to the environment.

As a result, AJE (0.050%) may be suitable as a feed additive for efficient feed utilisation and reduction of harmful gas emissions in broiler breeding without affecting growth performance when supplemented with a low-protein diet.

Acknowledgements

The Department of Animal Resource & Science was supported through the Research-Focused Department Promotion & Interdisciplinary Convergence Research Projects as a part of the University Innovation Support Program for Dankook University in 2022.

Conflict of interest

The Authors declare that there is no conflict of interest.

References

- Ao X., Kim I.H., 2020. Effects of *Achyranthes bidentata* polysaccharides on performance, immunity, antioxidant capacity, and meat quality in Pekin ducks. *Poult. Sci.* 99, 4884–4891, <https://doi.org/10.1016/j.psj.2020.06.026>
- AOAC International, 2000. Official methods of analysis of AOAC International. 17th Edition. Association of Official Analytical Chemists. Washington, DC (USA)
- Attia Y.A., Bovera F., Abd-El-Hamid E. Abd-El-Hamid, Abd-Elrazk E. Tag EL-Din, Al-Harhi, M. A., Nizza A., Elharidy R.M., 2017. Effect of dietary protein concentrations, amino acids and conjugated linoleic acid supplementations on productive performance and lipid metabolism of broiler chicks. *Ital. J. Anim. Sci.* 16, 563–572, <https://doi.org/10.1080/1828051X.2017.1301228>
- Babaei S., Rahimi S., Karimi Torshizi M.A., Tahmasebi G., Khaleghi Miran S.N., 2016. Effects of propolis, royal jelly, honey and bee pollen on growth performance and immune system of Japanese quails. *Vet. Res. Forum.* 7, 13–20
- Bang S.Y., Kim J.H., Kim H.Y., Lee Y.J., Park S.Y., Lee S.J., Kim Y., 2012. *Achyranthes japonica* exhibits anti-inflammatory effect via NF- κ B suppression and HO-1 induction in macrophages. *J. Ethnopharmacol.* 144, 109–117, <https://doi.org/10.1016/j.jep.2012.08.037>
- Dang D.X., Kim Y.M., Kim I.H., 2020. Effects of a root extract from *Achyranthes japonica* Nakai on the growth performance, blood profile, fecal microbial community, fecal gas emission, and meat quality of finishing pigs. *Livest. Sci.* 239, 104160, <https://doi.org/10.1016/j.livsci.2020.104160>
- Dozier W.A., Moran Jr E.T., Kidd M.T., 2000. Threonine requirement of broiler males from 42 to 56 days in a summer environment. *J. Appl. Poult. Res.* 9, 496–500, <https://doi.org/10.1093/japr/9.4.496>
- Forte C., Branciarri R., Pacetti D., Miraglia D., Ranucci D., Acuti G., Balzano M., Frega N.G., Trabalza-Marinucci M., 2018. Dietary oregano (*Origanum vulgare* L.) aqueous extract improves oxidative stability and consumer acceptance of meat enriched with CLA and n-3 PUFA in broilers. *Poult. Sci.* 97, 1774–1785, <https://doi.org/10.3382/ps/pex452>
- Galassi G., Colombini S., Malagutti L., Crovetto G.M., Rapetti L., 2010. Effects of high fibre and low protein diets on performance, digestibility, nitrogen excretion and ammonia emission in the heavy pig. *Anim. Feed Sci. Technol.* 161, 140–148, <https://doi.org/10.1016/j.anifeedsci.2010.08.009>
- Hashemi S.R., Davoodi H., 2010. Phytochemicals as new class of feed additive in poultry industry. *J. Anim. Vet. Adv.* 9, 2295–2304, <https://doi.org/10.3923/javaa.2010.2295.2304>
- Hoque M.R., Jung H.I., Kim I.H., 2021. Effect of yeast culture (*Saccharomyces cerevisiae*) supplementation on growth performance, excreta microbes, noxious gas, nutrient utilization, and meat quality of broiler chicken. *J. Poult. Sci.*, 58, 216–221, <https://doi.org/10.2141/jpsa.0190144>
- Huang C.W., Lee T.T., Shih Y.C., Yu B., 2011. Effects of dietary supplementation of Chinese medicinal herbs on polymorphonuclear neutrophil immune activity and small intestinal morphology in weanling pigs. *J. Anim. Physiol. Anim. Nutr.* 96, 285–294, <https://doi.org/10.1111/j.1439-0396.2011.01151.x>
- Jang G.Y., Kim H.Y., Lee S.H., Kang Y., Hwang I.G., Woo K.S., Kang T.S., Lee J.S., Jeong H.S., 2012. Effects of heat treatment and extraction method on antioxidant activity of several medicinal plants. *J. Korean Soc. Food Sci. Nutr.* 41, 914–920, <https://doi.org/10.3746/jkfn.2012.41.7.914>
- Kidd M.T., Maynard C.W., Mullenix G.J., 2021. Progress of amino acid nutrition for diet protein reduction in poultry. *J. Anim. Sci. Biotechnol.* 12, 45, <https://doi.org/10.1186/s40104-021-00568-0>
- Lan R.X., Lee S.I., Kim I.H., 2016. Effects of multistrain probiotics on growth performance, nutrient digestibility, blood profiles, faecal microbial shedding, faecal score and noxious gas emission in weaning pigs. *J. Anim. Physiol. Anim. Nutr.* 100, 1130–1138, <https://doi.org/10.1111/jpn.12501>
- Liu X., Kim I.H., 2021. Effects of long-term feeding of *Achyranthes japonica* Nakai extract as a supplement to diets with different protein levels diets on the growth performance and meat quality characteristics of growing-fattening pigs. *Anim. Feed Sci. Technol.* 279, 115030, <https://doi.org/10.1016/j.anifeedsci.2021.115030>
- Liu X., Lee S.I., Kim I.H., 2021. *Achyranthes japonica* extracts supplementation to growing pigs positively influences growth performance, nutrient digestibility, fecal microbial shedding, and fecal gas emission. *Anim. Biosci.* 34, 427–433, <https://doi.org/10.5713/ajas.20.0012>
- Naeemasa M., Alaw Qotbi A.A., Seidavi A., Norris D., Brown D., Ginindza M., 2015. Effects of coriander (*Coriandrum sativum* L.) seed powder and extract on performance of broiler chickens. *S. Afr. J. Anim. Sci.* 45, 371–378, <https://doi.org/10.4314/sajas.v45i4.3>
- Oso A.O., Suganthi R.U., Manjunatha Reddy G.B., Malik P.K., Thirumalaisamy G., Awachat V.B., Selvaraju S., Arangasamy A., Bhatta R., 2019. Effect of dietary supplementation with phytochemical blend on growth performance, apparent ileal digestibility of nutrients, intestinal morphology, and cecal microflora of broiler chickens. *Poult. Sci.* 98, 4755–4766, <https://doi.org/10.3382/ps/pez191>
- Park J.H., Kim I.H., 2020. Effects of dietary *Achyranthes japonica* extract supplementation on the growth performance, total tract digestibility, cecal microflora, excreta noxious gas emission, and meat quality of broiler chickens. *Poult. Sci.* 99, 463–470, <https://doi.org/10.3382/ps/pez533>
- Peng X., Hu L., Liu Y. et al., 2016. Effects of low - protein diets supplemented with indispensable amino acids on growth performance, intestinal morphology and immunological parameters in 13 to 35 kg pigs. *Animal* 10, 1812–1820, <https://doi.org/10.1017/S1751731116000999>

- Rostagno H.S., Albino L.F.T., Donzele J.L., Gomes P.C., Oliveira R.F.M., Lopes D.C., Ferreira A.S., Barreto S.L.T., 2011. Brazilian tables for poultry and swine - composition of feedstuffs and nutritional requirements. 3rd Edition. Universidade Federal de Vicosa, Departamento de Zootecnia. Vicosa, MG (Brazil)
- Saleh A.A., Amber K.A., Soliman M.M., Soliman M.Y., Morsy W.A., Shukry M., Alzawqari M.H., 2021. Effect of low protein diets with amino acids supplementation on growth performance, carcass traits, blood parameters and muscle amino acids profile in broiler chickens under high ambient temperature. *Agriculture* 11, 185, <https://doi.org/10.3390/agriculture11020185>
- Sun H.Y., Kim Y.M., Kim I.H., 2020. 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. *Poul. Sci.* 99, 5728–5735, <https://doi.org/10.1016/j.psj.2020.07.023>
- van der Aar P.J., Molist F., van der Klis J.D., 2017. The central role of intestinal health on the effect of feed additives on feed intake in swine and poultry. *Anim. Feed Sci. Technol.* 233, 64–75, <https://doi.org/10.1016/j.anifeedsci.2016.07.019>
- Williams C.H., David D.J., Iismaa O., 1962. The determination of chromic oxide in faeces samples by atomic absorption spectrophotometry. *J. Agric. Sci.* 59, 381–385, <https://doi.org/10.1017/S002185960001546X>
- Windisch W., Schedle K., Plietzner C., Kroismayr A., 2008. Use of phyto-genic products as feed additives for swine and poultry. *J. Anim. Sci.* 86, 140–148, <https://doi.org/10.2527/jas.2007-0459>
- Yan L., Meng Q.W., Kim I.H., 2011. The effect of an herb extract mixture on growth performance, nutrient digestibility, blood characteristics and fecal noxious gas content in growing pigs. *Livest. Sci.* 141, 143–147, <https://doi.org/10.1016/j.livsci.2011.05.011>
- Zeng Z., Zhang S., Wang H., Piao X., 2015. Essential oil and aromatic plants as feed additives in non-ruminant nutrition: a review. *J. Anim. Sci. Biotechnol.* 6, 7, <https://doi.org/10.1186/s40104-015-0004-5>
- Zhou L., Fang L., Sun Y., Su Y., Zhu W., 2016. Effects of the dietary protein level on the microbial composition and metabolomic profile in the hindgut of the pig. *Anaerobe* 38, 61–69, <https://doi.org/10.1016/j.anaerobe.2015.12.009>