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Influence of the supplementation of *Achyranthes japonica* extracts on the growth performance, nutrient digestibility, gas emission, fecal microbial, and meat quality traits of finishing pigs with different nutrition concentrations in the diet

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Abstract

The present study was conducted to investigate the effects of the supplementation of *Achyranthes japonica* extract (AJE) on overall performance levels with different nutrition concentrations in the diets of finishing pigs. Here, 140 finishing pigs with initial body weights of 50.18 ± 2.37 kg were used in a ten-week trial involving a 2 × 2 factorial design in two phases with the following factors: diet types with different nutrition concentrations (Positive control [PC] vs. Negative control [NC]) and 0 or 0.05% AJE supplementation to NC and PC diets. The PC diet was a basal diet, whereas the NC diet consisted of a 5% and 7% crude protein (CP) reduced basal diet during phase 1 (1 - 35 days) and phase 2 (36 - 70 days), respectively. A significant effect ($p < 0.05$) on the gain-to-feed ratio (G : F) was observed with interaction effects between the diets and AJE supplementation. However, during phase 1 and in all periods, the pigs fed the PC and NC diets with average daily feed intake (ADFI) tended to decrease compared to those fed diets with AJE. A significant effect ($p = 0.0380$) of *E. coli* was observed in pigs fed the PC and NC diets compared to AJE supplementation in phase 2 of the experiment. The backfat thickness (BFT) tended to decrease and the lean meat percentage (LMP) was significantly improved in phase 2 for pigs fed the PC and NC diets. In summary, BFT and LMP showed beneficial effects and fecal microbiota of *E. coli* counts were positively affected when pigs were fed diets with different nutrient concentrations.

Key words: *Achyranthes japonica* extracts, backfat thickness, fecal microbial, growth performance, lean meat percentage

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Introduction

The most important factor in the livestock industry is to provide adequate nutrients and to achieve efficient and profitable animal production. Protein and energy are the two most important components that generate a lot of curiosity and challenges for nutritionists. The provision of energy and protein from corn and soybean meals can account for up to 70% of feed costs and an expensive feed component (Saleh et al., 2004). Thus, energy and protein are determinants in evaluating the performance and production coefficients of farm animals. The incessant scarcity of feed raw materials with a consequential increase in the price of animal feed has attracted the attention of the nutritionist to address this issue of feed raw material scarcity and increased feed costs.

High antimicrobial and antioxidant activity along with their functional properties in meat products are the key factors for many herbs, spices, and their extracts (Rounds et al., 2012). Moreover, phytochemicals can be classified into several categories for their anti-microbial properties as phenolics/polyphenols, terpenoids/essential oils, alkaloids, and lectins/polypeptides (Windisch et al., 2008). *Achyranthes japonica* is commonly known as Japanese chaff flower and perennial herb belonging to the member of the *Achyranthes* genus in the *Amaranthaceae* family. It is available in a roadside area mainly in Japan, Korea, and East Asian countries. Lee et al. (2012) mentioned that *A. japonica* has different physiological and biochemical functions in the body, and also active components, like antioxidant phytochemicals and bioactive compounds. An important concern during the use of plant extracts is their toxicity. Some plants may contain toxic or allergic compounds that prevent them from being used as a medicinal element. In addition, Park et al. (2004) reported no toxic effect of *Achyranthes japonica* extract dose (400 $mg \cdot kg^{-1}$) for 25 days. Furthermore, Liu et al. (2020) showed the improvement of growth performance, fecal microbial and reduced gas emission in growing pigs.

Since there are limited studies on the efficacy of inclusion of AJE supplementation with different nutrient concentration diets, the objective of this study was to determine whether the AJE supplementation in different nutrient concentration diets could enable pigs to achieve similar performance to pigs fed standard control diets. And also, investigate if there would be any interaction between nutrient concentration diets and AJE supplementation.

Materials and Methods

The protocol of this experiment was reviewed and approved by the Animal Care and Use Committee of Dankook University (DK-1-2004), Cheonan, Republic of Korea, for animal experimentation.

Preparation of AJE

The AJE applied in this experiment was received from a commercial company (Synergen Inc., Bucheon, Korea). The roots were washed three times with clean water and then powdered in the mill (IKAM20, IKA, Staufen, Germany). The dried powder was extracted with distilled water at 80℃ and then refluxed for 6 h to obtain an initial extract. The residues were extracted with distilled water (1 : 5) at 80℃ for 2 h. The extract was filtered using high-velocity centrifugal machine under low temperature, and the useful parts were collected by column and eluted with ethanol. After cooling to room temperature (25℃) and filtering (Whatman No. 2, Whatman Ltd., Kent, UK), the samples were vacuum-dried at a temperature below 40℃, followed by complete drying in a freeze-drier AJE contains total flavonoid (1.15 $mg \cdot g^{-1}$), a total polyphenol (4.26 $mg \cdot g^{-1}$) and saponin (0.47 $mg \cdot g^{-1}$).

Animals and facilities

A total of 140 finishing pigs ([Landrace × Yorkshire] × Duroc) with an initial average body weight of 50.18 ± 2.37 kg were selected for a ten-week trial in a 2 × 2 factorial design in 2 phases with the following factors: diet types as a different nutrition concentrations (positive control [PC] vs negative control [NC]) and 0 or 0.05% AJE supplementation to NC and PC diets PC diet consisted of basal diet whereas NC diet consisted of 5% and 7% CP reduced basal diet during phase 1 (1 - 35 days) and phase 2 (36 - 70 days) respectively. The pigs were kept into a room temperature (25℃) with the slatted stone floor, and every pen has been equipped with one side self-feeder and nipple drinker. Each treatment consisted of 7 replicate pens with 5 pigs per pen (two barrows and three gilts). The basal diet of pigs was formulated to meet the nutritional needs recommended by the NRC (2012) (Table 1). Basal diet and 0.05% AJE supplement were mixed using feed mixer.

Table 1. Composition of finishing pig diets (as fed-basis).

Item	Phase 1		Phase 2	
	PC	NC (-5% CP down)	PC	NC (-7% CP down)
Ingredient (%)				
Corn	76.72	78.46	79.67	81.88
Soybean meal (48%)	15.32	13.41	12.81	10.37
Tallow	2.53	2.56	2.36	2.41
Molasses	2.00	2.00	2.00	2.00
DCP	1.35	1.38	1.15	1.17
Limestone	0.56	0.55	0.55	0.56
Salt	0.30	0.30	0.30	0.30
Methionine (99%)	0.07	0.08	0.07	0.08
Lysine	0.48	0.55	0.44	0.52
Threonine (99%)	0.14	0.17	0.13	0.17
Tryptophan (99%)	0.05	0.06	0.04	0.06
Mineral mix[y]	0.20	0.20	0.20	0.20
Vitamin mix[z]	0.20	0.20	0.20	0.20
Phytase	0.05	0.05	0.05	0.05
Choline (25%)	0.03	0.03	0.03	0.03
Total	100.00	100.00	100.00	100.00
Calculated value				
Crude protein (%)	14.00	13.30	13.00	12.09
Ca (%)	0.60	0.60	0.55	0.55
P (%)	0.55	0.55	0.50	0.50
LYS (%)	1.00	1.00	0.90	0.90
MET (%)	0.30	0.30	0.28	0.28
THR (%)	0.65	0.65	0.60	0.60
TRP (%)	0.20	0.20	0.18	0.18
ME ($kcal \cdot kg^{-1}$)	3,300.00	3,300.00	3,300.00	3,300.00
Fat (%)	5.39	5.45	5.28	5.37
Fiber (%)	2.34	2.30	2.31	2.26
Ash (%)	4.36	4.29	4.03	3.94

PC, positive control; NC, negative control; CP, crude protein; DCP, dicalcium phosphate; LYS, lysine; MET, methionine; THR, threonine; TRP, tryptophan; ME, metabolizable energy.

[y] Provided per kg diet: Fe, 115 mg as ferrous sulfate; Cu, 70 mg as copper sulfate; Mn, 20 mg as manganese oxide; Zn, 60 mg as zinc oxide; I, 0.5 mg as potassium iodide; and Se, 0.3 mg as sodium selenite.

[z] Provided per kilograms of diet: Vitamin A, 13,000 IU; vitamin D3, 1,700 IU; vitamin E, 60 IU; vitamin K3, 5 mg; vitamin B1, 4.2 mg; vitamin B2, 19 mg; vitamin B6, 6.7 mg; vitamin B12, 0.05 mg; biotin, 0.34 mg; folic acid, 2.1 mg; niacin, 55 mg; D-calcium pantothenate, 45 mg.

Sampling measurements

The individual body weight measurement was documented at initial, phase 1 and phase 2. Average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G : F) were calculated, during the experiment, the intake of feed has been noted on a pen basis. Chromium oxide (0.2%) was added in pig's diet as an indigestible marker for a period of 7 days prior to fecal collection at phase 1 and end of the experiment (phase 2) to determine the apparent digestibility of dry matter (DM), nitrogen (N), and digestible energy. Fresh fecal samples were randomly collected from one gilt and one barrow (at least two pigs) from each pen, and pooled. Then the fecal samples were stored in a freezer at -20℃ until analysis and the samples were dried at 70℃ for 72 h. After all fecal and feed samples were grounded and sieved using 1 mm screen sieve. DM and N digestibility are determined using the published methods of AOAC (2000). Chromium in the feeds and feces were analyzed by the UV absorption spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan) and the energy was analyzed by measuring the heat of combustion in the feed and feces samples, using Parr 6100 oxygen bomb calorimeter (Parr Instrument Co., Moline, IL, USA). Tecator ™ Kjeltec 2300 Analyzer used for a analyze the nitrogen. The apparent total tract digestibility was calculated by using the equations of Upadhaya et al. (2016).

The fecal microbial was calculated at initial, phase 1, and phase 2. The fresh fecal samples were randomly collected from 8 pigs (4 gilt and 4 barrow) per treatment and placed on ice for transport to the laboratory, where the microbial analysis was immediately carried out. The one gram of fresh fecal sample was taken and diluted with 9 mL of 1% peptone broth (Becton, Dickinson and Co., Frankline Lakes, USA), and mixed well with a vortex mixer. Viable bacterial counts in the fecal samples were conducted by plating serial of 10^3 to 10^7 dilutions (in 1% peptone solution) onto MacConkey agar plates and Lactobacillus medium III agar plates (Medium 638, DSMZ, Braunschweig, Germany) to isolate *E. coli* and *Lactobacillus* respectively. The lactobacilli medium III agar plates were incubated for 48 h at 39℃ under anaerobic condition. The MacConkey agar plates were incubated for 24 h at 37℃. After finishing the incubation time, the bacterial plates were immediately taken out and counted the colonies.

For the analysis of NH_3 and H_2S contents in feces, 300 g of fecal samples were collected from each pen, pooled well and stored in plastic containers (polyvinyl, 25 × 35 cm) as triplicates at phase 2, and the fecal sample was fermented at 25℃ for 7 days, as explained by Sampath et al. (2020). NH_3, H_2S, Methyl mercaptans, CO_2, and acetic acid concentrations were measured using the published method of Sampath et al. (2020).

Backfat thickness (BFT) and lean meat percentage (LMP) was assessed at phase 1 and phase 2. Piglog 105 (Carometec Food Technology, Smoerum, Denmark) used for measured the Backfat thickness at the P2 position (6.5 cm area on the left and right end frames). Eights pigs per treatment were slaughtered at day 70. The meat samples were chilled for 24 hours at -4℃. The reflectance spectrometry measurements such as lightness (L*), redness (a*), and yellowness (b*) values are determined by Employing a Minolta CR410 chromameter (Minolta Co., Osaka, Japan). The pH meter (Testo205, Testo, Hampshire, UK) was used to measured pH values for each sample. According to the published method of Sullivan et al. (2007), the cooking loss was determined. Drip loss was determined as a percentage of the original weight using 4 g of meat sample according to the published method of (Honikel, 1998). The water-holding capacity (WHC), was measured followed the guide by Kauffman et al. (1986). *Longissimus dorsi* muscle area (LMA) was measured by tracing the longissimus muscle surface at the 10th rib, which also used the abovementioned digitizing area-line sensor. The sensory evaluation of meat color, marbling, and firmness scores were evaluated according to NPPC (1991) standards.

Carcass grade, carcass weight, and backfat thickness (mm) are measured. The quality of pork carcasses was graded into "Grade 2", "Quality Grade 1," or "Grade 1+," based on qualities such as lean color, marbling, and conditions of belly streaks (KAPE, 2010). The backfat thickness of the carcass was adjusted to a live weight of 115 kg, as described previously by Ha et al. (2010).

Statistical analysis

All data were statistically analyzed as 2 × 2 factorial arrangement using the GLM procedure of the SAS program (SAS Inst. Inc., Cary, NC, USA). The model included the effects of PC, NC and AJE supplementation, as well as the interaction between AJE supplementation with positive control and negative control. Logarithmic conversion of the data was performed for statistical analysis of the microbial count. The data were expressed as the standard error of the mean (SEM) and results are considered significant at $p < 0.05$ level and $p < 0.10$ as trends.

Results and Discussion

Effects of nutrient concentration diets

In the current study, different levels of PC and NC (-5% crude protein [CP] or -7% crude protein) diets were used to investigate the different nutrient concentrations of diets. The effect of AJE supplementation to PC and NC diets on growth performance is shown in Table 2. The supplementation of AJE, as well as diet types, did not have any significant effect ($p > 0.05$) on growth performance parameter (BW) during phases 1, 2, and the overall period. However, during phase 1 and overall periods, the pigs fed PC and NC diets had decreased ADFI than those fed diets with AJE. Throughout the experiment, ADG was not affected with or without supplementation of AJE. Hong et al. (2016) reported that dietary energy density by protein level had no significant effects in BW, ADG, ADFI, and G/F ratio in growing-finishing pigs during the entire experimental period. Similarly, growing pigs fed diets with 8.3% reduced CP did not have any improvement in growth performance (Guay et al., 2006). Fang et al. (2019) indicated that ADFI was increased in weaning pigs by decreasing the diet energy level. Moreover, intake of amino acids and protein deposition is related to energy concentration (NRC, 1998). An important factor of feed intake is the energy concentration in feed. A lower energy level can decrease feed intake, while a high energy level can decrease protein deposition (Lewis and Southern, 2001). The diet supplementation with lower crude protein had no detrimental effects on growth performance (Tjong et al., 1972; Wahlstrom and Libal, 1983). Deficiency in intact protein or excess of free amino acids results in poor performance in pigs fed a very low protein diet. Moreover, the di- and tri-peptides hydrolyzed content from intact protein was reported to have a positive correlation with the activities of digestive enzymes (Shimizu, 2004). Induction of excessive oxidation of amino acids may be due to the more rapid absorption rates of free amino acids contributing to poor growth performance and decreased body protein deposition (Yen et al., 2004). The effect of AJE supplementation to PC and NC diets on nutrient digestibility is shown in Table 3. Throughout the experiment, no significant effects were found with or without supplementation of AJE diets did not significantly affect. Hong et al. (2016) indicated that when pigs were fed with reduced energy and crude protein, the digestibility of dry matter was not significantly improved. Likewise, in our study, nutrient digestibility did not have any effects on finishing pigs by the addition of different concentration diets. The type and proportion of ingredients and energy source in the diet affects easy digestibility (Beaulieu et al., 2006).

Table 2. The effect of different nutrient concentration diets and dietary *Achyranthes japonica* extract supplementation on growth performance in finishing pigs.

Item	PC (standard basal diet)		NC (CP reduced diet)		SEM	p-value		
	AJE-	AJE+	AJE-	AJE+		Diet	AJE	Diet × AJE interaction
Body weight (kg)								
Initial	50.20	50.15	50.17	50.18	0.02	0.9912	0.9887	0.9862
Phase 1[y]	78.17	78.87	77.72	78.03	0.33	0.8486	0.9506	0.8239
Phase 2[z]	110.47	112.43	108.97	110.23	0.82	0.5970	0.7264	0.5507
Phase 1[y]								
ADG (g)	799	821	787	796	10	0.5603	0.8023	0.4918
ADFI (g)	2,339	2,364	2,310	2,301	24	0.0546	0.7358	0.4725
G/F	0.342	0.347	0.341	0.346	0.002	0.1255	0.9788	0.0757
Phase 2[z]								
ADG (g)	923	959	893	920	15	0.2181	0.3158	0.1694
ADFI (g)	3,000	3,069	2,967	2,975	42	0.1331	0.3535	0.4548
G/F	0.308	0.312	0.301	0.309	0.003	0.6573	0.9858	0.0497
Overall								
ADG (g)	861	890	840	858	12	0.3061	0.4788	0.2616
ADFI (g)	2,670	2,716	2,639	2,638	31	0.0742	0.4331	0.4278
G/F	0.323	0.327	0.318	0.325	0.002	0.4379	0.9654	0.0498

PC, positive control; NC, negative control; AJE, *Achyranthes japonica* extract; SEM, standard error of means; ADG, average daily gain; ADFI, average daily feed intake; G/F, gain to feed ratio.

[y] PC, basal diet supplemented with or without 0.05% AJE; NC, 5% reduced basal diet supplemented with or without 0.05% AJE.

[z] PC, basal diet supplemented with or without 0.05% AJE; NC, 7% reduced basal diet supplemented with or without 0.05% AJE.

Table 3. The effect of different nutrient concentration diets and dietary *Achyranthes japonica* extract supplementation on nutrient digestibility in finishing pigs.

Item	PC (standard basal diet)		NC (CP reduced diet)		SEM	p-value		
	AJE-	AJE+	AJE-	AJE+		Diet	AJE	Diet × AJE interaction
Phase 1[y]								
Dry matter	75.18	76.16	74.23	75.00	0.78	0.2238	0.3099	0.9028
Nitrogen	73.33	75.13	72.78	73.55	0.77	0.1910	0.1205	0.5257
Digestibility energy	74.67	75.59	74.07	74.48	0.79	0.3101	0.4232	0.7586
Phase 2[z]								
Dry matter	72.71	74.03	74.07	73.16	1.01	0.4121	0.4467	0.8384
Nitrogen	70.30	72.09	71.90	70.75	1.08	0.3229	0.3046	0.6283
Digestibility energy	71.76	73.51	73.19	72.25	1.10	0.3431	0.3179	0.6691

PC, positive control; NC, negative control; AJE, *Achyranthes japonica* extract; SEM, standard error of means.

[y] PC, basal diet supplemented with or without 0.05% AJE; NC, 5% reduced basal diet supplemented with or without 0.05% AJE.

[z] PC, basal diet supplemented with or without 0.05% AJE; NC, 7% reduced basal diet supplemented with or without 0.05% AJE.

Caridi (2002) reported different species of *Lactobacillus* having an inhibitory effect on *E. coli*. *E. coli* count was reduced by nutrient use competition or by producing lactic acid in the digestive system. A significant effect (p = 0.0380) of *E. coli* was observed in pigs fed PC and NC diets compared with AJE supplementation at phase 2 of the experiment (Table 4). As a result, the alterations are alleviated in energy and crude protein diets in intestinal morphology stimulated by bacterial pathogens and maintain the absorption capacity of intestinal cells and normal digestion. The nutrient's concentration diets did not have any significant effect on gas emission. As shown in Table 5, the backfat thickness had a tendency (p = 0.0836) in decreased, and LMP was significantly improved (p = 0.0244) at phase 2 in pigs' diet of PC and NC diets. Boyd et al. (2002) discovered that BFT plays an important role in determining the amount of energy in pigs in short period of time. Roongsitthichai and Tummaruk (2014) insisted that using BFT, the sow reproductive performance was determined. In addition, Backfat thickness and LMP determine the quality of pork in the swine industries (Grzes et al., 2016). It has been reported that the meat color is an important factor in assessing meat quality since the relationship between color and freshness. Some other researchers indicated that different dietary crude proteins had no effects on meat quality parameters such as WHC and pH (Goerl et al., 1995; Alonso et al., 2010). Additionally, dietary crude protein positively affects meat color (L*, a*, b*) (Goerl et al., 1995; Suárez-Belloch et al., 2016). Castell et al. (1994) and Witte et al. (2000) demonstrated that drip loss percentage was not influenced by dietary nutrient density. However, PC and NC diets, supplementation did not have any adverse effects (p > 0.05) on the meat quality traits of finishing pigs. The meat quality of finishing pigs is shown in Table 6. The different nutrition concentration diets were not affected on a carcass grade (Table 7), consistent with the study of reducing dietary crude protein level (Kerr et al., 1995; Zhou et al., 2015; Suárez-Belloch et al., 2016). The functioning mechanism of nutrient concentration on finishing pigs remains unclear, and whether the effect may be due to the feed ingredients containing pigments requires further study.

Table 4. The effect of different nutrient concentration diets and dietary *Achyranthes japonica* extract supplementation on fecal microbial in finishing pigs.

Item ($log_{10}cfu·g^{-1}$)	PC (standard basal diet)		NC (CP reduced diet)		SEM	p-value		
	AJE-	AJE+	AJE-	AJE+		Diet	AJE	Diet × AJE interaction
Phase 1[y]								
Lactobacillus	9.41	9.49	9.34	9.41	0.07	0.2548	0.3039	0.9015
Escherichia coli	7.47	7.38	7.53	7.51	0.06	0.1179	0.3394	0.6092
Phase 2[z]								
Lactobacillus	9.55	9.63	9.52	9.57	0.04	0.2737	0.1052	0.7126
Escherichia coli	7.61	7.54	7.51	7.65	0.04	0.0380	0.2437	0.4404

PC, positive control; NC, negative control; AJE, *Achyranthes japonica* extract; SEM, standard error of means.
[y] PC, basal diet supplemented with or without 0.05% AJE; NC, 5% reduced basal diet supplemented with or without 0.05% AJE.
[z] PC, basal diet supplemented with or without 0.05% AJE; NC, 7% reduced basal diet supplemented with or without 0.05% AJE.

Table 5. The effect of different nutrient concentration diets and dietary *Achyranthes japonica* extract supplementation on backfat thickness and lean meat percentage (LMP) in finishing pigs.

Item	PC (standard basal diet)		NC (CP reduced diet)		SEM	p-value		
	AJE-	AJE+	AJE-	AJE+		Diet	AJE	Diet × AJE interaction
Phase 1[y]								
Backfat thickness (mm)	14.0	14.4	13.5	13.9	0.3	0.1330	0.2327	0.9120
Lean meat percentage (%)	57.6	57.7	57.6	57.5	0.2	0.6925	0.6925	0.5686
Phase 2[z]								
Backfat thickness (mm)	18.5	18.8	18.0	18.4	0.2	0.0836	0.2555	0.8978
Lean meat percentage (%)	55.2	55.3	55.8	54.9	0.2	0.0244	0.8605	0.5212

PC, positive control; NC, negative control; AJE, *Achyranthes japonica* extract; SEM, standard error of means.
[y] PC, basal diet supplemented with or without 0.05% AJE; NC, 5% reduced basal diet supplemented with or without 0.05% AJE.
[z] PC, basal diet supplemented with or without 0.05% AJE; NC, 7% reduced basal diet supplemented with or without 0.05% AJE.

Table 6. The effect of different nutrient concentration diets and dietary *Achyranthes japonica* extract supplementation on meat quality in finishing pigs.

Item	PC (standard basal diet)		NC (CP reduced diet)		SEM	p-value		
	AJE-	AJE+	AJE-	AJE+		Diet	AJE	Diet × AJE interaction
pH	5.84	5.74	5.93	5.82	0.10	0.3045	0.2338	0.9882
Longissimus muscle area (mm^2)	7,822	8,361	7,653	7,082	352	0.1890	0.5448	0.3699
Water holding capacity (%)	46.79	49.00	42.18	44.89	4.36	0.3104	0.5612	0.9516
Meat color								
L*	52.64	51.21	54.18	54.49	1.41	0.1323	0.7151	0.5708
a*	16.16	15.68	14.48	16.51	0.50	0.8445	0.4196	0.1165
b*	7.34	6.78	6.27	7.74	0.38	0.8378	0.6077	0.1972
Cooking loss (%)	35.24	34.47	33.02	35.38	2.42	0.9444	0.9234	0.3358
Sensory evaluation[z]								
Color	3.47	3.41	3.57	3.50	0.11	0.2661	0.4210	0.9409
Firmness	2.41	2.44	2.38	2.41	0.14	1.0000	0.4155	0.9868
Marbling	2.97	2.84	2.97	2.84	0.04	0.5552	0.5552	0.9810
Drip loss (%)								
d1	8.22	8.00	7.76	8.15	0.56	0.8292	0.9042	0.6670
d3	13.46	12.83	13.26	13.57	0.89	0.7950	0.8835	0.6613
d5	19.78	18.54	19.28	19.28	1.13	0.9301	0.6434	0.6434
d7	25.80	24.34	24.98	25.26	1.24	0.9727	0.6949	0.5621

PC, positive control; NC, negative control; AJE, *Achyranthes japonica* extract; SEM, standard error of means.

[z] Color: 1.0, pale pinkish gray to white; 2.0, grayish pink; 3.0, reddish pink; 4.0, dark reddish pink; 5.0, purplish red; 6.0, dark purplish red. Firmness: 1.0, soft; 2.0, firm; 3.0, very firm. Marbling: marbling score correspond to intramuscular lipid content.

Table 7. The effect of different nutrient concentration diets and dietary *Achyranthes japonica* extract supplementation on carcass grade in finishing pigs.

Item	PC (standard basal diet)		NC (CP reduced diet)		SEM	p-value		
	AJE-	AJE+	AJE-	AJE+		Diet	AJE	Diet × AJE interaction
Carcass weight (kg)	89.50	91.20	92.90	91.10	1.4	0.2414	0.9682	0.2185
Backfat thickness (mm)	18.70	19.30	19.00	18.70	0.5	0.7163	0.7584	0.3569
1+ (%)	34.29	37.14	22.86	31.43	-	-	-	-
1 (%)	34.29	34.29	28.57	34.29	-	-	-	-
2 (%)	31.43	28.57	48.57	34.29	-	-	-	-

PC, positive control; NC, negative control; AJE, *Achyranthes japonica* extract; SEM, standard error of means.

Effects of AJE supplementation

Plant extracts and herbs can be given as dietary supplements that are evaluated as growth promoters or immune enhancers and improve growth performance in swine production (Lin et al., 2000; Chen et al., 2009; Czech et al., 2009; Yan et al., 2011a). In this regard, AJE was used as a growth promoter in our study. The dietary inclusion of AJE supplementation did not have any significant effects on growth performance parameters such as BW, ADG, ADFI, and G : F. In contrast, Liu et al. (2020) stated that inclusion of AJE supplementation had beneficial effects on the growth performance of the growing pigs. Similarly, body weight gain of broilers was improved using the supplementation of herbal plant extracts (Park et al., 2014). In addition, Dang et al. (2020) reported that AJE had a linear increment in ADG and no significant effect on BW and

G : F ratio of growth performance in finishing pigs. The enhanced efficiency in AJE-fed pigs may be due to the bioactive phytochemicals, including saponin and polyphenol compounds. In addition, Liu et al. (2008) and Lee et al. (2012) mentioned that *A. japonica* has different physiological and biochemical functions in the body and active components, like antioxidant phytochemicals and bioactive compounds. Thus, contradictory was resulted in various experiments due to the variation in amount of AJE dosage and differences in dietary compositions. In a previous study, Liu et al. (2020) stated that AJE had a linear increment in DM of nutrient digestibility to the growing pigs. There were no significant effects ($p > 0.05$) observed on PC and NC diets and AJE supplementation on the gas emission (methyl mercaptans, NH_3, H_2S, CO_2, and acetic acid) of finishing pigs as shown in Table 8. The result agrees with (Nousiainen and Setala, 1993), who reported that the herbal plant mixture supplementation was not affected in nutrient digestibility during the 10-week trials for pigs. The absence of nutrient digestibility is due to the better development of digestive system as pigs become older (Thamaraikannan and Kim, 2021).

Table 8. The effect of different nutrient concentration diets and dietary *Achyranthes japonica* extract supplementation on gas emission in finishing pigs.

Item	PC (standard basal diet)		NC (CP reduced diet)		SEM	p-value		
	AJE-	AJE+	AJE-	AJE+		Diet	AJE	Diet × AJE interaction
Phase 2[z]								
Methyl mercaptans	5.18	5.95	4.58	4.75	1.00	0.4010	0.6540	0.7766
NH_3	1.58	2.10	0.98	1.68	0.45	0.3229	0.2415	0.8632
H_2S	5.20	5.40	4.20	4.50	0.70	0.2406	0.7376	0.9732
CO_2	16,500	18,750	15,000	16,500	2,239	0.3885	0.3885	0.8610
Acetic acid	8.40	9.30	8.00	8.30	0.70	0.2892	0.4119	0.6914

PC, positive control; NC, negative control; AJE, *Achyranthes japonica* extract; SEM, standard error of means.

[z] PC, basal diet supplemented with or without 0.05% AJE; NC, 7% reduced basal diet supplemented with or without 0.05% AJE.

The pigs fed with or without AJE supplementation had no effects on the *Lactobacillus* count. Chen et al. (2009) demonstrated that different dosages of *Achyranthes bidentata* extract supplementation decreased the diarrhea frequency of weaning pigs, which indicates the inhibition of gut pathogens. Liu et al. (2020) reported that reducing the bacterial count in growing pigs by addition of 0 to 0.10% AJE and Park and Kim (2019) stated that the *E. coli* count was reduced using the 0 to 0.25% of AJE supplementation in broilers. However, herbal plants might inhibit the growth of pathogenic microorganisms in the gastrointestinal tract. Therefore, animal's resistance capacity to different stress situations is enhanced (Windisch et al., 2008). In controversially, there was no statistical difference found in the *Lactobacillus* count and *E. coli* count in the dietary supplementation of AJE throughout the experiment in finishing pigs. An important predictor of carcass lean content and meat quality is pigs' backfat thickness (BFT) (De Jong et al., 2012). However, with AJE supplementation on finishing pigs, the effect on BFT and LMP was unclear. Previously, Mohankumar et al. (2020) stated that AJE supplementation had significant effects on the backfat thickness in finishing pigs. However, AJE supplementation with PC and NC diets were significantly not influenced. Throughout the experiment, no significant difference was found at phase 1. Dang et al. (2020) found that AJE to finishing pigs had reduced noxious gas emissions. Also, Park et al. (2019) reported that 0 to 0.1% AJE supplementation had reduced the gas emission in broilers. Previously, Yan and Kim (2011) stated that fecal noxious gas emission was associated with nutrient digestibility, as decreased digestibility may reduce the microbial fermentation substrate in the large intestine. Thus, decreased fecal noxious gas emission. In our study, nutrient digestibility is not affected in finishing pigs by adding AJE supplementation, and this may be the reason for the no effect on noxious gas emission.

Previously, several researchers have stated the beneficial effects of the plant extract feed supplementation on the chemical composition, physicochemical and sensory properties, and carcass grade (Nasir and Grashorn, 2010; Marcinˇcáková et al., 2011), Nevertheless some other authors demonstrated no effects on a plant extract (Koreleski and Swiatkiewicz, 2007; Kim et al., 2016). According to the Yan et al. (2011b) study, inclusion of plant extract supplementation had no effects on the meat color, drip loss, cook loss, pH, WHC, and sensory evaluation scores. Likewise, our study noted that dietary inclusion of AJE supplementation failed to show significant effects on meat quality, which include pH, LMA, WHC, L*, a*, b*, sensory evaluation (Color, Firmness, Marbling), and drip loss. Dang et al. (2020) demonstrated that AJE had reduced the drip loss. The lower level of drip loss indicates the improved meat quality. These inconsistent results are due to differences in experimental conditions, different dosage level, that has been used and the pig's genotype. The results indicate that the physical parameters of pork quality have no detrimental effect on AJE supplementation. On the other hand, further evaluation needs to know the effects of AJE supplementation with different diet concentrations. The supplementation of plant extract had no significant effect on carcass grade (Grela, 2000; Paschma and Wawrzynski, 2003). Carcass quality grade was highly correlated with marbling and meat color and backfat thickness to some extent when the grade was quantified. We found that "1+, %" carcass grade was higher in pigs fed AJE supplementation than PC and NC diets.

Interactive effects between AJE supplementation and nutrient concentration

Significant effects ($p > 0.05$) in G : F and no significant effects in ADG, ADFI, and BW were observed with the interaction effects between PC and NC diets and AJE supplementation. This may be due to the optimized microflora balance in the gut, which leads to an improvement in nutrient utilization. Moreover, the conversion of feed to body mass can be maintained by macrofloral balance and subsequently increase the total metabolism of nutrients and energy. Furthermore, there were no interactive effects between different nutrient concentration diets and AJE supplementation in other measurements such as nutrient digestibility, fecal microbial, gas emission, meat quality, and carcass traits.

Conclusion

The different nutrient concentration diets had beneficial effects on BFT and LMP. Moreover, fecal microbiota of *E. coli* counts was positively influenced. However, it failed to show significant growth performance effects, nutrient digestibility, gas emission, meat quality, and carcass traits. The different nutrient concentration diets with dietary AJE supplementation did not have any significant effects on all parameters. However, further research is needed to determine the perfect amount of AJE supplementation with different nutrition concentration diets in finishing pigs.

Conflict of Interests

No potential conflict of interest relevant to this article was reported.

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