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Effect of dietary *Achyranthes japonica* extract on growth performance of growing pigs and absorption rate of quercetin in blood

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Abstract

This study was done to investigate the effects of the incorporation of *Achyranthes japonica* extracts (AJE) in diet on the production parameters of growing pigs. Exp 1: Total, 105 cross-bred pigs (average body weight: 24.47 ± 2.46 kg) were used in a 6-week feeding trial. Pigs (seven replicates, five pigs per pen) were allotted randomly to three treatments. Dietary treatments: CON (basal diet); basal diet with 0.025% AJE, and basal diet + 0.050% AJE). Growth performance, nutrient digestibility, fecal microbial count, and fecal noxious gas were assessed in this study. Average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F) were not affected by the addition of up to 0.05% AJE. In the case of apparent total tract digestibility (ATTD), dry matter (DM), nitrogen (N), and digestible energy (DE) were not changed in 3rd and 6th weeks of the feeding trial through the addition of AJE up to 0.05% in the growing pig diet. In microbial count, *Lactobacillus* and *Escherichia coli* count at 3rd and 6th week was similar in all the treatment diets. The inclusion of AJE at levels up to 0.05% in growing pig diet had no effect on the production of NH3, H2S, acetic acid, and CO_2 in the feces. After ending the Exp 1, a total of nine pigs were divided into three treatment groups. Treatment diets were included, TRT1, basal diet + powder quercetin 30 g; TRT2, basal diet + powder quercetin 150 g; TRT3, basal diet + powder quercetin 300g. Rate of absorption in blood was increased with the higher dose of quercetin. The results suggested incorporation of AJE up to 0.05% has no significant effect on ADG, ADFI, and G:F, as well as DM, N, and DE digestibility, fecal microbial count, and fecal noxious gas emission in growing pigs, even though no negative effect was found.

Keywords: *Achyranthes japonica* extracts, Fecal microbial count, Fecal noxious gas emissions, Growth performance, Growing pigs, Nutrient digestibility

INTRODUCTION

Antibiotic growth promoters (AGP) are being used in livestock farms since their discovery to improve productivity and to assure animal immunity due to their antimicrobial properties [1]. But undisciplined use of AGP may result in antibiotic-resistant bacteria and harmful residues [2]. And the rising concern

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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: Hossain MM, Pang M, Kim IH.
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Ethics approval and consent to participate
The experiment was inspected by the Animal Care and Use Committee at Dankook University, and the relevant experimental procedure was accepted (Ethics Approval Number: DK-1-2111).

about the risk associated with antibiotics usage in pig production resulted in an increased interest in growing pigs without these AGP. But there is a significant increase in disease and a retardation in growth in antibiotic-free animals [3]. To prevent the adverse effects of antibiotics, increase consumer health, and reduce environmental impact, researchers are looking for alternatives to antibiotics. To respond to these challenges, studies have been done to find other feed additives that can be used instead of antibiotics. Alternative additives should have the ability to boost beneficial microbial counts and decrease detrimental ones without affecting feed efficiency or animal growth [4]. Phytochemicals (phenols, flavonoids, and tannins) present in medicinal plants have different anti-bacterial, anti-microbial, and anti-fungal properties, which are useful in the treatment as well as prevention of diseases [5].

Achyranthes japonica is a medicinal plant generally distributed in Japan, Korea, and China [6]. The root of *Achyranthes japonica* Nakai (AJN) contains different bioactive components like saponins, triterpenoids, phytoecdysteroids, 20-hydroxyecdysone, and inokosterone [7]. These medicinal plants have phytochemical properties such as flavonoids, tannins, and phenolics that can improve nutrient metabolism as well as the gut environment [8]. The addition of *Achyranthes japonica* extract (AJE) supplementation increased the growth performance of broilers [6]. The incorporation of 0.5% AJE has the ability to protect the gut against potentially harmful bacteria [9]. Flavonoid is a common bioactive compound found in many medicinal plants like *Achyranthes japonica* [10]. Total flavonoid contents in AJN extract were measured to be 26.27±3.95 quercetin equivalents μg/mg [11]. According to the epidemiological research, it has been shown that flavonoids may be essential health-promoting components in plant-based foods [12]. Quercetin is a carbohydrate-free flavonoid that is present in a variety of plant-based foods. The biological activities of flavonoids can be measured with the help of quercetin [13]. To understand the effect of a feed additive in animals, the absorption capacity or bioavailability should be evaluated in animal bodies. However, there is still need for improvement in our understanding of quercetin bioavailability in pigs.

Research about AJE supplementation to the growing pig diet as a phytogenic feed additive is still inadequate. We assumed that the addition of AJE to the diet could positively increase growth performance, nutrient digestibility, fecal microbial, and reduce fecal gas emissions in growing pigs. Thus, the focus of this investigation was to find out the impact of AJE on the growth performance, nutrient digestibility, fecal microbial count, and gas emission of growing pigs and to check the rate of absorption of quercetin in the blood of pig.

MATERIALS AND METHODS

The experiment was inspected by the Animal Care and Use Committee at Dankook University, and the relevant experimental procedure was accepted (Ethics Approval Number: DK-1-2111).

Experiment 1

Preparation of Achyranthes japonica extracts

In this feeding trial we used commercial AJE extract (Synergen, Bucheon, Korea). Plant roots were washed and milled (IKAM20, IKA, Staufen, Germany). After extraction, residues were extracted at 80℃ for 2 hours with 1:5 distilled water. The extract was then filtered and recovered using column and ethanol. After getting the samples cooled down (25℃) and filtering them with a Whatman No. 2 filter (Whatman, Kent, UK) then were vacuum-dried at temperatures lower than 40℃, and then dried in a freeze-dryer. AJE comprises flavonoids (1.15 $mg.g^{-1}$) and polyphenols (4.26 $mg.g^{-1}$) as well as saponin (0.47 $mg.g^{-1}$).

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Animals and facilities

A total of 105 crossbred ([Landrace × Yorkshire)]× Duroc) growing pigs (average body weight: 24.47±2.46 kg) were allocated to three treatments. All the animals were reared in a thermostatically regulated shed to maintain a temperature of 25℃ and had a slatted plastic floor, self-feeder, and nipple drinker. Pigs were given three treatment diets: CON (basal diet), basal diet with 0.025% AJE, and basal diet with 0.05% AJE. Each treatment has seven 5-pig pens (three gilts and two barrows). Basal diet was calculated to fulfill NRC [14] nutritional requirements (Table 1). Feed and water were provided on an *ad libitum* basis for the duration of the trial.

Sampling measurements

To calculate the growth performance, at the beginning of the feeding trial, in week 3, and in week 6, body weight was measured. During the experiment, average daily gain (ADG), average daily feed intake (ADFI), and gain to feed ratio (G:F) were all calculated, and the feed intake in each pen was observed.

In the 3rd and 6th weeks of the feeding trial, 0.5% of chromium oxide was mixed in the pig's diet. On the last day of the week, two pigs from each pen were randomly selected to collect fecal by

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

Item	
Ingredients (%)	
Corn	60.01
Soybean meal	16.07
Distillers dried grains with soluble	6.50
Rapeseed meal	2.50
Wheat	6.00
Tallow	3.00
Molasses	3.00
Dicalcium phosphate	1.08
Limestone	0.65
Salt	0.30
Lysine (98%)	0.19
Mineral premix[1)]	0.10
Vitamin premix[2)]	0.20
Choline (50%)	0.04
Calculated composition	
Crude protein (%)	15.50
Crude fat (%)	5.78
Lysine (%)	0.91
Calcium (%)	0.65
Phosphorus (%)	0.55
Digestible energy (kcal/kg)	3,428
Crude fiber (%)	3.43
Crude ash (%)	4.59

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

[2)]Provided per kg of complete diet: 1,103 IU vitamin D_3; 11,025 IU vitamin A; 44 IU vitamin E; 8.3 mg riboflavin; 50 mg niacin; 4.4 mg vitamin K; 4 mg thiamine; 29 mg D-pantothenic acid; 166 mg choline; 33 μg vitamin B_{12}.

massaging the rectum, brought to the lab, and frozen at −20℃. Before analysis, freeze-dried feed and fecal samples were dried at 105℃ for 48-h and ground and then sieved with a screen sieve (1 mm). Following AOAC [15] guidelines, the nutrient digestibility of dry matter (DM), N, and digestible energy (DE) was measured. Spectrophotometry (UV-1201, Shimadzu, Kyoto, Japan) based on UV absorption was used in order to determine chromium contents. Apparent total tract digestibility (ATTD) was measured with following equation, ATTD (%) = {1 − [(Nf × Cd) / (Nd × Cf)]} × 100

here, nutrient concentrations in fecal (Nf), dietary nutrient concentrations (Nd), dietary chromium concentrations (Cd), and fecal chromium concentrations (Cf) are all expressed in terms of percent dry matter.

At the end of weeks 3 and 6, two pigs' fecal were taken for microbiological analysis. After collection, the fecal were frozen and transferred to the lab. The fecal samples were then pooled on a per-pen basis. After diluting one gram of fecal sample with nine milliliters of peptone broth at a concentration of 10 grams per liter, the results ranged from 103 to 107 (1% chroma, Becton, Dickinson & Co., Franklin Lakes, NJ, USA). Culture media were used to culture certain microorganisms. *Lactobacillus* was incubated at 30℃ for 48 hours in De Man, Rogosa, and Sharpe medium (CM0361B; Thermo Fisher Scientific, Waltham, MA, USA), while *E. coli* was cultured at 37℃ for 24 hours in Violet Red Bile Glucose Agar (Thermo Fisher Scientific). Calculated CFU/g per gram of fecal were written as $\log_{10}$-transformed. The bacteria were identified based on the growth media instructions, colony structure, and color.

Three hundred g of fresh fecal from pigs' rectums were pooled and put in a 2.6-l airtight plastic crisper. They were then fermented for 24 hours at 25℃ to determine the effect of dietary AJE on fecal toxic gas emission between weeks 3 and 6. Before measuring, the crisper was lightly shaken to break up any scabs that had formed on the surface and make sure that all of the samples were the same size. After that, a gas sampling pump was used to collect 100 cc of higher air from the crisper. Gastec tubes were used to analyze H_2S, NH_3, and methyl mercaptan (No. 3La for NH_3, No. 4LK for H_2S, and No. 70 for mercaptans; Gastec, Kanagawa, Japan).

Experiment 2

After experiment 1, nine pigs were grouped into three treatment groups, with three pigs in each treatment. Treatment diets were, TRT1, basal diet + powder quercetin 30 g; TRT2**,** basal diet + powder quercetin 150 g; TRT3**,** basal diet + powder quercetin 300 g. In order to feed quercetin properly, 2,500 g of quercetin-containing feed was fed after a one-day fast. In this investigation, we used quercetin that was purchased from Synergen (Synergen, Bucheon, Korea), which had a purity level of 97%. According to the company this quercetin was extracted from the flower of *Sophora japonica*.

Blood sample was collected using a 5 mL K_3EDTA vacuum tube (Becton Dickinson Vacutainer Systems, Franklin Lakes, NJ, USA). A sterile needle was inserted into the jugular vein to draw blood, at 1 h, 2 h, 4 h, 8 h, 12 h, and at the end of the experiment (24 hours). After blood collection, 6 mL of methanol was added and then centrifuged for 10 minutes (4℃, 10,000×g).

Quercetin hydrate, Naringenin (internal standard, IS) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile, methanol, water (Tedia, Fairfield, CT, USA), ethyl acetate (J. T. Baker, Phillipsburg, NJ, USA), and formic acid (Sigma-Aldrich) were employed as HPLC or reagent grade solutions. In methanol, the stock solution of quercetin was produced at a concentration of 1 mg/mL. The stock solution was then diluted in methanol to a concentration of 10 g/mL. Around 10 μg/mL of quercetin solution was spiked into solutions of 10, 20, 50, 100, 200, 500, and 1,000 ng/mL to create working solutions. In acetonitrile, a stock solution of naringenin

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containing 1 mg/mL was produced. By diluting the stock solution with acetonitrile, the IS solution was diluted to a concentration of 20 ng/mL. During the analysis, both the stock solutions and the working solutions were stored at a temperature of −20℃.

Minor adjustments were made to original method of Wiczkowski et al. [16] for preparing blood samples. Following the addition of an aliquot of IS solution containing 20 ng/mL naringenin in acetonitrile to a plasma sample volume of 200 μL, 1 mL of ethyl acetate was then added to the mixture. The mixture was violently vortexed for five minutes and then centrifuged at 16,000×g for five minutes. After transferring 900 μL of the supernatant to a clean tube, it was evaporated with a Speed Vac at 100 mbar and 50℃ for 25 minutes (Christ RVC 2-25 CDplus, Martin Christ, Germany). The residue was diluted with 100 μL of mobile phase, and a 15 L aliquot of the resulting solution was injected directly into the LC-MS/MS apparatus. The LC-MS/MS system comprises of an Agilent 6470 triple quadrupole MS coupled with Agilent Infinity 1260 Infinite II HPLC (Agilent Technologies, Wilmington, DE, USA). Quercetin was separated chromatographically using a Synergi polar RP column (150 mm × 2.0 mm, 4 m; Phenomenex, Torrance, CA, USA). The mobile phase consisted of 0.1% formic acid-containing water and methanol (20:80, v/v). The column temperature was 30℃ with a 0.2 mL/min flow rate. Each injection ran for a total of 3.8 minutes. Quercetin was detected and quantified using an electrospray ionization (ESI) source in negative ion mode with MRM transitions at m/z 301.1→151.0

Statistical analysis

In Experiment 1 and Experiment 2 all the collected data were subjected to analysis of variance in a completely randomized block design (CRD) using SAS (SAS Institute, Cary, NC, USA). Duncan's multiple comparison tests were done to find out if the means were very different. When $p < 0.05$, the results are considered significant, and when $p < 0.10$, they are called a trend.

RESULT

Experiment 1

Growth performance in the feeding trial is shown in Table 2. ADG, ADFI, and G:F were not affected ($p > 0.05$) significantly through the addition of AJE in pig diet in week 0–3, week 3–6 and overall experimental period. However, both AJE supplemented groups showed slightly, but not significantly higher ADG and ADFI compared to the control diet in the overall experiment. ATTD of nutrient is shown in Table 3. Significant effects were not found ($p > 0.05$) in the ATTD of DM, N, and DE in all of the feeding trials when up to 0.05% of AJE was added to the diet of growing pigs. Numerical slightly higher (not significantly) ATTD of DM, N, and DE was found in AJE supplemented diet groups compared to the control group in the overall experiment, but this change was not constant throughout the experimental period. At the third and sixth weeks, the number of fecal microbial counts (*Lactobacilus* and *E. coli*) was not changed by the treatment diets (Table 4). *E. coli* count decreased numerically, but not significantly with the supplementation of AJE. But This change was not constant as the count increased in 0.05% AJE. Moreover, *Lactobacilus* count increased slightly ($p > 0.05$), not significantly in AJE supplemented diet group compared to the control group. Fecal gas emissions are shown in Table 5. The addition of AJE up to 0.05% to the food of growing pigs had no significant ($p > 0.05$) effect on the levels of fecal noxious gases (NH_3, H_2S, acetic acid, and CO_2). However slightly lower, but not significantly ($p > 0.05$) fecal gas emissions were found through the supplementation of AJE in pig diet. Even 0.05% AJE group showed lowest ($p > 0.05$) emission of gasses compared to the control and 0.025% AJE group.

Table 2. Effect of AJE supplementation on growth performance in growing pigs (Exp 1)

Items	CON	0.025% AJE	0.05% AJE	SEM	*p*-value
Week 0–3					
ADG (g)	611	625	620	12	0.685
ADFI (g)	1,391	1,387	1,388	5	0.808
G:F	0.442	0.451	0.447	0.009	0.776
Week 3–6					
ADG (g)	761	770	764	15	0.794
ADFI (g)	1,973	1,998	1,970	24	0.753
G:F	0.389	0.386	0.388	0.008	0.962
Overall					
ADG (g)	681	697	692	10	0.503
ADFI (g)	1,674	1,692	1,679	12	0.711
G:F	0.404	0.412	0.413	0.007	0.967

AJE, *Achyranthes japonica* extracts; CON, basal diet; ADG, average daily gain; ADFI, average daily feed intake; G:F, gain to feed ratio.

Table 3. Effect of AJE supplementation on nutrient digestibility in growing pigs (Exp 1)

Item (%)	CON	0.025% AJE	0.05% AJE	SEM[2]	*p*-value
Week 3					
Dry matter	83.98	85.13	84.93	0.97	0.764
Nitrogen	82.05	82.78	82.60	1.14	0.908
Digestible energy	83.21	84.45	83.68	1.04	0.785
Week 6					
Dry matter	84.06	84.14	84.57	0.52	0.586
Nitrogen	78.21	78.40	78.40	0.80	0.983
Digestible energy	84.63	85.00	84.74	0.55	0.878

AJE, *Achyranthes japonica* extracts; CON, basal diet.

Table 4. Effect of AJE supplementation on fecal microbial count in growing pigs (Exp 1)

Items (Log_{10}CFU/g)	CON	0.025% AJE	0.05% AJE	SEM	*p*-value
Week 3					
Lactobacillus	7.68	7.74	7.73	0.05	0.948
Escherichia coli	4.32	4.30	4.33	0.03	0.325
Week 6					
Lactobacillus	7.87	7.96	7.92	0.04	0.739
Escherichia coli	4.39	4.38	4.40	0.04	0.662

AJE, *Achyranthes japonica* extracts; CON, basal diet.

Experiment 2

Effects of dietary quercetin supplementation on absorption rate in blood are shown in Table 6. At the 4th hour after feeding quercetin, the quercetin absorption in the blood was higher in the 300 g-quercetin group, as compared to the 30 g and 150 g supplemented groups. However, after 12 hours of feeding quercetin, only quercetin was found in the blood of TRT3 group. And 24 hours after quercetin was given, there was no sign of quercetin absorption in any of the treatment groups at all.

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Table 5. Effect of AJE supplementation on fecal gas emission in growing pigs (Exp 1)

Items (ppm)	CON	0.025% AJE	0.05% AJE	SEM	*p*-value
Week 3					
Ammonia (NH_3)	5.8	5.5	5.3	0.83	0.874
Hydrogen sulfide (H_2S)	0.55	0.53	0.50	0.10	0.945
Acetic acid	4.3	3.8	3.5	0.76	0.874
Carbon dioxide (CO_2)	3,425	3,200	3,175	242	0.607
Week 6					
Ammonia (NH_3)	4.8	4.3	4.0	0.45	0.420
Hydrogen sulfide (H_2S)	0.53	0.50	0.45	0.10	0.841
Acetic acid	5.5	5.3	4.5	0.96	0.639
Carbon dioxide (CO_2)	3,350	3,300	3,200	232	0.812

AJE, *Achyranthes japonica* extracts; CON, basal diet.

Table 6. Effect of dietary quercetin supplementation on absorption rate in pig (Exp 2)

Items (ng/mL)	TRT1[1)]	TRT2	TRT3	SEM	*p*-value
0 H	0.00	0.00	0.00	0.00	-
1 H	0.68	2.50	3.27	0.63	0.241
2 H	1.47	2.33	4.03	0.55	0.148
4 H	3.14[b]	10.62[a]	12.05[a]	1.58	0.013
8 H	1.61	1.18	6.74	1.39	0.204
12 H	0.00	0.00	0.83	0.18	0.080
24 H	0.00	0.00	0.00	0.00	-

[1)]TRT1, basal diet + 30 g quercetin; TRT2, basal diet + 150 g quercetin; TRT3, basal diet + 300 g quercetin.

[a,b]Values with different subscript in the same row are significant different ($p < 0.05$).

DISCUSSION

The restriction on antibiotic use in livestock farming induced the research on medicinal herbs as a potential alternative in recent years. Due to the presence of antioxidant phytochemicals and bioactive compounds [6], AJE has been tested in several livestock diets to understand the capabilities of AJE. Dang et al. [17] observed that the addition of AJE up to 0.2% had a linear effect on ADG but had no effect on body weight or the G:F ratio of finishing pigs' growth performance. Additionally, Liu et al. [8] found that growing pigs diets supplemented with AJE caused a higher ADG and gain-to-feed ratio than the diets without supplementation. AJE supplementation up to 0.1% enhanced the ADG and G:F in growing pigs [18]. But the exact reasons how AJE is linked with improved growth performance in growing pigs is still unknown. AJE could improve the digestion of nutrients, therefore enhancing their growth performance in pigs [18]. But in this study, ADFI, ADG, and G:F were unaffected. Similar results were seen in the previous study [19] where 0.05% AJE was added to the diets of finishing pigs; and growth performance remained unaffected. Hanczakowska et al. [20] observed that the growth performance parameters of pigs fed a diet containing herbal extract mixture were not affected. The phytochemistry field is extensive, so the inconsistent growth performance responses to various plant extracts can therefore be related to differences in plant species, biochemical characteristics, extraction method, and dosage [21,22]. The low dose of AJE in this study may explain why it has no influence on the ADG and G:F in growing pigs in this study.

Pigs with AJE supplementation up to 0.05% had no effect on the ATTD of DM, N, or energy.

This finding was consistent with earlier work by Mohankumar et al. [19] where 0.05% AJE was supplied to a finishing pigs diets. Previously, Oanh et al. [23] found that the ATTD of nutrients was the same in pigs that were fed medicinal plant diets or rather, the control diet. On the other hand, Liu et al. [8] showed that adding AJE to a growing pigs diets improved the ATTD of DM. Sun et al. [24] demonstrated that the addition of AJE to broiler chicken diets increased their ATTD of nitrogen and DM effectively. These positive results might be because of the active ingredients present in herbal extracts that assist with digestion and nutrient metabolism, which makes the pigs grow faster [25]. In addition, earlier research demonstrated that phytogenic feed additions increase villus length and reduce crypt depth, indicating enhanced nutritional absorption [26,27]. However, the low dose of AJE could be the reason why ATTD was not affected compared to the control diet in this study.

In the present study growing pigs fed with AJE supplementation had no effect on the microbial count. On the other hand, Liu et al. [8] observed that the incorporation of 0.10 percent AJE reduced the bacterial count in growing pigs, while Park & Kim [6] found that the addition of 0.25 percent AJE reduced the *E. coli* count in broilers. This implies that herbs have the capability to limit the development of harmful germs in the digestive tract. Controversially, Mohankumar et al. [19] did not find a difference between the *Lactobacillus* count and the *E. coli* count in the diets of finishing pigs supplemented with AJE. Oanh et al. [23] found that medicinal diets did not reduce the number of pathogenic bacteria in pig fecal compared to the control diet. Arabski et al. [28] found that herbal plants did not inhibit *E. coli* from growing, and in fact, they helped bacteria grow in the guts of animals. Weaning pigs supplemented with herbal extract combination had no changes in intestinal microbiota or diarrhea compared to those fed the control diet [29]. The current results show that the addition of AJE up to 0.05% did not change the bacterial count in the guts of growing pigs.

In the pig industry, the major air pollutants are NH_3, H_2S, and total mercaptan. Ferket et al. [30] found that the emission of noxious gases from animal fecal is related to intestinal microbiota, especially harmful *Escherichia coli* populations [30,31]. Yan et al. [32] found that pigs' higher food digestibility could lower fecal noxious gas. The enhanced digestibility of nutrients might lead to a reduced substrate for microbial fermentation, reducing noxious gas emissions [33]. Liu et al. [18] reported that AJE lowered the fecal *E. coli* counts and hydrogen sulfide emissions with enhanced DM and energy digestibility. The decreasing fecal H_2S gas level is related to improved digestibility and lower coliform count. On the other hand, here we did not find any effect of AJE on fecal noxious gas emissions. Mohankumar et al. [19] showed no influence on fecal gas emission when supplementing 0.05% AJE to the finishing pigs diets. Moreover, medicinal plant extracts in weaning pigs diets failed to affect both the nutrient digestibility as well as fecal gas emissions [34]. The lower dose of AJE used in the study may be responsible for this result. Another possible cause is the similar microbial count in this study because the fecal noxious gas is related to the microbial fermentation in the lower intestine. Further study is needed to understand the specific processes between AJE and fecal gas emission. From this feeding trial we can understand that 0.05% AJE supplemented diets don't have the capability to improve the growth performance of the growing pigs. AJE cannot directly improve the growth performance of pigs. Because of the antimicrobial activity it helps in inhibiting the growth of harmful bacteria and ultimately helps the proliferation of beneficial lactic acid bacteria [9]. And through this the improved gut microflora helps in nutrient utilization and ultimately improves the growth performance of the pigs [24]. In this study, the lower dose of AJE failed to change the bacterial count in the gut and ultimately the growth performance was not improved. However, the previous experiment showed positive results in growth performance when 0.2% [17], 0.1% [6,24,33] of different types of AJE were used in

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animal feeding trial. As the results in this study are insignificant, we assume that higher dose of AJE in pig diets should be supplied for positive result in growth performance. In the previous study, 0.05% AJE failed to improve growth performance in finishing pigs [19], so it is understandable that at least 0.1% AJE must be supplied in growing pigs for improved growth performance parameters. However further study should be done to check the optimum dose and absorption mechanism of AJE for better understanding.

Quercetin is the primary flavonoid compound [35] in medicinal plants. Flavonoids from diets must be distributed throughout the body to impact on the body. However, we have a very limited understanding of the bioavailability of flavonoids. A drug's bioavailability can be described as the degree to which its active agent is released from its formulation, absorbed, and eventually present at the location where it is utilized [11]. This is measured in terms of both the amount of the release and the velocity with which it occurs. Therefore, the current research was conducted to obtain information on the absorption of quercetin which is one of the naturally most abundant and physiologically very efficient flavonoids. In our study, the absorption rate increased with increasing amounts of quercetin. Additionally, at the 4th hour of the study, the rate of absorption was highest. Guo and Bruno [36] noted that the site and mechanism of quercetin absorption depend on its chemical structure. In vitro experiments found that the concept of a glucose component used a transporter that usually pumps glucose through the intestinal membranes [37]. We are unable to make a direct comparison between our results and those of other research because of the limited number of studies on pigs. The findings from our study are preliminary and should be confirmed by further study.

CONCLUSION

The incorporation of AJE into a diets had no effect on the growth performance, nutrition digestibility, fecal microbial count, or fecal gas emission of growing pigs. However, none of the treatment diets showed negative effects. Furthermore, more research is needed to determine the optimal amount of AJE supplementation with different nutrient concentration diets in growing pigs.

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