Research Article

Effect of *Moringa oleifera* Leaf Extract on Growth Performance, Blood Indices, Diarrheal Rate, and Fecal Microbial Shedding in Weaned Pigs

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Abstract | *Moringa oleifera* leaf (MOL) has been used in traditional medicine for its multiple bioactive components beneficial to animals and humans. However, the effects of *Moringa oleifera* leaf extract (MOLE) supplementation on growth performance and intestinal morphology in weaned pigs are poorly understood. This study examined the effects of MOLE supplementation (250 mg/kg or 500 mg/kg diet) on 144 Duroc × Landrace × Large White weaned pigs over a 5-week period. The results of this study showed that dietary supplementation with MOLE for 5 weeks improved average daily weight gain, feed efficiency and reduced the incidence of diarrhea in weaned pigs. Blood hematological parameters, intestinal morphology, and fecal microbial shedding were assessed at the end of the experiment. MOLE supplementation did not significantly affect blood indices, except for a reduction in neutrophils and an increase in MCHC at 500 mg/kg diet (P=0.025 and 0.042, respectively). Villus height/crypt depth in the 250 mg/kg diet MOLE group was significantly higher than in the control group (P=0.002). Our study is the first to report that dietary supplementation with MOLE granules at 250 mg/kg improved growth performance, reduced diarrhea rate, and microbial shedding in weaned pigs.

Keywords | *Moringa oleifera* leaf extract; growth performance; blood indices; diarrheal rate; fecal microbial shedding; weaned pigs

Received | September 23, 2023; **Accepted** | October 20, 2023; **Published** | December 25, 2023
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Citation | Ketpanyapong W, Marupanthorn K (2023). Effect of *Moringa oleifera* leaf extract on growth performance, blood indices, diarrheal rate, and fecal microbial shedding in weaned pigs. J. Anim. Health Prod. 11(4): 410-419.
DOI | http://dx.doi.org/10.17582/journal.jahp/2023/11.4.410.419
ISSN | 2308-2801

INTRODUCTION

Weaning is a critical stage in piglet development, marking the transition from a milk-only diet to solid feed. This essential transition is accompanied by weaning stress, a complex physiological and psychological adaptation associated with separation from the sow, moving to a new environment, and dietary changes. Weaning stress can cause diarrhea, respiratory problems, and reduced weight gain in weaned pigs (Campbell et al., 2013). Antibiotic additives have been used in weaned pig diets to help reduce weaning stress (Barton, 2000). However, the overuse of antibiotics has led to antibiotic residues in animal products and the transmission of antibiotic-resistant bacteria to humans, posing a significant health hazard. The ban on the supplementation of antibiotics in animal feed has created an urgent need for safe alternatives that can better ensure the safety of animal products and human health. *Moringa oleifera* (MO) is a promising plant-based alternative for use as a key ingredient in pig feed due to its exceptionally high nutritional value (Sánchez-Machado et al., 2010). *Moringa oleifera* leaf meal (MOLM) is a highly nutritious protein

source, with a crude protein content exceeding 27.1% of leaf powder. It is also rich in vitamins, minerals, and essential phytochemicals, which have anticancer, antidiabetic, antioxidant, anti-inflammatory, and antimicrobial properties (Gopalakrishnan et al., 2016). Additionally, MO has been shown to have immune-modulating properties and can be a potential natural treatment for microbial infections (Gupta et al., 2010; Paikra et al., 2017). Moringa has been used to prevent malnutrition in developing tropical countries, and it is used in traditional medicine to treat inflammation, ulcers, and diarrhea. Moringa contains bioactive components similar to those found in medicinal herbs with antimicrobial properties. The most notable bioactive molecules identified in moringa are phenolic compounds, flavonoids, alkaloids, and tannins (Chelliah et al., 2017; Choudhary et al., 2013). Additionally, moringa contains isothiocyanates and glucosinolates, which inhibit bacterial conjugation and reduce bacterial pathogenicity. Isoquercitrin, kaempferol, niaziminin, and rhamnetin have direct beneficial effects against microbial eradication (Costa and Iraola, 2019; Guevara et al., 1999). Given moringa's notable nutritional value, robust biomass production, and potential health benefits, a previous study explored its utilization to enhance animal production in cows, goats, and sheep. MOL improve the intestinal health of broiler chickens by promoting a balanced microflora. The inclusion of MOLM in poultry diets does not induce adverse effects (Onunkwo, 2015). Cao et al. (2022) reported that aqueous MOLE curtails oxidative stress and the inflammatory cytokines expression triggered by Porcine Epidemic Diarrhea infection in Vero E6 cells. It also elevates the anti-apoptotic protein expression, resulting in reduced cell apoptosis.

Motivated by the high nutrient and bioactive compound content of MOLE, this study determined the impact of MOLE supplementation on growth potential, blood indices, intestinal morphology, and diarrheal rate of weaned pigs. We aimed to provide evidence for the use of MOLE in the swine industry to reduce antibiotic usage and promote sustainable agriculture.

MATERIALS AND METHODS

Moringa oleifera leaf extract

Fresh MOL were collected from Ayutthaya Province, Thailand. The leaves were cleaned promptly, minced, and dried at 40°C. The dried leaves were rendered into a powder and extracted using 95% ethanol using cold succinate extraction. The extract was weighed and then stored at subzero temperatures in a refrigerator. Wet granulation of the MOL extract was performed with pharmaceutical excipients, maltodextrin and microcrystalline cellulose PH101 (PC drug, Bangkok, Thailand), using the Moisture-Activated Dry Granulation technique (Shanmugam, 2015).

Analysis of phenolic compounds composition using high-performance liquid chromatography (HPLC)

High-performance liquid chromatography (HPLC) analysis was conducted with a Shimadzu model 20AT HPLC system (Shimadzu Corporation, Kyoto, Japan) operating in isocratic mode with a 20 µL injection loop. Signal detection was achieved using a UV-Vis detector model SPD-20A (Shimadzu Corporation, Kyoto, Japan). A methanol:water (70:30) mobile phase was used at a flow rate of 0.7 mL/min. A 20 µL sample was injected, and the analysis time was 15 minutes. The column used was an HPLC Column Hypersil GOLD (Thermo Scientific™, Massachusetts, USA) with dimensions of 4.6 x 250 mm and a particle size of 5 µm. The standards used for preparation were cryptochlorogenic acid (4-O-caffeoylquinic acid), isoquercetin (quercetin 3-β-D-glucoside), and kaempferol (kaempferol 3-β-D-glucopyranoside) (Sigma Aldrich, MO, USA). Sample preparation involved weighing 0.0020 g of the sample, dissolving it in 1 mL of ethanol, and then filtering the sample using a 0.22 µm syringe filter.

Analysis of volatile compounds using Gas chromatography-mass spectrometry (GC-MS)

The ethanol extract from MOL was analyzed using a GC-MS system consisting of a GC7890B gas chromatograph and an MSD 5977A mass detector (Agilent Technologies, Inc., California, USA). A helium carrier gas was used with a flow rate of 1 mL/min. A microliter of supernatant from the sample was injected into the GC. The GC oven temperature program was as follows: 80°C to 200°C at 15°C/min, followed by 200°C to 280°C at 5°C/min., with a final hold of 5 minutes at 280°C. The ion source temperature was set to 230°C and ionization voltage was 70 eV (Ibrahim et al., 2021).

Animals, general management, and experimental design

This study was conducted at the commercial swine farm in central part of Thailand. A total of 144 Duroc × Landrace × Large White piglets at the end of the fourth week with a mean body weight of 8.26 ± 0.536 kg were block-randomized by body weight and randomly assigned to one of three dietary treatments. Each dietary treatment group had six replicates, with eight piglets per replicate. The weaned pigs were fed diets containing different levels of MOL extract for 5 weeks. The control basal diet was fed to the first group, while the other two groups were fed basal diets supplemented with MOLE at 250 mg/kg diet and 500 mg/kg diet, respectively. The nutrient composition of the diets was calculated to meet the recommendations of the National Research Council (NRC, 1998), as shown in Table 1. The

Table 1: Feed ingredients and nutrient composition of the basal diet (% as fed basis).

Ingredient	% as fed basis
Broken rice	53.52
Full-fat soybean	20.00
Soybean meal (46% CP)	13.00
Fish meal (62% CP)	5.00
Skim milk	5.00
Dicalcium phosphate	1.30
Monocalcium phosphate	1.15
Sodium chloride	0.33
L-lysine monochloride (98%)	0.09
DL-methionine	0.31
L-threonine	0.05
Vitamin-mineral premix	0.25
Total	100
Calculated composition	
Crude protein (%)	22.30
Metabolizable energy, kcal/kg	3,362.81
Lysine (%)	1.40
Methionine (%)	0.73
Threonine (%)	0.92
Tryptophan (%)	0.30
Calcium (%)	0.91
Total phosphorus (%)	0.97
Analyzed composition	
Crude protein (%)	19.84
Ether extract (%)	4.86
Ash (%)	5.22

[1]Supplied (per kilogram diet): Vitamin A as retinol, 8,400 IU; Vitamin D3, 945 IU; Vitamin E, 0.0126 g; Vitamin K, 0.0021 g; Vitamin B1 (thiamine), 0.0011 g; Vitamin B2 (riboflavin), 0.0022 g; Vitamin B6 (pyridoxine), 0.0016 g; Vitamin B12 (cyanocobalamin), 0.02 mg; nicotinic acid, 0.0126 g; pantothenic acid, 0.063 g; folic acid, 0.0053 g; biotin, 0.0315 mg; choline, 0.175g; copper as $CuSO_4$, 0.126 g; iron as $FeSO_4$, 0.105 g; manganese, 0.021 g; cobalt, 0.0007 g; iodine, 0.0007 g; selenium as Na_2SeO_3, 0.00007 g

piglets' diets were administered thrice daily at 6:00 AM, 12:00 PM, and 5:00 PM. Throughout the experimental period, all piglets had *ad libitum* access to feed and water from a self-feeder and nipple drinker. During the experiment period, the piglets were housed in pens measuring 1.20 m × 2.40 m, with a stocking density of 0.36 m^2 per pig. The pens with half-slatted concrete floors were in a conventional open-housed environment. The experimental facility temperature was 20-23°C from 7:00 PM to 6:00 AM, and 29-33°C from 7:00 AM to 6:00 PM. To maintain optimal conditions for the piglets, each pen was furnished with a heating lamp and a hemp sack over the two-week post-weaning period. This arrangement ensured that the piglets remained within the required temperature range. Body weight (BW), average daily gain (ADG), and feed conversion ratio (FCR) were recorded to assess growth performance. All animal procedures used in this study were reviewed and approved by the Institutional Animal Care and Use Committee (IACUC), following the regulations of Rajamangala University of Technology Suvarnaphumi, as outlined in animal use protocol No. IAU-RUS63-006.

Sampling and measurements

At the termination of the experiment, two pigs were randomly sampled from each pen and jugular vein catheterized and blood samples were collected using sterile needle into a 3 mL ethylenediamine tetra-acetic acid (EDTA) tube for subsequent analysis. To analyze blood hematological parameters, including red blood cells (RBCs), hemoglobin, hematocrit, platelet, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), hematocrit (HCT), and white blood cells (WBCs), samples were assessed using a hematology analyzer (Cell Dyn 3700, Wiesbaden, Hesse, Germany).

For microbiological analysis, three individual pigs were randomly sampled, and fecal samples were collected from them by massaging the rectum. The pooled fecal samples were placed on ice for transportation to the laboratory and analyzed immediately upon arrival. Facal samples (1 g) from each pen were pooled and mixed with 9 mL of 1% peptone broth (Himedia Laboratory Pvt. Ltd., Maharashtra, India) and homogenized. Serial 10-fold dilutions of fecal samples in 1% peptone solution were plated onto MacConkey agar plates (Himedia Laboratory Pvt. Ltd., Mumbai, India) to determine viable bacterial counts and lactobacilli MRS agar plates (Himedia Laboratory Pvt. Ltd., Mumbai, India) for the isolation of *Escherichia coli* and Lactobacillus, respectively. Lactobacilli MRS agar plates were incubated anaerobically at 39°C for 48 hours. Simultaneously, the MacConkey agar plates were incubated aerobically at 37°C for 24 hours. Enumeration of *Escherichia coli* and Lactobacillus colonies was performed immediately after removal of the samples from the incubator.

Diarrheal score

Diarrheic piglets per pen were monitored daily throughout the five-week experiment at 07:00 according to the following criteria that fecal consistency was scored on a scale of 1 to 4, with 1 representing solid, well-formed feces and 4 representing watery diarrhea (Pedersen and Toft, 2011). Pigs with a fecal score of 3 or higher were classified as having diarrhea, while those with a fecal score of less than 3 were considered normal. Diarrhea symptoms and mortality, if any, were recorded daily for each pig throughout the trial. The diarrheal rate was calculated using the method described by Hu et al. (2013).

Measurement of intestinal morphology

On the last day of the experiment, two pigs per pen were randomly sampled. Intestinal histology was performed using the previous methodology (Zong et al., 2018). In brief, the jejunum of intestinal tissue was gradually dehydrated using varying concentrations of ethanol and then embedded in paraffin wax. Each section of embedded tissue was cut into three slices (5 µm thick) and placed onto microslides. Paraffin-embedded tissue sections were stained with hematoxylin and eosin (H&E). Villus height and crypt depth were measured at 10x magnification using an optical microscope (Leica Imaging Systems Ltd.). Villus height and crypt depth for each piglet were determined by averaging measurements taken from a minimum of 30 intact villi and their associated crypts.

Statistical analysis

A completely randomized design (CRD) was used in this study. Statistical analysis was performed using analysis of variance (ANOVA) at a significance level of 95%, following the guidelines of SAS Institute Inc. (1993). Tukey's multiple comparison test was used to compare the mean values of the treatment groups. The results are presented as least squares mean values for each treatment group, along with their corresponding standard error of the mean (SEM) values and P-values.

RESULTS

Moringa oleifera leaf extract

The percent yield for the MOLE was 26.05%. Analysis of the phenolic compound profile using chromatographic and spectroscopic techniques revealed that isoquercetin, astragalin, and cryptochlorogenic acid, the major active antioxidant constituents in MOL, were present at 1.126%, 0.921%, and 0.829% (w/w) of the MOL extract, respectively. Hydroxyprogesterone showed the highest peak area (75.34%). Gas chromatography analysis identified hydroxyprogesterone as the major volatile compound in the sample, with a peak area of 75.34%. Vitamin E, octadecatrienoic acid, and dianhydromannitol were also detected, with peak areas of 59.68%, 38.03%, and 32.30%, respectively.

Growth performance and diarrheal occurrence

Dietary supplementation with MOLE significantly improved BW, ADG, and FCR of weaned pigs ($P< 0.05$) (Table 2, Figure 1). Pigs fed a dietary supplement with 250 mg/kg MOLE exhibited the highest final BW and ADG. Pigs fed dietary supplements containing 250 mg/kg MOLE and 500 mg/kg MOLE had a significantly lower incidence of diarrhea from 0 to 5 weeks of the experiment than the control group ($P=0.028$). Figure 1 shows scatter plots and regression lines for each treatment group. Eight replicate experiments were conducted for each time point. Weight gain, feed intake, and FCR data were collected from 0 to 5 weeks, while diarrheal rate data were collected daily.

Blood characteristics

Dietary supplementation with MOLE did not significantly affect most hematological parameters (Table 3). However, MCHC was significantly higher in MOL extract-supplemented groups than in the control group ($P=0.042$). The percentage of neutrophils was significantly lower in the 500 mg/kg MOLE group ($P=0.025$). MOLE-supplementation treatment did not affect the percentages of monocytes, eosinophils, and basophils.

Fecal microbial shedding

Dietary MOLE supplementation significantly reduced the population of *E.coli* ($P=0.011$). The *E. coli* population was lower in pigs fed MOLE than in pigs fed the control diet. In contrast, MOLE supplementation did not significantly affect lactobacillus shedding in piglet feces versus the control diet ($P=0.672$) (Table 4).

Table 2: Effect of MOLE supplementation on growth performance and diarrhea occurrence in weaned pigs.

Items	T1	T 2	T 3	SEM	P-value
Body weight (kg)					
D 0	8.26	8.27	8.24	0.536	0.994
1 week	9.23	9.28	9.04	0.522	0.638
2 weeks	10.70	10.53	10.26	0.612	0.350
3 weeks	12.36	12.41	12.23	0.508	0.787
4 weeks	14.61[a]	15.20[a]	14.06[b]	0.747	0.004
5 weeks	17.46[b]	18.49[a]	17.09[b]	1.005	0.008
Average daily gain (g)					
0 to 1 week	139.46[a]	143.21[a]	113.04[b]	23.282	0.011
1 to 2 week	210.18	179.29	174.29	34.722	0.075
2 to 3 week	236.07	268.04	281.96	43.675	0.093
3 to 4 week	322.50[b]	399.46[a]	261.25[c]	66.510	<0.001
4 to 5 week	405.89	469.29	432.50	58.021	0.084
0 to 5 weeks	262.82[b]	291.86[a]	252.61[b]	30.812	0.022
Feed conversion ratio (feed/gain)					
0 to 1 week	1.46[b]	1.35[b]	1.76[a]	0.288	0.007
1 to 2 week	1.35	1.48	1.60	0.233	0.09
2 to 3 week	2.23[a]	1.55[b]	1.67[b]	0.436	0.004
3 to 4 week	1.67[a]	1.23[b]	1.76[a]	0.298	<0.001
4 to 5 week	1.37[a]	1.11[b]	1.11[b]	0.190	0.003
0 to 5 weeks	1.57[a]	1.32[c]	1.48[b]	0.177	0.009
Diarrheal rate (%)					
0 to 1 week	8.82	6.70	6.45	6.262	0.061
1 to 2 week	4.53	4.37	4.34	1.768	0.773
2 to 3 week	3.41	3.25	3.12	1.220	0.383
3 to 4 week	2.74	2.55	2.42	0.971	0.217
4 to 5 week	2.58	2.23	2.16	1.307	0.178
0 to 5 weeks	4.54[a]	3.90[c]	3.78[b]	3.689	0.028

T1, basal diet; T2, basal diet + 250 mg/kg MOLE; T3, basal diet + 500 mg/kg MOLE.
[a-c]mean values in the same row with different letters differ significantly at P≤0.05

Table 3: Blood hematology parameters from weaned pigs fed on diets with different levels of MOLE.

Items	T1	T2	T3	SEM	P-value
RBC, 10^6/µL	5.80	5.95	5.47	0.668	0.263
Hb, g/dL	10.48	10.80	10.66	1.109	0.824
HCT, %	36.40	36.69	35.84	3.639	0.873
MCV, fL	63.25	61.80	65.66	4.937	0.211
MCH, pg	18.19	18.19	19.54	1.509	0.056
MCHC, g/dL	28.79[b]	29.41[ab]	29.75[a]	0.905	0.042
Platelet, 10^3/µL	293.20	349.11	310.73	114.403	0.572
WBC, 10^3/µL	18.81	17.96	18.48	5.261	0.944
Neutrophil, %	64.89[a]	52.30[ab]	43.09[b]	18.441	0.025
Lymphocyte, %	22.11[b]	33.10[ab]	43.82[a]	18.774	0.029
Monocyte, %	13.80	12.44	12.45	4.341	0.738
Eosinophil, %	0.80	0.56	0.64	0.922	0.848
Basophil, %	0.00	0.00	0.00	0.000	-

T1, basal diet; T2, basal diet + 250 mg/kg MOLE; T3, basal diet + 500 mg/kg MOLE.
[a-c]mean values in the same row with different letters differ significantly at P≤0.05

Table 4: Effect of MOLE supplementation on fecal microbial shedding in weaned pigs (Log[10]CFU/1g chyme)

Microorganism	T1	T2	T 3	SEM	P-value
Escherichia coli	7.78[a]	7.37[b]	7.18[b]	0.431	0.011
Lactobacillus	8.72	8.58	8.50	0.488	0.672

T1, basal diet; T2, basal diet + 250 mg/kg MOLE; T3, basal diet + 500 mg/kg MOLE.
[a-c]mean values in the same row with different letters differ significantly at P≤0.05

Table 5: Effect of MOLE supplementation on jejunal mucosa epithelium structure

Item	T 1	T 2	T 3	SEM	P-value
Villus height (µm)	349.75	359.62	340.25	75.541	0.375
Crypt depth (µm)	169.59	150.25	164.46	46.366	0.059
Villus height/crypt depth	2.15[b]	2.63[a]	2.21[b]	0.819	0.002

T1, basal diet; T2, basal diet + 250 mg/kg MOLE; T3, basal diet + 500 mg/kg MOLE.
[a-c]mean values in the same row with different letters differ significantly at P≤0.05

Intestinal morphology

Supplementation with MOLE did not significantly affect villus height or crypt depth in the jejunum at post-weaning days (Table 5). Villus height:crypt depth was significantly higher (P < 0.05) in the jejunum of pigs fed 250 mg/kg MOL extract than in pigs fed any other treatment group. Representative images of jejunal morphology are shown in Figure 2.

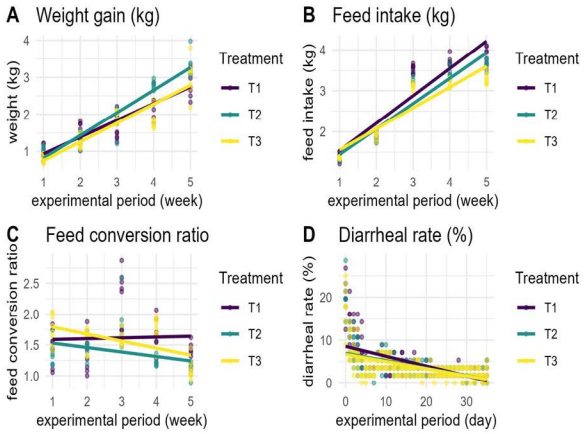

Figure 1: Scatter plot illustrating the regression for effects of MOLE supplementation on weight gain (A), feed intake (B), feed conversion ratio (C), and diarrheal rate (D) in weaned pigs. T1, basal diet; T2, basal diet + 250 mg/kg MOLE; T3, basal diet + 500 mg/kg MOLE.

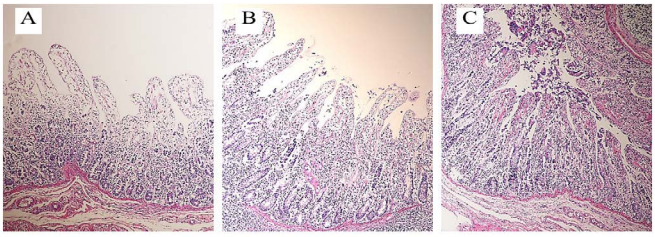

Figure 2: Representative histological images of the jejunum of weaned piglets feed (H&E, 40X). Weaned pigs were fed the basal diet (A), basal diet supplemented with 250 mg/kg MOLE (B), and basal diet supplemented with 500 mg/kg MOLE (C).

DISCUSSION

The medicinal plants and their extracts are commonly used to improve health and treat diseases (Li & Weng, 2017). Herbal feed additives have also garnered growing interest for their potential to enhance the nutritional status and overall health of farm animals (Abdallah et al., 2019). In addition to antibiotics, herbal medicines have also been shown to improve growth performance, nutrient digestibility, and meat quality in pigs, which can lead to cost savings in pig farming and the production of antibiotic-free pork products (Lin et al., 2020). It can be used in a variety of forms, including raw materials, extracts, and isolates. The herbal extracts are a promising approach for improving the efficacy of herbal feed additives because they can provide more consistent results than raw materials, can control the quantity and quality of active ingredients.

In this study, final BW and ADG were increased in pigs supplemented with 250 mg/kg MOLE. Similar results were obtained by Oduro-Owusu et al. (2015) and Serem et al. (2017), who also reported improved ADG in pigs fed MOLM. In contrast, ADG was not significantly affected by feeding finishing pig's diets with different levels of MOLM. (Mukumbo et al., 2014), and 10% MOLM supplementation did not significantly impact on ADG of pigs (Acda et al., 2010). The inconsistent findings in the literature suggest that the impact of MOL supplementation on growth performance may be influenced by a variety of factors, including the type of MOL product used (e.g., extract vs. meal), the dose of MOL supplementation, and the composition of the basal diet. The increased growth rate observed in pigs supplemented with MOLE in this study may be attributed to the high vitamin E content of MOLE, which can reach up to 59.68%. Vitamin E is a cel-

lular antioxidant, contributes to cell structure, and plays a role in reproduction (Mahan, 1991). Moreira and Mahan (2002) found that vitamin E supplementation significantly increased BW between days 7 and 35 in weaning pigs, compared to the control group (P<0.05). However, more research is needed to elucidate the mechanisms by which MOLE supplementation promotes growth in weaned pigs. Furthermore, MOLE supplementation has been shown to improve other aspects of pig health and production, such as feed efficiency. MOLE supplementation significantly reduced FCR, with the lowest value observed in the group receiving 250 mg/kg of MOLE. This finding contrasts with the study conducted by Mukumbo et al. (2014), which found that partial substitution of the weaner diet with 7.5% MOLM resulted in a less efficient FCR. The difference in FCR may be attributed to the use of MOLE in the present study, which may extract specific phytochemical components like isoquercetin, astragalin, and cryptochlorogenic acid. In contrast, MOLM may contain allergic compounds such as glycinin and anti-nutritional factors such as tannins, which can bind to proteins and decrease the bioavailability of nutrients for the animal (Moyo et al., 2011). Overall, the findings of the present study suggest that MOLE supplementation is an effective way to increasing weight gain and feed utilization in weaned pigs. The inclusion rate of 250 mg/kg of MOLE in the diet was the most appropriate level for the weaned pigs under the experimental conditions of this study.

Weaned pigs typically experience diarrhea within 1-2 weeks following weaning, primarily due to feed changes that disrupt their digestive system. Diarrhea results from reduced absorption capacity, characterized by shortened villi length, increased crypt depth, and diminished digestive enzyme activity. Proliferation of enterotoxic bacteria such as *E. coli* in the small intestine can impair nutrient absorption (Pedersen et al., 2015) and reduce fermentation of digestible nutrients in the large intestine (Goh et al., 2023), both of which can contribute to diarrhea in weaned pigs.In this study, we found that MOLE-supplemented diets significantly reduced the incidence of diarrhea compared to the control diet. This finding is consistent with that of Chikasa et al. (2022), who reported that piglets in the 0% MOLM group and 4.5% MOLM diet group had a higher incidence of scours than those fed the 8% MOLM diet. Moreover, this consistent with the findings of Falowo et al. (2016), who reported that MOLE exhibits strong antimicrobial activity against many pathogens, including Staphylococcus, Salmonella, and *E. coli*. The reduction in *E. coli* shedding in the feces of piglets fed MOLE supplemented diets is consistent with this antimicrobial activity. Oliver et al. (2015) observed that MO has regulation of immune function, promoting the early development of the piglet immune and digestive systems. The phytochemicals of MOL demonstrate efficacy against a wide range of pathogenic bacteria or beneficial additives that enhance pig health and performance characteristics (Li et al., 2021). Based on these findings, it is possible that piglets fed MOLE-based diets may have benefited from the antimicrobial and immunomodulatory properties of MOLE, which may have helped to reduce diarrheal incidence and promote early immune development. Our findings indicate that MOLE supplementation may reduce the need for antimicrobial use in the treatment of diarrhea in piglets, which could help to mitigate the development of antimicrobial resistance.

This study demonstrated that MOLE supplementation improved jejunal morphometric parameters in weaned pigs. More specifically, villus height was significantly greater in the 250 mg/kg MOLE supplementation group, which resulted in the highest villus surface area. Previous studies have shown that increased villus height and villus height:crypt depth ratio are correlated with increased epithelial cell proliferation and mitosis (Awad et al., 2009; Ashraf et al., 2013). Phytogenic feed additives have also been documented to modify intestinal villus morphology and affect growth performance (Namkung et al., 2004; Oetting et al., 2006). Therefore, it is possible that the piglet weight gain observed in this study was partly mediated by the taller villi induced by MOLE supplementation. This would increase the absorptive surface area of the intestine, allowing for more nutrients to be absorbed, leading to improved body weight gain.

Our findings suggest that dietary MOLE supplementation improved blood parameters, possibly due to a combination of its regulatory effects on metabolic pathways and its antioxidant properties (Al-Masruri et al., 2022; Al-Mufarji and Mohammed, 2022). Diet supplementation with 250 mg/kg MOLE in weaned pigs significantly increased MCHC (P=0.042). A supplementary diet containing 500 mg/kg MOLE reduced neutrophil percentages, this finding may be explained by the anti-inflammatory effects of isoquercetin, a flavonol glycoside that reduces polymorphonuclear cells such as neutrophils (Izuegbuna et al., 2020). Isoquercetin is a glucoside of quercetin that occurs naturally in plants, which has been shown to inhibit the production of inflammatory mediators, including prostaglandin E2 and leukotriene LTB4, in human neutrophils (Bouriche et al., 2005).Quercetin also inhibits the expression of cytokines and inducible nitric oxide synthase (iNOS) by inhibiting the NF-κB signaling pathway, which contributes to its ability to inhibit inflammation (Comalada et al., 2005). Quercetin may also modulate the inflammatory response by systemically inhibiting TNF-α (Hsieh et al., 2020).

CONCLUSION

This study aimed to evaluate the impact of MOLE supplementation on growth performance, blood indices, diarrheal incidence, intestinal morphology, and fecal microbiota composition in weaned pigs. MOLE supplementation improved growth performance and reduced neutrophil counts, suggesting an anti-inflammatory effect. MOLE also reduced diarrheal incidence, which was accompanied by an increase in villus height/crypt depth ratio and a decrease in fecal microbial shedding. These findings suggest that MOLE supplementation at 250 mg/kg diet significantly enhanced growth and reduced diarrheal rate in weaned pigs. Further research is needed to elucidate the mechanisms by which MOLE exerts its beneficial effects on weaned pigs, including its chemical composition and potential interactions with the gut microbiota.

ACKNOWLEDGMENTS

This research is partial funded by the Thailand Science Research and Innovation (TSRI) via Rajamangala University of Technology Suvarnaphumi, project no.2703.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest relevant to this article.

NOVELTY STATEMENT

Our study find that dietary MOLE supplementation reduces the incidence of diarrhea and fecal shedding of bacteria in weaned pigs. This is the first study to report this finding. Additionally, MOLE supplementation improves production efficiency in weaned pigs. These findings could be applied to weaned pig management and help reduce antibiotic use on pig farms.

AUTHORS CONTRIBUTION

Wisit K.: Conducted formal analysis, acquired funding and resources, developed methodology, performed validation, contributed to visualization, and engaged in review and editing of the manuscript.
Kulisara M.: Responsible for conceptualization, data curation, formal analysis, investigation, methodology development, resource management, software utilization, supervision, validation, writing of the original draft, and project administration.
The final manuscript was read and approved by all authors.

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