Journal of Animal Science and Veterinary Medicine
Volume 9(6), pages 361-368, December 2024
Article Number: 0285749D10
ISSN: 2536-7099
https://doi.org/10.31248/JASVM2024.504
https://integrityresjournals.org/journal/JASVM

Full length Research

Influence of *Justicia secunda* leaf meal, scent leaf meal, and bamboo charcoal on growth, nutrient digestibility and faecal microbial shedding of weanling pigs

Emmanuel Abiodun Adeola*, Babatunde Richard Oluwasegun Omidiwura and Adebisi Favour Agboola

Department of Animal Science, University of Ibadan, Ibadan, Oyo state, Nigeria.

*Corresponding author. Email: emmayem.dtb@gmail.com; Tel: +234(8)132129142

Received 5th November 2024; Accepted 10th December 2024

ABSTRACT: The discontinuous use of antibiotic growth promoters (AGPs) in swine production may have serious negative consequences on animals' health and performance due to impaired immunological response to pathogenic microbes, especially in newly weaned pigs, warranting changes in nutritional and management strategies. *Justicia secunda* Leaf Meal (JLM), Scent Leaf Meal (SLM), and Bamboo Charcoal (BC) are additives that could modulate the microbiota of pigs, thereby enhancing animals' health and performance. Therefore, the influence of JLM, SLM, and BC on growth, nutrient digestibility and faecal microbial shedding of pigs was assessed. Large White x Landrace male pigs (n=48), weighing 7.5±0.50kg, aged 6 weeks, were assigned to eight dietary treatments in a completely randomised design (r=3) for six weeks. Treatment 1 had No supplementation (control); while other treatments contained 3%JLM, 5%SLM, 1%BC, 3%JLM+1%BC, 5%SLM+1%BC, 3%JLM+5%SLM, and 3%JLM+5%SLM+1%BC, respectively. Growth performance indices were measured, and faecal samples were collected for nutrient digestibility, microbial load and volatile fatty acid concentration following standard procedures. Data were subjected to descriptive statistics and ANOVA at $\alpha_{0.05}$. Significant (P<0.05) differences were observed in the average daily gain (ADG) and gain-to-feed ratio (G/F) of the pigs. Pigs fed control had lower ADG and G/F (191.7 g/day and 0.355) which were similar to those fed 3%JLM+1%BC (217.9 g/day and 0.407). Crude protein digestibility by pigs was improved by supplementation of 3%JLM (73.5%), 5%SLM (74.6%), and 1%BC (74.2%). Pigs fed 3%JLM had lower faecal *Escherichia coli* (4.15) which was similar to those of pigs fed 1%BC (4.81) and 3%JLM+5%SLM+1%BC (4.61). Similarly, pigs fed a 3%JLM diet had lower lactic acid (4.52 mmol/100g), and total volatile fatty acid (51.47 mmol/100g) concentrations compared with other treatment groups. In conclusion, dietary inclusion of *Justicia secunda*, scent leaf, and bamboo charcoal at 3%, 5%, and 1% inclusion levels, respectively, improved growth performance of weanling pigs. Supplementation of *Justicia secunda* at a 3% inclusion level improved crude protein digestibility and reduced faecal microbial shedding. Thus, it is recommended that *Justicia secunda* leaf meal should be supplemented in the diets of weanling pigs at a 3% inclusion level for enhanced gut health and growth performance.

Keywords: *Justicia secunda*, scent leaf, bamboo charcoal, gut health, weanling pigs.

INTRODUCTION

In intensive swine production, the post-weaning period is very critical as the intestinal microflora and digestive capacity of weaned piglets are usually under-developed, thereby making the animals susceptible to enteric diseases such as diarrhoea and salmonellosis (Ferronato and Prandini, 2020), which results in gut disruptions, reduced performance, and impaired immune system of the animals (Omonijo *et al.*, 2018). The separation of piglets from the sow, sudden transition from liquid diet to less digestible feedstuffs, and other changes like transfer to a new facility aggravate the intricacy of the gastrointestinal tract (GIT) and may lead to impaired morphology and functions of the GIT (Rodrigues *et al.*, 2020).

Post-weaning diarrhoea is characterised by a reduction in benign microbes, including *Lactobacillus acidophilus* and *Lactobacillus sobrius*, and an increase in malign microbes such as *E. coli* (Bagaria *et al*., 2024). Feed intake needed for gut integrity and function maintenance may be affected and result in intestinal inflammation, which may damage the villus and crypt structure and consequently worsen feed efficiency and growth performance (Ferronato and Prandini, 2020). Furthermore, there is a disruption of electrolyte absorption and deterioration of the immune system, which makes the animals more susceptible to diseases such as diarrhoea (Engelsmann *et al*., 2023). The decrease in the population of *Lactobacillus* during weaning also leads to increased gut pH, thereby increasing disease susceptibility because low intestinal pH is bactericidal. As a result, researchers have been exploring different strategies to increase the population of benign microbes and reduce malign ones in the GIT.

Antibiotics have been used in swine production at sub-therapeutic levels to stabilise gut microflora and to enhance pig growth performance (Silva Júnior *et al*., 2020). However, it has been reported that the incessant use of antibiotics has resulted in antimicrobial resistance, which is detrimental to the health of both livestock and humans (Nguyen *et al*., 2020). This led the European Union to prohibit the use of antibiotics at sub-therapeutic levels in livestock production in 2006 (Murphy *et al*., 2017).

The discontinuous use of antibiotic growth promoters (AGPs) in swine production may have serious negative consequences on animals' health and performance due to impaired immunological response to pathogenic microbes, especially on newly weaned pigs, warranting changes in nutritional and management strategies. Phytogenic feed additives (PFAs) contain bioactive substances, including thymol, eugenol, carvacrol, cineole, capsaicin, and several others, which are known for their antifungal, antibacterial, anti-coccidial, and antiviral effects (Madhupriya *et al*., 2018). PFAs may directly destroy pathogens due to the high percentage of phenolic compounds and also due to their hydrophobicity. Furthermore, certain bioactive compounds in PFA may hinder the development of virulent structures in bacteria by disrupting the glycolipid walls of bacterial cells, which results in leakage and reduction of cytoplasmic constituents (Miklasińska-Majdanik *et al*., 2018).

Justicia secunda (bloodroot) at 300 mg/kg exhibited anti-inflammatory activity (Anyasor *et al*., 2019), supplementation of scent leaf at 3% in broiler chickens' diet improved growth performance (Omidiwura *et al*., 2021), and dietary bamboo charcoal supplementation at 0.6% enhanced the growth of finishing pigs (Chu *et al*., 2013). It has been suggested that synergistic effects could be induced when different plant components are mixed (Wani *et al*., 2021). Hence, the aim of this study was to evaluate the effects of *Justicia secunda* leaf meal, scent leaf meal, and bamboo charcoal on growth performance, nutrient digestibility, faecal microbial shedding, and volatile fatty acid production of weanling pigs.

MATERIALS AND METHODS

Experimental location

The feeding trial was carried out at the Pig Unit, Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria (GPS Coordinates: 7.4432oN, 3.9003ºE). University of Ibadan Animal Care and Use Research Ethics Committee approved experimental procedures before the commencement of the study (Approval ID: 23/067).

Preparation of test ingredients

Fresh leaves of *Justicia secunda* and *Ocimum gratissimum* were sourced from Atisbo Local Government, Tede, Oyo State, Nigeria. The leaves were dried at room temperature to constant weights and milled using the laboratory hammer mill. The milled leaves were stored in clean air-tight bags prior to use. Bamboo charcoal was prepared through the following process. Bamboo (*Bambusa vulgaris* S.) chips of 1 cm (width) x 2 cm (length) were sourced from Atisbo Local Government, Tede, Oyo State, Nigeria, and subjected to heat at 400°C in a muffle furnace for 72 hours and then allowed to cool to room temperature. The charcoal was ground using a hammer mill and sieved, then stored in clean air-tight bags prior to use. Identification and authentication of the plants were carried out at the Department of Botany, University of Ibadan.

Animal, experimental design and diets

A total of forty-eight Crossbred (Large White x Landrace) weanling male pigs (7.50±0.50 kg initial body weight) were sourced from the Pig Unit, Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria for a 6-week study. The pigs were randomly assigned to one of eight dietary treatments (Table 1). Each treatment was replicated three times, with two pigs per replicate. A completely randomised design was used for the study. Diets were formulated to meet the nutrient requirements recommended by the NRC (1998), and the treatments were as follows:

No supplementation (CON); 30 g/kg *Justicia secunda* leaf meal (JLM); 50 g/kg scent leaf meal (SLM); 10 g/kg bamboo charcoal (BC); 10 g/kg BC + 30 g/kg JLM; 10 g/kg BC + 50 g/kg SLM; 30 g/kg JLM + 50 g/kg SLM; 10 g/kg BC + 30 g/kg JLM + 50 g/kg SLM. The pigs were allowed *ad libitum* access to feed and water. All other routine management practices were carried out in accordance with standard practices.

Table 1. Gross composition (g/100g DM) of phytogenic-supplemented diets for weanling pigs.

Ingredients	CON	3%JLM	5%SLM	1%BC	3%JLM + 1%BC	5%SLM + 1%BC	3%JLM+ 5%SLM	3%JLM+ 5%SLM+ 1%BC
Maize	38.00	38.00	38.00	38.00	38.00	38.00	38.00	38.00
Palm Kernel Cake	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Soybean Meal	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00
Groundnut Cake	25.00	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Wheat Offal	13.00	10.00	8.00	12.00	9.00	7.00	5.00	4.00
Palm Oil	5.00	5.00	5.00	5.00	5.00	5.00	5.00	5.00
Justicia secunda	0.00	3.00	0.00	0.00	3.00	0.00	3.00	3.00
Scent leaf	0.00	0.00	5.00	0.00	0.00	5.00	5.00	5.00
Bamboo charcoal	0.00	0.00	0.00	1.00	1.00	1.00	0.00	1.00
Premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Methionine	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.10
Dicalcium Phosphate	1.30	1.30	1.30	1.30	1.30	1.30	1.30	1.30
Limestone	0.75	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Table salt	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Nutrients								
ME (kcal/kg)	2991.00	2994.00	3000.00	2964.00	2967.00	2975.00	3005.00	2978.00
Crude Protein	20.03	20.12	20.06	19.88	19.97	19.91	20.15	20.00
Lysine	1.22	1.18	1.17	1.21	1.18	1.16	1.14	1.13
Methionine	0.39	0.39	0.38	0.39	0.38	0.38	0.37	0.37
Calcium	0.65	0.65	0.65	0.65	0.65	0.65	0.64	0.64
Phosphorus	0.50	0.49	0.48	0.50	0.49	0.48	0.48	0.47

ME = Metabolisable energy; *Supplied per kilogram of diet: Vitamin A – 10,000 IU; Vitamin D_3 – 2,000 IU; Vitamin E – 20 IU; Vitamin K – 2.25 mg; Thiamine B_1 – 1.75 mg; Riboflavin B_2 – 0.5 mg; Pyridoxine B_6 – 2.75 mg; Niacin – 27.50 mg; Pantothenic acid – 7.50 mg; Biotin – 50 mg; Choline Chloride – 400 mg; Antioxidant – 0.125 ppm; Manganese – 80 mg; Zinc – 50 mg; Iron – 20 mg; Copper – 5 mg; Iodine – 1.2 mg; Selenium – 0.20 mg; Cobalt – 0.20 mg. CON = control diet; JLM = *Justicia secunda* leaf; SLM = scent leaf; BC = bamboo charcoal.

Sampling and measurements

The body weight and feed consumption of each pig were determined at the start of the experiment and on day 42 to calculate the average daily gain (ADG), average daily feed intake (ADFI) and gain-to-feed ratio (G/F). ADG was calculated by subtracting the initial weight from the final weight and dividing the value by 42. Feed intake was determined by subtracting leftovers from the feed offered. ADFI was determined by dividing the total feed consumed during the experiment by 42. G/F was computed by calculating the ratio of ADG to ADFI. At day 35, Titanium dioxide was added to the diets at 0.5% inclusion level as an indigestible marker for nutrient digestibility study. Four days were used for adaptation, and three days were used for faecal collection. Thoroughly mixed representative samples of the experimental diets and faeces from the digestibility study were analysed for proximate compositions as described

Table 2. Growth performance of weanling pigs fed phytogenic-supplemented diets.

Parameters	CON	3%JLM	5%SLM	1%BC	3%JLM+ 1%BC	5%SLM+ 1%BC	3%JLM+ 5%SLM	3%JLM+5%SLM + 1%BC	SEM	P-value
ADG (g/day)	191.67[b]	247.62[a]	230.95[a]	234.52[a]	217.86[ab]	230.95[a]	239.29[a]	223.81[a]	4.16	0.029
ADFI (g/day)	541.20	542.30	539.30	545.20	538.20	547.10	535.30	531.70	2.29	0.777
G/F	0.36[b]	0.46[a]	0.43[a]	0.43[a]	0.41[ab]	0.42[a]	0.45[a]	0.42[a]	0.01	0.037

Means in the same row with different superscripts differ (p<0.05); CON = control diet; JLM = *Justicia secunda* leaf; SLM = scent leaf; BC = bamboo charcoal; SEM = standard error of mean; ADG = average daily gain; ADFI = average daily feed intake; G/F = gain to feed ratio.

by AOAC (2019). Titanium dioxide levels were determined according to Jagger *et al*. (1992). Nutrient digestibility was calculated using this formula: Coefficient of apparent total tract digestibility= {1– [(Nf × Td) / (Nd × Tf)]} × 100. Where: Nf = nutrient concentration in faeces (% DM), Nd= nutrient concentration in diet (%DM), Tf = titanium concentration in faeces (% DM), Td = titanium concentration in diets (% DM).

At day 42, fresh faecal samples were collected via massaging the rectum from 1 pig randomly selected from each replicate for the analysis of faecal microbial shedding and volatile fatty acid and ammonia-nitrogen production. One gram of each pig's faecal sample was diluted with 9 ml of 1% peptone broth and homogenised. Viable counts of bacteria in the faecal samples were determined by plating serial 10-fold dilutions (in 1% peptone solution) onto MacConkey agar plate to isolate *Escherichia coli*, Lactobacilli medium III agar plate for *Lactobacillus spp*., and Salmonella-Shigella agar plate for *Salmonella spp* and *Shigella spp*. The agar plates were then incubated for 24 h at 37°C. The microbial colonies were counted immediately after removal from the incubator. Microflora enumerations were expressed as log10 cfu/g. Volatile fatty acids (VFAs), including acetate, propionate, and butyrate, were measured according to the procedure of Chen *et al*. (2013), while ammonia nitrogen was measured as described by Diao *et al.* (2015).

Statistical analysis

Data were analysed using the analysis of variance (ANOVA) procedure of SAS (2020). The differences among treatments were compared using Duncan's Multiple Range Test (Duncan, 1955) when the treatment effect was observed significance was taken at p<0.05.

RESULTS

Growth performance of weanling pigs fed phytogenic-supplemented diets

The growth performance of weanling pigs fed diets supplemented with *Justicia secunda*, scent leaf, and bamboo charcoal is presented in Table 2. Significant (p<0.05) differences were observed in the average daily gain (ADG) and gain-to-feed ratio (G/F) of the pigs. Pigs fed the CON diet had lower ADG (191.67 g/pig), which was statistically similar to that of pigs fed a 3%JLM+1%BC diet (217.86 g/pig). Similarly, pigs fed the CON diet had lower G/F (0.355), which was statistically similar to that of pigs fed 3%JLM+1%BC diet (0.407). However, average daily feed intake was comparable among all the treatment groups.

Nutrient digestibility by weanling pigs fed phytogenic-supplemented diets

Nutrient digestibility by weanling pigs fed diets supplemented with *J. secunda*, scent leaf, and bamboo charcoal is presented in Table 3. Significant (p<0.05) differences were observed in the digestibility of all the nutrients analysed except fibre. Lower dry matter digestibility was recorded for pigs fed CON (73.09%) and 3%JLM+5%SLM +1%BC (73.80%) diets, which was statistically similar to those of pigs fed 3%JLM (77.57%) and 3%JLM+1%BC (75.47%) diets. Similarly, lower crude protein digestibility was recorded for pigs fed CON (66.17%), 3%JLM+1%BC (64.40%) and 3%JLM+5%SLM+1%BC (64.88%) diets, which was statistically similar to that of pigs fed 5%SLM+1%BC (71.10%) diet. Pigs fed a 5%SLM diet had higher ash digestibility (69.85%) compared to other treatments.

Faecal microbial load of weanling pigs fed phytogenic-supplemented diets

The faecal microbial load of weanling pigs fed diets supplemented with *J. secunda*, scent leaf, and bamboo charcoal is presented in Table 4. Significant (p<0.05) differences were observed among the treatments for all the microbes analysed. Pigs fed 3%JLM diet had lower faecal *E. coli* (4.15) which was statistically similar to those of pigs fed 1%BC (4.81) and 3%JLM+5%SLM+1%BC (4.61) diets. Pigs fed a 1%BC diet had higher faecal *Salmonella spp*. (1.52) which was statistically similar to that of pigs fed CON diet (1.04).

Table 3. Nutrient digestibility by weanling pigs fed phytogenic-supplemented diets.

Parameters (%)	CON	3%JLM	5%SLM	1%BC	3%JLM+ 1%BC	5%SLM+ 1%BC	3%JLM+ 5%SLM	3%JLM+ 5%SLM+1%BC	SEM	P-value
Dry matter	73.09[c]	77.57[abc]	81.31[a]	80.41[ab]	75.47[bc]	81.37[a]	83.05[a]	73.80[c]	0.78	0.001
Crude protein	66.17[b]	73.45[a]	74.61[a]	74.15[a]	64.40[b]	71.10[ab]	75.83[a]	64.88[b]	0.97	0..001
Ash	54.75[c]	50.19[c]	69.85[a]	61.54[b]	51.21[c]	53.34[c]	50.81[c]	53.87[c]	1.17	0.000
Ether extract	79.84[bc]	88.10[a]	83.54[ab]	84.53[ab]	78.90[bc]	85.51[ab]	89.06[a]	76.15[c]	0.96	0.002
Crude fibre	42.85	45.05	44.68	47.70	43.93	44.90	42.95	46.09	0.76	0.815

Means in the same row with different superscripts differ ($p<0.05$); CON = control diet; JLM = *Justicia secunda* leaf; SLM = scent leaf; BC = bamboo charcoal; SEM = standard error of mean.

Table 4. Faecal microbial load of weanling pigs fed phytogenic-supplemented diets.

Parameters	CON	3%JLM	5%SLM	1%BC	3%JLM+ 1%BC	5%SLM + 1%BC	3%JLM+ 5%SLM	3%JLM + 5%SLM + 1%BC	SEM	P-value
Total bacteria count	5.41[a]	4.86[ab]	5.03[ab]	5.05[ab]	5.27[ab]	5.46[a]	5.59[a]	4.69[b]	0.085	0.084
Escherichia coli	5.17[a]	4.15[b]	4.97[a]	4.81[ab]	5.20[a]	5.31[a]	5.29[a]	4.61[ab]	0.091	0.008
Lactobacillus spp	5.24[a]	4.76[ab]	4.94[ab]	4.93[ab]	5.05[a]	5.29[a]	5.27[a]	4.30[b]	0.086	0.056
Salmonella spp	1.04[ab]	NG[d]	0.90[b]	1.52[a]	0.48[bcd]	0.30[cd]	0.85[bc]	NG[d]	0.097	0.000
Shigella spp	1.36[a]	NG[b]	NG[b]	1.18[a]	0.30[b]	NG[b]	1.30[a]	NG[b]	0.102	0.000

Means in the same row with different superscripts differ ($p<0.05$); CON = control diet; JLM = *Justicia secunda* leaf; SLM = scent leaf; BC = bamboo charcoal; SEM = standard error of mean; NG = no growth.

Faecal volatile fatty acid concentration of weanling pigs fed phytogenic-supplemented diets

Faecal volatile fatty acid concentration of weanling pigs fed diets supplemented with *J. secunda*, scent leaf, and bamboo charcoal is presented in Table 5. Significant ($P<0.05$) differences were observed in all the volatile fatty acids analysed. Pigs fed 5%SLM+1%BC diet had higher faecal acetic acid (9.24 mmol/100g), propionic acid (8.82 mmol/ 100g), and butyric acid (8.42 mmol/100g) concentrations which were statistically similar to those of pigs fed the CON diet (7.70 mmol/100g, 7.34 mmol/100g, and 7.01 mmol/100g, respectively). Pigs fed 3%JLM diet had lower lactic acid (4.52 mmol/100g) and total volatile fatty acid (51.47 mmol/100g) concentrations compared with other treatment groups. Faecal pH was comparable among all treatment groups.

DISCUSSION

Phytogenics have been proposed as an alternative to antibiotics in enhancing the growth and health of livestock. Supplementation of *J. secunda*, scent leaf, and bamboo charcoal and their combinations in the present study improved ADG and G/F of weanling pigs, with the exception of the pigs fed the combination of *J. secunda* and bamboo charcoal which was similar to the control group. Improved growth performance could be attributed to the phytochemicals such as phenol present in *J. secunda* and scent leaf exhibiting anti-inflammatory and antioxidative activities, and also the adsorptive property of BC, which serves as a binding site for pathogens, thereby modulating the gastrointestinal tract (Caprarulo *et al*., 2021). Similar to the present study, supplementation of *Eucommia ulmoides* leaf extracts at 0.3 g/kg in the diet of weanlings improved ADG (Ding *et al*., 2020). However, Nantapo and Marume (2022) observed no improvement in the growth performance of weanling pigs when their diets were supplemented

Table 5. Faecal volatile fatty acid concentration of weanling pigs fed phytogenic-supplemented diets.

Parameters (mmol/100g)	CON	3%JLM	5%SLM	1%BC	3%JLM+ 1%BC	5%SLM+ 1%BC	3%JLM+ 5%SLM	3%JLM + 5%SLM + 1%BC	SEM	P-value
Acetic acid	7.70[ab]	3.63[c]	6.18[b]	6.31[b]	6.39[b]	9.24[a]	5.78[b]	6.06[b]	0.31	0.000
Propionic acid	7.34[ab]	3.46[c]	5.90[b]	6.02[b]	6.09[b]	8.82[a]	5.51[b]	5.78[b]	0.30	0.000
Butyric acid	7.01[ab]	3.31[c]	5.63[b]	5.75[b]	5.82[b]	8.42[a]	5.26[b]	5.52[b]	0.30	0.001
Valeric acid	6.98[ab]	3.29[c]	5.61[b]	5.72[b]	5.79[b]	8.38[a]	5.24[b]	5.50[b]	0.30	0.001
Total VFA	109.13[ab]	51.47[c]	87.67[b]	89.43[b]	90.59[b]	131.04[a]	81.91[b]	85.92[b]	4.26	0.000
Lactic acid	9.58[ab]	4.52[c]	7.69[b]	7.85[b]	7.95[b]	11.50[a]	7.19[b]	7.54[b]	0.40	0.000
pH	6.67	7.75	8.19	6.50	7.61	7.12	7.27	7.12	0.30	0.902
NH_3-N (%)	0.11[bc]	0.17[a]	0.12[bc]	0.11[c]	0.11[c]	0.12[bc]	0.15[ab]	0.14[abc]	0.01	0.010

Means in the same row with different superscripts differ ($P<0.05$); CON = control diet; JLM = *Justicia secunda* leaf; SLM = scent leaf; BC = bamboo charcoal; SEM = standard error of mean; Total VFA = Total volatile fatty acid; NH3-N = ammonia nitrogen.

with an herbal mixture containing *Lonicera japonica* Thunb., *Astragalus membranaceus*, *Eucommia folium* and *Codonopsis pilosula* leaves. Part of plant used, plant age, time of harvest, method of extraction, inclusion level, and interactions with other ingredients in the feed matrix could have brought about the discrepancies in the observed effects of phytogenics supplementation in the diets of the pigs (Fresno Rueda *et al*., 2021).

Although PFAs have been reported to enhance the flavour and palatability of feed by stimulating taste buds and olfactory nerves (Costa *et al*., 2013), feed intake was not affected by phytogenic supplementation in the present study. Similarly, Zhang *et al*. (2012) documented that feed intake was not affected when phytoncide was supplemented in the diets of weanling pigs. The author attributed the observed effect to the reduced level of gustatory activity of alkaloids. Meanwhile, Adebiyi *et al*. (2014) observed reduced feed intake when garlic extract was supplemented in the diets of weanling pigs. Contrary to the present study, Li *et al*. (2012) observed improved feed intake with essential oil supplementation in the diets of weanling pigs and attributed it to the pleasant flavour and odour of essential oils, whereas Yan *et al*. (2012) reported decreased feed intake when essential oil was supplemented in the diets of weanling pigs. The organoleptic properties and inclusion levels of the phytogenic used could account for the variations reported in the effects of their supplementation on feed intake.

Feed additives have been employed in animal nutrition to stabilise the gut microbiota, thereby resulting in improved gut function, including enhanced nutrient digestion and absorption. Supplementation of *J. secunda*, scent leaf and bamboo charcoal singly in the present study improved the crude protein digestibility of the piglets. The improved nutrient digestibility could be attributed to the ability of phytogenics to stimulate the secretion of mucous in the gut (Kikusato, 2021). Furthermore, phytogenics have been observed to stimulate the production of digestive enzymes such as amylase, lipase and protease, thereby enhancing nutrient utilisation in the gut (Wang *et al*., 2020b). Similar to the present study, Yang *et al*. (2021) documented that the inclusion of rosemary extract (400 mg/kg) in the diets of weaned pigs enhanced protein digestibility. Enhanced nutrient absorption may reduce feed costs by allowing appropriate alterations to nutrient-dense diets (Upadhaya and Kim, 2017). Contrary to the result of the present study, Matoso *et al*. (2024) reported that supplementation of phytogenic additives in the diets of weanling pigs did not improve nutrient digestibility. The variations observed in the effects of phytogenic supplementation on nutrient digestibility could be attributed to the level of endogenous loss that was due to mucous secretion stimulated by the different phytogenics (Zeng *et al*., 2015).

The intestinal microbiota is essential for efficient feed digestion and nutrient absorption, and the development of a strong immune system that would serve as a barrier protection against harmful pathogens and antigens (Wang *et al*., 2020a). Phytogenic supplementation in the present study reduced faecal *E. coli*, *Lactobacillus spp.* and the total bacteria count of the pigs. Several mecha-nisms are involved in the antimicrobial activity of PFAs due to the various chemical components present in them. PFAs may directly destroy pathogens due to the high percentage of phenolic compounds and also due to their hydrophobicity.

Furthermore, certain bioactive compounds in PFA may hinder the development of virulent structures in bacteria by disrupting the glycolipid walls of bacterial cells, which results in leakage and reduction of cytoplasmic constituents (Miklasińska-Majdanik *et al*., 2018).

Similar to the present study, Papadomichelakis *et al*. (2023) observed a reduction in faecal *E. coli* count when a natural phytogenic formulation containing anethole, apigenin, alicin, and olive polyphenols was supplemented in weaned pigs' diet. However, there was an increase in *E. coli*, *Lactobacillus spp*. and total bacteria count when the phytogenics were paired. When mixtures of plant components are used, interactions among various phytochemical constituents could result in antagonistic or synergistic effects (Wani *et al*., 2021). It has been observed that, although some microbes affect the host directly, some microbes only interact with other microbes. They influence the gut microbiota by making environments conducive to the proliferation of some specific microbes in the GIT (Isaacson and Kim, 2012). Thus, it is speculated that the interactive effects of the phytogenics resulted in the decrease in the population of a butyrate-producing bacteria such as *Clostridium spp*., which provided a conducive environment for *E. coli* and *Lactobacillus spp*. to thrive due to less competition for nutrients.

Microbial fermentation of carbohydrates can occur in the GIT of animals, resulting in the production of short-chain volatile fatty acids (such as acetic acid and butyric acid). These volatile fatty acids are absorbed by the epithelia cells and serve as the energy source for animals (Ma *et al*., 2021), and also provides protection against harmful microbes (Li *et al*., 2019). Phytogenics have been observed to modulate the microbiota of animals, decreasing microbial fermentation which is responsible for volatile fatty acid production (Popov *et al*., 2023). Phytogenic supplementation in the present study reduced faecal volatile fatty acid production of the pigs with the exception of pigs fed the combination of scent leaf and bamboo charcoal, which was much higher than other groups. A similar trend was observed in the production of all the volatile fatty acids measured.

Conclusion

Supplementation of *J. secunda*, scent leaf and bamboo charcoal improved ADG and G/F of weanling pigs with the exception of pigs fed the combination of *J. secunda* and bamboo charcoal which was statistically similar to the control group. However, no synergistic effects were observed with the combinations of the test ingredients. Phytogenics supplementation had no influence on the feed intake of the pigs. Supplementation of the phytogenics singly in the present study improved the crude protein digestibility of the piglets. Furthermore, supplementation of phytogenics singly reduced microbial shedding in weanling pigs. However, there was an increase in faecal bacteria count when the test ingredients were paired. Also, phytogenic supplementation reduced faecal volatile fatty acid production of the pigs. It is thus recommended that *J. secunda*, scent leaf and bamboo charcoal can be supplemented singly in the diets of weanling pigs to enhance the growth of animals.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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