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Research Article

Journal of Veterinary and Animal Sciences
ISSN (Print): 0971-0701, (Online): 2582-0605
https://doi.org/10.51966/jvas.2025.56.3.469-475

Growth performance, nutrient digestibility and faecal microbial load of growing pigs fed blood root leaf meal, scent leaf meal, and bamboo charcoal[#]

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Citation: Adeola, E.A. and Omidiwura, B.R.O. 2025. Growth performance, nutrient digestibility and faecal microbial load of growing pigs fed blood root leaf meal, scent leaf meal, and bamboo charcoal. *J. Vet. Anim. Sci.* **56** (3):469-475

Received: 24.04.2025 Accepted: 14.07.2025 Published: 30.09.2025

Abstract

The indiscriminate use of antibiotic growth promoters in livestock contributes to antimicrobial resistance. Phytogenics are natural, safer and promising alternatives that could improve animal performance. Nevertheless, the effects of phytogenics, such as Blood Root Leaf Meal (BRLM), Scent Leaf Meal (SLM) and Bamboo Charcoal (BC), and their combinations in pig production, have not been adequately evaluated. Therefore, growth performance, nutrient digestibility and faecal microbial load of growing pigs fed combinations of BRLM, SLM, and BC were assessed. Large White × Landrace male growing pigs (n=48) weighing 28.40±1.46 kg were assigned to eight dietary treatments in a Completely Randomised Design (CRD, r=3) for 42 days. Dietary treatments included a basal diet (control) and basal diet to which one of the following was added: 3% BRLM, 5% SLM, 1% BC, 3% BRLM+1% BC, 5% SLM+1% BC, 3% BRLM+5% SLM, or 3% BRLM+5% SLM+1% BC. Average Daily Gain (ADG) and Gain to Feed Ratio (Gain/Feed) were computed. Faecal samples were collected to determine nutrient digestibility and bacterial load. Data were subjected to descriptive statistics and ANOVA at $\alpha_{0.05}$. Pigs fed with control, 1% BC, and 5% SLM+1% BC diets had higher ADG (275.00 g/pig, 292.00 g/pig, and 267.00 g/pig, respectively) than other treatment groups. Pigs fed 5% SLM diet had lower crude protein digestibility (56.28%) compared to other treatments. When faecal microbial load was analysed Escherichia coli count was lowest {4.00 cfu/g) in growing pigs fed 3% BRLM. In conclusion, dietary supplementation of blood root leaf meal, scent leaf meal and bamboo charcoal singly reduced faecal Escherichia coli count of growing pigs. However, combined treatments increased microbial loads, suggesting potential antagonistic interactions. The supplementation of blood root leaf meal, scent leaf meal, and bamboo charcoal in growing pigs' diets did not improve average daily gain or gain-to-feed ratio.

Keywords: *Blood root, scent leaf, bamboo charcoal, gut health*

Over the years, antibiotic growth promoters (AGP) have been administered at therapeutic and sub-therapeutic levels in swine production to improve the growth performance of animals and as a line of defence against pathogen invasion. However, the use of antibiotics to enhance animal growth had shown that they might affect the beneficial gut microorganisms along with potentially harmful ones (Xu *et al*., 2020). Moreover, concerns have been raised regarding the

[#]*Part of PhD thesis submitted to University of Ibadan, Ibadan, Nigeria*

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overuse of antibiotics in livestock production due to spread of AMR as it directly or indirectly affects public health.

Several alternatives have been developed to replace antibiotics as growth promoters in swine production. The use of phytogenics, bacteriophages, prebiotics, probiotics, eubiotics and organic minerals have been suggested by some researchers as potential alternatives to antibiotics in livestock production (Nguyen *et al*., 2020). Phytogenic compounds are secondary plant metabolites, which are responsible for the colour and odour of plants (Liu *et al*., 2018). They contain various bio-active substances such as thymol, eugenol, carvacrol, cineole, capsaicin and several others which are known for their antifungal, antibacterial, antioxidant and antiviral effects (Yang *et al.*, 2019).

Blood root (*Justicia secunda* Vahl) is a perennial plant that has been used folkloric medicine in treating hypertension, diabetes, anaemia, and gastro-enteritis (Mea *et al.*, 2017). Furthermore, blood root leaf has abundance of bioactive substances including tannins, saponins, quinones, flavonoids, anthocyanins, leucoanthocyanins and terpenoids (Arogbodo, 2020). These bioactive compounds usually possess some anthelmintic and antimicrobial properties, which can enhance nutrient utilisation by animals and consequently improve the growth performance of animals (Valdes *et al*., 2015).

The West African scent leaf (*Ocimum gratissimum*) is a perennial herb that possesses antifungal, anti-inflammatory, antioxidative, and antibacterial properties (Ugbogu *et al*., 2021). The essential oil extracted from scent leaf contains bioactive substances, including pinene, thymol, eugenol, camphor, and limonene, that contribute to its medicinal effects by decreasing the secretion of pro-inflammatory cytokines, thereby mitigating inflammation (Akpoghelie *et al*., 2022). Furthermore, scent leaf is rich in flavonoids (kaempferol, apigenin, rutin, cirsimaritin, xanthomicrol and vixen) and phenolic substances (trans-ferulic acid, caffeic acid, catechins, gallic acid, salvigenin, sinapic acid and rosmarinic acid) (Dharsono *et al*., 2022). Phenols and flavonoids were reported to shield cells from oxidative stress-induced damage (Hussain *et al*., 2019). They exhibited their antioxidative and anti-inflammatory activities through mechanisms such as scavenging free radicals, chelating metal ions, and inhibiting enzymes that produce free radicals (Nimse and Pal, 2015).

Charcoal products are produced with varying procedures; however, they possess high porosity and very high surface area. Due to its adsorptive properties, charcoal has been utilised in livestock nutrition to enhance feed intake, growth performance, egg laying, egg quality, meat quality, and immune function in livestock (Toth and Dou, 2016). Research indicated that blood root (*Justicia secunda*) at 300 mg/kg exhibited anti-inflammatory properties *in vitro* (Anyasor *et al*., 2019), while inclusion at 1% scent leaf (*Ocimum gratissimum*) in the diet of weanling piglets enhanced growth performance (Adedoyin *et al*., 2023). Similarly, dietary supplementation with 0.6% bamboo charcoal has been shown to improve growth parameters in finishing pigs (Chu *et al*., 2013). These natural alternatives offer multiple advantages, including low toxicity and the absence of harmful residues, and have been proven safe for both human and animal consumption (Zeng *et al*., 2015). Many studies have highlighted the efficacy of Phytogenic Feed Additives (PFAs). Nevertheless, the effects of phytogenics, such as blood root leaf meal, scent leaf meal and bamboo charcoal, and their combinations in pig production, have not been adequately evaluated. Therefore, the study aimed to evaluate the growth performance, nutrient digestibility, and faecal microbial shedding of growing pigs fed combinations of blood root leaf meal, scent leaf meal and bamboo charcoal.

Materials and methods

The feeding trial was carried out at the Pig Unit, Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria (GPS Coordinates: 7.4432°N, 3.9003°E). University of Ibadan Animal Care and Use Research Ethics Committee approved experimental procedures before the commencement of the study (Approval ID: 23/067). Fresh leaves of blood root (*Justicia secunda*) and scent leaf (*Ocimum gratissimum*) were sourced from Atisbo Local Government, Tede, Oyo State, Nigeria. The leaves were air-dried at room temperature for thirty days, milled using a laboratory hammer and stored in clean air-tight bags until used.

Bamboo charcoal was prepared through the following process. Bamboo (*Bambusa vulgaris*) 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, sieved and stored in clean air-tight bags before its use. Identification and authentication of the plants were carried out at the Department of Botany, University of Ibadan.

Forty-eight Crossbred (Large White x Landrace) growing male pigs (28.4±1.46 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 with six replications each. A completely randomised design was used for the study. Diets were formulated to meet the nutrient requirements recommended by NRC (2012) as presented in Table 1. The treatments evaluated were as follows: No supplementation (control); 3% Blood Root Leaf Meal (BRLM); 5% Scent Leaf Meal (SLM); 1% Bamboo Charcoal (BC); 1% BC+3% BRLM; 1% BC+5% SLM; 3% BRLM+5% SLM; and 1% BC+3% BRLM+5% SLM. The supplementation levels

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

Ingredients (%)	CON	3% BRLM	5% SLM	1% BC	3% BRLM+ 1% BC	5% SLM+ 1% BC	3% BRLM+ 5% SLM	3% BRLM+ 5% SLM+ 1% BC
Maize	37.00	37.00	37.00	37.00	37.00	37.00	37.00	37.00
Palm Kernel Cake	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00
Soybean Meal	17.00	17.00	17.00	17.00	17.00	17.00	17.00	17.00
Groundnut Cake	11.00	11.00	11.00	11.00	11.00	11.00	11.00	11.00
Wheat Offal	17.00	14.00	12.00	16.00	13.00	11.00	9.00	8.00
Palm Oil	4.00	4.00	4.00	4.00	4.00	4.00	4.00	5.00
Blood root leaf	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*	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Lysine	0.25	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	0.80	0.75	0.75	0.75	0.75	0.75	0.75	0.75
Table salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Calculated Nutrients								
ME (kcal/kg)	2917.80	2920.80	2928.80	2890.80	2893.80	2901.80	2931.80	2904.80
Crude Protein	18.44	18.53	18.47	18.29	18.38	18.32	18.56	18.41
Calcium	0.80	0.80	0.80	0.80	0.80	0.80	0.80	0.79
Phosphorus	0.29	0.28	0.28	0.29	0.28	0.27	0.27	0.27
Analysed Nutrients								
Dry matter	91.55	91.28	91.35	91.17	91.89	92.02	91.11	90.90
Crude protein	17.90	18.01	17.90	17.80	17.92	18.10	18.05	18.20
Ash	9.00	8.30	10.30	10.20	9.50	7.10	6.40	6.80
Ether extract	4.00	4.40	6.10	6.60	4.10	3.60	4.70	5.20
Crude fibre	4.52	4.64	4.74	4.63	4.92	5.10	5.05	5.21

CON = control; BRLM = blood root leaf meal; SLM = scent leaf meal; BC = bamboo charcoal; *Supplied per kilogram of diet: Cobalt – 0.20 mg; Selenium – 0.20 mg; Iodine – 1.2 mg; Copper – 5 mg; Iron – 20 mg; Zinc – 50 mg; Manganese – 80 mg; Antioxidant – 0.125 ppm; Choline Chloride – 400 mg; Biotin – 50 mg; Pantothenic acid – 7.50 mg; Niacin – 27.50 mg; Pyridoxine B6 – 2.75 mg; Riboflavin B2 – 0.5 mg; Thiamine B1 – 1.75 mg; Vitamin K – 2.25 mg; Vitamin E – 20 IU; Vitamin D3 – 2,000 IU; Vitamin A – 10,000 IU; ME = Metabolisable energy

of the additives were based on recommendation from previous *in vitro* and *in vivo* experiments. The pigs were allowed *ad libitum* access to feed and water. All other routine management practices were carried out following standard practices.

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). The ADG was calculated by subtracting initial weight from final weight and dividing the value by 42. Feed intake was determined by subtracting left over from the feed offered. The ADFI was determined by dividing the total feed consumed during the experiment by 42 and 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 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 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= $\{1-[(Nf \times Td) / (Nd \times Tf)]\} \times 100$. 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. 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. 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, with significance taken at P<0.05.

Results and discussion

Growth performance

The result of the growth performance of growing pigs fed diets containing blood root leaf meal, scent leaf meal, and bamboo charcoal is presented in Table 2.

Although phytogenics were documented to improve growth performance by enhancing nutrient digestibility, gut health, and immune function, phytogenics supplementation in the present study did not improve average daily gain or gain-to-feed ratio. Pigs fed control diet (275.00 g/pig) 1% BC (292.00 g/pig), and 5% SLM+1% BC (267.00 g/pig) diets had higher ADG than other treatment groups. Higher G/F values were recorded for pigs fed control (0.332) and 1% BC (0.344) diets, which were similar to that of pigs fed 5% SLM+1% BC diet (0.319).

Chu *et al*. (2013) reported that dietary bamboo charcoal supplementation in the diets of finishing pigs improved growth performance and attributed the positive result to enhanced nutrient utilisation by the pigs. Moreover, Adedoyin *et al*. (2023) documented that dietary scent leaf meat supplementation improved the growth performance of weanling pigs. Stage of growth of animals, 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 (Fresno Rueda *et al*., 2021).

Feed intake was not affected by the supplementation of blood root leaf meal, scent leaf meal, and bamboo charcoal during the growing phase. Similarly, Adegbenro *et al*. (2018) reported that supplementation of a composite leaf meal including scent leaf did not influence the feed intake of growing pigs. Contrary to the results of the present study, Adedoyin *et al*. (2023) observed improved feed intake of weanling pigs with dietary scent leaf meal supplementation. The organoleptic properties and inclusion levels of the phytogenic used could account for the variations in reported effects of their supplementation.

Nutrient digestibility

Nutrient digestibility in growing pigs fed on diets supplemented with blood root leaf meal, scent leaf meal, and bamboo charcoal is presented in Table 3. Higher value of dry matter digestibility was obtained from pigs fed

Table 2: Growth performance of growing pigs fed phytogenic-supplemented diets

Parameters	CON	3% BRLM	5% SLM	1% BC	3% BRLM+ 1% BC	5% SLM+ 1% BC	3% BRLM+ 5% SLM	3% BRLM+ 5% SLM+ 1% BC	SEM	P-value
ADG (g/pig)	275.00^{a}	210.00^{bc}	195.00^{c}	292.00^{a}	204.00^{bc}	267.00^{a}	230.00^{b}	205.00^{bc}	6.08	0.000
ADFI (g/pig)	827.00	830.00	822.00	849.00	840.00	838.00	829.00	802.00	14.72	0.998
G/F	0.332^{a}	0.253^{c}	0.237^{c}	0.344^{a}	0.243^{c}	0.319^{ab}	0.278^{bc}	0.256^{c}	0.01	0.000

^{a,b,c} Means in the same row with different superscripts differ (P<0.05); CON = control; BRLM = blood root leaf meal; SLM = scent leaf meal; BC = bamboo charcoal; G/F = gain to feed ratio; ADFI = average daily feed intake; ADG = average daily gain; SEM = standard error of mean

Table 3: Nutrient digestibility in growing pigs fed phytogenic-supplemented diets

Parameters (%)	CON	3% BRLM	5% SLM	1% BC	3% BRLM+ 1% BC	5% SLM+ 1% BC	3% BRLM+ 5% SLM	3% BRLM+ 5% SLM+ 1% BC	SEM	P-value
Dry matter	72.45^{ab}	67.48^{bc}	65.30^{c}	74.99^{a}	72.99^{ab}	72.10^{ab}	61.39^{c}	63.43^{c}	1.00	0.000
Crude protein	64.84^{a}	65.60^{a}	56.28^{b}	68.21^{a}	65.37^{a}	64.18^{a}	64.96^{a}	68.81^{a}	0.90	0.015
Ash	41.81^{bc}	37.11^{c}	48.10^{b}	55.90^{a}	37.81^{c}	55.03^{a}	40.91^{c}	43.98^{bc}	1.23	0.000
Ether extract	71.64^{bc}	78.54^{ab}	74.96^{abc}	80.40^{a}	76.31^{abc}	69.55^{c}	70.21^{c}	72.16^{bc}	0.91	0.009
Crude fibre	61.65^{a}	59.52^{a}	52.64^{b}	60.94^{a}	63.40^{a}	62.32^{a}	44.61^{c}	47.81^{bc}	1.23	0.000

^{a,b,c} Means in the same row with different superscripts differ (P<0.05); CON = control; BRLM = blood root leaf meal; SLM = scent leaf meal; BC = bamboo charcoal; SEM = standard error of mean

Table 4: Faecal microbial load of growing pigs fed phytogenic-supplemented diets

Parameters (cfu/g)	CON	3% BRLM	5% SLM	1% BC	3% BRLM+ 1% BC	5% SLM+ 1% BC	3% BRLM+ 5% SLM	3% BRLM+ 5% SLM+ 1% BC	SEM	P-value
TBC	5.27^{ab}	4.84^{bc}	4.97^{abc}	5.01^{abc}	5.18^{ab}	5.40^{ab}	5.51^{a}	4.56^{c}	0.07	0.011
Escherichia coli	5.05^{a}	4.04^{c}	4.72^{b}	4.73^{b}	5.13^{a}	5.28^{a}	5.23^{a}	4.49^{b}	0.07	0.000
LAB	5.27^{a}	4.84^{ab}	4.99^{a}	5.00^{a}	5.12^{a}	5.30^{a}	5.30^{a}	4.34^{b}	0.07	0.008
Salmonella spp	NG	NG	NG	NG	NG	NG	NG	NG		
Shigella spp	NG	NG	NG	NG	NG	NG	NG	NG		

^{a,b,c,d} Means in the same row with different superscripts differ (P<0.05); CON = control; BRLM = blood root leaf meal; SLM = scent leaf meal; BC = bamboo charcoal; LAB = *Lactobacillus spp.;* NG = no growth; TBC = total bacteria count; SEM = standard error of mean

1% BC (74.99%), which was statistically similar to those of pigs fed 3% BRLM+1% BC (72.99%), 5% SLM+1% BC (72.10%) and CON (72.45%) diets. Similarly, Kim *et al*. (2017) documented that organic medicinal charcoal increased dry matter digestibility *in vitro*. Pigs fed a 5% SLM diet had lower crude protein digestibility (56.28%) compared to other treatments. The result obtained could be due to the high amount of tannin present in scent leaf. High levels of tannin have been reported to denature digestive enzymes, thereby impeding nutrient digestion and absorption, which can worsen feed utilisation and impair animal growth (Nantapo and Marume, 2022).

However, Oanh *et al*. (2021) observed that the inclusion of herbal extract mixture in diet of growing pigs did not influence apparent nutrient digestibility. Meanwhile, Hsu *et al*. (2021) documented that supplementing mixtures of essential oils of carvacrol, cinnamaldehyde and thymol in growing pigs' diets improved crude protein digestibility. The variations in the reported observations on the effects of phytogenics on nutrient digestibility could be due to the differences in the chemical contents of the various phytogenics. Lower crude fibre digestibility (44.61%) was recorded for pigs fed 3% BRLM+5% SLM, which was statistically similar to that of pigs fed 3% BRLM+5% SLM+1% BC diet (47.81%). Gall *et al*. (2008) suggested that growing pigs have limited capacity for degrading dietary fibre when supplemented at high levels. Blood root and scent leaves contain appreciable levels of crude fibre. Hence, their combination could have elevated fibre level of the diet beyond the capacity of the pigs to degrade efficiently.

Faecal microbial load

Faecal microbial load of growing pigs fed diets supplemented with blood root leaf meal, scent leaf meal, and bamboo charcoal is presented in Table 4. Significant differences were observed in the faecal total bacteria count, *Escherichia coli* and *Lactobacillus spp*. concentrations among the treatment groups. Furthermore, pigs fed 3% BRLM had lower faecal *Escherichia coli* (4.04 cfu/g) compared to other treatment groups. However, pigs fed 3% BRLM+5% SLM+1% BC diet had lower faecal *Lactobacillus spp* (4.34 cfu/g), which was statistically similar to that of pigs fed 3% BRLM diet (4.84 cfu/g). Supplementation of blood root leaf meal, scent leaf meal and bamboo charcoal singly in the diets of growing pigs reduced the *Escherichia coli* level of the pigs. The presence of phenols and saponins in blood root leaf and scent leaf could induce anti-inflammatory and antimicrobial effects by damaging the external and internal structures of cytoplasm membranes of bacteria, resulting in an increased flow of propidium iodide into the microbial cells (Famuyide *et al*., 2020).

Furthermore, polyphenols have been observed to exhibit broad antimicrobial effect against pathogenic microbes including *Staphylococcus aureus*, *Escherichia coli* and *Salmonella spp*. (Bouarab-Chibane *et al*., 2019). Shimazu *et al*. (2019) documented that supplementation of wakame seaweed powder decreased gut *Escherichia coli* and *Lactobacillus spp*. levels in pigs. Furthermore, activated charcoal was observed to suppress *Escherichia coli* levels *in vitro* (Ilomuanya *et al*., 2011). Pore diameter and size of charcoal are essential factors that determine the binding capacity of charcoal to bacteria (Katwal *et al*., 2022).

However, there was an increase in *Escherichia coli*, *Lactobacillus spp,* and the total bacteria count when the phytogenics were paired compared to when they were supplemented singly. 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 for 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 reduction in the population of a butyrate-producing microbe, such as *Clostridium* spp., which provided a conducive environment for *Escherichia coli* and *Lactobacillus spp*. to thrive due to less competition for nutrients. No growth of *Salmonella spp* and *Shigella spp* was detected in the faeces of the pigs across all treatment groups.

Conclusion

The supplementation of blood root leaf meal, scent leaf meal, and bamboo charcoal in diet of growing pigs did not improve average daily gain or gain-to-feed ratio. Dry matter digestibility was enhanced with 1% bamboo charcoal supplementation, while high tannin content in scent leaf meal likely reduced crude protein digestibility. Fibre digestibility declined with combined blood root leaf meal and scent leaf meal, possibly due to excessive dietary fibre levels. Microbial analysis revealed that single phytogenic supplementation reduced *Escherichia* coli counts, likely due to antimicrobial phenolic compounds, but combined treatments increased microbial loads, suggesting potential antagonistic interactions. These findings indicate that the efficacy of phytogenics depends on inclusion levels, plant composition, and interactions within the feed matrix, highlighting the need for optimised formulations to achieve desired growth and health benefits in swine nutrition.

Conflict of interest

The authors declare that they have no conflict of interest.

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