



RESEARCH

Effects of Dietary Inclusion of Herbal-Mix Foliage Meals on Morphological Parameters, Haematological Indices, Ileal Microbial Counts, and Ham Muscle's Mineral and Lipid Profile of Pigs

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ABSTRACT

This study investigated the dietary effects of herbal-mix leaf meals on morphological and haematological indices, ileal microbial counts, ham muscle's minerals and lipid profiles of pigs. Fifty-four, eight-week-old Large White pigs of both sexes with mean weight of 14.83 ± 0.10 kg were divided into 3 treatments and replicated in triplicates of 6 pigs. Three diets (control, moringa-basil, and neem-basil) were formulated to meet the pigs' nutritional needs as recommended by National Research Council (NRC). Feed and water were supplied ad libitum throughout the 12 weeks. At the end of the study, 2 pigs per replicate whose weights were close to replicate means were fasted for 16 hours, blood samples were collected to determine haematological response, thereafter, slaughtered by neck decapitation. The left ham muscle was harvested for pork mineral and lipid profile determination. Data derived were analysed using one-way analysis of variance (ANOVA) as obtained in SAS. Results showed significantly higher packed cell volume, haemoglobin, and red blood cells in diet with moringa-basil. Significant lower monocyte and high-density lipoprotein were documented for pigs on neem-basil diet. Pigs on the neem-basil diet had higher potassium of 69.33 ± 3.35 mg/100g compared to 58.33 ± 1.79 mg/100g noted for pigs on moringa-basil diet. Conversely, pigs on moringa-basil diets had higher sodium and calcium values. Lower triglyceride values were obtained in pork-fed herbal-mix diets compared to those on basal diet. It can be concluded that pigs fed a moringa-basil diet showed higher haematological indices, sodium, calcium, and HDL levels, indicating improved nutritional status, metabolic efficiency, and physiological stability that may enhance growth. Moringa-basil leaf meal can partially replace costly plant protein sources in pig diets, reducing feed costs while maintaining optimal health and physiological functions in pigs.

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INTRODUCTION

Pig production is a vital sub-component of Nigerians agricultural sector that accounts for 4% of the country's total domestic livestock production, contributing significantly to the nation's economic development, food security, employment and social structure (Ezeibe, 2010). The pig enterprise has the potential to contribute immensely in ameliorating effect of inadequate protein intake of Nigerians which is one of the greatest problems confronting the nation's teeming population, as pigs possess some inherent abilities that distinguished them from other domestic animals. These inherent abilities include but not limited to high survival rate, high feed conversion efficiency that leads to higher returns per unit of input, high fecundity, short gestation and early maturity, and relatively small space requirement for rearing (Aminu and Akhigbe Ahonkhai, 2017; Onyekuru *et al.*, 2020).

However, the survival of the pig industry in Nigeria and developing countries of the world depends on the ease of sources and costs of feed ingredients since nutritional needs of pig accounts for 75-80% of the total cost of production (Esonu, 2006). The pig industry depends majorly on cereal grains as the main energy source and grain legumes and oil seed cake as the protein sources. These conventional feed ingredients are not sufficiently available to pig producers at all locations. They are expensive as most of them are imported and those grown locally are in direct competition with human and biofuel industry which limit their utilization in the livestock industry (Adesehinwa, 2016). As a result of this, animal nutritionist and scientist are currently investigating alternative feed ingredients that are cheap, readily available and nutritionally balance that can totally or partially replace these conventional feed ingredients in livestock nutrition especially in poultry and pigs' husbandry. This quest for alternatives to conventional feed ingredients has led to different studies among which the utilization of phytogenic plant parts is currently being explored as feed additives and performance enhancers in poultry and swine rations (Ekunseitan *et al.*, 2019; Njoku *et al.*, 2018a and b, 2021).

Phytogenic feed additives are plant derived materials from natural sources with minimal or no residual effects whose application in livestock nutrition is gaining massive acceptance (Ciftci *et al.*, 2015; Nghonjuyi *et al.*, 2015 ; Ahossi *et al.*, 2016). Phytogenic feed additives have been defined by Jacela *et al.* (2010) as non-woody flowering medicinal/herbal plants or parts (flowers, leaves, fruits and roots) with intensive smells or tastes that are added to human or animal diet to enhance their acceptability and palatability. The report by D'Mello *et al.* (1987) indicated that leaf meals are valuable sources of protein, vitamins, minerals, and oxy-carotenoids, which are responsible for yellow pigmentation of the skin, shank, and egg yolk in farm animals. Studies in monogastric animals have further demonstrated that 10–20% dietary inclusion of processed phytogenic plant leaf meals supports growth performance, feed efficiency, and health status comparable to conventional diets, without deleterious effects on nutrient utilization (Fasuyi *et al.*, 2005; Olabode *et al.*, 2022). Several phytogenic plants with pharmacological activities that are indigenous to the tropical environment include neem (*Azadirachta indica* A. Juss), moringa (*Moringa oleifera*), and basil (*Ocimum gratissimum*), among others (Osuji *et al.*, 1995).

Neem, basil and moringa are among other indigenous therapeutic plants adopted in tropics as animal feed, alternative growth promoter and medicinal plants (Deka *et al.*, 2019). The leaf meals of these herbal plants are not only cheaper alternatives to the conventional protein sources in livestock ration but possess an array of biochemical properties like antimicrobial (antibacterial, antifungal, antiviral), antiprotozoal, hepatoprotective, immune-modulatory and other properties (Kale *et al.*, 2003; Hameed *et al.*, 2020). Many studies reported the use of sole herbs such as neem, moringa and basil leaf as phytogenic feed additives in animal diets (Nworgu, 2016; Al-Juhaimi *et al.*, 2020; Egbeyale *et al.*, 2021). However, no study has determined the comparative efficacy of combining neem-basil or moringa-basil leaf meals as a partial replacement of protein sources in the diets of growing pigs. Hence, this study was designed to determine the

dietary inclusion of herbal-mix leaf meal on morphological parameters, haematological indices, ileal microbial counts, ham muscle's minerals and lipid profiles of growing pigs. The parameters assessed provided information on suitability, quality and safety use of novel sources of feed protein in pigs production enterprise, their impact on muscle transformation rate (tissue conversion efficiency), health, welfare and pork quality.

METHODOLOGY

MATERIALS AND METHODS

Experimental Sites

The Animal Care and Use Committees of the College of Animal Science and Livestock Production, Federal University of Agriculture, Abeokuta, approved the animal experiment (FUNAAB/COLANIM/AEWC/2026/02/01).

The protocols relating to the use of animals were in accordance with the animal welfare requirements for care and management of experimental animals, as contained in the guidelines of the Animal Welfare Committee of the Federal University of Agriculture, Abeokuta, Nigeria (FUNAAB, 2013). The study was conducted at the Piggery Unit of the Directorate of the University Farms (DUFARMS), Federal University of Agriculture, Abeokuta, Ogun State, Nigeria. The region lies between Latitude 7°10'N and Longitude 3°20'E (Google Earth, 2023). Abeokuta has an average annual rainfall of 1100 mm and a temperature range of 28°C in December to 36°C in February with a yearly average relative humidity of about 82%

Experimental diets

Fresh matured neem, moringa and basil leaves were harvested within the University environment and air dried until they became crispy to the touch but still retaining their greenish colouration. The dried leaves were blended into powder. The moringa-basil and neem-basil leaf meal mixtures were in the ratio of 5:1 (that is five per cent (5%) moringa or neem to one per cent (1%) basil). A 5:1 mixing ratio was adopted in this study owing to the limited availability of basil leaves as seasonal fluctuations affected the production

during the fieldwork period. The ground herbal-mix leaf meal was used to replace conventional protein sources in the diet on a weight-for-weight basis during feed manufacturing process. Three grower diets in mash form (control diet, diets with moringa-basil or neem-basil foliage meals) were formulated and offered to the experimental pigs, according to their respective treatment groups. The composition of the diets is shown in Table 1. This revealed that the diets had heterogeneous calories (2834.59, 2741.99, and 2805.99 Kcal DE/kg) and nitrogen contents (21.00, 19.00 and 18.00%). The feeding trial lasted for twelve weeks.

Experimental animal management and design

A total of fifty-four Large White pigs of 8 weeks old with mean weights range of 14.45 ± 1.74 – 16.18 ± 1.56 kg were obtained from a reputable farm at Abeokuta. On arrival, the growing pigs were quarantined and acclimatized for 7 days during which they were injected ivomec® (ivermectin) at the dosage of 1 ml to 33 kg live weight against ecto- and endo-parasites. The pigs were grouped on weight equalization and randomly assigned into 3 treatments in Completely Randomised Design (CRD), with each treatment having 3 replicates of 6 pigs each. The pigs in replicate groups were raised under an intensive system of management in a 4 m × 4 m floor pen dimension, furnished with concrete feeders and drinking troughs. Throughout the experimental duration, feed and water were provided ad libitum.

Table 1: Percentage composition of growing pigs' experimental diets

Ingredients	No Herbal	Moringa-Basil	Neem-Basil
Maize	50	50	50
Basil Leaves	0	2	2
Moringa-Basil Leaf Meal	0	10	0
Neem-Basil Leaf Meal	0	0	10
Palm Kernel Cake	10	10	10
Soya Bean Meal	18	8	8
Groundnut Cake	9	7	7
Fish Meal	2	2	2
Wheat Offal	6.9	6.9	6.9
Bone meal	3	3	3
Lysine	0.3	0.3	0.3
Methionine	0.25	0.25	0.25
*Premix	0.3	0.3	0.3
Salt (NaCl)	0.25	0.25	0.25
Total	100	100	100
<i>Calculated Analysis</i>			
Metabolizable energy (Kcal/kg)	2834.59	2741.99	2805.9
Crude Protein (%)	21.00	19.00	18.00
Ether extract (%)	4.56	4.84	4.49
Crude fiber (%)	4.68	5.52	6.40
Ash	3.05	3.10	3.54

*To supply the following per kg diets; Vit. A 12600 IU; Vit. D3 2800 IU; Vit. K3 2.8 mg; Vit B1 1.4mg; Vit. B2 5.6 mg; Vit. B12 0.014 mcg; Niacin 21 mg; Pantothenic Acid 14 mg; Folic Acid 1.40 mg; Biotin 0.028 mcg; Choline Chloride 70 mg; Manganese 70 mg; Zinc 140 mg; Copper 140 mg; Iodine 1.4 mg; Selenium 28 mg; Cobalt 0.7 mg; Antioxidant 168 mg

Data Collection

Three pigs with body weights close to the mean replicate weight were identified, tagged, and used for the determination of changes in morphological parameters. The initial body dimensions of the experimental pigs were assessed and documented with measuring tapes while body weight changes were determined with weighing scale. Subsequent weekly changes of these body parameters were determined as outlined by Njoku *et al.* (2013). The body length was evaluated as the distance between the anterior point of the shoulder to the posterior extreme of the pin bone of the body. The heart girth measures the body circumference perpendicular to the median vertebra. Loin girth measures the body circumference taken within the 4th lumbar vertebra. The measurement of the distance between the most dorsal point of the wither and the floor level indicates the height at the wither. Ear length entails the length of the external ear from the point of attachment

to the tip. Face length was measured as the distance from the anterior point of attachment of the head to the body to the tip of the snout. The length from the tip of the posterior vertebrae to the point of attachment to the body was tagged as tail length.

On the 84th day of the feeding trial, 2.5 ml of blood was collected from two pigs per replicate via the jugular vein into labelled EDTA (ethylenediaminetetraacetic acid) bottles after a 16-hour fast, making a total of nine blood samples. The pigs were fasted prior to blood collection to ensure that the parameters measured reflected baseline physiological status rather than postprandial fluctuations. The blood samples were analysed for haematological parameters such as packed cell volume, haemoglobin, white blood cell, red blood cell, lymphocytes, basophils, monocytes, eosinophils and neutrophil were determined. These were measured according to the methods of Bush (1991). Two pigs with an average weight from each replicate were

fasted for 16 hours to empty their gut, after which they were stunned by percussion method and bled via incision made through the jugular vein between the skull and the atlas bone. Complete bleeding and dehairing were ensured before the carcass were dissected according to procedures described by Barca *et al.* (2006). The digestive tract was obtained, the ileum section (10 cm to the ileo-caecal orifice) was excised and its content was collected into a universal bottle and taken to the laboratory for microbial count. The microbial assessment was done following the procedures described by Barrow and Feltham (1993). Serial dilution of the ileum content was done by pipetting about 1 ml of the ileum digesta into a sterile 15 ml test tube containing 9 ml of 0.1% peptone water and vortex. Serial dilution of the digesta was done to the dilution factor of 10^{-3} dilution level. One milliliter of the dilution was pipetted on plate count agar and MacConkey agar and incubated at 37 °C for 24 hours. Discrete colonies on the plates were quantified with a colony counter as an aid.

The ham muscles of the slaughtered pigs were separated by locating the division between the second and third scrotal vertebrae and saw perpendicularly along the axis of the ham. The ham muscle was then taken to the Meat Processing Laboratory of the Department of Animal Production and Health, Federal University of Agriculture, Abeokuta, where its lipid profile was determined according to methods described by Spackman *et al.* (1958). Mineral composition was determined using an atomic absorption spectrophotometer and Perkin Elma 5000 instruments, after wet ashing with a mixture of nitric, perchloric and sulphuric acids. Phosphorus was determined by the phosphovanamolybdate method of AOAC (1990). Sodium and potassium were determined using the flame photometer model -EEL, while calcium and magnesium concentrations were determined using the method of AOAC (1990).

Statistical Analysis

Data generated from the study were subjected to one way analysis of variance (ANOVA) using SAS (2004). The variations in means were separated using Duncans Multiple Range Test as contained in the same statistical package. The model of the study is as shown:

$$Y_{ijk} = \mu + T_i + \Sigma_{ij}$$

Where: Y_{ijk} = Individual Observation, μ = Overall Mean, T_i = Treatment Effect (Herbal-mix foliage meals), Σ_{ij} = Experimental error

RESULTS

The effect of herbal-mix feed additives on the morphological parameters of growing pigs is shown in Table 2. The body weight, body length, heart girth and face length were statistically ($P < 0.05$) influenced by herbal-mix feed additives. Body weight of pigs on control diet was significantly ($P < 0.05$) higher than documented values for pigs fed diet containing neem-basil leaf meal mix. Pigs on ration containing herbal-mix feed additive irrespective of the type had comparable mean values (2.38 ± 0.35 , 2.42 ± 0.20 cm) for body length that differed significantly from 3.40 ± 0.40 cm noted for those on a diet without herbal mix. The highest ($P < 0.05$) heart girth value (2.88 ± 0.21 cm) was observed for pigs on a control diet while the least heart girth value of 2.25 ± 0.22 cm was noted for growing pigs on a ration that contained neem-basil leaf meal. Pigs on a ration containing moringa-basil leaf meal had the least ($P < 0.05$) face length of 0.44 ± 0.07 cm while those on a ration containing neem-basil leaf meal recorded the highest face length value (0.68 ± 0.05 cm).

Table 2: Effect of herbal-mix feed additives on weekly changes in the morphological parameters of growing pigs

Parameters	No herbal-mix	Moringa-basil	Neem-basil
Body weight (kg)	59.44±2.86 ^a	54.23±2.17 ^{ab}	50.76±1.81 ^b
Body length (cm)	3.40±0.40 ^a	2.38±0.35 ^b	2.43±0.20 ^b
Height at wither (cm)	1.78±0.22 ^a	2.13±0.25 ^a	2.19±0.14 ^a
Heart girth (cm)	2.88±0.21 ^a	2.37±0.24 ^{ab}	2.25±0.22 ^b
Face length (cm)	0.56±0.07 ^{ab}	0.44±0.07 ^b	0.68±0.05 ^a
Ear length (cm)	0.39±0.05 ^a	0.30±0.06 ^a	0.23±0.07 ^a
Tail length (cm)	0.66±0.13 ^a	0.62±0.13 ^a	0.53±0.09 ^a
Thigh width (cm)	2.17±0.25 ^a	1.97±0.33 ^a	2.12±0.15 ^a

Data represents mean ± standard error of mean. Within the same row different superscripts are significantly ($P<0.05$) different

Haematological results revealed that packed cell volume, haemoglobin, red blood cell and monocytes values of pigs were significantly ($P<0.05$) influenced by herbal-mix feed additives (Table 3). Pigs fed control diets and those on a diet containing neem-basil leaf meal had statistically similar packed cell volume (33.50±0.62 and 35.17±2.36%) but differed significantly ($P<0.05$) from packed cell volume of 40.83±1.70% documented for pigs on a ration containing moringa-basil leaf meal. The lowest haemoglobin value of 10.93±0.30 g/dL was observed in pigs fed a control diet while those on a diet containing moringa-basil leaf meal recorded the highest haemoglobin value of 13.45±0.43 g/dL.

Statistically similar red blood cell values (5.70±0.09 and 6.15±0.28) were noted for pigs on control diet and those on a diet containing neem-basil leaf meal. Still, they differed significantly ($P<0.05$) from 6.97±0.25 obtained for pigs on a diet containing moringa-basil leaf meal. Pigs on the control diet and those fed a diet containing moringa-basil leaf meal had comparable higher monocytes values (1.97±0.58 and 1.10±0.28%) that varied significantly from 0.53±0.24% noted for pigs on a diet containing neem-basil leaf meal.

Table 3: Effect of herbal-mix feed additives on haematological parameters of growing pigs

Parameters	No herbal-mix	Moringa-basil	Neem-basil	+Reference value
Packed cell volume (%)	33.50±0.62 ^b	40.83±1.70 ^a	35.17±2.36 ^b	32-50
Haemoglobin g/dL	10.93±0.30 ^b	13.45±0.43 ^a	11.90±0.73 ^{ab}	10-16
Red blood cell (x10 ¹² /l)	5.70±0.09 ^b	6.97±0.25 ^a	6.15±0.28 ^b	5-7
White blood cell (x10 ⁹ /l)	11.63±0.32 ^a	12.40±0.72 ^a	13.28±0.83 ^a	7-20
Neutrophil (%)	3.13±0.43 ^a	3.25±0.76 ^a	3.38±0.19 ^a	2-15
Lymphocyte (%)	64.83±1.51 ^a	63.83±1.17 ^a	63.33±0.88 ^a	40-60
Eosinophils (%)	0.33±0.21 ^a	0.33±0.21 ^a	0.67±0.21 ^a	0-10
Basophils (%)	0.83±0.17 ^a	0.70±0.22 ^a	0.67±0.21 ^a	0-2
Monocytes (%)	1.97±0.58 ^a	1.10±0.28 ^a	0.53±0.24 ^b	2-10

Data represents mean ± standard error of mean. ^{ab} means within the same row with different superscripts are significantly ($P<0.05$) different.

*Adopted from Research Animal Resources (RAR; 2009)

The effect of herbal-mix feed additives on the ileal microbial count of pigs is shown in Table 4. Total bacterial count, *Lactobacillus*, *Escherichia coli* and *Clostridium* counts were significantly ($P<0.05$) influenced by dietary inclusion of herbal-mix feed additives. Statistically similar total bacterial count (1.80 ± 0.14 and $1.50\pm1.12 \times 10^6$ cfu/mL) were obtained in pigs fed diet containing moringa-basil and neem-basil feed additives which varied significantly ($P<0.05$) from the corresponding value ($2.37\pm0.12 \times 10^6$ cfu/mL) gotten from their counterparts fed control diet. Pigs fed a diet containing moringa-basil feed additive had the highest *Lactobacillus* spp count of $0.77\pm0.09 \times 10^6$ cfu/mL compared to those on a diet containing neem-basil leaf meal ($0.54\pm0.15 \times 10^6$ cfu/mL) and control diet ($0.33\pm0.05 \times 10^6$ cfu/mL). A higher *Escherichia coli* count of $0.70\pm0.09 \times 10^6$ cfu/mL was documented for pigs on the control diet compared to a lower value of $0.40\pm0.06 \times 10^6$ cfu/mL noted for pigs fed dietary inclusion of neem-basil leaf meal. Pigs fed a diet containing herbal-mix feed additives irrespective of the type had statistically similar *Clostridium* count that differed significantly ($P<0.05$) from the value of $1.13\pm0.07 \times 10^6$ cfu/mL observed for their counterparts on the control diet.

The effect of herbal-mix feed additives on pork mineral composition is shown in Table 5. Most of the pork minerals considered in the present study were not significantly ($P>0.05$) impacted by herbal-mix feed additives except for magnesium content. The potassium content of pork from pigs fed a diet containing neem-basil leaf meal as a feed additive was the highest while the least potassium content of 58.33 ± 1.79 mg/100g was obtained from the meat of pigs fed dietary inclusion of moringa-basil leaf meal. Statistically similar sodium contents (37.67 ± 1.10 and 35.33 ± 2.63 mg/100g) were observed in pork from pigs on a control diet and those on neem-basil leaf meal but differed significantly from the sodium value (44.00 ± 1.40 mg/100g) noted for pigs on moringa-basil leaf meal feed additives. The calcium contents of the pork followed the same trend with sodium content with the highest value noted in pork from pigs fed moringa-basil leaf meal whereas statistically similar calcium content was noted in pork from pigs on the control diet and those fed diet containing neem-basil leaf meal.

Table 4: Effect of herbal-mix feed additives on ileal microbial count of growing pigs

Parameters (x10 ⁶ cfu/mL in fresh matter basis)	No herbal-mix	Moringa-basil	Neem-basil
Total bacterial count	2.37±0.12 ^a	1.80±0.14 ^b	1.50±1.12 ^b
<i>Lactobacillus</i> spp.	0.33±0.05 ^c	0.77±0.07 ^a	0.54±0.14 ^b
<i>Salmonella</i> spp.	0.40±0.08 ^a	0.20±0.04 ^a	0.52±0.15 ^a
<i>Coliform</i> spp.	1.03±0.08 ^a	0.90±0.13 ^a	0.88±0.07 ^a
<i>Escherichia coli</i>	0.70±0.09 ^a	0.57±0.07 ^{ab}	0.40±0.06 ^b
<i>Clostridium</i> spp.	1.13±0.07 ^a	0.90±0.06 ^b	0.78±0.04 ^b

Data represents mean ± standard error of mean. Within the same row different superscripts are significantly ($P<0.05$) different

Table 5: Effect of herbal-mix feed additives on ham muscle's mineral composition

Parameters (mg/100g)	No herbal-mix	Moringa-basil	Neem-basil
Potassium	64.00±4.89 ^{ab}	58.33±1.79 ^b	69.33±3.35 ^a
Sodium	37.67±1.10 ^b	44.00±1.40 ^a	35.33±2.63 ^b
Calcium	4.53±0.16 ^b	5.13±0.16 ^a	4.47±0.23 ^b
Magnesium	17.67±1.67 ^a	20.33±1.34 ^a	17.67±1.77 ^a

Data represents mean ± standard error of mean. Within the same row different superscripts are significantly ($P<0.05$) different

Table 6: Effect of herbal-mix feed additives on ham muscle's lipid profiles

Parameters (mg/100mL)	No herbal-mix	Moringa-basil	Neem-basil
Total cholesterol	92.67±2.80 ^a	90.67±2.87 ^a	87.67±3.87 ^a
Triglyceride	72.00±4.82 ^a	53.00±1.85 ^b	61.00±2.03 ^b
High density lipoprotein	58.63±1.74 ^a	62.33±2.86 ^a	52.43±1.75 ^b
Low density lipoprotein	23.03±2.18 ^a	19.73±1.56 ^a	19.65±1.57 ^a

Data represents mean ± standard error of mean. Within the same row with different superscripts are significantly (P<0.05) different

Table 6 shows the effect of herbal-mix feed additives on the lipid profile of pigs. Triglyceride and high-density lipoprotein contents were significantly (P<0.05) influenced by herbal-mix feed additives. Pork derived from pigs on dietary inclusion of herbal-mix feed additives irrespective of the type had lower triglyceride composition (53.00±1.85 and 61.00±2.03) when compared to the triglyceride value (P<0.05) of 72.00±4.82 mg/dl noted in pork gotten from pigs fed control diet. Pork from pigs fed the control diet and those on the diet that contained moringa-basil leaf meal had comparable high-density lipoprotein levels which differed significantly (P<0.05) from the corresponding value noted for pork from pigs fed dietary inclusion of neem-basil leaf meal as a feed additive.

DISCUSSION

The observed decrease in the body weight of pigs fed diets containing different herbal leaf meals could be attributed to the presence of anti-nutritional factors which could inhibit nutrient digestibility and efficiency of nutrient utilization. It has been reported that leafy plants have high potentials to meet the dietary needs of farm animals but their inherent ability to synthesize myriads anti-nutritional factors inhibits their utilization in monogastric animal production (Agbede, 2005). The bioactive limonoids present in *Azadirachta indica*, particularly nimbolide and nimbidin, possess hypolipidemic and anti-inflammatory properties. These compounds have been reported to regulate lipid metabolism by modulating adipogenic and fatty acid synthesis pathways, enhancing lipid oxidation, and inhibiting pancreatic lipase activity, thereby reducing dietary fat digestion and absorption (Bhowmik et al., 2010; Gupta

et al., 2013 and Bhatia et al., 2014). Suppression of pro-inflammatory mediators by nimbidin further contributes to improved metabolic regulation (Chattopadhyay, 1999). Collectively, these mechanisms may decrease energy availability for tissue accretion and adipose deposition, resulting in lower weight gain. While beneficial for limiting excessive fat accumulation, such effects may compromise optimal growth performance if inclusion levels are not properly regulated. Similarly, isothiocyanates and phenolic compounds in moringa leaves enhance fatty acid oxidation, modulate adipogenic gene expression, and exhibit antioxidant and anti-inflammatory activities, thereby influencing nutrient partitioning and lipid accumulation (Leone et al., 2015; Waterman et al., 2014; Sreelatha and Padma, 2009). However, phenolic compounds may also reduce nutrient digestibility through enzyme and protein complex formation (Makkar and Becker, 1996), while secondary metabolites can affect feed intake and nutrient utilization efficiency (Vergara-Jimenez et al., 2017). The combined effects of enhanced lipid metabolism and modest reductions in nutrient availability may therefore explain the comparatively lower weight gain in pigs on herbal-mix leaf meal diets when compared with the weight of pigs fed control diet. The lower body weight gain of pigs on a diet containing herbal plants could also be linked to increasing bulkiness of the feed. Bulky feed has been associated with higher fibre and low nutrient concentration per unit volume, thus pigs on such a diet attain satiety quicker but slow body mass deposition due to poor intake of the feed. This observation is in line with the report of Adeyemi et al. (2012) who noted a negative growth rate and performance in birds on 30% inclusion level of cassava leaf meal: blood meal mix, the significant variation in weights was attributed to the adverse effect of

amino acid imbalance in the leaf meal-based diet. Aletor *et al.* (2000) asserts that ration with unbalanced amino acids is less palatable and tends to be consumed in smaller quantity which consequently in depressed growth performance of the animals placed on such ration. The observed decrease in final body weight of pigs on diets containing herbal leaf meals when compared to their counterparts on control diets in this study supports the earlier report of Ruckli and Bee (2016) that observed depressed feed intake and lower weight gains in finishing pigs on diets with higher inclusion levels of *Moringa oleifera* leaf meals. The significant decrease in body length and heart girth in pigs fed dietary inclusion of herbal leaf meals points to the significant lower body size or weight of those sets of pigs. This observation confirms the reports of Sulabo *et al.* (2006), Onyimonyi *et al.* (2009) and Njoku *et al.* (2013) who argued that changes in linear body dimensions are indications of tissue growth and development which tend to increase as the animal grows. Ige *et al.* (2006) affirmed that linear body dimensions' data can be used to assess the performance, productivity, and carcass quality of the animals. Sulabo *et al.* (2006) concluded that there is a positive correlation between morphological body measurements and body weight gains in pigs. The highest significant variation in the face length of the pigs on the diet that contained neem-basil leaf meal could be due to non-additive genetic effects rather than environmental effects. This is in line with the observation of Mavule *et al.* (2013), the effect of ear length on body weight is affected less by environmental factors but can be assessed more via non-additive genetic effects.

Changes in the components of blood are often used to determine the physiological status of the body and to assess stress resulting from environmental, nutritional, and pathological factors in farm animals (Afolabi *et al.*, 2010). According to Oyawoye and Ogunkunle (2004), haematological indices are veritable tool use for monitoring toxicity of feed, especially feed ingredients that affect the blood components and health status of farm animals. Haematological results of this present study revealed Packed cell volume, haemoglobin, red blood cell and monocytes values of pigs

were significantly influenced by dietary inclusion of herbal-mix foliage meals. These listed indices were within the normal physiological ranges for healthy pigs as reported by Research Animal Resources (RAR; 2009). This implies that the dietary inclusion of herbal-mix foliage meals (types and levels) used in this present study did not have any deleterious effects on the immunity and general well-being of pigs. Significant greater packed cell volume, haemoglobin and red blood cell values observed in pigs fed the diet containing moringa-basil leaf meals over their counterparts on control diets could point to the fact that moringa leaves are rich in minerals, vitamins and protein with eight essential amino acids that must have contributed a lot to the physiological, digestive and biosynthetic processes within the pigs' body, resulting to better haemopoietic processes of the pigs as reported by Makkar and Becker (1996) and Oloruntola *et al.* (2019). Also, Peters *et al.* (2011) enthused that packed cell volume, haemoglobin and mean corpuscular haemoglobin are the main indices for erythrocyte evaluation which serve as useful parameters to assess the capacity of bone marrow to produce red blood cells in animals. Reis *et al.* (2018) indicated that dietary inclusion of phytobiotics (cinnamic, thymol, pawpaw leaf and seed meals, turmeric etc.) in monogastric animal's ration significantly improved erythrocyte counts and haemoglobin value when compared to their corresponding values in the control treatment. The results obtained in this study are in tandem with the findings of Ebenebe *et al.* (2012) who reported an increase in red blood cell, packed cell volume and haemoglobin values of broiler chickens fed diets containing moringa leaf meals at inclusion levels of 10 and 15%. The findings disagree with the observation of Tang *et al.* (2017) who asserted that the use of prebiotics and probiotics as natural feed additives did not have any significant impact on the haematological parameters (packed cell volume, haemoglobin, red blood cell and mean cell volume). The potential antioxidant properties of moringa leaf meal have been attributed to the presence of vitamins A, C and E, selenium, chlorophyll, carotenoids, flavonoids, phenolics, xanthins etc, which must have contributed to the improvement in the health

status of farm animals feeding on the plants (Falowo et al., 2014; Moyo et al., 2016). The similarity in monocytes values of pigs on the control diet and those fed the diet that contains moringa-basil foliage meals indicates that moringa leaf meal had no harmful effects on the immunity of the pigs. The current finding aligns with the investigation of Olatunji et al. (2016) that showed monocytes and eosinophils values were not affected significantly by inclusion levels of moringa leaf meal in the diet of rabbits when compared to those in control diet. The significantly lower monocyte value obtained in pigs fed ration with neem-basil leaf meal compared to the values gotten for pigs on the control diet and diet containing moringa-basil leaf meal could be linked to blood purifying properties of neem as reported by Bhowmik et al. (2010). Monocytes are key mediators of innate immune and inflammatory responses, and reductions in their counts have been associated with diminished inflammatory stimulation (Jain, 1993; Weiss and Wardrop, 2010). Neem (*Azadirachta indica*) and basil (*Ocimum basilicum*) contain bioactive compounds such as flavonoids, terpenoids, and phenolics with documented anti-inflammatory and immunomodulatory effects (Biswas et al., 2002; Kwee and Niemeyer, 2011), which may suppress monocyte proliferation or recruitment. While lower monocyte values may indicate reduced immune activation and improved physiological stability, values below reference limits could also suggest potential immune suppression if prolonged (Merck Veterinary Manual, 2018). The current result is in line with the observations of earlier researchers who reported that total white blood cell counts and lymphocyte values decreased significantly in neem-treated groups compared with the control groups (Zanu et al., 2011; Bonsu et al., 2012).

Gut health is an essential component in livestock production since it can be used in prediction of the health status and nutrient utilization of the farm animal (Lalles et al., 2007). The significant reduction in total bacterial counts and *Escherichia coli* in pigs offered ration with different herbal-mix feed additives compared to those fed control ration could be ascribed to higher fibre contents in

the diets, the percentage increase in fibre contents in ration with moringa-basil and neem-basil leaf meals is 17.95% and 36.75%, respectively compared to the control diet. According to Hussein et al. (2021), the fermentation process of fibre in pig's gastrointestinal tracts enhanced the structure of intestinal microflora and depress the incidence of intestinal diseases. Also, it has been noted that improvement in dietary fibre usage in pig production promotes healthy growth of pigs, as well as reduces the use of antibiotics and antibacterial drugs thereby improving the profitability of the farmer by reducing the cost of production and increase in pork demands due to enhanced safety of the pork and its products (Agyekum and Nyachoti, 2017). The significant decrease in total bacterial, *Escherichia coli* and *Clostridium* Spp counts in pigs on diets with different herbal-mix leaf meals could also be linked to the antimicrobial effects derived from the bioactive agents (secondary metabolites) of the herbal plants. According to the reports of Ndhlala et al. (2014) and Sirsat et al. (2018), the antimicrobial activity of herbal plants can be associated with phenolic compounds, saponins and flavonoids inherent in the leaves. The results of this present study are in line with the observations of Ayoola et al. (2020) and Abu Hafsa et al. (2016) who affirmed that herbal extracts decreased the counts of total bacterial and *Escherichia coli* in their investigations. Also, Allison et al. (2013) reported that herbal extracts improved the weight gain and feed efficiency of poultry birds as well decreased the bacterial loads and oocyst counts of the birds. The significant decrease in *Escherichia coli* and *Clostridium* spp., points to the fact that the herbal plants used in this present study have the potential to control the proliferation of harmful microorganisms, as well as enhance the favourable growth of beneficial bacteria like *Lactobacillus* spp among the pigs' microflora. This observation is line with the report of Castillo et al. (2018) that dietary supplementation of moringa extracts inhibits Grams-negative and positive bacteria and fungi in quail production. Likewise, Djakalia et al. (2011) reported that herbal plants have some antimicrobial activity that could be used to control pathogenic bacteria. Abu Hafsa et al. (2019) stated that pigs on herbal extracts and essential oil

supplementations recorded higher amounts of probiotic bacteria such as *Lactobacillus*, *Bacillus*, *Bifidobacterium* etc., when compared with control groups.

Herbal plant leaves (moringa, neem and basil) used in this study have been reported to be rich in protein, fat, total carbohydrates, vitamins and minerals such as calcium, iron, magnesium, phosphorus, sodium and potassium (Qazi and Molvi, 2016). According to Eruvbetine (2003) minerals being inorganic substances present in all body tissues and fluids whose presence are essential for the maintenance of certain physicochemical processes vital for life. Significant improvements in mineral compositions (potassium, sodium and calcium) of pork obtained from pigs on dietary inclusion of different herbal-mix foliage meals compared to those fed control diets indicate that these herbal leaves can serve as an excellent source of nutritive ingredients in pig production. The active compounds inherent in the leaves of these herbal plants must have played a role in physiological and chemical function of digestive tracts. These active compounds in the herbs must have stimulated the release of digestive enzymes from the pancreas, this must have resulted to better digestion and absorption of plants' minerals by the pigs that ingested them thereby, improve their deposition in the body cells of pigs. According to the reports of Oleforuh-Okoleh *et al.* (2015) and Kappel *et al.* (2013), the leaves of herbal plants contain active phytochemicals compounds that enhanced nutrients metabolism of pigs. Likewise, Liu *et al.* (2017) reported that plants extract stimulates the secretions of digestive enzymes, thereby making nutrients available for absorption. The increase in potassium content in pork from pigs fed diet with neem-basil leaf meal over their counterparts on diet with moringa-basil leaf meal points to better bioavailability of potassium content in neem-basil ration, which could have resulted from interactions of the bioactive compounds in the composite leaf meals of neem and basil. Esonu *et al.* (2002) enthused that leaf meals of most tropical legumes and browse plants are rich in vitamins, minerals and carotenoids. However, Chukwuebuka and Chinenye (2015) noted that excessive amount of phytoconstituents

could cause a shortfall in normal digestion and absorption of nutrients. They have the ability to chelate with other mineral elements to form insoluble mineral complexes that cannot be utilized by monogastric animals like pigs and poultry birds. The sodium value of 44.00 ± 1.40 mg/100 g noted in pigs fed dietary inclusion of moringa-basil seed and leaf meals in this present study is in accordance with 45 mg/100 g reported by Lawrie and Ledward (2006) for raw pork meat. Potassium, sodium and chlorine have been reported to be vital in the maintenance of osmotic balance between cells and interstitial fluids in farm animals (Soetan *et al.*, 2010). Calcium is essential for bones and teeth development as well required for healthy muscle contraction. It has been reported in the literature that supplementation of poultry ration with *Moringa oleifera* improved eggshell weight and thickness as a result of phytochemical substances inherent in the plant which must have enhanced calcium deposition and intestinal secretions (Nadia *et al.*, 2008; Liu *et al.*, 2020). This present result is in line with the above observation where higher calcium was observed in pork from pigs fed ration containing moringa leaf meal over their counterparts on control and diet containing neem leaf meal. The resultant higher calcium could also be linked to the higher calcium content in moringa over that of neem plants, it has been reported by Mulyaningsih and Yusuf (2018) that dried moringa leaf contains about 3.65% whereas Singh and Maharaj (2011) noted 140.00 mg of calcium per 100 g in neem leaf powder.

Cholesterol is needed for effective nerve, cell membrane and hormonal functioning; however, high cholesterol increases the risk of cardiovascular disease in humans. In the current study, herbal-mix feed additives have shown significant impact on triglyceride and high-density lipoprotein profiles of pork. The reduction in triglyceride contents in pork of pigs fed diets containing herbal-mix feed additives points to the hypo-cholesterolemic function of the plant constituents. The bioactive compounds inherent in the herbal plants could have trigger off the cholesterol-lowering activities by decreasing intestine dietary cholesterol uptake and the rate of its deposition in the tissue (Jain *et al.*, 2010;

Maheshwari *et al.*, 2014). According to the report of Ahmed *et al.* (2010), the lipid-lowering effects and the inhibition of oxidation of low-density lipoprotein of herbal plants were ascribed to the sulphur-containing active constituents of the plants. Also, the high dietary fibre content of the ration with herbal-mix feed additive could as well resulted in a reduction in triglyceride levels in this current study. The increase in high-density lipoprotein contents of pork of pigs on the diet containing moringa-basil leaf meal is in line with the observation of Ekpo and Okon (2022) that reported phenolic content and antioxidant properties of *Jatropha tanjorensis* and *Vernonia amygdalina* reduced total cholesterol and triglyceride whereas increasing high-density lipoprotein in growing pigs. El-Hawy (2018) stated that an increase in the concentration of high-density lipoprotein particles is sturdily connected with declining accretion of atherosclerosis, increasing accumulation of atherosclerosis results in sudden plaque ruptures, cardiovascular diseases, and other vascular diseases.

CONCLUSIONS

Based on the results from this study, it can therefore be concluded that dietary inclusion of herbal-mix leaves meal as feed additives decreased body weight gain, body length and heart girth of pigs but dietary inclusion of moringa-basil leaves meal improved some haematological parameters (red blood cell, haemoglobin, packed cell volume and monocytes) values of pigs, which points to enhanced welfare indices in this set of pigs. Likewise, total bacterial counts, *Escherichia coli* and *Clostridium* spp decreased in pigs on diets with different herbal-mix leaves meal, which points to the pharmacological activities of the plants used in this study. The improvement in sodium, calcium and high-density lipoprotein contents, and lower triglyceride values in pork from pigs on different herbal-mix leaves leaf especially those on moringa-basil leaf meal could lead to higher consumers' acceptability of pork due to its health benefits. It can be recommended that pig farmers can use moringa-basil leaves meal mix as feed additives to improve the welfare and pork quality of pigs.

DATA AVAILABILITY STATEMENT

The data presented in this study are available on request from the corresponding author

DECLARATION OF COMPETING INTEREST

The authors of this manuscript assert that there is no known competing financial interest or personal relationships that could have influenced the results obtained in this study.

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