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Research Article

Influence of blood root leaf meal, scent leaf meal, and bamboo charcoal on haematological, serum biochemical and antioxidant parameters of growing pigs

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Abstract

The indiscriminate use of antibiotic growth promoters contributes to antimicrobial resistance in livestock and humans. Phytogenics are natural alternatives that could improve animal performance without exerting negative effects on the environment, livestock and human health. Therefore, the influence of Blood Root Leaf Meal (BRLM), Scent Leaf Meal (SLM), and Bamboo Charcoal (BC) on haematological, serum biochemical and antioxidant parameters of growing pigs was assessed. Large White x Landrace male pigs (n=48, weighing 28.4±1.46 kg) were allotted to eight treatments (r=3) in a completely randomised design for six weeks. Treatment 1 (control), while treatment 2 to treatment 8 contained 3%BRLM, 5%SLM, 1%BC, 3%BRLM+1%BC, 5%SLM+1%BC, 3%BRLM+5%SLM, and 3%BRLM+5%SLM+1%BC, respectively. At day 42, blood (5 mL) was sampled for haematological, serum biochemical and antioxidant parameters according to standard procedures. Data were subjected to descriptive statistics and ANOVA at α0.05. Pigs fed control had lower packed cell volume (30.50%), haemoglobin (9.65 g/dL), and red blood cells (4.77x10^{12}/L), and higher white blood cells (9.75x10^{9}/L) compared to other treatments. Pigs fed 3%BRLM+5%SLM+1%BC had lower alanine aminotransferase (36.50 U/L), and aspartate aminotransferase (62.00 U/L) which was similar to that of pigs fed 5%SLM+1%BC (70.00 U/L). Higher total antioxidant capacity was recorded for pigs fed 3%BRLM+5%SLM (16.45 μg/dL), while pigs fed control had lower glutathione peroxidase (8.21 units/mg protein). In conclusion, supplementation of blood root leaf meal, scent leaf meal, and bamboo charcoal increased red blood cell, haemoglobin, and packed cell volume values, decreased white blood cell values, and improved superoxide dismutase and glutathione peroxidase activities in pigs.

Keywords: Bamboo charcoal, Blood parameters, Blood root, Growing pigs, Scent leaf

Highlights

- Phytogenics supplementation increased PCV, Hb, and RBC, and decreased WBC of growing pigs
- Supplementation of blood root and scent leaf meals enhanced IgG of growing pigs
- Phytogenics supplementation enhanced GPx and SOD activities in growing pigs

INTRODUCTION

The application of Phytogenic Feed Additives (PFAs) to improve animals' growth in place of antibiotics has gained widespread acceptance in recent years (Alagawany *et al.*, 2016). PFAs are natural bioactive substances derived from plants, including botanicals, herbs and essential oils, which are responsible for the colour and odour of plants (Liu *et al.*, 2018), and their antimicrobial, antiviral, anti-inflammatory, antioxidative, growth-promoting, and immunomodulatory activities in livestock were documented(Yang *et al.*, 2019). The bioactive compounds of PFAs are phenolics (tannins), alkaloids (aldehydes, alcohols, ethers, ketones, lactones, esters, etc.), terpenoids (steroids, mono- and sesquiterpenes) and glycosides (Al-Yasiry and Kiczorowska, 2016).

Chu *et al.* (2013) observed that supplementation of bamboo charcoal at 0.6% improved the growth of pigs. Scent leaf meal supplementation in the diets of broiler chickens enhance growth performance of the birds (Omidiwura *et al.*, 2021). Anyasor *et al.* (2019) reported that blood root exhibited anti-inflammatory activity in an in vitro experiment. Huyghebaert *et al.* (2011) noted that phytogenic feed additives exhibit significant variability in their chemical composition due to biological factors (location and specie of plant), processing (distillation, stabilisation and extraction), and storage conditions (temperature and light). Factors influencing the efficacy of PFAs include the part of the plant used and its physical properties, age of the plant, the genetic composition of the plant, harvest time, extraction method, different dosage used and compatibility with other ingredients in the feed matrix (Yang *et al.*, 2009).

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Haematological and serum biochemical parameters are used by researchers and veterinarians in evaluating the conditions of animals' health (Zhang *et al*., 2022). These could be affected by several factors such as health status, sex, age, and breed of animals (Stevančević, *et al*., 2019). In addition, nutritional strategies such as iron supplementation could influence concentrations of blood parameters (Abeni *et al*., 2018). PFAs are natural, less toxic, contain no residues, and have been certified safe for use both in human and animal nutrition (Li *et al*., 2016).

Many studies have highlighted the efficacy of PFAs; however, there is need to conduct a study to assess the efficacy of combining different PFAs in order to enhance their utilisation in livestock production. In Nigeria, the effect of the combining phytogenics, such as blood root leaf meal, scent leaf meal and bamboo charcoal on pig production, has not been elucidated. This study therefore aimed at assessing the influence of blood root leaf meal, scent leaf meal, and bamboo charcoal on haematological, serum biochemical and antioxidant parameters of growing pigs.

MATERIALS AND METHODS

The feeding trial was conducted at the Pig Unit, Teaching and Research Farm, University of Ibadan, Ibadan, Nigeria (GPS Coordinates: 7.4432°N, 3.9003°E). 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 dried under 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*) 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 done at 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 assigned to one of eight dietary treatments. 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 NRC (1998), and the treatments were as follows: No supplementation (CON); 30 g/kg blood root leaf meal (BRLM); 50 g/kg scent leaf meal (SLM); 10 g/kg bamboo charcoal (BC); 10 g/kg BC + 30 g/kg BRLM; 10 g/kg BC + 50 g/kg SLM; 30 g/kg BRLM + 50 g/kg SLM; 10 g/kg BC + 30 g/kg BRLM + 50 g/kg SLM. The inclusion levels were based on recommendation from previous research works. The pigs were allowed *ad libitum* access to feed and water. All other routine management practices were done following standard practices.

At day 42, blood samples were collected from the jugular vein via vena cava puncture from all the pigs by 8.00 am following an overnight fast into non-heparinised tubes and vacuum tubes containing K_3EDTA to obtain serum and whole blood, respectively. Serum samples were centrifuged at 3000 rpm for 15 minutes at 24°C and the blood samples were stored at –20°C for subsequent analysis. The determination of lymphocyte, mean corpuscular haemoglobin concentration, packed cell volume, mean corpuscular haemoglobin, red blood cell, mean corpuscular volume, white blood cell and haemoglobin concentrations in blood samples was done with the aid of an automated haematology analyser, following the methodology outlined by Ewuola and Egbunike (2008). Additionally, levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, globulin, albumin, and total protein were quantified using an automated biochemical analyser (Shimadzu CL-7200, Shimadzu, Kyoto, Japan).

Serum concentrations of Immunoglobulin G (IgG) were measured using a swine-specific enzyme linked immunosorbent assay. The assay was performed in duplicate, and absorbance readings were obtained using a microplate reader. Additionally, serum levels of total antioxidant capacity, superoxide dismutase(SOD), glutathione transferase (GST), glutathione peroxidase (GPx), and malondialdehyde (MDA) were quantified using commercially available enzyme kits(Ransel, RANDOX/RS-504; Randox Laboratories, Crumlin, United Kingdom).

The data were analysed using the General Linear Model (GLM) procedure of SAS version 9.4 (SAS, 2020). Differences among treatment groups were evaluated using Duncan's Multiple Range Test (Duncan, 1955), with statistical significance set at $p < 0.05$.

RESULTS

Haematological responses of growing pigs fed diets supplemented with blood root leaf meal, scent leaf, and bamboo charcoal are presented in Table 1. Significant (P<0.05) differences were observed among the treatments for all the haematological parameters with the exception of MCH. Pigs fed CON diet had lower PCV (30.50%), haemoglobin (9.65 g/dL), and RBC (4.77 x10^{12}/L) values compared to other treatments. However, pigs fed CON diet had higher WBC (12.13 x10^9/L) compared to other treatment groups. Pigs fed 3% BRLM diet had higher lymphocyte (53.50%) compared to other treatments. However, lower neutrophil was recorded for pigs fed 3% BRLM diet (40.00%) which was statistically similar to that of pigs fed CON diet (43.50%). MCH was comparable among all treatment groups.

Serum biochemistry and immunoglobulin G concentration of growing pigs fed diets supplemented with blood root leaf meal, scent leaf, and bamboo charcoal is presented in Table 2. Significant (P<0.05) differences were observed among the treatments for ALT, ALP, BUN and IgG. Similarly, pigs fed % BRLM + 5% SLM + 1% BC diet had lower ALT (36.50 μ/L) compared to other treatments. However, lower IgG was recorded for pigs fed CON (1.66 mg/L) and 1% BC (1.76 mg/L) diets, which was similar to that of pigs fed 3% BRLM+1% BC diet (2.30 mg/L).

Serum antioxidant capacity of growing pigs fed diets supplemented with blood root leaf meal, scent leaf, and bamboo charcoal is presented in Table 3. Significant (P<0.05) differences were observed in all the serum antioxidant parameters analysed. Pigs fed 3% BRLM + 5% SLM diet had higher total antioxidant capacity (16.45 μg/dL) compared to other treatment groups. Furthermore, pigs fed 5% SLM had higher GST concentration (68.89 units/mg protein) compared to other treatment groups. However, pigs fed CON diet had lower GPx (8.21 units/mg protein) and SOD (1.23 units/mg protein) compared to other treatment groups.

DISCUSSION

Haematological and serum biochemical parameters are used by researchers and veterinarians in evaluating the conditions of animals' health (Zhang *et al*., 2022). Elevated red blood cells levels were observed with dietary supplementation of blood root leaf meal, scent leaf and bamboo charcoal in the present study. Similar observation was documented when *Coptis chinesis* was supplemented at 1.0 g/kg in the diets of pigs as red blood cells levels of the animals were increased (Zhou *et al*., 2013). The increased RBC production could be attributed to the presence of flavonoid and alkaloid in

Table 1. **Haematological responses of growing pigs fed phytogenic-supplemented diets**

Treatments	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*
PCV (%)	30.50^{c}	34.00^{ab}	33.00^{b}	34.00^{ab}	36.50^{a}	35.00^{ab}	35.00^{ab}	35.00^{ab}	0.36	0.001
Hb (g/dL)	9.65^{c}	10.75^{ab}	10.50^{b}	10.70^{ab}	11.60^{a}	11.25^{ab}	11.40^{ab}	11.30^{ab}	0.13	0.001
RBC (x10^{12}/L)	4.77^{c}	5.54^{ab}	5.27^{b}	5.22^{b}	5.86^{a}	5.87^{a}	5.64^{ab}	5.56^{ab}	0.07	0.000
WBC (x10^9/L)	9.75^{a}	6.55^{b}	6.25^{b}	5.75^{b}	6.28^{b}	5.20^{b}	6.90^{b}	5.70^{b}	0.26	0.000
PLAT (x10^9/L)	128.50^{a}	117.00^{b}	120.00^{ab}	106.00^{c}	131.00^{a}	113.50^{bc}	105.50^{c}	120.50^{ab}	1.74	0.000
LYM (%)	49.00^{b}	53.50^{a}	47.50^{bc}	48.00^{b}	46.50^{bc}	46.50^{bc}	44.00^{c}	46.00^{bc}	0.55	0.000
NEUT (%)	43.50^{bc}	40.00^{c}	45.00^{b}	50.50^{a}	46.00^{ab}	46.00^{ab}	50.00^{a}	47.50^{ab}	0.55	0.000
MON (%)	3.00	3.50	2.50	3.00	3.00	3.50	2.50	2.50	0.11	0.061
EOS (%)	4.50^{ab}	3.00^{d}	5.00^{a}	3.00^{d}	4.50^{ab}	4.00^{bc}	3.50^{cd}	4.00^{bc}	0.14	0.000
MCV (fl)	64.17^{ab}	61.38^{cd}	62.62^{bc}	65.13^{a}	62.51^{bc}	59.92^{d}	62.15^{bcd}	62.97^{abc}	0.33	0.001
MCH (pg)	20.34	19.41	19.93	20.50	19.88	19.24	20.25	20.32	0.15	0.335
MCHC (g/dL)	31.68	31.61	31.82	31.47	31.79	32.13	32.58	32.27	0.10	0.081

^{a,b,c,d} Means in the same row with different superscripts differ (P<0.05); CON = control diet; BRLM = blood root leaf meal; SLM = scent leaf meal; BC = bamboo charcoal; SEM = standard error of mean; PCV = packed cell volume; Hb = haemoglobin; RBC = red blood cells; WBC = white blood cells; PLAT = platelet; LYM = lymphocyte; NEUT = neutrophil; MON = monocyte; EOS = eosinophil; MCV = mean corpuscular volume; MCH = mean corpuscular haemoglobin; MCHC = mean corpuscular haemoglobin concentration.

Table 2. Serum biochemistry of growing pigs fed phytogenic-supplemented diets

Treatments	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*
TP (g/dL)	8.65	8.15	8.35	8.55	8.60	8.30	8.70	7.90	0.10	0.548
ALB (g/dL)	3.00	2.95	3.00	3.05	3.00	2.90	3.05	2.75	0.06	0.923
GLO (g/dL)	5.65	5.20	5.35	5.50	5.60	5.40	5.65	5.15	0.06	0.183
A.G. Ratio	0.53	0.57	0.56	0.55	0.54	0.54	0.53	0.53	0.01	0.418
AST (μ/L)	74.00	72.50	71.50	73.50	74.50	70.00	73.50	62.00	1.08	0.067
ALT (μ/L)	44.00^{a}	39.50^{b}	41.50^{ab}	44.00^{a}	43.50^{a}	41.50^{ab}	42.50^{a}	36.50^{c}	0.46	0.000
ALP (μ/L)	154.00^{a}	142.50^{b}	153.50^{a}	156.50^{a}	155.00^{a}	150.50^{a}	154.00^{a}	143.50^{b}	1.03	0.000
BUN (mg/dL)	9.55	9.10	9.50	9.75	10.25	9.80	10.00	9.25	0.12	0.254
CRT (mg/dL)	1.05	0.95	0.95	1.05	1.10	0.95	1.15	1.10	0.03	0.652
IgG (mg/L)	1.66^{c}	2.85^{ab}	2.94^{ab}	1.76^{c}	2.30^{bc}	3.50^{a}	3.12^{ab}	2.71^{ab}	0.14	0.001

^{a,b,c} Means in the same row with different superscripts differ (P<0.05); CON = control diet; BRLM = blood root leaf meal; SLM = scent leaf meal; BC = bamboo charcoal; SEM = standard error of mean; TP = total protein; ALB = albumin; GLO = globulin; A.G. Ratio = Albumin/globulin ratio; AST = aspartate aminotransferase; ALT = alanine aminotransferase; ALP = alkaline phosphatase; BUN = blood urea nitrogen; CRT = creatinine; IgG = immunoglobulin G

Table 3. Serum antioxidant capacity of growing pigs fed phytogenic-supplemented diets

Treatments	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*
TAC (μg/dL)	13.51^{c}	14.22^{bc}	15.19^{b}	13.96^{bc}	13.44^{c}	14.36^{bc}	16.45^{a}	13.67^{c}	0.19	0.000
GPX (units/ mg protein)	8.21^{d}	11.15^{ab}	10.39^{bc}	9.92^{c}	11.36^{a}	11.74^{a}	11.26^{a}	11.89^{a}	0.19	0.000
GST (units/ mg protein)	43.66^{de}	53.85^{bc}	68.89^{a}	50.33^{bcd}	45.32^{cde}	38.27^{e}	42.39^{de}	56.67^{b}	1.64	0.000
MDA (μmol/ mg protein)	1.44	1.81	2.52	2.05	2.25	2.10	2.27	2.01	0.09	0.069
SOD (units/ mg protein)	1.23^{c}	3.52^{b}	6.02^{a}	5.55^{ab}	4.23^{ab}	4.56^{ab}	4.48^{ab}	6.03^{a}	0.31	0.000

^{a,b,c,} Means in the same row with different superscripts differ (P<0.05); CON = control diet; BRLM = blood root leaf meal; SLM = scent leaf meal; BC = bamboo charcoal; SEM = standard error of mean; TAC = total antioxidant capacity; GPx = glutathione peroxidase; GST = glutathione transferase; MDA = malondialdehyde; SOD = superoxide dismutase

the *J. secunda* and scent leaf which could have enhanced erythropoietin synthesis in the kidney. Erythropoietin is a glycoprotein produced by the endothelial cells of the kidney's peritubular capillary which regulates erythropoiesis (Zhou *et al*., 2013). White blood cells play a critical role in immune response. An increase in WBC level could suggest an elevated inflammatory reaction to where the injury has occurred (Chen *et al*., 2017), although nutrition and exercise might also contribute to it (Czech *et al*., 2017). Blood root leaf, scent leaf, and bamboo charcoal exhibited immunomodulatory effect in this study as WBC levels were decreased with their supplementation in the diets of the pigs.

Alanine aminotransferase and aspartate aminotransferase levels provide information tissue injury and are estimated to diagnose liver damage (Hashem *et al*., 2019). Stress arising from inflammation and oxidation of cells, could damage liver cells. This could cause the enzymes of the cytoplasm to leak into the blood, thereby increasing the activities of plasma enzymes. The combination of blood root leaf meal, scent leaf meal and bamboo charcoal in the diets of pigs reduced the ALT levels of the animals in comparison with the control group. The hepato protective activity of blood root and scent leaf could be due to the bioactive compounds in the leaves which exhibited anti oxidative effect against damage of tissue by reactive oxygen species.

Blood creatinine and urea levels are determined to evaluate the functioning of the kidney; higher levels suggest reduced ability of the kidney to excrete waste products, implying kidney damage (Chinnappan *et al*., 2019). Although, polyphenols of plants have been reported to exhibit chemoprotective activity against damage of the kidney (Tungmunnithum *et al*., 2018), the blood creatinine and urea levels obtained from the phytogenic supplementation group were comparable to the control group. Serum immunoglobulin content can be an indicator for assessing the host's immune function. Immunoglobulin G can bind specifically to corresponding antigens and serves as the major elements of humoral immunity. Enhanced IgG levels was recorded with dietary supplementation of phytogenics except for pigs fed 1%BC. Similarly, essential oils supplementation in the diets of pigs was observed to elevate serum IgG level (Zeng *et al*., 2015). Nimmerjahn and Ravetch (2010) suggested that active compounds present in PFAs (such as terpenoids and phenols) can serve as extra ligands of Fc receptors, attaching to Immunoglobulin G, thereby triggering immune response. However, (Zhou *et al*., 2013) reported that *Coptis chinesis* supplementation in the diets of growing pigs had no influence on IgG levels of the animals.

There is a continuous production of (reactive oxygen species) ROS in cells during metabolism-related activities, irrespective of the stress status of the animal. There are mechanisms in place to regulate the level of ROS in the systems of animals, keeping oxygen radicals' formation and neutralisation in equilibrium (Barbosa *et al*., 2020). Antioxidative enzymes, including SOD and GPx, facilitate the conversion of oxygen radicals into molecular oxygen and water, thereby modulating gut health and enhancing the capacity of microvilli to absorb nutrients (Surai, 2016). Supplementation of blood root leaf meal, scent leaf meal and bamboo charcoal in the diets of growing pigs enhanced the activities of SOD and GPx.

Glutathione peroxidase is a key antioxidative enzyme that facilitates the conversion of reduced glutathione to oxidized glutathione, thereby converting lethal peroxides to harmless hydroxyl molecules and enhancing the dissolution of hydrogen peroxide. Consequently, the structural integrity and operations of cell membranes are protected from the destructive activities of peroxides (Ma *et al*., 2021). Similarly, there was an increased activity of SOD when the diets of stressed pigs were supplemented with oregano extract (Zhang *et al*., 2015). Synergistic effect was observed when blood root leaf meal and scent leaf meal were combined as total antioxidant capacity was increased. This could be due to the presence of phenols in blood root leaf and scent leaf which could serve as hydrogen donor to the ROS radicals, thereby hindering the formation of hydrogen peroxide.

In conclusion, phytogenic supplementation of blood root leaf meal, scent leaf meal, and bamboo charcoal increased red blood cell, haemoglobin, and packed cell volume values of the pigs while white blood cell values were decreased. Supplementation of blood root leaf meal and scent leaf meal enhanced serum immunoglobulin G level. Furthermore, phytogenic supplementation improved superoxide dismutase and glutathione peroxidase activities in pigs. In this study, the effects of different combinations of three PFAs on pig production were evaluated, but their interaction with other commonly used feed additives was not assessed. Future research could focus on exploring the synergistic effects of combining PFAs with probiotics, prebiotics, and organic acids.

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Author's contribution: EAA: Execution of study, data collection, data analysis, and preparation of draft manuscript; BROO: Design and conceptualisation of study and proof reading of manuscript; AFA: Methodology outline, supervision, and results interpretation.

Data availability statement: Data generated from the study is available upon request from the corresponding author.

Ethical approval: University of Ibadan Animal Care and Use Research Ethics Committee approved experimental procedures before the commencement of the study (Approval ID: 23/067).

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