

HAEMATOLOGICAL AND SERUM BIOCHEMICAL INDICES OF GROWING PIGS FED VARYING LEVELS OF BENISEED (*Sesamum indicum* L.) HULL IN REPLACEMENT FOR MAIZE

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ABSTRACT

A feeding trial was conducted to evaluate the nutrient content of beniseed hull (BSH) as a substitute for maize, and its effect on haematological and serum biochemical indices of growing pigs. Sixty 8-weeks old growing pigs (Landrace x Large white) were randomly allotted into five dietary treatments with three replicates of four animals per replicate in a completely randomized design. Five diets were formulated by incorporating BSH into the basal diet to replace maize at 0, 25, 50, 75 and 100% designated as D1 (control), D2, D3, D4 and D5, respectively and fed to growing pigs for ten weeks. Diets had no significant effect on the parameters measured, except White blood cell (WBC) and Lymphocytes. The highest WBC count ($\times 10^9/L$) ($P < 0.05$) of 23.70 was obtained in pigs fed 50% BSH-based diets compared with 15.55 (0% BSH), 14.00 (25% BSH), 21.45 (75% BSH) and 15.10 (100% BSH), respectively. The lymphocyte counts of pigs fed 25% BSH (60.00%) and 75% BSH (76.50%) were statistically similar to those on other dietary treatments. The cholesterol values recorded in pigs fed with the control diet (0% BSH) (125.00 mg/dL) and 25% BSH (122.50 mg/dL) were similar but significantly ($P < 0.05$) reduced to 119.50 mg/dL (50% BSH), 118.00 mg/dL (75% BSH) and 104.00 mg/dL (100% BSH), respectively, as the level of BSH increases. The High-density lipoprotein, HDL value (20.95 mg/dL) of pigs fed with control diet (0% BSH) was significantly ($P < 0.05$) lower than HDL values of pigs placed on other diets. The total protein (g/dL) 7.08 (0% BSH), 7.11 (25% BSH), 7.00 (50% BSH) were similar ($P > 0.05$) but significantly ($P < 0.05$) higher than 6.81 (75% BSH) and 6.66 (100% BSH). Beniseed hull poses no health challenge to the growing pigs as depicted by normal haematological and serum biochemical indices including lowest cholesterol at 100% BSH; beniseed hull could safely replace maize in pig's diets up to 100% level.

Key words: Beniseed hull, Performance, Haematology, Biochemical indices, Feeding trials, Grower pigs



INTRODUCTION

The use of unconventional feed ingredients could serve as alternatives to ameliorate the high cost of animal feed in Nigeria. Beniseed hull (BSH), a crop residue resulting from dehulling of sesame seeds is readily available and unlike maize, less competitive between man and animal. In many states of Nigeria, it is often treated as waste and discarded. Agiang *et al.*[1] reported that sesame seeds are rich in oil, containing high quality oleic and linoleic acids, 25% protein, rich in methionine and tryptophan. Treated forms of BSH have been used for livestock feeding at graded levels with no adverse effects on performance and carcass [2, 3]. Sesame seed hulls can be used up to 10% of broiler feed with possible benefits on animal performance without any negative effects on carcass and meat quality [4]. Reis de Souza *et al.*[5] reported that sesame meal can be used as a protein source for piglets and its consumption increases arginine intake; addition of phytase to the diet increases the apparent total tract digestibility of P and Ca. The best indicator of animal's wellbeing and its potential for production is its health status, which can be assessed through its haematological profile[6].

Haematological profiles are known to be influenced by diets. Blood examination gives the opportunity to investigate the presence of several metabolites and other constituents and helps detect conditions of stress, which can be nutrition, environmental or physical [7]. There is paucity of information on haematological and biochemical values of pigs fed BSH in place of maize in the diets. This study was conducted to evaluate the effect of feeding graded levels of beniseed hull in replacement for maize on haematological and biochemical parameters of growing pigs.

MATERIALS AND METHODS

Site of the experiment

The experiment was carried out at the Piggery Unit of the Teaching and Research Farm of Joseph Ayo Babalola University, Ikeji- Arakeji, Osun State, Nigeria. Ikeji-Arakeji is situated on 350.52 m above sea level at latitude 7° 25'N and longitude 5° 19'E. The area is of the rainforest vegetation characterized by hot and humid climate. The mean annual rainfall is 1524mm. The atmospheric temperature ranges between 28°C and 31°C and mean annual relative humidity is 80% [8].

Experimental animals, diets, management and design

Sixty pigs (Landrace × Large White) of about eight weeks old were purchased from a reputable commercial farm in Owo, Ondo State, Nigeria. The pigs were individually weighed and assigned to five dietary treatments of three replicates of four animals each in a completely randomized design. Pigs of each replicate were housed on cemented floor pen. The initial live weight of the growing pigs ranged between 7.33-7.67kg. Five experimental diets were prepared as presented in Table 1. Beniseed hull (BSH) was incorporated into the basal diet to replace maize at 0, 25, 50, 75 and 100%, designated as D1 which served as control, D2, D3, D4 and D5, respectively. Other ingredients in the experimental diets include Groundnut cake (GNC), Soyabean Meal, Palm kernel Cake (PKC), Brewer Dry Grain (BDG), Bone meal, Oyster shell, Growers' premix and Table Salt.



The pigs were fed graded levels of beniseed hull-based diets for the two weeks' pre-experimental period for acclimatization and thereafter, for ten weeks during which data were collected. Medications were administered routinely as scheduled and feed and water supplied to the pigs *ad libitum*.

Data collection

Data were collected on haematological and biochemical indices of the experimental animals. At the end of the 10 weeks feeding trial, thirty (30) animals at the rate of six per treatment were randomly selected for haematological and biochemical indices assay.

Blood sample collection and analysis

Blood samples for haematological and biochemical indices were collected from randomly selected pigs from each of the replicates using sterile bottles containing anti-coagulant (EDTA- ethylene diamine tetra-acetic acid) and other blood samples were collected without anti-coagulants for the determination of serum biochemical indices. The blood samples were analyzed for Red Blood Cells (RBC), Packed Cell Volume (PCV), Haemoglobin (Hb), Lymphocytes (LYM), Neutrophils (NEU), Monocytes (MONO) and Eosinophils (EOS). From the RBC, PCV and Hb concentration values, the mean cell volume (MCV), Mean cell haemoglobin (MCH) and Mean cell haemoglobin concentration (MCHC) were calculated. The coagulated blood samples were also subjected to standard serum separation for Total Protein (TP), and the determination of Alanine amino transferase (ALT), Aspartate aminotransferase (AST), Bilirubin, Creatinine, Cholesterol and Highdensity lipoprotein (HDL) as described by Sirios [9].

Haematology parameters

Haemoglobin concentration determination

Calorimetric method [10] was used for the determination of haemoglobin. One gramme (1g) of pure haemoglobin was ground in a porcelain mortar and 0.19g of it was weighed and dissolved in 100ml of distilled water using a glass rod to stir continuously until all the haemoglobin had been thoroughly dissolved. This solution is the haemoglobin standard. Thereafter, 0.02ml of each of the blood samples was put into labeled test tubes containing 4ml of Drabkins solution. To flush out the blood completely, Drabkins solution was poured repeatedly into the pipette and ejected until a complete flushing was observed. The test tubes were covered and the contents thoroughly mixed and left to stand for 5 minutes to allow full colour development. A standard solution was prepared the same way using haemoglobin standard. The calorimeter set at 540nm to read the samples. Haemoglobin concentration was determined by measuring absorbance at 540nm. A blank solution was inserted and the full scale zero was set. The standard dilution was read and recorded before each of the samples was inserted one after the other and their readings recorded accordingly. Haemoglobin concentration was calculated using the following formula:



$$\text{Haemoglobin Concentration (g/100ml)} = \frac{\text{Reading of test}}{\text{conc}} \times \text{Haemoglobin standard.}$$

Red blood cell (RBC) count

For the determination of RBC, properly mixed blood sample from EDTA bottles was drawn to 0.5 mark of a red cell pipette. The tip of the pipette was immersed and carefully drawn to the saline at exactly 10 marks; the diluted blood was mixed by shaking for about half a minute. About a quarter of the content was expelled before filling the counting chamber. The counting chamber was allowed to stand for about a minute to settle the red cell after filling. All the red cells were then counted using the microscope (x 40 objectives and x 8 eye piece) with the aid of a counter.

Calculation of absolute values

From the RBC counts, PCV and Hb concentrations, the following absolute values were calculated.

a. Mean cell volume (MCV)

This expressed the average red cell volume measured in cubic microns (μ^3) and calculated as the product of 10 and the ratio of PCV (%) to RBC (per mm^3).

$$\text{MCV } (\mu^3) = \frac{\text{PCV}}{\text{RBC}} \times 10$$

b. Mean cell haemoglobin (MCH)

This expresses the amount of haemoglobin in one red cell. The measurement is in pictograms (pg), while it was calculated as the product of 10 and the ratio of Hb (gm/100ml of blood) to RBC (per mm^3).

$$\text{MCH (pg)} = \frac{\text{Hbc}}{\text{RBC}} \times 10$$

c. Mean cell haemoglobin concentration

This represents the concentration of haemoglobin in the red cells, as compared to the concentration of haemoglobin in 100ml of whole blood. The MCHC was calculated by expressing the haemoglobin concentration of the blood as a percentage (%) of its PCV.

$$\text{MCHC (\%)} = \frac{\text{Hbc}}{\text{MCH}} \times 100 \text{ or } = \frac{\text{MCH}}{\text{PCV}} \times 100$$

Differential white blood count

Benjamin method [10] was adopted in WBC count determination. The smear of each blood sample in the bottle containing EDTA was made on a clean slide and air-dried for three minutes. The smear was stained in Giesma stain for 30 minutes, rinsed in water and air-dried, and the cell count for each sample was carried out. During the process, the slide was viewed under the microscope using x 100 objective lens. The first one hundred cells were counted. From the cells counted, the percentages of the



different cells (neutrophils, eosinophils, lymphocytes and monocytes) present were determined.

Chemical analysis

The chemical composition of beniseed hull (BSH) and experimental diets with respect to proximate composition was evaluated at the Animal Care Konsult Research Laboratory in Ogere, Ogun State, Nigeria This was according to the method of Association of Official Analytical Chemist [11] viz-a-viz AOAC Official Methods 988.05, 2003.06, 942.05, 958.06, 967.08 respectively for the determination of crude protein, crude fat, Ash, crude fibre and moisture. The NFE was by difference and metabolizable energy value according to the standard procedure [12].

Statistical analysis

Data obtained were subjected to analysis of variance using SAS statistical package [13] and mean separated using Duncan's Multiple Range Test (DMRT) of the same statistical software.

RESULTS AND DISCUSSION

Weights of the experimental animals

The initial live weight of the growing pigs ranged from 7.33-7.67kg. The starting average live weights (kg) per treatment were 7.67 (0% BSH), 7.33 (25% BSH), 7.33 (50% BSH), 7.33 (75% BSH), and 7.67 (100% BSH); while the final live weights (kg) were 30.67 (0% BSH), 30.00 (25% BSH), 29.33 (50% BSH), 27.33 (75% BSH), and 28.00 (100% BSH).

Proximate and metabolisable energy compositions of the experimental diets and BSH

The proximate and energy values of the experimental diets are presented in Table 1. The crude protein (%) increased from 20.27 for the control diet (0% BSH) to 20.54 (25% BSH), 20.80 (50% BSH), 21.06 (75% BSH) and 21.32 (100% BSH). The crude fibre followed the same trend. The metabolisable energy (kcal/kg) were 3047.26, 3006.45, 2965.65, 2924.86 and 2888.05, respectively for 0, 25, 50, 75 and 100% BSH-based diets. Table 2 shows the chemical composition of beniseed hull based on dry matter weight with crude protein of 11.82%, crude fibre (22.15%), Ash (19.95%), ether extract or fat (24.40%) and dry matter (96.04). The metabolisable energy value is 3155.00 kcal/kg DM. The crude protein and crude fibre increased and caloric components of the experimental diets decreased as BSH increased in the diets. The trends were reflections of the contents of nutrients and metabolisable energy of the beniseed hull and maize used in the formulation of experimental diets. The experimental diets were formulated to meet the nutrient requirements of growing pigs as recommended [14, 15]. The crude protein, ether extract, crude fibre, ash and metabolisable energy values obtained for the beniseed hull in this study were higher than the values obtained by others [16].



Haematological parameters of growing pigs fed graded levels of beniseed hull

Haematological parameters of the experimental animals are presented in Table 3. Diets had no significant effect on the parameters measured, except White blood cell (WBC) and Lymphocytes. The lowest WBC ($\times 10^9/L$) of 14.00 was obtained in pigs fed 25% BSH-based diets compared with 15.55 (0% BSH), 23.70 (50% BSH), 21.45 (75% BSH) and 15.10 (100% BSH), respectively. Lymphocyte counts of pigs placed on 75% BSH (76.50%) were significantly ($P < 0.05$) higher than those on 0% BSH (63.00), 25% BSH (60.00%), 50% BSH (64.00%), and 100% BSH (64.50%), respectively. Other haematological parameters, the Packed Cell Volume (PCV), Haemoglobin (Hb), Red Blood Cell (RBC), Neutrophils, Monocytes, Eosinophils, Mean Cell Volume (MCV), Mean Cell Haemoglobin (MCH), and Mean Cell Haemoglobin Concentration (MCHC) were not significantly ($P > 0.05$) affected by the dietary treatments. The WBC ($7.80-20.70 \times 10^9/L$) Lymphocytes (41.30-68.50%), Cholesterol (76.0-174.0 mg/dL) and Total Protein (4.80-10.30 g/dL) recommended for normal pigs compares favourably with the present study [17]. The nonsignificant values of most of the haematological parameters could indicate that the diets were safe for the experimental growing pigs. There is the possibility of residual anti-nutrients and high fibres in BSH responsible for the elevation of WBC and lymphocytes in D3 and D4. The increased crude fibre values with BSH in the diets did not cause significant decrease in weight gain across the dietary treatments. Navarro *et al.* [18] reported that the contribution of digestible and metabolizable energy from high-fibre. dietary ingredients was not affected by inclusion rate in mixed diets to growing pigs. Sesame meal contains 2.15% tannins [19]. The elevated WBC could be linked to the need for defense. High WBC may indicate that the immune system is working to destroy an infection. Elevation of WBC (leucocytes) in broilers was linked to challenge of the body by capsaicin in dried hot red pepper [20]. The elevated WBC was also linked with improved performance at a lower cost. These antibodies can fight foreign bodies, in the present study the residual anti-nutritional factors in BSH as the foreign bodies. The values of PCV, RBC, Haemoglobin and Eosinophil were not affected by the dietary treatments, which agrees with earlier reports [21]. The values for PCV, Haemoglobin and lymphocytes obtained in this study were similar to reported values [22]. The values for WBC, MCV and MCH were, however, higher than the values reported by the authors [23].

Serum biochemical indices of growing pigs fed graded levels of beniseed hull

Results of the serum biochemical parameters (Table 4) showed that the Cholesterol, High-density lipoprotein "HDL" and Total protein were significantly ($P < 0.05$) affected by the inclusion of BSH in the diets. The cholesterol level (125.00 mg/dL) recorded in pigs fed with the control diet (0% BSH) significantly ($P < 0.05$) reduced to 122.50 mg/dL (25% BSH), 119.50 mg/dL (50% BSH), 118.00 mg/dL (75% BSH) and 104.00 mg/dL (100% BSH) across the dietary treatments. The HDL value (20.95 mg/dl) of pigs fed with control diet (0% BSH) was significantly ($P < 0.05$) lower than those on other treatments. Other authors [24] reported that total protein and albumin are indicators of the total protein reserve in an animal for productive body functions. In this study, the total protein (g/dL) for treatments 0% BSH (7.08), 25% BSH (7.11) 50% BSH (7.00) were similar ($P > 0.05$) but significantly ($P < 0.05$) higher than those for 75% BSH (6.81) and 100% (6.66). However, alanine amino transferase (ALT), aspartate



amino transferase (AST), bilirubin and creatinine were not significantly ($P>0.05$) affected by the dietary treatments. Ileal digestibility of most of its amino acids declined as the inclusion of sesame oil meal increased in pig diets [24]. This agrees with other reports [21]. The values of bilirubin (0.14 – 0.61mg/dL) in the present study compares with the range (0 -10 mg/dL) reported by Susan [25]. Susan [25] reported higher ALT (55-78U/L), AST (95 – 153.4U/L), cholesterol (103 – 145 mg/dl); and lower creatinine (0.29 – 0.38 mg/dL) and total protein (5.55 – 7.28 mg/dL) than the respective values in the present study. The variations observed in the serum biochemical indices from other findings could have been occasioned by differences in diets, breed and environment.

CONCLUSION

Results of the study showed that beniseed hull (BSH) may not pose health challenges to the growing pigs as depicted by normal haematological and serum biochemical indices with reduced cholesterol on 100% BSH-based diet. Beniseed hull could, therefore, replace maize in pigs' diets up to 100% level without any deleterious effects.



Table 1: Gross composition of experimental diet (%)

Ingredients	Beniseed Hull inclusion level (%)				
	D1 0.0% BSH	D2 25.0%BSH	D3 50.0% BSH	D4 75.0% BSH	D5 100.0% BSH
Maize	58.50	43.87	29.25	14.63	0.00
Beniseed hull	0.00	14.63	29.25	43.87	58.50
SBM	10.00	10.00	10.00	10.00	10.00
GNC	11.00	11.00	11.00	11.00	11.00
BDG	14.00	14.00	14.00	14.00	14.00
Fish Meal	2.00	2.00	2.00	2.00	2.00
Bone Meal	1.00	1.00	1.00	1.00	1.00
Oyster shell	1.00	1.00	1.00	1.00	1.00
Grower Premix*	0.25	0.25	0.25	0.25	0.25
Methionine	0.25	0.25	0.25	0.25	0.25
Lysine	0.25	0.25	0.25	0.25	0.25
Table Salt	0.25	0.25	0.25	0.25	0.25
Vegetable Oil	1.50	1.50	1.50	1.50	1.50
Total	100.00	100.00	100.00	100.00	100.00
Calculated nutrients					
CP (%)	20.27	20.54	20.80	21.06	21.32
ME (Kcal/kg)	3047.26	3006.45	2965.65	2924.86	2884.05
CF (%)	5.19	8.15	11.10	14.05	17.01

*Contained vitamins A (10,000,000.00 IU); D3 (2,000,000.00 IU); E (20,000.00mg); K3 (2000.00mg); B1 (3000.00mg); B2 (5000.00mg); Niacin (45,000.00mg); Calcium pantothenate (10,000.00mg); B12 (20.00mg); Choline Chloride (300,000mg); Folic Acid (1000.00mg); Biotin (50.00mg); Manganese (300,000.00); Iron (120,000.00mg); Zinc (80,000.00mg); Copper (8,500.00mg); Iodine (1500.00mg); Cobalt (300.00mg); Selenium (120.00mg); Antioxidant (120,000.00) per 2.5kg. BSH = beniseed hull; SBM = soyabean meal; GNC = groundnut cake; BDG = brewer dried grain; D1-D5 = Diets; CP = crude protein; CF = crude fiber, ME = metabolisable energy

Table 2: Chemical composition of beniseed hull based on DM weight

Components	Values (%)
Crude Protein	11.82
Crude Fat	24.40
Dry matter	96.04
Crude fibre	22.15
Ash	19.95
Nitrogen free extract	17.72
Metabolisable energy (kcal/kg DM)	3155.00
Calculated values	
Dry matter	96.04
Organic matter	80.04
Protein/Fat	0.48
Fatty acid	19.52

Table 3: Haematological parameters of pigs fed varying levels of beniseed hull as replacement for maize

Parameters	Beniseed Hull inclusion level (%)					SEM
	D1	D2	D3	D4	D5	
	0.00%	25.00%	50.00%	75.00%	100.00%	
	BSH	BSH	BSH	BSH	BSH	
PCV (%)	47.00	49.00	48.00	45.50	48.50	0.71
Hb (g/dL)	15.60	16.30	15.95	15.25	16.15	0.24
WBC (x10 ⁹ /L)	15.55 ^{ab}	14.00 ^b	23.70 ^a	21.45 ^{ab}	15.10 ^{ab}	1.24
RBC(x10 ¹² /L)	5.31	5.45	5.45	5.00	5.40	0.09
Neutrophil(%)	34.50	35.50	33.00	22.00	33.00	1.73
Lymph (%)	63.00 ^{ab}	60.00 ^b	64.00 ^{ab}	76.50 ^a	64.50 ^{ab}	1.83
Monocytes(%)	2.00	2.00	3.00	1.50	2.00	0.31
Eosinophil(%)	0.50	0.00	0.00	0.00	0.50	0.11
MCV(fl)	88.30	89.85	88.15	91.10	89.70	0.56
MCH (pg)	29.30	29.85	29.30	30.50	29.85	0.02
MCHC (%)	33.15	33.25	33.20	33.45	33.25	0.04

^{ab}: Means within the same row with different superscripts differ significantly (P<0.05). PCV packed cell volume, Hb haemoglobin, WBC white blood cells, RBC red blood cells, Lymph lymphocytes, MCV mean corpuscular volume, MCH mean corpuscular haemoglobin, and MCHC mean corpuscular haemoglobin concentration.

Table 4: Serum biochemical indices of pigs fed varying levels of beniseed hull as a replacement for maize

Parameters	Beniseed Hull inclusion level					SEM
	(%)					
	D1	D2	D3	D4	D5	
	0.00%	25.00%	50.00%	75.00%	100.00%	
	BSH	BSH	BSH	BSH	BSH	
ALT (U/L)	65.20	71.40	68.97	69.30	70.76	0.18
AST (U/L)	124.65	122.35	123.25	131.05	129.70	6.29
Bili (mg/dL)	0.61	0.41	0.37	0.14	0.16	0.06
Creat(mg/dL)	0.38	0.43	0.38	0.29	0.27	0.02
Chol(mg/dL)	125.00 ^a	122.50 ^{ab}	119.50 ^b	118.00 ^b	104.00 ^c	3.87
HDL(mg/dL)	20.95 ^b	28.65 ^a	26.95 ^a	27.15 ^a	27.45 ^a	0.81
TP (g/dL)	7.08 ^a	7.11 ^a	7.00 ^a	6.81 ^b	6.66 ^b	0.18

^{abc}: Means within the same row with different superscripts differ significantly (P<0.05). ALT- alanine amino transferase, AST- aspartate aminotransferase, Bili- bilirubin, Creat -creatinine, Chol -cholesterol, HDL- high-density lipoprotein, TP- Total protein

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