Composite leaf meal: effects on haematology and biochemical indices of growing pigs

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SUMMARY

Twenty four weaner-pigs were used for this trial designed to study the effects of using composite leaf meal (product of mixture of five locally available tropical leafy vegetables: *Moringa oleifera, Ocinum gratissimum, Manihot esculenta, Telfaria occidentalis a*nd *Vernonia amygdalina*) as premix in the diets of pigs on their haematology and biochemical indices. Six diets were formulated in which composite leaf meal was added at 0, 10, 20, 30, 40 and 50g/kg to replace the commercial premix. The pigs were then assigned to these 6 dietary treatments of four replicates at one pig per replicate in a completely randomized design. The diets were fed to the pigs at 5% of their body weight throughout the period of the experiment while water was supplied *ad libitum*. At the end of the experimental period, blood samples was collected via the right saphenous vein for haematological studies and blood samples was collected at slaughtering for biochemical studies. All data were subjected to analysis of variance. Results shows that the most haematological and biochemical parameters were not influenced ($P > 0.05$) by the dietary treatments. However, cholesterol (mg/dl), alkaline phosphatase (IH/1) and total bilirubin (IU/1) were significantly ($P < 0.05$) influenced by the dietary treatments. The increasing level of composite meal leaf in the diet II to VI significantly decreased the cholesterol and alkaline phosphatase concentration in a dose dependent manner in plasma compared to control diet (diet I). By contrast, Bilirubin concentration increased ($P<0.05$) in the diets IV and V compared with the other diets and decreased ($P<0.05$) with diet VI. Generally, there was no deleterious effect of dietary leaf meal on the haematology and biochemical values of the growing pigs. Animals fed diet VI (5% CLM) had the highest packed cell volume (40.35±0.52%), haemoglobin concentration (13.45±0.17g/100ml) and mean cell volume (62.56±0.84μ^3).

Keywords: growing pigs, haematology, biochemical indices, composite leaf meal

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INTRODUCTION

The need to harness the potentials of numerous agro-industrial by-products and green vegetable plants as part replacements for the more expensive conventional feed ingredients have been variously expressed by Agbede and Aletor (2004), Adegbenro et al., (2012). The increasing demand and supply deficit coupled with a concomitant high cost of products such as maize, fish meal, premix especially in developing nations, have made the search for cheaper protein, energy, premix alternatives very compelling in monogastric feedings. Thus, there is a need to evaluate the effect of these alternative feed sources on the health status of livestock.

The blood contains several metabolites which provide useful information on nutritional status and clinical investigation of an individual; hence World Health Organisation (WHO) recommended the use of blood parameters for medical for medical and nutritional assessments (WHO, 1963, Egbunike et al., 2009, Adeyemo and Sani, 2013). Many studies investigating the effects of various feed on the haematology and serum biochemistry of livestock concluded that feed ingredients including alternative sources affect the physiology of animal.

Based on these theoretical and practical backgrounds, this study was carried out to evaluate the effects of feeding with different levels of composite leaf meal as a replacement of dietary premix on the haematology and serum biochemical parameters in growing pigs.

MATERIAL AND METHODS

Leaf composite meal production

The five selected leaves (Cassava, Moringa, Fluted pumpkin, African basil and Bitter leaves) after ''harvest'' were air dried to prevent the loss of some vital nutrients. The air-dried leaves were milled using hammer mill and stored in plastic container prior to use. Thereafter, the leaves were mixed together in the same ratio (1:1:1:1:1) to produce the composite leaf meal.

Experimental site

The feeding trial was carried out at the Livestock Section (Piggery Unit) of the Teaching and Research Farm of the Federal University of Technology, Akure, Nigeria.

Experimental diets

A basal diet was formulated to meet the requirement of swine (NRC, 1994). The quantity of the commercial premix in the basal diet (Diet I) was reduced by 0, 20, 40, 60, 80 and 100% and replaced with 0, 10, 20, 30 40 and 50 g/kg of composite leaf meal and designated Diets I - VI, respectively (Tables 1 and 2).

Table 1: The gross composition (g/kg) of the weaner diets

Ingredients	Diet I	Diet II	Diet III	Diet IV	Diet V	Diet VI
Maize	520	510.5	501	486.5	472	462.5
Wheat offal	20	20	20	20	20	20
Soybean meal	90	90	90	90	90	90
Groundnut cake	100	100	100	100	100	100
Palm kernel cake	90	90	90	90	90	90
Brewer's dried grain	150	150	150	150	150	150
Bone meal	15	15	15	15	15	15
Oyster shell	5	5	5	5	5	5
Premix	2.5	2.0	1.5	1.0	0.5	0
Composite leaf meal	0	10	20	30	40	50
Salt	2.5	2.5	2.5	2.5	2.5	2.5
Vegetable oil	5	5	5	10	15	15
Total	1000	1000	1000	1000	1000	1000
Calculated						
Crude protein (g/kg)	191.3	193.4	195.5	197.1	198.8	200.9
Metabolizable energy (MJ/kg)	11.87	11.74	11.60	11.58	11.56	11.42
Lysine (g/kg)	7.5	7.5	7.5	7.4	7.4	7.4

Table 2: The gross composition (g/kg) of the grower diets

Ingredients	Diet I	Diet II	Diet III	Diet IV	Diet V	Diet VI
Maize	560	550.5	541	526.5	512	502.5
Wheat offal	20	20	20	20	20	20
Soybean meal	65	65	65	65	65	65
Groundnut cake	100	100	100	100	100	100
Palm kernel cake	90	90	90	90	90	90
Brewer's dried grain	120	120	120	120	120	120
Bone meal	15	15	15	15	15	15
Oyster shell	5	5	5	5	5	5
Premix	2.5	2.0	1.5	1.0	0.5	0
Composite leaf meal	0	10	20	30	40	50
Salt	2.5	2.5	2.5	2.5	2.5	2.5
Vegetable oil	20	20	20	25	30	30
Total	1000	1000	1000	1000	1000	1000
Calculated						
Crude protein (g/kg)	176.8	178.8	180.9	182.6	184.2	186.3
Metabolizable energy (MJ/kg)	12.43	12.30	12.16	12.14	12.12	11.98
Lysine (g/kg)	6.7	6.6	6.6	6.6	6.5	6.5

Animals' arrangement and management

A total number of twenty four weanling pigs (Large White) were assigned to six dietary treatments of four replicates at one pig per replicate.

The design of the experiment was a completely randomized design. The weight of each pig (1 pig/replicate) was measured and recorded as initial weight for the animal. Each animal's weight was recorded and later balanced in order to get the average weight for all the treatments. Thereafter, their respective starter diets were fed at 5% of their body weight for the period of four weeks during which the weekly feed consumption and weight changes were measured and feed conversion ratio were calculated. Thereafter, the finisher diets were fed to their respective group from the fifth week for another four weeks at 5% of their body weight and the same parameters in starter phase were measured.

Slaughtering of animals, blood collection and analyse

During the last week of the experiment, blood samples were taken from the animals via the right saphenous vein for haematological analysis, using 19 gauge needles. Blood collected in this way was immediately introduced into a set of sterilized glass test tubes containing a speck of Ethylene Diamine Tetra-acetic Acid (EDTA) powder. At the end of the feeding trials, all the animals were starved over night and weighed. The animals were hanged with their hind limbs so that their heads were facing downward to allow for proper blood bleeding. The animals were then slaughtered for the determination of parameters through mechanical stunning by hitting their forehead and severance of the jugular vein and allowing free flow of blood into some test tubes for serum collection. These test tubes were allowed to stand in the test tube rack in the laboratory in a slanting position for 6 hours. The serum separated from each blood sample was then decanted after centrifugation at 2,000 rpm for 4 minutes.

Data collected were subjected to one-way analysis of variance using SPSS version 13 package and where significant differences are found; the means were compared using Duncan Multiple Range Test of the same package.

RESULTS

Haematological studies

Table 3 shows the effects of using different levels of composite leaf meal as premix on haematological indices of swine. Among all the parameters measured, none was significantly influenced (P>0.05) by dietary treatments. The erythrocyte sedimentation rate (ESR) (mm/hr) of animals fed diet V (6.67mm/hr) was the highest while the lowest ESR rate was recorded in diet VI (5.33mm/hr). Highest packed cell volume (PCV) (%) was recorded in animals fed diet VI (40.35%) while the lowest PCV was observed in animals fed diet V (38%). The highest values for red blood cell (RBC) ($\times10^6mm^{-3}$) was recorded in diet IV ($6.97\times10^6mm^{-3}$) while the lowest RBC was recorded in diet V ($6.15 \times 10^6 mm^{-3}$). The highest haemoglobin concentration (Hbc) (g/100ml) was recorded in diet VI (13.45g/100ml) while the lowest Hbc was recorded in diet V (12.67g/100ml). The mean cell haemoglobin concentration (MCHC) (%) values ranges from 33.29% in diet I to 33.34% in diet III. Also, for mean cell haemoglobin (MCH) (pg), the highest value (20.84pg) was recorded in diet VI while the lowest value (19.51pg) was recorded in diet IV. Mean cell volume (MCV) (μ^3) values also varied from 58.60μ^3 in diet IV to 62.56 μ^3 in diet VI.

Table 3: Effects of varying levels of composite leaf meal on haematological indices of swine

Parameters	Diet I	Diet II	Diet III	Diet IV	Diet V	Diet VI	±SEM
Erythrocyte sedimentation rates (mm/hr)	6.00	5.67	6.00	5.67	6.67	5.33	0.23
Packed cell volume (%)	39.33	39.35	40.00	40.33	38.00	40.35	0.52
Red blood cell ($\times 10^6 mm^{-3}$)	6.34	6.50	6.82	6.97	6.15	6.47	15.82
Haemoglobin concentration (g/100ml)	13.10	13.10	13.33	13.43	12.67	13.45	0.17
Mean cell haemoglobin concentration (%)	33.29	33.31	33.34	33.30	33.33	33.31	0.01
Mean cell haemoglobin (pg of Hb)	20.73	20.16	19.61	19.51	20.62	20.84	0.28
Mean cell volume (μ^3)	62.26	60.51	58.83	58.60	61.85	62.56	0.84

Biochemical Indices

Table 4 shows that among the parameters measured, cholesterol, alkaline phosphatase and total bilirubin were significantly ($P<0.05$) affected by dietary treatments. Animals fed diet VI (5% composite leaf meal) had the highest total protein value (41.28g/dl) while the lowest value (24.96g/dl) was recorded in diet 1. For the cholesterol, the levels of cholesterol in animals fed composite leaf meal diets were significantly ($P<0.05$) reduced and this follows a particular trend. The highest value (48.84mg/dl) was recorded in diet I while the lowest cholesterol value (22.34mg/dl) was recorded in diet VI. This diet (VI) significantly reduced the cholesterol level in comparison with the other diets from I to V. For creatinine, the lowest value (0.21mg/dl) was recorded in diet VI and the highest value (0.30mg/dl) was also observed in diet I. For alkaline phosphatase, the highest value (125.49IU/l) was recorded in diet I while diet VI had the lowest value (86.35IU/l); this diet significantly reduced alkaline phosphatase concentration compared with diet I.

Table 4: Effects of varying levels of composite leaf meal on swine's biochemical indices

Parameters	Diet I	Diet II	Diet III	Diet IV	Diet V	Diet VI	± SEM
Total protein (g/dl)	24.96	29.11	33.43	35.24	36.95	41.28	3.42
Albumin (g/dl)	20.88	19.53	20.85	12.48	18.97	22.79	2.58
Globulin (g/dl)	8.22	17.43	12.58	12.48	16.26	18.49	2.24
Cholesterol (mg/dl)	48.84^{b}	45.57^{ab}	40.08^{ab}	36.68^{ab}	28.83^{ab}	22.34^{a}	3.46
Creatinine (mg/dl)	0.30	0.26	0.24	0.25	0.23	0.21	0.01
Alkaline phosphatase (IU/l)	125.49^{b}	112.05^{ab}	112.45^{ab}	107.03^{ab}	106.40^{ab}	86.35^{a}	4.59
Aspartate aminotransferase (IU/l)	10.67	10.67	24.67	25.33	34.33	13.00	3.64
Alamine amino transferase	17.00	18.33	15.67	20.67	18.33	17.67	2.39
Total bilirubin (IU/l)	2.99^{bc}	3.60^{c}	2.25^{ab}	4.83^{d}	5.02^{d}	1.77^{a}	0.32

a-b: Mean within rows having different superscripts are significantly different ($P<0.05$)

DISCUSSION

Blood represents a means of assessing clinical and nutritional health status of animals in feeding trials (Aletor and Egberongbe 1992) and according to Togun and Oseni (2005), haematological indices such as RBC, WBC, PCV and Hb have been found useful for disease prognosis and for therapeutic and feed stress monitoring. In the present study, the value for Erythrocyte sedimentation rates (ESR), Packed cell volume (PCV), Red blood cell (RBC), Haemoglobin concentration (Hbc), Mean cell haemoglobin concentration (MCHC), Mean cell haemoglobin (MCH) and Mean cell volume (MCV) were not significantly affected by the dietary treatments. The ESR value of animals fed 4% composite leaf meal diet was the highest. With regard to the blood physical properties, the erythrocyte sedimentation rate (ESR) was similar for all the treatments. It seems that the frictional resistance of the surrounding plasma, which holds the cells in suspension and the gravitational pull on the erythrocyte, mostly determines the ESR (Agbede et al., 2011; Aro et al., 2012). The highest PCV value was recorded in animals fed diet VI (5% composite leaf meal). The PCV values for all treatments ranging between 38.00 and 40.35% were similar with those reported by Peter et al., (2002) and Oluwafemi et al., (2012). The RBC count of all the animals fed either the control or the test diets ranged between 6.15 and 6.97 x 10^{6} mm^{-3} and are in accordance with those described for pig by Oluwafemi et al., (2012) (5.70 – 6.85 x 10^{6} mm^{-3})

and Peter et al., (2002) (5 - 8 x 10^6 mm^{-3}). The reduction in the number of blood cells and their content of haemoglobin causes anaemia (Rebecca et al., 1998) while PCV value below normal range is an indication of anaemia (Radostis et al., 1994) and poor quality of protein of the diets (Awoniyi et al., 2000). Thus, with the values obtained in this study, it could be suggested that the nutritional quality of the test diets compared favourably with the control as it has been established that Hb could be a measure of nutritional adequacy of the pigs (Tewe, 1985). Udo (1987) reported that the concentration of haemaglobin (Hbc) in the cytoplasm of the red blood cells gives an indication of an oxygen carrying capacity of the blood of the individual. Since the Hbc of the animals (12.67 – 13.45g/100ml) investigated herein fell within the range of haemoglobin value for healthy pigs (g/100ml) as reported by Peter et al., 2002 (10 – 16g/100ml) this suggests that the animals had sufficient blood pigment for proper transportation of oxygen, with attendant healthy living. Reduction in the concentration of haemoglobin and packed cell volume suggest the presence of toxic factor such as haemagglutinin, which could have adverse effect on blood formation (Oyawoye and Ogunkunle, 1998). Also, abnormal high MCH, MCV and low MCHC suggest poor quality protein of the test diets (Tewe, 1985; Awoniyi et al., 2000). Normal range of 33-47%, 90-140% and 26-35% have been predicted for MCH, MCV and MCHC, respectively (Awoniyi et al., 2000; Akinmutimi, 2004; Ahamefule, et al., 2006) and 17 - 21pg, 50 – 68fl and 30 – 34g/dl, respectively as reported by Peter et al., (2002). Hence, the values obtained for MCH, MCV and MCHC in this study fell within these range (MCHC [33.27 – 33.37%], MCH [42.20 – 43.46 pg] and MCV [127.78 – 135.93 μ^3]. RBC, Hbc, PCV, MCH, MCHC and MCV values observed in this study are in agreement with the range reported by Aro, (2010).

The present study reveals that total protein, albumin, globulin, creatinine, aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) were not affected by the composite leaf meal inclusion in the diet and therefore suggests that composite leaf meal might not pose any serious deleterious health challenges to the animals, especially as it relates to liver, as increased activities of these enzymes in the serum are well-known diagnostic indicators of liver injury (Oboh and Akindahunsi, 2005; Agbede et al., 2011; Aro et al., 2012). High values of serum total protein are indicators of quality protein of the experimental diet (Tewe, 1985; Aletor et al., 1998). The higher the value of albumin, the higher the clotting ability of blood and hence prevention of haemorrhage (Robert et al., 2003). Decrease in total protein and albumin is an indication of poor quality of the experimental diets. High value of alkaline phosphatase is an indication of poor quality protein of the experimental diets (Eggum, 1970; Ologhobo et al., 1993; Awosanya et al., 1999). Total serum protein has been reported as an indication of the protein retained in the animal body (Akinola and Abiola, 1991; Esonu et al., 2001). The values for albumin recorded in the present study were not significantly different among the treatments. The highest value of 22.79g/dl was obtained in animals fed Diet VI while the lowest was recorded in Diet IV (12.48g/dl). Looking at the results from this study, it shows clearly that the use of this composite leaf meal in the diets of pigs will surely improve the quality of the animals health status.

Awojobi and Opiah (2000) observed that higher value of globulin, increase the ability of animals to fight against infection. Akinmutimi and Eburuaja (2010) also noted that low level of globulin could lead to high mortality. From the present study, the highest value for globulin (18.49g/dl) was recorded in diet VI and the lowest value (8.22g/dl) recorded in diet I which shows that animals fed diet VI will posses more ability to fight with infection as reported by Awojobi and Opiah (2000) and Akinmutimi and Eburuaja (2010). From the present study, the value for globulin concentration of animals fed diets with leaf meal (II-VI) was higher than that of control diet (I) although the difference was not significant. By contrast, the increasing level of composite meal leaf in the diet II to VI significantly decreased the cholesterol concentration in plasma compared to control diet (diet I). This observation agrees with the results by Ghasi et al. (1999) which reported that juice extracted from moringa leaves was found to be a potent hypocholesterolemic agent. In their study using Wister rats, they concluded that even when moringa juice extract was given at the relatively low dose of 1mg/g, co-administered with a high fat diet daily over a period of 30 days, cholesterol was reduced in the serum. This reduction in serum cholesterol level of pigs fed composite leaf meal diets could suggest a general decline in lipid mobilization and lipogenesis. It could be that composite leaf meal has some indirect inhibitory effects exerted at the levels of 3-hydroxy-3-methyl-glutaryl-COA reductase, a key enzyme in cholesterol biosynthesis. It may be suggested then that, composite leaf meal was capable of reducing serum cholesterol, and hence could assist in the reduction of deposition of cholesterol in the muscles. Reduction of depot fat accumulation in this way could increase pig consumption by patients prone to arthesclerosis. The reduction in the levels of cholesterol and creatinine as the leaf composite mix increases is in agreement with the reports of Esonu et al., (2001) and Iheuwumere et al., (2007).

This fall in serum cholesterol level of animals fed on composite leaf meal diets further suggests that composite leaf meal could be used to produce animal products with reduced cholesterol content. Hence, the production of such animals could in turn improve the health status of meat-eaters by boosting their immunity. This could contribute greatly towards prevention and reduction of neurodegerative diseases associated with lipid-rich diets. The observed cholesterol reduction is in agreement with earlier findings (Upadhyay, 1990; Oforjindu, 2006) which indicated that neem leaf meal in the diets of broiler birds and rats resulted in a decrease in the cholesterol and liver lipid levels. Also, there was a trend toward a reduction in the creatinine level when the inclusion of composite leaf in the diets increased; however the difference was not significant. The values obtained for Alkaline Phosphatase in this study were in agreement with the report of Modern Weng (2016) and Total Bilirubin values obtained were also in agreement with the report of Merck vet manual (2015) which shows that the dietary treatments had good nutritional values.

CONCLUSIONS

The health status of the animals illustrated by close range of blood parameters measured and compared with previous research works and zero mortality in all the experimental animals shows that the use of composite leaf meal produced from these five leaves in pigs diets will not cause in deleterious effects in the animals.

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