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Haematological indices of grower pigs fed low protein and low energy diets supplemented with multi-enzyme

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Abstract

One of the reliable information for evaluating nutritional status, performance and physiological state of farm animals to nutrient utilization is through haematological indices assessment. Haematological parameters of grower pigs fed low crude protein and low energy diets supplemented with multi-enzyme (enziblend plus+) were determined using 36 hybrid (Landrace x Large white) male pigs of 8-10 weeks old. Two metabolizable energy (3000 and 2600 Kcal ME/kg) and three crude protein levels (18, 16 and 14 % CP) were used to formulate Six dietary treatments; T1 (3000 Kcal ME/Kg; 18 % CP), T2 (3000 Kcal ME/Kg; 16 % CP + 1g of enzyme/kg diet), T3 (3000 Kcal ME/Kg; 14 % CP + 1g of enzyme/kg diet), T4 (2600 Kcal ME/Kg; 18 % CP + 1g of enzyme/kg diet), T5 (2600 Kcal ME/Kg; 16 % CP + 1g of enzyme/kg diet) and T6 (2600 Kcal ME/Kg; 14 % CP + 1g of enzyme/kg diet). The treatments were replicated three with two pigs per replicate. The experiment was a 2 x 3 factorial, and T1 served as the control. Blood samples were collected (3mls) via the ear vein from one pig in each replicate, three from treatment for haematological analysis. The results of this study recorded significant differences (P<0.05) in most of the hematological parameters evaluated, except in mean corpuscular haemoglobin concentration (MCHC) and basophile counts of the pigs. Grower pigs fed T2 and T4 diets recorded significantly (P <0.05) higher packed cell volume (PCV) (40.15% and 40.22%), hemoglobin (12.25g/dl and 12.00g/dl), red blood cell (7.95 x10^6/ µl and 8.00 x10^6/ µl), white blood cell (8.12 x10^3/ µl and 7.91 x10^3/ µl) and lymphocyte counts (5.26 x10^3/ µl and 5.00 x10^3/ µl). Grower pigs on T6 recorded significantly (P <0.05) lower mean values for PCV (32.55%), hemoglobin concentration (Hb) (9.82g/dl), red blood cell (RBC) (5.32 x10^3/ µl), white blood cell (WBC) (6.10 x10^3/ µl), lymphocyte (3.10 x10^3/ µl) and monocyte (0.31 x10^3/ µl) counts, respectively. Based on the findings of this study, feeding of growing pigs with low energy and low crude protein diets below 16 % CP with multi-enzyme supplementation adversely effected PCV, hemoglobin, RBC, WBC, lymphocytes and monocytes of grower pigs and therefore should be avoided in pig production.

Keywords: Crude protein; Grower pigs; Haematology; Metabolizable energy; Multi-enzyme.

Introduction

Consumers demand for livestock products has been on the high side, both in Nigeria and foreign markets as a result of rapid increase in population (Obike *et al*., 2017), but the current rate of production in Nigeria is relatively very low and inadequate to meet demand due mainly to high cost of conventional feed stuffs like soybean meal, groundnut cake and fish meal (Amaefule *et al*., 2009). This has led to the search for alternative feed stuffs such as agro industrial by-products which are not in direct competition with mans' dietary need.

However, the use of agro-industrial by-products such as maize offal, PKM and BDG in monogastric animal feeding is limited by high level of crude fibre and non-starch polysaccharides (NSPs) contained in their cell wall which may not be digested by the endogenous enzymes in monogastric animals (Dalolio *et al*., 2016). In order to improve the utilization of such feed stuffs, nutritionists have proposed the use of exogenous enzymes to help break down fibre and NSPs, thereby enhancing nutrient release from the agro-industrial by-products (Amaefule *et al*., 2009), especially energy and protein content of the feed ingredients. Protein and energy are two important components of feed that are determinants of the performance and productivity of farm animals (Dairo *et al*., 2010; Amaefule *et al*., 2019). Protein sources for animal feeding are generally more expensive than energy sources such that their reduced inclusion level in diets could save the cost of feeds and that of animal production (Ajayi, 2014; Amaefule *et al*., 2019). For pig production, the regime of dietary protein and energy has been established both in the tropics and temperate climates. The performance of growing pigs has evaluated by Unigwe *et al*. (2017) who stated that 3118 digestible energy x 20 % CP supplemented with multi-enzyme was adequate as the requirement for grower pigs, which I considered high. With the use of enzymes, it is possible to reduce nutrient levels in diets in order to maximize profit and reduce amount lost in faecal matter. In addition, nitrogenous compounds (proteins) in feeds can be reduced in diets to reduce environmental pollution (*Ajayi and Imouokhome, 2015).* Recently, lower energy and low protein diets have been tried in an attempt at resolving such problems of high nutrient density diet, and it has been revealed that overall performance is not totally affected in pigs and broilers (Amaefule and Onyejekwe, 2015; Amaefule *et al*., 2019). Therefore, this work is aimed at investigating the haematological indices of grower pigs fed low protein and low energy diets supplemented with multi-enzyme (enziblend plus+) in a humid tropical environment.

Materials and method

Experimental site

This research was conducted at the Piggery Unit, Teaching and Research Farm, School of Agriculture and Agricultural Technology, Federal University of Technology Owerri, Imo State; Nigeria.

Experimental pigs and management

A total of 36 hybrid (Landrace x Large white) male pigs of 8-10 weeks old were used to conduct this study. The pigs will be housed in a tropical-type, open-sided pig house roofed with asbestos roofing sheets. The open side of the house will be covered with iron nets to screen out flies and other insects. The pigs will be provided with experimental diets and water *ad libitum* for 99 days.

Experimental diets

Two metabolizable energy (3000 and 2600 Kcal ME/kg) and three crude protein levels (18, 16 and 14 % CP) were used to formulate Six dietary treatments; T1 (3000 Kcal ME/Kg; 18 % CP), T2 (3000 Kcal ME/Kg; 16 % CP + 1g of enzyme/kg diet), T3 (3000 Kcal ME/Kg; 14 % CP + 1g of enzyme/kg diet), T4 (2600 Kcal ME/Kg; 18 % CP + 1g of enzyme/kg diet), T5 (2600 Kcal ME/Kg; 16 % CP + 1g of enzyme/kg diet) and T6 (2600 Kcal ME/Kg; 14 % CP + 1g of enzyme/kg diet). The treatments were replicated thrice with two pigs per replicate. The experiment is a 2 x 3 factorial in a Complete Randomized Design (CRD), and T1 served as the control. The experimental design for this study was 2 x 3 factorial in a Completely Randomized Design with model;

$Y_{ijk} = \mu + E_i + P_j + (EP)_{ij} + e_{ijk}$

Where;

Y_{ijk}; Single observation

μ ; Overall mean

E_i; Energy effect

P_j; Protein effect

$(EP)_{ij}$; Energy x Protein interaction effect

e_{ijk}; Error term

Data collection

Blood samples were collected (3mls) via the ear vein from one pig in each replicate and were discharged into sample bottles containing Ethyldiamin-tetra-acetic acid (EDTA) as anticoagulant, gently mixed for haematological analysis. Data collected was subjected to analysis of variance (ANOVA) for a 2 x 3 factorial experiment in a Completely Randomized Design (Steel *et al.*, 1997). Statistical computation will be done using Statistical Package for Social Sciences (SPSS, version 25), while difference between treatments means will be separated using Duncan's Multiple Range test of the same Statistical Package.

Table 1: Composition of the multi-enzyme (Enziblend Plus ™)

Ingredients	Units
Serine Protease	7500.00 PRT/g
Endo-1,4-beta glucanase	64.00 U/g
Endo-1,3(4)-beta glucanase	56.00 U/g
Endo-1,4-beta-xylanase	216.00 U/g
Alfa-amylase	4.00 KNU/g
Anti-caking (Sepiolite, E562)	350,000 mg/kg
Calcium	17.90 %
Sodium	2.07 %
Magnesium	4.03%

Result and discussion

The effect of low energy diet supplemented with multi-enzyme on the hematological parameters of growing pigs is shown in Table 2. All the hematological parameters of grower pigs fed 3000 Kcal/kg diet recorded higher significant ($P < 0.05$) mean values difference than those fed with 2600 Kcal/kg diet, apart from neutrophils which is significantly higher ($2.59 \times 10^3/\mu l$) in grower pigs fed 2600 Kcal/kg diet.

There were higher significant differences ($P < 0.05$) in Packed cell volume (PCV), haemoglobin (Hgb), red blood cell, white blood cells, lymphocytes and eosinophils of grower pigs fed 16 % crude protein (CP) diet supplemented with multi-enzyme as presented in Table 3. Similar statistical trend was observed with growing pigs fed 18 % crude protein diet, while those placed on 14 % crude protein diet showed significant ($P < 0.05$) lower values on those parameters studied.

Apart from mean corpuscular hemoglobin concentration (MCHC) and basophils that showed no significant difference ($P > 0.05$) in the interaction effect of low energy and low crude protein diets supplemented with multi-enzyme on the hematological parameters of grower pigs as shown in Table 4, other indices studied differ significantly ($P < 0.05$). Grower pigs fed T2 diet and T4 diet recorded higher significant ($P < 0.05$) mean value difference for PCV, hemoglobin, red blood cell (RBC), white blood cells (WBC) lymphocytes and monocytes. Interaction effect of T6 diet on the hematological parameters of grower pigs showed lower significant mean values for PCV, hemoglobin, RBC, WBC, lymphocytes and monocytes. From the findings of this study, the significant

Table 2: Effect of low energy diets supplemented with multi-enzyme on haematological indices of grower pigs

Parameters	3000 Kcal/kg	2600 Kcal/kg	SEM
PCV (%)	38.91^{a}	36.99^{b}	0.78
Hemoglobin (g/dl)	11.42	11.02	0.26
Red blood cell (x10^{6}/ µl)	7.13	6.77	0.28
MCV (fl)	54.81^{a}	53.56^{b}	0.88
MCH (pg)	16.05	15.96	0.21
MCHC (g/dl)	2.93	2.98	0.08
White blood cells (x10^{3}/ µl)	8.01^{a}	7.27^{b}	0.21
Lymphocytes (x10^{3}/ µl)	5.09^{a}	4.36^{b}	0.22
Nutrophils (x10^{3}/ µl)	2.42^{b}	2.59^{a}	0.06
Monocytes (x10^{3}/ µl)	0.42^{a}	0.37^{b}	0.01
Eosinophils (x10^{3}/ µl)	0.15^{a}	0.07^{b}	0.01
Basophils (x10^{3}/ µl)	0.00	0.00	0.00

a,b,c Mean values within rows with different superscript letters are significantly different (P<0.05), SEM: standard error of mean

Table 3: Effect of low protein diets supplemented with multi-enzyme on haematological indices of grower pigs

Parameters	18 %	16 %	14 %	SEM
PCV (%)	39.46^{a}	39.17^{a}	35.22^{b}	0.78
Hemoglobin (g/dl)	11.51^{ab}	11.74^{a}	10.41^{b}	0.26
Red blood cell (x10^{6}/ µl)	7.32^{ab}	7.48^{a}	6.06^{b}	0.28
MCV (fl)	54.24	52.49	55.83	0.88
MCH (pg)	15.78	15.72	16.52	0.21
MCHC (g/dl)	2.91	2.99	2.96	0.08
White blood cells (x10^{3}/ µl)	8.01^{a}	7.97^{a}	6.95^{b}	0.21
Lymphocytes (x10^{3}/ µl)	5.05^{a}	5.13^{a}	4.01^{b}	0.22
Nutrophils (x10^{3}/ µl)	2.46	2.37	2.69	0.06
Monocytes (x10^{3}/ µl)	0.41	0.41	0.37	0.01
Eosinophils (x10^{3}/ µl)	0.06^{b}	0.16^{a}	0.11^{ab}	0.11
Basophils (x10^{3}/ µl)	0.00	0.00	0.005	0.00

a,b,c Mean values within rows with different superscript letters are significantly different (P<0.05), SEM: standard error of mean

differences in values observed in PCV, hemoglobin and RBC with decreased mean values for 14 % CP diet, T6 diet indicates the physiological and nutritional status of the pigs. These parameters being within the reference value for swine implies that the low energy and low protein levels of experimental diets has no adverse effect of the on the haematological parameters of the pigs used but performance were affected. As blood conveys nutrients and other materials to different parts of the body, which means that any factor that affects blood such as nutrition, may surely affect the entire body of the animal in relation to health, growth and reproduction. This is in line with the report of Oke, *et al*. (2007) who stated that animals with blood are likely to perform very well. The normal range is 32.0 – 50.0 for PCV, 10.0 – 16.0 for Hgb, 50.0 – 68.0 for MCV, 17.0 – 23.0 for MCH (Research Animal Resources 2009; Etim *et al*., 2013). This agrees with the report of Etim *et al*. (2013) who stated that low values in pig haematological parameters is thought to be due to malnutrition. The higher significant hemoglobin (12.25g/dl. 12.00g/dl and

Table 4: Interaction effect of low energy and low protein diets supplemented with multi-enzyme on the haematological indices of grower pigs

Parameters (g)	Energy levels	18 %	16 %	14 %	Mean	S.D
PCV (%)	3000 Kcal/kg	38.70^{ab}	40.15^{a}	37.90^{b}	38.91	1.16
	2600 Kcal/kg	40.22^{a}	38.20^{b}	32.55^{c}	36.99	3.57
	Mean	39.46	39.17	35.22		
Hemoglobin (g/dl)	3000 Kcal/kg	11.02^{ab}	12.25^{a}	11.00^{ab}	11.42	0.83
	2600 Kcal/kg	12.00^{a}	11.24^{ab}	9.82^{b}	11.02	1.04
	Mean	11.51	11.74	10.41		
Red blood cell ($x10^6$/ µl)	3000 Kcal/kg	6.65^{b}	7.95^{a}	6.80^{b}	7.13	0.77
	2600 Kcal/kg	8.00^{a}	7.01^{ab}	5.32^{c}	6.77	1.22
	Mean	7.32	7.48	6.06		
MCV (fl)	3000 Kcal/kg	58.20^{a}	50.50^{c}	55.74^{b}	54.81	3.57
	2600 Kcal/kg	50.28^{c}	54.49^{b}	55.93^{b}	53.56	2.67
	Mean	54.24	52.49	55.83		
MCH (pg)	3000 Kcal/kg	16.57^{a}	15.41^{bc}	16.18^{ab}	16.05	0.67
	2600 Kcal/kg	15.00^{c}	16.03^{abc}	16.87^{a}	15.96	0.86
	Mean	15.78	15.72	16.52		
MCHC (g/dl)	3000 Kcal/kg	2.85	3.05	2.90	2.93	0.28
	2600 Kcal/kg	2.98	2.94	3.02	2.98	0.38
	Mean	2.91	2.99	2.96		
White blood cells ($x10^3$/ µl)	3000 Kcal/kg	8.11^{a}	8.12^{a}	7.81^{a}	8.01	0.20
	2600 Kcal/kg	7.91^{a}	7.82^{a}	6.10^{b}	7.27	0.92
	Mean	8.01	7.97	6.95		
Lymphocytes ($x10^3$/ µl)	3000 Kcal/kg	5.10^{ab}	5.26^{a}	4.92^{b}	5.09	0.17
	2600 Kcal/kg	5.00^{ab}	5.00^{ab}	3.10^{c}	4.36	0.98
	Mean	5.05	5.13	4.01		
Nutrophils ($x10^3$/ µl)	3000 Kcal/kg	2.43^{ab}	2.30^{b}	2.54^{ab}	2.42	0.13
	2600 Kcal/kg	2.50^{ab}	2.44^{ab}	2.84^{a}	2.59	0.27
	Mean	2.46	2.37	2.69		
Monocytes ($x10^3$/ µl)	3000 Kcal/kg	0.42^{a}	0.41^{a}	0.44^{a}	0.42	0.02
	2600 Kcal/kg	0.40^{a}	0.41^{a}	0.31^{b}	0.37	0.05
	Mean	0.41	0.41	0.37		
Eosinophils ($x10^3$/ µl)	3000 Kcal/kg	0.12^{b}	0.22^{a}	0.11^{b}	0.15	0.05
	2600 Kcal/kg	0.00^{c}	0.10^{b}	0.12^{b}	0.07	0.05
	Mean	0.06	0.16	0.11		
Basophils ($x10^3$/ µl)	3000 Kcal/kg	0.00	0.00	0.01	0.00	0.00
	2600 Kcal/kg	0.00	0.00	0.00	0.00	0.00
	Mean	0.00	0.00	0.00		

a,b,c Mean values within rows with different superscript letters are significantly different (P<0.05), S.D: standard deviation, PCV: packed cell volume, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, MCHC: mean corpuscular hemoglobin concentration

11.24 g/dl P <0.05) value for growing pigs fed T2, T4 and T5 could reflect increased oxygen transportation to body tissue for energy release for different body functions through oxidation of nutrients, as well as transportation of carbon dioxide out of the body. The result of this study is corroboration with the report of Isaac *et al.* (2013). Immune defense system is the role of WBC, lymphocytes, neutrophils, monocytes and eosinophils which significantly differ (P <0.05) among the treatment diets at both the main effect and interaction effect. The increased in viscosity has been reported (Pourvet, 2010) to cause problems in the smooth digestion of fat, protein and carbohydrate and this can be overcome by addition of enzyme, this could be attributed to the better utilization of the energy and protein content of the diets by the grower pigs, as they lack endogenous enzyme that can handle fibre at this stage of growth. Lower mean values were observed from pigs fed 2600 kcal/kg x 14 % CP on WBC, lymphocytes and monocytes with higher neutrophils mean value could be attributed to the crude protein level of the diet. As reported by Etim *et al*. (2014b) abnormal increase or decrease of these immune defense systems could be attributed to parasitism and malnutrition, which are usually corrected after rehabilitation. The significant values recorded among these immune defense indices indicates that the energy, crude protein levels and the feeding pattern were adequate and had no effect on the immune system of the growing pigs used for this study.

Conclusion

The study showed that feeding growing pigs with low energy and low crude protein diets did not show any adverse effect on the haematological indices of grower pigs, but the performance of pig fed T3 and T6 were affected. Diets of 14% CP with or without multi-enzyme supplementation should be avoided in pig production.

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