

Growth performance and blood parameters of grower pigs fed diet supplemented with multi-enzyme complex

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Abstract

Maize and soya bean meal are commonly used in pig diets. The high cost of these ingredients however may not be sustainable and economically viable in the long run. Alternative feedstuff such as wheat bran, rice husk and palm kernel cake are cheaper and readily available but pose challenges due to their high fibre content. This study evaluated the impact of multi-enzyme-supplemented diets on the performance, haematology and serum parameters of grower pigs. In a completely randomised design, forty-five (45) crossbred pigs (Large White × Landrace) were allotted to three (3) dietary groups: T1 (control), T2 (Basal diet + AVB Multi-enzyme (400g/tonne) and T3 (Basal diet + Enziblend Energy (500g/tonne) in a feeding trial which lasted 9 weeks. Performance parameters were determined and blood samples were collected to assess haematology and serum parameters. The results showed that multi-enzyme supplementation had no significant ($P < 0.05$) effect on pig performance. Average daily feed intake of pigs on the different dietary treatments did not differ significantly either. Same trend was observed for average daily weight gain and feed conversion ratio. There were no significant ($P < 0.05$) differences in PCV, Hb, RBC, platelet count, WBC and leukocyte differentials across the treatment groups. No significant differences were observed for the serum parameters assayed except the cholesterol levels. In conclusion, grower pigs can tolerate the fibre level in the diet and still thrive well without enzyme supplementation.

Keywords: Blood, Enzyme, Growers, Performance, Pig

Problem Statement

Globally, maize and soya bean meal are the major feedstuff in pig and poultry diets and are the major sources of energy and protein respectively. However, these feedstuffs are usually expensive and may not be economical and sustainable for pig production. Although cereal byproducts such wheat bran, corn bran, rice husk, distiller's grains etc are utilised as alternative feedstuff to reduce the feed

cost in pig diets, the high fibre content which is resistant to degradation in the small and large intestines may limit the efficiency of nutrient utilisation [1]. The role of dietary fibre in the diet of livestock especially pigs and poultry cannot be overemphasized as a certain amount of dietary fibre must be present in their diets in order to maintain the proper physiological functions of the digestive tract [2]. Dietary fibre may promote gut

health, heighten satiety, influence behaviour and generally improve animal well-being [3]. However, high dietary fibre content is linked to poor net energy values and impaired nutrient utilisation which is a major concern when incorporating fibre in diets for monogastric animals [4]. Furthermore, the fibre characteristics determine the detrimental effects of dietary fibre on nutrient utilisation and net energy value. Dietary fibre content and composition vary greatly within feedstuff [5]. Over the years, diverse technologies have been employed towards improving the nutritional value of fibrous feedstuffs in pigs. Physical treatments such as grinding and pelleting improve the accessibility of fibrous components while chemical treatments like alkali or acid hydrolysis reduce anti-nutritional factors [6]. Fermentation has also been used to predigest fibre through microbial activity thereby improving its nutritional value [7]. However, enzyme supplementation is one of the most effective strategies. Fibre-degrading enzymes such as xylanase and β -glucanase hydrolyse non-starch polysaccharides (NSPs), a process which enhances nutrient digestibility [8]. Multi-enzyme complexes, which combine multiple enzyme activities, improve feed efficiency by targeting a broad spectrum of NSPs [8]. In addition to improving digestibility, enzymes significantly impact animal health [9]. By reducing intestinal viscosity and enhancing gut microbial balance, they promote better nutrient absorption and overall health. Enzyme supplementation also affects haematological and serum biochemical

parameters such as glucose and serum protein levels, reflecting enhanced metabolic and physiological status [10].

The effect of enzymes on fibrous feedstuffs in pig diets, focusing on both single enzymes and multi-enzyme complexes have been studied [11, 12]. Slominski [13] demonstrated that the addition of single enzymes like xylanase and cellulase improved the digestibility of fibre in pig diets, leading to better feed efficiency and growth performance. Similarly, a study by Adeola and Cowieson [8] showed that multi-enzyme complexes which combine xylanase, cellulase and β -glucanase enhanced the overall digestibility of fibrous feedstuffs and improved nutrient absorption in pigs. Furthermore, Kiarie *et al.* [14] observed that multi-enzyme supplementation reduced intestinal viscosity in broiler chickens which was also beneficial in pigs, leading to improved nutrient uptake and overall growth. Other studies such as that by Boontiam *et al.* [15] found that multi-enzyme complexes resulted in higher serum protein levels and better overall health in pigs fed fibrous diets. This study evaluated the effect of two different multi-enzyme complexes on the growth performance and blood parameters of grower pigs.

Materials and Methods

Experimental site

This study was carried out at AK Research Farm, Eleyele, Ibadan. Ibadan is in southwest Nigeria and lies on the geographical coordinates of 7° 23' 16"N and 3° 53' 47" E. Ibadan has a mean annual rainfall of 1382mm, annual mean temperature range of 21.3 – 31.2°C,

relative humidity of 60 – 90% and ranges in elevation between 150m and 275m above sea level [16].

Experimental diets

Three experimental diets as shown in Table 1 were formulated. The dietary treatments were T1 (basal diet only), T2 (basal diets + AVB Multi-enzyme at 400g/tonne) and T3 (basal diet + Enziblend energy plus at 500g/tonne). AVB enzyme contains phytase, protease, mannanase and carbohydrase while

Enziblend Energy Plus contains xylanase, beta-glucanase and amylase. The enzymes were added to the diets at the manufacturers' recommended dosages.

Experimental animals

Forty-five (45) crossbred pigs (Large white × Landrace) with an average weight of 27.52 kg were assigned to 3 treatments with 3 replicates each. Each replicate had 5 animals. The experimental design was completely randomised design. The experiment lasted 9 weeks.

Table 1: Ingredient composition of experimental diets

Ingredient (Kg)	T1	T2*	T2 ‡
Maize	150	150	150
Corn bran	100	100	100
Rice bran	100	100	100
Groundnut cake	110	110	110
Bone meal	20	20	20
Oyster shell	12	12	12
PKC (local)	480	480	480
Lysine	2.5	2.5	2.5
Salt	3	3	3
Poultry meal	20	20	20
Premix	2.5	2.5	2.5
Total	1000	1000	1000
Calculated Analysis			
Crude Protein (%)	17.6	17.6	17.6
Crude Fibre (%)	9.1	9.1	9.1
Energy(Kcal/kg ME)	2437	2437	2437

*AVB Multi-enzyme was added to diet 2 at 400g/tonne of feed

‡Enziblend Energy Plus was added to diet 3 at 500g/tonne of feed

Evaluation of growth trials and collection of blood samples

Performance parameters monitored were daily feed intake, weekly body weight and weight gain. Feed conversion ratio (FCR) was calculated. Blood samples were collected from 2 animals per replicate at the end of the feeding trial from the anterior vena cava using hypothermic needles and syringes. 10mls

of blood was sampled from each animal. For haematological analysis, 5 mls of the blood was immediately dispensed into labeled sample bottles containing ethylene diaminetetraacetic acid (EDTA) as anti-coagulant, covered and turned gently for the blood to mix with the anti-coagulant to prevent coagulation. The other 5mls of the blood was dispensed into plain sample bottles (without anti-

coagulant) for serum analysis. The blood samples were centrifuged and the serum was separated. The samples were assayed for aspartate aminotransferase (AST), alanine aminotransferase (ALT), total protein, albumin, globulin, glucose, high density lipoprotein (HDL) and creatinine using methods described by Sirios [12]. The haematological indices examined were packed cell volume (PCV), haemoglobin concentration (Hb), red blood cell (RBC) counts, white blood cell (WBC) counts, platelets, lymphocytes, neutrophils, monocytes and eosinophils.

Statistical Analysis

Data collected were subjected to analysis of variance (ANOVA) using the general linear model of SAS [17]. Duncan's New Multiple Range Test was used in separating the significant means.

Results and Discussion

Performance Parameters

Results of the effect of the multi-enzyme complex supplementation on the performance of the grower pigs are shown in Table 2. The pigs fed basal diet supplemented with Enziblend energy multi-enzyme complex (T3) had an average daily feed intake of 1.40 kg while the average daily feed intake for pigs in T2 fed basal diet with AVB multi-enzyme (T2) was 1.29 kg and that of pigs fed the basal diet only (T1) was 1.31 kg. No significant differences were observed between the average daily feed intake of pigs on the basal diet and those on enzyme-supplemented diets. The weight

gain of the pigs on the different dietary treatments {T1 (20.22 kg), T2 (21.43 kg) and T3 (21.43 kg)} did not differ significantly despite multi-enzyme supplementation of diets 2 and 3. A similar trend was observed for the final body weight of the pigs: T1 (47.43 kg), T2 (48.44 kg) and T3 (49.77 kg).

Enzymes might not improve the performance of grower pigs if the diet is already highly digestible or low in problematic non-starch polysaccharides (NSPs). This could explain the observed similarities in the performance parameters of the pigs across the treatment groups despite the supplementation of diets T2 and T3 with enzymes. There may also be fewer NSPs for the enzymes to break down; leading to no noticeable improvement in performance compared to pigs on the unsupplemented diet. Ravindran [18] reported that a challenge faced by users of exogenous enzymes is that animal responses to feed enzyme addition are not entirely predictable. The author stated that the factors contributing to these inconsistencies are complex and could involve age, sex and individual animal variation. This current study corroborates the findings of Lindberg [19] who found that the inclusion of enzyme in the diet of grower pigs did not improve its performance while the findings of Len *et al.* [11] and Omogbenigun *et al.* [20] highlighted positive effect of enzyme complex supplementation on the growth response of weaner pigs.

Table 2: Effect of multi-enzyme complex supplementation on the growth indices of grower pigs

Parameters	T1	T2	T3	SEM
Initial Weight (Kg)	27.21	27.01	28.34	2.39
Final Weight (Kg)	47.43	48.44	49.77	3.18
Weight Gain (Kg)	20.22	21.43	21.43	0.93
Feed Intake (Kg)	82.19	81.59	88.34	5.16
ADFI (Kg)	1.31	1.29	1.40	0.08
ADWG (Kg)	0.24	0.25	0.25	0.01
FCR	4.06	3.80	4.12	0.12

*Means without superscripts along the same row are not significantly different ($P < 0.05$)

T1 - Control/Basal diet, T2 - Basal diet + AVB Multi-enzyme (400g/tonnes), T3 - Basal diet + Enziblend Energy (500g/tonnes). ADFI - Average daily feed intake, ADWG - Average daily weight gain, FCR - Feed conversion ratio.

Haematological indices of grower pigs fed diets supplemented with multi-enzyme complex

Results of the effect of multi-enzyme complex supplementation on the haematological parameters of the grower pigs are shown in Table 3. Haematological characteristics of livestock have been observed as factors determining the response of livestock to the diet they are fed (Madubuike *et al*) [21]. The evaluation of haematology in pigs can be valuable in the treatment or prognosis of many diseases (Eze *et al.*) [22] and contribute to the early detection of pathological conditions and reflect metabolic disorders due to nutrient deficiencies (Sanchez *et al.*) [23]. None of the parameters measured differed significantly among the pigs on the different dietary treatments. With the exception of lymphocyte values, all the other parameters fell within the normal range for healthy growing pigs according to Merck [24]. The haematological parameters falling within the normal reference range in grower pigs generally indicate good health and a stable physiological state, suggesting that the

diets were adequate and the pigs were not experiencing adverse effects from nutritional deficiencies or severe infections. Red blood cell (RBC) count, haemoglobin (Hb) and packed cell volume (PCV) falling within normal reference range $\{(5.5 - 9.1 \times 10^6 \mu\text{L}), (10 - 16 \text{ g/dl}) \text{ and } (29 - 40\%)\}$ respectively suggests sufficient oxygen transport and overall cellular health. Optimal PCV levels are critical for oxygen transport in animals, influencing growth and metabolic activities [25, 26]. Adequate Hb levels are essential for cellular respiration and energy production, particularly in growing animals [27]. The observations from this study suggest that the addition of AVB and Enziblend Energy Plus did not compromise blood volume and Hb concentration. Also, the enzyme levels used in this study did not impact erythrocyte production and oxygen distribution to tissues. This result aligns with prior studies in which enzymes were added to pig diets and RBC counts remained stable [28].

White blood cell (WBC) count and the values of the differentials falling within normal range indicate the absence of

significant inflammatory responses. The WBC count is indicative of the immune status of the animals. WBC counts were not significantly ($P < 0.05$) different among the treatments. The absence of significant ($P < 0.05$) differences in WBC counts across treatments suggests that enzyme supplementation did not induce immune responses or physiological stress [28]. Results of this study are consistent with that of previous research that found no significant alterations in WBC counts with enzyme supplementation in grower pigs [29]. The same trend was observed for all leukocyte differentials such as

lymphocytes, neutrophils, monocytes and eosinophils.

The higher-than-range values observed for the lymphocytes in apparently healthy grower pigs can stem from ongoing immune stimulation by environmental pathogens like viruses, bacteria or mycotic antigens. While it does not always indicate disease, persistent elevated lymphocyte levels can reflect an active immune response to these antigens Merck [24]. In all, the pigs fed enzyme-supplemented diets did not show any superior haematological indices to those fed the unsupplemented diet.

Table 3: Effect of enzyme supplementation on haematology of grower pigs

Parameters	T1	T2	T3	SEM	Reference Range
PCV (%)	35.83	36.67	35.62	0.85	29 - 40
Hb (g/dL)	12.00	12.08	11.66	0.31	10 – 16
RBC ($\times 10^6 \mu\text{L}$)	5.74	6.00	5.83	0.16	5.5 – 9.1
WBC ($\times 10^3 \mu\text{L}$)	7750.00	7283.33	8031.25	357.93	4500 – 11000
Platelets ($\times 10^9 /\text{L}$)	143.83	134.00	144.63	3.74	33.4 – 181
Lymphocytes (%)	65.33	65.83	64.25	0.73	39 – 62
Neutrophils (%)	31.66	30.83	32.75	0.74	30 – 45
Monocytes (%)	1.17	2.00	1.63	0.20	0.5 – 4
Eosinophils (%)	1.83	1.33	1.38	0.24	1.95 – 3.33

Means without superscripts along the same row are not significantly different ($P < 0.05$)

T1 - Control/Basal diet, T2 - Basal diet + AVB Multi-enzyme (400g/tonnes), T3 - Basal diet + Enziblend Energy (500g/tonnes). PCV-Packed Cell Volume, Hb-Haemoglobin, RBC-Red Blood Cell Count, WBC-White Blood Cell Count

Serum biochemical indices of grower pigs fed diets supplemented with multi-enzyme complex

Results of the effects of enzyme supplementation on serum parameters in grower pigs are shown in Table 4. The

chemistry of serum is routinely used for detection of organ disease in domestic animals and the amount of available protein in the diets [30]. They provide insights into protein synthesis, tissue development and overall physiological

functions [24]. Except the cholesterol levels, all the parameters measured did not differ significantly among pigs on the different dietary treatments. It was also observed that the parameters that fell within the normal reference ranges were total protein (TP) {4.4 – 9.21 g/dl}, albumin {1.9 – 4.0 g/dl}, globulin {2.0 – 4.20 g/dl} and creatinine {0.5 – 2.7 mg/dl}. Pigs on T1 and T2 had lower-than-range values for alanine aminotransferase (AST). All the pigs on the different diets had lower-than-range values for glucose while the values for high density lipoprotein (HDL) were higher than range.

Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) are key enzymes used to assess liver function and hepatocellular integrity. Alanine aminotransferase (AST) values were observed to be lower in pigs on T1 and T2 than the normal reference range {32 – 84 IU/L} for healthy grower pigs. Lower-than-range AST in otherwise healthy pigs is generally not considered a significant clinical issue and is often not clinically significant on its own [31]. More often, lower AST values simply reflect a pig in good health. Values of alanine aminotransferase (ALT) were also lower than the normal range {25 – 55 IU/L} for healthy pigs. Lower-than-range ALT in pigs is not a primary concern and is not usually a sign of illness although it can be linked with vitamin B6 deficiency [32]. Higher-than-range values however are a cause for concern as elevated levels indicate hepatic damage or stress. In most cases, low ALT in healthy pigs is often not a red flag. Results of this study suggest that enzyme supplementation did not exert hepatotoxic effects or cause cellular damage in the pigs.

Total protein is a measure of the protein content in the blood and reflects the balance between protein synthesis and

degradation. Total protein values (T1-4.98, T2 – 5.38, T3 – 5.27) fell within the normal range for healthy growing pigs (4.4 – 9.21) but did not differ significantly among the pigs on the different diets. This suggests that the diets offered the pigs were adequate for the pigs irrespective of enzyme supplementation as enzyme inclusion did not alter hepatic protein synthesis or renal protein loss which are key contributors to serum protein levels. This agrees with the findings of Yeom *et al.* [33]. Blood glucose did not differ significantly although the values were lower than the reference values {75 – 150 mg/dl} for healthy growing pigs. Exogenous enzymes such as carbohydrases and proteases break down complex carbohydrates and proteins which improves the digestibility and absorption of nutrients [34]. This enhanced nutrient uptake can lead to a more efficient conversion of feed into energy potentially lowering blood glucose levels if the energy is used or stored efficiently as reported by [35]. On the other hand, lower-than-range glucose levels in otherwise healthy grower pigs can be caused by several factors including insufficient energy intake from the diet, increased energy demands from growth and activity, environmental stress like chilling or competition.

The cholesterol levels of pigs on T2 and T3 were significantly ($p < 0.05$) higher than that of pigs on T1. This suggests that enzyme supplementation in this study affected lipid digestion, absorption and/or metabolism which is reflected in the serum cholesterol. The findings in this study are in agreement with that of Amaefule *et al.* [28] and Yeom *et al.* [33] who reported that there was a significant ($P < 0.05$) increase in the level of cholesterol in growing pigs in response to enzyme supplementation in low energy diet. According to Wu [36], high

cholesterol in grower pigs fed exogenous enzymes can stem from the increased energy and nutrient availability from the enzymes which may lead to increased feed intake and consequently higher fat deposition and cholesterol levels.

The high density lipoprotein (HDL) levels were not significantly different but were higher than the normal range for healthy grower pigs. High HDL levels in grower pigs can stem from genetics, diet or stress but they are generally associated with better lipid profile rather than health problems [36]. The overall implications are less clear than in humans with some studies showing a correlation with better growth and lipid metabolism. Many factors influence these values so the

figures should be considered indicative rather than definitive.

Conclusion and recommendation

Supplementation of grower pigs' diet with AVB Multi-enzyme and Enziblend Energy did not show any improvement on the performance, health status and haematology and serum indices of the pigs compared to those on the unsupplemented diets although the enzymes did not have negative effects. This suggests that the diet (without enzyme) was adequate for the pigs and that the pigs could digest the diet without enzyme supplementation. In subsequent studies, the fibre content of the diet could be increased so as to find out if the enzymes would elicit any positive effects on the performance of grower pigs.

Table 4: Effect of enzyme supplementation on Serum Biochemical indices of grower pigs

Parameters	T1	T2	T3	SEM	Reference Range
AST (IU/L)	25.10	30.98	33.35	3.24	32 – 84
ALT (IU/L)	18.87	22.44	22.39	0.78	25 -55
Total Protein (g/dL)	4.98	5.38	5.27	0.16	4.4 – 9.21
Albumin (g/dL)	2.77	3.28	2.79	0.11	1.9 – 4.0
Globulin (g/dL)	2.21	2.09	2.29	0.16	2 - 4.20
Glucose (mg/dL)	74.80	60.47	59.25	3.39	75 – 150
Cholesterol (mg/dL)	118.92 ^b	132.76 ^a	143.89 ^a	3.44	80 - 130
Creatinine (mg/dL)	1.56	1.67	1.58	0.05	0.5 – 2.7
HDL (mg/dL)	54.49	56.44	56.78	1.97	35 - 45

^{a, b} Means with same superscript along the same row are not significantly different ($P>0.05$). T1 - Control/Basal diet, T2 - Basal diet + AVB Multi-enzyme (400g/tonnes), T3 - Basal diet + Enziblend Energy (500g/tonnes); AST - Aspartate aminotransferase; ALT - Alanine aminotransferase, HDL - Highdensity lipoprotein cholesterol.

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