



## Effects of Enzyme in Palm Kernel Meal-Based Diet on Blood, Carcass, and Organ Weights in Weaners Pigs

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**Abstract** | Supplementing palm kernel meal (PKM)-based diet with an exogenous enzyme can help to improve the utilization and minimize the negative consequences of the high fibre and antinutritional factors on performance and health of animals. Forty weaned male pigs with an average initial weight of  $7.85 \pm 0.31$  kg were used to determine the effects of supplementing a PKM-based diet with enzyme inclusion, on blood parameters, carcass and organ characteristics. Pigs were allotted to four diets which include the control diet containing 55% PKM while the addition of 1, 2 and 3 g/kg enzyme cocktail was tested in a complete randomized design trial that lasted seven weeks. Feed and water were provided *ad-lib* and blood sampled via jugular vein after the trial. Pigs fed diets containing high PKM with or without enzyme, did not manifest any significant deviation in their haematology or serum variables. Enzyme inclusions reduce cholesterol concentrations ( $p < 0.05$ ), but no linear or quadratic effect of the enzyme was observed on alkaline phosphatase or creatinine concentration ( $p > 0.05$ ). Eviscerated weight increased ( $p < 0.05$ ) as enzyme inclusion increased but other carcass traits were not affected. Pancreas and empty stomach weights reduced quadratically with enzyme inclusion ( $p < 0.05$ ) and this may ostensibly be due to improved digestive activities of the organs. This study confirms that the inclusion of enzyme in diets containing high proportion of PKM does not have any negative physiological effect on growing pigs.

**Keywords** | Carcass, Digestion, Enzyme, Haematology, High fibre

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## INTRODUCTION

Palm kernel meal (PKM) is an abundant agro-industrial by-product from the oil processing mill with potential nutritive value for livestock feeding. The chemical composition of PKM showed that it has crude protein (CP) concentration of 14–18% and moderate energy value (10.6 MJ/kg DM) (Kim et al., 2016; Sathitkowitzchai et al., 2018). These values are dependent on processing techniques which affect the ether extract and crude fibre

content (Ojediran et al., 2020). However, PKM has high levels of non-starch polysaccharides (NSP) such as mannan, xylan which limits its utilisation as feed for non-ruminant animals (Adeola and Cowieson, 2011; Sharmila et al., 2014). The cell wall components of PKM consists of 58% mannan, 12% cellulose and 4% xylan (Sathitkowitzchai et al., 2018). Furthermore, PKM is coarse-textured, gritty in appearance and high viscosity which tend to decrease nutrient absorption (Ojediran et al., 2020).

The  $\beta$ -mannan and non-starch polysaccharide (NSP) present in PKM have anti-nutritional properties that hinder the full utilization of nutrients in pigs (O’Shea et al., 2014; Oladokun et al., 2016). The cell wall of PKM contains (1–4)-linked  $\beta$ -mannose, (1–6)-linked  $\alpha$ -galactose (galactomannan) which reduces feed efficiency by 20–30% (Düsterhöft et al., 2006). Despite the limitations to the use of PKM, studies have shown that it can sustain maintenance and moderate growth in pigs and can be used as partial substitute of maize and soybean in pigs and poultry diet (Alshelmani et al., 2017). Previous studies have shown that the feeding value of PKM for monogastric can be improved through solid-state fermentation (Mirnawati et al., 2011; Alshelmani et al., 2017), enzyme supplementation (Kononenko and Gorkovenko, 2011; Ravindran and Son 2011) among others. The addition of enzymes that digest the  $\beta$ -mannan can improve animal weight gain and feed efficiency (Adeola and Cowieson, 2011; Choe et al., 2017). Mannanase enzyme aids in the hydrolysis of linear mannans, resulting in increased digestion of NSPs, improved nutrient utilization and decreased intestinal viscosity of digesta (O’Shea et al., 2014; Ojediran et al., 2020).

The use of an enzyme to improve the digestibility of NSPs and other antinutritional factors in feed could enhance, not just the growth performance of the pigs, but also the health and immune status of the animals as reflected in haematology and serum biochemical parameters (Ao et al., 2011; Stephenson et al., 2014; Zuo et al., 2015). Furthermore, feeding high fibre feeds like PKM to pigs can have confounding effects on body weight as compared to carcass weight because of enlarge digestive organs causing higher relative organ weights (Agyekum et al., 2012). However, there are limited studies on the effect of a cocktail of enzymes on the blood parameters, carcass traits and relative organ weight in growing pigs. Beside the effect of fibre on nutrient digestion, the impact of enzyme inclusion on the overall physiology of the animals consuming high fibre diets may provide further justification for increasing the use of it in pig production systems. Therefore, the objective of this study was to evaluate the effect of a cocktail of different enzymes, supplemented to a PKM-based diet, on the haematology and serum biochemical parameters, as well as carcass characteristics and organ weights of weaned piglets.

## MATERIALS AND METHODS

### ETHICS STATEMENTS

Animal care and all experimental procedures were approved by the Research Ethics Committee of the Ladok Akintola University of Technology with approval number: LAUTECH/EC/2019/09/011.

## ANIMALS AND EXPERIMENTAL DESIGN

The trial was conducted at the Piggery section of the Research Farm of the University and located on latitude 8° 10.398’N and longitude 4° 16.272’E. Forty (40) male weaner pigs (Large white x Landrace) with an average initial body weight of 7.85 ± 0.31 kg were grouped by weight and from each group, randomly assigned to one of four dietary treatments in a 7-week trial with 10 pigs per treatment allocated to individual pens. The dietary treatments include a control diet which contained PKM, and with 1, 2, 3 g/kg enzyme supplementation (Table 1). The enzyme source is Polyzyme, a commercial enzyme product containing a proprietary cocktail of mannanase, xylanase, cellulase, glucanase, phytase, amylase, pectinase, lipase, galactosidase, and protease enzymes (Zeus Biotech Company, Mysore-Karnataka, India). The powdered product was incorporated at the dosage of 2 g/kg feed DM, into the complete ration with a vertical mixer, and weekly batches of each feed was produced and fed to the respective animals, all through the trial. All the pigs were housed in concrete floor pens of 2 m<sup>2</sup>/pen, 1.5 m walls and iron roofing. Pigs were adapted to the respective diets for seven days, in two equal instalments daily using one-sided plastic feeders at 08h00 and 14h00. Thereafter, both feed and water were provided *ad libitum* throughout the trial period which lasted additional seven weeks.

**Table 1:** Ingredient and Chemical Composition of Experimental Diet (g/kg DM)

Ingredients	Diet
Maize	150
Fish meal	30
Full fat Soya	90
Palm kernel meal	550
Wheat offal	150
Bone meal	15
Limestone	10
<sup>1</sup> premix	2.5
Salt	2.5
Total	1000
Nutrient composition (g/kg)	
Crude Protein	199
Ether Extract	63.5
Crude Fibre	87
ME (MJ/kg)	11.4
Calcium	9.5
<sup>2</sup> Lysine (%)	0.87
<sup>2</sup> Methionine (%)	0.4

<sup>1</sup>supplied the following (per kg feed): vitamin A, 12 500 IU; vitamin D3, 5 000 IU; vitamin E, 40 mg; vitamin K3, 2 mg; vitamin B1, 3 mg; vitamin B2, 5.5 mg; niacin, 55 mg; calcium pantothenate, 11.5 mg; vitamin B6, 5 mg; vitamin B12, 25

mg; folic acid, 1 mg; biotin, 50 mg; choline chloride, 500 mg; manganese, 300 mg; iron, 120 mg; zinc, 80 mg; copper, 85 mg; iodine, 1.5 mg; cobalt, 3 mg; selenium, 1.2 mg; anti-oxidant, 120 mg. <sup>2</sup>Calculated composition.

## DATA COLLECTION

At the end of the 7-week feeding period, 5 mL blood samples were collected via the jugular vein into both EDTA vacuum tubes and plain vacutainer tubes. Six animals were randomly sampled per treatment, tubes kept in a cooler box immediately after collection and then analysed for haematological and serum biochemical variables as described in Ojediran et al. (2019a). Thereafter, all the pigs were transferred to the slaughterhouse and slaughtered using conventional procedures. After evisceration, the primal cuts and organs were weighed and expressed in relation (percentage) to the live weight of the respective animal.

## STATISTICAL ANALYSIS

All data were subjected to the Proc Mixed procedure of SAS (Statistical Analysis System, version 9.4., SAS Institute Inc., Carry, NC, USA). The model statement included dietary treatment as a fixed effect, animals as random effect as shown:

$$y_{ijk} = \mu + A_i + T_j + \varepsilon_{ij}$$

where  $y_{ijk}$  = individual observation;  $\mu$  = overall mean;  $A_i$  = animal effect (random);  $T_j$  = treatment effect (fixed), and  $\varepsilon_{ij}$  = random error. Single degree of freedom contrast was used to compare i) control versus average of enzyme-treated diets, ii) the linear effect of increasing enzyme inclusion, iii) the quadratic effect of increasing enzyme inclusion.

## RESULTS

Table 2 showed that the red blood cell (RBC), mean corpuscular haemoglobin concentration (MCHC), and lymphocyte counts in pigs were not affected by enzyme inclusion in the palm kernel meal-based diet ( $p > 0.05$ ). In contrast, enzyme supplementation in the diets affected the haemoglobin (Hb), haematocrit (HCT), total white blood cell (WBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and platelet count in the pigs ( $p < 0.05$ ). While there was lower HCT and Hb concentration in enzyme supplemented animals, their WBC and platelet counts were higher ( $p < 0.05$ ). There was a linear increase in WBC count with increasing enzyme inclusion while platelet counts increased both linearly and quadratically with increasing inclusion levels ( $p < 0.05$ ).

Comparing pigs in the control diets versus the average of the enzyme supplemented pigs, albumin, total protein, urea, and creatinine concentration was not affected by enzyme inclusion ( $p > 0.05$ ) (Table 3). However, globulin,

cholesterol, triglyceride, and glucose concentration were affected by enzyme supplementation of the palm kernel meal-based diet ( $p < 0.05$ ). A quadratic increase in globulin, total protein and cholesterol concentration in pigs was associated with increasing enzyme inclusion while a linear increase, as well as quadratic increase in glucose and creatinine concentration, was associated with increasing enzyme inclusion in the pig diets ( $p < 0.05$ ). Furthermore, alkaline phosphatase (ALP) concentrations were neither affected by the average effects nor by the increasing inclusion levels of enzyme supplementation in the pig diets ( $p > 0.05$ ).

Table 4 shows the carcass characteristics of weaner pigs fed PK-based diet with or without enzyme supplementation. The bled weight, eviscerated weight, head, jowl, loin, picnic shoulder, buston butt and belly were not different between animals on the control diet and the average of the enzyme-supplemented diets ( $P > 0.05$ ). Ham, spare rib and trotters were different between animals on the control diet and the average of the enzyme supplemented group ( $p < 0.05$ ). Furthermore, increasing inclusion levels of enzyme linearly reduced carcass weight but linearly increased ham weight ( $p < 0.05$ ).

The spleen and kidney weights were not different in the pigs across the dietary treatments ( $p > 0.05$ ) (Table 5). However, Liver weight was lower in the enzyme supplemented pigs compared to the control group ( $p < 0.05$ ). Pancreas weight was equally lower in enzyme supplemented pigs and increasing inclusion levels resulted in a linear and quadratic decrease in pancreas weight. Empty stomach weight and lung weight were not affected by diet differences. There was a linear and quadratic change in heart weight across the enzyme inclusion levels.

## DISCUSSION

Previous data published showed that there were no significant differences among the pigs in terms of Average daily intake, average daily gain (ADG) and feed conversion ratio (FCR) ( $P > 0.05$ ). Average daily intake ranged from 620- 800 g/d while ADG ranged between 240- 290 g/d (Ojediran et al., 2020). Haematological values are of diagnostic importance in practical husbandry, which is in response to the environment and dietary changes and reveal adverse conditions even when without obvious signs of ill health (Eze et al., 2010). The result of the effect of the experimental diets on haematological parameters indicates that the experimental diets had no detrimental effects on the health status of the weaner pigs as most of the parameters measured were within the reference values prescribed by Mitruka and Rawnsley (1977) as well as Thorn, (2000). Abnormal deviations in haematological variables in pigs are thought to be due to malnutrition or dietary influence

**Table 2:** Haematological Parameters of Weaner Pigs Fed Palm Kernel Meal-Based Diet, with Graded Levels of Enzyme Supplementation

Parameters	Enzyme supplementation (g/kg Diet)				SEM	<sup>1</sup> Contrast P-values		
	Control (0)	1	2	3		C vs Enzy	Enzy <sup>L</sup>	Enzy <sup>Q</sup>
Red blood cell (×10 <sup>6</sup> /μl)	7.58	7.20	7.07	7.20	0.11	0.144	0.249	0.319
Haemoglobin (g/dl)	10.3	9.40	8.85	9.90	0.15	<0.01	0.04	<0.01
Haematocrit (%)	46.0	43.3	40.7	44.4	0.65	0.025	0.147	0.016
White blood cells (×10 <sup>3</sup> /μl)	17.3	20.0	21.5	21.8	0.60	0.01	<0.01	0.266
Mean corpuscular volume (fl)	60.8	60.4	57.6	61.6	0.40	0.125	0.906	<0.01
Mean corpuscular haemoglobin (pg)	13.6	13.2	12.6	13.8	0.14	0.09	0.877	<0.01
Mean corpuscular haemoglobin concentration (g/dl)	22.4	21.8	21.8	22.3	0.11	0.15	0.879	0.039
Platelets (×10 <sup>3</sup> /μl)	235	348	423	363	21.1	<0.01	<0.01	0.065
Lymphocytes (×10 <sup>3</sup> /μl)	10.2	11.8	11.7	10.5	0.29	0.115	0.01	0.233
Red cell distribution width _SD (fl)	45.7	44.1	37.5	43.0	0.85	0.01	0.01	0.01
RDW_CV (%)	21.3	20.8	17.7	19.9	0.39	0.01	0.01	0.03

<sup>1</sup>Contrast analysis across treatments: C vs. Enzy, control diet vs. average of enzyme treated diets; Enzy<sup>L</sup>, linear effect of enzyme levels; Enzy<sup>Q</sup>, quadratic effect of enzyme levels. SEM, standard error of means.

**Table 3:** Serum Biochemistry Parameters of Weaner Pigs Fed PKM-Based Diet and with Increasing Levels of Enzyme Supplementation

Parameters	Enzyme supplementation (g/kg Diet)				SEM	<sup>1</sup> Contrast P-values		
	Control (0)	1	2	3		C vs Enzy	Enzy <sup>L</sup>	Enzy <sup>Q</sup>
Albumin (g/dl)	2.71	2.65	2.81	2.36	0.06	0.531	0.123	0.083
Globulin (g/dl)	0.91	1.21	1.97	1.07	0.13	0.01	0.06	<0.01
Total protein (g/dl)	3.62	3.85	4.78	3.43	0.17	0.08	0.483	<0.01
Cholesterol (mg/dl)	170	109	160	144	6.21	<0.01	0.552	0.015
Alkaline phosphatase (U/L)	29.1	27.9	31.6	29.6	0.72	0.698	0.703	0.319
Urea (mg/dl)	7.31	11.3	6.77	7.07	0.66	0.452	0.313	0.162
Triglyceride (mg/dl)	341	298	192	260	22.3	0.04	0.056	0.129
Glucose (mg/dl)	156	84.0	163	171	8.47	0.024	<0.01	<0.01
Creatinine(mg/dl)	0.77	0.72	0.79	0.95	0.11	0.103	<0.01	0.01

<sup>1</sup>Contrast analysis across treatments: C vs. Enzy, control diet vs. average of enzyme treated diets; Enzy<sup>L</sup>, linear effect of enzyme levels; Enzy<sup>Q</sup>, quadratic effect of enzyme levels. SEM, standard error of means.

**Table 4:** Carcass Characteristics of Weaner Pigs Fed PKM-Based Diet and with Increasing Levels of Enzyme Supplementation

Parameters (%)	Enzyme supplementation (g/kg Diet)				SEM	<sup>1</sup> Contrast P-value		
	Control (0)	1	2	3		C vs Enzy	Enzy <sup>L</sup>	Enzy <sup>Q</sup>
Bled weight	94.70	95.59	94.64	96.64	0.26	0.103	<0.01	0.01
Eviscerated weight	69.72	70.40	71.05	71.11	0.25	0.44	0.011	0.387
Carcass weight	53.34	52.49	52.82	51.91	0.30	0.055	0.039	0.445
Head	8.34	8.37	7.38	8.97	0.22	0.295	0.225	0.702
Jowl	2.62	1.91	4.44	3.00	0.28	0.61	0.219	0.289
Loin	12.60	10.91	11.53	10.09	0.27	0.682	0.021	0.363
Ham	14.03	12.93	13.28	13.98	0.17	<0.01	<0.01	0.818
Picnic shoulder	9.52	11.52	10.03	8.21	0.32	0.117	0.421	0.024
Buston butt	10.70	10.47	10.18	10.71	0.11	0.341	0.01	<0.01

Spare rib	3.10	2.22	2.73	5.06	0.38	0.047	0.32	0.003
Belly	3.19	2.69	2.95	2.56	0.10	0.768	0.046	0.035
Trotters	1.76	1.38	1.82	1.70	0.06	0.047	0.062	0.81

<sup>1</sup>Contrast analysis across treatments: C vs. Enzy, control diet vs. average of enzyme treated diets; Enzy<sup>L</sup>, linear effect of enzyme levels; Enzy<sup>Q</sup>, quadratic effect of enzyme levels. SEM, standard error of means.

**Table 5:** Organ Weight of Weaner Pigs Fed Palm Kernel Meal-Based Diet with or without Enzyme Supplementation

Parameters (%)	Enzyme supplementation (g/kg DM)				SEM	<sup>1</sup> Contrast P-values		
	0	1	2	3		C vs Enzy	Enzy <sup>Q</sup>	Enzy <sup>L</sup>
Spleen	0.12	0.12	0.13	0.13	0.00	0.186	0.773	0.097
Kidney	0.43	0.44	0.39	0.40	0.01	0.443	0.377	0.504
Liver	2.88	2.59	2.71	3.01	0.04	0.028	<0.01	0.848
Pancreas	0.23	0.23	0.24	0.19	0.01	0.01	<0.01	<0.01
Empty stomach	1.29	1.28	1.33	1.17	0.09	0.27	0.762	0.024
Lungs	0.92	1.30	0.95	0.87	0.19	0.424	0.917	0.019
Heart	0.45	0.43	0.41	0.45	0.07	0.022	0.01	<0.01

<sup>1</sup>Contrast analysis across treatments: C vs. Enzy, control diet vs. average of enzyme treated diets; Enzy<sup>L</sup>, linear effect of enzyme levels; Enzy<sup>Q</sup>, quadratic effect of enzyme levels. SEM, standard error of means.

(Yan et al., 2012; Dlamini et al., 2017; Min et al., 2019). In a similar study, the inclusion of enzyme in the pig diets did not have any negative effect on the metabolic processes of the growing and fattening pig youngsters but rather, there was increased haemoglobin concentration in pigs supplemented with endoxylanase enzyme in a high NSP-triticale diet (Kononenko and Gorkovenko, 2011).

Only a slight increase in WBC count was observed in animals supplemented with the enzyme. Although Akintunde et al. (2011) observed that supplementing a diet containing 30% PKM inclusion had no effect on the haematological parameters such as Hgb, RBC, WBC, MCV, MCH and MCHC, however, the current study observed marked differences in only RBC and WBC. Overall, the result of the current study showed that the pigs were not anaemic nor their immunity compromised. Low and high WBC beyond the normal ranges is indicative of impaired immune function (Akanmu et al., 2020). While exogenous enzymes function primarily to complement the activities of digestive enzymes, the gut microbiota has been known to affect the integrity of the intestinal barrier against colonization by pathogens (Jandhyala et al., 2015), and via anti-inflammatory immune enhancement (Wu and Wu, 2012). For example, dietary probiotics resulted in increased production of immunoglobins and act as an immune adjuvant in pigs (Naqid et al., 2015).

Multi Enzyme product containing xylanases did not affect blood profile and carcass characteristics in finishing pigs in the study reported by Min et al. (2019). However, enzyme supplementation in the current study seemed to reduce blood cholesterol concentration, and this is similar to the observation of Józefiak et al. (2011) who reported re-

duced cholesterol concentration in broilers. Furthermore, Kilic et al. (2006), reported that enzyme supplementation significantly reduced abdominal fat deposition in broilers. Increased fibre digestibility through enzyme supplementation may have modulated the synthesis of cholesterol and other fats in the enzyme supplemented pigs. Increased metabolic activity by visceral organs has been associated with increased cellular turnover and may affect haematological parameters of the pigs (Adejoro et al., 2013). Some antinutritional factors are known to irritate the gut, and this may be easily detected from serum biochemical parameters (Eze et al., 2010; Adejoro et al., 2013; Alshelmani et al., 2017). Elevated creatinine concentration is an indication of muscular wastage (Akanmu et al., 2020). Although slightly higher at 0.3 g/kg enzyme inclusion, creatinine concentration was within the normal range as described by Thorn, (2000).

The result of the eviscerated weight in this study also shows that the enzyme supplemented groups recorded significantly higher eviscerated weight than the control group. The higher eviscerated weight in the treated groups agrees with the findings of Wang et al. (2011) with keratinase enzyme. In contrast, O'Shea et al. (2014) and Choe et al. (2017) did not observe any improvement in pig carcass traits as a result of enzyme supplementation. Ojediran et al. (2019b) attributed changes in carcass characteristics like jowl, loin ham, picnic shoulder and belly to feed utilization. Nkosi et al. (2020) reported that enzyme supplementation did not affect most of the carcass characteristics and the current study agrees with this.

Supplementation of enzyme to high fibre diet did not affect relative organ weight as observed in the current study

and that is consistent with the observation of Agyekum et al. (2012) in crossbred pigs fed distillers dried grains (DDGS). It appears that the metabolic strain on the digestive process associated with high fibre diet was not ameliorated by enzyme supplementation which aimed to improve fibre digestion. Furthermore, O'Shea et al. (2014) noted that the addition of protease to the diets of growing pigs did not affect carcass characteristics (carcass weight, back fat, lean meat, and dressing percentage) and this is consistent with the present study.

The mechanism of action of exogenous enzymes on the gastrointestinal tract (GIT) and the physiological effect on pigs when consuming PKM-based diets alone or in combination with enzymes are not well understood. Previous studies showed that high fibre diets increased organ weight in pigs (Nyachoti et al., 2000; Unigwe et al., 2017). It has been noted that feeding high fibre diets like PKM to pigs may affect gastrointestinal tract activity due to gut weight and gut fill, thereby confounding bodyweight changes as against carcass weight at slaughtering (Njoku et al., 2015). The study by Agyekum et al. (2012) observed that gut mass and digestive organs such as liver, pancreas and spleen were larger in pigs consuming DDGS diets without enzyme. The implication of this is the increase of energy requirement for maintenance in the animal. High fibre diets increase secretion of digestive fluids for the breakdown of fibre (Wenk, 2001) and the enlargement of the digestive organs. Any exogenous factor that aids fibre digestion will be savings on energy cost and the secretory activities of the organs and GIT to break down these high-fibre diets (Zamora et al., 2011; Agyekum et al., 2012). This, along with the improved feed utilisation may justify the inclusion of enzyme in pig diets containing high fibre concentration.

## CONCLUSION

Weaned pigs fed PKM-based diet supplemented with enzyme did not manifest any significant change in haematological or serum biochemical variables. Furthermore, carcass characteristics and organ weights did not follow any specific pattern but a tendency for increase in eviscerated weight and reduced empty stomach weight was observed. This justifies the inclusion of enzyme up to 3% dietary inclusion in pig diets containing a high proportion of PKM.

## CONFLICT OF INTEREST

All the authors declare that there are no conflicts of interest in carrying out this study.

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## AUTHORS CONTRIBUTION

**Conceptualization:** TKO, RAA. **Methodology:** TKO, RAA, IAE. **Data acquisition and analysis:** RAA, FAA, OEA. **Writing and editing:** RAA, FAA. **Project Supervision:** TKO, IAE.

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