Effect of Supplementation of Palm Kernel Cake (PKC) with Enzyme *Xylanase* on Performances and Gut Microbiota of Broiler Chickens

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Abstract

Recent development of antibiotics-resistance pathogens in poultry which poses threat to human health has necessitated the search for alternative to Antibiotic Growth Promoters (AGPs) to improve gut microflora in poultry diets. One of the alternatives to AGPs is probiotics which are beneficial organisms. Prebiotics, are by-products of digestion of polysaccharides which poultry do not have enzymes to digest are food for probiotics. Advent of enzymes makes this digestion possible. The prebiotic potentials of enzyme supplemented High Fibre Feedstuffs (HFFs) are not known. This study was conducted to assess the prebiotics potential of *xylanase* enzyme supplemented Palm Kernel Cake (PKC) on broiler chickens (*in-vivo*). In the absence of *xylanase* supplementation, there was significant increase in feed intake ($P>0.05$), significant decrease in weight gain ($P<0.05$) and significant increase in feed conversion ratio ($P>0.05$). There were significant interaction between dietary levels of Palm Kernel Cake and enzyme supplementation on Feed Intake, Weight Gain and Feed Conversion Ratio parameters ($p<0.05$). Enzyme supplementation irrespective of dietary level of PKC caused a reduction in cost of raising 1kg of broiler Chickens. It can be deduced that enzyme supplementation of PKC helped in increasing and improving protein, ether extract and fibre digestibility. The result obtained for the weights of vital organs showed that the birds were in good health conditions. Analysis of gut microflora (Fungi and Bacteria) showed that dietary levels of PKC (10%, 20% or 30% inclusion) with supplementation of enzyme *xylanase* enhanced the growth of beneficial microbes which resulted in inhibition or elimination of the opportunistic/ pathogenic microbes. The result of the cost benefit analysis also showed that 10% inclusion level of PKC supplemented with *xylanase* enzyme resulted in reduction of the cost of production with the best improved broiler performance. Use of enzymes is therefore recommended when HFFs are required as prebiotic source in the gut of broilers.

Key words: prebiotics, enzymes, intestinal microflora, digestibility, broiler nutrition

1. Introduction

Palm Kernel Cake (PKC) is a major byproduct from the palm kernel oil industry in several tropical countries including Malaysia, Indonesia, Thailand and Colombia. Its composition contains about 15.4% crude protein and 16.4% crude fibre out of which about 20 to 40% of the fibre is in the form of β-mannans (Yadi and Yana, 2011; Adrizal et al., 2011). This abundant by-product of palm oil industry is used as

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animal feed, although its use is limited to ruminants due to its high hemi-cellulose (mannan and galactomanan) and low essential amino acids content (Sakamoto and Toyohara, 2009).

Though the palm kernel meal is not widely used in the poultry industry because of its high fibre and low energy contents; the use of PKC in poultry diets has been reported by several researchers (Onwudike, 1986; et al., 2003; Mustafa et al., 2004). It has also been reported that enzymes could break down the non-starch polysaccharides (mannans) into manno-oligosaccharides to improve its nutritional quality (Dusterhoft et al., 1993a, b, c; Daud et al., 1997).

The use of antibiotics in the feed to improve animal performance has been in practice for decade but this has recently been banned in the European Union since 2006. This was due to the development of antibiotics resistance by pathogens such as *Salmonella*, *E. coli* and *Clostridium perfringens* and the presence of antibiotics residues in livestock/ human consumers of livestock products. This has resulted in the search for alternative growth promoters in livestock production. Prebiotics is reported as one of the alternatives to the use of antibiotics in animal feed as Mannan can be hydrolyzed into manno-oligosaccharides (MOS), a type of prebiotics (Reid and Robert, 2002; Samadi, 2002; Ohimain and Ofongo, 2013). Thus, this study was aimed at evaluating the prebiotics potential of Palm kernel cake supplemented with *xylanase* in the gut content of broiler chickens.

2. Materials and methods

2.1 Sourcing and Management of birds

A total of one thousand nine hundred and twenty **(**1,920) day old broiler chicks of Arbor Acre strain purchased from Yammfy Farm hatchery at Ilemona, Kwara State were used for this experiment. The birds were housed in an electrically heated battery cage and fed the experimental diet shown in Table 1. *Xylanase* enzyme used is a bacteria *xylanase* feed enzyme (Nutrase, a pure endo-1, 4-beta-*xylanase*) produced by *Bacillus subtilis* to break down the arabinoxylan fraction into shorter polysaccharide (xylose monomers) with a decrease of viscosity, liberation of nutrients and improved zoo technical performances. It was supplied by Nutrex, Belgium. The study was conducted following the guidelines of the Research Policy of the University of Ilorin on Animal Welfare and Ethics.

2.2 Experimental design

Birds were fed a control diet (50% maize) or diets in which palm kernel cake was added at 10%, 20%, or 30% replacing maize in the control diet. Each of these diets was given with or without 100 PPM *xylanase* enzyme in a 4x2 factorial combination. Thus, there were 8 treatments each with 8 replicate cages of 30 birds. The experiment was conducted for a period of 5 weeks. Live weight was recorded weekly while feed intake was recorded daily in grams and excreta samples collected over a 72 hour period. Nutrient retention trial was done at the third week of the feeding trial using a total collection method. Excreta samples were oven dried at 70^{o} C, weighed and ground prior to chemical analysis. The experimental diets and excreta samples were analyzed for their chemical constituents using the procedures outlined by AOAC (2008).

At the end of the experiment, 10 birds per replicate from each treatment was randomly selected and euthanized by bleeding through the carotid artery for collection of digesta from the gastro-intestinal tract (GIT) to determine the microbial profile. The carcasses were subsequently opened and the entire GIT removed using aseptic techniques. It was excised and the luminal contents of the crop, jejunum, duodenum, ileum and caecum were collected and pooled together according to the procedure described by Kalantar et al. (2014). This was done to determine population and profile of microorganisms present in the broiler gut. Five grams of each sample was put into sterile universal bottle containing 100 ml of sterile water and shaken

thoroughly. The mixture was allowed to settle and the supernatant decanted into separate test tubes; this serves as stock solution. Serial dilutions of the stock were made and 0.1 ml aliquot was spread on Potato Dextrose Agar (PDA) plate containing 1% streptomycin (to inhibit bacterial growth). The plates were incubated at room temperature (27-31^{o} C) for 48 hours (Bengmark, 2001). After incubation, representative fungal colonies were picked from each plate and purified on fresh PDA (for other fungi) and YPDA (Yeast Peptone Dextrose Agar) plates for yeast. The purified isolates were transferred to PDA and YPDA slants incubated at room temperature for 48 hours and stored at 4^{o} C. The isolates were identified according to the scheme of Chio et al. (1994). In isolation of bacteria, identification was based on the basis of their Gram's reaction and biochemical tests with reference to Bergey's manual of determinative bacteriology. The direct culturing of the samples was carried out by pipetting 1ml of the dissolved sample in sterile distilled water asceptically on sterile Petri-dishes. Pour plate technique was used with the NA (Nutrient Agar) plates incubated at 35^{o} C for 2-3 days. Bacteria were identified in their unique colonies. They were subcultured by streaking out on Nutrient Agar. Bacterial cells on NA took 2-3 days at 35^{o} C before their pure cultures were stored on slants containing Nutrient agar for bacteria and later stored at 4^{o} C. These slants served as stock culture for bacteria. Other tests were carried out to identify the morphological and biochemical characteristics of the microbes such as Gram's staining reaction, spore staining, motility test, catalase test, Voges-Proskauer test, methyl-red test, starch hydrolysis, casein hydrolysis, gelatin hydrolysis, oxidase test, indole test, nitrate reduction, sugar fermentation test, growth at different temperature, NaCl concentration, anaerobic growth, citrate utilization test, hydrogen sulphide production, urease test, acid production, sporulation test, growth in liquid medium at varying temperature, NaCl solution, and deamination of amino acid test.

Table 1: Composition of experimental diet (%).

Ingredients	1	2	3	4	5	6	7	8
Maize	50	50	40	40	30	30	20	20
Palm kernel cake	0	0	10	10	20	20	30	30
Xylanase (ppm)	0	100	0	100	0	100	0	100
Basal ingredients	50	50	50	50	50	50	50	50
Total	100	100	100	100	100	100	100	100

*Basal diets: Groundnut cake (GNC)-26%, corn bran-1%, soybean meal-12%, fishmeal (72%)-4%, palm oil-2%, oyster shell-2%, bone meal-2%, salt-0.25%, methionine-0.25%, lysine-0.25% and vitamin premix-0.25%.(*Vitamin/ mineral premix contained the following: (Univit. 15 Roche) Vit A, 1500 I.U, Vit D, 3000 I.U, Vit E, 3.0 g, Vit K, Vit. B_2 0.3 g, Vit.B_6, 8.0 mg, Vit.B_{12}, 8.0 g, Nicotinic Acid, 3.0 g, Ca-Pantothenate, 50 mg, Fe, 10.00 g, Al, 0.2 g, Cu, 3.5 mg, Zn, 0.15 mg, I, 0.02 g, CO_2, 0.01 g, Se).*

2.3 Cost-benefit Analysis

Cost-benefit analysis was carried out, taking into consideration the cost of maize, Palm Kernel Cake and the enzyme (*xylanase*) as they related to the performance of birds.

2.4 Statistical analysis

All Data collected were subjected to two way analysis of variance (ANOVA) using the PRO GLM (General Linear Model) of SAS (2008) at 5% level of significance. All significantly different means were separated using the Duncan's Multiple Range Test of the same software package.

3. Results and Discussion

Table 2 shows the effects of dietary levels of PKC with or without enzyme supplementation on the performance of broilers. Increase in dietary levels of Palm Kernel Cake from 0% to 30% had a significant effects on the feed intake, weight gain and feed conversion ratio ($p<0.05$). Feed intake by birds fed the control was significantly lower than those of birds fed diets with dietary levels of PKC ($p<0.05$). Dietary levels of PKC had significant increased effects on the feed intake ($p<0.05$). Birds fed the control diet gained more weight than those of birds fed dietary levels of PKC ($p<0.05$). Dietary levels of PKC had significant decreased effects on the weight gain ($p<0.05$). Feed gain ratio of birds fed the control diet was significantly lower than those of birds fed diets with dietary levels of PKC ($p<0.05$). The dietary levels of PKC had increased significant effects on the feed conversion ratio (FCR) of the birds. Thus, birds fed the control diet had a better feed gain ratio compared to dietary levels of PKC ($p<0.05$). There were significant effects by enzyme supplementation on performance of the broilers. Enzyme supplementation had significant increase effect on weight gain ($p<0.05$) but decrease effect on feed intake and feed/gain ratio ($p<0.05$). There were significant interaction between dietary levels of Palm Kernel Cake and enzyme supplementation on Feed Intake, Weight Gain and Feed Conversion Ratio parameters ($p<0.05$). The details of interactions are shown in Table 3. In the absence of *xylanase* supplementation, there was significant increase in feed intake ($p>0.05$), significant decrease in weight gain ($p<0.05$) and significant increase in feed conversion ratio ($p>0.05$). The reverse was the case respectively when the diet was supplemented with *xylanase*. The effects of dietary level of PKC with or without enzyme supplementation on nutrient retention are shown in Table 2. Crude protein of birds fed the control diet was significantly higher than those of birds fed diets with dietary levels of PKC ($p<0.05$). Dietary levels of PKC had a significant decrease effects on the protein retention by the birds. Crude fibre by birds fed the control diet and birds fed diet with10% PKC were comparable ($p>0.05$), but significantly higher than those of birds fed diets with 20% or 30% PKC ($p<0.05$). Dietary levels of PKC had significant increase effects on the Crude Fibre ($p<0.05$). Ether extract by birds fed the control diet and birds fed diet with 10% PKC were comparable ($p>0.05$), but significantly higher than those of birds fed diets with 20% or 30% PKC ($p<0.05$). Ether extract by birds fed diets with 20% and 30% PKC were comparable but significantly lower than those of birds fed diet with 10% PKC ($p<0.05$). Enzyme supplementation had significant increased effects on nutrient retention ($p<0.05$).

Table 2: Effects of dietary levels of Palm Kernel Cake with or without *Xylanase* supplementation on performance of broilers (0-5wks) and digestibility.

PKC (%)	Feed consumed (g/bird/day)	Weight gain (g/bird/day)	FCR	Crude protein (%)	Crude fibre (%)	Ether extract (%)
0	55.40^{d}	29.70^{a}	1.80^{d}	83.90^{a}	66.10^{a}	60.10^{a}
10	56.10^{c}	28.10^{b}	2.00^{c}	76.90^{b}	65.10^{a}	66.50^{a}
20	57.50^{b}	25.90^{c}	2.20^{b}	69.50^{c}	61.80^{b}	58.60^{b}
30	59.60^{a}	24.40^{d}	2.50^{a}	62.00^{d}	56.10^{c}	59.80^{b}
SE	0.11	0.16	0.01	0.44	0.58	1.66
Enzyme Supplementation (ES) (ppm)						
0	58.60^{a}	26.80^{b}	2.20^{a}	70.50^{b}	57.70^{b}	59.40^{b}
100	55.70^{b}	27.30^{a}	2.10^{b}	76.00^{a}	67.20^{a}	63.20^{a}
SEM±	0.08	0.12	0.01	0.31	0.41	1.17
PKCxES	NS	NS	NS	S	S	NS

Column means with different superscripts are significantly different ($p<0.05$), NS: not significant, S: Significant.

There was significant interaction between enzyme supplementation and dietary levels of PKC on Crude Protein and Crude Fiber ($p<0.05$). The detail of interactions are shown in Table 3.Thus, enzyme supplementation of the control diet resulted in decrease in crude protein retention and crude fibre retention but reverse was the case with addition of PKC irrespective of levels ($p<0.05$).

Table 3: Details of interaction on feed consumed, weight gain, feed conversion ratio, crude protein and crude fibre.

Enzyme supplementation			Dietary Palm Kernel Cake Supplementation (%)			
ES (100ppm)			0	10	20	30
Feed	0		56.40^{d}	58.20^{c}	59.20^{b}	60.50^{d}
Consumed		100	54.40^{f}	54.10^{f}	55.70^{e}	58.60^{c}
Weight	0		29.80^{a}	28.30^{b}	25.70^{c}	23.40^{d}
Gain	100		29.70^{a}	27.90^{b}	26.10^{c}	25.50^{c}
Feed		0	1.90^{e}	2.10^{d}	2.30^{b}	2.60^{a}
Conversion	100		1.80^{f}	1.90^{e}	2.10^{c}	2.30^{b}
Crude		0	86.63^{a}	76.59^{c}	66.32^{e}	56.43^{f}
Protein		100	81.25^{b}	77.15^{c}	72.60^{d}	67.49^{e}
Crude		0	67.81^{b}	57.81^{e}	57.20^{e}	51.34^{f}
Fibre100			64.45^{c}	76.59^{c}	66.32^{e}	56.43^{f}

Column means with different superscripts are significantly different ($p<0.05$).

Table 4 shows the cost benefit analysis for replacing maize with PKC with or without enzyme *xylanase* supplementation. Increase in dietary level of PKC reduced the cost of feed. Addition of *xylanase* to each level of PKC automatically increased the cost of the feed. Increase in dietary level of PKC caused an increase in the cost of producing 1 kg of broiler irrespective of enzyme supplementation. Enzyme supplementation irrespective of dietary level of PKC caused a reduction in cost of raising 1 kg of broiler chickens.

Table 4: Cost benefit analysis for replacing Maize with Palm Kernel Cake with or without enzyme *xylanase* supplementation.

Source of Variation	Cost of Producing/kg (N)	Percentage reduction in price of feed	Cost of Raising 1 kg of Broilers (N)	Percentage reduction to Raise 1 kg of Broilers (%)
Enzyme*Treatment				
Non- Inclusion 0	111.49	0.00	211.83	0.00
Non- Inclusion 10	106.49	4.70	218.30	-3.06
Non- Inclusion 20	101.15	10.22	233.66	-10.30
Non- Inclusion 30	96.50	15.53	249.94	-17.99
Inclusion	114.49	-0.03	209.52	1.09
Inclusion	109.49	1.82	212.41	-0.27
Inclusion	104.15	7.06	221.84	-4.72
Enzyme*Treatment	99.50	12.05	228.85	-8.03

Table 5 shows the effects of dietary levels of PKC with or without enzyme supplementation on organs and body parts weight. Increase in dietary levels of wheat offal from zero to 30% had significant effects on the weights of head, legs, thigh, drumsticks, wings, breast, back, neck, liver, heart, crop, proventriculus, abdominal fat, full and empty gizzard ($p<0.05$), but no effects on spleen weight and weight of bursa of fabricius ($p>0.05$). Weight of the head of birds fed the control diet and birds fed diet with 10%

PKC were comparable ($p>0.05$), but significantly lower than those of birds fed diets with 20% or 30% PKC. The head weight of birds fed diet with 30% PKC was significantly higher than those fed diets with 10% or 20% PKC. Legs weight of birds fed the control diet and birds fed diet with 10% PKC were comparable ($p>0.05$), but significantly higher than those of birds fed diets with 20% or 30% PKC. Weight of legs of birds fed diets with PKC irrespective of levels were comparable ($p>0.05$). Thigh weight of birds fed the control diet and birds fed diets with 10% or 20% PKC were comparable ($p>0.05$), but significantly higher than those of birds fed diets with 30% PKC. The thigh weight of birds fed diet with 30% PKC was significantly lower than those of birds fed diets with 10% or 20%PKC. Weight of drumsticks of birds fed the control diet and birds fed diets with 10% or 20% PKC were comparable ($p>0.05$), but significantly higher than those of birds fed diets with 30% PKC ($p<0.05$). The drumsticks weight of birds fed diet with 30% PKC was significantly lower than those of birds fed diets with 10% or 20% PKC ($p<0.05$). Weight of wings of birds fed the control diet and birds fed diets with 10% or 20% PKC were comparable ($p>0.05$), but significantly higher than those of birds fed diets with 30% PKC ($p<0.05$). Weight of wings of birds fed diets with PKC irrespective of levels were comparable ($p>0.05$). Breast weight of birds fed the control diet was significantly higher than those fed diets with dietary levels of PKC ($p<0.05$).

Breast weight of birds fed diet with 20% and 30% PKC were comparable but significantly lower than those of birds fed diet with 10% PKC ($p<0.05$). Weight of back of birds fed the control diet and birds fed diets with 10% or 20% PKC were comparable ($p>0.05$), but significantly higher than those of birds fed diets with 30% PKC ($p<0.05$). Back weight of birds fed diets with 30% PKC was significantly lower than those of birds fed diets with 10% or 20% PKC ($p<0.05$). Weight of neck of birds fed the control diet was significantly higher than those of birds fed with dietary levels of PKC ($p<0.05$). Weight of neck of birds fed diets with PKC irrespective of levels were comparable ($p>0.05$). Weight of Liver of birds fed the control diet was significantly higher than those of birds fed diet with 10% PKC ($p<0.05$), and comparable with those of birds fed diets with 20% or 30% PKC ($p>0.05$). Liver weight of birds fed diet with 30% PKC was significantly higher than birds fed diet with 10% PKC ($p<0.05$), but comparable with birds fed diet with 20% PKC ($p>0.05$). Heart weight of birds fed the control diet was significantly lower than those of birds fed with dietary levels of PKC ($p<0.05$). Heart weight of those of birds fed diets with PKC irrespective of levels were comparable ($p>0.05$).Crop weight of birds fed the control diet was significantly lower than those of birds fed with dietary levels of PKC ($p<0.05$).

Crop weight of birds fed diets with 10% and 20% PKC were comparable ($p>0.05$), but significantly lower than those of birds fed diet with 30% PKC ($p<0.05$). Proventriculus weight of birds fed the control diet was significantly higher than birds fed diet with 20% PKC ($p<0.05$), but comparable with those of birds fed diets with 10%or 30% PKC ($p>0.05$). Proventriculus weight of birds fed diet with 30% PKC was significantly higher than those of birds fed diets with 10% or 20% PKC ($p<0.05$).). Abdominal fat weight of birds fed the control diet was significantly higher than those fed diets with dietary levels of PKC ($p<0.05$). Abdominal fat weight of birds fed diet with 30% PKC was significantly lower than birds fed diet with 20% PKC ($p<0.05$), and comparable with those of birds fed diet with 10% PKC ($p>0.05$).Weight of full gizzard of birds fed the control diet was significantly lower than those of birds fed with dietary levels of PKC ($p<0.05$). Dietary levels of PKC had significant increased effect on the weight of full gizzard ($p<0.05$). Empty gizzard weight of birds fed the control diet was significantly lower than those of birds fed with dietary levels of PKC ($p<0.05$). Empty gizzard weight of birds fed diet with 30% PKC was significantly higher than those of birds fed diets with 10% or 20% PKC ($p<0.05$). There was no significant effect of enzyme supplementation on weights of the head, thigh, wings, breast, heart, crop and proventriculus. However, there were significant increased effects on weights of the Legs, drumsticks, back, neck, liver and spleen ($p<0.05$), but decreased effects on the weights of abdominal fat, bursa of fabricius, full and empty gizzard ($p<0.05$). There was no significant interaction between enzyme supplementation

and dietary levels of Palm Kernel Cake on all the parameters ($p>0.05$), except for spleen and Empty gizzard ($p<0.05$). The details of interaction are shown in Table 6.

The interaction between enzyme supplementation and dietary levels of Palm Kernel Cake on the weight of the spleen is given in Table 6. Enzyme supplementation of the control diet and diets with 20 or 30% PKC caused a decrease in spleen weight. The reverse was the case at 10% PKC inclusion level. There was decrease in empty gizzard weight with enzyme supplementation of diet with 30% PKC. However, there were marginal but no significant effect of the enzyme supplementation at the control and lower dietary levels of PKC.

Table 7 illustrates the effects of dietary levels of PKC with or without enzyme supplementation on the microbial gut profile of broilers. Increase in dietary levels of wheat offal from 0% to 30% had significant effect on all the parameters except FCC ($p<0.05$). TVC of birds fed the control diet was significantly lower than those of birds fed with dietary levels of PKC ($p<0.05$). TVC of birds fed diets with PKC irrespective of levels were comparable ($p>0.05$). TCC of birds fed the control diet was significantly higher than birds fed diet with 20% PKC ($p<0.05$), but comparable with those of birds fed diets with 10% or 30% PKC ($p>0.05$). TCC of birds fed diets with PKC irrespective of levels were comparable ($p>0.05$). LBC of birds fed the control diet and birds fed diet with 10% PKC were comparable ($p>0.05$), but significantly lower than those of birds fed diets with 20% or 30% PKC ($p<0.05$). LBC of birds fed diet with 30% PKC was significantly higher than those of birds fed diets with 10% or 20% PKC ($p<0.05$). FC of birds fed the control diet was significantly lower than those of birds fed with dietary levels of PKC ($p<0.05$). FC for those of birds fed diets with 20% or 30% PKC was significantly higher than birds fed diet with 10% PKC ($p<0.05$). pH of birds fed the control diet was significantly lower than birds fed diet with 30% PKC ($p<0.05$), but comparable with those of birds fed diets with 10% or 20% PKC ($p>0.05$). pH of birds fed diet with 30% PKC was significantly higher than those of birds fed diets with 10% or 20% PKC ($p<0.05$). There was no significant effects by enzyme supplementation for total viable count (TVC), total colony count (TCC), fungi colony count (FCC), fecal count (FC) and pH ($p>0.05$). However, there was significant increased effect for *Lactobacillus* count (LBC) ($p<0.05$). There was no significant interaction between enzyme supplementation and dietary levels of Palm Kernel Cake on all the parameters ($p>0.05$).

Table 8 shows the summary of the identified fungi in the crop, ileum and caecum pooled together. Dietary levels of Palm Kernel Cake with or without *xylanase* supplementation had an effect on the number of the fungi in the crop, ileum and caecum. Dietary levels of PKC from 0 to 30% without *xylanase* supplementation had no effect on the number of beneficial fungi identified in the gut but a numerical increase effect on the number of opportunistic/pathogenic identified in the gut of the broiler chickens. *Saccharomyces cerevisiae* was the only beneficial fungus identified in the gut of bird fed the control diet and those birds fed diets with dietary levels of PKC without *xylanase* supplementation. Two species of opportunistic/pathogenic fungi namely; *Rhizopus stolonifer and Aspergillus niger* were identified in the gut of birds fed the control diet without *xylanase* supplementation while Four species of opportunistic/pathogenic fungi namely; *Fusarium solani, Rhizopus stolonifer, Aspergillus niger* and *Aspergillus flavus* were identified in the gut of those birds fed diets with dietary levels of PKC without *xylanase* supplementation.

Table 5: Effects of dietary levels of Palm Kernel Cake with or without enzyme *xylanase* supplementation on organs and body parts expressed in percentage live body weight of broilers.

	Head	Legs	Thigh	Drum sticks	Wings	Breast	Back	Neck	Liver	Heart	Crop	Proven tri-culus	Abdo-minalf at	Spleen	Bursa of fabrici u-s	Full gizzar d	Empty gizzar-d
PKC (%)																	
0	2.50^{c}	4.60^{a}	11.40^{a}	10.70^{a}	8.00^{a}	21.30^{a}	12.10^{a}	5.50^{a}	2.20^{a}	0.60^{a}	0.60^{c}	0.70^{ab}	2.40^{a}	0.10	0.10	2.80^{d}	1.60^{c}
10	2.60^{bc}	4.30^{ab}	10.80^{a}	10.80^{a}	7.50^{ab}	19.50^{b}	13.00^{a}	4.50^{b}	1.90^{b}	0.40^{b}	0.90^{b}	0.50^{bc}	0.50^{bc}	0.10	0.20	3.20^{c}	2.00^{b}
20	2.70^{b}	4.20^{b}	11.00^{a}	10.40^{a}	7.50^{ab}	16.60^{c}	12.40^{a}	5.00^{b}	2.10^{ab}	0.40^{b}	0.90^{b}	0.50^{c}	0.70^{b}	0.10	0.20	3.60^{b}	2.10^{b}
30	2.90^{a}	4.00^{b}	8.00^{b}	9.10^{b}	7.40^{b}	16.10^{c}	11.00^{b}	4.50^{b}	2.20^{a}	0.40^{b}	1.20^{a}	0.70^{a}	0.30^{c}	0.10	0.20	4.20^{a}	2.50^{a}
SE	0.06	0.13	0.30	0.29	0.20	0.54	0.33	0.08	0.07	0.02	0.06	0.04	0.12	0.01	0.02	0.11	0.06
ES (100ppm)																	
0	2.63	3.61^{b}	10.36	9.82^{b}	7.58	17.88	11.30^{b}	4.65^{b}	1.99^{b}	0.42	0.86	0.61	1.18^{a}	0.09^{b}	0.17^{a}	3.72^{a}	2.19^{a}
100	2.73	4.97^{a}	10.75	10.65^{a}	7.64	18.86	12.90^{a}	4.91^{a}	2.20^{a}	0.42	0.88	0.57	0.80^{b}	0.11^{a}	0.14^{b}	3.21^{b}	1.89^{b}
SE	0.04	0.09	0.21	0.21	0.14	0.38	0.23	0.06	0.05	0.01	0.04	0.03	0.08	0.01	0.02	0.08	0.04
	0.0899	<0.0001	0.2174	0.0119	0.7613	0.0889	0.0002	0.0073	0.0051	0.8775	0.8410	0.3021	0.0050	0.0950	0.1574	0.0004	0.0002
PKC*ES	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	S	NS	NS	S

Column means with different superscripts are significantly different (p<0.05), NS: not significant, S: Significant.

Table 6: Details of interaction on spleen, and empty gizzard weight.

Enzyme supplementation	Dietary Palm Kernel Cake Supplementation (%)				
	ES (100ppm)	0	10	20	30
Spleen	0	0.07^{de}	0.16^{a}	0.06^{e}	0.08^{cde}
	100	0.11^{cd}	0.07^{e}	0.12^{bc}	0.15^{ab}
Gizzard	0	1.70^{de}	1.90^{cd}	2.00^{bc}	3.10^{a}
Weight	100	1.50^{e}	2.10^{bc}	2.20^{b}	2.10^{bc}

Column means with different superscripts are significantly different (p<0.05).

Table 7: Effects of dietary levels of Palm Kernel Cake with or without enzyme supplementations on the microbial gut profile of broilers.

	TVC (10^7cfu/ml)	TCC (10^7cfu/ml)	FCC (10^7cfu/ml)	LBC (10^7cfu/ml)	FC (10^5cfu/ml)	pH
PKC (%)						
0	5.00^{b}	4.70^{a}	1.60	2.60^{c}	2.40^{c}	4.90^{b}
10	6.30^{a}	3.70^{ab}	1.08	2.50^{bc}	3.70^{b}	5.00^{b}
20	7.00^{a}	3.30^{b}	1.18	2.80^{b}	3.30^{a}	5.20^{b}
30	6.80^{a}	3.70^{ab}	1.15	1.80^{a}	2.80^{a}	5.10^{a}
±SEM	0.24	0.35	0.29	0.12	0.09	0.10
Enzyme (100ppm)						
0	6.10	2.20	1.50	1.80^{b}	1.80	5.00
100	6.70	1.20	1.00	3.50^{a}	3.00	5.10
±SEM	0.17	0.24	0.21	0.08	0.06	0.07
PKC*ES	0.16	0.07	0.71	0.08	0.01	0.45

Column means with different superscripts are significantly different ($p<0.05$), Total Viable Counts (TVC), Total Coliform Counts (TCC), Feacal Coliform Counts (FCC), Lactobacillus Counts (LBC) and Fungi Counts (FC).

Dietary levels of PKC with *xylanase* supplementation had numerical effect on the number of beneficial fungi identified in the gut of broiler chickens. *Saccharomyces cerevisiae* was also the only beneficial fungus identified in the gut of birds fed the control diet with *xylanase* supplementation and also four species of opportunistic/pathogenic fungi namely; *Fusarium solani, Rhizopus stolonifer, Aspergillus niger* and *Aspergillus flavus* were identified. The number of beneficial fungi identified in the gut of those birds fed diets with dietary levels of Palm Kernel Cake with *xylanase* supplementation had increased to two species namely: *Saccharomyces cerevisiae* and *Penicilium chrysogerium.* For birds fed diet with 10% PKC with *xylanase* supplementation, *Aspergillus niger* was the only opportunistic/pathogenic fungi identified in the gut while in the gut of those birds fed diets with 20% or 30% PKC with enzyme supplementation, two species namely; *Aspergillus niger* and *Aspergillus flavus* were identified.

Table 8 shows the summary of the identified bacteria in the crop, ileum and caecum pooled together. Replacement of Maize with Palm Kernel Cake with or without *xylanase* supplementation had an effect on the number of bacteria identified in the crop, ileum and caecum. In the gut of birds fed diets with dietary levels of PKC from 0 to 30% without *xylanase* enzyme supplementation; *Lactobacillus* sp. was the only beneficial bacterium identified while the pathogenic bacteria identified were six genera namely; *E. coli, Streptococcus, Staphylococcus, Clostridium, Campylobacter* and *Salmonella* species. Enzyme supplementation of the dietary levels of PKC had increase effects on the number of beneficial bacterial and reduces effects on the number of the pathogenic bacteria identified. With enzyme *xylanase* supplementation, in the gut of birds the control diet *Lactobacillus* sp. was still the only beneficial bacteria identified but the pathogenic bacteria identified had reduced to five genera namely; *E. coli, Clostridium* sp., *Streptococcus* sp., *Salmonella* sp. *and Staphylococcus* and in the gut of those birds fed diets with 10% or 20% or 30% PKC the beneficial bacteria had increased to three genera namely; *Lactobacillus sp. Bacillus subtilis* and *Bifidobacterium* sp. while the number of pathogenic bacteria had reduced to two (*E. coli, Clostridium* sp. and *Campylobacter* sp.).

Table 8: Identified microbes (fungi and bacteria) in the gastrointestinal tract of broiler chicken with or without enzyme supplemented Palm Kernel Cake.

Without Enzyme	Beneficial Fungi	Opportunistic/pathogenic Fungi	Beneficial Bacteria	Pathogenic Bacteria
Control	*Saccharomyces cerevisiae*	*Rhizopus stolonifer, Aspergillus niger*	*Lactobacillus plantarum*	*E. coli, Streptococcus sp, Staphylococcus sp,Clostridium spp, Campylobacter, Salmonella spp.*
10%	*Saccharomyces cerevisiae*	*Fusarium solani, Rhizopus stolonifer , Aspergillus niger, Aspergillus flavus*	*Lactobacillus plantarum*	*E. coli, Streptococcus sp, Staphylococcus sp,Clostridium spp, Campylobacter, Salmonella spp.*
20%	*Saccharomyces cerevisiae*	*Fusarium solani, Rhizopus stolonifer, Aspergillus niger, Aspergillus flavus*	*Lactobacillus plantarum*	*E. coli, Streptococcus sp, Staphylococcus sp,Clostridium spp, Campylobacter, Salmonella spp.*
30%	*Saccharomyces cerevisiae*	*Fusarium solani, Rhizopus stolonifer, Aspergillus niger, Aspergillus flavus*	*Lactobacillus plantarum*	*E. coli, Streptococcus sp, Staphylococcus sp,Clostridium spp, Campylobacter, Salmonella spp.*
With Enzyme				
Control	*Saccharomyces cerevisiae*	*Fusarium solani, Rhizopus stolonifer, Aspergillus niger, Aspergillus flavus*	*Lactobacillus plantarum*	*E. coli, Streptococcus sp, Staphylococcus sp,Clostridium spp, Salmonella spp.*
10%	*Penicilium chrysogenum, Saccharomyces cerevisiae*	*Aspergillus niger*	*Bacillus subtilis, Lactobacillus plantarum, Bifidobacterium*	*Clostridium sp. Campylobacter sp.*
20%	*Penicilium chrysogenum, Saccharomyces cerevisiae*	*Aspergillus niger, Aspergillus flavus*	*Bacillus subtilis, Lactobacillus plantarum, Bifidobacterium*	*Clostridium sp. Campylobacter sp.*
30%	*Penicilium chrysogenum, Saccharomyces cerevisiae*	*Aspergillus niger, Aspergillus flavus*	*Bacillus subtilis, Lactobacillus plantarum, Bifidobacterium*	*Clostridium sp. Campylobacter sp.*

Palm Kernel Cake (PKC) is a by-product of the African palm oil industry, which can be fed to poultry because of its availability and low cost (Perez, et al., 2000). World report (2003) indicated Nigeria as the third largest world producer and exporter of PKC after Malaysia and Indonesia. The inclusion of PKC in the diets was less practiced in monogastric animals particularly in poultry due to its high fibre content. But the nutritive value of PKC may be improved through exogenous supplementation of enzymes to breakdown the non-starch polysaccharides.

According to the findings of this study, Feed intake increased with increasing PKC levels in the diets without enzyme *xylanase* supplementation, with broilers fed the control (0% PKC) diet without *xylanase* having significantly ($p<0.05$) least feed intake which was close to feed intake of birds on 10% PKC diet without *xylanase*. The feed intake was also increasing with PKC inclusion level without *xylanase*

supplementation. This is in agreement with Sundu et al., (2005) who reported increase in feed intake with increase in PKC inclusion level in the diets of broilers. This increase in daily feed intake with inclusion level could be due to energy dilution of the diet by PKC leading to the broilers consuming more feed to meet their energy requirement (Onifade and Babatunde, 1998). However, the addition of enzyme *xylanase* reduced the feed intake. This is supported by Lawal et al., (2010) and Soltan (2009) who recorded similar observation and it might be due to better feed utilization in the presence of exogenous enzymes.

Replacement of maize with PKC without enzyme supplementation caused a significant ($P<0.05$) reduction in weight gain when compared with the control. This is in agreement with Ezieshi and Olomu (2004) and Ojewola and Ozuo (2006) who reported that birds fed on diets containing 10%, 15% and 20% of PKC instead of soybean meal reduced the body weight gain when compared with control. With *xylanase* supplementation, the body weight gains were apparently improved. The moderate improvement in weight gain might be due to improved fibre digestibility by the exogenous enzyme (*xylanase*) which is in consonance with the findings of Ojewola et al., (2003) and Soltan (2009).

The feed/gain ratio of broilers was significantly affected with the inclusion levels of PKC without enzyme supplementation compared to control confirming the earlier report of Panigrahi and Powel (1991) and Hosamain et al., (2001) who recorded significant ($P<0.05$) differences in feed efficiency due to inclusion of higher levels of PKC in the diets of broiler chickens. Birds fed PKC diets with *xylanase* showed better feed/gain ratio. It might be due to better utility of nutrients from Non-starch polysaccharides (NSP) by the effect of *xylanase* leading to better feed efficiency (feed/gain ratio). This result is in conformity with the finding of Lawal et al. (2010) who reported improvement in feed conversion ratio in birds fed biodegraded PKC. Feed to gain ratio was better among enzyme supplemented diets and the control compared with all other diets without enzyme supplementation. This is similar to the reports of Atteh (2000) and Esuga et al., (2008) who separately observed improvements in weight gain and feed: gain ratio in birds fed enzyme supplemented diets. Esuga et al., (2008) also observed lower weight of birds fed increasing levels of PKM without enzyme supplementation. PKC inclusion resulted in a lower cost of raising broiler chickens to 1 kg live weight without detrimental effect, hence shows its economic advantage. This was as a result of an expensive high energy feedstuff source (maize) being replaced by PKC (a cheaper non-conventional feedstuff).

The reduction in CP, CF, and EE digestibility in the PKC diets without *xylanase* supplementation was attributed to the effect of replacement of highly digestible carbohydrate source, maize by PKC which was of low digestibility. The higher crude fibre content of the PKC diets may have adversely affected digestion. This is in line with McDonald et al., (1983) who reported that PKC in diets has been implicated in low digestibility of due to its high crude fibre level which is estimated at 150 g/kg of PKC. Based on this trial an improved nutrient retention is observed when PKC was supplemented with *xylanase*. The enhanced nutrient digestibility was due to the breakdown of the NSPs in PKC by exogenous enzyme. Fibrous feeds decreased the digestibility of crude protein and the possibility that the presence of the fibre may speed up the rate of passage of feed through the simple stomach of monogastric thereby leaving little time frame for nutrient to be utilized by the birds; this was reported by Jorgensen et al. (1996); but enzyme supplementation helps to improve the nutrient availability in monogastric (Schingoethe et al., 1999) such that the crude protein, crude fibre and ether extract are release in a way that is more readily available for utilization by the birds (Iyayi et al., 2005; Iyayi and Davies, 2005). This is also in agreement with Adams (1993) and Atteh (2000) who observed that the use of *xylanase* enhanced nutrient utilization. Birds fed with diets supplemented with enzymes had better crude fibre retention as compared to those fed diets without enzyme supplementation. This may also be as a result of the effect of the enzyme supplementation on the feedstuffs as exogenous enzyme supplementation has been reported to improve the digestibility of fibrous agricultural products (Tuleun et al., 1998; Rotter et al., 1999; Viveros et al., 2000). The effect of dietary treatments on ether extract as observed in this study may be attributed to the fact that enzyme supplementation improves ether extract availability and utilization for monogastric.

The result of absolute weight of the body parts and the organs is in conformity with the result of Bai (2002) who reported no significant difference in carcass yield of broiler when normal maize was replaced by quality protein maize, is also in conformity with the findings of Zand and Froudi (2011) who uses corn snacks waste on the performance of broilers. There was a significant difference on the wings, neck and liver. The wings increased with increased level of dietary PKC which may be as a result of the birds in constant use of their wings probably to gain balance while struggling to eat in order to meet their energy requirement. The significant difference in the treatments may also be as a result of their feeding habit. The significant effect of enzyme and treatments on the liver maybe as a result of the level of work load placed on the liver due to the level of anti-nutritional factors such as high dietary fibre as those with enzyme supplementation at 0% PKC level have higher value than those with enzyme supplemented PKC (which have similar effect) but the percentage weight of the liver increased as the level of PKC increased in diets without enzyme supplementation. This study shows that the liver increased with increased fibre level in the absence of enzyme but the reverse was the case in enzyme supplemented diets probably due to the activity of the enzyme supplemented i.e. breaking down the anti-nutritional factors to reduce or eliminate the anti-nutritional effect of the fibre in the PKC thereby reducing the level of detoxification that the liver will handle. Liver functions in the process of detoxification; when work load reduces on the liver, liver tends to reduce as organs are known to enlarge when used over time. Atteh (2004) reported increase in percentage liver weight in the absence of enzyme.

This is in consonance with the observation made based on enzyme supplementation, there was a significant difference in the percentage weight of legs, drum sticks, back, neck and liver. Birds fed enzyme supplemented diets had a significant higher percentage weight as compared to diets without enzyme supplementation. This can be traced to the positive effect of enzyme supplementation as it aided significantly lower feed conversion ratio in birds fed to and hence, a superior body size and body weight (Yunusa et al., 2014). But the significantly higher value for the liver is conflicting to the report of Nadeem et al., 2005. Hosseini-Vashan (2010) reported that the relative weight of heart will increased when broiler chickens were fed diets containing higher concentration of ME and digestible CP.

This might be as a result of high level of fibre which acts as diluent thereby preventing excess fat deposition in the body of the animals. This is in agreement with Babatunde, et al. (1975) who reported that fibrous feeds, because of their lower content and availability of energy tend to promote better carcasses with higher lean meat and lower fat contents. The lower abdominal fat at 0% enzyme treated PKC diet might be as a result of enzyme action i.e. breaking down the nutrients for better utilization and performance.

The increased weight of the full gizzard might be as a result of the bulkiness of the feed as PKC is more bulky than maize. It might also be as a result of the time interval with which the birds last consumed their feed. The significant variation in the empty gizzard was as a result of the quantity of feed removed from the gizzard of the birds. It might also be as a result of the bulkiness of the diets as 30% PKC without enzyme supplementation had the highest empty gizzard weight. Based on enzyme supplementation, the significant lower percentage weight of the abdominal fat, full gizzard and empty gizzard in enzyme supplemented diets as compared to diets without enzyme supplementation can be attributed to the effect of the enzyme supplementation on the feedstuffs as exogenous enzyme supplementation has been reported to improve the digestibility of fibrous agricultural products (Tuleun et al., 1998; Rotter et al., 1999; Viveros et al., 2000) and as such utilizes nutrient adequately (resulting in less fat deposition) and reduces the bulkiness of the feed (feed and gizzard weight reduces).

This is in agreement with Hosseini-Vashan (2010). The full gizzard increased significantly with increased level of PKC in the diets. This is due to the bulkiness of PKC as compared to maize. The effect of the level of PKC inclusion for crop and empty gizzard was similar at 10% and 20% PKC inclusion level but significantly higher than that of 0% and lower than that of 30% PKC inclusion level. This can also be

attributed to the bulkiness of the feed they accommodate in these organs. Olorede (1998) reported that weight of crop is expected to increase at graded level of dietary fibre. The percentage weight of the abdominal fat at 10% PKC inclusion had similar effects with 20% and 30% PKC inclusion level but significantly lower than 0% PKC inclusion level. This might be as a result of high level of fibre which acts as diluent thereby preventing excess fat deposition in the body of the animals. This is in agreement with Babatunde et al. (1975) who reported that fibrous feeds, because of their lower content and availability of energy tend to promote better carcasses with higher lean meat and lower fat contents.

The result from microbial loads might be attributed that enzyme supplementation aided the release of prebiotic oligosaccharides in PKC. This resulted in the growth of lactobacillus in the enzyme supplemented diet. Though the lower population of the faecal coliform bacteria in enzyme supplemented diets was not of significant effect, the significant higher value of lactobacillus population in enzyme supplemented diets can be said to have suppressed the growth of faecal coliform bacteria. This corresponds with the work of Chen et al., 2015 who reported that enzyme treatment releases the prebiotic oligosaccharides from palm kernel expeller. This shows that PKC has prebiotic properties. The significant rise in lactobacillus population and numerical reduction in the faecal coliform count may be as a result of the prebiotic property of PKC as it produced more probiotics (lactobacillus) thereby suppressing the growth of non-beneficial bacteria (faecal coliform bacteria e.g. *E. coli*). The enhancement of beneficial oligosaccharides could be due to inhibition of pathogenic bacteria colonization (Djouzi and Andrieux, 1997; Bruggencate et al., 2004, Van Meer et al., 2008) and/or the adherence of numerous grams negative bacteria to animal cells is specifically inhibited by mannose or its derivatives through the competitive absorption to the mannose specific type 1 fimbriae, thereby limiting their colonization in intestinal epithelium (Ofek and Beachey, 1978). Baurhoo et al., (2007) also reported a significant increase in *Lactobacillus* and *Bifidobacterium* population using mannose oligosaccharide. This further strengthens the work of Chen et al. (2015) although he reported that this property may be enhanced by enzyme supplementation which was also observed in this study. Increased level of dietary PKC which implies that the acidity of the diets reduced with increased inclusion level of PKC. This may be the reason why birds fed diets with little or no PKC inclusion level performed better than those with higher PKC inclusion level in their diets as acidic condition has been reported to aid digestion (most especially fibre) e.g. hydrochloric acid.

According to Hetland et al. (2005), coarse feed particles, such as those provided by the fibrous feeds; remain longer in the upper part of the GIT and as such, birds with lower pH (more acidic) in the upper part of the GIT will be able to digest and utilize fibre more than those with higher pH. The pH value in this study was lower (more acidic) than that reported by Mabelebele et al. (2014) who reported a crop pH value of 6.08 for broiler chickens although he reported similar value for Venda chickens (4.90 pH value). The workers also reported that gizzard pH values of broiler and indigenous Venda chickens were 3.47 and 2.97, respectively stating that it might be the reason why indigenous chickens tend to digest fibre better than broiler chickens as it stimulates gizzard activities, and hence increases the production of hydrochloric acid and refluxes between the proventriculus and gizzard (Duke, 1986; Hetland et al., 2005; Mabelebele et al., 2014).

The results obtained from this study showed that replacement of maize with Palm Kernel Cake irrespective of levels supplemented with 100 ppm *xylanase* enzyme caused a reduction in feed intake and an increase in weight gain and better FCR. In all these parameters, it is observed that birds fed diet with 10% PKC supplemented with *xylanase* enzyme out-performed birds fed diets with 10% or 30% PKC supplemented with *xylanase* enzyme and closer to the birds fed the control diet which was with better FCR. It can be deduced that enzyme supplementation of PKC helped in increasing and improving protein, ether extract and fibre digestibilities. The result obtained for the weights of vital organs in this trial showed that the birds were in good health conditions during the trial period. The result of identification of microbes (Fungi and Bacteria) in this study showed that dietary levels of PKC (10%, 20% or 30% inclusion) with supplementation of enzyme *xylanase* enhanced the growth of beneficial microbes which resulted in

inhibition or elimination of the opportunistic/pathogenic microbes. The result of the cost benefit analysis also showed that 10% inclusion level of PKC supplemented with *xylanase* enzyme gave the best result of a beneficiary reduction in the cost of production with the best improved broiler performance.

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