



ORIGINAL ARTICLE

The Apparent Metabolisable Energy Values of Palm Kernel Cake as Influenced by Enzymes and Cocktails

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Abstract

This study was conducted to quantify the effects of enzymes and their cocktails on the apparent metabolisable energy (AME) value of palm kernel cake to maximise enzymes' advantages. There were eight treatments, each with three replicates in a completely randomised design with T1 as control. The experimental treatments had Xylanase, Multipurpose and phytase enzymes respectively for T2, T3 and T4, and a cocktail of xylanase and multipurpose, a cocktail of xylanase and phytase, cocktail of multipurpose and phytase, and cocktail of xylanase, multipurpose and phytase for T5, T6, T7 and T8. The feeding trial was done using the intubation method. Gross energy values were determined with calorimeter while calculated AME values were subjected to analysis of variance using Statistical Analysis System and treatment means separated by Duncan Multiple Range Test. Results show that individual enzymes, apart from phytase, significantly ($p>0.05$) improved the AME value of palm kernel cake. Treatment T8 was significantly ($p>0.05$) better than the other treatments. Each cocktail was significantly better than its respective individual enzymes except cocktail of multipurpose enzyme and phytase that was not significantly different from multipurpose enzyme but significantly ($p>0.05$) better than phytase. It was concluded that enzymes and cocktail of enzymes could be used to enhance the energy content of nonconventional feedstuffs thereby serving as a replacement to conventional energy feedstuffs.

Keywords: Cocktail, Enzyme, Intubation, Metabolizable, Quantification

Introduction

Energy is the most essential item in poultry nutrition that affects the feed intake of an animal. Energy is derived mainly from carbohydrates. However, carbohydrates in the form of structural cell walls, otherwise known as crude fibre, are not digestible monogastric like poultry. This has limited the utilisation of some feed stuffs in poultry nutrition. Therefore, this has necessitated the use of exogenous enzymes to assist in the utilisation of these fibrous feed stuffs. The efficacy of exogenous enzymes on poultry feed stuffs has been widely reported (Atteh, 2003; Adeola and Cowieson, 2011; Adeniji and Jimoh, 2007). Esuga (2007) observed that Maxigrain, a multipurpose

enzyme, improved the retention of protein, fat and Nitrogen Free Extract of palm kernel cake. Jimoh (2018) reported an increase in the In-vitro degradability of palm kernel cake with individual enzymes and cocktails.

However, there is a need to quantify these effects to maximise the advantage of enzyme supplementation in the practical feeding of poultry species. This will facilitate the inclusion of the enzyme's impact in least-cost feed formulation. Exogenous enzymes are of different profiles and activity. Considering the complexity of crude fibre, it may be impossible for one enzyme to achieve complete breakdown of crude fibre of the feed stuff. Therefore, because of the difference in profile of exogenous enzymes and the complex nature of crude fibre, there is a proposal for adding several enzymes on the same feedstuff to see whether this improves the digestibility of the feed stuff beyond the effect of the individual enzyme. This phenomenon, known as enzyme cocktail, is still a subject of research. Jimoh and Atteh (2018) reported that a cocktail of enzymes performed significantly better than individual enzymes in their effects on the apparent metabolisable energy value of Brewer Dried Grains (BDG).

Palm kernel cake (PKC) is obtained after extracting most of the oil from palm kernel seeds. According to Onifade and Babatunde (1998), the extraction method, which could be either solvent or hydraulic press, determines the level of residual oil left after extraction, which eventually affects the proximate composition and quality of the cake. It is readily available, cheap and palatable and has considerable potential as a carbohydrate and protein source. However, large amounts of PKC are often discarded due to its low fibrous content, low nutritive value, grittiness, and potential for deterioration in unhygienic conditions. It can create environmental problems in the future (Sundu et al., 2005). Palm kernel cake has the advantage of being with no known antinutritional factor. Thus the use of exogenous enzymes may be considered to enhance its nutritional value in poultry. Therefore, this study was designed to quantify the effect of individual enzymes and cocktails on the apparent metabolisable energy value of Palm kernel cake.

Materials and Methods

Experimental Location

The experiment was conducted in the Department of Animal Production, University of Ilorin, Ilorin, Nigeria. Ilorin has coordinates of 8.479° N and 4.541° and is in the north-central part of Nigeria.

Experimental Design and Materials

A completely randomised design was used in this study. Eight treatments were comprising of one control and seven experimental treatments, as shown in Table 1. The treatments were named T1, T2, T3, T4, T5, T6, T7 and T8. Three enzymes namely xylanase, multipurpose and phytase were used individually and as cocktails of two or three.

Table 1: Composition of Experimental Treatments

TEST MATERIAL	TREATMENTS							
	T1	T2	T3	T4	T5	T6	T7	T8
Palm kernel cake (%)	100	100	100	100	100	100	100	100
Xy ¹ (ppm)	--	100	--	--	100	100	--	100
Mp ² (ppm)	--	--	150	--	150	--	150	150
Ph ³ (ppm)	--	--	--	150	--	150	150	150

1: Xylanase enzyme 2: Multipurpose enzyme 3: Phytase enzyme

T1= No enzyme, T2=Xylanase enzyme alone, T3=Multipurpose enzyme alone, T4=Phytase enzyme alone, T5=Cocktail of Xylanase and Multipurpose, T6=Cocktail of Xylanase and Phytase, T7=Cocktail of Multipurpose and Phytase, T8 =Cocktail of Xylanase, Multipurpose and Phytase.

The enzymes were included according to the manufacturers' recommendation. For Cocktails the enzymes were included at ratio of 100 ppm: 150 ppm: 150 ppm (Xylanase: multipurpose: phytase). Palm kernel was obtained from a commercial feed mill in Ilorin. It was ground into mash form. The exogenous enzymes were obtained from a feed additives' distributor in Lagos, Southwestern Nigeria.

Management of Birds and Feeding Trial

Twenty-four adult black cockerels of approximately equal weight (about 2.2Kg) were used in this study. They were randomly allocated to the battery cage with one bird in a cell representing a replicate. There were eight treatments each with three replicates. The birds were provided with feed and water ad libitum before the experiment. The feeding trial was done using the intubation method as described by Sibald (1976). Feed was withdrawn from all the birds for 21 hours prior to the administration of the treatment to empty the digestive system. At exactly 21 hours, a cockerel was removed from its cell and a tube of about 8mm internal diameter was inserted into the crop of the cockerel via the oesophagus. A Plastic funnel was placed on top of the tube. Sixty grams of the treatment in form of mash was placed in the funnel and gently pushed down with the aid of a glass rod. Water was then added to rinse the treatment off the funnel and the tube. After this procedure the fed bird was returned to the cell and this procedure was repeated for each of the birds. The time for the intubation for each bird was recorded. Faecal collection tray was placed under the individual cell immediately after the intubation for each bird and faecal samples were collected over a period of 24 hours after the intubation from all the cockerels. Adequate water was provided for all the birds prior to and after the intubation. The faecal sample collected was weighed and prepared for gross energy determination.

Gross Energy Determination

Gross energy values of the feed stuff (palm kernel) and faecal samples from each replicate were determined using a bomb calorimeter (Gallenkamp Ballistic Bomb Type). Apparent metabolisable energy value for each treatment was calculated using the formula below.

$$AME \text{ (kcal/g)} = \frac{(GE_f \times X) - (y_{ef} \times y)}{X}$$

Where,

AME	=	Apparent metabolizable energy value of experimental diet
GE _f	=	Gross energy value of experimental feedstuff in Kcal/g
X	=	Weight of feed intake in gram
y _{ef}	=	Gross energy of faeces of birds fed with experimental feedstuff in Kcal/g
y	=	Weight of faeces voided by fed birds in gram

Statistical Analyses

Apparent metabolisable energy values as well as percentage increase in apparent metabolisable energy values were subjected to analysis of variance suitable for a completely randomised design using general linear procedure of Statistical Analysis Software (SAS, 2002). Significant differences between treatments' means were determined by the procedure of Duncan Multiple Range Test (Duncan, 1955).

Results and Discussion

All the enzymes irrespective of the type and combination improved the apparent metabolisable energy (AME) value of palm kernel cake compared to the control (T1). The effect of the phytase enzyme (T4) on AME of PKC is significantly lower (P>0.05) than the effects of each of the other two individual enzymes (T2 and T3). Palm kernel cake (PKC) is one of the alternative feedstuffs that are being used in poultry feeds since it virtually has no competition between man and farm

animals. According to Onuh *et al.* (2010), agro-industrial by-products such as PKC could be used to spare conventional feed ingredients such as maize in poultry diets because of their relatively low pricing and availability. However, the low nutritive value is limiting its utilisation in poultry nutrition. There is a reduction in feed digestibility as the inclusion level of palm kernel cake increases. Osei and Amo (1987) observed that addition of PKC to broiler diets had no significant influence on feed consumption and body weight ($P < 0.05$) up to 8 weeks of age. Feed conversion efficiency, in contrast, significantly declined as PKC levels reached 12.5% of the diet or higher.

Alshelmani *et al.* (2016) observed that 15% inclusion of PKC in the feed of broilers led to a significant decrease ($P < 0.05$) in nutrient digestibility compared with the control group. Although the use of PKC considerably reduced feed costs, profit over production costs nevertheless favoured the control diet containing no PKC. This has necessitated the use of exogenous enzymes to improve its utilisation. Several works have been conducted to improve the nutritional value of PKC as one of the measures to reduce and/or eliminate the constraints of utilising PKC in poultry diets. Esuga (2007) reported that *Maxigrain*, a multipurpose enzyme improved the Metabolisable Energy value of PKC with broiler chicks when PKC was included at graded levels up to 30%. Saenphoom *et al.* (2011) reported that the composition of crude fibre, NDF, ADF, hemicellulose and cellulose contents of enzyme-treated PKC was significantly decreased with the addition of exogenous enzymes by approximately 34.6%, 26%, 20%, 35.7% and 22.1%, respectively, showing that exogenous enzymes used to treat PKC effectively broke down complex hemicellulose and cellulose.

The findings by Saenphoom *et al.* (2011) have been corroborated by the findings of this present study indicating the effectiveness of exogenous enzymes in hydrolysing the structural carbohydrates into monosaccharide sugars which eventually resulted in increased metabolisable energy. The multipurpose enzyme (T3) is significantly best among the three individual enzymes (T2, T3 and T4) as shown in Table 2. The multipurpose enzyme used in this study has more carbohydrase effect than others and this can create an additional effect for the enzyme. The effect of the multipurpose enzyme is superior to the single purpose enzyme because of the difference in activity as it has more xylanase activity than that of the single purpose Xylanase (26000units/g vs. 9000units/g). Also, according to Bastawde (1992), fungal xylanase has three or more substrate binding sites compared to bacterial xylanase. The multipurpose enzyme is a fungal enzyme while the Xylanase is a bacterial enzyme. This may be one of the reasons responsible for the higher percentage increase in the AME due to the multipurpose enzyme compared to the Xylanase enzyme (Figure 1).

Table 2: Effects of enzymes and cocktails on apparent metabolisable energy value of palm kernel cake

PARAMETERS	TREATMENTS								SEM
	T1	T2	T3	T4	T5	T6	T7	T8	
AME (Kcal/Kg)	1867.2 5 ^f	2136.6 7 ^e	2344.08 c	1921.3 3 ^f	2692.6 7 ^b	2236.71 d	2394.08 c	2862.9 2 ^a	70.54
AME Increment (%)	0.00 ^f	14.43 ^e	25.54 ^c	2.90 ^f	44.20 ^b	19.79 ^d	28.21 ^c	53.32 ^a	3.73

a, b, c, d, e, f: means in the same row with same superscript are not significantly different ($P < 0.05$)

T1= No enzyme, T2=Xylanase enzyme alone, T3=Multipurpose enzyme alone, T4=Phytase enzyme alone, T5=Cocktail of Xylanase and Multipurpose, T6=Cocktail of Xylanase and Phytase, T7=Cocktail of Multipurpose and Phytase, T8 =Cocktail of Xylanase, Multipurpose and Phytase.

According to Jimoh (2018), exogenous xylanase, phytase and multipurpose enzymes individually and as cocktails improved the *in vitro* digestibility of PKC compared to the control. However, this effect can only be converted to practical application through quantification. Abdollahi *et al.* (2016) reported a significant ($p < 0.05$) decrease in AME of feed with PKC inclusion of 24%. However, addition of a multipurpose enzyme containing β -mannanase and NSP-degrading enzymes namely β -mannanase, xylanase, amylase, protease, cellulase and β -glucanase improved the AME from 13.37Kj/kg to 13.43 Kj/kg. Phytase has the least effect on AME among the individual enzymes in this study with a value of 1921.33 Kcal/Kg. The positive effect of phytase on AME as observed in this study may be attributed to the binding effect of phytate on proximate components of the feedstuff. Phytate is known to bind with minerals like calcium and magnesium and nutrients like carbohydrate, protein and ether extract (Ravindran *et al.*, 1999).

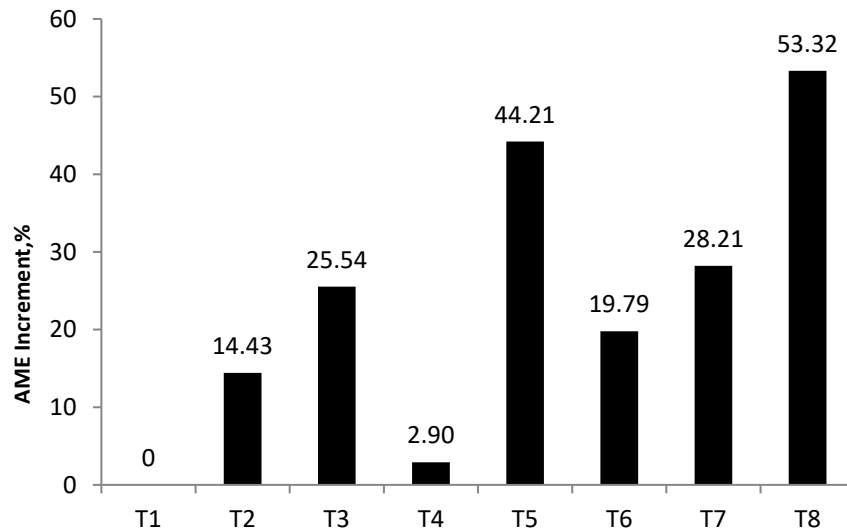


Figure 1: Percentage Increase in Apparent Metabolisable Energy Value of Palm Kernel Cake Due to Addition of Enzymes and Cocktails

AME=Apparent metabolisable energy, T1= No enzyme, T2=Xylanase enzyme alone, T3=Multipurpose enzyme alone, T4=Phytase enzyme alone, T5=Cocktail of Xylanase and Multipurpose, T6=Cocktail of Xylanase and Phytase, T7=Cocktail of Multipurpose and Phytase, T8 =Cocktail of Xylanase, Multipurpose and Phytase.

Therefore, when the phytate is broken down by phytase, other nutrients bound to phytate are also released. There is also the fact that exogenous enzymes include the presence of other activities apart from the enzyme for which the complex is named. This is known as side chains. According to McCleary (2001), phytase enzymes are known to have traces of other side active enzymes and these enzymes may contribute to the effect of the phytase on other nutrients. Ravindran *et al.* (2000) reported that the effect of phytase on AME is about 5% which is relatively higher than the value obtained in this study (2.90%). The activity of the phytase used may be an important factor in this regard as well as the quantity of phytate in the feedstuff.

The values of AME obtained in this study for the individual enzymes are either due to the calorific effect of xylanase and multipurpose enzymes or 'extra phosphoric effect' of Phytase as the enzyme is originally meant for breakdown of phytate. Findings of this study are like that obtained by Atteh (2001) where a bacterial endoxylanase improved the AME of wheat offal and Brewers Dried Grains by 56% and 26% respectively. It could be inferred that exogenous enzymes allow birds to utilise the hitherto undigested crude fibre better thereby producing simple sugars that can generate energy for the monogastric animal. The effectiveness of this action depends on

the profile and activity of the enzymes as seen in this study where multipurpose enzyme with several activities (glucanase, xylanase, cellulase etc.) performed significantly better than the xylanase enzyme with single purpose activity. This explains why treatment T3 improved the AME of PKC better than each of the other individual enzymes (Nutrase Xyla and Phytase).

The AME values of treatment T8 (cocktail of the three enzymes) and treatment T5 (cocktail of xylanase and multipurpose enzymes) were comparable and were not significantly different ($P>0.05$) from each other (Table 2). There were no significant differences between the AME values for treatment T6 (cocktail of xylanase and phytase) and treatment T7 (cocktail of multipurpose and phytase enzymes). Treatment T8 (Cocktail of the three enzymes) had the highest AME value of 2862.92Kcal/Kg while treatment T4 (phytase enzyme) had the least AME value of 1921.33 Kcal/Kg and this was not significantly ($P>0.05$) different from treatment T1 (Control) with AME value of 1867.25 Kcal/Kg. This study reveals that complementary effect of the enzymes plays prominent role in the values obtained for the cocktails as differences exist between the individual enzymes and the cocktails. Such effect was however not prominent for cocktails involving phytase (T6 and T7). For instance, xylanase improved AME by 14.43 % while cocktail of xylanase and phytase (T6) resulted in increment of 19.79% indicating that phytase contributed only 5.36% to the effect of the cocktail (Figure 1). Complementary effect could be due to the number of active sites of an enzyme. Where an enzyme's active sites are exhausted and there is still the presence of substrate, the presence of another similar enzyme with more active sites may increase the overall digestibility of such feed stuff (David and Michael, 2004). This explains why there are more improvements when xylanase and multipurpose enzymes combined than when either of the two combined with phytase enzyme.

Conclusion

Quantification of effect of exogenous enzymes is essential to ensure that feed formulation account for anticipated effect of enzyme in the digestive system of the animal. Findings of this study reveal that apparent metabolisable energy value of palm kernel cake can be improved by exogenous enzymes. In addition, different enzymes have varying effects on the digestibility of the feedstuff. This could be attributed to the difference in profile of the enzymes. This study also revealed that cocktail of enzymes is better than individual enzymes. The AME values obtained in this study have placed palm kernel cake close to conventional energy supplements like maize especially when cocktail of the three enzymes was used and these values can be used in feed formulation when the respective enzymes or cocktails are available.

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