Original scientific paper
DOI: /10.5513/JCEA01/19.1.2030
Journal of Central European Agriculture, 2018, 19(1), p.114-125

Effects of enzyme cocktails on in vitro digestibility of palm kernel cake

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Abstract

The variable nature of crude fibre and the difference in the profile of exogenous enzymes have necessitated the need to investigate the effects of enzyme cocktail on some high fibre feed stuffs. Whether enzyme cocktail will perform better than individual enzyme is still a subject of research. This research was conducted to test the hypothesis that cocktail of enzymes will perform better than individual enzymes on palm kernel cake using in vitro technique. Three different exogenous enzymes namely xylanase, multipurpose and phytase were used individually and as cocktails in a completely randomized design. There were eight treatments comprising of a control (with no enzyme) and seven experimental treatments with either individual enzymes, pairwise combination of enzymes or combination of the three enzymes. Each treatment was replicated thrice. In vitro technique was carried out and values of nutrients' digestibility obtained were analyzed using Statistical Analyses Software. Treatments means were separated using Duncan's multiple range test. This study revealed that multipurpose enzyme was significantly ($P<0.05$) better than other individual enzymes in their effects on digestibility of dry matter, crude fibre and fibre fractions with the exception of acid detergent lignin where there was no significant difference ($P>0.05$) between the treatments. Phytase gave the least improvement in the measured parameters among the individual enzymes. Cocktails of enzymes were significantly better ($P<0.05$) than individual enzymes in their effects on digestibility of dry matter, crude fibre and fibre fractions while cocktail of the three enzymes was significantly ($P<0.05$) the best among the cocktails. The study concluded that cocktail of exogenous enzymes holds better potential than individual enzymes in improving the utilization of palm kernel cake for poultry species.

Keywords: enzyme cocktails, in vitro digestibility, palm kernel cake, poultry nutrition

Introduction

There has been considerable interest in the use of exogenous enzymes in improving the digestibly of nonconventional feedstuffs in poultry feeding. Exogenous enzymes available in the market have different profile although most are produced for nonstarch polysaccharides (NSP) digestion (Alam et al., 2003). Enzyme

supplementation is well documented as effective in breaking polymeric chains of NSPs and improve the nutritive value of fibrous feedstuffs (Adeniji and Jimoh, 2007; Elwakeel et al., 2007; Geraldo et al., 2008). However, due to variation in the chemical structure of these feedstuffs, a combination of various non-starch polysaccharide enzymes has been recommended to enhance better digestibility of nonstarch polysaccharides. Nonstarch polysaccharides are not chemically uniform constituents of the feed and their profile varies from feedstuff to feedstuff (Van Soest, 1967). Thus an enzyme that can achieve a good digestibility in a feedstuff may not be able to achieve the same level in another feedstuff. For instance, wheat offal has 46% neutral detergent fibre, 14% acid detergent fibre, 10.2% cellulose, 32% hemicellulose and 3.8% acid detergent lignin. Maize offal also has 50.88% neutral detergent fibre, 28.5% acid detergent fibre, 24.4% cellulose, 22.38% hemicellulose and 4.1% acid detergent lignin (Onifade and Babatunde, 1998). Because of this, a growing body of literature advocating the use of multi-enzyme preparation has emerged particularly of those with multicarbohydrase activities (Slominski, 2000).

Palm Kernel cake is obtained after extracting most of the oil from palm kernel seeds. The extraction method used (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 has no known antinutritional factors (Onifade and Babatunde, 1998). Its nutritional value in poultry feeding is hampered by its high fibre content as poultry lacks the enzyme to digest nonstarch polysaccharides. Furthermore, several studies have demonstrated the efficacy of enzymes in degrading fibre components of some feedstuffs and enhancing their utilization by poultry species. However, there is the need to investigate the efficacy of cocktail of enzymes on Palm kernel cake before they are fed to the animal.

In vitro experiment can be used to predict the response of livestock to exogenous enzymes as well as to investigate the mode of action of exogenous enzymes and to know enzyme-substrate specificity (Morten et al., 2004). It will also assist in identifying the enzyme and combination which possess the best potential for improving the utilization of the palm kernel cake as a feed stuff for poultry. This study was designed to test the hypothesis that cocktail of enzymes will give better digestibility than individual enzyme using in vitro technique. The study investigated the extent of degradation of the proximate and fibre components of palm kernel cake with enzymes and cocktail of enzymes as well as the complementary effects or otherwise of enzyme combinations on palm kernel cake.

Materials and methods

Experimental design

Completely randomized design was used in this experiment. There were eight treatments comprising of one control treatment (no enzyme) and seven experimental treatments as shown in Table 1. Each of the treatments was replicated thrice given a total of twenty-four experimental units. The treatments were randomly assigned to the experimental units. Three different exogenous enzymes were used for the experiment. They were Xylanase (a bacterial endo-xylanase), multipurpose enzyme (fungal enzyme containing xylanase, glucanase, hemicellulase and cellulase among

others) and a phytase (derived from *Aspergillus niger*). The enzymes were used individually, in pair wise combination as well as the three together. The enzymes were included at manufacturers' recommended inclusion level of 100 ppm for xylanase, 150 ppm for multipurpose enzyme and 150 ppm for phytase.

The xylanase used in this study had 9,000 units of xylanase activity per gram as stated by the manufacturer. It was extracted from *Bacillus substilis* and it is powdery in nature and creamy colored. It has wheat flour as its carrier and the recommended inclusion level is 100 ppm. The multipurpose enzyme used was produced from *Trichoderma viride*. It is a granular and odorless solid preparation. The enzyme complex has 5-10% active enzyme and the manufacturer's recommended inclusion level is 150 ppm. It has 26,000 units/gram of endo 1,4-β-xylanase, 18,000 units/g of endo 1,3(4)-glucanase, 8,000 units/g of endo 1,4 β-glucanase, 8,000 units/gram of cellulase and traces of pectinase, hemicellulase, α-amylase and others as stated by the manufacturer. The phytase used is 3-phytase enzyme obtained from *Aspergillus niger*. It is granular in nature and it has activity of 5,000 FTU/gram as stated by the manufacturer. One FTU (phytase unit) is the amount of enzyme which liberates 1 µmol of inorganic phosphate per minute from sodium phytate at pH of 5.5 and temperature of 37 ^{o}C. The phytase was included at 150 ppm.

Table 1. Composition of experimental treatments

	Treatments							
Test material	NE	Xy	Mp	Ph	Xy+Mp	Xy+Ph	Mp+Ph	Xy+Mp+ Ph
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; NE=No enzyme; Xy=Xylanase enzyme alone, Mp=Multipurpose enzyme alone, Ph=Phytase enzyme alone, Xy+Mp=Cocktail of xylanase and multipurpose, Xy+Ph=Cocktail of xylanase and phytase, Mp+Ph=Cocktail of multipurpose and phytase, Xy+Mp+Ph =Cocktail of xylanase, multipurpose and phytase.

In vitro techniques

In vitro digestion technique was carried out in line with the procedure of Boisen and Fernadez (1997) with some modifications. The two-step digestion procedure simulates the chicken's gastric and intestinal pancreatic digestions. Palm Kernel Cake was milled to pass through 1 mm sieve and mixed with the appropriate enzyme or cocktail. Five gram of each treatment was put in a 50 ml flask and 10 gm of pepsin

in 10 ml of 0.1 M HCl (aq.) was added. The content was incubated for 30 minutes at 40 ^{o}C and pH of 2. Neutralization followed with 0.2 M NaOH (aq.) and 10 gm of pancreatin in 10 ml of buffer solution was added and incubated for additional 2 hours at temperature of 40 ^{o}C and pH of 7. The two stages of the incubation were accompanied with shaking with the aid of a mechanical shaker. At the end of the incubation stages, the content of the flask was filtered using a weighed filter paper. The filtrate was discarded while the residue was weighed and oven dried for 24 hours at 72 ^{o}C. The oven dried residue was used for proximate analysis and fibre partitioning.

Chemical analyses

The proximate composition of Palm Kernel Cake and the oven-dried residue was determined in accordance with the procedure of A.O.A.C (2005). Fibre fractions assay was done according to the procedure of Van Soest (1967). Nutrient digestibility was calculated using the formula below

$$\text{Nutrient digestibility (\%)} = \frac{\text{Nutrient in sample (g)} - \text{Nutrient in residue (g)}}{\text{Nutrient in sample (g)}} \times 100$$

Statistical analyses

Values of nutrient digestibility obtained were statistically analyzed using one-way ANOVA procedure of Statistical Analysis System Version 8 (SAS, 2002). Significant differences between treatments means were determined using Duncan's multiple range test (Duncan, 1955).

Results

The proximate analysis and fibre fraction of palm kernel cake are shown in Table 2 while the effects of the treatments on in vitro digestibility of proximate components of palm kernel cake are shown in Table 3. All the enzymes, except phytase, individually and as cocktails significantly ($P<0.05$) improved the digestibility of dry matter compared to the control.

Table 2. Proximate composition and fiber fractions of palm kernel cake

Parameters	Amount (% of air dried sample)
DM (%)	90.64
CP (%)	18.25
CF (%)	16.27
EE (%)	7.98
Ash (%)	4.47
NFE (%)	43.67
GE (KJ/Kg)	18,475.6
NDF (%)	66.8
ADF (%)	42.67
HEMI. (%)	24.13
CELL. (%)	36.07
ADL (%)	6.6

DM=Dry Matter, CP=Crude Protein, CF=Crude Fibre, EE=Ether Extract, NFE=Nitrogen Free Extract, GE=Gross Energy, NDF=Neutral Detergent Fibre, ADF= Acid Detergent Fibre, HEMI.=Hemicellulose, CELL.=Cellulose, ADL=Acid Detergent Lignin.

The multipurpose enzyme and cocktail of the three enzymes gave the highest effect on dry matter digestibility and these were not significantly different ($P>0.05$) from each other. There were significant differences ($P<0.05$) between the individual enzymes on dry matter digestibility and multipurpose enzyme gave the highest effect. There were no significant differences ($P>0.05$) between the cocktails in their effects on ether extract digestibility except cocktail of multipurpose enzyme and phytase which is significantly lower than other cocktails. Among the individual enzymes, there were significant differences in their effects on ether extract digestibility and phytase gave the highest effect while xylanase and multipurpose enzymes gave effects that were significantly lower ($P<0.05$) than the control. All the enzymes individually and as cocktails improved crude fibre digestibility of palm kernel cake compared to the control. Cocktail of the three enzymes gave the best crude fibre digestibility with a value of 71.16% and this was significantly different ($P<0.05$) from other cocktails and other treatments. There was no significant difference ($P>0.05$) between the cocktail of xylanase and multipurpose and the cocktail of multipurpose enzyme and phytase on crude fibre digestibility. Xylanase gave the significantly highest effect on crude protein digestibility and this is followed by the effect of phytase (76.44%). Xylanase, phytase and cocktail of xylanase and phytase improved digestibility of crude protein

significantly compared to the control while other enzyme treatments did not improve the digestibility of crude protein compared to the control.

Table 3. Effects of enzymes on proximate composition of palm kernel cake using in vitro technique

	Treatments								
Parameters	NE	Xy	Mp	Ph	Xy+Mp	Xy+Ph	Mp+Ph	Xy+Mp+Ph	SEM
DM (%)	48.95^{e}	55.66^{b}	62.31^{a}	49.78^{e}	54.49^{c}	53.37^{d}	52.50^{d}	62.58^{a}	1.011
CF (%)	0.24^{h}	31.62^{f}	58.67^{d}	10.51^{g}	67.12^{bc}	39.07^{e}	65.83^{c}	71.16^{a}	5.3
EE (%)	68.25^{b}	67.48^{c}	67.16^{d}	68.53^{a}	68.44^{a}	68.55^{a}	68.23^{b}	68.55^{a}	0.161
CP (%)	75.76^{d}	76.6^{a}	75.25^{f}	76.44^{b}	75.52^{e}	76.01^{c}	75.63^{de}	75.31^{f}	0.141

^{abcdefgh}Means in the same row followed by the same superscript are not significantly different; (P>0.05); DM=dry matter, CF=crude fibre, EE=ether extract, CP=crude protein, NE=no enzyme, Xy=xylanase enzyme alone, Mp=multipurpose enzyme alone, Ph=phytase enzyme alone, Xy+Mp=cocktail of xylanase and multipurpose, Xy+Ph=cocktail of xylanase and phytase, Mp+Ph=cocktail of multipurpose and phytase, Xy+Mp+Ph =cocktail of xylanase, multipurpose and phytase.

Table 4 shows the effects of the treatments on digestibility of fibre fractions of palm kernel cake. All the treatments have significantly different (P<0.05) effects on digestibility of neutral detergent fibre and acid detergent fibre with cocktail of the three enzymes having the best values of 74.35% and 48.54% for neutral detergent fibre and acid detergent fibre, respectively. The treatments have significantly different (P<0.05) effects on cellulose digestibility of palm kernel cake with the exception of cocktail of xylanase enzyme and multipurpose enzyme (Xy+Mp) and cocktail of multipurpose enzyme and phytase (Mp+Ph) which were not significantly different from each other (46.75 vs. 44.51). All enzymes individually and as cocktail did not improve the digestibility of Acid Detergent Lignin compared to the control. Treatments with cocktail of xylanase and multipurpose enzymes (Xy+Mp) and that of cocktail of multipurpose enzyme and phytase (Mp+Ph) were not significantly different in their effects on digestibility of hemicellulose. However, all other treatments were significantly different (P<0.05) in their effects on hemicellulose digestibility. All enzymes individually and as cocktails improved hemicellulose digestibility compared to the control while cocktail of the three enzymes (Xy+Mp+Ph) had the highest effect on hemicellulose digestibility.

Table 4. Effects of enzymes on fibre fractions of palm kernel cake using in vitro technique

	Treatments								
Parameters	NE	Xy	Mp	Ph	Xy+Mp	Xy+Ph	Mp+Ph	Xy+Mp+Ph	SEM
NDF (%)	0.28^{f}	29.97^{e}	56.61^{c}	8.28^{b}	60.06^{bc}	39.13^{d}	64^{b}	74.35^{a}	5.27
ADF (%)	0.06^{h}	9.87^{f}	30.25^{d}	4.59^{g}	40.14^{b}	16.39^{e}	36.41^{c}	48.54^{a}	3.52
CELL. (%)	0.06^{g}	9.98^{e}	38.51^{c}	4.92^{f}	46.75^{b}	18.01^{d}	44.51^{b}	58.99^{a}	4.33
HEMI. (%)	0.07^{f}	29.59^{d}	64.42^{b}	13.21^{e}	69.39^{ab}	44.74^{c}	69.57^{ab}	74.37^{a}	5.57
ADL (%)	0.06^{a}	0.06^{a}	0.06^{a}	0.06^{a}	0.06^{a}	0.06^{a}	0.06^{a}	0.05^{a}	0.01

^{abcdefgh}Means in the same row followed by the same superscript are not significantly different (P>0.05); NDF=neutral detergent fiber, ADF=acid detergent fibre, CELL.=cellulose, HEMI.=hemicellulose, ADL=acid detergent lignin, NE=no enzyme, Xy=xylanase enzyme alone, Mp=multipurpose enzyme alone, Ph=phytase enzyme alone, Xy+Mp=cocktail of xylanase and multipurpose, Xy+Ph=cocktail of xylanase and phytase, Mp+Ph=cocktail of multipurpose and phytase, Xy+Mp+Ph=cocktail of xylanase, multipurpose and phytase.

Discussion

Dietary fibre is generally defined as the plant polysaccharides and lignin that are resistant to hydrolysis by digestive enzymes (Mongeau and Brassard, 1979). Fibre is not a chemically uniform substance and the components vary from plant to plant. It exerts antnutritive effects in monogastric animals. Because fibre cannot be digested by monogastric animals' exogenous enzymes are now been added to poultry feedstuffs to aid in the digestion of the fibre (Atteh, 2003).

Complementary effects were noticed when enzymes were used as cocktails. This was observed when the multipurpose enzyme combined with phytase for cellulose digestibility of palm kernel cake. Complementary effects may be attributed to the difference in activity of enzymes and difference in profile of the enzymes. The study observed that complementary effects were noticed more when phytase combined with either of xylanase or multipurpose indicating that the effect of phytase on crude fibre fractions was minimal. The improvement of crude fibre digestibility due to phytase may be attributed to two reasons. Firstly, phytate can also chelate with other nutrients including crude fibre (Ravindran et al., 2000). Thus a breakdown of phytate by the phytase will lead to the release of the nutrients and make them available to the respective enzymes. Secondly, no exogenous enzyme is wholly single purpose. In the course of manufacturing enzymes and based on the profile of the substrate used in the inoculation, commercial enzymes used as feed supplements contain more than a single enzyme, rather they are preparations of a variety of enzymes. A large number of carbohydrase, protease, phytase and lipase are available but in varying percentages (McCleary, 2001). Therefore, there is tendency that phytase could also have traces of alpha-amylase, hemicellulase, cellulase and protease and these are called side active enzymes. Several authors have also reported positive effect of

phytase on other nutrients in addition to its effect on phosphorus. Ravindran et al. (2000) observed that added phytase (400 units/kg diet) increased apparent metabolisable energy value of wheat by 5.34%. Rutherford et al. (2002) observed that microbial phytase (Ronozyme P) resulted in improved amino acids digestibility in addition to a significantly greater phytate phosphorus disappearance when rice bran was included in the diet. Also, Parkkonnen et al. (1997) has observed that xylanase increases the permeability of the aleurone layer of wheat which is the site of phytic acid storage thereby improving the accessibility of phytase to the phytate. Effects of phytase observed in this study where phytase improved the digestibility of crude protein and ether extract compared to the control also corroborates the findings of Parkkonen et al. (1997). This is known as 'extra phosphoric effect'. This 'extra phosphoric effect' of phytase on non-Phytate components of a feed stuff will depend on the type of phytase used. Non-genetically modified phytase product has side active enzymes like carbohydrase (α-amylase, hemicellulase and cellulase) and protease. This is in contrast with genetically modified phytase product since the organism's capacity to produce phytase has been reinforced by gene modification.

The spectrum of activity of an enzyme is also very important in its efficacy. This may have favored the multipurpose enzyme among the three enzymes studied individually while the complementary effect is responsible for the highest effect observed in treatment with the cocktail of the three enzymes (Xy+Mp+Ph) for Palm Kernel Cake. Hemicellulose is more digestible than cellulose but less digestible than starch polysaccharides (McDonald et al., 2010). The digestibility values obtained for hemicellulose in this study varied from enzyme to enzyme. The multipurpose enzyme has traces of hemicellulase and this will have additional effect on the efficacy of the multipurpose enzyme on hemicellulose digestibility. The digestibility values obtained for cellulose in this study could be attributed to several reasons. Cellulase is present in the multipurpose enzyme. Cellulase, though a single enzyme carry series of glycosidase activities which depolymerize cellulose into glucose. These activities are endo-1, 4-β-glucanase, cellobiohydrolases and β-glucosidases (Adeola and Cowieson, 2011). This explains why the multipurpose enzyme was significantly better than each of the other two enzymes individually in their effects on cellulose digestibility. Also, there may be traces of cellulase in other enzymes in the course of manufacturing and this may have resulted in improved cellulose digestibility observed with other enzymes compared to the control.

Juanpere et al. (2005) observed that the use of multiple carbohydrase activities may produce greater benefit than each of the enzymes acting individually. However, the hydrolytic activity of carbohydrase may be limited by the presence of protease which can digest carbohydrase. This can cause negative synergistic effect. Beneficial interactions among carbohydrases (Choct et al., 2004) and between carbohydrase and phytase (Diebold et al., 2004) have been reported. Xylanase has an affinity for insoluble arabinoxylans giving soluble hydrolytic products and consequently decreasing viscosity. Furthermore, the hydrolysis of hemicellulose is the rate-limiting step for subsequent hydrolysis of other nutrients that may be trapped in the cell wall. This may be the reason for the highest digestibility values obtained with the multipurpose enzyme among the three enzymes individually. The activity of the enzyme constituent in each enzyme complex is also an important factor in the level of digestion (Fang et al., 2007).

In this study, an increase in digestibility values for dry matter, neutral detergent fibre, acid detergent fibre, cellulose and hemicellulose for palm kernel cake indicates the positive effect of these enzymes and the cocktail. The observed enhanced digestibility of the nonstarch polysaccharides by the combined enzymes may be attributed to positive synergy between the activities of individual enzymes in the various enzyme mixtures. Enzyme synergism has been demonstrated in other works including Sreenath et al. (1999) where a significant degree of synergism of enzymes in the saccharification of alfalfa fibre was observed when it was treated with mixture of commercial cellulase and pectinase compared to treatments with individual enzymes. Jimoh and Atteh (2015) also reported significant improvement in digestibility of crude fibre and fibre fraction of wheat offal when cocktail of xylanase and multipurpose enzyme was used compared to the control and the individual enzymes. The quantity of fibre fractions like NDF (mainly hemicellulose, cellulose and lignin) and ADF (mainly cellulose and lignin) are often negatively correlated with dry matter digestibility especially in monogastric animals (Van Soest et al., 1991). The results also showed that none of the enzymes or their combinations has significant improvement on the digestibility of acid detergent lignin. Acid detergent Lignin is a polymer that originates from three derivatives of phenyl propane (McDonald et al., 2010). Acid detergent lignin also renders some nutrients unavailable during digestion. The recalcitrance of lignin is as a result of its unique structure. The predominant types of linkages between monomers are alpha- and beta-aryl ether bonds which cannot be broken by these enzymes. It also exerts negative effect on cellulose digestibility.

To maximize the benefits of enzyme supplementation and obtain optimum responses, efforts have been made to derive matrix values for energy and specific nutrients in enzyme-supplemented diets. This is based on the premise that enzymes release nutrients that are otherwise unavailable, and thus, it is possible to make allowance for such released nutrients in feed formulation. Zhou et al. (2009) demonstrated the need for making allowances for contribution of energy from carbohydrases in diets using decreasing amount of AME in diet supplemented with a multi-enzyme containing xylanase, α-amylase and protease. The greatest response to the multi-enzyme was observed at the least energy concentration, indicating that enzyme supplementation is more beneficial when ME is suboptimal. Cowieson and Ravindran (2008) noted that supplementation of a nutritionally marginal diet with multi-enzyme containing xylanase, α-amylase and protease restored performance to that of a nutritionally adequate diet.

Conclusions

Findings of this study have confirmed the hypothesis that cocktail of enzymes is better than individual enzymes. In comparison, the multipurpose enzyme performed significantly best among the three enzymes when used individually. As cocktails, cocktail of the three enzymes gave the best result in most of the parameters although it was not significantly different from cocktail of xylanase and multipurpose enzyme in some parameters. This study also revealed that effect of phytase enzyme is not limited to phytate alone although its effects on those parameters are small.

Finally, quantification of these effects through feeding trials will be necessary in order to develop matrix value (nutrient-equivalent value) for enzyme product or the cocktail in least cost feed formulation. It will also allow for building models and adjusting feed formulation to meet the conventional values in anticipation of enzyme actions. The results indicate the potential for inclusion of cocktail of enzymes in poultry feeding as a means of improving the nutritive value of Palm kernel cake as a feed stuff for poultry production.

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