BAP -10

In Vitro Digestibility of Maize Offal with Enzyme Cocktails

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Abstract
In vitro trial was conducted to compare the effects of cocktails of enzymes and individual enzymes on digestibility of maize offal. Three exogenous enzymes were used in a completely randomized design comprising of eight treatments and three replicates per treatment. The treatments were controlled with no enzyme, maize offal with either a xylanase , a multipurpose enzyme, a phytase, cocktail of xylanase and phytase, cocktail of xylanase and multipurpose, cocktail of phytase and multipurpose or cocktail of xylanase, phytase and multipurpose. Parameters measured were *in vitro* digestibility values for proximate components and fibre fractions. Enzymes, individually and as cocktails, increased the digestibility values of the measured parameters. There were significant differences ($p<0.05$) between the individual enzymes in their effects on the parameters. Cocktails of the enzymes were significantly better ($p<0.05$) than individual enzymes in their effects on the parameters especially fibre fractions and crude fibre. It was concluded that cocktail of enzymes is better than individual enzymes in their effects on *in vitro* digestibility of maize offal. Feeding trial is recommended to quantify these effects.

Key words: Maize offal, fibre fractions, exogenous enzymes

Introduction

Exogenous enzymes have been used to improve the utilization of fibrous feedstuffs. The use of exogenous enzymes in poultry feed is aimed at assisting the poultry species in degrading components of the feedstuff that cannot be digested such as fibre. Exogenous enzymes are of different types and their efficacy depends on factors like the profile, the concentration, the activity and the presence of the respective substrate. However, crude fibre is not a chemically uniform substance but a variable mixture the major components of which are cellulose, hemicelluloses and lignin. The proportion of these components varies from feedstuffs to feedstuffs and has the greatest influence on its digestibility (McDonald *et al.,* 2010). Commercially available exogenous enzymes are produced to target crude fibre. Exogenous carbohydrate enzymes have activities like xylanase, β-glucanase, cellulase, pectinase among others in varying amounts and presence. However the variable nature of crude fibre and the lignocellulose content of feedstuffs necessitate the addition of several enzyme complexes on the same feedstuff to achieve better digestibility. This is known as cocktail.

Fibre fractions are composed of different types of monosaccharides (monomers) as well as different types of bond linking the monosaccharide units together. Thus it may be practically impossible to have an enzyme complex which can degrade hemicellulose, cellulose, pectin and the oligosaccharides at the same time. Because of this, a growing body of literature advocating the use of multi-enzymes preparation has emerged particularly for those with carbohydrase activities (Slominski, 2000).

This study was conducted to test the hypothesis that enzyme cocktails are better than individual enzymes using *in vitro* digestibility trial with maize offal. Maize offal is obtained as the by-product of maize grain processing using a locally fabricated machine which produces the polished grain used for human consumption. It contains the testa, aleurone layer, some quantities of broken endosperm and most of the germ of the maize grain (Vantsawa *et al.,* 2007).

Materials and Methods

Three different exogenous enzymes were used for the experiment in a completely randomized design. *In vitro* digestion technique was carried out in line with the procedure of Boisen and Fernadez (1997) with some modifications. It was done at the Central Research Laboratory of the University of Ilorin. Proximate analyses of the residues after filtration were done in accordance with the procedure of A.O.A.C (1990). Fibre fractions assay was done according to the method of Van Soest (1967).

Nutrient digestibility was calculated and values obtained were statistically analyzed using one way ANOVA procedure of Statistical Analysis System (SAS) Version 8. Significant differences between treatments means were separated using Duncan Multiple Range Test (Duncan, 1955).

Results and Discussion

Table 1 shows the effect of the treatments on digestibility of maize offal. All the enzymes individually and as cocktail improved the dry matter digestibility compared to the control. Cocktail of xylanase and multipurpose resulted in the highest effect on Dry matter digestibility while multipurpose enzyme gave the highest effect among the individual enzymes on all the parameters measured.

Table 1: Effects of enzymes on proximate composition of maize offal using *in vitro* technique

	Treatments								
Parameters	NE	Xy	Mp	Ph	Xy+Mp	Xy+Ph	Mp+Ph	Xy+Mp+Ph	SEM
DM (%)	35.90^{f}	51.29^{c}	59.45^{b}	44.83^{e}	63.11^{a}	48.83^{d}	59.07^{b}	59.19^{b}	1.80
CF (%)	1.26^{g}	35.94^{e}	54.73^{c}	13.27^{f}	69.89^{a}	41.45^{d}	59.90^{b}	73.46^{a}	5.08
EE (%)	63.11^{f}	63.37^{e}	64.06^{a}	63.08^{f}	63.82^{b}	63.65^{c}	63.53^{d}	63.78^{b}	0.08
CP (%)	57.49^{c}	57.31^{c}	64.83^{a}	57.90^{bc}	64.42^{a}	57.41^{c}	58.23^{b}	58.51^{b}	0.62

a, b, c, d, e, f, g: 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.

There were significant differences ($p<0.05$) in the effects of the treatments on the digestibility of fibre fractions namely neutral detergent fibre, acid detergent fibre, cellulose and hemicellulose (Table 2). All the enzymes individually and as cocktail improved the digestibility of these fractions. Among the cocktails, cocktail of the three enzymes gave the highest effect in all the mentioned parameters. The multipurpose enzyme gave the best effect followed by xylanase and then phytase. There was no significant difference among the treatments on acid detergent lignin digestibility.

Table 2: Effects of enzymes on fibre fractions of maize offal using *in vitro* technique

	TREATMENTS								
PARAMETERS	NE	Xy	Mp	Ph	Xy+Mp	Xy+Ph	Mp+Ph	Xy+Mp+Ph	SEM
NDF (%)	0.24^{g}	33.50^{e}	51.74^{c}	10.61^{f}	52.37^{c}	39.52^{d}	68.89^{b}	77.21^{a}	5.35
ADF (%)	0.04^{f}	4.73^{e}	16.67^{d}	1.28^{f}	24.71^{b}	5.90^{e}	21.72^{c}	27.75^{a}	2.17
CELL. (%)	0.03^{g}	7.47^{e}	23.75^{c}	4.03^{f}	31.68^{b}	10.33^{d}	29.38^{b}	37.64^{a}	2.78
HEMI. (%)	3.14^{g}	34.83^{e}	57.71^{c}	9.78^{f}	64.15^{b}	41.97^{d}	66.98^{ab}	71.56^{a}	5.11
ADL (%)	0.03^{a}	0.04^{a}	0.04^{a}	0.03^{a}	0.03^{a}	0.03^{a}	0.03^{a}	0.03^{a}	0.02

a, b, c, d, e, f, g, h: means in the same row followed by the same superscript are not significantly different ($p>0.05$). NDF=Neutral detergent fibre, 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.

Exogenous enzymes like any typical enzymes act by recognizing and binding to its substrate and usually act on only one specific substrate. Enzymes are not used up during the binding and are released unchanged after the formation of new products. Generally enzymes function through the lock-and-key model (McDonald *et al.,* 2010). Active site of an enzyme is shaped to recognize and hold the specific substrates. New substances are formed from the reaction and the new molecule is separated from the enzyme. Enzyme-substrate specificity is achieved by complementary shapes, charge and hydrophilic/hydrophobic characteristics of the substrate. An improved amount of enzyme will increase the rate of reaction with enzyme. However, once past a certain point, the rate of reaction will level out because the active sites available have stayed constant. This

brings about enzyme saturation. An enzyme is saturated when the active sites of all the molecules are occupied (David and Michael, 2004). Thus the addition of one enzyme can increase the active sites in the reaction system thereby increasing digestibility of the substrate (Jimoh and Atteh, 2015).

The crude fibre digestibility values obtained in this study for the feedstuff shows the difference in the activity of the enzymes either as individual or as cocktail. The best performance due to multipurpose enzyme may be attributed to several reasons like profile and activity .It has 26,000 units/gram of endo 1,4,-β-xylanase , 18000 units/g of endo 1,3,(4)-glucanase, 8000 units/g of endo 1,4 β-glucanase and 8000 units/gram of cellulase among others compared to 9000 units of xylanase activity per gram for the xlanase enzyme used. The presence of cellulase in the multipurpose enzyme also contributed to its effect on the digestibility of cellulose. 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.

Complementary effects of enzymes may be attributed to several reasons. For instance, endo-xylanase and exo-xylanase enzymes work synergistically. Endo-xylanase enzyme specialize in splitting the glycosidic bonds within polysaccharide thereby causing a decrease in viscosity and as well as production of smaller fragments of oligosaccharides each with a terminal reducing sugar. Exo-xylanase enzymes specialize in breaking down the terminal sugar molecule of the oligosaccharides and polysaccharides (Chesson, 1993).Therefore, a complementary effect is expected from a combination of dietary endo- and exo- acting xylanase when the diet contains high levels of non-starch polysaccharide for monogastric animals. Also, the amount of enzyme unit can bring a complementary effect on the other.

The use of multiple carbohydrase activities may produce greater benefit than each of the enzymes acting individually (Juanpere *et al*., 2005). Beneficial interactions among carbohydrases (Choct *et al.*, 2004) and with phytase (Diebold *et al.*, 2004) have been reported. The multipurpose enzyme increased protein digestibility in the test feedstuff better than the other enzymes. This may be attributed to its effect on crude fibre digestibility. For instance reduction in nitrogen digestibility has been attributed to neutral detergent fibre and acid detergent fibre content and an increase in digestibility of these fibre fractions will lead to an increase in digestibility of protein. The significant difference between the control (no enzyme) and treatment with phytase especially in digestibility of fibre fractions in all the feedstuffs studied may be attributed to two reasons. Firstly, encapsulating effect of phytate is not limited to phosphorus and other minerals alone because it can also form complex with other nutrients in the feedstuff notably carbohydrate, protein, ether extract *etc*. Therefore a breakdown of phytate to release phosphorus will also lead to the release of these nutrients which will also make them more available to the respective digestive enzymes (Farrel *et al*., 1993; Sebastian *et al.,* 1997). Secondly, no exogenous enzyme is wholly single purpose (McCleary, 2001).A typical enzyme complex has other side active enzymes. This has been demonstrated by Rutherford *et al*. 2002).

Conclusion and Recommendation

In conclusion, results of this study have shown the efficacy of enzymes individually and as cocktails on *in vitro* digestibility of maize offal. In comparison, the multipurpose enzyme performed significantly best among the three enzymes when used individually. This study has also shown that cocktail of enzymes is better than individual enzymes. Among the 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 has revealed that effect of phytase enzyme is not limited to phytate alone although its effects on those parameters are small.

Based on the outcome of this *in vitro* study, it is recommended that quantification of these effects through feeding trials will be essential so as to develop matrix value (nutrient-equivalent value) to be assigned to 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.

References

Adeola, O. and Cowieson, A.J. (2011). Opportunities and Challenges in using exogenous enzymes to improve non ruminant animal production. *Journal of Animal Science*, 89: 3189-3218

AOAC (1990). Official methods of analysis, 15th ed. Association of Official Analytical Chemists, Washington DC

Boisen, S. and Fernadez, J.A. (1997). Prediction of the total tract digestibility of energy in feedstuffs and pig diets by *in vitro* analyses. *Animal Feed Science Technology*, 68: 277-286.

Chesson A. (1993). Feed enzymes. *Animal Feed Science and Technology,* 45: 65-79.

Choct, M., Kocher, D., Waters, L.E.D., Pettersson, D. and Ross, G. (2004). A comparison of three xylanases on the nutritive value of two wheats for broiler chickens. *British Journal of Nutrition,* 92: 53–61.

David, L.N. and Michael, M.C. (2004). *Lehninger principles of biochemistry,* Fourth Edition. W.H. Freeman, USA.

Diebold, G., Mosenthin, R., Piepho, H.P. and Sauer, W.C. (2004). Effect of supplementation of xylanase and phospholipase to a wheat-based diet for weanling swine on nutrient digestibility and concentrations of microbial metabolites in ileal digesta and feces. *Journal of Animal Science,* 82: 2647–2656.

Duncan, D.B. (1955). Multiple Range and Multiple F- tests. *Biometrics,* 11: 1-42.

Farrel, D.J., Betts, M., Sudam, N.A. and Thompson, E. (1993). The beneficial effects of a microbial phytase in diets of broiler chickens and nutrition. Journal? 69:278-283

Jimoh, A. and Atteh, J.O. (2015). Efficacy of enzyme cocktails on *in vitro* digestibility of wheat offal. In: Adebiyi, E.A., Ogunwole, O.A., Babayemi, O.J. and Iyayi, E.A. (Eds.). *Proceedings of the 20th Annual Conference of Animal Science Association of Nigeria (ASAN),* 6th -10th Sept. 2015, Ibadan, Nigeria. Pp: 493-499.

Juanpere, J.A., Perez-Vendrell, E.A. and Brufau, J. (2005). Assessment of potential interactions between phytase and glycosidase enzyme supplementation on nutrient digestibility in broilers. *Poultry Science,* 84: 571-580.

McCleary, B.V. (2001). Analysis of feed enzymes. In: M.R. Bedford and G.G. Partridge (Eds.). *Enzymes in farm animal nutrition.* CAB International, Wallingford, UK.

McDonald P., Edwards R.A., Greenhalgh J.F.D., Morgan C.A., Sinclair L.A. and Wilkinson R.G. (2010). *Animal nutrition,* Seventh edition. Pearson Education Limited, Gate Harlow, Edinburgh, United Kingdom

Rutherford, S.M., Chung, T.K. and Moughan, P.J. (2002). The effect of microbial phytase on ileal phosphorus and amino acid digestibility in the broiler chicken. *British Poultry Science*, 44: 598-606.

Sebastian, S., Touchbourn, S.P., Chawez, E.R. and Lague, P.C. (1997). Apparent digestibility of protein and amino acids in poultry chicken fed a corn-soybeans diet supplemented with microbial phytase. *Poultry Science,* 76:1760-1769

Slominski, B.A. (2000). A new generation of enzymes for animal feeds. Proc. 21st Western Nutrition Conference, Winnipeg, Manitoba, Canada.

Statistical Analysis System SAS (2002). Statistical Analysis System SAS user's guide statistics, Version 8, SAS Institute Inc. Cary, NC, USA.

Van Soest P.J. (1967). Development of a comprehensive system of feed analysis and its application to forages. *Journal of Animal Science,* 26: 119-128.

Vantsawa P.A., Ogundipe S.O., Dafwang I.I. and Omage J.J. (2007). Replacement value of Dusa (locally processed maize offal) for maize in the diet of egg type chicks (0-8Weeks). *Pakistan Journal of Nutrition*, 6(6): 530-533.