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Effects of Enzymes and Cocktail of Enzymes on *In vitro* Digestibility of Brewers' Dried Grains

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Authors' contributions

This work was carried out in collaboration between both authors. Author AJO designed the study. Author JA performed the laboratory trial, the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors JA and AJO managed the analyses of the study. Author JA managed the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

The effects of three exogenous enzymes were determined individually and in cocktails on *in vitro* digestibility of Brewers' Dried Grains (BDG). It was aimed at testing the hypothesis that cocktail of exogenous enzymes will perform better than individual enzymes. A Xylanase enzyme, a multipurpose enzyme and a phytase enzyme were used individually and in cocktails in a Completely Randomized Design comprising of seven experimental treatments and one control (no enzyme). The *in vitro* experiment was conducted at the Central Research Laboratory of University of Ilorin, Nigeria. It lasted for 48 hours.

In vitro digestibility values were calculated for dry matter, crude protein, ether extract, crude fibre and fibre fractions. Digestibility values obtained were analyzed in a one way Analysis of Variance (ANOVA) with the aid of Statistical Analysis System. Treatments' means were separated using Duncan Multiple Range Test.

Findings of the study showed that among the individual enzymes the multipurpose enzyme was significantly (P= .05) best in its effects on digestibility of dry matter, crude protein, ether extract and crude fibre with values of 52.75%, 75.87%, 69.18% and 73.45% respectively. Each of the cocktails was significantly better than the respective individual enzymes in their effects on digestibility of

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fibre fractions of BDG. Additive effects was observed especially for the digestibility of fibre fractions with 27.91% for acid detergent fibre digestibility for cocktail of xylanase and phytase compared to 18.29% and 9.21% respectively for xylanase and phytase. Positive synergistic effect was observed on neutral detergent fibre digestibility with 50.88% for cocktail of xylanase and phytase compared to 32.41% and 16.37% for xylanase and phytase respectively. Furthermore complementary effects were observed when xylanase and multipurpose enzymes were used as cocktail.
It is concluded that cocktail of different exogenous enzymes is better than individual enzymes in improving the digestibility of Brewers' Dried Grains.

Keywords: Additive effect; synergistic effect; complementary effect; fibre fractions; in vitro.

1. INTRODUCTION

Brewers' Dried Grain (BDG) is a solid waste from the brewery industries. It is available and cheap but difficult to dry to low moisture content for easy storage and use, especially during the wet season. Breweries in Nigeria use maize, barley and sorghum in combinations, which vary from one brewery to another. This, therefore, has resulted in the production of BDG with variable physical and chemical composition [1]. Brewers' dried grain is a good source of quality protein and digestible fibre, with a good amino acid profile and high mineral and B-vitamin content [2]. It does not form food for humans and does not have any other industrial use for now. It is usually dried and sold as feedstuff for livestock. Brewers' dried grain is reported to have better available protein, energy and ash composition than maize offal and wheat offal [3,4]. According to [5] broilers fed BDG–based diet had poor efficiency of feed utilization and this was attributed to its very high crude fibre. The use of BDG in poultry nutrition is limited because of its fibre content though it has a relatively high protein value of about 23%.To enhance its use as a feedstuff in poultry exogenous enzymes are added. Also, [6] reported that substitution of maize with 20% BDG supplemented with Grindazyme® enzyme resulted in better performance and gave a higher net profit compared with other treatments and could be adopted to alleviate the problem of high cost of maize. Birds fed BDG-based diet supplemented with enzyme (Avizyme) had significantly ($p<0.05$) higher live weights than those on the basal diets [7].

However, because of the difference in profile of enzymes and the complex nature of crude fibre the need for cocktail of enzymes has been suggested. This is borne out of the fact that no single enzyme can achieve complete degradation of crude fibre. Therefore this study was designed to test the hypothesis that combination of different enzymes will perform better than individual enzymes on Brewers' Dried Grains using *in vitro* techniques. The study investigated the effects of the enzymes and cocktails on parameters like dry matter, crude fibre, crude protein and ether extract.

2. MATERIALS AND METHODS

2.1 Experimental Design

Three different exogenous enzymes were used in a completely randomized design for the study. The enzymes were xylanase (a bacterial endo-xylanase), multipurpose enzyme (fungal enzyme containing xylanase, glucanase, hemicellulase and cellulase among others) and phytase.

The enzymes were used individually, in pair wise combination as well as the three together. Thus there were eight treatments comprising of one control treatment (no enzyme) and seven experimental treatments as shown in Table 1. The enzymes were included at manufacturers' recommended inclusion level of 100 ppm for xylanase, 150 ppm for multipurpose enzyme and 150ppm for phytase. For Cocktails the enzymes were included at ratio of 100 ppm: 150 ppm: 150 ppm (Xylanase: multipurpose: phytase). Each treatment was replicated thrice giving a total of twenty four experimental units.

The xylanase used in this study has 9000 units of xylanase activity per gram as stated by the manufacturer with number EC3.2.1.8 - endo-1,4-β-xylanase. It was produced from *Bacillus substilis* and it was powdery in nature and cream colored. The complex has wheat flour as its carrier.

The multipurpose enzyme used was produced from *Trichoderma viride*. It was a granular and odorless solid preparation. It has the identifications endo-1,4-β-xylanase (EC 3.2.1.8), endo-1,3(4)-β-glucanase (EC 3.2.1.6) and

Table 1. Composition of experimental treatments

Test material	Treatments							
	NE	Xy	Mp	Ph	Xy+Mp	Xy+Ph	Mp+Ph	Xy+Mp+Ph
BDG(%)	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

BDG: Brewers' Dried Grains,1: Xylanase enzyme,2: Multipurpose enzyme, 3. Phytase enzyme
NE = No enzyme, Xy = Xylanase enzyme only, Mp = Multipurpose enzyme only, Ph = Phytase enzyme only, 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

endo-1,4-β-glucanase (EC 3.2.1.4). The enzyme complex had 5-10% active enzyme. 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, 8000 units/gram of cellulase and traces of pectinase, hemicellulase, α-amylase and others as stated by the manufacturer.

The phytase used was a 3-phytase enzyme obtained from *Aspergillus niger* with identification number EC 3.1.3.8. It was granular in nature and had activity of 5000FTU/gram as stated by the manufacturer. One FTU (phytase unit) is the amount of enzyme which liberates 1 micromole (1 µmol) of inorganic phosphate per minute from sodium phytate at pH 5.5 and 37°C. The enzyme inclusion level was 150 ppm.

2.2 *In vitro* Techniques

In vitro digestion technique was carried out in line with the procedure of [8] with some modifications. The two step digestion procedure simulates the chicken's gastric and intestinal pancreatic digestions. Brewers' Dried Grains was milled to pass through 1.00 mm sieve. One kilogram of each treatment was prepared and five gram of each was put in the respective 50 ml flask and 10 ml of pepsin in 0.1 M HCl (aq) was added. The content was incubated for 30 minutes at temperature of 40°C and P^H of 2.0. Neutralization followed with 0.2 M NaOH and 10 ml of pancreatin in a buffer solution was added and incubated for additional 2 hours at temperature of 40°C and P^H of 7.0. The two stages of the incubation were accompanied with shaking with the aid of a mechanical shaker. At the end of the digestion stages, the content of the flask was filtered using a weighed filter paper. The filtrate was discarded while the residue was prepared for proximate analyses and fibre partitioning. The *in vitro* digestion took place at the Central Research Laboratory of the University of Ilorin while proximate analyses and fibre partitioning were done at the Department of Animal Production Laboratory, University of Ilorin.

2.3 Chemical and Statistical Analysis

The proximate composition of the residues after filtration was determined in accordance with the procedure of [9]. Fibre fractions assay was done according to the method of [10].

Nutrient digestibility was calculated using the formula below

Nutrient Digestibility (%) =

$$\frac{\text{Nutrient in sample (g) - Nutrient in Residue (g)}}{\text{Nutrient in Sample (g)}} \times 100$$

Values of nutrient digestibility obtained were statistically analyzed using one way ANOVA procedure of [11]. Significant differences between treatments means were separated using [12].

3. RESULTS

3.1 Effects of Enzymes and Cocktails on *In vitro* Digestibility of Brewers' Dried Grains

Table 2 shows the effects of the enzymes and their cocktails on *in vitro* digestibility of brewers' dried grains (BDG). All the enzymes individually and as cocktails improved dry matter digestibility of brewers' dried grains compared to the control. There were significant differences (P= .05) in the effects of the treatments on dry matter digestibility of brewers' dried grains except for phytase and cocktail of xylanase and multipurpose enzyme which were not significantly different from each other (48.99 vs. 49.03). There were significant differences

(*P*= .05) between the cocktails in their effects on dry matter digestibility and cocktails of the three enzymes had the highest effect of 56.02%. There were significant differences in the effects of the treatments on crude protein digestibility with multipurpose enzyme giving the highest effect of 75.87%. Phytase, multipurpose enzyme and cocktails of multipurpose and phytase and that of the three enzymes improved crude protein digestibility compared to the control. Xylanase enzyme was significantly lower than the control in its effect on crude protein digestibility. Xylanase enzyme and cocktail of xylanase and multipurpose enzymes were not significantly different in their effects on crude protein digestibility (51.87% vs. 50.84%). All the enzymes individually and as cocktails improved crude fibre digestibility compared to the control. Among the three enzymes used individually, the multipurpose enzyme performed significantly (*P*= .05) best followed by xylanase on crude fibre digestibility while phytase effect was the least. There was no significant difference (*P*= .05) between the cocktail of xylanase and multipurpose enzymes and cocktail of the three enzymes on crude fibre digestibility of brewers dried grains (77.50 vs. 77.36). All the enzymes individually improved significantly ether extract digestibility compared to the control. However effect of cocktail of xylanase and multipurpose enzymes was not significantly different (*P*= .05) from the effect of the control on ether extract digestibility. Effects of the individual enzymes were significantly different and were also significantly better than the control on ether extract digestibility.

3.2 Effects of Enzymes and Cocktails on *In vitro* Digestibility of Fibre Fractions of Brewers' Dried Grains

All the enzymes individually and as cocktails improved the digestibility of fibre fractions namely neutral detergent fibre, acid detergent fibre, cellulose and hemicellulose compared to the control. There were significant differences *(P= .05)* between the treatments in their effects on the digestibility of the fibre fractions (neutral detergent fibre, acid detergent fibre, cellulose and hemicellulose) with the control having the least value while cocktail of the three enzymes have the highest value in all these parameters (Table 3). However there were no significant differences between the treatments in their effects on digestibility of acid detergent lignin and none of the enzymes and the cocktails was able to improve the digestibility of lignin compared to the control.

Table 2. Effects of enzymes on *In vitro* digestibility of brewers dried grains

Parameters	Treatments								
	NE	Xy	Mp	Ph	Xy+Mp	Xy+Ph	Mp+Ph	Xy+Mp+Ph	SEM
DM (%)	41.74^{f}	44.78^{e}	52.75^{b}	48.99^{c}	49.03^{c}	47.01^{d}	53.15^{b}	56.02^{a}	0.92
CF (%)	4.51^{d}	54.11^{b}	73.45^{a}	15.76^{c}	77.50^{a}	57.25^{b}	74.32^{a}	77.36^{a}	5.62
EE (%)	63.58^{d}	64.53^{c}	69.18^{a}	67.78^{b}	59.79^{e}	64.37^{c}	64.65^{c}	64.04^{d}	0.55
CP (%)	53.97^{d}	51.87^{e}	75.87^{a}	57.81^{c}	52.88^{d}	50.84^{e}	56.98^{c}	60.00^{b}	1.57

a, b, c, d, e, f: Means in the same row followed by the same superscript are not significantly different. (P= .05)
DM = Dry matter, CF = Crude fibre, EE = Ether extract, CP = Crude protein, NE = No enzyme, Xy = Xylanase enzyme only, Mp=Multipurpose enzyme only, Ph=Phytase enzyme only, 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 3. Effects of enzymes on *In vitro* digestibility of fibre fractions of brewers dried grains

Parameters	Treatments								
	NE	Xy	Mp	Ph	Xy+Mp	Xy+Ph	Mp+Ph	Xy+Mp+Ph	SEM
NDF (%)	1.15^{g}	32.41^{e}	54.84^{d}	16.37^{f}	62.52^{c}	50.88^{d}	69.02^{b}	74.57^{a}	5.10
ADF (%)	0.22^{h}	18.29^{f}	43.63^{d}	9.21^{g}	51.95^{c}	27.51^{e}	58.53^{b}	65.82^{a}	4.71
CELL. (%)	0.02^{f}	9.04^{d}	39.15^{c}	2.51^{e}	43.82^{b}	10.82^{d}	41.14^{bc}	51.75^{a}	4.12
HEMI. (%)	2.04^{g}	34.73^{e}	48.31^{c}	9.05^{f}	57.26^{b}	39.93^{d}	51.80^{c}	62.92^{a}	4.32
ADL (%)	0.09^{a}	0.08^{a}	0.08^{a}	0.08^{a}	0.08	0.08^{a}	0.08^{a}	0.08^{a}	0.01

a, b, c, d, e, f, g, h: means in the same row followed by the same superscript are not significantly different. (P= .05)
NDF = Neutral detergent fibre, ADF = Acid detergent fibre, CELL.= Cellulose, HEMI. = Hemicellulose, ADL = Acid detergent lignin, NE = No enzyme, Xy = Xylanase enzyme only, Mp = Multipurpose enzyme only, Ph = Phytase enzyme only, 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

4. DISCUSSION

Dietary fibre is generally defined as the plant polysaccharides and lignin that are resistant to hydrolysis by digestive enzymes [13]. Fibre is not a chemically uniform substance and the components vary from plants to plants. It exerts antnutritive effects in monogastric animals. Because fibre is not digestible by monogastric animals exogenous enzymes are now being added to poultry feedstuffs to aid in the digestion of the fibre. Components of crude fibre have varying degrees of digestibility values.

This *in vitro* trial has revealed that crude fibre digestibility of BDG can be improved with exogenous enzymes and that cocktail of enzymes is better than individual enzymes. Effects of enzymes on feedstuffs can be synergistic (positive or negative), complementary or additive [14]. Positive synergistic effects were noticed in this study and in this situation the effects of the cocktail is more than the addition of the effects of the individual enzymes while additive effects is due to a situation where the effect of the of the cocktail is equal to the addition of the effects of the individual enzymes when used individually. Instance of positive synergistic effect was seen in the digestibility of neutral detergent fibre fraction of Brewers' Dried Grains where the cocktail of xylanase and phytase produced effect that was higher than the addition of the effects of xylanase and phytase when used individually. Additive effect was observed with neutral detergent fibre when Xylanase and phytase enzymes were combined. Positive synergistic effects may be attributed to the difference in activity of enzymes and difference in profile of the enzymes. This experiment revealed that positive synergistic effect was noticed when phytase combined with Xylanase indicating that the effect of phytase on crude fibre fractions was minimal.

The *in vitro* trial also shows the matrix effects of the enzymes. Multipurpose enzyme increased the digestibility of crude protein in brewers' dried grains. This effect is also observed with ether extract where xylanase and multipurpose improved ether extract digestibility in BDG. These improvements are in addition to their effects on crude fibre digestibility which the enzymes are primarily designed for. Matrix effects may be attributed to encapsulation effect of nonstarch polysaccharides. For instance the quantity of fibre fractions like neutral detergent fibre (mainly hemicellulose, cellulose and lignin) and acid detergent fibre (mainly cellulose and lignin) are often negatively correlated with dry matter digestibility especially in monogastric animals [15]. Therefore a breakdown of crude fibre will lead to an increase in the availability of these nutrients and also allow their respective enzymes to act on them.

The *in vitro* dry matter digestibility (IVDMD) observed in this study suggests the efficacy of the enzymes individually and the synergistic effect of the enzyme cocktails to digest fibre. All the treatments with enzymes have DM digestibility value higher than the control. The digestibility values due to the enzymes may be attributed to the difference in profile of the enzymes as well their activities. For example the multipurpose enzyme has higher activity of xylanase than the single purpose xylanase used (26,000 units per gram vs. 9,000units per gram) in addition to the presence of other enzymes that would have complemented its activities. The profile of the multipurpose enzyme may have favored it among the three enzymes studied individually while the complementary effect is responsible for the highest effect observed in treatment with the three enzymes combined in this study. Hemicellulose is more digestible than cellulose but less digestible than starch polysaccharides [16]. 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 [14]. This explains why the multipurpose enzyme was significantly better than each of the other two enzymes individually. Also, there may be traces of cellulase in other enzymes in the course of manufacturing [14] and this explains why there is improved cellulose digestibility observed in this study compared to the control.

Combination of enzymes is expected to elicit one of several effects and these include additive effect, synergistic effect or complementary effect. [14]. It is important to understand the optimum combination of enzymes to use in animal diets especially where there are differences in

chemical composition of the diets/feedstuffs. Beneficial effects may be dependent on diet composition [17] or enzyme profile [18]. In this experiment, improvements due to enzyme cocktails are due to either synergistic or additive effects. In case of additive effect, it is expected that the sum of effects due to individual enzymes will be the same as the effect when the enzymes are used in combinations. For positive synergistic effects, it is expected that the effect of the enzymes when used in cocktails will be more than the addition of the effects of the individual enzymes [19]. In this study the enzyme cocktails have both positive synergistic effects and additive effects.

Positive synergistic 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. Exo-xylanase enzymes act by sequential removal of sugar units from chain ends [20]. Therefore, a positive synergism 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. Where the active sites in an enzyme are saturated, the presence of another similar enzyme with more active sites may lead to improvement in the digestibility of the substrate far and above the effect of the first enzyme. Thus the positive synergistic effect observed with neutral detergent fibre digestibility may be attributed to the presence of more active sites in xylanase compared to phytase. The effect of phytate is not restricted to phosphorus only. Phytic acid also chelates(form complex compound) with other minerals like calcium, magnesium, zinc and copper as well as other nutrients like protein and carbohydrate thereby preventing them from the action of digestive enzymes. According to [21], xylanase increases the permeability of the aleurone layer of wheat which is the site of phytic acid storage. Xylanase by itself will not target phytate but a cocktail of xylanase and phytase will be mutually beneficial [14]. Thus the breaking of the phytate complex will release these nutrients thereby making them available to other specific enzymes. This may be responsible for the improvement observed when other enzymes were added to Phytase in this study. This is an example of matrix effect of enzyme.

The use of multiple carbohydrase activities may produce greater benefit than each of the enzymes acting individually [22]. However, the hydrolytic activity of carbohydrase may be limited by the presence of protease which can digest carbohydrase. This can cause negative synergistic effect which was observed in this study with cellulose digestibility when cocktail of xylanase and phytase was used. Beneficial interactions among carbohydrases [23] and with phytase [19] 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 behind the better 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 [24].

The multipurpose enzyme increased protein digestibility better than each of the other enzymes used in this study. This may be attributed to its effects 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. This is evident in crude protein digestibility obtained in this study where the multipurpose enzyme gave the highest effect among the three enzymes individually. The multipurpose enzyme also improved ether extract digestibility of the feedstuff individually and as cocktail. This is part of the matrix effect of exogenous enzymes. This effect may be attributed to presence of the specific enzyme or due to encapsulating effect of the antinutritional factor that was broken down by the exogenous enzyme.

The significant difference between the control treatment (no enzyme) and treatment with phytase especially in digestibility of fibre fractions in this study 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 available to the respective digestive enzymes. Improved nitrogen digestibility due to added phytase has been reported in several studies with poultry [25,26]. Improved crude protein digestibility due to phytase was also observed in this study with Brewers dried Grains. Secondly, no exogenous enzyme is wholly single purpose. In the course of manufacturing enzymes and based on the constituents of the substrate used in the inoculation, commercial exogenous enzymes used as feed supplements contain more than a single enzyme, rather they are preparations of a variety of enzymes. A large number of carbohydrases, protease, phytase and lipase are available but in varying percentages [27]. 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 apart from phosphorus. According to [28] added phytase (400 units /Kg diet) increased apparent metabolisable energy value by 5.34%. Also, [29] 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. A cocktail of phytase and xylanase will not only improve phytate phosphorus digestibility but also improve the digestibility of nutrients encapsulated by phytate. This is responsible for some of the results in this study especially where phytase combination with each of xylanase and multipurpose enzyme improved digestibility more than each of the individual enzymes in the parameters measured. However the effect of the phytase enzyme used in this study may be limited by the duration of time it was exposed to the feedstuff as well as the pH condition. By the simulation procedure, it is expected that the enzyme will act in its favorable acidic medium for just twenty minutes. This is because the pH was changed at about 30 minutes to neutral in line with the condition for pancreatic digestion of the chicken. The phytase used in this experiment is derived from *Aspergillus niger* and needs acidic medium for its action.

In this study an increase in digestibility values for dry matter, neutral detergent fibre, acid detergent fibre, cellulose and hemicellulose for Brewers Dried Grains 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 synergy between the activities of individual enzymes in the various enzyme cocktails. Enzyme synergism has been demonstrated in other works including [30] where a significant degree of synergism of enzymes in the saccharification of alfalfa fibre was observed when it was treated with combined mixture of commercial cellulase and pectinase compared to treatment with 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 [15].

Furthermore, the findings of this study also showed that none of the enzymes or their cocktails has significant effect on lignin degradability. Lignin is a polymer that originates from three derivatives of phenyl propane [16]. 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.

5. CONCLUSION

Findings of this study have shown the efficacy of enzymes individually and as cocktails on *in vitro* digestibility of test feedstuffs. 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 is not significantly different from cocktail of xylanase and multipurpose enzyme in some parameters. This study has revealed that the effect of phytase enzyme is not limited to phytate alone although its effects on those parameters are small.

Finally, this study is in addition to the body of knowledge in the use of exogenous enzymes in improving the digestibility of high fibre feed stuffs for monogastric animals. It will be appropriate to quantify the effects in all the nutrients rather than limiting it to crude fibre and its fractions. This can enhance the maximization of the economic advantage of enzyme supplementation in poultry feeding. Quantification of these effects through

feeding trials will lead to the development of a 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. The results indicate the potential for inclusion of cocktail of enzymes in poultry feeding as a means of improving poultry production through the enhancement of the nutritive value of high fibre feed stuffs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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