IMPROVING THE METABOLISABLE ENERGY VALUE OF BREWERS’ DRIED GRAINS WITH ENZYME COCKTAILS IN POULTRY NUTRITION

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**Abstract:** The determination of the positive effects of exogenous enzymes is essential to ensure their inclusion in poultry feed formulation. This study was conducted to determine the effect of enzymes on the apparent metabolisable energy (AME) value of brewers’ dried grain (BDG). Xylanase, phytase and multipurpose enzymes were used in a completely randomized design to determine the effects of individual exogenous enzymes and their cocktails on poultry metabolisable energy using adult cockerels. There were eight treatments comprising a control and seven experimental treatments with BDG and one, two or three enzymes. The AME values were determined using the intubation method. Data collected were analyzed using the statistical analysis system. Enzymes individually and as a cocktail improved the AME value of BDG compared to the control. An increase in the AME value was 3.48%, 5.39%, 5.92%, 14.29%, 18.13%, 23.21% and 29.58% respectively for phytase, xylanase, cocktail of xylanase and phytase, multipurpose, cocktail of multipurpose and phytase, cocktail of xylanase and multipurpose and cocktail of xylanase, phytase and multipurpose. Cocktails of enzymes were significantly better (P˂0.05) than individual enzymes in their effects on apparent metabolisable energy of BDG. Phytase gave a marginal increase in AME of the studied feedstuff. It has been concluded that the cocktail of enzymes is better than individual enzymes in their effects on AME of BDG. If different enzymes are available, it is recommended that the enzyme with higher units should be used.

**Key words:** cockerel, enzyme, cocktail, metabolisable, energy, intubation.

**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 raining/wet season. Breweries in Nigeria use cereals such as wheat, maize, millet and sorghum in different combinations, which vary from one brewery to another. This, therefore, has resulted in the production of BDG with variable physical and chemical composition (Oluponna et al., 2002). Brewers’ dried grain is a good source of quality protein and dietary fibre for the poultry, with a good amino acid profile, high mineral and B-vitamin content (Ironkwe and Bamgbose, 2011). It does not form food for humans nor 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 and wheat offal (Aletor, 1986; Babatunde, 1989).

The nutritional value of BDG in poultry feeding is limited by its fibrous nature though it has a relatively high protein value of about 19–25% (Kwari et al., 1999). Onifade and Babatunde (1998) reported that broilers fed BDG-based diet had poor efficiency of feed utilization and this was attributed to its high crude fibre. The presence of this high crude fibre with non-starch polysaccharide (NSP) limits its desirability in poultry diets (Kwari et al., 1999; Oluponna et al., 2002). Brewers’ dried grains have 33.02% of hemicellulose and 34.20% of cellulose and these are polymers of xylans and arabinans. Xylans play an important role in the integrity of the plant cell wall and increase cell wall recalcitrance to enzyme digestion (Faik (2013). Comwell et al. (1993) reported that dietary inclusion of BDG was normally limited to 5–10% in swine and poultry diets. Brewers’ dried grain has 10–22% crude fibre, metabolisable energy value of 7379.82 Kj/kg (Dogari, 1985) and gross energy value of 19339.54 Kj/kg (Amaefule et al., 2009). High fibrous feedstuff like BDG has energy locked up in the fibre content and this cannot be digested by the monogastric animal. Therefore, to enhance its nutritional value as a poultry feeding, exogenous enzymes have been added. Olajide et al. (2013) reported that substitution of 20% of maize with BDG supplemented with *Grindazyme*® enzyme resulted in better performance and gave a higher net profit compared with other treatments. It was concluded that BDG with enzyme supplementation could be adopted to alleviate the problem of high costs of maize. In addition, because of the difference in profile of enzymes and the complex nature of crude fibre, the need for the cocktail of enzymes has been suggested.

Jimoh and Atteh (2017) reported that cocktails of enzymes were significantly better than the respective individual enzymes in their effects on *in vitro* digestibility of fibre fractions of BDG. It was reported that the enzyme cocktails significantly improved the measured parameters, namely crude fibre, fibre fractions and crude protein compared to individual enzymes and the control treatment. It was, therefore, the objective of this study to determine the effect of exogenous enzymes individually and as a cocktail on the apparent metabolisable energy of brewers’ dried grains in adult cockerels.

**Materials and Methods**

Experimental design

Twenty-four (24) Harco black adult cockerels of twenty-six weeks of age and about 2.2 kg each were randomly allotted to the experimental treatments. There were eight treatments comprising one control and seven experimental treatments in a completely randomized design as shown in Table 1. Each treatment was replicated three times with one bird per replicate. Three enzymes, namely xylanase, multipurpose enzyme and phytase were used individually and as cocktails following the manufacturers’ recommended inclusion levels. The choice of xylanase and multipurpose enzymes was based on the cellulose and hemicellulose content of brewers’ dried grains. BDG has 33.02% hemicellulose and 34.20% cellulose which are mainly composed of xylans and arabinans. Phytase was included to investigate its ability to release nutrients bound to phytate, especially carbohydrates (Ravindran et al., 1995). For cocktails, the enzymes were included at ratio of 100 ppm: 150 ppm: 150 ppm (xylanase: multipurpose: phytase).

The xylanase is a bacterial enzyme preparation obtained from *Bacillus substilis* and contains 9000 units of endo-1, 4-β-xylanase enzymes per gram. It is recommended for rations with high content of arabinoxylans which are present in grains and their by-products. As an endoxylanase, it specializes in splitting the glycosidic bonds within the polysaccharide chain. This causes a dramatic decrease in digesta viscosity and increases the liberation of entrapped nutrients. It also produces a large number of smaller fragments of oligosaccharides, each with a reducing terminal monosaccharide.

The multipurpose enzyme is derived from *Trichoderma viride*. Each gram of the enzyme complex has 8000 units of cellulase, 18000 units of β-glucanase and 26000 units of xylanase.

The phytase used is a 3-phytase enzyme obtained from *Aspergillus niger*. It is granular in nature and it has activity of 5000 FTU/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 37oC. Phytate usually chelates with cations, proteins and carbohydrate (Ravindran et al., 1995). Thus, a breakdown of the phytate will release other nutrients like protein, carbohydrate and minerals, in addition to phosphorus. This explains why phytase could lead to improvement in the digestibility of other nutrients apart from phosphorus. The use of microbial phytase in poultry diet has increased in response to concerns over phosphorus pollution of effluents from intensive animal farming operations and the skyrocketing price of inorganic phosphates. Phytic acid, also known as 1, 2, 3, 4, 5, 6, hexakisdihydrogen phosphate is an organic phosphate and is abundant in plant seeds. Its salt is known as phytate.

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 |
| Xy1 (ppm) | -- | 100 | -- | -- | 100 | 100 | -- | 100 |
| Mp2(ppm) | -- | -- | 150 | -- | 150 | -- | 150 | 150 |
| Ph3(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.

Feeding trials

The cockerels were randomly allocated to the battery cage system with one bird in a cell representing a replicate. The birds were provided with *ad libitum* feed and water before the experiment. Brewers’ dried grain was obtained from a commercial feed mill in Ilorin, north central Nigeria. It was ground into mash form. The exogenous enzymes were obtained from appointed distributors of the feed additives in Lagos, Nigeria. The feeding trial was done using the intubation method as described by Sibbald (1976) with some modifications. Feed was withdrawn from all the birds for 21 hours prior to the administration of the treatment so as to empty the digestive system. At exactly 21 hours, a cockerel was removed from its cell and a tube of about 8mm of 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 (BDG plus a respective individual enzyme or a cocktail) in the 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 feedstuff 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. Immediately after the feeding for each bird, an excreta collection tray was placed under the individual cell and excreta samples were collected over a period of 24 hours after the intubation of all the cockerels. Adequate water was provided for the birds prior to and after the intubation. At exactly 24 hours post intubation, the excreta collection tray was removed from each of the cells. The sample was collected, weighed, oven dried at 720C for 24 hours and weighed again.

Gross energy determination

Gross energy determination of the excreta sample was done using bomb calorimeter (Gallenkamp Ballistic Bomb calorimeter).

Calculations

Apparent metabolisable energy for each treatment was calculated using the formula below:

AME (kj/g) = (GEf × X) – (yef-y), ......................................................Eq.1
 X

where:

AME = Apparent metabolisable energy of experimental diet;

GEf = Gross energy of experimental feedstuff in Kj/g;

X = Weight of feed given to the cockerel (60g);

yef = Gross energy of feaces of birds fed with experimental treatment in Kj/g;

y = Weight of feaces voided by fed birds in g;

Percentage increase in AME (%**)** =

AME value for treatment - AME value for control X 100.

 AME Value for control

Statistical analyses

Values obtained for apparent metabolisable energy and percentage increase in apparent metabolisable energy values were subjected to analysis of variance suitable for a completely randomized design using a general linear procedure of the statistical analysis system (SAS, 2002). Significant differences between treatments’ means were determined using Duncan multiple range test (Duncan, 1955).

**Results and Discussion**

All the enzymes individually and as a cocktail improved the AME of BDG compared to the control (Table 2). There were significant differences (P˂0.05) between the individual enzymes and cocktails in their effects on AME of BDG. Among the individual enzymes, multipurpose enzyme gave the highest AME while phytase gave the lowest AME value of 10236.03 Kj/kg, meanwhile, a cocktail of the three enzymes gave the highest AME (12817.64 Kj/kg) and this was significantly different (P˂0.05) from other cocktails. However, there was no significant difference (P˃0.05) between the effects of xylanase enzyme and that of the cocktail of xylanase and phytase enzymes.

There was no significant difference between xylanase and phytase in their effects on AME increment (5.39% vs. 3.48%). There was also no significant difference (P˃0.05) between xylanase and the cocktail of xylanase and phytase in their effects on AME increment of BDG (5.39% vs. 5.92%). The cocktail of the three enzymes gave the highest increment on AME of BDG (29.58%) and this was significantly different from other treatments.

Table 2. Effects of enzymes on apparent metabolisable energy of brewers’ dried grains.

|  |  |
| --- | --- |
| Parameters | Treatments |
| NE | Xy | Mp | Ph | Xy+Mp | Xy+Ph | Mp+Ph | Xy+Mp+Ph | SEM |
| AME, Kj/Kg | 9891.68g | 10424.43e | 11304.95d | 10236.03f | 12187.57b | 10477.61e | 11684.86c | 12817.64a | 49.62 |

a, b, c, d, e, f Means in the same row with the same superscript are not significantly different (P˃0.05) AME = Apparent metabolisable energy, 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.

Onifade and Babatunde (1998) have reported that the increased inclusion level of brewers’ dried grains in broiler diets is known to increase the feed intake but without a corresponding increase in weight gain. This has been attributed to the high fibre level of the feedstuff prompting the birds to eat more so as to satisfy their energy requirement. The excessive use of high fibre sources like brewers’ dried grains in the diet may increase the viscosity of the intestinal content with a resulting decrease in the digestion and bioavailability of nutrients which adversely affect body weight gain. Results of the present study showed that enzyme supplementation resulted in the improved AME of brewers’ dried grains. Similar results have been reported by Iyayi and Tewe (1998) in layers as well as Alabi et al. (2014) in broilers. Alabi et al. (2014) reported that an increase in the dietary level of BDG without commercial enzyme supplementation significantly decreased weight gain, and increased feed conversion ratio and nutrient digestibility of broilers. Hypertrophy of the digestive organs was also observed in the birds fed brewers’ dried grain without enzyme supplementation, but this effect was ameliorated with the inclusion of commercial enzymes.

For energy to be derived from nonstarch polysaccharides, the polysaccharides like cellulose, xylans, arabinans, glycans must be broken down first to oligosaccharides and then to monosaccharides that are absorbable by the animal. Polysaccharides are sugar polymers containing twenty or more monosaccharide units and some have hundreds or thousands of units. Polysaccharides, also called glycans, differ from each other in the identity of their recurring monosaccharide units, in the length of their chains, in the types of bonds linking the units, and in the degree of branching (David and Michael, 2004). Endo-xylanase enzymes specialize in breaking down polysaccharides from within the polymer, and this results in the production of many oligosaccharides each with terminal reducing sugars. However, oligosaccharides cannot be absorbed except if they are broken down further. Exo-xylanase enzymes are then involved in the breakdown of the oligosaccharides into monosaccharides that are absorbable by the villus of the animal. Values obtained for AME in this study are indicative of the efficacy of the individual enzymes and their combinations in performing this role. This implies that the non-starch polysaccharides in the brewers’ dried grains have been broken down to oligosaccharides and eventually to absorbable monosaccharides. Furthermore, this ability of the enzymes varies from enzyme to enzyme depending on the profile and activity and this explains the variations obtained in the performance of the individual enzymes. Jimoh and Atteh (2017) reported that the cocktail of xylanase and multipurpose enzymes performed significantly better than the individual enzymes in their effects on *in vitro* digestibility of proximate components and fibre fractions of brewers’ dried grains. The efficacy of these enzymes in this regard will depend on the profile and activity of the respective enzymes (McDonald et al., 2010). Thus, among the three enzymes used in this study, the multipurpose enzyme has the highest of the carbohydrase enzymes. The values obtained in this study are indicative of this attribute of the enzyme complex.

Figure 1. Percentage increase in apparent metabolisable energy value of brewers’ dried grains following addition of enzymes.

AME = Apparent metabolisable energy, 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.

The marginal effect observed for the phytase enzyme in this study was probably due to the matrix effect of enzymes. The enzyme is primarily designed for digestion of phytate. Phytic acid is known to bind with minerals such as calcium, magnesium and other nutrients like protein, carbohydrate and ether extract (Lesson, 1993), and it is, therefore, obvious that phytate-bound nutrients will also be released along with the free phosphorus. This effect known as an ‘extra phosphoric effect’ resulted in improved digestibility of crude fibre, protein and ether extract attributed to phytase compared to the control (Shelton et al., 2004). Thus, it could be inferred that even if the phytase is used for the primary aim (to release phosphorus), its positive effect on fibre, crude protein and ether extract must also be taken into consideration. Therefore, the 3.48% increase in AME of BDG in this study may be ascribed to the ‘extra phosphoric effect’ of phytase. This value is significant to any poultry business and practical application can lead to substantial reduction in feed cost.

Furthermore, results of this study show that the fungal enzyme (the multipurpose) was better than the bacterial enzyme (the xylanase), both of which are carbohydrases. Fungal enzymes have more active sites than bacterial enzymes (Krisana et al., 2005; Kar et al., 2006) and this effect may have manifested in the better digestibility of the nonstarch polysaccharides in addition to the higher activity of the fungal enzyme (18,000,000 units of xylanase for the multipurpose compared to 9,000 units for the xylanase). Adeniji and Jimoh (2007) also reported that the multipurpose enzyme was better than the single purpose xylanase enzyme in their effects on the digestibility of the bovine rumen content used as a replacement for maize in the diets of pullets.

**Conclusion**

Generally, in this study, exogenous enzymes improve energy digestibility and this effect is more pronounced with NSP-degrading enzymes than the phytase enzyme. An expanded enzyme matrix containing xylanase, cellulase, hemicellulase and β-glucanase combinations gave better results than the individual enzymes. The values of AME obtained in this study are practically useful in the presence of the respective enzymes and its cocktails. The practical application of these values in feed formulation will ensure that the anticipated effects of exogenous enzymes in poultry feed are taken into consideration even during formulation. In conclusion, the cocktails of enzymes in degrading NSP have a future to improve nutrient utilization of agricultural by-products in poultry.

**Acknowledgements**

The authors are grateful to members of the academic and laboratory staff of the Department of Animal Production, University of Ilorin for allowing their teaching and research facilities to be used for the research.

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Received: June 26, 2018

Accepted: November 30, 2018

UNAPREĐIVANJE VREDNOSTI METABOLIČKE ENERGIJE PIVARSKOG SUŠENOG TROPA ENZIMSKIM KOKTELIMA U ISHRANI ŽIVINE

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R e z i m e

Utvrđivanje pozitivnih uticaja egzogenih enzima je suštinsko kako bi obezbedilo njihovo uključivanje u formulaciju hraniva za živinu. Ovo istraživanje je sprovedeno kako bi se odredio uticaj enzima na vrednost očigledne metaboličke energije (engl. *apparent metabolisable energy* ‒ AME) pivarskog sušenog tropa (engl. *brewers’ dried grain* ‒ BDG). Ksilanaza, fitaza i višenamenski enzimi korišćeni su u potpuno slučajnom dizajnu kako bi se odredili uticaji pojedinačnih egzogenih enzima i njihovih koktela na metaboličku energiju živine korišćenjem odraslih petlića. Bilo je osam tretmana uključujući kontrolu i sedam eksperimetalnih tretmana sa BDG i jedan, dva ili tri enzima. Vrednosti AME određene su pomoću metode intubacije. Prikupljeni podaci su analizirani pomoću sistema statističke analize. Enzimi pojedinačno ili kao koktel poboljšali su vrednost AME pivarskog sušenog tropa u poređenju sa kontrolom. Povećanje vrednosti AME bilo je 3,48%, 5,39%, 5,92%, 14,29%, 18,13%, 23,21% odnosno 29,58% za fitazu, ksilanazu, koktel ksilanaze i fitaze, višenamenskog enzima, koktel višenamenskog enzima i fitaze, koktel ksilanaze i višenamenskog enzima i koktel ksinalaze, fitaze i višenamenskog enzima. Kokteli enzima su bili značajno bolji (P˂0,05) nego pojedinačni enzimi u svojim uticajima na očiglednu metaboličku energiju BDG. Fitaza je dovela do marginalnog povećanja AME proučavanih hraniva. Zaključeno je da je koktel enzima bolji nego pojedinačni enzimi u svojim uticajima na AME pivarskog sušenog tropa. Ukoliko su različiti enzimi dostupni, preporučuje se da se koriste enzimi sa višim jedinicama

**Ključne reči:** petlić, enzim, koktel, metabolička, energija, intubacija.

Primljeno: 26. juna 2018.

Odobreno: 30. novembra 2018.

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