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EFFECT OF LABORATORY PRODUCED XYLANASE FROM *Aspergillus niger* ON FIBRE DIGESTIBILITY OF RICE HUSK AND GUINEA GRASS

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Abstract
The efficacy of laboratory produced xylanase enzyme from Aspergillus niger on highly fibrous feeds and feedstuffs was investigated. Guinea grass (Panicum maximum) from grass hay and rice husk (Oryza sativa) from crop by-product characterised with high fibre content served as substrates (Feeds and Feedstuffs) for the experiment. The enzyme xylanase was prepared from Aspergillus niger (fungal extracts). The two substrates were treated with three levels of aqueous enzymes xylanase (0.02 ml/g, 0.04 ml/g, 0.08 ml/g). Substrate with no enzyme treatment (0 ml/g) served as control. Substrates were subjected to xylanase enzyme treatment at different time interval to determine the optimum incubation period for maximum fibre digestibility. Rice husk and guinea grass were analysed to determine the level of digestibility of fibre fractions such as crude fibre, hemicellulose, acid detergent fibre, neutral detergent fibre and lignin. The results indicate an improvement in fibre digestibility for rice husk and guinea grass treated with enzyme when compared with the control. There was no significant different in the fibre digestibility of substrate treated different levels of xylanase enzyme. Results obtained on the period of application showed no significant different across the time intervals (0 hr, 24 hr, 48 hr and 72 hr). The results therefore, suggest that optimum fibre digestibility could be obtained at level 0.02 ml/g of xylanase treatment at any time interval.

Key Words: *Aspergillus niger, Xylanase, Rice husk, Guinea grass, Laboratory*

Introduction

An improvement in livestock production can be attributed to an improved nutrition. The viability of any Livestock enterprises is a function of type of feed and feeding strategy adopted by farmers. During the dry season, highly fibrous feeds and feedstuffs that are low in nutritive quality and less costly are available in abundance. Most feeds and feedstuffs contain non-digested part such as hemicellulose, lignin and some anti-nutritive factors which inhibit feed intake utilization and growth (Briat, 2000) and non-starch polysaccharides which have negative effect on feeding digestibility (Bedford, 2000). Agricultural and industrial by-products have the potential of meeting the nutritional needs as small ruminant livestock feeds, if properly harnessed (Okoruwa *et al*., 2014). Reports shown that xylanase has been

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successfully used in the diet of monogastric animals that lack enzymes for hydrolysis of NSPs (Choct *et al*., 1995) and as such necessitate the need to reciprocate same in the diet of ruminant animals. Many microbial strains such as fungi, bacteria and actinomycetes can produce xylanase (Kalogeries *et al*., 2003). Feng *et al*. (1992) reported that adding fibrolytic enzymes to grass hay directly before feeding to cattle improved voluntary intake and digestibility (ruminal and total tract). Xylanase can break down the fibre content and improve the nutritive value of feeds and feedstuffs. Therefore, the objectives of this study were to produce the xylanase locally from *Aspergillus nige*r, investigate its efficacy in improving feeds and feedstuffs and to determine the optimal level of locally produced xylanase enzyme and incubation time for optimum digestibility of feeds and feedstuffs.

Materials and Methods

Enzyme Xylanase Preparation

The fungal strain used in this study was *Aspergillus niger* with wheat bran as the carbon source. This combination of fungus and carbon source was based on the work of Okafor *et al*. (2007), where this combination produced the highest amount of xylanase. *Aspergillus niger* used in this work was obtained from stock culture from the Microbiology Laboratory, University of Ilorin, Nigeria. Wheat bran was purchased from Oja Gboro market in Ilorin. The fungus was grown on Potato Dextrose Agar (PDA) at 25 ^{o}C for 72 hours and stocked at 4 ^{o}C. A litre of xylanase production media contained 3 g of $NaNO_3$, 0.5 g of KCl, 1 g of KH_2PO_4, 0.5 g of $MnSO_4.7H_2O_4$, 0.01 g of $FeSO_4.7H_2O$ and 10 g of Wheat Bran was used as Carbon source. One ltre of the media was supplemented with 1 ml of trace solution containing (per litre of distilled water) 1.0g of $ZnSO_4$ and 0.5 g of $CuSO_4.5H_2O$. The pH of the medium was adjusted to 5.6. 100ml of liquid medium was put into 250 ml and sterilized by autoclaving at 121^{o}C for 15 mins. The solution was cooled and inoculated with 10 discs of 5mm diameter of the organism from PDA culture plates using a sterile cork borer. The cultures were harvested in triplicates daily and centrifuged at 1000 rpm for 30mins over a period of 7 days. The supernatants obtained were the crude extracellular enzyme source.

Partial Purification of Crude Xylanase

Exactly 65% (w/v) of $NaSO_4$ was added to crude enzyme and centrifuged at 10,000 rpm. The pellet was suspended in 0.005M Na_2HPO_4 at pH of 6.0 and the purified enzyme was used thereafter.

Fibre Digestibility Tests

Two samples of feeds and feedstuffs (substrates) were sourced from two classes of feedstuffs. Rice husk from Crop by-product and guinea grass from grass hay. The two substrates were dried to a constant weight in a forced air oven at 55 ^{o}C. Samples were weighed at 1g into test tube with three replicates per sample. Substrates were treated with four different levels of aqueous enzyme concentrations at 0.02 ml/g, 0.04 ml/g, 0.08 ml/g and 0 ml/g as control. This is in accordance with the report of Beauchemin *et al*. (1995) that aqueous solution of enzyme sprayed at 10 LT^{-1} (0.01 ml/g) were found to improve feed efficiency numerically. The aqueous enzymes were applied on to the substrates at four regular time intervals such as 0, 24, 48 and 72 hours to determine the optimum incubation period

for maximum fibre digestibility. Substrates were treated with enzyme at different levels of concentrations under optimum conditions for incubation with pH of 6.5, temperature at 56 ^{o}C as determined by Latif *et al*. (1996) and Aslam (1999).

Statistical Analysis

The experiment was a 4 x 4 factorial design. An aqueous xylanase enzyme of four different levels: 0.02 ml/g, 0.04 ml/g, 0.08 ml/g and 0.0 ml/g as control (no enzyme) were used as treatments on two substrates sourced from two classes of feedstuffs and at four different time intervals: 0, 24, 48 and 72 hr(s) to determine the optimum incubation period. Experiment was carried out in a Factorial Experimental design with three replicates for each treatment with three interactions for each substrate. Excel plots were used to show the results.

Results and Discussion

The results of improved fibre degradation of both rice husk and guinea grass are shown in figure 1 to figure 4. They indicated that the substrates treated with xylanase enzyme differed significantly in terms of dry matter and neutral detergent fibre degradation ($P<0.05$). Rice husk had the highest soluble fraction and closely followed by guinea grass. Dry matter degradability for rice husk was 82.1% from 87.1% and 62.0% from 88.5% for guinea grass. Effective neutral detergent fibre degradation for rice husk was also high at 39.8% from 62.5% and guinea grass had the least at 43.8% from 63.7%. The dry matter and neutral detergent fibre digestibility were high for rice husk and guinea grass at different levels of xylanase concentrations when compared to control at 0 level of concentration. In agreement with the results obtained, Feng *et al*. (1996) reported improved dry matter, neutral detergent fibre and acid detergent fibre digestibility when fibrolytic enzymes were applied to grass hay before feeding to cattle. The result obtained is also in agreement with Feng *et al*. (1992) who reported that voluntary intake and total track digestibility of dry matter and neutral detergent fibre were increased by treating hay with fibrolytic enzymes directly before feeding, but not when applied to fresh or wilted forage. The observed variation was mainly due to the differences in chemical composition and substrate type. This is in accordance with Van Soest (1994), who stated that dry matter and neutral detergent fibre degradability of forages is associated with chemical composition of substrates. The rice husk and guinea grass treated with 0.02 ml/g concentration of xylanase showed a significant difference ($p< 0.05$) when compare to substrates with no enzyme treatment. There was no significant difference in fibre digestibility of rice husk and guinea grass treated with 0.04 ml/g and 0.08 ml/g concentration of enzyme compared to those treated with 0.02 ml/g. This explains that the lower enzyme concentration can be used in the treatment of highly fibrous feeds and feedstuffs to obtain optimum fibre digestibility (Kongbuntad *et al*., 2006). This is in line with Beauchemin *et al*. (2004) who stated that larger amount of enzyme supplementation can be less effective than smaller amounts and that the optimum amount of enzyme supplementation depends on the diet. The lack of response to low concentrations of enzymes supplementation indicates an insufficient supply of enzyme activity. The results obtained based on the

duration of application of enzymes had no significant difference across the time intervals from 0 hr, 24 hr, 48 hr and 72 hr. Application of exogenous enzymes with respect to duration did not have a significant effect on dry matter and neutral detergent fibre degradation of rice husk and guinea grass (P>0.05). This showed that enzyme could be applied at 0 hour to obtain optimum degradation of fibre content with enzyme interacting with the target substrates. This is in accordance with Tricarico and Dawson (1999) who reported that an addition of xylanase and cellulase enzyme preparations directly into feed improved the in vitro rumen digestion. The neutral detergent fibre of rice husk had the highest soluble fraction (from 62.50% to 39.87%) and neutral detergent fibre of guinea grass had an appreciable soluble fraction (from 63.70% to 43.89%) treated with 0.02ml/g of xylanase enzyme concentration. Dry matter degradability for rice husk was from 87.09% to 81.89% and 88.45% to 62.12% for Guinea Grass. Agosin and Odier (1985) reported that *in vitro* digestibility of wheat straw improved from 38% to 68% when treated with lignolytic fungal strains. There was a significant difference ($p< 0.05$) across all fibre fractions in both rice husk and guinea grass treated with 0.02 ml/g of xylanase enzyme concentration when compared to those without enzyme treatment (0 ml/g). This finding training agrees with the report of Heldt-Hansen (1997) that the xylanase can be an improved growth performance due to xylanase functioning in the hydrolysis of arabinoxylan and starch.

Alabi *et al*. (2014) reported that birds fed with rice husk diets supplemented with commercial enzymes did better in all parameters measured than those fed with rice husk diets without commercial enzyme. In this work, there was no significant difference recorded on those treated with 0.04 ml/g and 0.08 ml/g of xylanase enzyme.

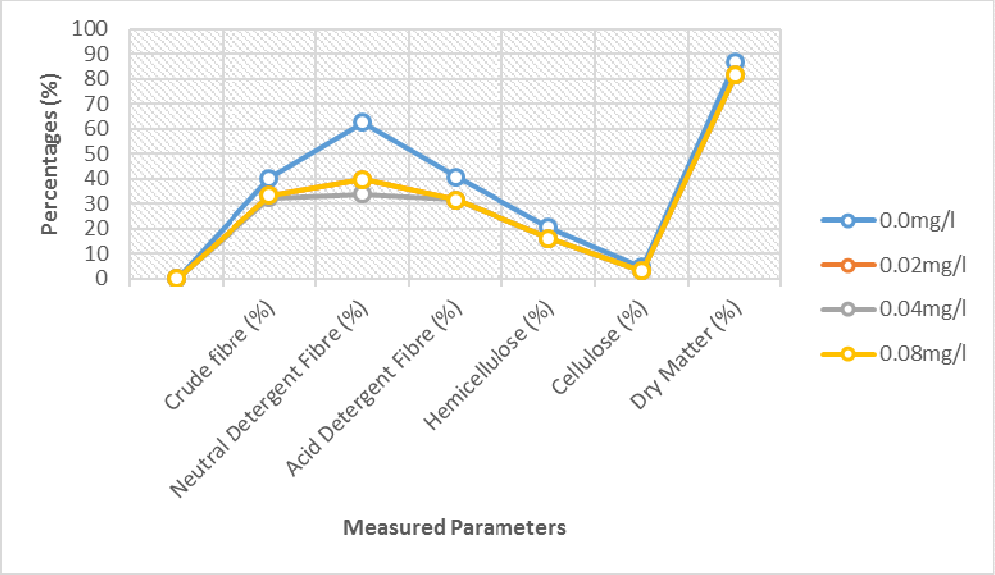

Figure 1: Impact of Concentration of Laboratory Produced Xylanase on Fibre Digestibility of Rice Husk.

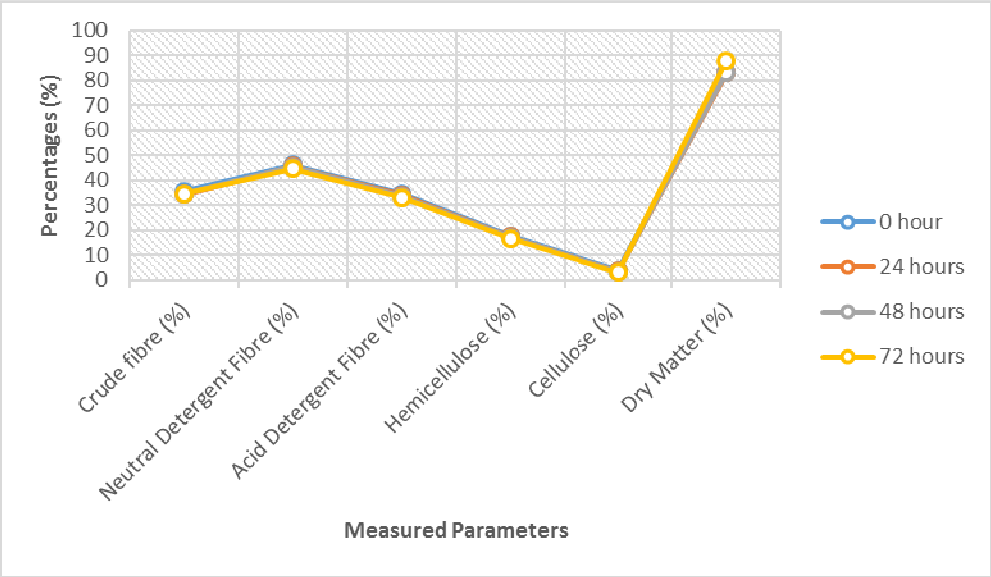

Figure 2: Impact of Time of Exposure of Laboratory Produced Xylanase on Fibre Digestibility of Rice Husk

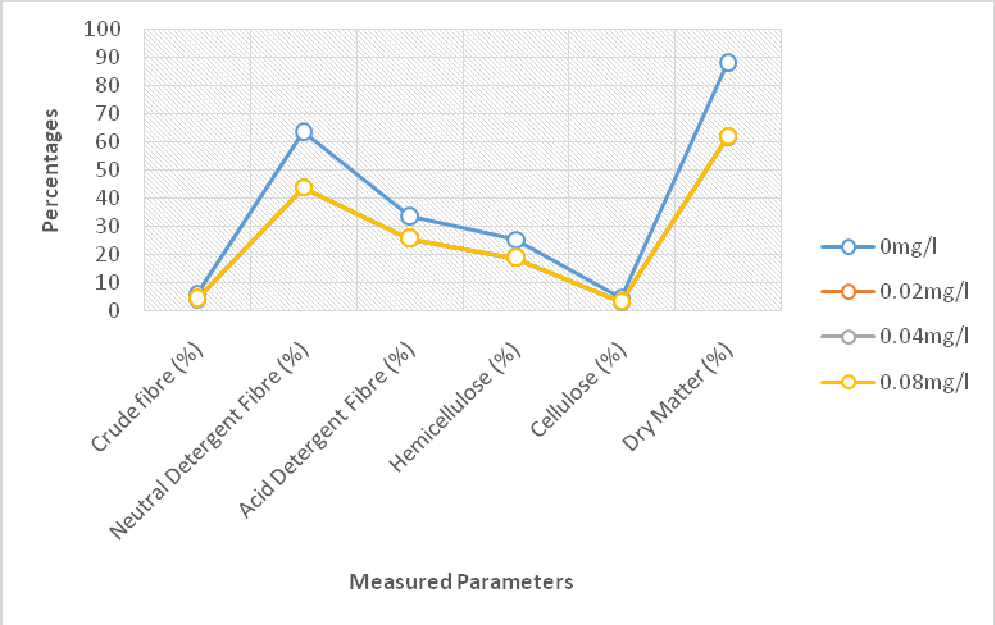

Figure 3: Impact of Concentration of Laboratory Produced Xylanase on Fibre Digestibility of Guinea Grass

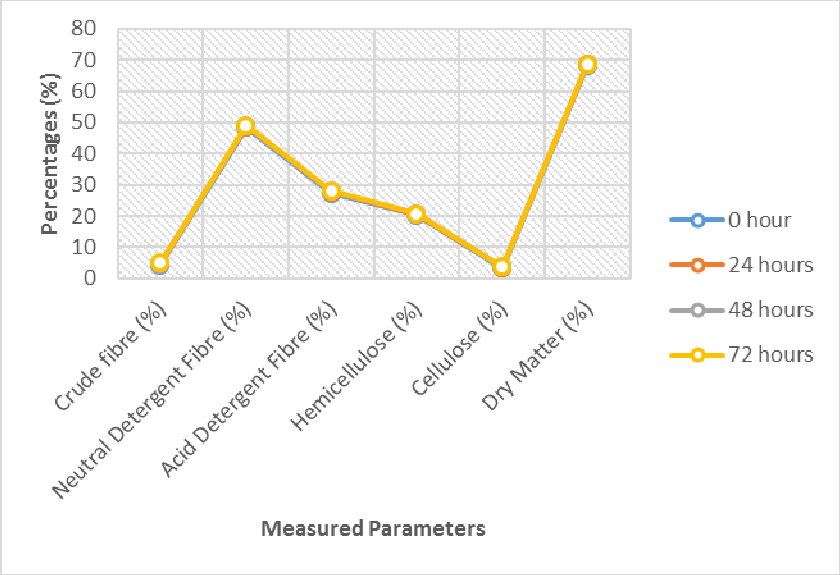

Figure 4: Impact of Time of Exposure of Laboratory Produced Xylanase on Fibre Digestibility of Guinea Grass

Conclusion

Xylanase supplementation improved fibre digestibility of rice husk and guinea grass because of an increase in fibre digestibility of substrates. Time of application of xylanase had no significant effect on substrates. Digestibility of rice husk and guinea grass seemed to be independent of time of application. Further studies to clarify the mode of action of xylanase enzyme supplementation in ruminant diets will be done.

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