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Extraction of Enzymes from Four Fungi and Their Use to Improve the Nutritive Value of Groundnut Pod for Broiler Feeding

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Abstract: Enzymes were extracted from four fungi: *Aspergillus niger, Trichoderma viride, Rhizopus stolonifer* and *Mucor mucedo*. The purified enzyme extracts were used to degrade Groundnut Pod (GNP) in solid state. Undegraded GNP-, degraded GNP- and Roxazyme G2G-based diets were fed to starter and finisher broilers at the rates 70 and 100 g kg^{-1} of diet, respectively. There was a production of a broad spectrum of enzymes from the 4 fungi. Treatment of the GNP with the fungal enzyme extracts caused a more significant ($p<0.05$) reduction in the crude fibre and complex carbohydrate fractions and an increase in the crude protein, metabolizable energy and phosphorus in the GNP compared to the undegraded and Roxazyme treated GNP. The amounts of glucose, fructose, galactose and sucrose in the GNP were significantly ($p<0.05$) increased on biodegradation with the fungal enzyme extracts. Enzyme extracts from *M. mucedo* and *R. stolonifer* were more superior in this regard compared to extracts from the other fungi. Diets containing the degraded GNP resulted in significantly ($p<0.05$) reduced viscosity, better apparent nutrient digestibility and performance in broilers compared to the other diets. Results suggest the possibility of production of a multienzyme complex from some common tropical fungi. These enzyme complexes are more effective in biodegrading complex carbohydrates of by-products like GNP than Roxazyme which is specific for cereal based diets.

Key words: Fungi, enzyme extracts, groundnut pod, biodegradation, broilers, performance

INTRODUCTION

Increase in the cost of conventional energy and protein ingredients in developing countries have necessitated the need to find some alternative measures to alleviate the feed supply situation. Poultry farmers in Nigeria for example, have been put under pressure to use some Agro-industrial By-products (AIBs) in formulating feed. Large amounts of AIBs from plants are produced by the food and agro allied industries (Balagopalan, 1996; Peter *et al*., 2001). Groundnut Pod (GNP) which is produced after the removal of the seeds from groundnut is a valuable resource that can be useful for poultry feeding after further processing. According to Siulapwa and Simukoko (2005) the crude protein, fibre, calcium and phosphorus in GNP are 10.4, 31.2, 0.93 and 0.87%, respectively. The nutrients in GNP especially its energy are present as intracellular compounds or together with lignin as cell wall material consisting mostly of non Starch Polysaccharides (NSPs). The use of GNP for poultry feeding is limited by the occurrence of the NSPs in it, being associated with its viscous nature, physiological and morphological effects on the digestive tract and the interaction with microflora of the gut (Vahouny, 1982). Nevertheless, enzymes supplied exogenously have been reported (Bedford, 1995; Balagopalan, 1996) to split the β-1,4 linkages in hemicellulolytic xyloglucans of NSPs. Efficient utilization of GNP carbohydrate therefore is only possible after conversion of its NSP component to monosaccharides (Choct and Annison, 1992; Essers *et al*., 1994). Fungal enzymes have been reported (Iyayi and Lösel, 2000) to be effective in breaking down the NSPs in AIBs under solid state fermentation to increase their nutritive values. The objectives of this study are two-fold: I) to extract and purify enzymes from 4 fungi namely *Aspergillus niger, Trichoderma viride, Rhizopus stolonifer* and *Mucor mucedo* and use the enzymes to biodegrade GNP, ii) to incorporate the biodegraded GNP in broiler diets and compare its effects with that of a commercial feed enzyme on the performance of the birds.

MATERIALS AND METHODS

Isolation of fungi and enzyme extraction: Obtained from the culture bank of the Department of Botany and Microbiology, University of Ibadan. A sterile wire loop was used to collect the spores and the mycelia of the actively growing fungi. The spores of *A. niger, T. viride, R. stolonifer* and *M. mucedo* were inoculated on Potato Dextrose Agar (PDA) in a lamina flow cabinet. The inoculated plates were incubated at 37°C in a Gallenkamp incubator. The PDA plates were examined for growth of fungi after 48 h and the mycelia of pure cultures of the fungi put on slants of sterile PDA. Fifty grams of milled GNP was autoclaved at 121°C for

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15 min and moistened with 20 ml of basal medium consisting of KNO_3, 5.0 mg; KH_2PO_4, 2.0 g; $MgSO_4.7H_2O$, 0.5 g; Tryptone, 0.5 g; $FeSO_4.4H_2O$, 3.5 mg; Nicotinic acid, 0.5 mg; Thiamine, 0.05 mg and Biotin, 0. 05 mg per litre of distilled H_2O. The inoculum of each isolate was prepared by pouring 10 ml of sterile distilled water into spores of each agar slants and using sterile wire loop to wash the spores into the water. The filtrate of each isolate was subsequently diluted with more sterilized distilled water until a spore count of approximately 2.85 x 10^6 per ml was obtained using the Haemocytometer (Onilude and Oso, 1999). Each flask was inoculated with 1.0 ml of an aqueous spore suspension of each isolate. All the flasks were tightly sealed and incubated at 37°C for 7 days after which 100 ml of 0.1 M phosphate buffer, pH 7.2 was added to the solid culture of the mycelia on the GNP substrate, mixed thoroughly and then filtered through a muslin material. The culture residue was further rinsed in more washes of the same buffer. The filtrate was collected in chilled 500 ml flask, placed in ice blocks and then centrifuged at 4°C 3000 rpm for 15 min in a refrigerated centrifuge. The supernatant containing the dialized enzyme was decanted and dialyzed using a Gallenkamp magnetic stirrer against distilled water at 4°C for 12 h to obtain the purified enzyme.

Experimental diets, housing and management of birds: Groundnut pods obtained from a local feed market were dried to a constant weight, milled and autoclaved at 121°C for 15 min. The dialized enzyme extract from each fungus was applied to the autoclaved GNP at the rate of 250 ml kg^{-1} of the GNP, using a spray gun. The bags containing the GNP were then tightly sealed. At the end of 7 days, the degraded GNP was oven dried at 60°C for 24 h to stop further action of the enzymes. The GNP obtained was tagged dGNP. An equal amount of milled GNP was also autoclaved at 121°C for 15 min but without enzymes added. The undegraded GNP was tagged uGNP.

The uGNP and dGNP were used to formulate diets for both starter and finisher broilers. In the basal diet (Diet 1), the uGNP was incorporated in the starter and finisher diets at the rates of 7 and 10g kg^{-1} respectively. In diets 2, 3, 4 and 5 GNP degraded with enzymes from *A. niger*, (An), *T. viride* (Tv), *R. stolonifer* (Rs) and *M. mucedo* (Mm) were incorporate in the starter and finisher diets at the same rates. In Diet 6 a commercial feed enzyme Roxazyme G2G commonly used by poultry farmers in the country was incorporated in the starter and finisher diets at the manufacturer's recommended rate of 0.15 g kg^{-1}, respectively (Table 1). The uGNP, dGNP and diets were analyzed for proximate composition by the methods of AOAC (1995). The Acid Detergent Fibre (ADF), Neutral Detergent Fibre (NDF) hemicellulose, Acid Detergent Lignin (ADL), cellulose and pectin were determined using the procedures of Van Soest and Queen (1995). Soluble sugars were determined by the method of Somogyi (1945).

A total of 252 day-old Ross chicks obtained from Agritek Farms, Ibadan were weighed and distributed into 36 compartments in a standard poultry house. After an initial adjustment period of 2 days on a commercial starter feed, the birds in each compartment were weighed to obtain the initial weights for each replicate. Each of the 6 dietary treatments was then randomly assigned to 6 compartments containing 7 birds each. Feed and water were offered *ad libitum* for a period of 28 days after which the birds were weighed and their feeds switched to finisher feeds and fed for a further 28 days. At the end of the finisher phase, the birds were weighed to obtain their final weights. Records of feed intake were taken weekly by calculating the difference between quantity of feed offered and the total of the refusals for each week.

In order to determine the apparent digestibility of nutrients, consisting of 5 birds in each of 4 replicates were randomly assigned to each of the 6 starter and

Table 1: Gross composition (g/kg) of experimental diets containing groundnut pod for starter and finisher broilers

	Starter phase						Finisher phase					
Ingredients	Control uGNP	GNP +An	GNP +TV	GNP +Rs	GNP +Mn	GNP +RG2G	Control uGNP	GNP +An	GNP +TV	GNP +Rs	GNP +Mn	GNP +RG2G
Maize	570.0	560.0	560.0	560.0	560.0	559.85	570.0	560.0	560.0	560.0	560.0	560.0
Undegraded GNP	70.0	0.0	0.0	0.0	0.0	0.0	100.0	0.0	0.0	0.0	0.0	0.0
Degraded GNP	0.0	70.0	70.0	70.0	70.0	70.0	0.0	100.0	100.0	100.0	100.0	100.0
Groundnut cake	133.0	110.0	110.0	110.0	110.0	110.0	100.9	110.3	110.4	110.4	112.9	112.85
Soyabean meal	160.0	193.0	193.0	193.0	193.0	193.0	162.1	162.7	162.6	162.6	160.1	160.0
Fish meal	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0	30.0
Bone meal	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0	20.0
Oyster shell	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Premix	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Salt	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Lysine	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Methonine	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Roxazyme G2G	0.00	0.00	0.00	0.00	0.00	0.15	0.00	0.00	0.00	0.00	0.00	0.15
Total	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000	1000

An = *Aspergillus niger*, Tv = *Trichoderma viride*, Rs = *Rhizopus stolonifer*, Mm = *Mucor mucedo*, RG2G = Roxazyme G2G

Table 2: Proximate and detergent fibre of undegraded and degraded groundnut pod (g/100 gDM)

Parameter	(uGNP)	GNP+An	GNP+Tv	GNP+Rs	GNP+Mm	GNP+ RG2G	SEM	P-Value
Dry matter	89.54	88.76	89.84	88.64	88.32	88.10	3.71	
Crude protein	4.57^{d}	17.86^{a}	16.96^{b}	15.76^{c}	16.56^{b}	15.02^{c}	2.51	0.0041
Crude fibre	28.61^{a}	14.32^{d}	13.92^{d}	16.32^{c}	10.56^{e}	20.22^{b}	2.85	0.0022
Ether extract	2.02	3.12	2.37	2.74	3.12	2.95	0.05	0.401
Ash	19.74^{a}	11.24^{b}	19.06^{a}	18.76^{a}	18.87^{a}	18.32^{a}	2.65	0.0032
NFE	45.06^{d}	53.46^{a}	47.69^{c}	46.42^{cd}	50.89^{b}	43.49^{e}	4.72	0.0022
Phosphorus	0.47^{c}	0.54^{b}	0.59^{b}	0.71^{a}	0.73^{a}	0.72^{a}	0.02	0.0001
NDF	49.82^{a}	37.67^{c}	39.32^{b}	38.24^{bc}	39.76^{b}	42.05^{b}	3.15	0.0001
ADF	25.32^{a}	15.97^{c}	19.67^{a}	16.38^{c}	18.74^{b}	23.88^{b}	2.05	0.0002
Hemicellulose	24.50^{a}	21.70^{c}	19.65^{d}	21.86^{c}	21.02^{c}	22.07^{b}	1.81	0.0001
ADL	10.95^{a}	5.26^{c}	9.32^{b}	4.76^{c}	8.12^{b}	9.852^{a}	0.44	<0.001
Cellulose	14.38^{a}	10.71^{c}	10.35^{d}	11.62^{c}	10.62^{cd}	12.81^{b}	0.97	0.0037
ME (kcal/kg)	1562.63^{e}	2414.85^{a}	2153.70^{b}	2051.88^{c}	2259.89^{b}	1918.50^{d}	10.0	0.0065
Pectin	12.67^{a}	4.76^{d}	5.24^{cd}	6.78^{c}	3.84^{d}	9.20^{b}	0.71	0.001

Means with different superscripts along the same row are significantly different (p<0.05).
An = *Aspergillus niger*, Tv = *Trichoderma viride*, Rs = *Rhizopus stolonifer*, Mm = *Mucor mucedo*, RG2G = Roxazyme G2G

Table 3: Performance of broiler starter fed diet containing undegraded and degraded groundnut pod

Parameters	uGNP	GNP +An	GNP +Tv	GNP +Mm	GNP +Rs	GNP +RG2G	SEM	Pvalue
Initial weight (g)	39.50	38.90	39.60	38.99	39.40	39.20	0.32	
Final weight at 4 weeks (g)	469.50^{c}	634.94^{a}	607.44^{a}	598.99^{ab}	615.92^{ab}	522.48^{b}	19.1	0.0026
Feed intake (g)	977.48^{d}	1206.80^{a}	1137.08^{b}	1016.68^{c}	1063.72^{c}	997.08^{bc}	20.41	0.303
Feed conversion ratio	1.81^{a}	2.02^{a}	2.00^{a}	1.81^{b}	1.84^{ab}	2.06^{a}	0.10	0.0027
Weight gain (g)	430^{a}	596.04^{b}	567.84^{ab}	560.00^{b}	576.52^{b}	483.28^{c}	3.09	0.0062
%DM digestibility.	61.91^{c}	65.93^{a}	65.00^{a}	63.75^{b}	64.50^{a}	62.09^{b}	0.42	0.0001

Means with different superscripts along the same row are significantly different (p<0.0).
An = *Aspergillus niger*, Tv = *Trichoderma viride*, Rs = *Rhizopus stolonifer*, Mm = *Mucor mucedo*, RG2G = Roxazyme G2G

finisher diets. The birds were housed in stainless steel metabolic cages with facilities for collection of faeces. They were allowed an initial 3 day adjustment period to the cage environment followed by a 5 day collection of droppings. Faecal droppings were collected daily, weighed, bulked according to pen, stored in airtight containers and kept in a freezer until needed for analysis. Data were analyzed using the ANOVA procedure (SAS, 1999). Significant means were separated using the Duncan Multiple Range test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

The results of proximate composition and analyzed fibre components in the dGNP and uGNP are presented in Table 2. Fungal Enzyme Extracts (FEE) caused a significant increase (p<0.05) in the Crude Protein (CP), Metabolizable Energy (ME) and Phosphorus (P) levels in the dGNP. The Crude Fibre (CF), NDF, ADF, hemicellulose, ADL, cellulose and pectin levels in the dGNP were significantly (p<0.05) reduced by treatment of the GNP with the FEE. Treatment of GNP with FEE gave better results in terms of increase in levels of CP and reduction of non Starch Polysaccharides (NSPs) than with Roxazyme G. The results of soluble sugars in the dGNP and uGNP are presented in Fig. 1. Treatment of GNP with FEE resulted in higher (p<0.05) levels of glucose, fructose, galactose and sucrose than in the undegraded GNP. The FEEs were also significantly (p<0.05) better than RG in the production of these

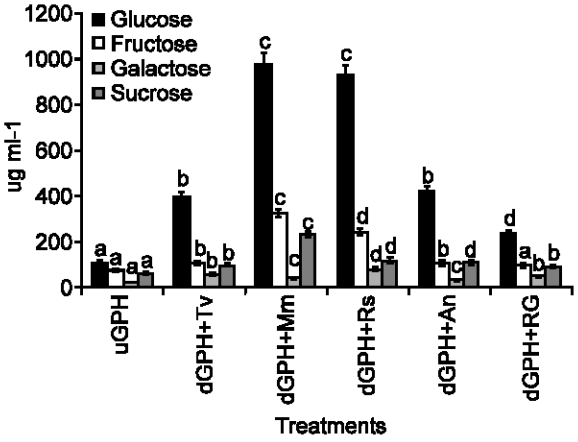

Fig. 1: Sugar levels of undegraded and degraded groundnut pod (µg/ml)

sugars. Enzyme extracts from *M. mucedo* and *R. stolonifer* were consistently better in the production of these sugars from the GNP.

The results of performance of the birds at both starter and finisher phases are presented in (Table 3 and 4), respectively. Feed intake, weight gain, FCR and Dry Matter (DM) digestibility were significantly (p<0.05) higher in birds fed diets containing dGNP than those on diets with uGNP and RG both at the starter and finisher phases. The results of apparent nutrient digestibility at starter and finisher phases are presented in (Table 5

Table 4: Performance of broiler finisher fed diets containing undegraded and degraded Groundnut pod

Parameters	uGNP	GNP +Tv	GNP +An	GNP +Rs	GNP +Mm	GNP +RG2G	SEM	P value
Final weight at 8 weeks (g)	1321.12^{d}	1631.42^{a}	1532.37^{a}	1516.10^{b}	1497.10^{b}	1397.20^{c}	2.41	0.0001
Feed intake (g)	2972.76^{c}	3665.76^{a}	3371.76^{a}	3278.52^{b}	3155.04^{b}	3090.92^{c}	40.14	0.0026
Feed conversion ratio	3.48	2.2	3.55	3.49	3.49	3.49	0.12	0.003
Weight gain (g)	852.04^{c}	1035.16^{a}	950.32^{b}	939.68^{b}	903.84^{b}	885.92^{c}	25.21	0.004
% DM digestibility	78.81^{b}	81.86^{a}	81.35^{a}	80.46^{a}	78.96^{b}	78.91^{b}	0.54	0.00052

Means with different superscripts along the same row are significantly different (p<0.05)
An = *Aspergillus niger*, Tv = *Trichoderma viride*, Rs = *Rhizopus stolonifer*, Mm = *Mucor mucedo*, RG2G = Roxazyme G2G

Table 5: Apparent nutrient digestibility in broiler starters fed degraded and undegraded groundnut pod-based diets

Parameters (%)	uGNP	GNP+Tv	GNP+An	GNP+Rs	GNP+Mm	GNP+RG2G	SEM	P value
Dry matter	65.56^{e}	77.05^{a}	75.61^{ab}	75.32^{ab}	68.85^{d}	70.51^{c}	0.56	0.0006
Crude protein	76.20^{c}	82.92^{a}	80.71^{a}	80.26^{a}	78.10^{b}	78.77^{b}	0.41	0.0001
Crude fibre	14.99^{d}	25.81^{a}	23.20^{bc}	22.91^{c}	23.41^{bc}	25.26^{b}	0.22	0.0024
Ash	23.82^{c}	26.82^{a}	25.78^{a}	25.14^{b}	24.25^{bc}	24.70^{bc}	1.02	0.001
Ether extract	45.14^{d}	73.01^{a}	70.85^{b}	70.06^{b}	65.52^{c}	67.55^{c}	1.05	0.0001
Nitrogen free extract	64.35^{d}	70.29^{a}	70.17^{a}	68.45^{b}	68.56^{b}	66.73^{c}	0.25	0.0025
NeutralDetergent fibre	37.76^{b}	38.71^{ab}	39.24^{a}	39.88^{a}	39.27^{a}	38.22^{ab}	1.56	0.0068
AcidDetergent Lignin.	35.81^{b}	37.88^{a}	36.71^{ab}	36.50^{ab}	36.10^{ab}	36.61^{ab}	1.27	0.0025
AcidDetergent Lignin	31.85^{a}	32.77^{a}	32.82^{a}	32.35^{a}	32.77^{a}	31.91^{a}	0.58	0.001
Hemicelluloses	42.15^{c}	45.45^{a}	44.95^{a}	43.22^{b}	43.92^{b}	43.88^{b}	0.62	0.0001
Cellulose	40.33^{a}	40.60^{a}	40.91^{a}	40.82^{a}	40.71^{a}	40.56^{a}	0.81	0.001

Means with different superscripts along the same row are significantly different (p<0.05)
An = *Aspergillus niger*, Tv = *Trichoderma viride*, Rs = *Rhizopus stolonifer*, Mm = *Mucor mucedo*, RG2G = Roxazyme G2G

Table 6: Apparent nutrient digestibility of broiler finisher fed diets containing undegraded and degraded groundnut pod

Parameters	uGNP	GNP + Tv	GNP + An	GNP + Rs	GNP + Mm	GNP + RG2G	SEM	P value
Dry matter	78.81^{b}	81.86^{a}	81.35^{a}	80.46^{a}	78.96^{b}	78.91^{b}	1.44	0.0026
Crude protein	79.14^{d}	83.71^{a}	83.71^{a}	82.81^{a}	80.22^{c}	79.62^{d}	1.81	0.001
Crude fibre	52.45^{d}	45.32^{c}	55.67^{bc}	57.22^{d}	56.25^{b}	55.91^{b}	0.78	0.0011
Ash	23.82^{c}	26.82^{a}	25.78^{a}	25.14^{b}	24.25^{c}	24.70^{a}	0.95	0.0032
Ether extract	68.92^{d}	81.82^{a}	80.92^{a}	79.11^{b}	79.85^{b}	62.25^{c}	0.66	0.0001
Nitrogen free	75.65^{c}	76.27^{b}	78.56^{a}	77.22^{a}	76.29^{b}	78.96^{c}	1.45	0.0004
Neutral Detergent fibre	54.99^{d}	61.82^{b}	65.79^{a}	57.21^{c}	59.92^{b}	56.89^{c}	1.88	0.0001
Acid Detergent Lignin.	5071^{e}	55.65^{c}	62.71^{b}	68.02^{a}	53.86^{d}	56.65^{c}	0.89	0.0001
Acid Detergent Lignin	38.82^{b}	39.00^{a}	39.22^{a}	39.54^{a}	38.45^{b}	38.98^{a}	2.25	0.0001
Hemicelluloses	57.23^{c}	67.57^{b}	67.85^{a}	68.21^{a}	69.93^{a}	67.59^{b}	0.74	0.001
Cellulose	61.37^{d}	65.58^{a}	64.34^{b}	63.85^{c}	63.91^{c}	64.32^{b}	0.64	0.0025

Means with different superscripts along the same row are significantly different (p<0.05)
An = *Aspergillus niger*, Tv = *Trichoderma viride*, Rs = *Rhizopus stolonifer*, Mm = *Mucor mucedo*, RG2G = Roxazyme G2G

and 6) respectively. Digestibility of CP and CF, NDF, ADF and hemicellulose was significantly (p<0.05) increased in the dGNP diets compared with the uGNP and RG diets. But enzyme extract from *A. niger* and *M. mucedo* had no significant advantage over RG in the digestibility of CF. Digestibility of cellulose and ADL were not significantly affected at the starter phase (Table 5). Digestibility of CP, CF and fibre components were significantly higher in the diets containing dGNP than those containing uGNP and RG. Digestibility of the nutrients was higher at the finisher than starter phase due most probably to the ability of older birds to utilize fibrous feeds more efficiently than younger ones. Digesta viscosities were significantly (p<0.05) lower in the dGPH diets than in the uGNP and RG diets in the crop, gizzard, small and large intestines (Table 7).

Microorganisms including fungi, bacteria and actinomycetes constitute the natural sources of protein compounds that are used for the production of feed enzyme cocktails. The results of the present study showed that the fungi *A. niger, T. viride, R. stolonifer* and *M. mucedo*, were capable of producing an enzyme system with either cellulase or xylanase properties or both. The procedure described in the production of the enzyme extracts was based on the principle that cellulases and xylanases are inducible enzymes (Saloheimo *et al*., 1998) and that cellulose-rich material and xylan or xylan-rich material are the best carbon sources for the production of high levels of cellulases and xylanases by many microorganisms (Ryu and Mandels, 1980; Biely, 1993). The high CF (28.6%) of GPH indicates that its cellulose and hemicellulose carbon are a good substrate which elicited the production of the enzymes from the fungi. The treatment of GNP with the FEE produced a significant reduction in the CF and NSPs with a corresponding increase in the levels of soluble sugars and CP. Similar results have been reported by other workers. Bachtar (2005) reported increases in CP when *A. niger* was inoculated on sago fibre, cassava fibre and cocoa shell resulting in 16.5, 18.5 and 21.9% increases, respectively. Iyayi and Aderolu (2004) reported 31, 36 and 41% increases in CP in brewer's dried grain, maize offal and wheat offal, respectively after 14 days inoculation with *A. niger, A.*

Table 7: Viscosities of digesta (mPa.s) in broiler finishers on experimental diets

Parameters	Control	GNP+An	GNP+Tv	GNP+Rs	GNP+Mm	GNP+RG2G	SEM	P Value
Crop	4.30^{a}	3.50^{b}	3.44^{b}	3.66^{b}	3.86^{b}	4.00^{a}	0.012	0.0004
Gizzard	3.00^{a}	2.35^{b}	2.11^{c}	2.51^{b}	2.77^{ab}	2.81^{a}	0.031	0.0021
Large intestine	2.02^{a}	1.96^{a}	1.86^{b}	1.92^{a}	1.95^{a}	2.00^{a}	0.022	0.0001
Small intestine	2.55^{a}	1.54^{b}	1.53^{b}	1.54^{b}	1.55^{b}	2.30^{a}	0.015	0.0001

Means with different superscripts along the same row are significantly different ($p<0.05$)
An = *Aspergillus niger*, Tv = *Trichoderma viride*, Rs = *Rhizopus stolonifer*, Mm = *Mucor mucedo*, RG2G = Roxazyme G2G

flavus and *Penicillium sp*. The ability of fungal enzymes to reduce CF and increase the CP and soluble sugars in cassava peel, a fibrous agro by-product has also been reported (Iyayi and Lösel, 2000). There is usually the incorporation of some of the carbon atoms from the break down of the complex carbohydrate into single cell protein by the microbes with increase in CP in the substrate-enzyme mixture. The improvement in the ME content of the GNP was a direct result of the significant increases in the soluble sugars produced by FEE. Steenfeldt *et al*. (1988) and Oldale and Hoffman (1996) have reported higher ME values for enzyme supplemented diets and feed materials, respectively. These results point to the ability of fungi to produce enzyme complexes for the degradation of the cell wall carbohydrate in the GNP material. As is typical of most fungi, the aerobic fungi used in this study generally produced extracellular cellulases and hemicellulases because according to Groleau and Forsberg (1981), Gilbert and Hazelwood (1993) and Bhat and Bhat (1997), they produce these enzymes in form of multienzyme aggregated complexes, with xylanases and mannanases debranching enzymes as integral components of the complex. According to Gilbert and Hazelwood (1993), this is a characteristic of these species of fungi. The resultant improvement of the ME in the GNP was due to the reduction in the levels of the non Starch Polysaccharides (NSPs). According to Oldale and Hoffman (1996), added enzyme increased the ME of wheat offal as it led to an increase in the digestibility of cell wall components with an enhancement of starch digestibility.

Treatment of GNP with the FEEs resulted in significant reduction in viscosity. Similar results with *Trichoderma logibriachiatum* have been reported by Bedford (1995). The NSPs of fibrous agro by-products usually contain water soluble and insoluble fractions. The water fraction is virtually undigested in the bird's alimentary tract (Hasselman and Aman, 1986; Onilude and Oso, 1999). The soluble NSPs elicit anti-nutritive activities in poultry diets, which are closely related to their polymeric nature and ability to increase digesta viscosity. With high viscosity as reported in the uGNP, the ability of the gut to physically mix the contents is severely compromised (Edwards *et al*., 1988) leading to a situation of reduced nutrient digestion. There are indications that high digesta viscosity affects performance by altering gut-enterocyte turnover rates, endogenous-enzyme synthesis rates, microfloral and coccidial populations, and litter quality (Choct *et al*., 1995; Morgan and Bedford, 1995; Smithard and Silva, 1996). Enzymes used in viscous diets act according to Bedford (1995) by simply breaking up the structure of the soluble viscous gel by binding and cleaving both the endo (center) and exo (side) ends of the NSP structure. Results of the present study showed that the performance of the birds at both starter and finisher phases were better on the dGNP and RG diets than on the uGNP diets. Microbial enzyme supplementation of diets has been reported by various authors as capable of enhancing the nutrient digestibility in poultry and pigs. Dänicke *et al*. (1995) reported increased fat digestibility with enzyme supplementation in a tallow-based diet resulting in enhancement in broiler growth. Bedford (1995) reported increased ileal digestibility amino acids, energy and protein in wheat-based diets supplemented with xylanase. Beneficial effects on ileal digestibility of nutrients and enhanced performance in pigs have been reported by Li *et al*. (1996) and Omogbenigun *et al*. (2004). The reduction in viscosity and the increased nutrient digestibility reported in this study were responsible for the observed better performance of birds on the dGNP diets. It is of importance to note that phosphorus levels were significantly increased in the biodegraded GNP; an indication that the fungi used produced phytase. The efficacy of microbial phytase in enhancing P release from phytate complexes and P digestibility has been reported (Harper *et al*., 1997; Jendza *et al*., 2006; Pillai *et al*., 2006; Brana *et al*., 2006).

Conclusion: In conclusion, results of the study showed that multi-enzyme extracts can be obtained from fungi using a high fibre agro by-product such GNP as substrate. Treatment of GNP with such enzymes significantly enhanced its nutritive value by increasing the CP, soluble sugars, ME and P levels and reducing the CF, NSPs and viscosity. The apparent digestibility of nutrients was increased with the use of dGPH in the diets resulting in better performance of birds on such diets. Multi enzyme extracts from the fungi were more superior to RG due to the specific nature of RG as an enzyme product for wheat based diets compared to the FEEs which contained a broader spectrum of enzymes.

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