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Biodegradation of Palm Kernel Cake with Multienzyme Complexes from Fungi and its Feeding Value for Broilers

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Abstract: Palm Kernel Cake (PKC) was used as a substrate to elicit the production of polysaccharidases from *Aspergillus niger*, *Trichoderma viride*, *Rhizopus stolonifer* and *Mucor mucedo*. The extracted enzymes produced were purified and used to ferment PKC in solid state at the rate of 250 ml/kg of the material for 7 days. Unbiodegraded and enzyme degraded PKC were used to formulate broiler starter and finisher diets at the rates of 70 g kg^{-1} and 100 g kg^{-1}, respectively. A 6th diet was formulated in which Roxazyme G2G, a commercial enzyme was used to supplement the unbiodegraded PKC at the recommended inclusion level of 0.15 g kg^{-1}. A total of 360 1-d-old broiler chicks were randomly allocated to the 6 treatments of 6 replicates each with each replicates having 10 birds. Cellulose and hemicellulose were significantly ($p<0.05$) reduced in the biodegraded PKC compared with the unbiodegraded PKC and PKC supplemented with Roxazyme G2G. The level of soluble sugars increased in a similar trend. Crude protein, phosphorus and energy increased significantly ($p<0.05$) in the biodegraded PKC compared to that treated with Roxazyme G2G and the unbiodegraded PKC. Apparent digestibility of nutrients was significantly improved ($p<0.05$) in birds that received the diets based on the biodegraded PKC than those on the unbiodegraded PKC and Roxazyme G2G supplemented diets. Feed conversion and weight gain in birds were significantly ($p<0.05$) higher in birds on the diets based on the biodegraded PKC compared to those on diets based on the unbiodegraded PKC and Roxazyme supplemented diets. Results of the study showed that PKC can act as a substrate for the production of a multienzyme complex from the 4 fungi. The enzyme complexes so produced were more efficacious in breaking down the cellulose and hemicellulose in it compared to Roxazyme G2G which is an enzyme product specific for cereal-based diets.

Key words: Palm kernel cake, fungal enzymes, biodegradation, broiler, performance

INTRODUCTION

Increasing demand for conventional protein feed ingredients like soy bean by man and industry has continued to lead to increase in the cost of finished feeds for poultry in Nigeria. In addition, there are periods when maize and other conventional energy feed ingredients become scarce and at such times production schedules in many poultry farms suffer. The overall consequence of this is increase in cost of poultry products. It seems that the use of non-conventional alternative sources to these conventional ingredients will bring stability to the supply and cost of poultry feeds in the country. Palm Kernel Cake (PKC) is currently used at moderate levels in poultry feeds. Despite the considerable amounts of energy and protein present in it (Dusterhoff, 1993), its use in poultry feeding is limited because it is high in fibre (Iyayi and Aderolu, 2004). Another reason is that poultry birds do not have the full complement of endogenous enzyme that can break down the fibre made up of insoluble non Starch Polysaccharides (NSPs). Microbial enzymes have been reported (Bachtar, 2005) to be able to act on both the endo and exo sites of NSPs, breaking both the ß-1,4 and ß-1,6 linkages with the production of soluble carbohydrates. Multi enzyme complexes can be produced from fungi. Such enzyme extracts have the potential to breakdown the NSPs in PKC thereby enhancing its nutritive value for broiler feeding. Iyayi and Aderolu (2004) have reported the nutritional improvement of agro-industrial by-products including PKC by solid state fermentation with fungi for laying hens. In the presented study it was the objective to obtain purified enzyme extract from four fungi namely *Aspergillus niger, Trichoderma viride, Rhizopus stolonifer* and *Mucor mucedo*. A further objective was to investigate the efficacy of the enzyme extract to break down the complex carbohydrate fractions in PKC compared with a commercial enzyme (Roxazyme) used by poultry farmers and to investigate the performance of broilers on the PKC degraded and Roxazyme supplemented diets.

MATERIALS AND METHODS

Isolation of fungi and enzyme extraction: A. *niger, T. viride, R. stolonifer* and *M. mucedo* were obtained from the culture bank of the Department of Botany and

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Microbiology, University of Ibadan. A sterile wire loop was used to collect the spores and the mycelia of the actively growing fungi. The spores and mycelia were then inoculated on sterile Potato Dextrose Agar (PDA) in a lamina flow cabinet. The inoculated plates were incubated at 34°C in an incubator. After 48 h when growth of the fungi on the plates was fully established, their pure cultures were then obtained and the mycelium put on slants of sterile PDA.

Fifty grams of the milled PKC was autoclaved at 121°C for 15 min and then 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 34°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 PKC 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 crude enzyme was decanted and dialyzed using a magnetic stirrer against distilled water at 4°C for 12 h to obtain the purified enzyme (Fig. 1).

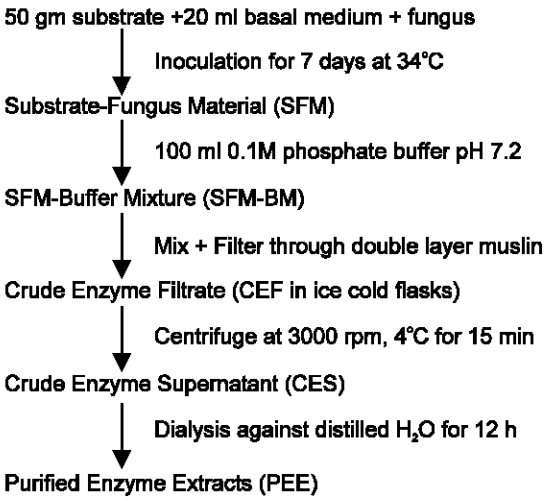

Fig. 1: Flow chart of production of enzyme from isolated fungus inoculated on substrate

Enzyme application on palm kernel cake, diet formulation and animal management: Palm kernel cake obtained from a local feed shop was autoclaved at 121°C for 15 min. The enzyme extracts were aseptically applied on the PKC at the rate of 250 ml/kg using a spry gun. The bags containing the PKC were then tightly sealed and allowed to stay for 7 days. At the end of d 7, the degraded PKC samples were oven dried at 60°C for 24 h to stop further action of the enzymes. The degraded and undegraded PKC were used to formulate diets for both starter and finisher broilers. In the basal diet (Diet 1), the undegraded PKC was incorporated in the starter and finisher diets at the rates of 70 and 100 g kg^{-1} respectively. In diets 2, 3, 4 and 5 PKC 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, as in the basal diets. In Diet 6 a commercial feed enzyme Roxazyme G2G (RG) used by poultry farmers in the country was incorporated in the starter and finisher diets at the rate of 0.15 g kg^{-1} (Table 3). The degraded PKC, undegraded PKC 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 360 1-day-old Ross chicks were obtained from Agritek Farms, Ibadan. They were weighed and distributed into 36 compartments in a standard poultry house. Each of the 6 dietary treatments was then randomly assigned to 6 compartments containing 10 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. On d 28 of the finisher phase, 5 birds were randomly selected from each of the replicates and killed by intraperitoneal injection of sodium pentobarbital. Their abdomens were immediately opened, the intestinal tracts cut open and the digesta in each part flushed with distilled water into containers and pooled on cage basis. The viscosity of the digesta was measured in centipoises according to the method of Steenfeldt *et al.* (1998).

In order to determine the apparent digestibility of nutrients in the birds, another set of day-old chicks consisting of 5 birds in each of 4 replicates were randomly assigned to each of the 6 starter and 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 replicate, stored in airtight containers and kept in a freezer until needed for analysis. Data collected were analyzed using the ANOVA procedure (SAS, 1999). Significant means were separated using the Duncan Multiple Range test (Steel and Torrie, 1980).

RESULTS

Results of proximate, NDF and ADF components in the degraded and undegraded PKC are presented in Table 1. There were significant ($p<0.05$) increases in the crude protein by 42.13, 43.49, 38.88, 35.6 and 32.7% when enzymes from *A. niger, T. viride, R. stolonifer, M. mucedo* and Roxazyme G2G respectively were used to degrade the PKC with the highest increase obtained with *T.viride*. The crude fibre content in the PKC was significantly ($p<0.05$) reduced by the fungal enzymes compared to the undegraded and Roxazyme G2G supplemented PKC. Enzyme extract from *A.niger* caused the highest reduction in the crude fibre from 20.21-11.32 g/100g representing 44.0% reduction. This was followed by *R. stolonifer, M. mucedo, T. viride* and Roxazyme G2G with 41.7, 39.0, 38.8 and 14.6%, respectively. There was a significant ($p<0.05$) increase in the ash and P contents and a significant ($p<0.05$) reduction in the NFE in the PKC treated with fungal enzymes. The highest value of 0.96 for phosphorus was obtained in the *R. stolonifer* degraded sample. The cellulose contents in the PKC significantly ($p<0.05$) decreased by 51, 30.6, 13.1, 14.3 and 11.0% with *A.niger, T.viride, R. stolonifer, M. mucedo* and Roxazyme G2G, respectively. Results of contents of soluble sugars in undegraded and biodegraded PKC are presented in Table 2. The contents of the soluble sugars in the PKC were significantly ($p<0.05$) increased when PKC was treated with the enzyme extracts and with Roxazyme G2G. *A.niger* produced the highest amounts of glucose, *T. viride* of galactose and sucrose and Roxazyme G2G of fructose. The results of performance and apparent nutrient digestibility in the birds at the starter phase are presented in (Table 4 and 5). The apparent nutrient digestibility, feed conversion ratio and body weight gain were significantly ($p<0.0$) better in birds on the degraded PKC diets than those on the undegraded PKC and RG2G diets. Apparent nutrient digestibility and weight gain at the finisher phase (Table 6 and 7, respectively) were significantly ($p<0.05$) higher in birds on the degraded PKC diets than those on the undegraded and RG2G diets.

DISCUSSION

Enzyme extracts from the fungi investigated were able to break down the cellulose and hemicellulose causing significant reductions in the contents of these NSPs in

Table 1: Proximate and detergent fibre of undegraded and degraded palm kernel cake (gDM)

Parameters	Undegraded PKC	PKC+An	PKC+Tv	PKC+Rs	PKC+Mm	PKC+ RG2G	SEM	p-value
Dry matter	88.43	88.9	90.0	88.8	88.2	88.3	3.41	
Crude protein	12.008^{c}	20.7^{a}	21.2^{a}	19.6^{ab}	18.6^{b}	17.8^{b}	1.85	0.001
Crude fibre	20.2^{a}	11.3^{c}	12.4^{c}	11.8^{c}	12.3^{c}	17.3^{b}	1.92	0.0001
Ether extract	3.97	4.07	3.99	4.26	4.32	3.99	0.20	0.30
Ash	13.9^{c}	18.9^{a}	19.6^{a}	19.8^{a}	19.3^{a}	14.1^{b}	2.41	0.32
NFE	49.9^{a}	45.0^{b}	42.8^{cd}	44.2^{c}	40.5^{d}	46.8^{b}	2.85	0.0002
Phosphorus	0.50^{c}	0.92^{a}	0.85^{ab}	0.96^{a}	0.77^{b}	0.71^{b}	0.04	0.0004
Pectin	13.3^{a}	5.08^{c}	4.86^{d}	3.96^{d}	5.28^{c}	7.88^{b}	0.35	0.0020
NDF	43.1^{a}	39.8^{b}	41.3^{bc}	31.9^{c}	40.3^{b}	42.8^{a}	2.22	0.0022
ADF	25.7^{a}	17.5^{d}	23.1^{b}	17.6^{d}	21.3^{c}	24.2^{a}	1.79	0.0024
ADL	15.5^{a}	9.37^{d}	11.5^{c}	3.09^{f}	7.63^{e}	13.2^{b}	0.41	0.0001
Hemicellulose	24.0^{a}	22.2^{b}	18.2^{c}	14.3^{d}	19.0^{c}	23.1^{a}	1.33	0.004
Cellulose	16.6^{a}	8.16^{c}	11.6^{c}	14.5^{b}	13.7^{d}	14.8^{b}	1.02	0.0016
ME (kcal/kg)	2129.08^{d}	2282.71^{b}	2273.48^{b}	2286.08^{b}	2470.54^{a}	2230.74^{c}	1.18	0.0067

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

Table 2: Levels of soluble sugars (µg/ml) in undegraded and degraded palm kernel cake

Soluble sugars	Undegraded PKC	PKC+Tv	PKC+Mm	PKC+ Rs	PKC+An	PKC+ RG2G	SEM	p-value
Glucose	197.54^{e}	957.76^{a}	352.41^{c}	334.21^{c}	654.59^{b}	245.36^{d}	0.07	0.001
Fructose	120.03^{d}	256.50^{a}	108.04^{b}	122.40^{b}	134.80^{b}	860.11^{c}	0.04	0.001
Galactose	100.34^{e}	872.71^{a}	210.50^{c}	420.30^{b}	760.80^{a}	140.02^{d}	0.01	0.0242
Sucrose	34.60^{e}	224.26^{a}	104.67^{c}	106.88^{c}	176.89^{b}	54.75^{d}	0.04	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 = *Mucur mucedo*, RG2G = Roxazyme G2G

Table 3: Gross composition (g kg^{-1}) of experimental diets

	Starter						Finisher					
Ingredients	Control	PKC +An	PKC +Tv	PKC +Rs	PKC +Mn	PKC +RG2G	Control	PKC +An	PKC +Tv	PKC +Rs	PKC +Mn	PKC +RG2G
Maize	570.0	560.0	560.0	560.0	560.0	560.0	570.0	560.0	560.0	560.0	560.0	560.0
Undegraded PKC	70.0	70.0	70.0	70.0	70.0	70.0	100.0	100.0	100.0	100.0	100.0	100.0
Degraded PKC	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Groundnut cake	133.0	110.0	110.0	110.0	110.0	110.4	100.9	110.3	110.3	110.3	110.3	112.9
Soyabean meal	160.0	193.0	193.0	193.0	193.0	192.5	162.1	162.7	162.7	162.7	162.7	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

Table 4: Performance of broiler starters fed diets containing undegraded and degraded palm kernel cake

Parameters	Control	PKC+An	PKC+Tv	PKC+Rs	PKC+Mm	PKC+ RG2G	SEM	p-value
Initial weight.(g)	35.10	34.89	35.00	34.70	34.91	35.21a	0.39	
Final weight. (g)	665.31^{e}	802.50^{a}	790.23^{a}	770.73^{b}	762.61^{c}	759.35^{d}	4.41	0.001
Wt. gained at 4th wk	630.21^{e}	767.61^{a}	755.23^{a}	736.03^{b}	727.70^{c}	724.14^{d}	2.05	0.0056
Ave. final feed intake (g/b)	1218.00^{d}	1365.00^{a}	1321.88^{b}	1302.28^{c}	1316.00^{c}	1282.21^{d}	6.15	0.0065
Daily feed intake (g)	43.50^{d}	48.75^{a}	47.21^{b}	46.51^{c}	47.00^{b}	45.52^{cd}	0.21	0.0015
Body weight gain (g)	22.50^{e}	27.41^{a}	26.97^{a}	26.30^{b}	25.99^{c}	25.11^{d}	0.50	0.0061
Feed conversion ratio	1.83^{a}	1.70^{b}	1.67^{c}	1.69^{b}	1.72^{b}	1.80^{a}	0.03	0.0022
Dry matter digestibility (%)	56.21^{c}	68.83^{a}	61.62^{b}	60.42^{b}	59.56^{b}	57.35^{c}	0.44	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

Table 5: Apparent nutrient digestibility (%) of nutrients in broiler starters fed degraded and undegraded PKC-based diets

Parameters	Control	PKC+An	PKC+Tv	PKC+Rs	PKC+Mm	PKC+RG2G	SEM	p-value
Dry matter	56.21^{d}	68.83^{a}	61.62^{b}	60.42^{b}	59.56^{b}	57.08^{c}	0.32	0.0014
Crude protein	72.22^{d}	87.21^{a}	85.62^{b}	85.00^{b}	80.80^{c}	78.82^{cd}	0.46	0.0008
Crude fibre	29.42^{c}	34.68^{b}	33.48^{b}	36.65^{a}	35.51^{a}	32.81^{c}	0.67	0.001
Ether extract	54.56^{d}	64.41^{b}	62.21^{a}	65.67^{a}	62.21^{c}	61.45^{c}	0.35	0.001
Ash	35.58^{d}	44.52^{a}	43.55^{b}	43.01^{b}	39.82^{c}	38.41^{c}	1.07	0.0001
Nitrogen free extract	65.65^{e}	76.65^{a}	75.21^{b}	75.00^{b}	73.56^{c}	70.32^{d}	0.81	0.0022
Neutral detergent fibre	53.24^{d}	54.22^{c}	56.69^{a}	53.82^{d}	55.73^{b}	54.48^{c}	1.02	0.0025
Acid detergent fibre	52.38^{e}	62.72^{c}	63.55^{b}	61.32^{c}	65.28^{a}	53.61^{d}	2.11	0.0032
Acid detergent lignin	27.75^{b}	28.44^{a}	28.31^{a}	29.01^{a}	28.95^{a}	29.92^{b}	1.10	0.0024
Hemicellulose	65.71^{d}	67.21^{b}	66.82^{c}	68.25^{a}	67.75^{b}	65.99^{d}	0.22	0.0041
Cellulose	60.35^{d}	63.68^{b}	61.76^{c}	64.42^{a}	63.31^{b}	61.39^{c}	1.56	0.0068

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

the PKC. This is an indication of the efficacy of these enzymes. Iyayi and Aderolu (2004) reported reduction in crude fibre content of brewer's dried grain, maize offal and wheat offal when A. *niger*, *A. flavus* and *Penicillium sp* were cultured on them. According to the authors, crude fibre contents in the Agro-industrial By-products (AIBs) were significantly (p<0.05) reduced by all the fungi after 14 days with *A niger* producing the highest reduction in crude fibre in all the AIBs followed by *A. flavus* and *Penicillium sp*. The highest percentage reduction by *A. niger* was achieved in wheat bran with 36.51% followed by brewer#s dried grain with 35.87% and maize bran with 35.80%. The ability of fungi to degrade crude fibre has also been reported by Ofuya and Nwajiuba (1990), Iyayi and Losel (200l). Earlier works of the latter showed successful degradation of cassava peel by *Rhizopus spp*. The authors reported that over 35% of the original cellulose content of the substrate was biodegraded in solid-state fermentation with the fungi. *A. niger* grown on rye-grass straw (Han and Anderson, 1975) produced similar results as obtained in this study. Hamlyn (1998) opined that fungi have the ability to produce a variety of enzymes. The author reported that cellulase, hemicellulase, pectinase and xylanases among other enzymes were produced by fungi, which helped to degrade the NSPs in the substrate.

After biodegradation of PKC with fungal enzymes, there was a significant improvement in the ME content of the PKC. These results agree with that of Steenfeldt *et al.*

Table 6: Performance of broiler finishers fed diets containing undegraded and degraded PKC-based diets

Parameters	Control	PKC+An	PKC+Tv	PKC+Rs	PKC+Mm	PKC+RG2G	SEM	p-value
Initial weight. (g)	665.31^{e}	802.50^{a}	790.23^{a}	770.73^{b}	762.61^{c}	759.50^{d}	0.65	0.0001
Final weight.(g)	1899.75^{d}	2281.78^{a}	2206.65^{a}	2158.81^{b}	2106.72^{b}	1996.56^{c}	3.42	0.001
Daily feed intake (g)	110.82^{d}	136.48^{a}	130.65^{b}	128.58^{b}	120.41^{c}	115.65^{cd}	2.67	0.0022
Body weight gain (g)	44.08^{d}	52.83^{a}	50.58^{b}	49.57^{b}	48.00^{c}	44.18^{d}	2.85	0.023
Feed conversion ratio	2.27	2.30	2.26	2.27	2.22	1.64	0.51	0.310
Final feed intake (g)	4320.96^{d}	5186.40^{a}	4980.08^{b}	4902.52^{d}	4670.48^{c}	3284.02^{cd}	15.60	0.0047
Weight gain at 8th week. (g)	1234.44^{c}	1479.28^{a}	1416.42^{a}	1388.08^{b}	1344.11^{b}	1237.06^{c}	8.32	0.0034
Dry Matter digestibility (%)	68.42^{c}	72.42^{a}	71.82^{a}	70.91^{b}	69.29^{b}	69.29^{b}	1.85	0.0004

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 7: Apparent nutrient digestibility (%) of nutrients in broiler finishers fed diets containing undegraded and degraded palm kernel cake

Parameters	Control	PKC+An	PKC+Tv	PKC+Rs	PKC+Mm	PKC+RG2G	SEM	p-value
Dry matter	68.42^{c}	72.42^{a}	71.82^{a}	70.91^{b}	70.36^{b}	69.29^{b}	0.38	0.0025
Crude protein	68.40^{e}	80.40^{a}	75.56^{b}	74.42^{c}	73.12^{c}	71.56^{d}	0.62	0.0001
Crude fibre	50.71^{d}	56.96^{b}	54.91^{c}	58.82^{a}	56.76^{b}	54.88^{c}	0.26	0.0034
Ether extract	63.85^{f}	72.21^{c}	75.70^{ab}	77.82^{a}	70.82^{d}	68.81^{e}	1.28	0.0002
Ash	55.32^{d}	66.71^{a}	65.92^{a}	65.00^{a}	62.42^{b}	60.00^{c}	0.91	0.005
Nitrogen free extract	70.35^{f}	90.02^{a}	88.81^{b}	85.53^{c}	82.65^{d}	80.46^{e}	2.06	0.0073
Neutral detergent fibre	58.11^{b}	59.82^{a}	59.55^{a}	59.34^{a}	58.98^{a}	59.10^{a}	1.82	0.0002
Acid detergent fibre	55.35^{b}	56.21^{a}	56.49^{a}	56.01^{a}	55.71^{ab}	55.62^{ab}	1.02	0.00031
Acid detergent lignin	32.86^{c}	34.56^{a}	34.21^{a}	33.65^{ab}	33.98^{ab}	33.10^{b}	0.44	0.0034
Hemicellulose	72.35^{c}	74.66^{a}	74.31^{a}	74.83^{a}	73.43^{b}	73.85^{b}	1.11	0.001
Cellulose	64.48^{c}	65.55^{a}	65.10^{a}	65.89^{a}	64.91^{b}	65.32^{a}	1.68	0.0005

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

(1988) who reported higher ME values for enzyme supplemented diets. Oldale and Hoffman (1996) also reported higher ME for enzyme supplemented feed materials. The cell contents of PKC are digestible but the cell walls consist primarily of cellulose and hemicellulose and they are poorly digested being also highly lignified (Felt-Well and Fox, 1978; Classen, 1996). From the result obtained in this study, the addition of enzyme has the ability to increase the nutritional value of the PKC. Adding exogenous enzymes to poultry diets according to Naveed *et al*. (1999) allows inexpensive ingredients of low nutritive value to be incorporated in such diets. Oldale and Hoffman (1996), reported that enzyme supplementation increased the ME of wheat offal as it led to an increase in the digestibility of starch and cell wall components. Cell wall containing β-glucans and arabinoxylans also act as physical barrier to endogenous enzymes of poultry and therefore reduce the utilization of starch and protein encapsulated within endospermal cells (Hesselman and Aman, 1986). Breakdown of β-glucan via enzyme addition results in increased digestibility of starch in the small intestine, which may result in more efficient starch utilization and hence increase metabolizable energy. The higher values of soluble sugars obtained in the biodegraded PKC agree with the observed decrease in the contents of crude fibre and NSPs. This correlation indicates that NSPs in PKC which are high molecular weight substances are broken down by the fungal enzymes into soluble sugars, which are easily absorbed. Iyayi and Aderolu (2004) have reported similar results of continuous increase in sugar production in the substrates until d 14. The increase in crude protein value of the degraded PKC was partly due to ability of the enzymes to increase the bioavailability of the protein hitherto encapsulated by the cell walls. According to Liu and Baidoo (2005), the fungal enzymes have the potentials of improving not only the NSPs but also of protein as well as other dietary components, such as fatty acids. Secretion of proteinase by fungi invariably increases the protein content of feed materials. Many workers have reported similar increase in protein content. In his work, Bachtar (2005) reported increase in crude protein when *A. niger* was inoculated on sago fibre and cassava fibre resulting into 16.5% and 18.5% protein increase respectively. The author reported a 21.9% increase in the protein of cocoa shell when inoculated with *A niger*. In their work, Iyayi and Aderolu (2004), reported increases in crude protein of 31, 36 and 41% with A. niger, 26, 33 and 38% with A. flavus and 27, 36 and 32% with *Penicillium* sp in brewer's dried grain, maize offal and wheat offal, respectively after 14 days of their biodegradation. Similar results have been reported by Ofuya and Nwajiuba (1990) when they cultured cassava peels with *Rhizopus* sp. The authors reported a 185% increase in the protein of the peels. Such high increase can be attributed to the fact that cassava peels are less fibrous than PKC. Results of other workers

(Smith *et al*., 1996; Balagopalan and Gregory, 1985; Mikani *et al*., 1982; Manilal *et al*., 1985) suggest the ability of fungi inoculated on low quality feed ingredients ti increase the protein levels in such ingredients by the conversion of the carbon atom of the broken down carbohydrates into mycelia protein.

The enhanced nutrient digestibility at both the starter and finisher phases were due to the breakdown of the NSPs in PKC. Iyayi and Aderolu (2004) have reported that PKC treated with fungi in solid state fermentation and fed to laying hens resulted in better egg production than hens fed undegraded PKC. Bedford *et al*. (1991) reported that high fibrous ingredients supplemented with enzymes improved digestibility of CP and AAs resulting in improved performance of broilers. Apart from the CP and AAs, Dänicke *et al*. (1995) also reported improved fat and energy digestibility in broilers. From the results of the present study, it is evident that phytase in the fungal enzymes was able to act on the PKC and therefore release the phytate bound phosphorus. The ability of microbial phytase to increase phosphorus availability in feed and enhance its digestibility has been reported by various workers (Pillai *et al*., 2006; Brana *et al*., 2006).

Conclusion: In conclusion, results of the study have shown that PKC can act as a substrate to elicit the production of multienzyme complex in fungi. The enzyme so produced when extracted, purified and used to ferment the PKC in solid state results in the degradation of the complex carbohydrate fractions in the PKC to increase its CP, energy and phosphorus contents. Feeding biodegraded PKC to broilers resulted in a significant increase in digestibility of nutrients, improved feed conversion and increased body weight gain in the birds.

REFERENCES

AOAC, 1995. Official methods of Analysis of Association of Analytical Chemist International (16th Edn.) Washington D.C.

Bachtar, B., 2005. Improvement of nutritive of crop by products using bioprocess technique and their uses for animals. J. Feed Technol., 21: 1-5.

Balagopalan, C. and K.F. Gregory, 1985. Fermentation of cassava starch for single cell protein production. Seminar on post harvest technology of cassava held in Trivandrum, Kerala, India.

Bedford, M.R., H.L. Classen and G.L. Campbell, 1991. The effect of pelleting, salt and pentosanase on the viscosity of intestinal contents of the performance of broilers fed rye. Poult. Sci., 70: 1571-1577.

Brana, D.V., M. Ellis, E.O. Castaneda, J.S. Sands and D.H. Baker, 2006. Effect of novel phytase on growth performance, bone ash and mineral digestibility in nursery and grower-finisher pigs. J. Anim. Sci., 84: 1839-1849.

Classen, H.L., 1996. General grain starch and exogenous enzymes in poultry diets. Anim. Feed Technol, 62: 21-27.

Dänicke, S., S. Simon, H. Jeroch and M.R. Bedford, 1995. Effect of fat and xylanase supplementation on the intestinal viscosity of rye-fed birds. In: van Hartingsveldt, W.M. Hessing, J.P. van der Lugt, W.A.C. Sommers (Eds) Proceeding of the Second European Symposium on Feed Enzymes, 25-27 October, Noordwijkerhout, Netherlands: TNO Nutrition and Food research Institute, Zeist Netherlands, pp: 102-107.

Dusterhoff, E.M., 1993. Characteristics and enzymic degradation of non-Starch polysaccharides in lignocellulosic by-products, A study on sunflower meal and palm kernel meal, pp: 93-97.

Felt-Well, R. and S. Fox, 1978. Fuel supplies in the concept of energy. J. Feed Technol., 10: 15-24.

Han, Y.W. and A.W. Anderson, 1975. Semi-solid fermentation of rye grass straw. Applied Microbial., 30: 930-934.

Hamlyn, P.F., 1998. Fungal biotechnology. British Mycological Society Newsletter, May 1998.

Hesselman, K. and P. Aman, 1986. Inhibition absorption by dietary fibre. J. Anim. Feed Technol., 15: 83-93.

Iyayi, E.A. and Z.A. Aderolu, 2004. Enhancement of the feeding value of some agro industrial by products for laying hens after their solid state fermentation with *Trichoderma viride*. Afr. J. Biotech., 3: 182-185.

Iyayi, E.A. and D.M. Lösel, 2001. Changes in carbohydrates fractions of cassava peel following fungal solid state fermentation. J. Food Technol. Afr., 6: 101-103.

Liu, Y.G. and S.K. Baidoo, 2005. Exogenous enzymes for Pigs diets: An overview. Proc. Of Asian conf. on Feed Biotechnol., pp: 67-70.

Mikani, Y., K.F. Gregory, W.L. Levadoux, C. Balagopalan and S.T. Whitewill, 1982. Factors affecting yield and safety of protein production from cassava by *Cephalosporium eichhorniae*. Applied Environ. Microbiol., 43: 403-411.

Manilal, V.B., C.S. Narayanan and C. Balagopalan, 1985. Amyloglucisidae and cellulose activity of *Aspergillus niger* in cassava starch factory wastes. In proceedings of the natural symposium and production and utilization of tropical crops held at Trivandrum Nov. 27-28, 1995, pp: 211-213.

Naveed, R.F.R., Durrani and M. Farooq, 1999. Broiler production status in district Chakwal. Unpublished report. Poultry Science Department NWFP, Agricultural University Peshwar, Pakistan.

Onilude, A.A. and B.A. Oso, 1999. Effect of fungi enzyme mixture supplementation of various dietary fibres contains diets fed broiler chicks 1 performance and carcass characteristic. World J. Microbiol. and Biotechnol., 15: 17-19.

Ofuya, C.O. and C.J. Nwajiuba, 1990. Microbial degradation and utilization of cassava peel. World J. Microbiol. Biotechnol., 6: 144-148.

Oldale, P.M.D. and F. Hoffman, 1996. Roche Nigeria seminar held at Sheraton Hotel, Lagos. 9th March. 1996.

Pillai, P.B., T. O'Connor-Dennie, C.M. Owens and J.L. Emmert, 2006. Efficacy of an *E. coli* phytase in broilers fed adequate or reduced phosphorus diets and its effect on carcass characteristics, Poult. Sci., 85: 1737-1745.

SAS Institute inc., 1999. SAS/STAT. SAS Institute Inc. Cary, NC USA.

Smith, J.P., A.J. Rinzema, H.M. Tramper, Van Sonsbeck and W. Krol, 1996. Fibre degradation and microbiology of anabolic zoosponiz fungi critical Reviews in Microbiol., 19: 99-115.

Somogyi, M., 1945. A new reagent for the determination of sugars, J. Biol. Chem., 160: 61-68.

Steel, R.G.D and J.H. Torrie, 1980. Principles and Procedures of statistics. Mc Graw-Hill Book Co; New York, NY.

Steenfeldt, S., A. Mullertz and J. Fins Jensen, 1988. Enzyme supplementation of wheat based diets for broilers (1). Effect on growth performance, intestinal viscosity AME and nutrient digestibility. Anim. Feed Sci. and Technol., 75: 27-64.

Van Soest, P.J. and R.W. Mc Queen, 1995. The chemistry and estimation of fibre. Proc. Nutr. Soc., 32: 123-130.