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Fungal biodegradation of plantain (Musa paradisiacal) peel through solid state fermentation for broiler finisher feeding: *In vitro* digestibility, performance, haematological and serum parameters

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ABSTRACT

The objective of this study was to investigate the changes in *in-vitro* dry matter digestibility (IVDMD) of plantain peel (PPL) after its biodegradation with *Aspergillus niger* and also to determine the effect of degraded PPL on the performance, nutrient digestibility, weights of internal organs , haematological and serum parameters of broiler finishers. A total of 165 unsexed broiler finishers were used. The design was Completely Randomized. *Aspergillus niger* was used for the biodegradation of PPL. There were five dietary treatments of 33 birds each. There were 0 % inclusion level of PPL, 7% inclusion level of undegraded PPL (UPPL) and 3,5 and 7% inclusion levels of degraded PPL (DPPL).Weight gained was significantly ($P<0.05$) higher with the birds fed degraded PPL. Feed conversion ratio was significantly ($P<0.05$) highest in birds placed on 7% UPPL (3.38). There were significant ($P<0.05$) differences in the digestibility of dry matter, crude protein and crude fibre. Crop, gizzard and abdominal fat were significantly ($P<0.05$) affected. The values of Packed Cell Volume (PCV), Total Protein, Cholesterol and Glucose were significantly ($P<0.05$) different in Haematological and serum biochemical parameters. Fungal biodegradation of PPL using *A.niger* has the potential of enhancing feed intake, nutrient digestibility and the body weight gain of broiler.

Introduction

Agro-industrial by-products in most developing countries vary from primary processing of farm produce wastes to wastes from agro allied industries. Some of these wastes are left unutilized, promoting environmental pollution and hazard. Those that are utilized are not maximally harnessed. Example of agro-industrial by-products that can be of good use in feeding animals is plantain peel. Plantain peel is a by-product from plantain (*Musa paradisiacal*). It is the left-over after plantain has been removed. Its inclusion in diets for monogastric animals is possibly limited by its fibrousness (Ezieshi *et al.* 2004). Ofuya and Nwajiuba (1990) observed that the residues of agro-industrial by-products still contains considerable amount of energy and protein which are present intracellularly in the fibrillian complex. The residue represents potential valuable and renewable resources which find application in various areas that include use as animal feed. Onilude and Oso (1999) reported that a waste (agricultural or industrial) refers to a material which at production has low content of protein or energy , low digestibility or bioavailability, low acceptability or palatability and or toxic. It may contain chemical and microbial contaminants and generally has low feeding or nutritional value. The use of fungi in biodegradation of AIBs has the potential of increasing productivity, efficiency and quality output in agro–industrial processing operations in many developing countries and it has the potential of reducing the cholesterol level which may be present in them. Onilude and Oso (1999) also observed that some of the commercial feeds in Nigeria lead to increase in lipids and cholesterol levels. White *et al.* (1973) opined that in humans, cholesterol is known as the cause for arteriosclerosis and it has the potential of leading to hepatic heart failure. However, Petterson and Aman (1989) reported that degraded dietary fibre fed to the birds reduced serum cholesterol concentration by maintaining a drain of the bile acid pool of the enterohepatic system. *Aspergillus niger* belongs to the family group generally described as black mould .It is capable of utilizing an enormous variety of substance for food because of the number of enzymes they produce. Iyayi and Losel (2000) stated that the use of solid state fermentation (SSF) for protein enrichment of lignocellulosic residues has been a focus of attention due to its direct application on the degraded product for animals as feed ingredients. The objective of this study was to investigate the ability of *A.niger* to biodegrade plantain peel and its subsequent utilization by broilers feeding.

Materials and Methods

Isolation of microorganisms

The isolation of *A.niger* was carried out on potatoes dextrose agar (PDA) using the samples obtained from decaying PPL. Samples of the fungus were suspended in 10ml of sterile distilled water and shake vigorously for 10 minutes. This was diluted with sterile water until a spore count of approximately $3X10^6$ per ml was obtained. Spore count was monitored by using the Hawksley Haemocytometer. Later, 1.0ml of the resulting liquid was spread on the surface of PDA and incubated at 37^0C

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for 7 days. The fungal isolates formed were subcultured to purity.

Solid-state fermentation (SSF) cultivation

The plantain peel used in this experiment was collected from plantain processing centre. The process of biodegradation, which lasted for 7 days, was carried out according to the method of Iyayi and Aderolu (2004) using the fungus *Aspergillus niger* .The PPL was autoclaved at 121^0C for 15 minutes after which it was allowed to cool down and then inoculated with the fungus culture and moistened with distilled water at the rate of 300 ml per kg of PPL. After a period of 7 days, the action of the fungus was stopped by oven drying the substrates at 80^0C for 24 hours. The dried material was then incorporated into the diets.

Chemical and data analyses

Undegraded and degraded PPL, feed and faecal samples were subjected to proximate analysis using the method of AOAC (1995).All data generated were subjected to one-way analysis of variance using the SAS statistical package (1999) and the treatment means of each variable were separated using the Duncan multiple range test (Steel and Torie ,1980).

Experimental Birds

One hundred and sixty five unsexed broiler chicks of Anak strain (1 day old) were used for this study. The chicks were reared together for 4 weeks and were placed on the same type of broiler diet. At the end of the 4th week, they were randomly divided into five groups of 33 birds and each group was assigned to one of the five dietary treatments in a Completely Randomized Design. Each group was further subdivided into three replicates and each replicate was kept on litter. The birds were fed from 4-8 weeks with formulated rations. Feed and water were provided *ad libtum* and uniform light was provided 24hours daily. Feed intake and body weight were recorded weekly. Vaccination programme were strictly followed .Feed consumption, weight gain and feed conversion ratio were used as measures of birds` performance.

Metabolic Studies

The determination of nutrient digestibility involved the collection of faecal samples from each of the replicate in a metabolic cage for four successive days during the 4th week of the experiment. Six birds per treatment (2birds /replicate) with close average weight were used for this purpose. The birds were allowed three days of acclimatization to the cage before faecal collection. Feed intake was measured during the same period of 3 days at 24 hours interval. The faecal sample for each replicate was weighed and placed in aluminum foil and dried in ovum to constant weight at 80 ^{0}C for 24 hours. The metabolizable energy (ME) of degraded and undegraded PPL, feeds and faecal samples were determined by the use of Pauzenga (9) method: ME=37x%CP+81.8x%Fat+35.5x%NFE

Blood sample collection

At the end of the 4th week, the birds were restricted from feed for 10hrs. Approximately 10 ml of fresh blood was collected. 5 ml for biochemical analysis while the other part was poured in bottle containing measured quantities of EDTA (anticoagulant for haematological analysis). Haematological parameters were determined as follows: Packed cell volume (PCV), red blood cell count (RBC), white blood cell (WBC) and haemoglobin were determined using Wintrobe microhaematrocrit and improved Neubauer haemocytometer and cynomethaemoglobin indices namely: mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) were derived as outlined by Jain (1986) .The serum metabolite protein ,total albumin, globulin, cholesterol and glucose were determined as described by Kaneko (1989).

Results

The results of the proximate and detergent fibre analysis of plantain peel before and after biodegradation are presented in table 1. *A. niger* was able to improve the crude protein content of PPL from 8.92 to 13.86% which is 35.64% increase. Besides, depolymerization of PPL led to reduction in crude fibre content. *A. niger* succeeded in reducing the crude fibre drastically from 14.81 to 10.27 which is 54.53% reduction. There was also improvement in Ash (6.47 to 7.95) which is 18.62% increase. The energy improved from 4.28 to 5.25kcal/kg. However, just like the crude fibre, the detergent fibre content also reduced after biodegradation of PPL with *A.niger*. The data for the performance and nutrient digestibility is shown in table 3. Data for average daily weight gain (ADWG) and average daily feed intake (ADFI) revealed significant ($P<0.05$) differences. The highest final feed intake value (3604.72g/b) was found in treatment with 7% UPPL while the highest value for ADWG was 46.39g/b and it was also found in treatment 5. There were significant ($P<0.05$) differences also in the feed conversion ratio (FCR) of the treatments and the highest value (3.38) was found in treatment with 7% Undegraded PPL (UPPL). There were no significant ($P>0.05$) differences in the digestibility of ether extract and ash. However, there were significant ($P<0.05$) differences in the digestibility of crude protein and crude fibre. Besides, crop, gizzard and abdominal fat in relative organ weights were significantly ($P<0.05$) affected as shown in table 4. Table 5 shows the data on the treatment means for some haematological and serum biochemical parameters investigated. White Blood Cell (WBC), Red Blood Cell (RBC), Mean Cell Haemoglobin (MCH), Mean Cell Volume (MCV) and Mean Corpuscular Haemoglobin Concentration (MCHC) were not significantly ($P>0.05$) affected by the dietary treatments.

Discussion

The proximate composition of the undegraded /degraded plantain peel and feed samples is shown in table 1. It was observed that the degraded PPL is higher in crude protein, ash, dry matter and nitrogen free extract but lower in crude fibre, NDF, cellulose, hemicellulose, ADF and NDL after degradation with *A.niger* The nutritional improvement observed in degraded PPL may be explained on the basis that the PPL served as the medium for metabolism and subsequent growth of the inoculated organism. Besides, the organism (*A.niger*) depolymerised the crude fibre and the detergent fibres therein and then converted the products to other useful components such as protein and other useful nutrients (Okon and Ogunmodede, 1996; Liu and Baidoo *et al*., 2005; Sabu *et al*., 2006).There was improvement in the Gross energy content of DPPL due to hydrolysis of the crude fibre into component disaccharides and monosaccharide which can be digested, made bioavailable and utilizable for energy liberation (Onilude and oso, 1999). Performance of the birds is shown in Table 3. There were no significant ($P>0.05$) differences in Average initial weights but there were significant ($P<0.05$) differences in the Average final weights, Average final feed intake, Feed conversion ratio and nutrients digestibility except for ether extract and Ash. The improvements observed in Feed conversion by birds placed on degraded PPL can be explained by the effect of fungal enzymic degradation conferred on target substrate. The birds fed degraded PPL had higher body weights probably because of the

biodegradation of the PPL which has led to the digest of the long chain carbohydrate molecules which bacteria often use to colonize the gastro intestinal tract. This shall indirectly alter the bacterial population of the different regions of the tract (Gunal and Yasar, 2004).This significantly reduces the microbial population and by implication increases the quantity of amino acids digested and available in the precaecal section of the tract since the microfloral population in the viscous fibre-based diets do affect digestibility of amino acids in the ileum (Iyayi and Davies, 2005). The decreased viscosity of the digested materials and the eventual release of nutrients via the enzymatic disruptions of cell wall aided the better growth of the birds placed on degraded PPL. Increase in feed consumption by birds on treated PPL may be due to production of vitamin B complex and flavour compounds as a result of fermentation (Sabu *et al*. 2006). The crop is the receptacle of the feed after it has been ingested. According to Aderemi et al. (1999), the weight of the crop increases when bulky feed is consumed by birds. The results obtained here is in line with the work of this author because the weights of the crops of birds placed on UPPL is higher than those placed on DPPL. The weight of the gizzards in the birds fed undegraded PPL is also higher probably due to more grinding work that the gizzard had to do (Ofuya and Nwajuiba 1990). Excess energy supplied to animal could be stored in form of fat. Feeding of UPPL like most of other fibrous agro-industrial by-products, can cause a dilution in feed energy content and this may explain why birds on diet 2 had highest abdominal fat. The haematological values show that the PCV of birds fed UPPL were low when compared with birds on DPPL. However, the values for haemoglobin and PCV were within normal range for normal broiler as reported by Mitruska and Rawnsley (1977). This implies that the birds fed UPPL and DPPL were not anaemic. The highest value of total protein observed in the birds fed 7% DPPL probably explains the enhanced growth of the birds. This high value of 8.21g/dl suggests good quality protein of this test diet since the higher the value of the total protein ,the better the quality of protein of the test feed stuff (Eggum, 1980). According to Abiola (2001), increase in urea value is an indication of poor protein quality, hence, from the results, treatment 5 (7%DPPL) gave the best value and the least was given by treatment 2 (7%UPPL). Onifade (1999) opined that, the higher the value of serum globulin, the better the ability to fight against diseases, hence the results show that treatment 5 has the highest ability to combat the body diseases. The increase in glucose level of birds fed UPPL could be due to inhibition of glycolysis by the presence of glycol-proteins and possibly other antinutritional factors which may have some adverse effect on regulation of insulin from pancreatic β-cells and on blood sugars. Cholesterol level was lower in the birds fed DPPL. This is in agreement with the findings of Onilude (1999). He reported that the cholesterol and Triglycerides consistently reduced in the blood of birds fed degraded fibrous feed. He adduced that the low cholesterol could be as a result of slight reduction in lipogenesis.

Conclusion

The use of solid state fermentation (SSF) to upgrade the nutritive value of PPL can enhance better performance of the broiler finisher birds. This is reflected in improvement in Crude protein, Ash and Gross energy of the PPL. It is also seen in feed consumption and body weight gained. The results from haematological and serum parameters also show that the performance of birds placed on DPPL is better than those placed on UPPL treatments.

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Table 1: proximate and Detergent fibre analysis of test ingredients and diets (g/100gDM)

UPPL=Undegraded plantain peel, DPPL= degraded plantain peel.

Parameters	UPPL	DPPL	DIET 1 0% PPL	DIET 2 7%UPPL	DIET 3 3%DPPL	DIET 4 5%DPPL	DIET 5 7%DPPL
%Dry matter	88.67	86.92	87.81	88.26	87.56	87.14	88.53
% Crude protein	8.92	13.86	18.11	18.23	18.38	18.27	18.14
% Crude fibre	14.81	10.27	3.81	3.41	3.85	3.53	3.16
% Ether extract	6.22	5.42	5.22	5.31	6.03	6.23	5.01
% Ash	6.47	7.95	17.65	17.73	18.20	17.89	18.22
(%)Nitrogen free extract	44.08	46.19	56.32	56.22	56.14	56.11	57.22
Gross energy (kcal/g)	4.28	5.25	3.68	3.51	3.22	3.61	3.76
Neutral detergent fibre	40.81	38.21	35.24	35.51	35.81	36.01	34.89
Cellulose	24.66	15.22	6.19	6.21	6.14	6.81	6.11
Hemicellulose	16.15	12.43	5.21	5.14	5.81	5.29	5.02
Acid detergent fibre	10.35	8.12	4.44	4.91	5.12	4.38	4.61
Neutral detergent lignin	14.31	11.61	8.18	8.71	8.22	8.42	8.43

Table 2: Gross composition of experimental diets for broiler finisher using degraded and undegraded plantain peel

INGREDIENTS	Diet 1 (0% PPL)	Diet 2 (7%UPPL)	Diet 3 (3% DPPL)	Diet 4 (5% DPPL)	Diet 5 (7% DPPL)
Maize	50.00	49.00	49.00	49.00	56.00
Degraded plantain peel	-	-	3.00	5.00	7.00
Undegraded plantain peel	-	7.00	-	-	-
Corn offal	6.30	10.80	9.30	6.30	5.30
Groundnut cake	9.00	8.50	9.00	9.00	11.00
Soyabean meal	21.00	22.00	21.00	22.00	22.00
Fish meal	3.00	3.00	3.00	3.00	3.00
Bone meal	2.00	2.00	2.00	2.00	2.00
Oyster shell	1.00	1.00	1.00	1.00	1.00
Premix (broiler)	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25
Lysine	0.10	0.10	0.10	0.10	0.10
Methionine	0.10	0.10	0.10	0.10	0.10
Total	100.00	100.00	100.00	100.00	100.00

Table 3: Performance of broiler finishers fed undegraded and degraded plantain peel

Parameters	(Control) containing	7% undegraded PPL	3% degraded PPL	5% degraded PPL	7% degraded PPL	SEM
Initial body weight(g/b)	410.00^{d}	390.00^{c}	480.00^{c}	510.00^{b}	560.00^{a}	10.15
Daily weight gained(g/b)	39.66^{d}	38.05^{d}	40.74^{c}	42.96^{b}	46.39^{a}	4.23
Final body weight(g/b)	1520.40^{d}	1455.44^{e}	1620.64^{c}	1712.76^{b}	1858.92^{a}	15.28
Weight gained at 4th week	1110.40^{c}	1065.44^{d}	1140.64^{b}	1202.76^{a}	1298.92^{a}	12.14
Daily feed intake (g/b)	115.77^{b}	128.74^{a}	110.41^{c}	106.53^{c}	102.99^{d}	5.16
Final feed intake (g/b)	3241.56^{b}	3604.72^{a}	3091.48^{c}	2982.84^{c}	2883.72^{d}	19.12
Feed conversion ratio	2.92^{ab}	3.38^{a}	2.71^{b}	2.48^{b}	2.22^{b}	0.46
Nutrients digestibility (%)						
Dry matter	80.11^{c}	83.61^{b}	83.78^{b}	85.00^{ab}	88.72^{a}	3.23
Crude protein	68.45^{b}	68.13^{b}	72.36^{c}	75.49^{a}	76.80^{a}	2.95
Crude fibre	41.24c	43.24^{b}	45.24^{a}	42.57^{b}	46.81^{a}	2.67
Ether extract	64.82	64.81	64.74	64.58	64.21	0.00
Ash	52.79	53.10	52.79	52.79	53.11	0.00

Table 4: Relative organ weights of broiler finishers fed degraded plantain peel and the undegraded plantain peel

Parameters	(Control) containing 0% PPL	7% undegraded PPL	3% degraded PPL	5% degraded PPL	7% degraded PPL	SEM
Crop (%)	0.81[b]	0.98[a]	0.78[c]	0.76[c]	0.76[c]	0.065
Proventiculus (%)	0.78	0.78	0.77	0.77	0.77	0.058
Gizzard (%)	2.80[b]	3.14[a]	2.65[c]	2.66[c]	2.65[c]	0.241
Small intestine weight (%)	2.51	2.50	2.50	2.51	2.52	0.310
Large intestine weight (%)	1.64	1.65	1.64	1.64	1.65	0.043
Caecum (%)	0.57	0.56	0.56	0.57	0.56	0.041
Heart (%)	0.48	0.48	0.47	0.47	0.47	0.033
Spleen (%)	0.10	0.10	0.11	0.10	0.11	0.021
Abdominal fat (%)	0.88[a]	0.89[a]	0.64[b]	0.65[b]	0.64[b]	0.045

Table 5: Haematological and serum biochemical parameters for broiler finishers fed undegraded and degraded plantain peel

Parameters	(Control) containing 0% PPL	7% undegraded PPL	3% degraded PPL	5% degraded PPL	7% degraded PPL	SEM
PCV (%)	28.11[b]	26.20[c]	29.14[b]	29.82[b]	20.51[a]	0.31
Haemoglobin (%)	20.44	20.50	20.32	21.54	21.60	0.043
RBC (%)	2.63	2.96	2.80	2.97	2.99	0.03
WBC($x10^3$/ µl)	25200	25985	25223	25431	25511	9.07
MCV(fl)	121.12	121.94	122.34	121.35	122.50	10.53
MCH(Pg)	42.11	41.54	42.36	42.45	42.01	0.46
MCHC (%)	33.14	33.19	32.46	32.51	32.22	1.38
Urea (ng/dl)	36.65[a]	31.38[b]	30.52[b]	25.11[b]	25.42[c]	1.22
Total Protein(g/dl)	6.32[c]	6.11[c]	6.54[c]	7.24[b]	8.21[a]	0.06
Albumin (g/dl)	2.81	2.85	2.80	2.82	2.80	0.09
Globulin(g/dl)	2.11[c]	2.12[c]	2.01[c]	2.92[b]	3.61[a]	0.04
Arginine(g/dm)	0.78	0.81	0.82	0.81	0.79	0.003
Cholesterol (g/dl)	2.14[a]	2.80[a]	1.14[b]	1.25[b]	1.31[b]	0.03
Triglyceride(mg/dl)	15.27[b]	17.85[a]	14.45[c]	11.87[d]	11.51[d]	0.05
Glucose(mg/dl)	130.23[b]	141.11[a]	125.32[c]	125.42[c]	126.91[c]	0.08

PCV=Packed cell volume, RBC=Red blood cell, WBC=White blood cell, MCV=Mean corpuscular volume, MCH=Mean corpuscular haemoglobin and MCHC=Mean corpuscular haemoglobin concentration.