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Performance, Nutrient Utilization, Haematological and Serum Indices of Layers Fed *Aspergillus Niger* Degraded Citrus Pulp

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ABSTRACT

The objective of this study was to determine the effect of *Aspergillus niger* (*A. niger*) degraded citrus pulp on the performance, nutrient utilization, haematological and serum biochemistry on layers. A total of two hundred and fifty two (252) laying birds that were thirty (30) weeks old were randomly allocated to the diets. Degraded and undegraded citrus pulps were used to formulate rations for laying birds for six weeks. There were seven treatments and treatment 1 was the control with 0% citrus pulp (CTP) inclusion level. The degraded and the undegraded CTP were used at 3, 5 and 7% inclusion levels. Thirty six birds were allocated to each of the diets with three (3) replicates at 12 birds each. *A. niger* mycelium was inoculated on milled citrus pulp by solid state fermentation method (SSF) for seven (7) days and the product was used as the degraded sample. The crude protein, gross energy, NFE and ash of the degraded citrus pulp rose from 14.10 to 16.14%, 2.88 to 3.79 Kcal/kg, 58.21to 61.31% and 4.61 to 5.11% respectively while the crude fibre content reduced from 8.15 to 6.41%. Result on performance of birds revealed that there were significant ($P<0.05$) differences in egg production and feed intake. The highest egg production (89%) was recorded by the birds placed on 7% inclusion level of degraded citrus pulp (DCTP) while the least value (84%) was found in birds placed on birds fed 7% undegraded CTP (UCTP). The highest feed consumption (4.8 kg/bird/week) was found in treatment with highest level of UCTP (7%). In egg quality parameter, it was observed that there were significant ($P>0.05$) differences in weight of egg, weight of yolk, albumen and shell thickness. In the haematological and serum biochemistry, it was observed that there were significant ($P<0.05$) differences in parked cell volume, mean corpuscular volume and total protein.

Introduction

In Nigeria and other Sub Saharan part of the world, the state of nutrition of the populace is predominantly characterized by inadequate protein intake in both quantity and quality. This shortage has given rise to high prices of animal protein. The high costs of egg, meat and meat products are mainly due to high costs of raw materials for feed industry especially maize. Consumption of too low protein is the major factor responsible for the occurrence of child/infant mortality, kwashiorkor, low level of productivity and short life span. The consumption of animal protein at present is 4.82g/caput/day (Aderemi et al, 2006) as against the minimum 35g recommendation of FAO (FAO 2001). Large scale egg production is one of the ways to ensure better availability of animal protein. The prices of conventional feed ingredients continue to rise unabated because of the competition between man and livestock and their increasing populations. The cost of feeds is as high as 70 to 75% of the total cost of production (Oluremi et al, 2006). In a bid to overcome this major constraint, several studies have been carried out on the utilization of agro industrial by products and crop residues. Hence the need to consider the identification and the possibility of using agro industrial by products (AIBs) one of which is citrus pulp. Agro industrial by products are secondary products derived from processing of agricultural products, i.e. the waste products which are got after farm produce are processed (Dafwang et al, 1996 and Salami, 2009). However, agro industrial by-products are characterized by low digestibility, low levels of nitrogen, protein and minerals. Therefore, to improve the digestibility and the nutritive value of these by-products, various processing methods have emerged, including biodegradation via solid state fermentation. Fungi can improve the protein; soluble sugars reduce the complex carbohydrates of these wastes. Citrus pulp is now mainly discarded as waste once the juice has been extracted from the orange. Industries producing citrus pulp have in time past incurred expenses for their proper disposal. It is a highly fermentable energy source with sweet taste and aroma.

Materials and methods

Solid State Fermentation of *citrus pulp*

Aspergillus niger was obtained from stock culture cultivated on Potato Dextrose Agar (PDA) slant at Microbiology Laboratory, Bowen University, Iwo, Osun State. A piece of mycelia of *A. niger* was subcultured on potato dextrose agar (PDA) in Petri dishes and incubated at 30^0C for 4 days under aseptic condition as described by Iyayi and Aderolu (2004).

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Citrus pulp as substrate

150g of milled citrus pulp was placed in each of four, 250ml conical flasks corked with cotton wool and sterilized at 121^0C for 15 minutes. Sterile water was added to raise the moisture of the meals as described by Ofuya and Nwajuiba (1990). About 10mm of innocula (*Aspergillus niger*) was used to inoculate each of the CTP containing flasks under aseptic condition and then incubated in the oven for 5 days at 30^0C. Each of these 150g samples were then poured into 10kg sterilized and moistened citrus pulp and mixed thoroughly. It was then incubated in the bigger bag for 7 days. The sample was oven dried after 7 days at 70^0C and the dried sample was then kept for the preparation of diets and laboratory analysis.

Experimental birds

A total of two hundred and fifty two (252) laying birds that were thirty (30) weeks old were randomly allocated to the diets. Degraded and undegraded citrus pulps were used to formulate rations for laying birds for six weeks. There were seven treatments and treatment 1 was the control with 0% citrus pulp (CTP) inclusion level. The degraded and the undegraded CTP were used at 3, 5 and 7% inclusion levels. Thirty six (36) birds were allocated to each of the diets with three (3) replicates at 12 birds each. Feed intake and body weights were recorded weekly. Vaccination programme were strictly followed. Isocaloric and isonitrogenous diets were formulated .All diets were supplemented with methionine and lysine to ensure that the requirements of the birds are met. The chemical composition of the undegraded and degraded CTP is shown in table 1while table 2 shows the gross composition of the experimental diets.

Metabolic Studies

At the end of the fifth week of the experiment, metabolic trial was carried out. The birds were offered known quantities of the respective experimental diets. Water and feed were offered *ad libitum.* Nine birds per treatment (3 birds per replicate) with close average weights were used for this purpose. The birds were allowed three days of adjustment to the cage before faecal collection. Feed intake was measured during the same period of 3 days at 24 hours interval. Faeces for each replicate were weighed and placed in aluminum foil and dried to constant weight at 80^0C. Proximate composition of the diets and faeces was analyzed by the methods of AOAC (1995).Apparent nutrient digestibility (%retention) was calculated using the following equation:

$$\frac{\text{Nutrient intake} - \text{nutrient output X100}}{\text{Nutrient intake}}$$

Haematological and Serum parameters

At the end of 6^{th} week, 10 ml of blood was collected from 3 birds in a replicate. 5ml for biochemical analysis while the other 5ml was poured in blood bottle containing measured quantities of Ethylene diamine tetra acetic acid (anticoagulant for haematological analysis).Haematological parameters were determined as follows: Packed cell volume (PCV), red blood counts (RBC), white blood cells (WBC) and haemoglobin were determined using Wintrobe's micro-haematocrit and improved Neubauer haematocytometer and Cynomethaemoglobin methods respectively. Erythrocytic indices, namely mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH) were derived as outlined by Jain (1986). Serum protein and albumin were determined as described by Kaneko (1989).

Data analysis

Data collected were analyzed statistically using the Analysis of Variance (ANOVA) technique of Steel and Torie (1980). Where statistical significant differences were observed , the treatment means were compared according to Statistical Analysis System (SAS, 1999).

Results and Discussion

Table 1 shows the chemical composition of undegraded and the degraded citrus pulp. The crude protein, gross energy, nitrogen free extract and ash increased from 14.10 to 16.40%, 2.88 to 3.79kcal/kg,58.21 to 61.31% and 4.61 to 5.11% respectively while the crude fibre content reduced from 8.15 to 6.41%. Ability of fungi to degrade crude fibre has also been reported by Ofuya and Nwanjimba (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 lost during the solid-state fermentation. Hamlyn (1998) opined that fungi have the ability to produce a variety of enzymes. The author reported that cellulase, hemicellulase, pectinase and xylanases among others were produced by fungi, which could degrade the non-starch polysaccharides (NSPs) in the substrate.

This result is in line with that of Lawal et al (2010). They reported higher ME values for fungi degraded AIBs. The cell contents of AlBs are digestible but for the cell walls which consist primarily of cellulose and hemicellulose and they are poorly digested also because they are highly lignified (Classen, 1996). This may explain the result obtained in the present study as low ME was recorded for undegraded CTP. From the result obtained in this study, fungal biodegradation has the ability to increase the nutritional value of CTP. The increase in crude protein value of the degraded CTP was partly due to ability of *A. niger* to increase the bioavailability of the protein hitherto encapsulated by the cell walls. According to Liu *et al* (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. In his work, Bachtar (2005) reported increase in crude protein when A. *niger* inoculated on sago fibre and cassava fibre resulting into 16.5% and 18.5% protein increase respectively. The author did it for cocoa shell and 21.9% increase in crude protein was recorded. According to Iyayi and Aderolu (2004), there was increase in protein because there was bioconversion of the sugar into mycelia protein. They reported increase in crude protein in brewer dried grain, maize offal and wheat offal after 14 days of biodegradation of the mentioned AIBs as 31%, 36% and 41% was obtained respectively with *A. niger*: 26%, 33% and 38% with *A. flavus* and 27%, 36% and 32% with *Penicillium* sp. The ability of fungi to reduce crude fiber component of AIBs has been reported by other workers (Iyayi and Aderolu, 2004; Iyayi and Losel, 2001; Lawal et al, 2010). Table 2 shows the proximate composition of the experimental diets. Diets were calculated to be Isocaloric and isonitrogenous. Table 4 shows the performance characteristics of birds fed varying levels of UCTP and DCTP. It was observed that there were significant ($P<0.05$) differences in egg production and feed intake, but there were no significant ($P>0.05$) differences in body weights and feed conversion ratio. The highest percentage (89%) of egg production was recorded for diet 4(7%DCTP). The least percentage (84%) was observed in treatments 7 containing 7% UCTP. These values confirm the values given by Oluyemi and Roberts (2000) that layers peaked at about 85% hen day production. Besides, the enrichment occasioned by degradation of CTP could be factor responsible for the better hen day production. Increase in feed consumption by birds on DCTP may be due to production of vitamin B complex and flavour compounds as a result of fermentation (Sabu et al, 2006). The feed intake decreased as the level of UCTP inclusion increased due to poor utilization of the UCTP and presence of higher level of dietary fibre in the diets. Other authors (Moges and Peter 2005; Eruvbertine and Oguntonna 1997) reported a decreasing daily feed intake as the level of undegraded cassava peel increases. Besides, there was decrease in the feed intake as the fibre content increased probably due to the gut fill effect. Lawal et al (2010) reported that fibrous feed leads to viscosity of feed materials in the GIT. This encourages decrease in the transit time of feed or digesta in the GIT. Table 5 shows the nutrients digestibility by birds fed degraded and undegraded CTP. There were significant ($P<0.05$) differences in digestibity of dry matter, crude fibre, ash and NFE. There were no significant ($P<0.05$) differences in the digestibility of ether extract . Significance of nutrients digestibility of birds fed DCTP from those fed UCTP shows that fermentation enhances utilization of CTP by layers. The results compares well with the results obtained from the control diet. The birds fed DCTP could digest and possibly utilize the CTP better. Improved digestibility could be attributed to degradation of cell wall of CTP resulting in better accessibility to intracellular nutrients. Also, direct utilization of hydrolysis products like glucose elimination of antinutritional factors; reduction of intestinal viscosity could be responsible for the better nutrient digestibility (Emiola et al, 2007; Gunal and Yasar, 2004; Sabu et al, 2006; Prakashan et al, 2006).The results indicate that digestibility of nutrients decreased with increased level of inclusion of UCTP. This is consistent with affirmation of several authors that increasing level of dietary fibre in the diet reduces nutrient utilization (Onilude and Oso, 1999; Lawal et al 2010; Aderemi et al, 1999). The increase in nutrients utilization as a result of increasing level of DCTP showed that fermentation enhanced digestibility and utilization of nutrients in the AIBs. Significant ($P<0.05$) differences were observed in the egg quality parameters. There were significant ($P<0.05$) differences in weights of eggs, weights of yolk and albumen and shell thickness. Increase in weights of eggs obtained from birds fed DCTP may be as a result of better availability, digestibility and utilization of nutrients by the birds. Shells obtained from eggs placed on DCTP were thicker. This shows better availability of minerals like calcium and phosphorus. In the work of Lawal (2007), it was reported that *A. niger* has the ability to release the enzyme phytase and this enzyme hydrolyses phytate thereby releasing the bound mineral or minerals. Phytate, like oxalates and tannins is an organic compound (myo-inositol hexaphosphate) which occurs in all plants and serves as the storage form of phosphorus in the living plant. Phytate is a potent chelator of minerals and thus its presence in a feed will strongly dictate the outcome of minerals associated with the molecule. Table 7 shows the haematological parameters while table 8 shows the serum biochemistry indices. In table 7, packed cell volume and mean corpuscular volume revealed significant ($P<0.05$) differences while significant ($P<0.05$) differences were observed in total protein in table 8. Onifade (1998) reported that blood examination is a good way of assessing the health status of animal as it plays a vital role in the physiological, nutritional and pathological status of an animal. Birds placed on dietary treatments 3, 4, 2 and 1 had the best values of packed cell volume (PCV) followed by treatments 5 and 6 while treatment 7 was worst. Treatment 7 also had the worst result for the mean corpuscular volume (MCV). This observation may suggest that the dietary treatment 7 mildly suppressed haemopoetic tissues with a resultant effect on the haematology of the affected birds. Total protein is composed of the albumin and globulin. Birds placed on dietary treatment 3 had the best values of total protein followed by treatments 4,2,1,5 and 6 were worst. This observation may suggest poor balance of protein biosynthesis and catabolism by the birds.

Conclusion

This investigation revealed that solid state fermentation had beneficial impact on the nutrient composition of the citrus pulp by converting non-metabolizable components to other useful products (protein and energy). Reduction in fibre content resulted in better utilization of degraded citrus pulp compared to undegraded CTP. Biodegradation has also aided nutrient digestibility and this positively reflected in the better egg production.

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Table1: Chemical Composition of Undegraded and Degraded Citrus Pulp (g/100gDM)

Composition	Undegraded Citrus pulp	Degraded Citrus Pulp
Dry Matter	89.24	94.17
Crude Protein	14.10	16.14
Ether Extracts	4.17	3.74
Crude Fibre	8.15	6.41
Ash	4.61	5.11
NFE	58.21	61.31
Calcium	3.21	4.14
Phosphorus	0.64	0.72
Gross Energy (Kcal/kg)	2.88	3.79

Table 2: The Gross Composition of the Experimental Diets

Ingredients	Control	3% DCTP	5% DCTP	7% DCTP	3% UCTP	5% UCTP	7% UCTP
Maize	41	38	36	35	39	27	36
Rice bran	30	30	28	26	31	29	27
GNC	10	10	10	10	10	10	10
Fish meal	2.6	2.6	2.6	2.6	2.6	2.6	2.6
Wheat offal	6	6	8	9	4	6	7
Undegraded CP	-	-	-	-	3	5	7
Degraded CP	-	3	5	7	-	-	-
Oyster shell	7.5	7.5	7.5	7.5	7.5	7.5	7.5
Bone meal	2.2	2.2	2.2 0.55	2.2	2.2	2.2	2.2
Premix	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Lysine	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Methionine	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Cal-Energy	2628.2	2639.4	2615.1	2618.2	2638.5	2635	2629
Cal-Protein	15.56	15.89	15.75	15.86	15.83	15.75	15.70

Premix Composition per kg

Vit. A - 4,000,000 IU, Vit. D_3 - 1, 200,000 IU, Vit.E - 3,200IU, Vit. K - 800mg, Vit. B_1 - 800mg, Vit.B- 2,200mg, Vit. B_6 - 480mg, Vit. B_{12} - 4,800mg , Biotin - 12mg, Niacin - 4,000mg, Panthothenic acid -2,800mg, Folic Acid -240mg, Choline Chloride - 2000, 000mg, Vit. C. - 4,000mg, Fe - 24,000mg, Mn - 32,000mg, Mg - 40,000mg
Cu - 3,200mg, Zn - 20,000mg, C - 180mg, Se - 40mg.

Table 3: Chemical Analysis Of The Experimetal Diets

Composition (%)	T_1 Control	T_2 3% DCTP	T_3 5% DCTP	T_4 7% DCTP	T_5 3% UCTP	T_6 5% UCTP	T_7 7% UCTP
Dry matter	90.46	90.11	91.00	91.02	90.10	89.76	89.12
Crude Protein	16.65	16.21	16.20	16.11	16.01	16.12	16.02
Ether Extract	4.08	4.22	3.14	3.98	4.21	3.14	4.22
Crude Fibre	7.41	7.02	0.65	6.44	7.23	7.81	7.64
Ash	10.92	9.81	10.41	10.24	9.40	9.81	9.75
NFE	51.4	52.85	54.60	54.25	53.25	52.91	51.49
Calcium	4.21	3.98	3.99	4.01	3.99	3.92	3.74
Phosphorus	0.67	0.66	0.66	0.64	0.70	0.58	0.61
Gross Energy Kcal/kg	3.14	3.12	3.42	3.21	3.41	3.22	3.14

Table 4: performance characteristics of the birds fed with undegraded and degraded citrus pulp

PARAMETERS	Control	3% DCTP	5% DCTP	7% DCTP	3% UCTP	5% UCTP	7% UCTP	SEM
Egg Production (%)	88.00^{a}	87.00^{a}	87.00^{ab}	89.00^{a}	87.00^{ab}	85.00^{b}	84.00^{b}	2.03
Feed intake (kg/bird/week)	4.65^{ab}	4.63^{ab}	4.72^{ab}	4.80^{a}	4.61^{b}	4.50^{b}	4.41^{c}	0.04
Feed intake (kg/bird/day)	0.66	0.66	0.66	0.69	0.69	0.67	0.69	0.005
Body wt (kg/ bird/week)	1.97	1.99	1.99	1.97	1.97	1.99	2.04	0.014
Mortality	0	0	0	0	0	0	0	0.00
Feed Conversion Ratio	2.60	2.33	2.32	2.46	2.40	2.37	2.37	

Means with different superscripts along the same row are significantly (P<0.05) different. DCTP=degraded citrus pulp and UCTP=undegraded citrus pulp

Table 5: Dry matter, crude protein, ash and nfe digestibility and ether extract retention of birds fed with undegraded and degraded citrus pulp

Composition (%)	Control	3% DCTP	5% DCTP	7% DCTP	3% UCTP	5% UCTP	7% UCTP	SEM
Dry matter	87.21^{a}	82.38^{c}	84.71^{b}	84.28^{b}	79.57^{cd}	76.16^{d}	76.24^{d}	1.20
Ether Extract	8.80	8.67	8.73	8.74	8.62	8.67	8.61	0.04
Crude Fibre	48.22^{b}	50.14^{a}	51.82^{a}	51.21^{a}	46.45^{c}	46.27^{c}	45.75^{c}	0.50
Ash	44.21^{a}	41.16^{b}	42.24^{ab}	44.56^{a}	42.71^{ab}	41.38^{b}	40.00^{b}	0.22
NFE	14.02^{c}	17.79^{b}	18.51^{b}	20.23^{a}	18.21^{b}	20.16^{a}	18.12^{b}	0.22

Means with different superscripts along the same row are significantly (P<0.05) different. DCP=degraded citrus pulp and UCP=undegraded citrus pulp

Table 6: Egg quality parameters

Composition (%)	Control	3% DCP	5% DCP	7% DCP	3% UCP	5% UCP	7% UCP	SEM
Weight of Egg (g)	51.42^{ab}	50.48^{b}	50.80^{b}	52.61^{a}	49.91^{ab}	47.63^{c}	47.20^{c}	3.10
Yolk Length (cm)	4.50	4.50	4.51	4.55	4.22	3.81	3.80	0.49
Wt of Yolk and Albumen (g)	49.47^{a}	48.60^{ab}	48.81^{ab}	50.61^{a}	48.06^{ab}	45.68^{b}	45.32^{b}	2.65
Wt of Yolk (g)	32.70	32.00	32.11	33.72	31.75	30.17	30.11	1.73
Wt of Albumen (g)	16.77	16.60	16.70	16.89	16.31	15.51	15.21	4.58
Shell thickness (mm)	0.34^{a}	0.31^{ab}	0.33^{a}	0.35^{a}	0.31^{ab}	0.3^{ab}	0.27^{c}	0.01
Albumen Percentage (%)	67.37	67.12	67.13	67.90	67.32	67.43	67.78	2.45
Yolk Percentage (%)	36.41	36.61	36.80	35.91	36.38	36.65	36.21	2.12

Means with different superscripts along the same row are significantly (P<0.05) different. DCTP=degraded citrus pulp and UCTP=undegraded citrus pulp

Table 7: Haematological indicies for layers fed undegraded and degraded citrus pulp

PARAMETERS	Control	3% DCTP	5% DCTP	7% DCTP	3% UCTP	5% UCTP	7% UCTP	SEM
PCV (%)	26.00^{a}	26.50^{a}	27.00^{a}	26.50^{a}	25.50^{ab}	25.60^{ab}	25.00^{b}	0.06
Haemoglobin (g/100ml)	7.63	7.83	7.34	7.50	7.85	8.05	7.73	0.03
Red Blood Cell (10^6/µl)	2.36	2.47	2.46	2.40	2.56	2.62	2.59	0.12
MCV (fl)	97.46^{a}	95.14^{b}	91.60^{c}	91.46^{c}	91.80^{c}	89.43^{d}	88.80^{d}	2.13
MCHC (gm/100ml)	33.17	33.32	31.23	33.33	33.40	34.25	33.61	0.47
MCH (pg)	32.33	31.70	29.84	31.30	30.66	30.73	29.85	2.21
Total WBC ($\times 10^3$/µl)	8.45	9.50	8.20	8.75	9.05	9.15	8.90	0.75

Means with different superscripts along the same row are significantly (P<0.05) different. DCTP=degraded citrus pulp and UCTP=undegraded citrus pulp

MCV= Mean Corpuscular Volume: PCV/RBC×10

MCHC= Mean Corpuscular Haemoglobin Concentration: Haemoglobin/PCV×100

MCH= Mean Corpuscular Haemoglobin: Haemoglobin/ RBC ×10

WBC= White Blood Cell

Table 8: Serum biochemistry indicies for layers fed undegraded and degraded citrus pulp

PARAMETERS	control	3% DCP	5% DCP	7% DCP	3% UCP	5% UCP	7% UCP	SEM
Total Protein (g/dl)	7.01[ab]	7.49[ab]	90.30[a]	8.01[ab]	6.63[b]	6.54[b]	5.24[c]	0.09
Albumin (g/dl)	2.41	3.02	2.04	2.70	2.06	2.54	2.86	0.27
Creatinine (mg/dl)	1.88	2.15	2.20	1.98	2.68	2.73	2.58	0.03
AST (I.µ/L)	33.59	37.13	37.13	49.51	45.09	40.66	37.13	2.74
ALT (I.µ/L)	8.15	7.57	7.38	7.81	8.85	9.57	10.00	1.07

Means with different superscripts along the same row are significantly ($P<0.05$) different. DCP=degraded citrus pulp and UCP=undegraded citrus pulp

AST= Aspertate aminotransferase

ALT= Alanine aminotransferase