

FAECAL CHARACTERISTICS OF MICROBE-FERMENTED CASSAVA TUBER WASTE OF DIFFERENT MIXING RATIOS AS FEED INGREDIENTS IN ISA BROWN LAYER'S FEED

*¹Kehinde-Olayanju, O.A., ²Awojobi, K.T., ³Aro, O.P., ²Aro, S.O., ²Azeez, A. A. and Oboh, G.³

¹Federal College of Agriculture, Akure, Nigeria.

²Department of Animal Production & Health, Federal University of Technology, Akure, Nigeria.

³Functional Foods & Nutraceutical Unit, Department of Biochemistry, Federal University of Technology, Akure, Nigeria.

*Corresponding author: tolulopekingsley1@gmail.com

Abstract

This study evaluated the nutritional modification of cassava wastes through *Aspergillus niger* (ATCC: 16404) fermentation and its faecal characteristics on ISA Brown Layers. Cassava peels and stumps were microbially fermented at three different mixing ratios for 5 days and incorporated into ISA Brown layers' diets. Dietary treatments (T1-T4) comprised the control and three different mixing ratios of microbe-fermented cassava stump (MFCST) and peel (MFCP): 0:0 (T1, the control), 66.67:33.33 (T2), 33.33:66.67 (T3) and 50:50 (T4), respectively. Two hundred point-of-lay ISA Brown layers were randomly allotted to the four dietary treatments with 10 birds per replicate and five replicates per treatment in a completely randomized experiment that lasted for six weeks. The faecal characteristics of the birds were taken as response criteria. Results showed that all faecal parameters like total faecal output, faecal dry matter, faecal moisture, volume, density and consistency did not differ significantly ($p > 0.05$) from the control. Faecal bacterial isolates showed that *Salmonella* spp., *Pseudomonas aureginosa*, *E. coli* and *Staphylococcus aureus* had 50%, 75%, 75% and 100% frequency of occurrence in the faecal samples of the birds fed T1-T4 while the fungal isolates showed that *Penicillium* spp., *Aspergillus niger* and *Aspergillus flavus* had 25%, 100% and 100% respectively. The fermented cassava tuber waste products were thus able to inhibit the faecal growth of *Penicillium* spp. than in the conventional feed which conversely was able to mitigate faecal bacterial growth better than the cassava tuber waste products. In conclusion, the results suggest that these fermented cassava tuber wastes as potential feed ingredients warrants further investigation of their mycotoxin mitigation capabilities.

Keywords: Cassava tuber wastes, Feed ingredient, Faecal parameters, Fermentation, Microbial growth.

INTRODUCTION

The bio-safety of the conventional and non-conventional livestock feed ingredients demands a holistic approach to all safety and quality control standards in animal nutrition and in the feed milling industries. The health and welfare of livestock could be compromised easily if adequate bio-safety measures are not put into consideration in the act of sourcing, collection, processing and value addition to these feed ingredients. One of the most affordable methods of adding value to our non-conventional livestock feed ingredients is through solid substrate microbial fermentation either naturally or through the use of candidate organisms (Aro and Aletor, 2012). Most of the attentions have been directed at nutritional enhancement of these agro-waste products while neglecting the health and welfare concern that could ensue from the use of these diverse microbial fermentation protocols. It becomes imperative to subject this emerging value added agro-waste products to rigorous bio-safety standards to ensure their wholesome qualification as tested livestock feed ingredients. This therefore calls for *in vivo* trials though animal experimentation to validate the possible nutritional and health benefits of these emerging alternative livestock feed resources. Two of such products derived from different mixing ratios of cassava peels and stumps are the focus of these research. The objective of this study is to evaluate the potentials of microbe-fermented cassava peels and stumps of different mixing ratios as alternative feed ingredients in poultry nutrition by investigating the faecal characteristics as well as the faecal microbial load in ISA Brown layers fed fermented cassava peels and stumps of different mixing ratios.

MATERIALS AND METHODS

Experimental Site and Materials

The experiment was conducted at the Layer's Unit of the Teaching and Research Farm (7°10'N, 5°05'E) of the Federal University of Technology, Akure (FUTA), Nigeria. Fresh cassava peels (CP) and cassava stumps (CS) were obtained from the Arable Crops and Processing Section of the Federal College of Agriculture, Akure, Ondo State. All microbial procedures, including inoculation and fermentation, were performed under sterile conditions at the Department of Animal Production and Health Laboratory, FUTA.

Production of Microbe-Fermented Cassava Wastes

The microbially fermented cassava tuber wastes were produced following the method described by Aro and Alerto (2012). Fresh cassava peels and stumps were sun-dried for 3-5 days depending on environmental

temperature and solar intensity. The dried materials were crushed, packed (in polythene sacks) and stored in a cool place prior to microbial inoculation.

Experimental Animals, Experimental Design and Animal management.

Two hundred (200) point-of-lay ISA brown layer chickens were used for the study. The birds were housed in a battery cage system, with each cage (47cm/34cm/37cm in Length, Width and Height respectively) accommodating 3-4 birds. The birds were randomly allotted to four treatment diets of different mixing ratios of microbe-fermented cassava stumps (MFCST) and peels (MFCP) i.e. 0 MFCST:0 MFCP (T1, the control), 66.67 MFCST:33.33 MFCP (T2), 33.33 MFCST:66.67MFCP(T2) and 50 MFCST:50 MFCP (T4), respectively with 10 birds per replicate and five replicates per treatment in a completely randomized design. Standard management practices like vaccination and medication were observed. Feed and drinking water were served as required throughout the experimental period of six (6) weeks. The gross composition of the experimental diets is presented in Table 1.

Table 1: Gross composition of the experimental diets

Ingredients	T1	T2	T3	T4
Maize	46.50	9.30	9.30	9.30
2/3 CST + 1/3 CP	0.00	37.20	0.00	0.00
1/3 CST + 2/3 CP	0.00	0.00	37.20	0.00
1/2 CST +1/2 CST	0.00	0.00	0.00	37.20
SBM	7.00	7.00	7.00	7.00
GNC	9.00	9.00	9.00	9.00
Wheat offal	24.50	24.50	24.50	24.50
Bone meal	6.00	6.00	6.00	6.00
DCP	3.00	3.00	3.00	3.00
Fish meal	2.00	2.00	2.00	2.00
Methionine	0.50	0.50	0.50	0.50
Lysine	0.50	0.50	0.50	0.50
Salt	0.50	0.50	0.50	0.50
Layers premix	0.50	0.50	0.50	0.50
Total	100.00	100.00	100.00	100.00
Calculated content:				
Crude protein (%)	20.39	19.69	19.00	19.19
M. E (Kcal/kg)	2781.65	2750.00	2780.00	2750.00
Lysine	1.52	1.45	1.45	1.45
Methionine	0.82	0.77	0.77	0.77
Calcium (%)	1.24	1.23	1.23	1.23
Phosphorus	0.95	0.86	0.86	0.86

CST=Cassava stump; CP= Cassava peel; SBM= Soya bean meal; DCP= Di-calcium phosphate; GNC= Groundnut cake; T1 =Control diet; T2= diet supplemented with 2/3Cassavastumpand1/3cassava peels; T3 = Diet supplemented with 1/3cassavastumpand2/3cassava peels; T4= Diet supplemented with 1/2 cassava stump and 1/2 cassava peels.

Assessment of Faecal Characteristics

Faecal samples were analysed for weight/output, dry matter, moisture content and faecal consistency following standard procedures (Standard procedure, year) while faecal volume and density determined through the procedure described by Aro and Alerto (2012).

Isolation of Faecal Microbes.

Faecal microbial evaluation was conducted using standard isolation procedures for both bacterial and fungal populations. Bacterial characterization was performed following the procedure of Aisyah *et al.* (2024), including gram staining, catalase test, indole production test and sugar fermentation tests.

Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA) using SPSS version 23.0. Differences between treatment means were separated using Duncan's Multiple Range Test.

RESULT

Faecal evaluation and microbial population parameters of Isa brown layers fed microbe-fermented CTW diets.

Faecal evaluation and microbial population parameters of Isa brown layers fed microbe-fermented CTW diets are presented in Table 2. The parameters evaluated were not significantly ($p>0.05$) influenced by the treatments.

Bacterial isolates from the faecal samples of Isa brown layers fed microbe-fermented CTW (Cassava tuber waste) diets

The distribution of the bacterial isolates from the faecal samples of the experimental animals is as shown in Table 3. Five bacteria species were isolated from the ISA Brown faecal samples. The control treatment had the least bacterial biodiversity as only 2 bacteria species were isolated from it while both T3 and T4 had the highest bacteria biodiversity with 5 bacterial species isolated from them.

Fungal isolates from the faecal samples of Isa brown layers fed microbe-fermented CTW (Cassava tuber waste) diets

The Fungal species isolated from the faecal samples of ISA Brown layers fed microbe-fermented CTW (Cassava tuber waste) diets is presented in Table 4. Three different fungi were isolated from the faecal samples with the control diets having the largest fungal biodiversity (3 of the 3 fungal isolates), 2 fungi each from T2, T3 and T4 diets.

Table 2: Faecal evaluation and microbial population parameters of Isa brown layers fed microbe-fermented CTW (Cassava tuber waste) diets

Faecal Parameters	T1	T2	T3	T4	±SEM	p-value
Output (g)	645.30	744.10	695.70	786.67	25.78	0.254
Dry matter (g)	231.03	257.23	209.97	218.60	12.95	0.659
% dry matter	35.30	34.30	30.28	27.27	1.37	0.170
% moisture	64.70	65.70	69.72	72.73	0.42	0.738
Volume (cm ³)	44.67	49.33	46.33	45.50	0.91	0.320
Density (gcm ⁻³)	1.12	1.01	1.08	1.11	0.02	0.325
Consistency	1	0	2	1	0.03	0.488
Fungi (x 10 ⁶ CFU/ml)	12.67	9.67	3.33	21.67	3.55	0.364
Bacteria (x 10 ⁶ 10 ⁶ CFU/ml)	17.67	14.67	19.00	26.00	3.41	0.746

SEM = Standard error of mean; CFU = Colony forming unit.

T1 = control diet; T2 = 2/3 cassava stump 1/3 cassava peel; T3 = 2/3 cassava peel 1/3 cassava stump; T4 = 1/2 cassava stump 1/2 cassava peel.

Table 3: Bacterial isolates from the faecal samples of Isa brown layers fed microbe-fermented CTW (Cassava tuber waste) diets

Isolates	T1	T2	T3	T4	Freq	% occurrence
<i>Salmonella spp.</i>	-	-	+	+	2	50.00
<i>Pseudomonas aureginosa</i>	-	+	+	+	3	75.00
<i>E.coli</i>	-	+	+	+	3	75.00
<i>Staphylococcus aureus</i>	+	+	+	+	4	100.00
<i>Streptococcus faecalis</i>	+	+	+	+	4	100.00
Total isolate	2	4	5	5		

Freq = frequency, (-) = isolate absent, (+) = isolate present

T1 = control; T2 = 2/3 cassava stump 1/3 cassava peel; T3 = 2/3 cassava peel 1/3 cassava stump; T4 = 1/2 cassava stump 1/2 cassava peel.

Table 4: Fungal species isolated from the faecal samples of Isa brown layers fed microbe-fermented CTW (Cassava tuber waste) diets

Isolates	C-CHA	T1	T2	T3	T4	Fre	% occurrence
<i>Penicillium spp</i>	Blue mould growth	+	-	-	-	1	25.00
<i>Aspergillus niger</i>	Black mycelia growth	+	+	+	+	4	100.00
<i>Aspergillus flavus</i>	Yellow mycelia growth	+	+	+	+	4	100.00
<i>Total organism</i>		3	2	2	2		

C-CHA = Cultural characteristics, (-) = isolate absent, (+) = isolate present, Freq = Frequency; T1 = control; T2 = 2/3 cassava stump 1/3 cassava peel; T3 = 2/3 cassava peel 1/3 cassava stump; T4 = 1/2 cassava stump 1/2 cassava peel.

Discussion

Faecal evaluation and microbial population parameters of Isa brown layers fed microbe-fermented CTW (Cassava tuber waste) diets

The increased faecal output in birds fed T4 might be related to the higher intake of cassava tuber waste or changes in the gut processing efficiency due to dietary fermentation. This suggests that T4 may have a higher fibre content or different digestibility compared to other treatments. Fermentation using lactic acid bacteria, such as *Lactobacillus delbrueckii* and *Lactobacillus coryneformis*, in combination with the fungus *Aspergillus fumigatus*, has been shown to enhance colonic faecal volume. This increase in volume expands the surface area of the digesta, thereby facilitating greater microbial degradation (Aro *et al.*, 2017).

Fungal species isolated from the faecal samples of Isa brown layers fed microbe-fermented CTW (Cassava tuber waste) diets

The dietary treatment with the highest gastro-intestinal fungal biodiversity was the control treatment where three different fungi were isolated from the faecal samples of Isa brown layers fed microbe-fermented CTW (Cassava tuber waste) diets. The presence of *Penicillium spp.* solely in the control group indicates that the introduction of microbe-fermented cassava tuber waste (in T2–T4) may have inhibited the growth of this fungus. This suggests that the fermentation process or the cassava waste itself could reduce the proliferation of certain moulds like *Penicillium* in the birds' digestive tracts. Notably, the fungal population across treatments was relatively low compared to the bacterial population. This difference may be due to competitive exclusion of fungi by bacteria, as bacteria can out-compete fungi for substrates, produce antimicrobial metabolites that inhibit pathogens, and compete for attachment sites in the gastrointestinal tract (Aro *et al.*, 2017).

CONCLUSION

Though incorporation into ISA Brown layer diets showed no negative impact on faecal characteristics, the high fungal counts observed across treatments highlight the need for mycotoxin control measures. These findings suggest that while fermented cassava waste holds promise as a sustainable feed ingredient, addressing safety concerns is essential for its successful inclusion in poultry nutrition.

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