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Performance, storability and microbiological assay of pelletized and un-pelletized cassava based diets fed to Muturu calves

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This study was carried out to determine performance, digestibility, storability and microbiological assay of pelletized and un-pelletized cassava based diets. Four experimental diets were used as follows: T_1, T_3 contained 0 g/kg foliage with 600 g/kg peels each while T_2 and T_4 contained 200 g/kg foliage with 400 g/kg peels. T_1, T_2 were pelletized, T_3, T_4 un-pelletized. Other ingredients (g/kg) that made up each of the treatments were as follows: Cassava sieviate, 205; molasses, 100; sulphur, 3; corn bran, 80; salt, 7; vitamins/minerals Premix, 5. Twelve (12) Muturu calves were fed the experimental diets for 12 weeks to monitor feed intake, digestibility and haematological indices. Media used were *Salmonella shigella* agar, potato dextrose agar, manitol salt agar and eosine methylene blue agar. Samples (1 g) were taken at intervals of 0, 21, 42 and 63 days for microbial analysis. Data generated were subjected to one way analysis of variance using completely randomized design (CRD). There were significant ($P<0.05$) differences in dry matter (DM) intake, body weight changes, nutrient digestibility and haematological analysis. No visible colour change, caking and mould growth was observed. Most of the bacteria and fungi isolated (*Staphylococcus aureus, Enterobacter* spp., *Mucour* spp., *Aspergillus* spp.) were persistent throughout the storage period. There was no significant ($P>0.05$) difference between microbial profile and total viable counts of the diets forms. Pelletizing had no effect on spoilage compared to the microbial load with that of un-pelletized feed form. Thus, feeding animals with the un-pelletized feed either readily or after storage is recommended as the cost of pelletizing increased total cost of production.

Key words: Cattle, cassava, pelletizing, storability, microbes.

INTRODUCTION

A major constraint to smallholder ruminant production in tropical Africa is the un-availability of good quality feed or forage throughout the year. Therefore, the use of various agro-industrial by-products is being promoted to fill the gap during the dry season and reduce the competition between man and animals' grains as feed. Nigeria is the highest producer of cassava in the world (FAO, 2011). With the pursuance of the millennium development

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goals, the country will boast of a further stimulation in the production of this commodity. Cassava leaves, peels, as well as other by-products which are produced in commercial quantities and regarded as wastes, can be useful and cheap sources of feed for livestock. According to Alli-Balogun et al. (2003), cassava foliage contains 91.25% dry matter (DM), 18.55% crude protein (*CP)*, 31.41% neutral detergent fibre (NDF), 29.3% Ash and 14.14% Lignin.

In order to explore the benefits from cassava as ruminant feed resources, as well as increase the use of cassava tops and also to reduce the effects of hydrocyannic acid (HCN), different methods can be used for preservation, for example sun drying could be exploited. Previous studies (Oduguwa et al., 2007) have also shown that cassava foliage with low concentration of HCN can be produced by ensiling.

Some of the objectives for processing cassava for livestock feeding include extending the shelf life of the crop for storage, improving the acceptability and palatability. It is also to maximize nutritive value and reduce toxins and contaminants. Sun-drying is the cheapest means of preserving non-conventional feedstuffs like cassava and its products for animal feeding; however, prolonged drying due to bad weather creates favourable conditions for bacteria and fungi build up producing moulds and mycotoxins. A not well adopted means of preservation of cassava for animal feed in the developing countries is pelleting. Pelleting confers the advantage of reduction in dustiness of cassava products. Shelf life in room temperature for flour is 4 to 5 months, while chips can be stored for up to 8 months. Pelleted products last for between 8 and 12 months, depending on if they are soft or hard pellets (Ravindran, 1993).

Thus, it has been documented that pelletizing of certain feed raw-materials will enhance both palatability and storability of such feedstuffs. Although an additional cost is required for this aspect of processing, it may be worthwhile and necessary to produce safe and wholesome diets as this will enhance performance of the animals through reduction in the incidence of diseases due to contamination with microbes and other toxins. The microbial contamination of other cassava products such as *gari*, *fufu* and *lafun* has been reported by researchers (Obadina et al., 2007, 2009). However, pelletizing of the aforementioned cassava based diets have not been reported. Thus, this study was designed to investigate the effect of pelletizing and sun-drying on the performance, digestibility, storability and microbiological assay of cassava based diets for Muturu calves.

MATERIALS AND METHODS

Experimental site

The experiment was carried out at the Teaching and Research Farm Directorate (TREFAD) and Microbiology laboratory in the Department of Microbiology, College of Natural Sciences, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria.(7°15'N,3° 25'E) during the early- rainy season in southern Nigeria.

Sourcing for materials

Cassava (mixed varieties) leaves and peels used for the experiment were collected from the TREFAD farms and other neighbouring farms located around the University. Molasses was obtained from Dangote Sugar Industries, Apapa, Lagos, Nigeria while other materials (cassava sieviate, corn bran, salt, sulphur, vitamin/mineral premix) were obtained from the reputable sources.

Performance evaluation

Twelve (12) weaner Muturu calves were used for the experiment. The animals were dewormed shortly before the commencement of feeding trial and were adapted for a period of 4 weeks during which they were fed freshly cut and wilted *Panicum maximum* and concentrate supplements with gradual withdrawal of the grass. At the end of the adaptation period, the animals were divided into four dietary treatments of three calves per treatment, and balanced for body weight. The animals were fed at 5% of their body weight.
Four (4) experimental diets were used. The dietary treatments were as follows:

Diet 1 (T_1): 0% cassava foliage and 60% cassava peel,
Diet 2 (T_2): 20% cassava foliage and 40% cassava peel,
Diet 3 (T_3): 0% cassava foliage and 60% cassava peel,
Diet 4 (T_4): 20% cassava foliage and 40% cassava peel.

Diets 1 and 2 were pelletized.

Other ingredients (g/kg) that made up each of the treatments were as follows: Cassava sieviate, 205; molasses, 100; sulphur, 3; corn bran, 80; salt, 7; vit./min. Premix, 5 (Table 1). Each animal was treated as a replicate. The animals were weighed before and at the end of the experiment.

Chemical analysis

Feed samples, and faeces were milled through a 1 mm sieve in a laboratory mill. Prior to milling, samples were oven-dried at 60°C for 96 h while dry matter (DM) was determined by oven-drying 2 gm of respective samples at 100°C for 24 h. Samples were mixed separately and sub-sampled for further analyses.

Chemical analyses were done according to the standard methods of AOAC (1995) and these included, ash (ID; 942.05), crude protein (CP; ID 984.13), ether extract (EE; ID 963.15), crude fibre (Foss fibertec 3428), neutral detergent fibre (NDF), acid detergent fibre (ADF; including ash; ID 973.18) and acid detergent lignin (ADL). NDF and ADL (by solubilization of cellulose with sulphuric acid) were determined by the methods of Van Soest and Robertson (1985). NDF was analysed using sodium sulphite and amylase and expressed with residual ash. Gross energy (mJ/kg) composition of diets and faecal samples were determined using a bomb calorimeter (Adiabatic bomb, Parr Instrument Co., Moline, IL, USA).

Collection of blood samples

Blood collection was carried out at the 8th week of the experiment. There were three calves per treatment in which a total of 12 calves were bled via the jugular vein. The blood samples were collected into the sample bottles containing ethylene diamine tetra- acetic acid (EDTA).

Table 1. Gross composition of pelletized and unpelletized cassava based diets fed to Muturu calves (%).

Parameter	Pelletized		Unpelletized	
Feed component (%)	T_1	T_2	T_3	T_4
Dried cassava foliage	0	40	0	40
Dried cassava peels	60	20	60	20
Cassava sievate	20.5	20.5	20.5	20.5
Corn bran	8	8	8	8
Molasses	10	10	10	10
Common salt	0.7	0.7	0.7	0.7
Sulphur	0.3	0.3	0.3	0.3
Vit/Min. premix	0.5	0.5	0.5	0.5
Total	100	100	100	100

Cost of pelleting ₦2, 300 naira per 100 kg feed; 1 USD = ₦150.

Storability studies

Preparation of media used

Media used were *Salmonella shigella* agar, potato dextrose agar, Manihot salt agar and eosine methylene blue agar. They were weighed following the manufacturer's description and dispensed into conical flasks (250 ml). Distilled water was added to the media and then placed into the water bath so as to dissolve easily after which the conical flasks were cotton plugged and then autoclaved for 15 min at 121°C.

Laboratory procedures

Serial dilution

The samples (1 g) of each diet (treatment) were taken at intervals of 3 weeks for 2 months (63 days). These were analyzed separately, using properly homogenized samples. Sterile pipettes were used on each assay. One gram (1 g) of each sample was aseptically suspended into 9 ml of sterile water in a test tube which was then shaken together.

Pour plate method

Into already sterilized Petri dishes, aliquots of 0.1 ml of the diluted diet samples were dispensed using a pipette and the plates were labelled properly. Thereafter, sterilized media were added accordingly onto the diet samples and swirled gently. This method allows for the anaerobic and facultative anaerobic organisms to grow.

Total bacteria count (CFU/g)

The total bacteria count for each sample was determined using the pour plate technique as described previously. The plates were incubated for 24 h at 37°C. All colonies that appeared at the end of the incubation period were counted and the counts were expressed in colony forming unit per gram (CFU/g) of the diet sample.

Identification of isolates

Bacteria isolates

The bacteria isolated were identified using both morphological-culture characteristics; colour, consistency, shape, size, elevation, edge, opacity, and biochemical tests; citrate, coagulase motility, indole and sugar fermentation test.

Fungi isolates

The fungi isolates were identified using cultural/morphological characteristics such as filamentous, rough, raised, conidiospore, sexual, and non septate.

Statistical analysis

Data generated were subjected to one way analysis of variance using CRD.

RESULTS AND DISCUSSION

Chemical composition of pelletized and unpelletised cassava based diets shows that crude protein content of the diets was enhanced with the inclusion of cassava leaves (Table 2). Neutral detergent fibre and acid detergent fibre fractions of the diets were within the same range irrespective of the processing involved. Table 3 shows the performance characteristics of Muturu calves fed pelletized and sundried cassava based diets containing 0 and 20% cassava foliage for each respectively. The average weight gain of 285.71, 337.13, 247.14 and 200 g were obtained from T1, T 2, T3 and T4, respectively. Animals fed T2 had the highest ($P<0.05$) average body weight gain of 337.14 g compared to others. This agrees with Babayemi et al. (2010), who reported that the feed intake, growth rate and the feed efficiency were improved when diets containing cassava foliage were fed to ruminant animals.

The feed efficiency ratios obtained were 0.21, 0.24, 0.16 and 0.12 respectively for animals fed T1, T2, T3 and T4 respectively. The animal fed T2 had the highest feed efficiency ratio. This makes T2 a better diet than any of the other diets; since lower value of feed conversion (higher value of feed efficiency) shows superiority of diet (Ogbonna et al., 2002).

Table 2. Chemical composition of pelletized and unpelletized cassava based diets fed to Muturu cattle (%).

Parameter	T_1	T_2	T_3	T_4
Dry matter	91.27	92.05	88.90	88.68
Neutral detergent fiber	51.33	51.44	52.13	51.66
Acid detergent fiber	38.51	38.29	39.08	38.88
Crude protein	5.74	15.72	6.87	15.5
Ether extract	7.50	7.81	7.86	7.46
Ash	9.67	9.46	8.97	9.27

Table 3. Performance characteristics of Muturu cattle fed pelletized and sundried cassava based diets.

Parameter	T_1	T_2	T_3	T_4	SEM
Average initial body weight(kg)	60.00	62.50	61.30	62.29	7.2
Average final body weight (kg)	64.67	68.00	65.33	65.67	6.4
Average body weight gain (g/day)	55.60^{b}	65.48^{a}	47.97^{c}	40.23^{d}	5.81
Average feed intake (g/day)	4485.7^{d}	4657.1^{c}	5228.6^{b}	5700^{a}	55.4
Dry matter intake (g/day)	2865.88^{c}	3000.83^{b}	3253.74^{a}	3259.03^{a}	19.4
Neutral detergent fibre intake (g/day)	1474.21^{c}	1540.33^{b}	1696.17^{a}	1683.61^{a}	10.8
Acid detergent fibre intake (g/day)	1097.35	1155.62	1271.56	1267.11	8.5
Feed efficiency ratio	0.21	0.24	0.16	0.12	0.05

'a, b, c, d: means with different superscripts are significantly different (P<0.05).

Table 4. Apparent nutrient digestibility (%) of Muturu cattle fed pelletized and sundried cassava based diets.

Parameter	T_1	T_2	T_3	T_4	SEM
Dry matter	79.03^{b}	79.61^{b}	83.12^{a}	76.63^{bc}	0.47
Neutral detergent fiber	68.08^{b}	69.57^{b}	73.44^{a}	66.83^{c}	0.63
Acid detergent fiber	67.88^{c}	69.64^{b}	73.40^{a}	67.96^{c}	0.91
Crude protein	68.74^{c}	80.72^{a}	66.87^{d}	77.05^{b}	2.32
Ether extract	78.50	77.81	77.87	77.46	0.12
Ash	66.18	66.46	68.73	66.27	0.14

'a, b, c, d: means with different superscripts are significantly different (P<0.05).

However, this is not the case with T4 which has the same composition as T2 but sundried. This is in accordance with Oni et al. (2010), who recorded that feed intake in ruminants is also influenced by a taste related factor-palatability. Beyond nutritional composition animals tend to consume more of palatable diet.

Table 4 shows the nutrient digestibility of Muturu calves fed pelletized and sundried cassava based diet. Only T2 and T3 had high dry matter digestibility ranging from 79.61 to 83.12% while the remaining two diets: 0% cassava foliage and 60% cassava peels pelletized and 20% cassava foliage and 40% cassava peels sundried had low dry matter digestibility ranging from 79.61 to 76.63%. This shows that cassava leaf is not a very palatable feed ingredient (Bunyeth and Preston, 2006) probably due to the anti-nutritional factor (Bunyeth and Preston, 2006). The pelletizing of T2 restricted the animal's ability to select against the composition of the feed and therefore increased the intake and digestibility of T2 over T4. Table 5 shows the haematology of blood samples with respect to haemoglobin concentration (Hb), red blood cell counts (RBC), white blood cell counts (WBC), packed cell volume (PCV) of the blood, glucose and urea.

Haemoglobin content in g/dl ranged from 10.9 to 14.4, this is within the normal ranges of 8.0 to 15.0 reported by Blood et al. (2007). The aim of estimating the Hb content is to assess the oxygen carrying capacity of the calves' circulatory system. Having a low oxygen carrying capacity indicates that such calves can easily succumb to stress factors that may lead to respiratory problems, while those with high level of Hb content can be regarded as having high level of oxygen capacity and therefore, likely to withstand respiratory stress (Oni et al., 2012).

Table 5. Haematological analysis of blood collected from Muturu cattle fed cassava-based experimental diets.

Parameter	Pelletised		Sun-dried	
	T_1	T_2	T_3	T_4
PCV (%)	32.0 ± 2.0[a]	33.3 ± 3.1[a]	40.3 ± 1.5[b]	39.3 ± 6.4[a]
Hb (g/dl)	10.9 ± 0.8[a]	11.4 ± 1.0 [b]	14.4 ± 1.4[c]	12.3 ± 1.6[ab]
WBC ($Cumm^3$)	9133.3 ± 451.0[a]	9666.7 ± 3786.0[a]	14300 ± 500.0[b]	18100 ± 5351.0[c]
RBC (x 10^{12}/L)	7.2 ± 0.7[a]	7.8 ± 0.9 [a]	9.5 ± 0.7[b]	8.5 ± 1.2[b]
Glucose (mg/dl)	58.8 ± 2.7[a]	47.6 ± 5.1[b]	62.0 ± 8.3[a]	41.9 ± 3.0[c]
Urea nitrogen (mg/dl)	29.1 ± 5.8	32.9 ± 10.7	42.5 ± 6.8	41.1 ± 8.9

RBC counts ranged from 7.2-9.5 × 10^6 /ul, this is within the normal ranges of 5 - 10 ×10^6 /ul (Blood et al., 2007). Since it is the red blood cells that carry the respiratory pigments (haemoglobin), a decrease in the quantity of the circulating RBC imply a decrease in the quantity of haemoglobin and thus decrease in the oxygen carrying capacity of the calves. The WBC play prominent role in disease resistance especially with respect to the generation of antibodies. The WBC of calves fed sun-dried cassava based diet was significantly higher than that of calves fed pelletized cassava-based diet. This finding differed from the report from Akinfala and Tewe (2001) who found that cassava-based diet did not have significant effect on the WBC of the animals. In this study, the WBC was higher than the normal ranges of 4000 to 12000 Cum^3 (Blood et al., 2007). It means that these groups of animals were responding to extraneous factors which are likely present in the feed (sundried cassava based diets). These factors are likely to be pathogens that have built up due to compositional changes that occurred during the slow drying process which would have allowed build-up of microorganisms some of which are pathogenic in nature.

The PCV is the measure of the ratio of the volume occupied by the red blood cells to the volume of the whole blood in a sample of capillary or arterial blood. The result of the analysis showed that PCV ranged from 32 to 40%, this is within normal ranges of 24 to 46% (Blood et al., 2007). The PCV values did not follow any particular trend. Blood glucose is the amount of sugar present in the blood of the animal. High level of blood sugar is a condition (hyperglycaemia) when excessive blood sugar circulates in the blood plasma. This can lead to kidney damage and neurological damage. Low blood sugar (hypoglycaemia) is characterized by in coordination and confusion while even lower plasma glucose levels result in lethargy, coma, convulsions and eventually death (Ganong, 2005). In this study, the result of the analysis showed that blood glucose level was higher than the normal range of 35 to 55% (Blood et al., 2007). Significant increase was observed in calves fed sun-dried cassava-based diet at 0% leaves and 60% cassava peels (62 ± 8.3). This may be due to the concentration of carbohydrates in the diet of these animals which were converted to their sugar (mono and dissacharide) derivatives in the blood. This scenario probably explains the likely transient high glucose level in the blood which will become largely normal when the seemingly excess glucose concentration will be used up through various pathways taking place in the animal.Urea blood level is the measure of nitrogen in the blood. It is a substance secreted by the liver, and removed from the blood by the kidneys. The result showed that blood urea nitrogen of all the treatment groups was higher than the normal values of 6 to 27% (Blood et al., 2007). The concentration of urea circulating in the blood is dependent on the dietary intake of nitrogen and its level of utilization by the ruminant animal. High level of urea nitrogen in the system of a ruminant animal may turn out to be beneficial in the long run if the urea nitrogen finds its way back into the rumen where it becomes an important substrate with which the rumen symbiotic organisms produce good quality microbial protein that will eventually be available to the host for digestion, absorption and assimilation.

The result of the microbiological studies during 0 to 63 days of storage is presented in Table 6. During the storage period, there were no visible colour changes even as the length of days increased. Caking and mould growth that are normally associated with progressive deterioration were also not visible. The only physical change observed during storage was insect infestations like weevils. Most of the bacteria and fungi isolated were common and persistent throughout the storage period that is from day 0 to day 63. *Staphylococcus aureus, Enterobacter* spp*, Mucour* spp*., Aspergillus* spp*., E. coli, Proteus* spp*. and Fusarium* spp. which are known to be virulent and pathogenic were isolated throughout the period of storage. However, the insignificant increase in their numbers within the storage period and apparent lack of toxicity effect on experimental animals during feeding trials suggests that the diets were durable and safe. It has been established that high moisture content of dry feeds and feedstuffs encourage proliferation of microbes such as bacteria and fungi (Wei et al., 2007). Microbial propagation on the surface of product can be controlled by the application of a biopolymer-based edible coating (Wunwisa and Jaruporn 2008). The trend of microbial concentration in this study shows that it was constant

Table 6. Microorganisms isolated from stored cassava based diets in different forms from day 0 to day 63

DPS	Diets	Microbes														
		ECO	ENTR	SHIG	SAM	CITR	KLEB	BACI	STAP	PROT	MUCO	ASPN	ASPF	SACC	PEN	RHIZ
0	T_1-T_4	+	+	+	+	+	+	-	+	-	+	+	+	+	+	+
21	T_1-T_4	+	+	-	+	-	+	+	+	-	+	+	+	-	-	-
42	T_1-T_4	+	+	+	+	+	+	+	+	-	+	+	+	-	-	+
63	T_1-T_4	+	+	+	+	-	+	-	+	+	+	+	+	+	-	-

DPS, Day post storage; ECO, *Escherichia coli*; ENTR, *Enterobacterspp;* SHIG, *Shigellae* spp.; SAM, *Salmonella* spp.; CITR, *Citrobacterspp*; KLEB, *Klebsiella* spp.; BAC, *Bacillus* spp.; STAP, *Staphylococcus* spp.; PROT, *Proteus* spp.; MUCO, *Mucor* spp.; ASPN, *Aspergillus niger*; ASPF, *Flavus* spp; SACC, *Saccharomyces* spp.; PEN, *Penicillium* spp.; RHIZ, *Rhizopus* spp.

Table 7. Microbial count of the experimental diets.

Days/agar	SSA		EMBA		MSA		PDA	
	10^{+2}	10^{+4}	10^{+2}	10^{+4}	10^{+2}	10^{+4}	10^{+2}	10^{+4}
$0T_1$	48	44	32	38	261	263	4	2
$0T_2$	64	68	264	268	284	288	8	11
$0T_3$	22	26	182	178	300	300	16	18
$0T_4$	-	-	62	68	122	128	18	15
$21T_1$	-	-	10	10	300	300	14	4
$21T_2$	2	2	8	8	300	300	17	20
$21T_3$	61	58	248	252	300	300	18	20
$21T_4$	38	34	300	300	300	300	16	14
$42T_1$	4	1	3	4	234	245	24	6
$42T_2$	5	6	11	11	300	300	25	24
$42T_3$	75	67	300	300	300	300	26	26
$42T_4$	54	50	300	300	300	300	24	13
$63T_1$	7	2	6	6	272	281	28	24
$63T_2$	8	7	13	15	300	300	32	28
$63T_3$	64	68	264	268	284	288	8	11
$63T_4$	62	57	257	286	300	300	28	34

SSA, *Salmonella Shigella* agar; EMBA, eosine methylene blue agar; PDA, potato dextrose agar; MSA, manihot salt agar.

from the species isolated and also in their numbers/concentration with the length of storage (Table 7). This can be attributed to the processing methods the feeds were subjected (drying) to before storage, which has likely inhibited microbial activities. Thus, it further suggests that processing of feeds under hygienic condition enhances storability. When the experiment was conducted, there was no significant difference ($P>0.05$) between microbial profile of the cassava based diet forms used, thus, ensuring its safety for animal consumption. Also, the insignificant increase in the total viable count could be due to low moisture content which is a vital condition for the growth of the spoilage organisms. The isolated fungi and bacteria that were isolated are similar to the findings by Obadina et al. (2009) who isolated some fungi from fufu flour stored at different relative humidity at ambient temperature. Microbial load of the cassava based diets did not increase consistently as opposed to the findings of Babarinde and Fabunmi (2009) who reported a consistent increase in microbial load with storage.

In conclusion, inclusion of 20% cassava foliage can be used to improve the crude protein content of cassava based diets. Pelleting of the diet did not have appreciable significant improvement on the utilisation of the nutrients in the feed. With the increase in length of days of storage, there was no significant increase in the microbial count. Hence, cassava based diets can be stored for about three months either in pelletized or unpelletized forms based on the outcome of this study. Unpelletized feed is however recommended for economic reasons.

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