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RUMEN FERMENTATION CHARACTERISTICS AND BLOOD PROFILE OF WEST AFRICAN DWARF GOATS FED UREA-TREATED CROP BY-PRODUCTS IN THE DRY SEASON

*Adebayo, K.O., Usman, A.O., Aderinboye, R.Y., Adelusi, O.O. Akinbode, R.M. and Onwuka, C.F.I.
Animal Nutrition Department, Federal University of Agriculture, Abeokuta, Ogun State, Nigeria
E-mail: yomowumi@gmail.com

ABSTRACT
A study was conducted for eighty-four days to determine the rumen microbial population, total volatile fatty acids, ammonia nitrogen, pH and blood parameters of West African Dwarf goats fed urea-treated crop by-products based diets. A total of twenty four West African Dwarf goats were alloted to four dietary treatments containing six goats in a Completely Randomized Design. The experimental diets consist of diet 1. Urea-treated cassava peel based diet (UTCP), diet 2. Urea-treated maize cob based diet (UTMC), diet 3.urea-treated sweet potato peel based diet (UTSP) and diet 4. Urea-treated maize husk based diet (UTMH). At the beginning and end of the experiment blood and rumen fluid were collected from the animals to determine rumen microbial population and blood parameters and in the last three days of the experiment rumen fluid was collected from four goats in each treatment for the determination of rumen pH, total volatile fatty acids and ammonia nitrogen production. Results showed that there was no significant difference ($p>0.05$) in bacteria and fungi count of the goats before and after the experiment, although there was appreciable increase in bacteria count at the end of the study. The diets significantly ($p<0.05$) affected the protozoa count with highest (5.17 x102 cell/ml) value obtained in goats fed UTSP and the lowest statistically similar value obtained in goats fed UTMC and UTMH. Rumen ammonia nitrogen was influenced by the experimental diet. The highest value (7.41mg/dl) was obtained from goats on UTSP and the lowest value obtained on goats fed UTMC and UTMH. Serum glucose, ALT and AST were significantly influenced ($p<0.05$) by the diets. Glucose was lowest in UTMH. All the experimental diets have no deleterious effect on the rumen environment and health status of the goats thus can be fed to them for a normal rumen environment and maintenance of normal health status especially during the period of forage scarcity.

Introduction

The constraints of dry season feed shortage for ruminants in Nigeria have been a matter of concern for ruminant nutritionists. During this period, grass which form the bulk of ruminants feed becomes dry and of low nutritive value (Yusuf *et al*., 2017). This results in seasonal pattern of wet season weight gain and dry season live-weight loss (Oduguwa *et al*., 2013). According to Bampidis and Robinson (2006), ruminant feeding system based on locally available by-product feedstuffs are often a practical alternative because the rumen microbial ecosystem can utilize by-product feedstuffs which often contain high levels of structural fibre to meet their nutrient requirements for maintenance, growth, reproduction and milk production. Some of such by-products include cassava peel, maize cob, sweet potato peel and maize husk. With Nigeria being the largest producer of cassava worldwide (Daniels *et al*., 2011), cassava harvesting and processing produce large amounts of leaves and peels and are generally considered to contribute significantly to environmental problems when left in the surroundings of processing plants or carelessly disposed off. Maize cob is an energy source for ruminants, has low palatability and poor in nutrients. Due to its nutrient deficiency, it must be fortified with other feed materials that furnish these important nutrients (Ibhase *et al*., 2014). Munthali *et al*., (2000) stated that less than 50%

of total maize husk is actually consumed by livestock. Intake and digestibility of maize husk can be improved by physical, chemical and biological treatment / processing. Sweet potato (*ipomea batatas*) peel is classified as an agro-industrial by-product that is rich in energy for livestock. Urea has been widely used in the treatment of crop residues and agro-industrial by-products to increase crude protein content and improve digestibility of fibre (Yusuf, et al., 2016). Research effort has concentrated on determining the effect of these by products on growth parameters. The study therefore aim to assess the rumen environment parameters (pH, ammonia nitrogen, total volatile fatty acids and microbial count) and blood profile of West African dwarf goats fed urea-treated crop by-product based diet

Materials and Methods

Experimental site

The experiment was conducted at the Directorate of University farm (DUFARMS) of the Federal University of Agriculture Abeokuta, Ogun State. The region lies between the latitude 7°5.5'-7° 8'N and latitude 3°11.2'-3°2.51' and is within the rainforest of south- western Nigeria. The annual mean temperature and humidity are 34.7° C and 82% respectively. Seasonal distribution of rainfall is approximately 44.96mm in the late dry season (January- march), 212.4mmin the early wet season (April- June), 259.3mm in the late wet season (July-September) and 48.1mm in the early dry season (October-December) (Google Earth, 2014).

Collection and processing of test ingredients

The by-products (Cassava peel, Sweet potato peel, Maize cob and husk) were collected from different sources in and around the University. Each of the by-products was treated with urea solution. 1kg of urea was dissolved in 25litres of water to make 4% urea solution. This was used to soak 25kg of each of the by-product for ten minutes after which the solution was drained and the content sealed in a black polythene bag and left to ferment for 48hours. After 48hours, the bags were opened and the content was sundried for 3-5 days. They were analysed for their chemical composition (Table 1) and used in formulating four diets (Table 2). Crude protein, crude fibre, ether extract, ash and nitrogen free extract were determined according to AOAC (2000). Neutral detergent fibre, acid detergent fibre and acid detergent lignin were determined according to Van Soest *et al*. (1991).

Experimental Animals and Management

Twenty –four (24) growing West African Dwarf (WAD) goats weighing 6.5-8.5kg were used for the experiment. Prior to the arrival of the animals at the experimental the pens, drinking and feeding troughs were thoroughly washed and disinfected. On arrival the goats were quarantined for a period of two weeks before being transferred into individual pens. They were treated against internal and external parasites, using Ivomectin injection (1ml/50kg BW), Cypermetrin (1ml/10kg BW), bacterial infection by using Oxytec L.A (1ml/10kg BW) and mineral supplements using Vitaflash injection (2-5ml/goat). After the adaptation period, animals were divided into four groups of six animals on weight equalization basis. Each group of animals was randomly assigned to one of the four experimental diets. The feeding trial lasted for eighty-four days.

Determination of Rumen microbes

At the beginning of the experiment rumen fluid was collected from the rumen of all the animals in each treatment. The fluid was immediately taken to the Veterinary Microbiology Laboratory, College of Veterinary Medicine, Federal University of Agriculture, Abeokuta for analysis, counting and identifying the microorganism present in the rumen. The total direct count of bacteria, protozoa and fungi zoospores were according to Galyean (1989). Microbial analysis of the rumen was repeated at the 84th day in order to determine the variation in the microbial count.

Collection and Analysis of Blood Samples

10ml of blood sample were drawn from each animal via jugular vein puncture using hypodermic syringe before feeding the animals. Blood collection was done at the commencement (day 1) and termination of the experiment (day 84). The blood sample from each of the goats was divided into two and one-half was emptied into labelled bottles containing Ethylene diamine tetra acetic acid (EDTA). The bottles were quickly capped and the content was gently mixed by rocking the bottles. Sample bottles were labelled and taken to the laboratory for haematological analysis such as packed cell volume (PCV), Haemoglobin, Red blood cell (RBC), or White blood cells (WBC) etc. while the second half was emptied into plain bottle containing no EDTA for serum analysis such as serum total protein, urea

levels and liver enzymes. The packed cell volume (PCV) was measured for each animal within 24hours of collection using heamatocrit centrifuge at 12,000rpm for 15 minutes. The packed cell volume was estimated using haematocrit reader and Haemoglobin concentration (Hb) was obtained by measuring the amount of oxygen which can combine with haemoglobin, using Van Slyke apparatus as stated in Benjamin (1978). RBC was measured with the aid of Neubaur counting chamber after appropriate dilution (haemocytometer). Blood smears were used for total WBC counts (Tavares-Dias *et al.,* 2008). Glucose was determined after enzymatic reaction in the presence of glucose oxidase (Bauer *et al*., 1974). Total serum protein was measured in serum for individual animal using biuret method. Aspartate aminotransferase (AST) was read using spectrophotometer and concentration was determined from the AST activity table and Alanine aminotransferase (ALT) was also read using spectrophotometer and concentration was determined from the ALT activity table (Randox® Test Kits).

Determination of Rumen Fermentation Parameters

In the last three days of the study, rumen fluid was collected before feeding and six hours (6 hrs) post feeding through a suction tube from four goats per treatment to determine the pH, volatile fatty acids and rumen ammonia nitrogen. Immediately after collection, it was freed from coarse particles by filtration through three-layered cheese cloth. After which the pH was determined immediately through the use of pH meter. The fluid was then divided into two for each animal and poured into two well-labelled sample bottles. One part was acidified with 1ml of 5% orthophosphoric acid for the determining volatile fatty acids using steam distillation technique. The second portion was used for the determination of rumen ammonia-nitrogen as described by Lanyansunya *et al.,* (2007).

Statistical analysis

All data collected were subjected to one-way analysis of variance for a Completely Randomized Design using version 9.1 of SAS software (SAS, 2003) with the following model $Y_{ij} = \mu + T_i + eij$, Where Y_{ij}= Observed variation, μ = Population mean, T_i = effect of ith diets (1-4), eij = error term. Significant means were separated using Duncan's procedure of the same software (Duncan, 1955). Mean differences were considered significant at $P< 0.05$

Table 1: Chemical composition (%) of untreated and urea-treated cassava peel, maize cob, sweet potato peel and maize husk

Parameters	UNCP	UTCP	UNMC	UTMC	UNSP	UTSP	UNMH	UTMH
Dry matter	90.83	89.53	93.00	92.47	92.23	88.17	91.30	90.60
Crude protein	5.25	9.98	1.05	7.88	5.78	12.78	1.23	4.01
Crude fibre	10.00	7.00	44.00	40.00	7.00	4.00	42.50	38.00
Ether extract	4.50	4.00	4.00	2.33	4.50	3.17	5.85	5.00
Ash	7.33	7.33	3.75	3.60	9.00	8.83	5.25	5.00
Nitrogen free extract	63.75	61.22	39.67	39.19	65.95	59.39	33.49	38.59
Neutral detergent fibre	57.00	37.00	87.00	86.00	67.00	43.00	85.00	82.00
Acid detergent fibre	28.00	22.00	59.00	54.00	20.00	17.00	51.00	49.00
Acid detergent lignin	12.00	9.00	19.00	15.00	10.00	9.00	22.00	20.00

UNCP-untreated cassava peel, UTCP- urea-treated cassava peel, UNMC- untreated maize cob, UTMC-urea-treated maize cob, UNSP- untreated sweet potato peel, UTSP- urea-treated sweet potato peel, UNMH- untreated maize husk, UTMH- urea-treated maize husk

Table 2 Ingredients and chemical composition of experimental diets (g/kg)

Ingredients composition g/kg	T1	T2	T3	T4
Cassava peels	400	-	-	-
Maize cob	-	400	-	-
Sweet potato peels	-	-	400	-
Maize husk	-	-	-	400
Wheat offal	300	300	300	300
Dried brewer's grain	100	100	100	100
Palm kernel meal	165	165	165	165
Bone meal	20	20	20	20
Salt	10	10	10	10
Premix	5	5	5	5
Total	**1000**	**1000**	**1000**	**1000**
Chemical composition g/kg				
Dry matter	916.0	915.7	893.0	922.0
Crude protein	145.6	137.2	156.8	120.4
Ether extract	70.0	55.0	58.0	43.5
Crude fibre	100.0	180.0	90.0	185.0
Ash	106.7	123.3	148.3	141.7
Nitrogen Free Extract	493.7	420.2	439.9	431.4
Neutral detergent fibre	550.0	730.0	500.0	730.0
Acid detergent fibre	230.0	320.0	260.0	380.0
Acid detergent lignin	120.0	150.0	60.0	200.0
Metabolizable Energy (Kcal/Kg)	3063.06	3471.75	2970.13	3397.65

T1- Urea-treated cassava peel based diet, T2- Urea-treated maize cob based diet, T3- Urea-treated sweet potato based diet and T4- Urea-treated Maize husk based diet

Results and Discussion

Rumen pH, total volatile fatty acids (TVFAs) and ammonia nitrogen (NH_3-N) production of the experimental animals before feeding the experimental diets and 6hrs (post-feeding) were presented in table 3. There were no significant differences ($p>0.05$) in the values of pH and TVFAs in the pre-feeding and post-feeding phases. Rumen NH_3-N was significantly ($P<0.05$) influenced by the experimental diets at post-feeding phase. It was highest (7.41mg/dl) in urea-treated sweet potato peel based diet (UTSP) and lowest in urea-treated maize cob based diet (UTMC) and urea-treated maize husk based diet (UTMH) with statistically similar values. The values were however within the range of 5-20 mg/dl reported by Leng and Nolan (1984) as being suitable for microbial activities. The highest value recorded in UTSP could be attributed to its greater buffering capacity (Muck *et al*., 2003). It is also a reflection of the degradation of the soluble fraction of supplemental protein in the rumen (Amole *et al*., 2013). Ammonia nitrogen is an indicator of proteolysis in the rumen. Rumen microbes work best in the presence of adequate energy. UTSP had the highest concentration of soluble energy which is reflected by the nitrogen free extract (439.9g/kg) thus making energy readily available for bacteria to break down feed protein into ammonia which is used by microbes to make their own protein. This invariably means that proteolytic activity is higher in UTSP.

Presented in table 4 is the rumen microbial count of the experimental animals before and after the experiment. The diets increased bacteria count from a range of 1.15 -1.40 $x10^6$ cfu/ml before the feeding of the experimental diets to a range of 3.45 -3.73 $x10^6$ cfu/ml at the end of the study. No significant difference ($p>0.05$) was observed in the values. This is an indication that all the four experimental diets contain adequate nutrients needed for bacterial activity. Bacteria are the principal agents for fermenting cell wall carbohydrates and are the largest population of microorganism in the rumen (Preston and Leng 1987). Fungi count remains almost the same before and after the study without any significant difference observed. This shows that the diets have no effect on the fungi count. However, protozoa count was significantly ($p<0.05$) influenced by the diets with a ranged of 2.05 -4.15 $x10^2$ protozoa

cell/ml before the study and 2.07 – 5.17 $x10^2$ protozoa cell/ml at end of the study. There was an increase in protozoa count of goats fed UTCP and UTSP by the end of the study. However, the protozoa count of goats fed UTMC and UTMH decreased by the end of the study. This is also connected to the high concentration of sugar and low fibre in UTCP and UTSP as reflected in their NFE and NDF content (Table 2). Protozoa population are usually high when animals are fed diet high in starch or sugars. According to Preston and Leng (1987) some protozoa are cellulolytic but the major substrates appear to be sugar and starches which are rapidly assimilated and stored as poly-dextran; this is mobilised as required to provide energy for the growth and maintenance of the protozoa. In this way they buffer

There were no significant differences in the values obtained for all the haematological parameters measured before the study and after feeding the experimental diets (Table 5). Serum glucose, urea, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were significantly ($p<0.05$) influenced by the experimental diets. While the highest and statistically similar values were obtained in UTCP, UTMC and UTSP, the lowest value (70.65mg/dl) was obtained in UTMH. This might be due to high fibre content of UTMH which would result in the production of less propionate, a major substrate of gluconeogenesis in the liver. However, the values obtained in all the diets are within the normal range of values suggested by Fraser and Mays (1986) for glucose. This means that all the diets are able to generate enough glucose for vital body functions. Urea had significantly ($p<0.05$) highest value (20.00mg/dl) in UTSP and statistically similar lowest value in UTCP, UTMC and UTMH. Eggum (1970) reported that higher urea level is indicative of poor protein quality which means that all the protein in UTSP was broken down by microbes in the rumen thereby generating more NH_3-N. This can be correlated with higher ammonia- nitrogen values obtained in UTSP (Table 3) when compared with other diets. Ammonia is used by microbes to produce their own microbial protein and excess is absorbed across the rumen wall and transfer to the liver where it is converted to urea and transported out of the body through urine while some are recycled through blood back to the rumen. The values were however within the range of 12.6-25.5mg/dl reported as normal for goats (Fraser and Mays, 1986). AST and ALT are liver enzymes, their activities increased by the end of the study as indicated in their values before and after feeding the experimental diets. They are parameters conventionally used in diagnosing human and domestic animal hepatic damage (Silanikove and Tiomkin, 1992). They were within the normal range of 66-230u/l and 15.3-52.3u/l reported by Daramola *et al*. (2005) and Fraser and Mays (1986) for AST and ALT respectively. The values obtained therefore indicated that there was no liver damage and the enzyme secretion mechanism of the goats was not hindered by the diets.

Table 3: Rumen environment parameter of West African Goats fed experimental diet at pre- feeding and post feeding phases

Parameters	T1	T2	T3	T4	SEM
Pre-feeding (0hr)					
PH	6.64	6.72	6.76	6.78	0.05
Ammonia-Nitrogen (mg/dl)	5.37^b	4.71^c	6.12^a	4.94^c	0.54
Total Volatile Fatty Acid (mM/100ml)	11.5	11.7	11.5	11.5	0.02
Post feeding (6hrs)					
PH	6.82	6.76	6.91	6.83	0.07
Ammonia Nitrogen (mg/dl)	6.11^b	5.43^c	7.41^a	5.38^c	0.58
Total Volatile Fatty acid (mM/100ml)	16.6	16.7	17.2	16.8	0.09

Means in the same row with different superscripts are significantly different ($p<0.05$). T1- Urea-treated cassava peel based diet (UTCP), T2- Urea-treated maize cob based diet (UTMC), T3- Urea-treated sweet potato peel based diet (UTSP) and T4- Urea-treated Maize husk based diet (UTMH)

Table 4: Rumen Microbial population of West African dwarf goats fed experimental diets

Parameters	T1	T2	T3	T4	SEM
Before the experiment					
Bacteria count ($\times 10^6$cfu/ml)	1.20	1.40	1.25	1.15	0.11
Fungi count ($\times 10^6$cfu/ml)	0.10	0.15	0.10	0.10	0.08
Protozoa ($\times 10^2$cell/ml)	2.05^c	2.25^c	3.80^b	4.15^a	0.45
After the experiment					
Bacteria count ($\times 10^6$cfu/ml)	3.45	3.73	3.47	3.67	0.02
Fungi count ($\times 10^6$cfu/ml)	0.10	0.13	0.10	0.10	0.02
Protozoa ($\times 10^2$cell/ml)	4.00^b	2.07^c	5.17^a	3.27^c	0.45

Means in the same row with different superscripts are significantly different ($p<0.05$). T1- Urea-treated cassava peel based diet (UTCP), T2- Urea-treated maize cob based diet (UTMC), T3- Urea-treated sweet potato peel based diet (UTSP) and T4- Urea-treated Maize husk based diet (UTMH)

Table 5: Haematological and serum biochemical parameters of West African dwarf goats fed urea-treated crop by-products based diets

Haematological parameters	[a] Normal Values	At the start of experiment (Day 0)					At the end of experiment (Day 84)				
		UTCP	UTMC	UTSP	UTMH	SEM	UTCP	UTMC	UTSP	UTMH	SEM
Packed cell volume (%)	22-38	28.00	28.33	28.33	30.00	0.51	33.00	32.55	31.00	33.60	0.42
Haemoglobin (g/dl)	8-12	8.65	9.30	9.73	10.47	0.50	11.00	10.85	10.30	11.20	0.47
Red blood cell ($x10^{12}$/l)	8-18	11.52	11.69	11.72	12.81	0.62	13.55	12.57	13.65	13.34	0.29
White blood cell ($x10^{9}$/l)	4-13	11.65	11.07	10.97	10.83	0.56	11.51	11.35	11.30	11.05	0.06
Serum parameters											
Glucose (mg/dl)	48.2-76	69.15^{c}	72.63^{b}	72.73^{b}	74.80^{a}	2.13	75.35^{a}	75.75^{a}	75.55^{a}	70.65^{b}	1.51
Total protein (g/dl)	6.1-7.45	6.70	5.50	6.27	6.70	0.20	7.40	6.95	6.65	6.75	0.18
Urea (mg/dl)	12.6-25.8	8.50^{c}	8.33^{c}	13.00^{a}	10.33^{b}	3.30	17.65^{b}	18.11^{b}	20.00^{a}	17.50^{b}	1.30
AST (u/l)	66-230	66.50^{b}	68.33^{b}	69.67^{b}	72.33^{a}	1.16	74.00^{b}	76.50^{a}	78.00^{a}	78.50^{a}	2.07
ALT (u/l)	15.3-52.3	12.00	12.00	14.00	15.00	0.69	33.50^{b}	37.00^{a}	29.50^{c}	32.50^{b}	2.42

a, b, c, show means within the row that are significantly different ($p < 0.05$), UTCP- Urea-treated cassava peel, UTMC- Urea-treated maize cob, UTSP- Urea-treated sweet potato peel and UTMH- Urea-treated maize husk, AST- Aspartate aminotransferase, ALT- Alanine aminotransferase, [a] Normal values according to Fraser and Mays (1986)

Conclusion

All the experimental diets; UTCP, UTMC, UTSP and UTMH did not have any negative effect on the fermentation characteristics and blood profile of the goats and therefore could be fed to them during the dry season when forage is scarce. However, UTMC and UTMH can be subjected to further processing to reduce their fibre content and improve their utilization or their quantity in the diets can be reduced and replaced by less fibrous ingredient.

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