

Effect of supplementation with different protein sources on digestibility and rumen metabolites of Yankasa rams

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Targeted Audience: Researchers, Students, Ruminant nutritionists

Abstract

A study was conducted to evaluate the dietary inclusion of cottonseed cake (CSC), sun dried poultry litter (SDPL) and dried cassava leaf meal (DCLM) on digestibility and rumen metabolites of Yankasa rams. Eighteen (18) grower Yankasa rams, weighed 11.5 to 15.5 kg were used to measure the rumen metabolites, nutrient digestibility and nitrogen balance of the rams. The chemical analysis result showed that treatment COSM or POUT diets had higher values of crude protein than CASM diet. Total dry matter intake was similar ($P>0.05$) for all the treatments. Rumen parameters were significantly ($P<0.05$) affected by the nitrogen sources in such a way that rumen pH 4 h after feeding was highest (6.50) in animals fed CASM diet and lowest for the COSM fed animals. Temperature values before feeding was highest (37.38°C) in POUT fed animals and lowest (34.38°C) in COSM fed animals, the total volatile fatty acid values 4 h after feeding was 42.75 and 28.25 for animals fed POUT diet and COSM diet, respectively. Nutrient digestibility was significantly ($P<0.05$) different on digestibility of nitrogen free extract. Nitrogen intake was significantly ($P<0.05$) higher in rams fed COSM (52.71 g/d) or POUT (48.72 g/d) diet compared to their counterpart fed CASM diet. Nitrogen balance and nitrogen absorbed were statistically similar ($p>0.05$) for all the groups. It could be concluded that CSC, SDPL and DCLM is a safe nitrogen sources for rams without any determinant effects.

Keyword: cottonseed cake, poultry litter, cassava leaf meal, Yankasa rams and digestibility

Description of Problem

Dry season is a critical period for livestock in the tropical region as it is characterized by shortage of feed both in quality and quantity. Feed resources for ruminants are mainly from native rangelands, the grasses that exist during the dry season are dry and highly lignified. Ruminant animals are unable to meet their nutrient requirements for maintenance due to inadequate quality and quantity of feed especially, during the dry season and will begin to lose weight as body reserves are depleted (1). Although, ruminant animals have ability to feed on poor quality forages

and fibrous feeds. In order to combat this feed scarcity, crop residues are useful source of ruminant supplementary feed in the dry season in the tropics (2). Sheep are able to use marginal lands and crop residues as feed and are kept in Nigeria mainly for meat (3). They are ranked second after cattle in terms of meat production (4). (5) reported sheep to contribute 16% of the total domestically produced meat in Nigeria. Of the four breeds of sheep in Nigeria, Yankasa sheep are perhaps the most widely and most numerous breed in the Northern part of the country, they are found in the Sahel, Sudan and Guinea Savannah zones of the Country

(6). The main nutritional constrain to the use of crop residues as ruminant feed is the rapid decline in quality, slow rate of digestion due to high lignin content and relatively poor nutrient content (7). Efforts to alleviate these constraints and reduce high cost of feeds have been concentrated primarily on harnessing feeds formulated from locally available, cost affordable and nutritionally adequate alternatives supplemental feeds and /or unconventional feedstuffs of no nutritional value in human diets (8). Poultry litter is one of such unconventional feedstuffs.

Dried poultry litter can be successfully included in the feed of ruminants and non-ruminants (9). Poultry litter contains about 28-30% crude protein (CP), of which 36-50% is true protein (10). The use of poultry litter in feed for ruminants decreases the cost and reduces their polluting effects on environment. They provide some of the animal's requirement for protein, energy and micronutrients. Cotton Seed Cake (CSC) is high in protein (26-48% CP), a good source of fiber and phosphorus.

In CSC however, the method of processing, the duration of heat treatment or processing, and the extent of oil extraction affect its gossypol content. Ruminants have the ability to detoxify large amounts of gossypol within the rumen (11) reported a decrease in dry matter intake (33-59 g/kg live weight) and increase in Organic matter (65-75%) and protein digestibility (41-73%) when increasing level of cotton seed meal with low quality forage were fed to male Sidama goats. The aim of this study therefore, is to evaluate the different protein sources on digestibility and rumen metabolites of Yankasa rams fed basal diet of *Digitaria smutsii* hay supplemented with one of concentrate mixtures containing CSC, SDPL or DCLM.

Materials and methods

Experimental Site

The experiment was conducted at the Experimental Unit of the Small Ruminant Research Programme of the National Animal Production Research Institute (NAPRI), Ahmadu Bello University, Shika-Zaria, Nigeria. Shika is situated in the Northern Guinea Savanna ecological Zone of Nigeria between latitudes 11° 8' 19.56"N and longitudes of 7° 45' 51.22"E at an elevation of 640m above sea level (12). The average annual rainfall of 1100mm starts from early-May to mid-October. The mean minimum and maximum environmental temperature ranges from 12 to 28°C during the cold (harmattan) season and 20 -36°C in the hot season. The mean relative humidity is about 72% during the rainy season. The dry season begins with a period of dry cool weather known as harmattan that lasts from October to January. The harmattan is followed by a dry hot weather from February to April. The relative humidity at this period is 21% (13).

Experimental Diets

Cottonseed cake (CSC), sun-dried poultry litter (SDPL) and dried cassava leaf meal (DCLM) were used as the main sources of protein to formulate and compound three different diets which contained, in addition, maize offal, wheat offal, bone meal and common salt. The three diets designated as COSM, POUT and CASM contained cotton seed cake, sun-dried poultry litter and dried cassava leaf meal, respectively, as the main source of protein (Table 1). The diets were iso- nitrogenous and contained 15% crude protein.

Collection and processing of cassava leaves and Poultry litter

Cassava leaves were obtained from local farmers around the study area after harvesting of cassava roots. The fresh

cassava leaves were harvested and sun-dried on a flat concrete floor for 3 to 4 days depending on the intensity of the sun to reduce the hydrocyanic acid present in the leaves. It was intermittently turned to prevent scorching and fermentation as recommended by (14). Thereafter, the leaves were crushed into a meal using a mortar and a pestle. The poultry litter (Layer litter) was sourced from Poultry Research Programme NAPRI, Shika- Zaria and sun dried for 3 days to reduce the effect of pathogenic organisms, and also checked to remove metals and sharp objects. Cottonseed Cake, wheat offal, maize offal, bone meal and common salt were obtained from NAPRI and incorporated into the supplementary concentrate diets.

Experimental design, animals and management

Eighteen (18) Yankasa rams aged 9-12

months weighing 11.5-15.5kg were randomly allotted to three dietary treatments in a completely randomized design (CRD), with six animals per treatment. The concentrate diet contained COSM, POUT or CASM along with other ingredients in the supplementary concentrate mixtures with each nitrogen source serving as a treatment designated earlier (Table 1). Prior to the commencement of the experiment, the animals were treated against ecto and endo parasites using Acaricide and albendazole[®], respectively. The animals were housed in individual pens and fed the supplementary concentrate diets at 2% of their body weight and *Digitaria smutsii* (wooly grass stent) hay was offered *ad libitum* as basal diet. The animals were weighed fortnightly and the quantities of feed offered were adjusted accordingly. Fresh clean water was provided *ad libitum*. The growth trial lasted for 90 days, excluding the adjustment period.

Table 1: Ingredient composition of the Experimental Diets

Ingredients	COSM	POUT	CASM
Maize Offal	48.88	43.65	36.44
Wheat Offal	24.07	21.50	17.95
Cotton Seed Cake	23.05	-	-
Poultry Litter	-	30.85	-
Dried cassava leaves	-	-	41.61
Bone Meal	2.5	2.5	2.5
Common Salt	1.5	1.5	1.5
Total	100.00	100.00	100.00

Rumen fluid sampling

About 50mls of rumen fluid samples were drawn from four (4) animals in each of the treatment groups at the end of the feeding trial using Stomach Suction Tube before feeding and at 4 h and 8 h after feeding. The tube was about 150 cm long with a metallic strainer attached at the end, this was passed through a pipe placed in the mouth of the ram into the rumen and a suction pump was used to draw out the rumen fluid. Immediately after collection, temperature of

the rumen liquor were taken using digital hand thermometer and also its pH were recorded using Henna Digital Hand pH meter (model 9409) immediately.

The rumen fluids were strained through double layers of cheese cloth and 20 mls aliquot of the filtrate was taken and put into plastic bottles containing an equal volume of 0.1N H₂SO₄ saturated with MgSO₄ to acidify, deproteinize and reduce bacterial activity, respectively. The mixture was centrifuged at 3000 rpm and allow to stand

for 10 min. Twenty (20) milliliters of the supernatant was decanted in to plastic bottles and kept frozen at -20°C until when required to be analyzed for volatile fatty acids (VFAs) and rumen ammonia- nitrogen ($\text{NH}_3\text{-N}$). The rumen ammonia concentrations were determined by steam distillation into boric acid and back titrated with 0.01N hydrochloric acid following the procedure described by (15).

Metabolism Trial

At the end of the growth trial, four rams were randomly selected from each treatment group and housed in individual metabolism crates ideal for easy and separate total fecal and urine collection, as described by (16). The animals were allowed 4days adjustment period to the conditions in the metabolic crates since the diet remained the same before the commencement of the collection period, which lasted for nine days. Known weight of the experimental diets was offered daily and water was provided *ad libitum*. Daily total fecal output were weighed fresh and 10% of each day collection were sub-sampled and oven dried at 60°C for 48hrs for the determination of dry matter. This was later bulked, milled, sub- sampled and stored in plastic bottles, until when required for laboratory analysis.

Daily total urine output for each ram were collected in 5 litres plastic jericans containing 100mls of 0.1N H_2SO_4 to prevent ammonia loss from the urine this was placed under the metabolic crate. A 10% aliquot of the total daily urine output was taken from each ram, bulked and stored in the refrigerator pending analysis

Chemical Analysis

Dried cassava leaf meal, sun dried poultry litter, cotton seed cake, experimental concentrate diets, *Digitarias mutsihay* and faecal samples were analyzed for dry matter

(DM), crude protein (CP), crude fibre (CF), ether extract (EE), ash and nitrogen free extract (NFE), according to the Method of (17). Acid detergent fiber (ADF) and neutral detergent fibre (NDF) were determined according to the method described by (18). Hemicelluloses content could be obtained by difference between NDF and ADF values (19). Urine samples were analyzed for nitrogen using the simple micro-Kjeldahl distillation method of (17). The energy contents of the diets were analyzed using bomb calorimeter model e2k. ME =estimated from GE determined with bomb calorimeter following (20) recommendation of : 100 Mcal GE =76 Mcal DE =62 Mcal ME = 35 Mcal NE

Statistical Analysis

Data collected on rumen metabolites was subjected to repeated measures analysis using PROC MIXED (24 version 9.0). The statistical model used was: $Y_{ijk} = \mu + t_i + b_j + p_k + (tp)_{ik} + e_{ijk}$, where Y_{ijk} is the response of animal j in treatment i at time k, μ is the overall mean, t_i is a fixed effect of i_{th} treatment ($i = 1,2,3$), b_j is the random effect of the j_{th} animal ($j = 1,2,3,4,5,6$) nested within the i_{th} treatment, p_k is the fixed effect of k_{th} time (1,2,3,-----13), tp_{ik} is the interaction between the i_{th} treatment and the k_{th} time, e_{ijk} is the random error.

For each variable analyzed using this model, animal as a subject nested within treatment was subjected to the appropriate covariance structure which gave the smallest Akaike's Information Criterion (AIC) and met convergence criteria. Kenward – Roger correction for degree of freedom was used. The use of Kenward – Roger correction reduces the probability of type I error.

Data on digestibility of nutrients and nitrogen retention were analyzed with ANOVA using General linear model procedures of (19) in accordance with the

following model: $Y_{ij} = \mu + t_i + e_{ij}$, where Y_{ij} is the response of animal j in treatment i ($j = 1,2,3,4,5,6$), μ = is the overall mean, t_i is a fixed effect of the i th treatment ($i = 1,2,3$),

e_{ij} = the random error. The least squares means were separated using contrast statement of SAS.

Table 2: Chemical composition of experimental diets

Parameters (%)	Dietary treatment			
	COSM	POUT	CASM	Digitaria smutsii
Dry matter	88.42	89.94	91.16	92.63
Organic matter	79.69	80.29	83.05	88.73
Crude protein	16.81	16.81	16.67	7.50
Crude fiber	24.11	16.09	18.93	31.17
Ether extract	14.61	16.88	14.61	7.77
Ash	8.73	9.65	8.11	3.90
NFE	24.16	30.51	32.84	42.29
ADF	22.68	19.01	28.08	39.01
NDF	38.72	41.17	36.06	73.42
TDN%	74.88	77.42	69.54	
GE MJ/kg	14.31	21.23	14.31	
ME MJ/kg	8.8722	13.1626	8.8722	

NFE=Nitrogen free extract, ADF= Acid detergent fiber, NDF= Neutral detergent fiber, TDN= total digestible nutrient, GE MJ/kg= gross energy mega joule per kilogram, ME = metabolizable energy

Result and discussion

Chemical composition of Diet

The result of the chemical composition of the dietary ingredients is shown in Table 2.

Dry matter contents of the diets are comparable to 83.13% - 88.21% reported by (21) for molasses urea multi-nutrients blocks. The observed dry matter value is an indication of the shelf life of the diets. The higher the dry matter the longer the shelf life and the more resistance to the growth of mould. Organic matter contents are comparable to 77.86 to 80.53% reported by (22) who fed cassava peels supplemented with nitrogen sources to WAD goats. The values of organic matter obtained in this study might be due to the nature of supplemental nitrogen used in this study. Crude protein (CP) contents are lower than 20.56% - 21.61% reported by (23), but comparatively higher than 9.95 to 11.89 CP % reported by (22). The crude protein content observed in this study may be

attributed to the sources of supplemental nitrogen in the diets. Crude fibre (CF), ether extract (EE), NDF and ADF content values are higher than the values reported by (22). Ash contents are comparable to 8.89% - 11.00% reported by (23), nitrogen free extract NFE) values are lower than values reported by (22). Chemical composition values observed for the supplementary concentrate diets in this study are comparable to those reported by (22). This is an indication that the diets can be potential supplements to grazing small ruminant animals as well as those being fed other crop residues in the dry season. Apparently, the diets may assist in improving the activities of rumen microbes by generating high level of ammonia in the rumen that will promote efficient digestion process (24). Also the fibre fraction level obtained in this study was lower than the safe upper limit of 60% reported by (25) for guaranteed forage intake by ruminant. The fibre fractions showed that the diets have the potentials to support

intestinal movement and proper rumen function. This may imply that the fibre fractions of the diet have the potential to improve fermentation in the fore stomach of the animals. (26) stated that excess fibre fractions especially NDF reduces the rate of fermentation and feed intake, but little fibre leads to rapid rumen fermentation.

Rumen metabolites

Animals fed CASM diet had a significantly ($P<0.05$) higher pH 4 h after feeding than those fed COSM or POUT diet. (Table 3). The ruminal pH value of rumen fluid before feeding was slightly higher than the range 6.7 – 7.1 reported by (27) as the optimal ruminal pH for fibre digestion or cellulolytic activity. The lower pH value observed 4 h post feeding could be due to the production of VFA's which tends to lower the Rumen pH (28). The lower rumen pH value 4 h post feeding, fell within the optimum range (6.2 – 6.8) for maximum cellulolysis reported by (29). The pH of a medium is known to have marked effect on enzymatic activity. Orskov, (30) indicated that ruminal pH below 6.0 – 6.2 would reduce the activity of cellulolytic bacteria and could reduce forage fibre digestibility. Ruminant animals depend on cellulolytic ruminal bacterial to digest cellulose; hence the higher pH observed in animals fed CASM diet fell within the range of 6.0–7.0 reported by (31) as the optimum pH values for microbial growth and fiber digestion but higher when compare to 6.1 below which cellulosis is inhibited(32). (33) explained that the major consequence of ruminal pH at pH less than 6 is that fibre digestion declines dramatically. There was a significant effect ($P<0.05$) of nitrogen sources on the mean rumen temperature of the weaner rams in this study. Rams fed COSM diet had significant lower rumen temperature before feeding. Heat stress in ruminant animals has

been shown to cause a rise in rumen temperature (34). This is consistent with the report of (35) who reported that supplementation of cottonseed meal in the ration of ruminants decreases heat increment by lowering body temperature and reduces heat stress with superior values of fattening performance (36). One of the main factors influencing the growth and activity of ruminal microbial populations is temperature, the values of temperature recorded in this study are generally lower than 39 to 39.5 °C reported by (37) as the temperature at which the rumen is maintained and may increase up to 41 °C immediately after the animal eats because the fermentation process generates heat (38). Rumen ammonia nitrogen concentration of the animals in this study did not differ ($P>0.05$) significantly, probably due to the similar crude protein concentration.

There was a significant ($P<0.05$) effect of nitrogen sources on volatile fatty acid production. Animals fed POUT or CASM diet were significantly higher in total VFA concentration than COSM diet 4 h post feeding. Volatile Fatty Acids, being the primary source of metabolizable energy for the ruminant animals, they need sufficient time for feed degradation through enhanced production of rumen microbes (39). The higher concentration of volatile fatty acids in animals fed POUT diet might have been as a result of an increase in the molar proportion of propionic acid, (40) reported that the presence of non-structural carbohydrates (starch and sugars) such as concentrates normally fermented faster than forage, resulting in elevated propionate levels. The amount of VFA production during fermentation is influenced by the nature and quality of feed and poor quality feed contains high crude fibre which does produces high amount of gas per unit fermented substrate (41).

Table 3: Rumen metabolites of Yankasa rams fed cottonseed cake, poultry litter or dried cassava leaf meal.

Parameters	Dietary treatment			SEM	LOS
	COSM	POUT	CASM		
PH					
0h	7.35	7.10	7.33	0.08	NS
4h	6.07 ^b	6.20 ^{ab}	6.50 ^a	0.08	*
8h	6.52	6.50	6.78	0.08	NS
Temperature°C					
0h	34.38 ^b	37.38 ^a	36.63 ^{ab}	0.52	*
4h	37.75	39.38	38.75	0.52	NS
8h	39.00	40.13	38.89	0.52	NS
NH ₃ -N (g/100g)					
0h	0.09	0.11	0.10	0.01	NS
4h	0.13	0.12	0.16	0.01	NS
8h	0.17	0.14	0.17	0.01	NS
TVFAs (Umol/l)					
0h	26.50	22.50	27.25	1.86	NS
4h	28.25 ^b	42.75 ^a	37.00 ^{ab}	3.94	*
8h	32.75	27.50	23.75	3.03	NS

^{a,b,c} means with different superscripts along the same row differ significantly. * significant difference at (P<0.05) , SEM= Standard error of the mean, NS= not significant at 5%, LOS= level of significant, TVFA= Total volatile acid.

Nutrient intake

The daily nutrient intake of Yankasa rams fed cottonseed cake, poultry litter or dried cassava leaf meal presented in table 4. Animals fed COSM or POUT diet had significantly higher nutrients intake (DM, CP, CF, EE, NFE and NDF) than animals fed CASM diet. The reason for the lower intakes of animals fed CASM diet was probably due to the higher residual level of anti nutritional factors such as cyanogenic glucosides and tannins which have been reported to negatively influence the nutritional permeability in the gut wall of the sheep (42). The result obtained in this study agree with the report of (43) who used *Moringa oleifera*, *Gliricidia sepium* and cassava foliage to feed West African Dwarf (WAD) goats. The researchers posited that the least DM intake was recorded for cassava foliage. The lower nutrient intake of the animals fed CASM diet might be due to the

type of the diet and length of time spent on the diet (44). (45) also posited that feed intake is greatly influenced by the palatability of the feed. Therefore, the value of nutrient intake recorded in this study could be attributed to the palatability and acceptability of the diet to the animals. More so, the aromatic smell of the POUT diet might have conferred better palatability and acceptability, thereby resulting in better intake.

Nutrient digestibility

Daily nutrient digestibility of Yankasa rams fed cottonseed cake, poultry litter and dried cassava leaf meal Table 5. Apparent digestibility values obtained in this study for all the dietary treatments were not significantly different from each other with the exception of the digestibility of NFE, this could be attributed to the adequate protein supplementation of the diets. (46) reported

that protein supplementation enhanced digestibility of feeds in animals. The higher NFE content in animals fed COSM or POUT diet in this study indicates that there was

appreciable fermentable carbohydrate for energy production in COSM or POUT diet than in CASM diet.

Table 5: Daily nutrient digestibility of Yankasa rams fed cottonseed cake, poultry litter and dried cassava leaf meal.

Parameters (g)	Dietary treatment			SEM	LOS
	COSM	POUT	CASM		
Dry matter	65.28	64.48	63.53	2.94	NS
Organic matter	70.73	69.02	65.68	2.61	NS
Crude protein	63.74	71.79	63.27	3.06	NS
Crude fiber	80.69	82.19	81.41	1.96	NS
Ether extract	60.02	65.72	58.46	3.75	NS
Nitrogen free extract	69.91 ^a	67.06 ^a	58.66 ^b	2.16	*
Acid detergent fiber	76.16	71.95	66.44	3.5	NS
Neutral detergent fibre	74.86	71.86	74.82	2.55	NS

^{a,b,c} means with different superscripts along the same row differ significantly. * significant difference at (P<0.05) SEM=Standard error of the means, LOS=Level of significant, TDN= Total digestible nutrient,

Nitrogen balance

Nitrogen balance in Yankasa rams fed diets containing cottonseed cake, poultry litter and dried cassava leaf meal in presented in Table 6. The higher nitrogen intake of the animals fed COSM or POUT diet might be due to the higher DM and crude protein intake of the animals in these treatments compared to the intake in the CASM treatment. The higher faecal N in CASM fed animals might be due to the effect of tannin resulting in dietary N being excreted in the faeces as tannin-protein complexes, this is in accordance with the report of (47) that the tannin protein complexes are formed from the dietary protein and voided undigested through faeces of ruminant animals.

The reason for high urinary N in POUT fed animals could be due to low available

energy in poultry litter and when the amount of energy is less than the required rumen microbe, the animals will be unable to convert ammonia into structural protein and the ammonia entered into urea cycle and gets excreted in urine. (48) reported that the concentration of urea in the urine likely correlates with N ingestion. Nitrogen balance, N retention and total N output were not significantly different from each other, this is similar to the report of (49).

The higher N retained as percent of nitrogen intake could be ascribed to efficient N utilization of the animals fed POUT or COSM diet. This explains why animals in POUT or COSM treatment had better weight performance than those in CASM treatment. The values of N retained in this study indicate that all the animals are in positive N balance.

Table 6: Nitrogen balance in Yankasa rams fed diets containing cottonseed cake, poultry litter and dried cassava leaf meal

Parameters (g/day)	Dietary treatment			SEM	LOS
	COSM	POUT	CASM		
Nitrogen intake	52.71 ^a	48.72 ^{ab}	46.80 ^b	1.83	*
Faecal N loss	21.06 ^{ab}	14.73 ^b	23.79 ^a	2.52	*
Urinary N loss	3.10 ^{ab}	5.14 ^a	2.79 ^b	0.63	*
Total N output	25.03	19.87	26.58	2.69	NS
N balance	27.67	28.85	20.22	3.18	NS
N. Absorbed	31.65 ^{ab}	33.10 ^a	23.01 ^b	3.04	*
N Retained (as % intake)	52.55	58.77	43.07	5.69	NS

^{a,b,c} means with different superscripts along the same row differ significantly. * significant difference at (P<0.05), SEM=Standard error of the means, LOS=Level of significant

Conclusion and Applications

From the results of this study it could be concluded that:

1. Concentrate diet containing cottonseed cake or poultry litter is an excellent supplement of crude protein for a better rumen metabolites, nutrient digestibility and nitrogen retention of Yankasa ram lambs, they did not impose harmful effect on their health.
2. It was shown that the sources of nitrogen used in this study were safe nitrogen sources without any detrimental effects on lamb's health.

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