Rumen Metabolites and Microbial Load in Fattening Yankasa Rams Fed Urea and Lime Treated Groundnut (*Arachis Hypogeae*) Shell in a Complete Diet

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ABSTRACT

The study was conducted to determine effect of treated groundnut (*Arachis hypogaea*) shell in a complete diet on blood metabolites and microbial load in fattening Yankasa rams. Each kilogram of groundnut shell was treated with 5% urea and 5% lime for treatments 2 (UTGNS) and 3 (LTGNS) respectively. For treatment 4 (ULTGNS), 1 kg of groundnut shell was treated with 2.5% urea and 2.5% lime, but shell in treatment 1 was not treated (UNTGNS). Sixteen Yankasa rams were used and randomly assigned to the four treatments diets with four animals per treatment in a completely randomized design (CRD). The diet was formulated to have 14% crude protein (CP) content. Rumen fluid was collected from each ram at the end of the experiment at 0 and 4 hours post feeding. The samples were then put in a 30 ml bottle and acidified with 5 drops of concentrated sulphuric (0.1N H_2SO_4) acid to trap ammonia. The results of the blood metabolites showed that the mean values of NH_3-N differed significantly ($P<0.05$) among the treatment groups with rams in ULTGNS diet having the highest significant value (31.96 mg/L). TVFs were significantly ($P<0.05$) higher in rams fed UNTGNS diet, and higher in total nitrogen, Effect of sampling periods revealed that NH_3N, TVFs and TP were significantly ($P<0.05$) higher in rumen fluid collected 4hrs post feeding among the rams across the treatment groups, but rumen fluid pH was significantly ($p<0.05$) higher in 0 hour post feeding in all the rams in the treatment diets. In the treatment and sampling period's interaction effects, animals on ULTGNS diet had the highest mean values of NH_3N in both 0 and 4 hours post feeding and were significantly ($P<0.5$) higher compared to rams on the other treatment diets. Rams on UTGNS diet had the highest bacteria load of $4.96X10^5$/ml which was significant ($P<0.05$). However, protozoa counts were significantly ($P<0.05$) higher in rams fed UTGNS diet then followed by ULTGNS diet. The results showed that there was no significant difference ($P>0.05$) in the bacteria count of the animals at both 0 and 4 hours post feeding. But rumen fungi and protozoa load at 0 hour were significantly ($P<0.05$) higher than at 4 hours post feeding. The use of untreated ground groundnut shell in the diet of fattening Yankasa ram was therefore recommended.

Key Words: Rumen metabolites, microbial load, volatile fatty acid, ammonia, total protein

INTRODUCTION

Feed is one of the critical factors that limit livestock production in the tropics especially during the dry season when high quality forages are scarce (Adebowale and Taiwo, 1996). The cost of livestock feeds and feed ingredients in recent years has increased tremendously. Hence, the cost of feeding has become a major problem of livestock production in the developing countries. Aduku (1993) reported cost of feed accounts to 70% of the total cost of animal production. This therefore necessitates the need and interest in exploring neglected or underutilized feedstuff materials, such as groundnut shells which are left after the groundnut was processed and are very much available in the north western zone of the country. Several researches were conducted in this area; however, not much has been done to evaluate nutritive potentials of groundnut shell in the diet of sheep in attempt to reduce the cost of sheep production.

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Sheep can use marginal lands and crop residues as feed and are kept in Nigeria mainly for meat (Bello, 2007). They are ranked second after cattle in terms of meat production (FDLPCS, 1992). FAO (1982) reported sheep to contribute 16% of the total domestically produced meat in Nigeria. Of the four breeds sheep in Nigeria, Yankasa sheep are perhaps the most widely and most numerous breed in the Northern part of Nigeria, they are found in the Sahel, Sudan and Guinea Savannah zones of the Country (Gefu, 2002).

Increasing demand for rams and bucks as slaughtered animals for meat can be satisfied through fattening. The primary objective of fattening is to increase the live weight of the animal and the quality of meat in relatively shorter period (Osuhor, 2002). Animal for fattening can completely be confined while all feeds and water are provided throughout the fattening period, though it can be achieved in a semi-intensive system, where they are offered more feed supplements than the rest of the flock before or after released for grazing.

Groundnut shells were reported to contain 65.7% cellulose, 21.2% carbohydrates, 7.3% protein, 4.5% minerals and 1.2% lipids, since the processed shells from shelling machines contain bits and skins of nuts, the actual protein and lipid contents of this waste material are probably much higher (Abdurrazak *et al.,* 2014).

Information on the utilization of groundnut shell as ingredient for feeding ruminants such as sheep is very scarce. Most of the earlier researches conducted were on groundnut haulms (Malau-Aduli *et al.,* 2003 and Arslan, 2005). The residue is left in the processing area after the nut has being removed, constituting environmental problem (Bello, 2018). Utilization of groundnut shell in the diet of ruminant animals will not only reduce cost of production but also helps in reducing its negative environmental impact. The study aimed to determine carcass percentage and carcass characteristics of fattening Yankasa rams fed a complete diet containing untreated, urea, urea plus lime treated groundnut shell.

MATERIALS AND METHOD

The study was conducted at the Teaching and Research Farm (Small Ruminants Unit of Animal Science Department, Faculty of Agriculture, Ahmadu Bello University, Zaria. It is located in the Northern Guinea Savannah of Nigeria, on Latitude $11^{0}12^{I}$ N and Longitude 7^{0} 33^{I} E at an altitude of 610m above sea level (GPS, 2012). Annual rainfall of 1100 -1200mm and temperature that fluctuates within the range of 14.5-39.5^{0}C (IAR Metrological Service Unit, 2015)

Groundnut shell used in the experiment was obtained from groundnut processing unit in Wanke, Zamfara state, Nigeria. It was ground using a hammer mill machine fitted with 2.5 seive to ease mixing with other ingredients. In treatment 1 the shell was left chemically untreated, therefore served as untreated (UNTGNS).

Each kilogram of groundnut shell was treated with 5% urea and 5% lime for treatments 2 (UTGNS) and 3 (LTGNS) respectively. For treatment 4 (ULTGNS), 1 kg of groundnut shell was treated with 2.5% urea and 2.5% lime. The urea and lime were diluted in water at 2kg in 20 litres of water and sprayed on 40kg of the groundnut shell. The treated shells were ensiled in Persue cowpea improved sacks (PICS) for two weeks) then dried for a day and packed into other bags till the period of the feed formulation.

A total of 16 intact Yankasa rams weighing 24±4kg were purchased from Sheme market of Katsina state. They were randomly allotted to the four treatments at four animals per treatment, in a completely randomized design (CRD). Four treatment diets were formulated with other ingredients to contain 14% CP with 40% inclusion of UNTGNS, UTGNS, LTGNS and ULTGNS respectively. Table 3.2 shows the ingredients composition of the fattening diet.

A daily allowance of complete diet at the rate of 4% body weight per head was offered. The rams were quarantined for two weeks in which prophylactic treatments were given. Dewormed with Albendazole 10% 3ml/20kg orally, sprayed with Ametic(Ascaracide) 20ml/15lts of water, injected with Tetracycine L.A 1ml/10kg (im), Tylosin L A1ml/10kg(im) and Ivomectine 1ml/50kg (sc). Fresh and clean water was provided *ad libitum*, good sanitation practice was adhered to. The fattening trial lasted for 90 days. The animals were weighed bi-weekly to determine weight gain. Daily records of feed intake were taken throughout the experimental period by weighing the feed offered and the left over (orts) in the following day. Daily intake of

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the diets was calculated for each animal by subtracting the left over from the quantity already served to the animals. Daily water intake and temperature were also recorded.

Weight of the individual animals was measured at the onset of the trial after an overnight fasting to obtain their initial weights and subsequently at 2weeks intervals between 8:00-9:00am throughout the feeding trial. Weight gain was determined by subtracting the initial weight from the final weight within the periods. Feed conversion ratio was also determined, dividing the feed intake by weight gain.

Rumen Liquor Collection

Rumen fluid was collected from each ram at the end of the experiment at 0 and 4 hours post feeding. Suction tube was used in the rumen fluid collection. The pH of the rumen fluid was determined using digital pH meter immediately after collection in the farm. Particulate matter was removed by filtering the sample through 4 layers of cheese-cloth. The samples were then put in a 30 ml bottle and acidified with 5 drops of concentrated sulphuric (0.1N H_2SO_4) acid to trap ammonia and stored in a refrigerator (-4^0 c) for the analysis of total and ammonia nitrogen using simple micro Kjeldahl distillation (AOAC, 2005) procedure, and total volatile fatty acids was determined by Gas Chromatography (Cottyn and Bonque, 1968). Microbial count was also carried out using Boyne *et al.* (1957) procedure at the Microbiology Laboratory of College of Veterinary Medicine of Ahmadu University, Zaria to determine microbial counts of the rumen fluid.

Rumen liquid collected was analysed for rumen metabolites and microbial loads were subjected to analysis of variance (ANOVA), (SAS, 2002). Differences among treatment means, were compared using the Duncan Multiple Range Test (DMRT) of SAS software.

The following model was used:

$$\mathbf{X_{ij}=\mu+t_i+e_{ij}}$$

Where:

X_{ij}=Observation measured

μ= Population mean

t_i=Treatment effect (control, urea, lime, urea/lime treatment)

e_{ij}=Experimental error

Repeated measure model was used to statistically analyse samples that were collected twice at 0 and 4 hours post-feeding i e blood and rumen liquor.

Repeated measure model

$$Y_{ijk} = \mu + \tau_i + \delta_{ij} + t_k + (\tau * t)_{ik} + \varepsilon_{ijk}$$

where:

Y_{ijk} = Observation ijk

μ = The overall mean

τ_i= The effect of treatment i (i= 1,2,3,4)

δ_{ij}=Treatment effect within the animals

t_k = the effect of period k (k= 0 & 4hrs)

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$(\tau*t)_{ik}$ = The effect of interaction between treatment i and period k

ε_{ijk} = random error with the mean 0 and variance σ2, the variance between measurements within animals

RESULTS AND DISCUSSION

Characteristics of Rumen Metabolites in Fattening Yankasa Rams.

Tables 2 presents results of treatments, sampling periods and interaction effects respectively at 0 and 4hrs post feeding measurements of rumen ammonia nitrogen (NH_3-N), volatile fatty acids (VFAS), total nitrogen (TN) and pH. The results showed that the mean values of NH_3-N differed significantly ($P<0.05$) among the treatment groups with rams in ULTGNS diet having the highest significant value (31.96 mg/L). TVFs were significantly ($P<0.05$) higher in rams fed UNTGNS diet, and higher in total nitrogen, though not significantly different ($P>0.05$) among the rams fed UNTGNS, LTGNS and ULTGNS diets which were significantly ($P<0.05$) higher than those rams on UTGNS diet. Effect of sampling periods revealed that NH_3N, TVFs and TP were significantly ($P<0.05$) higher in rumen fluid collected 4hrs post feeding among the rams across the treatment groups, but rumen fluid pH was significantly ($p<0.05$) higher in 0 hour post feeding in all the rams in the treatment diets.

In the treatment and sampling period's interaction effects, animals on ULTGNS diet had the highest mean values of NH_3N in both 0 and 4 hours post feeding and were significantly ($P<0.5$) higher compared to rams on the other treatment diets. Rams on UNTGNS diet followed similar trend. However, rams on UNTGNS diet were significantly ($P<0.05$) high in VFAs in both collection periods compared with those on the other treatment diets. Surprisingly, rams on LTGNS diet had the highest mean values of total nitrogen in the both collections and were significantly ($P<0.05$) higher than the rams on the other treatment diets. No statistical difference ($P>0.05$) was observed among the rams across the treatment diets in rumen pH at the both 0 and 4 hours post feeding periods.

Rumen Microbial Load of the Fattening Yankasa Rams

Results of total microbial counts in rumen fluid at 0 and 4hrs post feeding are presented in Tables 5, 6 and 7 for treatment, sampling period and treatment and sampling period's interaction respectively. The results showed the presence of bacteria, fungi and protozoa in the collections.

Table 5, shows the results of the treatment effect. Rams on UTGNS diet had the highest bacteria load of $4.96X10^5$/ml which were significantly ($P<0.05$) higher than microbial load of animals fed UNTGNS, LTGNS **a**nd ULTGNS diets. Rams on LTGNS diet were significantly ($P<0.05$) higher compared to those fungi load in rams fed UNTGNS, ULTGNS and UTGNS diets. However, protozoa counts were significantly ($P<0.05$) higher in rams fed UTGNS diet then followed by ULTGNS diet. The least were those on UNTGNS diet.

Results of sampling period on microbial load are presented in Table 6. The results showed that there was no significant difference ($P>0.05$) in the bacteria count of the animals at both 0 and 4 hours post feeding. But rumen fungi and protozoa load at 0 hour were significantly ($P<0.05$) higher than at 4 hours post feeding.

The interaction effect is shown in Table 7 and the results revealed that rams on UTGNS diet had higher significant ($P<0.05$) values at both rumen fluid samplings periods (0 and 4 hours). However, at 0 hour pot feeding rams on UNTGNS and LTGNS diets had significant ($P<0.05$) higher bacteria count than those on ULTGNS diet and the bacteria counts of the rams on UTGNS diet was significantly ($P<0.05$) higher than those on ULTGNS, UNTGNS and LTGNS diets at 4 hours post feeding. Fungi load was significantly higher ($P>0.05$) in rams fed LTGNS diet at both 0 and 4 hours post feeding. Protozoa counts were significantly ($P<0.05$) higher in animals fed UTGNS diet at 0 hour, but at 4 hours post feeding animals on LTGNS diet were significantly ($P<0.05$) higher than protozoa load of the animals on the other treatment diets.

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CONCLUSION AND RECOMMENDATION

It was observed from the study that urea and lime treatment increases nitrogen absorption and nitrogen retained as percent intake of the fattening rams.

Sampling periods have effect on the rumen metabolites (NH_3N, TVFs and TP) as were higher in rumen fluid collected 4 hours post feeding but rumen pH was higher in 0 hour collection in all the rams along the treatment diets.

Protozoa and load were higher in rams on urea treated diet but more of fungi were observed in rams on lime treated diet.

From results of the fattening study farmers can be advised to feed their rams with the untreated diet that achieved the highest total weight gain with least total cost and recorded the highest apparent profit.

Farmers should be encouraged to ground/crushed/chopped groundnut shell and incorporated it in diet formulation for growing and fattening rams, treated or untreated.

The treatment of groundnut shell with urea and lime in formulating diet for fattening ram could be encouraged.

Other treatment materials cheaper than urea and lime should be sourced and tried in order reduce total production cost experienced in the urea and lime treated diets.

Further researches should be carried out to investigate potentials and anti-nutrition properties of groundnut shell if exist.

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LIST OF TABLES

Table 1. Ingredients composition of fattening experiment diets.

Ingredient (%)	Treatments diets			
	Control	Urea	Lime	Urea/Lime
Groundnut shell	40.00	40.00	40.00	40.00
Maize offal	20.50	33.50	23.00	28.50
C S C	37.50	24.50	35.00	29.50
Bone meal	1.25	1.25	1.25	1.25
Salt	0.50	0.50	0.50	0.50
Premix	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
Calculated CP	14	14	14	14
CSC-Cotton Seed Cake, CP-Crude Protein				

Table 2. Rumen metabolites of fattening Yankasa rams fed UNTGNS, UTGNS, LTGNS and ULTGNS in a complete diet.

Parameters	Treatment diets				SEM	Normal value
	UNTGNS	UTGNS	LTGNS	ULTGNS		
NH_3 N mg/100l	31.22^b	30.85^c	30.30^d	31.96^a	0.15	28.40-33.23
TVFAs mg/L	14.33^a	13.98^b	14.05^b	13.78^c	0.08	10.00-20.50
Total Nitrogen %	2.88^b	2.48^b	2.86^a	2.87^a	0.03	2.50-3.45
Rumen Ph	6.53	6.60	6.57	6.52	0.18	6.0-7.00
Means within the same rows with different superscripts differ significantly (P<0.05) SEM=Standard error of means NH_3N=Ammonia nitrogen TVFTs=Total volatile fatty acids						

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Table 3. Rumen metabolites of fattening Yankasa rams fed UNTGNS, UTGNS, LTGNS and ULTGNS in a complete diet.

Parameters	Period		SEM	Normal value
	0 Hr	4Hr		
NH_3 N mg/100l	30.83^{b}	31.27^{a}	0.15	28.40-33.23
TVFAs mg/L	13.72^{b}	14.39^{a}	0.08	10.00-20.50
Total Nitrogen %	2.61^{b}	2.94^{a}	0.03	2.50-3.45
Rumen Ph	6.86^{a}	6.23^{b}	0.06	6.00-7.00
Means within the same rows with different superscripts differ significantly (P<0.05) SEM=Standard error of means				

Table 4. Treatment and period interaction effect on rumen metabolites characteristics of fattening Yankasa rams fed UNTGNS, UTGNS, LTGNS and ULTGNS in a complete diet.

Parameters	Treatment diets/Period in Hour								SEM
	UNTGNS		UTGNS		LTGNS		ULTGNS		
	0hr	4hr	0hr	4hr	0hr	4hr	0hr	4hr	
NH_3 N mg/100/l	30.8^{b}	31.6^{b}	30.7^{b}	30.9^{c}	30.1^{c}	30.4^{d}	31.6^{a}	32.4^{a}	0.15
TVFAs mg/L	14.0^{a}	14.6^{a}	13.7^{b}	14.1^{b}	13.4^{d}	14.6^{a}	13.6^{c}	14.0^{b}	0.08
Total Nitrogen %	2.1^{d}	2.7^{d}	2.7^{c}	3.01^{c}	2.9^{a}	3.2^{a}	3.2^{b}	3.5^{b}	0.03
Rumen Ph	6.8	6.2	6.8	6.3	6.9	6.2	6.8	6.1	0.18
Means within the same rows with different superscripts differ significantly (P<0.05) SEM= of means									

Table 5. Rumen microbial load of fattening Yankasa rams fed UNTGNS, UTGNS, LTGNS and ULTGNS in a complete diet.

Parameters	Treatment diets				SEM	Normal value
	UNTGNS	UTGNS	LTGNS	ULTGNS		
Bacteria ($X10^5$/ml)	4.12^{b}	4.96^{a}	3.93^{b}	4.06^{b}	0.11	$8.55\text{-}8.18x10^5$
Fungi ($X10^5$/ml)	17.67^{b}	15.50^{d}	25.17^{a}	16.60^{c}	0.48	$6.32\text{-}2.87x10^5$
Protozoa ($x10^5$/ml)	8.37^{c}	11.67^{a}	10.21^{b}	10.36^{b}	0.31	$3.95\text{-}5.34x10^5$
Means within the same rows with different superscripts differ significantly (P<0.05) SEM=Standard error of means						

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Table 6. Period effect on rumen microbial load of fattening Yankasa rams fed UNTGNS, UTGNS, LTGNS and ULTGNS in a complete diet

Parameters	Period		SEM	Normal value
	0 Hr	4Hr		
Bacteria ($X10^5$/ml)	4.37	4.18	0.11	$8.55\text{-}8.18x10^5$
Fungi ($X10^5$/ml)	20.67^a	16.81^b	0.48	$6.32\text{-}2.87x10^5$
Protozoa ($X10^5$/ml)	10.33^a	9.94^b	0.31	$3.95\text{-}5.34x10^5$
Means within the same rows with different superscripts differ significantly (P<0.05) SEM= of means				

Table 7. Treatment and period interaction effect on rumen microbial load of fattening Yankasa rams fed UNTGNS, UTGNS, LTGNS and ULTGNS in a complete diet.

Parameter	Treatment				SEM
	UNTGNS	UTGNS	LTGNS	ULTGNS	
	0hr 4hrs	0hr 4hrs	0hr 4hrs	0hr 4hrs	
Bacteria (CFU/ml)	4.24^b 4.00^b	5.29^a 4.64^a	4.15^b 3.71^c	3.81^c 4.43^a	0.11
Fungi (Spores/ml)	18.33^b 17.00^c	19.00b 12.00d	30.33a 20.00a	15.00c 19.00b	0.48
Protozoa (Count/ml)	8.72^c 8.02^d	13.13^a 10.21^b	8.71^c 11.67^a	10.44^b 9.85^c	0.31
Means within the same rows with different superscripts differ significantly (P<0.05) SEM=Standard error of means					