



**MICROBIAL PROTEIN PRODUCTION AND RUMEN METABOLITES
PARAMETERS OF YANKASA RAMS FED DIETS CONTAINING DIFFERENT
NITROGEN SOURCES WITH *BALANITES AEGYPTIACA* LEAF POWDER AS
RUMEN BUFFER**

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ABSTRACT

Experiment was carried out to evaluate the response of growing Yankasa rams to complete sorghum stover based diets containing varying levels of different nitrogen sources with rumen buffer plant on digestible organic matter fermented in the rumen (DOMR), microbial nitrogen (MN) and rumen metabolites. Complete diets containing 16% crude protein (CP) was formulated for the experiment. Urea was incorporated into the diets at 0, 0.5, 1, 1.5 and 2% with cottonseed cake at 20, 15, 10, 5 and 0% and designated as diet 1, 2, 3, 4, and 5% respectively. Twenty growing Yankasa rams were used for the experiment and randomly allocated to five treatment groups of four animals each in completely randomized designed (CRD). Rams were fed experimental diets and water *ad libitum*. The result reveals ($P < 0.05$) difference in DOMR and MN. Higher mean values of 0.37, 0.36 and 0.34 kg/d DOMR were recorded in animal fed diet 2, 3 and 5 with the least value 0.22 kg/day recorded in animals fed diet 4. MN production were higher 11.72, 11.65 and 10.81 g/day in animals fed diet 2, 3 and 5 while the least 7.03 g/day in animals fed diet 4. ($P < 0.05$) difference were observed in rumen pH 0 hour before feeding, total volatile fatty acids (TVFA), acetate, propionate, butyrate and rumen $\text{NH}_3\text{-N}$ 0 hour before and 4 hours after feeding across the diets. $\text{NH}_3\text{-N}$ was within the optimal levels for microbial activities. Inclusion of urea at graded levels of up to 2% in a complete diet has no detrimental effect on performance, DOMR, MN production and rumen parameters. Diet 3 is therefore recommended for growing Yankasa rams.

Keywords: Microbial protein, *Balanites aegyptiaca*, Leaf powder, Rumen ammonia nitrogen, Yankasa ram.

INTRODUCTION

The digestive process of ruminants is affected by a variety of factors, among them the proportion of degradable dietary N needed to support ruminal microbial activity. The rumen microorganisms ferment ingested organic matter (OM) to obtain energy for maintenance and growth and produce volatile fatty acids (VFAs) and ammonia (Khattab *et al.*, 2013). According to (Russell *et al.*, 1992; Khattab *et al.*, 2013) microbial growth is proportional to the intake and extent of fermentation of carbohydrates as long as an adequate N source is available to the bacteria. Mehrez *et al.* (1977) observed that the optimum microbial growth depends on the maximum rate of fermentation, which depends on the maximum ammonia concentration and the minimal ammonia concentration for maximal rate of fermentation was estimated to be 23.5mgdl⁻¹ rumen fluid. Urea is widely used as a dietary supplement for ruminants because it



is an inexpensive nitrogenous compound and has long been accepted as a replacement for some of the degradable true protein in diets (Pinos-Rodríguez *et al.*, 2010).

Urea is rapidly and extensively degraded in the rumen yielding maximum ammonia concentrations within the first few hours of ingestion (Puga *et al.*, 2001). Urea can provide NH₃ and so promote efficient utilization of fibrous roughages, if the rumen pH does not fall below about 6.0 (Ørskov and Ryle, 1990). Trees and shrubs play an important role in dry season ruminant feeding in many tropical and subtropical environments around the world. They have been identified as a source of feeds or feed ingredients for ruminants because of their high nutritive value and positive effects on rumen function (Fasae *et al.*, 2010; Arif *et al.*, 2016). These trees possess many advantages such as abundance, adaptability, accessibility, high protein content and quality in terms of available energy, minerals and vitamins (Ramírez, 1998). *Balanites aegyptica* is a medicinal plant and commonly known as hingot or desert date; belongs to Zygophyllaceae or Balanitaecae family (Kumawat *et al.*, 2012). *B. Aegyptica* has some properties such as anti-inflammatory, anthelmintic, antioxidant, anti-nociceptive, antiviral, antimicrobial, anticancer, anti-diabetic and anti-asthmatic effect in various animals. (Ajayi and Ifedi, 2014; Saboo *et al.*, 2014).

However, a major constraint to the use of these trees is the presence of toxic and plant secondary metabolite constituents; which have different but adverse effects on animal performance including loss of appetite and reductions in dry matter intake and protein digestibility (Das *et al.*, 2010). The present study was undertaken with an objective of assessing the effects of varied sources of nitrogen on haemato-biochemical parameters and rumen metabolites in growing Yankasa sheep.

MATERIALS AND METHODS

The Study Area

The research will be carried out at the Livestock Teaching and Research Farm, University of Maiduguri, Borno State. Maiduguri lies between Latitude 11⁰51' and 12⁰N and longitude 13⁰05' and 14⁰E. It falls within the Sahel savannah zone of West Africa, which is characterized by 4-5 months of rainfall with an average of 500-600mm. The ambient temperature ranges between 27-45⁰C, while the Relative humidity varies between 19 to 78% and remains at 45% during the wet season (Mayomi and Mohammed, 2014).

Collection and preparation of experimental feed materials

Sorghum stover was collected from harvested sorghum farms within Maiduguri and its environs after harvest and removal of the seeds. The stover was milled to about 3-5cm using a stover milling machine which are then bagged and stored before usage. Leaves of *Balanites aegyptiaca* (Desert date) were obtained by pruning branches of the trees. The pruned materials were sun-dried for 2-3 days before collecting the leaves. The leaves powder was produced by grinding using mortar and pestle. The powder was packed in polyethylene bags before usage. Other feed ingredients; urea graded fertilizer, cottonseed cake, wheat offal, sorghum panicle, molasses, bone meal and salt were purchased from Gamboru and Cattle market at Maiduguri.

Experimental animals and their management

Twenty (20) growing Yankasa rams were used for the experiment. The animals were purchased from Kasuwan Shanu at Maiduguri with an average weight of 18.23 kg. The animals were quarantined for 14 days during which they were tagged, vaccinated against *pestes despetit ruminant*, dewormed with Albendazole at 12.5mg/kg body weight for internal parasites and injected intramuscularly with antibiotic, i.e., Oxytetracycline L.A (Alfasan®) 20%, at 1ml per 10kg body weight. During this period the animals were fed with groundnut haulms and maize

offal for adaptation. The animals were divided randomly into five dietary groups of four animals each in a completely randomized design and allocated to their respective diets. All the animals were housed in well-ventilated pens (2 m × 2 m) pens that were disinfected using Dettol at the rate of 10mls/4litres of water as a fumigant used before the arrival of the experimental animals to maintained healthy surroundings and proper cleanliness during the experimental period.

Experimental diets

Complete diets containing 16% crude protein (CP) were formulated for the experiment. Urea was incorporated into the diets at 0, 0.5, 1, 1.5 and 2% alongside with cottonseed cake at 20, 15, 10, 5 and 0% and designated as diet 1, 2, 3, 4, and 5%, respectively. Ingredient and compositions of the experimental diets was presented in Table 1.

Table 1: Ingredients and chemical composition (%) of experimental diets

Ingredients	Diets				
	1	2	3	4	5
Wheat offal	26.93	32.73	38.52	44.32	50.11
Sorghum panicle	27.32	26.02	24.73	23.43	22.14
Urea	0.00	0.50	1.00	1.50	2.00
Cotton seed cake	20.00	15.00	10.00	5.00	0.00
Milled sorghum stover	20.00	20.00	20.00	20.00	20.00
Molasses	3.00	3.00	3.00	3.00	3.00
Bone meal	2.00	2.00	2.00	2.00	2.00
Salt	0.50	0.50	0.50	0.50	0.50
<i>B. aegyptiaca</i> leaf powder	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
Parameters					
Dry matter	93.01	93.2	92.33	93.87	93.07
Organic matter	86.32	88.13	90.08	88.32	89.06
Crude protein	16.04	16.13	16.02	16.18	15.95
Ash	6.69	5.07	2.25	5.55	4.01
Neutral detergent fibre	37.34	35.41	36.54	33.87	36.39
Acid detergent fibre	28.43	25.42	22.16	24.52	23.98
Hemicellulose	8.91	9.99	14.38	9.35	12.41

Diet 1 = 0% with cottonseed cake (CSC) at 20%, Urea, Diet 2 = 0.5% Urea with CSC at 15%, Diet 3 = 1.0% Urea with CSC at 10%, Diet 4 = 1.5% Urea with CSC at 5%, Diet 5 = 2.0% Urea with CSC at 0%

The respective diets were offered at 7.00 hr and 14.00 hr daily. During the feeding trial, animals were offered weighed quantities of respective complete diets *ad libitum*. Residues, if any, were weighed on the next day morning before offering of feed in order to calculated daily feed intake of animals. Fresh clean drinking water was provided to the animals and daily water intake is recorded. For growth performance, initial body weight, weekly weights and final body weights of the animals were measured in order to ascertain the growth performance. The experimental period lasted for 84 days (including 14 days adaptation period).

Faecal collection

Three animals were randomly selected from each treatment and moved to metabolic cages after feeding trial. The animals were fed their respective diets and fresh clean water daily. Faeces was collected and bulked for 7 days period and the amounts of feed offered and



individual refusals and faeces were weighed daily. After the 7 days faeces collection period a pooled representative sample was collected for laboratory analysis after thorough mixing of the faeces from each selected animal.

Rumen fluid collection

Rumen liquor was collected in the morning (0 hour before feeding) and (4 hours after feeding) at the last week of the experiment with a suction tube as describe by Abubakar *et al.* (2010). The tube was inserted from the mouth into the rumen where 20mls of rumen liquor was withdrawn from three (3) animals in each treatment. pH of each sample was immediately determined by using pH meter. The rumen liquor was strained through layers of surgical gauge to remove particulate matter and taken to laboratory for the analysis of total volatile fatty acids, individual volatile fatty acids and rumen ammonia-nitrogen before and after feeding.

Chemical analysis

Feed samples were ground through a 1 mm mesh sieve and analyzed for dry matter (DM) (55°C for 24 h), Nitrogen (or CP) content was determined by the automated Kjeldahl method (AOAC, 1990, ID No 945.01), neutral detergent fiber (NDF) and acid detergent fiber (ADF) by procedure of Van Soest *et al.* (1991) without amylase, sodium sulfite, or correction for residual ash. Rumen Ammonia N concentrations were determined by Kjeldahl distillation according to AOAC (1997; #954.01). Total and individual VFAs were analysed according to Erwin *et al.* (1961) using a gas chromatograph (Agilent 6890 N; Agilent Technologies, New York, NY, USA).

Chemical analysis calculation of DOMR and Microbial N (MN)

Feed and faeces samples were ground through a 1 mm mesh sieve and analyzed for dry matter (DM) (55°C for 24 h), Nitrogen (or CP) content was determined by the automated Kjeldahl method (AOAC, 1990, ID No 945.01), neutral detergent fiber (NDF) and acid detergent fiber (ADF) by procedure of Van Soest *et al.* (1991) without amylase, sodium sulfite, or correction for residual ash.

1) Calculation of DOMR

$$\text{DOMR} = \text{Feed intake} \times \text{DM content} \times \text{OM content} \times \text{OM digestibility} \times 0.65$$

2) Calculation of Microbial N (MN) yield:

$$\text{MN} = 32\text{g/kg DOMR (ARC 1984)}$$

Experimental design

The experimental design was a completely randomized design (Steel and Torrie, 1980), with five treatments of four animals each.

Statistical analysis

Data collected were subjected to Analysis of variance (ANOVA) as described by Steel and Torrie (1980) using MINITAB 16 (2014). Where significant differences existed between means, Duncan's new multiple range test (DMRT) was used to separate them.

RESULTS AND DISCUSSION

There was significant ($P < 0.05$) difference in TFI g/day as presented in Table 2. Rams fed diet 2 and 3 recorded the Highest TFI of 1177.63 and 1181.39g/day while those fed diet 4 had the least 961.50 g/day.

Table 2: Microbial protein production by Yankasa rams fed diets containing different nitrogen sources with *Balanites aegyptiaca* leaf powder as rumen buffer

Parameters	Diets					SEM
	1	2	3	4	5	
Total feed intake (g/day)	1076.10 ^b	1177.63 ^a	1181.39 ^a	961.50 ^c	1144.71 ^{ab}	31.93*
ADG (g)	106.86 ^b	132.00 ^{ab}	155.57 ^a	81.43 ^c	98.14 ^b	9.86*
OMD (%)	51.05 ^b	58.26 ^a	57.00 ^a	42.37 ^c	54.78 ^{ab}	2.23*
DOMR (kg/d)	0.29 ^b	0.37 ^a	0.36 ^a	0.22 ^c	0.34 ^a	0.02*
Microbial N (g/d)	9.17 ^b	11.72 ^a	11.65 ^a	7.03 ^c	10.81 ^a	0.58*

^{a,b}Means within each row with different superscripts are significantly different, *(P<0.05), DOMR= digestible organic matter fermented in the rumen, ADG=Average daily weight gain, OMD= Organic Matter Digestibility, Diet 1 = 0% with cottonseed cake (CSC) at 20%, Urea, Diet 2 = 0.5% Urea with CSC at 15%, Diet 3= 1.0% Urea with CSC at 10%, Diet 4 = 1.5% Urea with CSC at 5%, Diet 5 = 2.0% Urea with CSC at 0%, SEM standard error mean, NS= Not significant

The average daily weight gain (ADG) was significantly (P<0.05) different across the diets. Higher daily weight gain 155.57 g/day was recorded for animals fed diets 3 while the least 81.43 g/day was recorded in animal fed diet 4. Significant (P<0.05) difference was observed in dry matter digestibility (DMD) of animals fed experimental diets. Dry matter digestibility was higher 61.60 and 59.94% in animals fed diets 2 and 3, followed by 55.72 and 58.18% in those fed diet 1 and 5 while the least 46.99% in those fed diet 4.

Significant (P<0.05) difference was recorded in digestible organic matter fermented in the rumen (DOMR) and Microbial nitrogen (MN). The result reveals that higher mean values of 0.37, 0.36 and 0.34 kg/d DOMR were recorded in animal fed diet 2, 3 and 5 with the least value 0.22 kg/day recorded in animals fed diet 4. The DOMR in animals fed diet 1 was 0.29 kg/d. The mean value for MN were higher 11.72, 11.65 and 10.81 g/day in animals fed diet 2, 3 and 5 while the least 7.03 g/day was recorded in animals fed diet 4. It can be observed in this study increase in DOMR and MN is related to increase in daily weight gain.

The range of 34.58 to 36.16 g/kg DOMR reported by Dipu *et al.* (2008) fell within the range of higher values 0.34 to 0.37kg/d DOMR obtained in the present study. The values in this study concurs with the range of 14 to 49 g/kg DOMR specified by ARC (1984). The higher value of 11.72, 11.65 and 10.81 g/d microbial N recorded in this study were lower than 44.80 g/day reported by Misra *et al.* (2006), but greater than 4.99 g/day reported by Khattab *et al.* (2013). It was also observed by Khattab *et al.* (2013) microbial N synthesis (g/day) increased linearly with increasing urea supplementation which will also reflect in increased purine derivatives (PD). The documented range value of 9.90 to 10.83 MN g/d by Dipu *et al.* (2008) was lower than the reported value in this study. According to Dipu *et al.* (2008) if, a dietary regime has a high nitrogen conversion efficiency (NCE), less nitrogen is excreted in urine and more microbial protein is produced which coincides with the results in this study

Rumen metabolites of Growing Yankasa Rams Fed Diets Containing different Nitrogen Sources with *Balanites Aegyptiaca* Leaf Powder as Rumen Buffer

The results of rumen metabolites parameters of growing Yankasa rams fed diets containing different nitrogen sources with *Balanites aegyptiaca* as rumen buffer plants. The result revealed significant (P<0.05) difference in rumen pH 0 hour before feeding as presented in Table 3. No significant (P>0.05) difference in rumen pH after feeding.

Table 3: Rumen metabolites of growing Yankasa rams fed diets containing different nitrogen sources with *Balanites aegyptiaca* leaf powder as rumen buffer

Parameters	Diets					SEM
	1	2	3	4	5	
pH						
0 hours (before feeding)	6.67 ^b	6.67 ^b	6.8 ^b	6.83 ^b	7.43 ^a	0.09*
4 hours (after feeding)	6.23	6.63	6.33	6.63	6.43	0.08 ^{NS}
TVFA (mmol/L)						
0 hours (before feeding)	122.72 ^b	129.14 ^a	130.54 ^a	124.55 ^b	118.17 ^c	1.27*
4 hours (after feeding)	147.22 ^c	176.39 ^a	170.74 ^b	178.27 ^a	174.99 ^a	3.09*
Acetate (mmol/L)						
0 hours (before feeding)	60.48 ^b	61.29 ^b	65.89 ^a	57.87 ^d	59.10 ^c	0.75*
4 hours (after feeding)	77.49 ^e	93.82 ^b	86.86 ^d	97.64 ^a	90.16 ^c	1.84*
Propionate (mmol/L)						
0 hours (before feeding)	38.39 ^c	40.25 ^b	38.43 ^c	43.68 ^a	40.91 ^b	0.54*
4 hours (after feeding)	43.34 ^e	50.88 ^c	53.97 ^b	49.33 ^d	57.45 ^a	1.27*
Butyrate (mmol/L)						
0 hours (before feeding)	23.85 ^c	27.60 ^a	26.22 ^b	23.00 ^c	18.16 ^d	0.88*
4 hours (after feeding)	26.39 ^c	31.69 ^a	29.91 ^b	31.30 ^a	27.38 ^c	0.58*
NH₃-N (mg/L)						
0 hours (before feeding)	69.28 ^c	80.60 ^a	76.70 ^b	66.84 ^d	70.44 ^c	1.37*
4 hours (after feeding)	70.58 ^c	81.90 ^a	78.00 ^b	68.14 ^d	71.74 ^c	1.37*

^{a, b}. Means within each row with different superscripts are significantly different, *(P<0.05), Diet 1 = 0% with cottonseed cake (CSC) at 20%, Urea, Diet 2 = 0.5% Urea with CSC at 15%, Diet 3 = 1.0% Urea with CSC at 10%, Diet 4 = 1.5% Urea with CSC at 5%, Diet 5 = 2.0% Urea with CSC at 0%, SEM standard error mean, NS= Not significant, TVFA= Total volatile fatty acids.

Significant (P<0.05) difference was observed in total volatile fatty acids (TVFA), acetate, propionate, butyrate and rumen NH₃-N 0 hour before and 4 hours after feeding across the diets. Rumen pH values of the animals before feeding and after feeding in this study were 7.43 and range of 6.23 to 6.63. The value before feeding was lower than 7.52 reported by Abdullahi (2021) for growing Yankasa rams fed poultry litter ensiled sesame chaff diet with various supplements. Van Soest (1994) stated the pH range for optimal microbial activity as 6.2 to 7.2 which were in accordance with the value range after feeding in this study. The values recorded after feeding fell within the range of 6.00 to 7.20 suitable for the growth and activities of microbes reported by (Jallow and Hsia, 2011).

The higher TVFA values of 129.14 and 130.54 mmol/L recorded in this study were higher than 15.33 mmol/dl reported Jokthan by *et al.* (2013) and 13.10 mmol/dl recorded for Yankasa rams fed with sun dried broiler litter (SDBL) (Abubakar *et al.*, 2010). It was also higher than the range of 6.19 to 11.59 mmol/dl reported by (Ngele, 2008) and 14.02 to 15.67mmol/dl by (Elemam *et al.*, 2009).

The TVFA of rumen fluid in this study fell within the range of 31 to 196 mmol/L documented by (Woyengo *et al.*, 2004). The fluid TVFA concentrations in this study before feeding were within the normal range of 70 to 150 mmol/L (McDonald *et al.*, 1995). The molar proportions of acetate 65.89 and 97.64 mmol/L before and after feeding in this study were above the range of 14.00 to 56.11 mmol/land 47.8 to 56.4% molar proportions of TVFA documented by Abdullahi (2021) and Woyengo *et al.* (2004). The propionate in ruminal fluid recorded in this study 43.68 and 57.45 mmol/L before and after feeding were within the range of 39.16 to 70.15 mmol/l by Abdullahi (2021) for Yankasa supplemented with Groundnut cake fed with treated sesame basal diet and above the range of 12.5 to 18.9% documented by



Woyengo *et al.* (2004). Similarly, the butyrate in ruminal fluid recorded in this study 27.60 and 31.69 and 31.30 mmol/L before and after feeding was also above the range of 9.7 to 13.5% documented by Woyengo *et al.* (2004). The butyrate in this study fell within the range of 8.86 to 28.96 mmol/l and 7.18 to 24.89 mmol/l reported by Abdullahi (2021). Production of VFA depends on the availability of fermentable OM in the feed (Oosting, 1993).

The ruminal NH₃-N concentration value 80.60 mg/L in this study 0 hour before feeding was above the range of 41.31 to 63.62 mg/L before feeding reported by Abdullahi (2021). Urea can provide NH₃ and so promote efficient utilization of fibrous roughages, if the rumen pH does not fall below about 6.0 (Ørskov and Ryle, 1990). Ruminal NH₃-N concentration figures in this study fell within the range of 9 to 234 mg/L documented by Woyengo *et al.* (2004). The value reported was also higher than the range of 14.50 to 27.60 reported by (Singh *et al.*, 2010). The ruminal NH₃-N value 81.90 mg/L 4 hours after feeding in this study was above the range of 52.99 to 64.85 mg/100L after feeding reported by Abdullahi (2021). The values also fell above range of 21.5 to 37.5mg/100L reported by Abubakar *et al.* (2010) in nutritional evaluation of different sources of nitrogen on rumen metabolites in growing Yankasa sheep. The NH₃-N concentration for all the diets in this study was above the minimum concentration of 50mg/l for microbial growth and 23.80mg/L for optimum microbial fibre digestibility as reviewed by (Singh *et al.*, 2010). Estimates of the critical level of ammonia in the rumen fluid for efficient digestion have been reported to be as low as 50 mg N/l or as high as 200 mg N/l (Leng, 1990). The NH₃-N concentration in ruminal fluid for all diet were within the range of 40 to 250 mg/L, which was reported by Erdman *et al.* (1986) to be optimal for digestion depending on the potential digestibility of feed. Ruminal fluid NH₃-N concentration depends on quantity and degradability of N in ingested feed, the rate of incorporation of NH₃-N into microbial protein and rates of passage and absorption of NH₃-N from the rumen (Streeter and Horn, 1984; Oosting, 1993).

CONCLUSION AND RECOMMENDATIONS

Inclusion of varied levels of different nitrogen sources in diet 3 with 1% urea and 10% cottonseed cake in sorghum stover based diet which gave the best results in terms of average daily weight gain of 155.57 g/day, produces high DOMR (0.36 kg/d) and production of microbial nitrogen (11.65 g/d) as compared to other diets. The diet is therefore recommended for growing Yankasa rams.

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