

# RUMINAL AMMONIA AND BLOOD UREA METABOLISM IN SHEEP FED HAY WITH OR WITHOUT CONCENTRATE SUPPLEMENT

T.A. ADEGBOLA

*Department of Animal Science  
University of Nigeria, Nsukka, Nigeria.*

*(Received 29 June 1988; accepted for publication 18 July 1988)*

## ABSTRACT

Metabolism of ruminal ammonia and blood urea was investigated in West African Dwarf ewes and wethers, fed a low quality hay with or without concentrate supplementation, using single injection of [ $^{15}\text{N}$ ] ammonium chloride ( $^{15}\text{HN}_4\text{Cl}$ ) or [ $^{15}\text{N}$ ] urea into the rumen and blood respectively.

The percentages of  $^{15}\text{N}$  administered intraruminally as  $^{15}\text{HN}_4\text{Cl}$  recovered in the urine, faeces and milk of the ewes were 4.3, 9.0 and 3.1. Also 32.3 and 28.7% of [ $^{15}\text{N}$ ] urea administered into the blood were recovered in the urine of the wethers.

Ruminal ammonia contributed 50.6% of protozoal-N in sheep fed hay and 14.2, 78.7 and 35.0% respectively in sheep fed hay and concentrate. Also, 59.9 and 7.9% of ruminal ammonia-N was derived from blood urea of sheep fed hay and hay plus concentrate respectively.

The inclusion of concentrate in the diet increased the extent of ruminal bacteria protein synthesis but not that of the protozoa. However, the contributions of ruminal ammonia to blood urea synthesis and of blood urea to ruminal ammonia were sharply decreased in the presence of the concentrate.

**Key Words:** Ruminal Ammonia, Blood Urea, Metabolism, Sheep

## INTRODUCTION

One of the factors limiting ruminant production in the tropics is the seasonal variation in the availability and composition of forages. The abundant and high quality forages of the rainy season of 4 to 7 months duration, deteriorates rapidly in the dry season with increased fibre and decreased protein content, resulting in reduced feed intake and digestibility. It is usual to supplement the poor quality forages with energy and protein concentrates. Such a supplementation could affect the nitrogen conservation mechanism by reducing blood urea recycled

into the rumen, which could be appreciable in sheep fed low N roughage (Cocimano and Leng, 1967; Norton *et al*, 1982). It could also affect the extent of utilization of ruminal ammonia-N for microbial protein synthesis, which could be as low as 20% (Salter *et al*, 1979) or as high as 100% (Neutze *et al*, 1986).

The extensive work done on ruminal ammonia metabolism in ruminants by isotopic tracer techniques (Cocimano and Leng, 1967, Salter *et al*, 1979; Norton *et al*, 1982) is limited primarily to the Western countries. No comparative figures are available for indigenous breeds of ruminants in Nigeria.

The present work was therefore undertaken to study the extent of utilization of ruminal ammonia-N for urea, microbial and milk protein synthesis and, the contribution of blood urea to ruminal ammonia in sheep fed hay or hay plus concentrate supplement.

### MATERIALS AND METHODS

Four West African dwarf wethers, 2 to 2<sup>1</sup>/<sub>2</sub> years old and weighing 26 to 31 kg, fitted with permanent rumen cannula and two non-fistulated lactating ewes of 24 kg live weight were used. They were housed individually in metabolism cages. The wethers received their daily rations in equal amounts every hour but the ewes were fed twice daily at 0730 and 1700 h. Water (100 ml) was orally or intraruminally administered to each sheep after each feeding time.

The basal diet (Diet 1) was Giant star (*Cynodon nlemfuensis*) hay (7.7% crude protein). Diet 2 was the same hay supplemented with a concentrate of cassava and groundnut meal (2:1) at 17.5% crude protein. Two of the four wethers (A and B) were maintained on 360g of hay and the other two (C and D) were supplemented with 360g of concentrate. The two lactating ewes were presented individually with 360g hay and 360g concentrate.

On the day of isotope administration, sheep A and C received a single injection of [<sup>15</sup>N] ammonium chloride (250 mg, 99 atom % excess) dissolved in 100 ml water into the rumen and dispersed into the various sections of the rumen by means of a plastic tube. Sheep B and D were given a single intravenous injection of [<sup>15</sup>N] urea (250 mg, 95 atom % excess) dissolved in 10 ml isotonic saline. The two ewes (E and F) were orally dosed with aqueous solution (100 ml) of [<sup>15</sup>N] ammonium chloride (200 mg, 99 atom % excess).

Samples of rumen fluid were obtained at intervals by suction through a plastic tube

and strained through muslin. Bacteria and protozoa in the rumen fluid were separated (Blackburn and Hobson, 1960) washed twice with saline and stored at -10°C. Blood samples were collected in heparinized tubes and centrifuged at 2000 rpm for 20 min. The blood plasma was also stored at -10°C. Urine and faecal samples were collected for five days and the ewes were hand-milked before each feeding time. Samples were stored at -10°C.

The proximate analysis of the feeds were determined (AOAC, 1970). The ruminal ammonia, blood urea, urine, faeces, and the protozoal and bacterial fractions were prepared for <sup>15</sup>N analysis (Nolan and Leng, 1972), except that sec-octyl alcohol was used to prevent frothing of samples during distillation. The <sup>15</sup>N analysis was by mass spectrometry.

Ammonia in the ruminal fluid and urea in the blood were determined (Fawcett and Scott, 1960) ad modified (Chaney and March, 1962).

### Calculation

Enrichment with time curves for ruminal ammonia N, blood urea N, bacterial N and protozoal N after a single injection of [<sup>15</sup>N] ammonium chloride into the rumen of [<sup>15</sup>N] urea into the blood, were obtained and two exponential components were extracted from each curve (Van Liew, 1967). The area under each curve was calculated by integrating the equation to the curves (Nolan and Leng, 1972). The proportion of secondary pool (blood urea-N, bacterial N, protozoal N) derived from the primary pool (ruminal ammonia N), was obtained by dividing the area of the secondary pool by that of the primary pool.

### RESULTS

The results of Dry matter (DM), organic matter (OM) intake, digestibility and N-

balance of the sheep, are presented in Table I. The DM, OM and N intakes of sheep on Diet 1 were low but digestibility values were high. The animals on Diet 1 were on negative N balance. The intakes of DM, OM and N were higher on Diet 2 and nutrient digestibility was also higher.

The percentages of  $^{15}\text{N}$  administered as [ $^{15}\text{N}$ ] ammonium chloride or [ $^{15}\text{N}$ ] urea, recovered in the faeces, urine and milk are shown in Table 2. Of the  $^{15}\text{N}$  administered as [ $^{15}\text{N}$ ] urea, 32.3 and 28.7% were recovered in the urine. Much lower percentages (6.9 and 5.2%) of  $^{15}\text{N}$  of [ $^{15}\text{N}$ ] ammonium chloride intraruminally administered was recovered in the urine of the wethers; 4.4 and 4.8% were recovered in the urine of the ewes. There was no recovery of  $^{15}\text{N}$  in the faeces of the wethers but 8.5 and 9.6% were recovered in the faeces of the ewes. Also, 3.9 and 3.2% of  $^{15}\text{N}$  administered intraruminally to the ewes were recovered in the milk.

The ruminal ammonia-N and blood urea N levels and their utilization for microbial protein synthesis, are shown in Table 3. Ruminal ammonia-N and blood urea N levels were depressed by supplementation of hay with concentrate. The percentages of ruminal ammonia-N derived from blood urea N were 59.9 and 7.9% for animals on Diets 1 and 2 respectively. Also the percentage contributions of ruminal ammonia-N to blood urea N and bacterial N were much higher for sheep fed, Diet 1 than Diet 2. The contribution of ruminal ammonia N to protozoal N was similar for both diets.

## DISCUSSION

The DM and OM intakes of the sheep on Diet 1 were less than 3 to 5% of body weight (BW) (Olubajo and Oyenuga 1974). The intake of DM and OM was higher for sheep on Diet 2 but the intake of DM was less than 2.75% of BW obtained by Adu and

Olaloku (1976) for similar diet. The digestible organic matter (DOM) intake by sheep on Diet 1, was less than the maintenance requirement (0.036 kg per kg of  $\text{BW}^{0.73}$ ) for West African Dwarf sheep (Adegbola *et al.*, 1980) but DOM intake by animals on Diet 2, was sufficient for maintenance. The low intake of tropical forages in the dry season has been reported by Oyenuga (1957), who showed that the decline in nutritional quality was due to increased fibre and decrease crude protein content. Animals subsisting on such forages are prone to weight losses.

Nitrogen intake was also low in the sheep fed Diet 1 and the N intake was not sufficient for maintenance, as indicated by the negative N balance. The sheep on Diet 2 were in positive N balance.

The digestibilities of DM, OM and N were high for both diets. The values were slightly higher than those obtained for similar diets (Adegbola *et al.*, 1977).

The percentages of  $^{15}\text{N}$  of ammonium chloride recovered in the urine of the wethers and ewes were low compared with values of 38.6% (Piva and Silva, 1968) and 26% (Nolan and Leng, 1972) following intraruminal injection of [ $^{15}\text{N}$ ] ammonium chloride. The high level of  $^{15}\text{N}$  retained by sheep could reflect utilization in the rumen and exchange within the animals' body. Also,  $^{15}\text{N}$  was not recovered in the faeces after intra-ruminal administration of [ $^{15}\text{N}$ ] ammonium chloride to the wethers but some (8.5 and 9.6%) were recovered in the faeces of the ewes. The hourly feeding regime adopted for the wethers might have resulted in more extensive digestion of  $^{15}\text{N}$ -labelled microbes with very little loss of  $^{15}\text{N}$  in the faeces. The  $^{15}\text{N}$  recovered in the faeces of the ewes, was similar to 7.9% reported elsewhere (Piva and Silva, 1968).

There was a high retention of  $^{15}\text{N}$  administered as [ $^{15}\text{N}$ ] urea. The high retention of intravenously administered [ $^{15}\text{N}$ ] urea has also been reported (Mugerwa and Conrad,

Table 1  
The percentage of [ $^{15}\text{N}$ ] ammonium chloride or [ $^{15}\text{N}$ ] urea recovered in the faeces, urine and milk of sheep

$^{15}\text{N}$ Animals	$^{15}\text{N}$ recovered Diet	% Tracer administered	$^{15}\text{N}$ administered (mg)	Pool to which administered	$^{15}\text{N}$ recovered (%)			
					Faeces	Urine	Milk	Total
A	1	[ $^{15}\text{N}$ ] ammonium chloride	65.5	rumen ammonia	0	6.9	—	6.9
B	1	[ $^{15}\text{N}$ ] urea	116.7	blood urea	0	32.3	—	32.3
C	2	[ $^{15}\text{N}$ ] ammonium chloride	65.5	rumen ammonia	0	5.2	—	5.2
D	2	[ $^{15}\text{N}$ ] urea	116.7	blood urea	0	28.7	—	28.7
E	2	[ $^{15}\text{N}$ ] ammonium chloride	52.4	rumen ammonia	8.5	4.4	3.9	16.8
F	2	[ $^{15}\text{N}$ ] ammonium chloride	52.4	rumen ammonia	9.6	4.8	3.2	17.6

Table 2  
Summaries of dry matter (DM), organic matter (OM) and N intakes and digestibility values by sheep fed hay or hay plus concentrate supplement

Diets	Live weight (kg)	DM intake (kg/d)	OM intake (kg/d)	DM digestibility (kg/d)	OM digestibility	Digestible OM intake	N intake (g/d)	Urinary N (g/d)	Faecal N (g/d)	N digestibility	N in milk (g/d)	N balance (g/d)
1	26.1	0.189	0.175	76.4	77.5	0.136	2.33	2.15	0.71	69.5	—	-0.53
2 <sup>+</sup>	30.9	0.531	0.495	82.9	87.1	0.430	10.16	3.05	2.26	77.8	—	4.85
2 <sup>++</sup>	24.1	0.536	0.504	76.4	80.2	0.404	9.44	0.49	2.23	76.4	1.55	5.17

<sup>+</sup> Mean values for the wethers.

<sup>++</sup> Mean values for the ewes.

Table 3  
Ruminal ammonia and blood urea metabolism in West African dwarf sheep

Diets	Ruminal ammonia-N (mg/100 ml)	Blood urea N (Mg/100ml)	Ruminal NH <sub>3</sub> -N from blood 3 urea-N (%)	Blood urea-N from ruminal (%)	Bacterial-N from ruminal ammonia-N (%)	Protozoal-N from ruminal ammonia-N (%)
1	12.44	11.35	59.91 <sup>+</sup> (14.38) <sup>++</sup>	50.50 (34.50)	60.44 (24.44)	35.61 (13.76)
2	6.19	5.67	7.94 (2.66)	14.20 (12.99)	78.73 (19.18)	35.01 (12.47)

<sup>+</sup> Value at infinity.

<sup>++</sup> Value at t = 10 h.

1971), Nolan and Stachiw, 1979). Retention of  $^{15}\text{N}$  of urea is inversely related to dietary N intake (Cocimano and Leng, 1967).

The percentages of  $^{15}\text{N}$  recovered in the milk protein of the ewes after intraruminal injection of  $[^{15}\text{N}]$  ammonium chloride were less than 5.6 obtained by Piva and Silva (1968). Land and Virtanen (1959) noted that the proportion of ammonia used for milk protein synthesis decreased with liberal protein feeding and increased with the level of milk production. Milk production of the ewes was low (0.30 litre/day).

The non-recovery of  $^{15}\text{N}$  in the faeces of the wethers after intravenous injection of  $[^{15}\text{N}]$  urea seems to indicate that urea was recycled into the rumen and effectively fixed into microbial protein, which was extensively digested; or there was almost a complete re-absorption of blood urea secreted into the hind gut. Nolan and Leng (1972) observed that just 2% of  $^{15}\text{N}$  administered as  $[^{15}\text{N}]$  urea was recovered in the faeces of sheep.

The percentages of rumen ammonia-N derived from blood urea-N of hay-fed sheep was higher than values of 12% (Nolan and Leng, 1972) and 11% (Nolan *et al.*, 1976). These investigators fed lucerne chaff to sheep. Higher values of 39 to 64% were reported (Nolan and Stachiw, 1979). The value for Diet 2 was low probably due to low blood urea level, since blood urea recycling to the rumen varies directly with blood urea concentration (Mathison and Milligan, 1971).

The blood urea-N derived from ruminal ammonia-N for hay (50.6%) is comparable with 45% (Nolan and Leng, 1972), 52% (Norton *et al.*, 1982) and 56% to 65% (Neutze *et al.*, 1986). The high proportion of blood urea derived from ruminal ammonia would indicate high rate of transfer of ruminal ammonia into the blood.

The ruminal bacteria used ammonia as major source of N. The value recorded for sheep fed was Diet 1, high but comparable with values of 62 and 64% (Pilgrim *et al.*,

1970), 50 to 65% (Mathison and Milligan, 1971), and 51 to 68% (Nolan and Stachiw, 1979). The percentages of protozoal-N derived from ruminal ammonia N are similar to 35% and 41% (Pilgrim *et al.*, 1970) and 31 to 55% (Mathison and Miligan, 1971). The proportion of ruminal microbial N derived from ammonia varies with the protein content of the diet and may be as low as 20% (Salter *et al.*, 1979) and as high as almost 100% (Neutze *et al.*, 1986).

It is evident that blood urea contributed significantly to ruminal ammonia in sheep fed low quality hay. However, since a substantial proportion of blood urea was derived from ruminal ammonia, it would seem that there was appreciable transfer of N from the rumen into the blood and from the blood into the rumen in sheep fed low quality hay. Quantitative estimates of N transfer in and out of the rumen could indicate whether there was a net loss or gain of N into the rumen. This could not be accurately determined with  $^{15}\text{N}$  due to the recycling of N. Quantitative estimates of N transfers have been made using  $[^{14}\text{C}]$  urea (Norton *et al.*, 1982). The higher incorporation of ammonia N into ruminal bacterial N in the sheep supplemented with concentrate may be due to adequate supply energy, which could be limiting in the rumen of sheep fed on low quality forage. However, the marked depression in ruminal ammonia in sheep supplemented with concentrate, indicates that low ruminal ammonia could limit microbial protein synthesis. Inclusion of urea could have a beneficial effect by raising the ruminal ammonia level of sheep fed Diet 2.

## REFERENCES

- ADEGBOLA, T.A., ADELEYE, A.A. and YOILA, J.D. (1980) Requirement of young ewes fed Guinea grass (*Panicum maximum*) for maintenance and gain. *Niger. J. Anim. Prod.* 7(2):

- 117-121.
- ADEGBOLA, T.A., MBA, A.U. and OLUBAJO, F.O. (1976) Edogenous losses of nitrogen and protein requirement for maintenance of sheep. *Niger. J. Anim. Prod.* 3(2): 156-162.
- ADEGBOLA, T.A., MBA, A.U. and OLUBAJO, F.O. (1977) Studies on West African dwarf sheep; fed on basal hay or hay plus concentrates of varying protein contents. 1. Dry matter and crude protein digestion and utilization. *Trop. Agric. (Trinidad)* 54(3): 235-243.
- ADU, I.F. and OLALOKU, E.A. (1976) Studies on feed intake in west African dwarf sheep. *East African Agric. For. J.* 42: 224-230.
- AKINSOYINU, A.O., MBA, A.U. and OLUBAJO, F.O. (1976) Crude protein requirement of West African dwarf goat for maintenance and gain. *Journal of the A.A.A.S.A.* 3(1): 75-82.
- A.O.A.C. (1970) Official Methods of Analysis. Association of Official Analytical Chemists. 1st ed. Washington D.C.
- BLACKBURN, T.H. and HOBSON, P.N. (1960) The degradation protein in the rumen of the sheep and the re-distribution of the protein nitrogen after feeding. *Br. J. Nutr.* 14: 445-456.
- CHANÉY, A.L.A. and MARBARCH, E.P. (1962) Modified reagents for analysis of urea and ammonia. *Clin. Chem.* 8: 130-132.
- COCIMANO, M.R. and LENG, R.A. (1967) Metabolism of urea in the sheep. *Br. J. Nutr.* 21: 353-371.
- ELLIOTT, R.C. and TOPPS, J.H. (1963) Studies on protein requirement of ruminants. 1. Nitrogen balance trials on two breeds of African cattle given diets adequate in energy and low in protein. *Br. J. Nutr.* 17: 531-547.
- FAWCETT, J.K. and SCOTT, J.E. (1960) A rapid precise method for rapid determination of urea. *J. Clin. Pathol.* 13: 156-159.
- HOUPT, T.R. (1959) Utilization of blood urea in the ruminants. *American J. Physiol.* 197: 115-120.
- LAND, H. and ARRTURI VIRTANEN (1959) Ammonium salts as nitrogen source in the synthesis of protein by the ruminant. *Acta Chemica Scandinavia* 13: 489-496.
- LEWIS, D., HILL, K.J. and ANNISON, E.F. (1957) Studies on the portal blood of sheep. 1. Absorption of ammonia from the rumen of the sheep. *Biochem. J.* 66: 587-592.
- MATHISON, G.W. and MILLIGAN, L.P. (1971) Nitrogen metabolism in the sheep. *Br. J. Nutr.* 25: 351-366.
- MCDONALD, I.W. (1948) The absorption of ammonia from the rumen of the sheep. *Biochem. J.* 42: 584-587.
- MUGERWA, J.S. and CONRAD, H.R. (1971) Relationship of dietary non-protein nitrogen to urea kinetics in dairy cows. *J. Nutr.* 101: 1331-1342.
- NEUTZE, S.A., KELLAWAY, R.C. and FAICHNEY, G.J. (1986) Kinetics of nitrogen transfer across the rumen wall of sheep given a low protein roughage. *Br. J. Nutr.* 56: 497-507.
- NOLAN, J.V. and LENG, R.A. (1972) Dynamic aspect of ammonia and urea metabolism in sheep. *Br. J. Nutr.* 27: 177-194.
- NOLAN, J.V., NORTON, B.W. and LENG, R.A. (1976) Further studies of the dynamics of nitrogen metabolism in the sheep. *Br. J. Nutr.* 35: 127-147.
- NOLAN, J.V. and STACHIW, S. (1979) Fermentation and nitrogen dynamics in Merino sheep given low quality roughage diet. *Br. J. Nutr.* 42: 63-80.
- NORTON, B.W., MACKINTOSH, J.B. and ARMSTRONG, D.G. (1982) Urea synthesis and degradation in sheep given pelleted- grass diets containing flaked barley. *Br. J. Nutr.* 48: 249-264.
- OLUBAJO, F.O. and OYENUGA, V.A. (1974) The yield, intake and animal production of four tropical grass species grown at Ibadan. *Niger. J. Anim. Prod.* 1(2): 217-224.
- OYENUGA, V.A. (1957) The composition and agricultural value of some grass species in Nigeria. *Emp. J. Exp. Agric.* 25: 237-255.
- PILGRIM, A.F., GRAY, F.V., WELLER, R.A. and BELLING, C.B. (1970) Synthesis of microbial protein from ammonia in the sheep's rumen and the proportion of dietary nitrogen converted into microbial protein. *Br. J. Nutr.* 24: 589-598.

PIVA, G. and SILVA, S. (1968) Utilization of  $^{15}\text{N}$ - Diamonium phosphate by ruminants to produce milk and meat proteins. In Proc. Symposium: Isotope Studies on the Nitrogen chain, IAEA/FAO, Vienna, 1967.

SALTER, D.N., DANESVAR, K. and SMITH, R.H. (1979) The origin of nitrogen incorporated into compounds in the rumen bacteria of steers given protein- and urea-containing diets. *Br. J. Nutr.* 41: 197-209.

VAN LIEW, H.D. (1967) Graphic Analysis of Aggregates in linear and exponential processes. *J. Theoret. Biol.* 16: 43-53.