

## **The effect of source and level of nitrogen supplementation on feed intake, microbial protein synthesis and nitrogen metabolism in mature heifers fed poor quality hay**

**Paul Sebastian Mlay<sup>1\*</sup>, Apolinaria Pereka<sup>1</sup>, Sakurani Baltazary<sup>1</sup>, Torben Hvelplund<sup>2</sup>, Martin Riis Weisbjerg<sup>2</sup>, and Jørgen Madsen<sup>3</sup>**

<sup>1</sup>*Department of Physiology, Biochemistry, Pharmacology and Toxicology, Sokoine University of Agriculture, Morogoro, Tanzania*

<sup>2</sup>*Department of Animal Nutrition and Physiology, Danish Institute of Agricultural Sciences, Research Centre Foulum, Tjele, Denmark*

<sup>3</sup>*Department of Animal Science and Animal Health, The Royal Veterinary and Agricultural University, Copenhagen, Denmark*

---

**MLAY, P. S., A. PEREKA, S. BALTAZARY, T. HVELPLUND, M. R. WEISBJERG, J. MADSEN: The effect of source and level of nitrogen supplementation on feed intake, microbial protein synthesis and nitrogen metabolism in mature heifers fed poor quality hay. Vet. arhiv 75, 137-151, 2005.**

### **ABSTRACT**

The effect of nitrogen supplementation of poor quality hay on dry matter (DM) intake and digestibility, nitrogen balance and microbial protein was investigated using 5 ruminally fistulated mature heifers (1/2 Boran x 1/2 Friesian). The experimental set-up was 5x5 Latin square with 5 treatments and five periods, each of 28 days. In each period, 14 days were allotted to adaptation to treatments, a 7-day *in vivo* digestibility trial and total urine collection. Treatments were: poor quality hay only (HO) which was the control; hay with a low urea (32g/day) level (HLU); hay with a high urea (64g/day) level (HHU); hay with low soya bean meal (210g DM/day) (HLS), and hay with high (420g DM/day) soya bean meal (HHS). At a given level of supplementation, urea and soya bean meal was iso-nitrogenous. Hay was provided *ad libitum* with 10-15% refusal. Drinking water was provided *ad libitum* through automatic drinkers coupled to water flow meters. It was found that nitrogen supplementation led to significant increases in DM and OM intake and digestibility. Nitrogen retention and microbial protein synthesis were also significantly improved with nitrogen supplementation. Although soya bean meal showed a certain superiority in the improvement of these parameters, its cost would most likely hinder in its widespread use by smallholder farmers in Tanzania. Hence, it would be better to encourage the wide use of urea as a cheap

---

\* Contact address:

Dr. Paul. SSebastian Mlay (BVM, MVM, PhD), Sokoine University of Agriculture, P. O. Box 3017, Morogoro, Tanzania. Phone: +255 23 2604 978; +0744 601 151 E-mail: panejo@yahoo.com

source of nitrogen supplementation, especially for low- to medium-yielding animals in the smallholder dairy production system in Tanzania.

**Key words:** low quality hay, intake, nitrogen supplementation, microbial protein

---

## Introduction

In Tanzania, as in most tropical developing countries, poor quality forages are in most cases the sole diets of ruminants (PRESTON and LENG, 1987) especially during the dry season. One of the greatest limiting factors for efficient utilisation of low quality forage is the low nitrogen content that limits digestibility and intake (MAWUENYEGAH et al., 1997). The success of the Tanzanian Government's efforts to ensure food security and household income for smallholder farmers will depend on improved agricultural and livestock production (ANONYMOUS, 2000). For the livestock sector in general and the dairy sub-sector in particular, productivity could be greatly improved through cost effective strategic supplementation of the poor quality roughage, using inexpensive and locally available nitrogen and energy-rich concentrates. Reports show that when nitrogen supplementation is done, energy availability is improved through increased digestion of the fibre component of the diet (LENG, 1990; ØRSKOV, 1998).

The effect of a given nitrogen supplement on rumen functions differs with respect to the source, which influences the rate and extent of protein degradation in the rumen, as well as to the amount offered (CHEN et al., 1987; ROBINSON et al., 1998; DRIEDGER and LOERCH, 1999). Reports show that true proteins are superior to non-protein nitrogen (NPN) sources in improving intake and digestibility of poor quality forages (KROPP et al., 1977a; JELANTIK, 2001) although there are conflicting views as to the actual cause of such superiority (LENG, 1990). The aim of this study was to investigate further the responses in feed intake and digestibility, microbial protein synthesis, and nitrogen metabolism and supplemental protein cost when either soya bean meal (true protein) or urea (NPN) are used as nitrogen supplements in animals fed poor quality forage under Tanzanian feeding conditions.

## Materials and methods

*Animals feeds and feeding.* Five-rumen fistulated, mature non-pregnant crossbred heifers (1/2 Boran and 1/2 Friesian) were used in 5 x 5 Latin square. The experimental period lasted for 28 days, including 14 days of a preliminary period followed by 7 days for in vivo digestibility determinations and one day for rumen fluid sampling. The experimental work was carried out during the dry season (June-November) in the year 2000 at Magadu Farm belonging to Sokoine University of Agriculture in Morogoro, Tanzania.

Five treatments were compared. These were: hay only (HO) (control); hay with low (32g/day) urea level (HLU); hay with high (64g/day) urea level (HHU); hay with low (210g

DM/day) soya bean meal; hay with high (420g DM/day) soya bean meal (HHS). At a given level of supplementation, urea and soya bean meal was iso-nitrogenous. With urea, sodium sulphate was also included so as to give N:S ratio of 10:1 (SILVA and ØRSKOV, 1988).

Poor quality mixed hay cut at an advanced stage of maturity was used as a basal diet. The predominant plant species in the hay were *Cynodon dactylon* and *Panicum maximum*. The hay was chopped to small size (1-3 cm) to minimise selection. The animals were fed individually *ad libitum*. Feeding was adjusted everyday to be 10-15% in excess of the *ad libitum* intake using the previous day's level of intake. Half of the estimated daily hay and supplements for each animal were given at 7.30 am and the remaining half at 4 pm. All feeds and orts were weighed daily, sampled, and prepared for subsequent analyses. The DM determination was carried out everyday in both feeds and orts. Fresh, clean water was provided from an automatic drinker system equipped with flow meters where a reading was made at 8 am each day.

Urea mixed with sodium sulphate and mineral powder was put directly into the rumen through the cannula.

*Sampling procedures.* Faecal materials were collected manually from 7 am in the morning to seven 7 am the next day, when it was weighed to obtain the amount of fresh faecal material voided per day for each animal. Faecal material for each animal was then thoroughly mixed in large plastic basins and a 5% sample taken. The samples were immediately frozen at -15 °C. At the end of the collection period (7 days), samples were de-frozen and pooled for each animal. The pooled samples were then thoroughly mixed and a sub-sample of 500 g taken for each animal. Samples were oven dried at 60 °C to constant weight to determine DM. Samples intended for rumen incubation were then ground through a 2.0 mm sieve, while those for other chemical analyses were ground through a 1.0 mm sieve.

Daily urine output was effected by the use of special urine funnels held onto the vulva. Each funnel was linked by a pliable tube to large plastic collecting container (50 l capacity) where 500 ml of 17% sulphuric acid was initially added to maintain the urine pH values below 3 to avoid microbial destruction of purine derivatives. Values of purine derivatives obtained in the assay were corrected for the dilution effect of the added acid. About 2% of daily collection for each cow was sampled and filtered through two layers of surgical gauze to remove large particles. Daily samples were then deep frozen at -20 °C which at the end of the collection period were thawed and bulked for each animal. A sub-sample of 50 ml per animal was taken and deep-frozen until purine derivative assay.

*Ruminal fluid pH, ammonia and Volatile Fatty Acids (VFAs) concentration.* On the 23<sup>rd</sup> day of each period, 24-h rumen fluid samplings were taken for determination of daily mean ruminal fluid pH, and for concentration of ammonia and volatile fatty acids (VFAs). Sampling times were 7:30 am (before morning feeding), 9 am, 12 noon, 3 pm, 6 pm, 9 pm,

midnight, 3 am, 6 am, and 8 am the following morning. At the indicated sampling hours, materials from the ventral rumen sac were scooped by hand and squeezed through four layers of cheesecloth into a clean beaker. About 100 ml of the liquid was taken. The pH was immediately taken using a portable pH meter. Then, 30 ml of the fluid was decanted into a 50 ml sample bottle and acidified to pH 4.0-4.5 by adding 3 ml of 5M sulphuric acid to limit loss of ammonia from the sample. Corrections were made for the dilution effect of the 3 ml sulphuric acid in the final estimates of ammonia and VFAs. Samples were placed in an ice packed cool box and taken to the laboratory. In the laboratory the samples were centrifuged at 3000 rev/min for 20 min so as to precipitate feed particles and the supernatant was put into 20 ml test tubes, then deep frozen at -20 °C until VFAs and ammonia assay.

*Chemical analysis.* Dry matter and organic matter analyses were carried out using the procedure as outlined by the ANONYM. (1990). All samples analysed for NDF were carried out according to the methods described by VAN SOEST et al. (1991). In the analyses, however, sodium sulphite and  $\alpha$ -amylase were omitted since the feeds contained negligible amounts of starch that could not interfere with NDF analysis.

The concentration of ammonia was estimated by standard procedure as described by CHANEY and MARBACH (1962) using alkaline hypochlorite/phenol nitroprusside. Concentration of VFAs in ruminal fluid were determined by using gas chromatography (HP 6890 GC) coupled to peak integrator (HPGC Chemostation, Hewlett and Packard, 1990-1998) by the method of RICHARDSON et al. (1989), with some minor modifications.

Allantoin in the urine samples was analysed based on the use of a spectrophotometer (Cecil Instruments Serial No. 125 142 Model CE 2041, Cecil Instruments Ltd. Milton Technical Centre, Cambridge, CB4 6AZ, England), by the procedures outlined in FAO/IAEA, (1997). Uric acid was determined by the Uricase method as described by FUJIHARA et al. (1987). Total purine derivatives output (Y) was the sum of allantoin and uric acid as the contribution of xanthine and hypoxanthine in cattle to daily purine output is negligible (VERBIC et al., 1990).

*In vitro* organic matter digestibility was carried in accordance with the procedure outlined by TILLEY and TERRY, (1963).

## Calculations

*Microbial protein synthesis from purine derivatives.* Daily purine derivatives absorption (X) in mmoles per day was calculated based on the equation derived by VERBIC et al. (1990):

$$X = Y - (0.385W^{0.75})/0.85$$

Where Y = Daily purine excretion in mmoles per day

W = Body mass in kg

X was then calculated from using the Y obtained from urine analysis and the weight (W) of the animals. Microbial N synthesis was then calculated as: Microbial N (gN/day) = X mmol/day \*70/0.116\*0.85\*100 = 0.727X.

The equation is based on four assumptions. (i) Digestibility of microbial purines of 0.85; (ii) N content of purines is 70 mg N/mmol; (iii) Ratio of purine N to total N in mixed rumen microbes taken as 11.6:100, and (iv) Purine excretion in the cross-bred heifers will be similar to the temperate breeds from which the equation was derived.

*Cost of supplemental protein for common protein/nitrogen supplements in sources in Tanzania.* Supplemental protein cost of common protein sources available to smallholder farmers in Tanzania was calculated using maize bran as standard for energy, according to the equation: Energy cost of feed (Tsh/ME)-Energy cost standard feed (Tsh/ME) divided by protein concentration in the feed (g/ME) – protein concentration in standard feed (g/ME) (ØSTERGAARD, 1969).

*Statistical analyses.* Statistical analysis was carried out using the General linear Model (GLM) to test the differences between the means of the various parameters using four models.

$$Y = I + T + C + P + \varepsilon \text{ (Model I)}$$

$$Y = I + C + P + \beta_1 U + \beta_1 S + \varepsilon \text{ (Model II)}$$

$$Y = I + C + P + \beta_1 U + \beta_2 S + \beta_3 U^2 + \beta_4 S^2 + \varepsilon \text{ (Model III)}$$

$$Y = I + C + P + \beta_1 N + \varepsilon \text{ (Model IV)}$$

Where Y = dependable variable

I = Intercept

T = treatment (HO, HLU, HHU, HLS, HHS)

C = cow (cow 1-5)

P = period (period 1-5)

U = urea level (0, 32, 64 g/day)

S = soya bean meal level (0, 210, 420 g/day)

N = N supplementation (0, 15, 30 g/day)

$\beta_1 - \beta_4$  = regression coefficients

$\varepsilon$  = Random error

Means were compared by GLM PDIF and also by Duncan Multiple Range Test. Results are reported as least square means for each treatment with standard error of the least square means. Type II test (SAS, 1988) were used, implying that the mean effect of the treatments was tested without the interaction term in the model.

## Results

*Feeding and chemical composition of hay and soya bean meal.* The DM and chemical composition of the hay and soya bean meal used during the experiment are shown in Table 1. The hay CP and NDF (%DM) were 4.7 and 73.5, respectively.

Soya bean meal CP content, EE and NDF were 43.8, 17.1, 15.8%, respectively. The soya bean meal had high rumen degradation of both dry matter and nitrogen.

Table 1. Chemical composition and N degradation of soya bean meal and hay used during the experiment

Component	Type of feed	
	Hay	Soya bean meal
%DM	93.5	93.5
Dry matter composition (%DM)		
ASH	7.9	6.4
OM	92.1	93.6
CP	4.7	43.8
EE	1.8	17.1
NDF	73.5	15.8
CHO	85.6	32.7
K	1.06	NA
Ca	0.26	NA
Mg	0.14	NA
P	0.10	NA
Na	0.20	NA
IVOMD	35.6	83.5

NA: Not analysed, IVOMD: *In vitro* organic matter digestibility, DM: dry matter

*Feed intake and digestibility and rumen parameters.* Hay DM, total DM and ash intake were significantly ( $P < 0.001$ ) increased with nitrogen supplementation (Table 2).

Total DM intake was very highly correlated (linear  $r^2 = 0.89$  and  $0.92$  for soya bean meal and urea, respectively) to the CP content of the diet (Fig. 1).

Equations defining the relationships were  $Y = 0.55X + 3.2$  and  $Y = 0.44X + 3.7$ , for soya bean meal and urea, respectively, where  $Y = \text{DM intake (kg)}$  and  $X = \text{CP content in}$

Table 2. Effect of source and levels of nitrogen supplementation on dry matter intake, digestibility, Volatile Fatty Acids (mM), ruminal pH and ammonia concentration

Parameter	Treatment					SEM	P-value		
	HO	HLU	HHU	HLS	HHS		Treat	*Urea	*Soya
DM intake (kg/day)	5.7 <sup>b</sup>	6.5 <sup>c</sup>	6.9 <sup>b</sup>	7.0 <sup>ab</sup>	7.3 <sup>a</sup>	0.14	<0.001	<0.001	<0.0003
Apparent digestibility									
DM	45.0	47.8	50.0	49.1	49.9	1.29	0.1	0.02	0.01
OM	46.1	48.7	50.2	50.3	50.8	1.24	0.1	0.05	0.01
N	10.1 <sup>b</sup>	50.6 <sup>a</sup>	56.1 <sup>a</sup>	54.7 <sup>a</sup>	56.9 <sup>a</sup>	7.2	0.002	0.003	0.01
Ash	30.3 <sup>b</sup>	37.2 <sup>b</sup>	46.6 <sup>a</sup>	33.3 <sup>b</sup>	37.1 <sup>b</sup>	2.94	0.02	0.001	0.1
<sup>1</sup> Digested CHO (kg)	1.75 <sup>c</sup>	1.99 <sup>b</sup>	2.10 <sup>ab</sup>	2.12 <sup>ab</sup>	2.19 <sup>a</sup>	0.04	<0.001	<0.001	<0.0001
<sup>2</sup> App. digested OM (kg)	2.5 <sup>c</sup>	2.9 <sup>b</sup>	3.2 <sup>ab</sup>	3.3 <sup>a</sup>	3.4 <sup>a</sup>	0.10	<0.0002	<0.001	<0.001
Rumen indices									
VFA (mM)	38.0	44.0	47.6	47.6	52.7	4.46	0.3	0.1	0.02
pH	6.9	6.8	6.8	6.9	6.9	0.04	0.42	0.1	1.0
Ammonia (mg N/100ml)	1.02 <sup>a</sup>	3.58 <sup>b</sup>	5.62 <sup>c</sup>	1.89 <sup>d</sup>	1.98 <sup>c</sup>	0.378	<0.0001	<0.0001	0.1

HO: hay only; HLU: hay + low urea; HHU: hay + high urea; HLS: hay + low soya bean meal; HHS: hay + high soya bean meal \* Linear

<sup>1</sup>Calculated using the method described by WEISBJERG and HVELPLUND (1993)

<sup>2</sup>Calculated as OM intake-Fecal OM

Means within rows with different superscripts are significantly different (P<0.05)

the diet. It was clear that per unit change in diet CP content, soya bean meal had a greater impact on DM intake compared to urea.

There were significant linear increases in apparent DM digestibility (P = 0.05), with increased levels of urea and (P = 0.01), with increased levels of soya bean meal (Table 2). Nitrogen supplementation, irrespective of source, significantly improved both DM and OM digestibility (Model IV). There were significant (P = 0.01) increases in apparent N digestibility with both urea and soya bean meal supplementation compared to the control (Table 2).

*Rumen fermentation.* There were significant linear increases in the concentrations of individual and total acids with soya bean meal supplementation and also total VFAs with

urea supplementation (Table 2). The CP in the diet (%DM) and total VFAs were highly correlated (linear,  $r^2 = 0.97$  and  $0.98$  for soya bean meal and urea, respectively). The equations relating the two parameters were  $Y = 5.2X + 14.2$ , for soya bean meal and  $Y = 3.4X + 22.4$  for urea (Fig. 2) where  $Y = \text{Total VFAs (mM)}$  and  $X = \text{CP content of diet}$

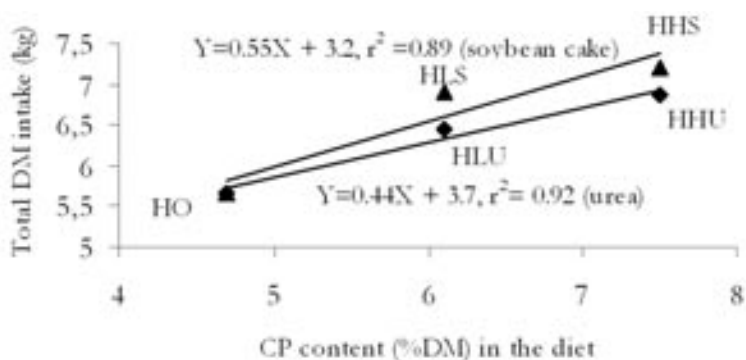


Fig. 1. Relationship between total DM intake (kg/day) with the CP content (%DM) of the diet

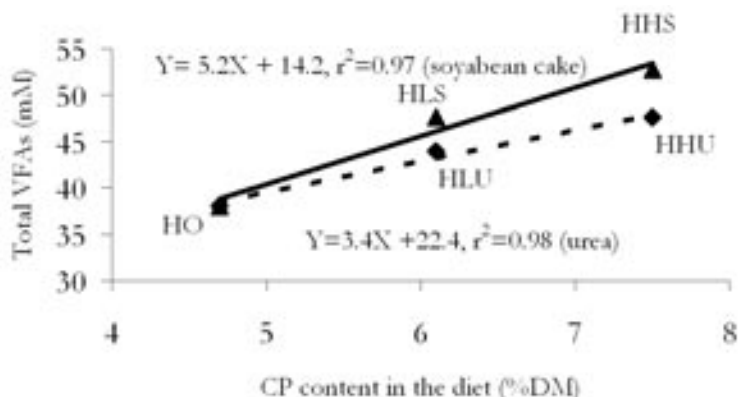


Fig. 2. Relationship between total VFAs with the CP content in the diet (%DM) under the various treatments

(%DM). It was evident that for a unit change in CP content in the diet, soya bean meal caused a greater increase in total VFA concentration in rumen fluid compared to urea. There were significant increases in ruminal concentration of propionate ( $P = 0.01$ ), butyrate ( $P = 0.04$ ) and total acids ( $P = 0.03$ ) with nitrogen supplementation (Model IV).



Mean daily ruminal fluid pH seemed insignificantly affected by treatments, although urea supplementation tended to cause a slight depression of pH, especially during the late night hours. Mean ruminal fluid ammonia concentration was significantly ( $P<0.0001$ ) higher with both levels of urea compared to the levels of soya bean meal and the control (Table 2).

Mean daily ruminal fluid ammonia was significantly higher ( $P<0.001$ ) in both levels of urea compared to HO, in HHU compared to HLS and HHS, and significantly ( $P<0.01$ ) higher in HLU compared to HLS and HHS. The differences were less during the night. Soya bean meal supplementation increased the rumen ammonia N, but the difference was not significant compared to the control.

Table 3. Effect of source and levels of nitrogen supplementation on urine output and composition and microbial protein synthesis estimated from purine derivative excretion

	Treatments					SEM	P-value		
	HO	HLU	HHU	HLS	HHS		Treat	*Urea	*Soya
Urine output (kg per day)	6.4	6.6	8.4	6.8	8.5	0.77	0.22	0.05	0.04
CP (%)	1.6	2.1	2.7	2.0	2.2	0.35	0.3	0.03	0.2
Allantoin (mmols/l)	7.6 <sup>b</sup>	13.0 <sup>a</sup>	10.5 <sup>ab</sup>	13.8 <sup>a</sup>	14.0 <sup>a</sup>	1.07	0.01	0.3	0.2
Uric acid (mmols/l)	1.8	1.7	1.4	1.3	1.6	0.15	0.1	0.03	0.2
Allantoin output (mmoles/day)	47.3 <sup>b</sup>	79.5 <sup>a</sup>	81.3 <sup>a</sup>	85.4 <sup>a</sup>	99.3 <sup>a</sup>	6.75	0.002	0.01	0.0003
Uric acid output (mmoles/day)	11.0	12.4	11.7	8.2	13.8	2.2	0.5	0.5	0.5
Total purines (mmoles/day)	58.3 <sup>b</sup>	91.8 <sup>a</sup>	93.0 <sup>a</sup>	93.6 <sup>a</sup>	113.1 <sup>a</sup>	8.1	0.01	0.01	0.001
Purine absorbed (mM/day)	29.7 <sup>b</sup>	68.3 <sup>a</sup>	69.8 <sup>a</sup>	70.6 <sup>a</sup>	92.7 <sup>a</sup>	9.45	0.01	0.01	0.001
Microbial N (g/day)	21.6 <sup>b</sup>	49.7 <sup>a</sup>	50.7 <sup>a</sup>	51.3 <sup>a</sup>	67.4 <sup>a</sup>	6.87	0.01	0.01	0.001
Microbial CP (g/day)	134.6 <sup>b</sup>	310.6 <sup>a</sup>	316.9 <sup>a</sup>	320.6 <sup>a</sup>	421.2 <sup>a</sup>	42.25	0.01	0.01	0.001
gMN/kg CHO	12.3 <sup>b</sup>	25.3 <sup>a</sup>	24.6 <sup>a</sup>	24.4 <sup>a</sup>	32.0 <sup>a</sup>	3.46 <sup>a</sup>	0.02	0.07	0.004

HO: hay only; HLU: hay + low urea; HHU: hay + high urea; HLS: hay + low soya bean meal; HHS: hay + high soya bean meal \* Linear. Means within rows with different superscripts are significantly different ( $P<0.05$ )

*Urine output and composition and microbial protein synthesis.* Urine output increased linearly with urea ( $P = 0.05$ ) and soya bean meal ( $P = 0.04$ ) supplementation (Table 3). Concentration of allantoin in urine ranged from 7.6 to 14 mmoles/l and was significantly ( $P = 0.01$ ) higher with urea and soya bean meal supplementation compared to the control (Table 3). Microbial nitrogen (MN) synthesis and efficiency of the synthesis estimated from purine derivatives were significantly improved with both urea and soya bean meal supplementation (Table 3). Efficiency of synthesis (g MN/kg CHO) estimates from purine derivatives showed linear ( $P = 0.01$ ) increases with soya bean meal supplementation and curve-linearly changes (Model III) with urea supplementation.

Nitrogen retention was significantly ( $P = 0.01$ ) improved by nitrogen supplementation, ranging from 12.8 with low urea to 21 g/day with a high level of soya bean meal supplementation (Table 4). While there was a sharp change in N retention from the control to the first levels of supplementation, the change from the first to the second levels were more gradual with soya bean meal supplementation, showing higher retention of N at all levels compared to urea.

Table 5 shows the calculated cost of supplemental protein of the commonly used protein/nitrogen sources in Tanzania. Cost in Tsh per g CP was 3.3 for soya bean meal, 2.5 for fishmeal, 0.2 for both sunflower and cottonseed cake, and 0.1 for urea.

Table 4. Effect of source and levels of nitrogen supplementation on nitrogen (N) metabolism

Parameter	Treatments					SEM	P-Value		
	HO	HLU	HHU	HLS	HHS		Treat	*Urea	*Soya
Total N intake (g/day)	42.3 <sup>c</sup>	68.9 <sup>b</sup>	80.6 <sup>a</sup>	64.9 <sup>b</sup>	80.4 <sup>a</sup>	1.07	<0.0001	<0.0001	<0.0001
Faecal N (g/day)	38.2	31.2	35.3	29.3	32.7	3.4	0.4	0.8	0.4
Urinary N (g/day)	15.9	18.9	29.2	18.6	26.5	3.21	0.5	0.01	0.01
Total N excreted (g)	54.2	50.1	64.5	48.0	59.2	5.2	0.2	0.10	0.3
N-balance (g/day)	-11.9 <sup>b</sup>	12.8 <sup>a</sup>	16.1 <sup>a</sup>	17.0 <sup>a</sup>	21.2 <sup>a</sup>	5.55	0.01	0.01	0.002

HO: hay only; HLU: hay + low urea; HHU: hay + high urea; HLS: hay + low soya bean meal; HHS: hay + high soya bean meal \* Linear.

Means within rows with different superscripts are significantly different ( $P < 0.05$ )

Table 5. Estimates of the costs of supplemental protein of the various sources of protein supplements available to smallholder farmers in Tanzania

Feed	CP (g/kg DM)	ME (MJ/kg DM)	<sup>1</sup> Price/kg DM (Tsh)	Price/ME (Tsh)	CP/ME (g)	Energy cost difference (Tsh)	CP/ME difference (g)	Price of supplemental protein (Tsh/gCP)
<sup>2</sup> Maize bran	109.0	11.8	54.40	4.60	9.2	-	-	-
Soyabean meal	438.0	14.7	1068.38	72.68	29.8	68.07	20.6	3.3
Sunflower cake	236.0	8.0	74.30	9.35	29.7	4.74	20.4	0.2
Cotton seed cake	358.9	8.55	105.82	12.38	42.0	7.77	32.7	0.2
Fishmeal	633.0	11.0	1363.64	123.97	57.3	119.36	48.0	2.5
Urea	2880	-	280.00	-	-	-	-	0.10

<sup>1</sup>Obtained from dealers of animal feeds in Morogoro in the year 2000.

<sup>2</sup>Used as standard feed for comparison with the others

## Discussion

The low CP, and ME, content in DM, low in vitro organic matter digestibility, and the high fibre content of the hay used during this trial confirms the characteristics of poor quality roughage fed to ruminants in the tropics for a large part of the year. This kind of hay, if fed as a sole diet to ruminants, is unable to meet the maintenance requirements of the animals, let alone support production requirements (MADSEN et al., 1997; JETANA et al., 2000; JELANTIK, 2001). Therefore, in order to improve animal production, supplementation of the basal roughage with the deficient nutrients is essential. Since the most limiting factor for microbial growth is N, it is most likely that when N availability is improved more energy will be made available from the roughage.

In this study, DM and OM intake and digestibility, volatile fatty, microbial protein synthesis and nitrogen digestibility and retention increased with N supplementation. This was mainly due to the positive effect of N supplementation, which enhanced microbial growth through increased ammonia availability. Higher microbial growth was possibly the major factor that contributed to the observed higher microbial protein yield, nutrient (plant fibre and nitrogen) digestibility, which in turn led to higher volatile fatty acids. MGHENI et al. (1994) reported that a large part of nitrogen in tropical grasses is bound to the cell walls. Thus, improved cellulolysis in the rumen leads to increased exposure of

the bound nitrogen compounds to enzymes that digest them to ammonia which is made available to the microbes. This study proved that the CP content of the diet has a strong impact on the DM intake and volatile fatty acids levels in the rumen as seen from Fig. 1 and 2, respectively.

The superiority of soya bean meal compared to urea in improving intake and rumen fermentation of feeds observed in this study was in agreement with other studies (KROPP et al., 1977a; KROPP et al., 1977b). This was possibly due to synchronisation of ammonia availability and fibre degradation, thereby limiting excessive ammonia absorption and loss through urine. There is also a wider range of absorbed amino acids from microbial and rumen by-pass proteins digested in the small intestine as opposed to urea, where the major source of amino acids to the animal would have been microbial proteins. True proteins, like soya bean meal, also provide an extra source of energy and minerals (JELANTIK, 2001).

Despite the proven superiority of true proteins, supplemental protein costs will most probably limit their wide use by smallholder farmers in developing countries like Tanzania. Most animals kept by smallholders in Tanzania are moderate to medium producers (5-10 L/day) (LEKULE et al., 1998; MLAY et al., 2001). Such a level of production can easily be supported by an improvement in rumen fermentation through urea supplementation as opposed to high producing animals (>20 litres per day), where an additional source of rumen bypass protein is required (MADSEN and HVELPLUND, 1988; MADSEN et al., 1995).

The use of urea for treating forages (MGHENI et al., 1994) and Urea Molassed Blocks (PLAIZIER et al., 1998) has been done during on-station and farm trials, but so far the adoption of this technique by smallholder farmers is almost non-existent. Multi-nutrient blocks containing urea, molasses and minerals have been in use in some developing tropical countries with highly significant achievements in improvement of ruminant productivity (PRESTON and LENG, 1984). Perhaps it is high time that more research be carried out on how to prepare urea-enriched supplements in a form that is safe and easy to apply by smallholder farmers in Tanzania.

## **Conclusion**

Low nitrogen in poor quality forages was found to be a major limiting factor in their utilisation. Nitrogen supplementation improved the intake and digestibility of hay and microbial protein synthesis and nitrogen retention. It will be interesting to observe the effects of higher levels of nitrogen supplementation on the measured parameters.

In most measured parameters, soya bean meal was superior to urea possibly due to relatively slow degradation, thereby matching ammonia availability with the slow degradation of the fibre component in the diet. However, it would be cheaper to use urea as a nitrogen supplement compared to most sources of true proteins available in Tanzania.

---

### Acknowledgements

The authors wish to thank M. Mbwana, Kibirige, W. of Sokoine University and Ejner Serup, Edith Olsen and Hanne Hansen from the Danish Institute of Agricultural Sciences at Foulum, Denmark for their Technical Support. We also express our sincere thanks to the Danish Government for sponsoring this work through DANIDA

### References

- ANONYMOUS (2000): Food Security and Household Income for Smallholder Farmers in Tanzania. Project Overview. Collaborative Research under the Tanzania Agricultural Research Project Phase 2 (TARP II), Directorate of Research and Postgraduate studies, Sokoine University of Agriculture pp. 21.
- ANONYMOUS (1990): Associations of Official Agricultural Chemists. Official Methods of Analysis. 15<sup>th</sup> Edition AOAC Washington D.C. Volume 1 pp. 62.
- CHANEY, A. L., A. P. MARBACH (1962): Modified reagents for determination of urea and ammonia. *Clin. Chem.* 8, 130-132.
- CHEN, G., C. J. SNIFFEN, J. B. RUSSELL (1987): Concentration and estimated flow of peptides from the rumen fluid of dairy cattle. Effects of protein quantity, protein solubility, and feeding frequency. *J. Dairy Sci.* 70, 983-999.
- DRIEDGER, L. J., S. C. LOERCH (1999): The effect of protein concentration and Source on nutrient digestibility by mature limit-fed high-concentrate diets. *J. Anim. Sci.* 77, 960-966.
- FAO/IAEA, (1997): Estimation of rumen microbial protein production from purine derivatives in urine. A laboratory manual for FAO/IAEA Co-ordinated Research Programme on the Development, Standardization and Validation of Nuclear Based Technologies for Measuring Microbial Protein Supply in Ruminant Livestock for Improving Productivity. AIEA, Vienna, Austria pp. 49.
- FUJIHARA, T., E. R., ØRSKOV, P. J. REEDS (1987): The effect of protein infusion on the urinary excretion of purine derivatives in ruminants nourished by intragastric nutrition. *J. Agric. Sci. (Camb.)* 109, 7-12.
- JELANTIK, N. (2001): Improving Bali Cattle (Bibos bateng Wagner) Production Through Protein Supplementation. PhD Thesis. The Royal University Veterinary and Agricultural University, Copenhagen, Denmark pp. 120.
- JETANA, T., A. N. HALIM, R. A. S. JALALUDIN, Y. W. HO (2000): Effects of energy and protein supplementation on microbial-N synthesis and allantoin excretion in sheep fed guinea grass. *Anim. Feed Sci. Technol.* 84, 167-181.
- KROPP, J. R., R. R. JOHNSON, J. R. MALES, F. N. OWENS (1977a): Microbial protein synthesis with low quality roughage rations: Isonitrogenous substitution of urea for soyabean meal. *J. Anim. Sci.* 46, 837-843.
- KROPP, J. R., R. R. JOHNSON, J. R. MALES, F. N. OWENS (1977b): Microbial protein synthesis with low quality roughage rations: Level and source of nitrogen. *J. Anim. Sci.* 46, 844-854.

- LEKULE, F. P., S. V. SARWATT, P. H. PETERSON (1998): The SURUDE heifer-in-trust scheme A preliminary assessment. Proceedings of the 25<sup>th</sup> Scientific Conference held in AICC, Arusha, Tanzania, August 5-7, 1998. TSAP Conference Series, Vol.25, (1998) pp. 13-21.
- LENG, R. A. (1990): Factors affecting the utilisation of poor quality forages by ruminants particularly under tropical conditions. *Nutr. Res. Rev.* 3, 277-303.
- MADSEN, J., T. HVELPLUND (1988): The influence of different protein supply and feeding level on pH, ammonia concentration and microbial protein synthesis in the rumen of cows. *Acta Agric. Scand.* 38, 115-125.
- MADSEN, J., T. HVELPLUND, M. R. WEISBERG, J. BERTILSSON, I. OLSSON, R. SPORN-DLY, O. M. HARSTAD, H. VOLDEN, M. TUORI, T. VARVIKOT, P. HUHTANEN, B. I. OLAFSSON (1995): The AAT/PBV protein evaluation system for ruminants. A revision. *Acta Agric. Scand. Suppl.* 19, 1-37.
- MADSEN, J., T. HVELPLUND, M. R. WEISBJERG (1997): Appropriate methods for the evaluation of tropical feeds in ruminants. *Anim. Feed Sci. Technol.* 69, 53-66.
- MAWUENYEGAH, P. O., M. N. SHEM, L. WARDY, T. FUJIHARA (1997): Effects of supplementary feeding with protein and energy on digestion and rumination behaviour in sheep on straw diets. *J. Agric. Sci. (Camb.)* 129, 479-484.
- MGHENI, D. M., T. HVELPLUND, M. R. WEISBJERG (1994): Intestinal digestibility of rumen undegraded dietary protein from tropical roughages estimated by the mobile bag technique. *Acta Agric. Scand., Section A, Anim. Sci.* 44, 230-235.
- MLAY, P. S., J. MADSEN, A. E. PEREKA, T. HVELPLUND, M. R. WEISBJERG, (2001): Smallholder Livestock Production Trends in Urban and Peri-urban areas of Morogoro. *TVJ Volume 21 (1)* 15-26.
- ØRSKOV, E. R. (1998): Feed evaluation with emphasis on fibrous roughages and fluctuating nutrient supply: A review. *Small Rumin. Res.* 28, 1-8.
- ØSTERGAARD, V. (1969): Tabeller over priser jå suppleringsproteins I krodffoder. 10 med.fra handonomisk Forsøgslaboratorium. p. 43.
- PLAIZIER, J. C. B, R. NKYA, M. N. SHEM, N. A. URIO, B. W. MCBRIDE (1998): Dry season supplementation of dairy cows with nitrogen molassed mineral blocks and molassed urea mix in the Morogoro region in Tanzania. Paper presented in the third Research Council Meeting (RCM) on feed supplementation strategies for smallholder farms in Africa held in Vienna, from 7-11 September 1998.
- PRESTON, T. R., R. A. LENG (1984): Supplementation of diets based on fibrous residues and by-products. In: *Straw and other fibrous by-products as feed*. Amsterdam, Elsevier Press pp. 373-413.
- PRESTON, T. R., R. A. LENG (1987): Matching ruminant production system with available resources in the tropics. Penambur Books, Armidale, NSW, Australia. p. 459.
- RICHARDSON, A. J., A. G. CALDER, C. S. STEWART, A. SMITH (1989): Simultaneous determination of volatile and non-volatile acidic fermentation products of anaerobes by capillary gas Chromatography. *Appl. Microbiol.* 9, 5-8.

- ROBINSON, P. H., D. M. VEIRA, M. IVAN (1998): Influence of supplemental protein quality on rumen fermentation, rumen microbial yield, forestomach digestion, and intestinal amino acid flow in late lactation Holstein cows. *Canadian J. Anim. Sci.* 78, 95-105.
- SAS (1988): Statistical Analysis System Institute, SAS Institute Inc, Cary, NC, USA
- SILVA, A. T., E. R. ØRSKOV (1988): The effect of five different supplements on the degradation of straw in sheep given untreated barley straw. *Anim. Feed Sci. Technol.* 19, 289-298.
- TILLEY, J. M. A., R. A. TERRY (1963): A two stage technique for the *in vitro* digestion of forages. *J. Brit. Grassl. Soc.* 18, 104-111.
- VAN SOEST, P. J., J. B. ROBERTSON, B. A. LEWIS (1991): Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. *J. Dairy Sci.* 74, 3583-3597.
- VERBIC, J., X. B. CHEN, N. A. MACLEOD, E. R. ØRSKOV (1990): Excretion of purine derivatives by ruminants: Effects of microbial nucleic acid infusion on purine derivatives excretion by steers. *J. Agric. Sci. (Camb.)* 114, 243-248.
- WEISBJERG, M. R., T. HVELPLUND (1993): Estimation of net energy content (FU) in feeds for cattle. Report No.3/1993. National Institute of Animal Science, Denmark. p. 39.

Received: 22 August 2003

Accepted: 1 March 2005

---

**MLAY, P. S., A. PEREKA, S. BALTAZARY, T. HVELPLUND, M. R. WEISBJERG, J. MADSEN: Utjecaj izvora i razine dodanog dušika na unos hrane, sintezu mikrobnih bjelančevina i metabolizam dušika kod spolno zrelih junica hranjenih sijenom loše kvalitete. *Vet. arhiv* 75, 137-151, 2005.**

**SAŽETAK**

Utjecaj dodanog dušika sijenu loše kvalitete na unos suhe tvari i probavljivost, ravnotežu dušika i mikrobnje bjelančevine, istražen je u pet spolno zrelih junica. Junice su bile križanci boran (1/2) i frizijske pasmine (1/2), s učinjenim fistulama na buragu. Istraživanje je oblikovano kao 5x5 latinski kvadrat, s 5 različitih tretmana u 5 različitih vremenskih razdoblja. Trajanje pojedinog vremenskog razdoblja iznosilo je 28 dana. Prvih 14 dana svakog razdoblja iskorišteno je za pripremu junica, a zatim je slijedilo 7 dana istraživanja probavljivosti *in vivo*, te skupljanja ukupnog urina. Tretmani su se razlikovali prema hranidbi: samo sijenom loše kvalitete - kontrola, sijenom s niskom razinom dodane ureje (32 g/dan), sijenom s visokom razinom dodane ureje (64 g/dan), sijenom s niskom razinom dodane sojine sačme (210 g suhe tvari/dan) i sijenom s visokom razinom dodane sojine sačme (420 g suhe tvari/dan). Pri istoj razini dodavanja, ureja i sojina sačma imali su jednaki sadržaj dušika. Sijeno je davano *ad libitum*, a gubitak pri hranjenju iznosio je 10-15%. Voda za piće je ponuđena *ad libitum* u automatskim pojilicama s mjerачem protoka. Utvrđeno je da dodatak dušika dovodi do značajno povećanog unosa suhe tvari i OM te povećane probavljivosti. Također, značajno su poboljšani zadržavanje dušika i sinteza mikrobnih bjelančevina. Iako je sojina sačma, u odnosu na ureju, pokazala određene prednosti pri poboljšanju promatranih pokazatelje, cijena ovog dodatka će vjerojatno ograničiti njegovu širu primjenu u malim gospodarstvima Tanzanije. Zbog toga bi bilo bolje poduprijeti uporabu ureje, kao jeftinog izvora za dodavanje dušika stočnoj hrani, posebice kod niže i srednje produktivnih životinja uzgajanih u manjim sustavima za proizvodnju mlijeka u Tanzaniji.

**Ključne riječi:** sijeno loše kvalitete, unos, dodatak dušika, mikrobnje bjelančevine

---