Tropical Agricultural Research Vol. 11 1999, 211-220

Effects of Supplementation with Undegradable Dietary Protein in Urea Molasses Bolus in Rice Straw Based Rations on Digestibility and Nitrogen Metabolism in Sheep

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ABSTRACT. *An experiment was conducted to study the effect of adding undegradable dietary protein (UDP) in urea molasses bolus on the digestibility and nitrogen metabolism in sheep. Eighteen male sheep were allotted to 6 groups. The control (T_0) urea molasses bolus was prepared by mixing molasses, urea, rice bran, salt and mineral mixture at the rate of 350, 120, 480, 30 and 20 g/kg respectively. Five sources of UDP namely local fish meal (T_1), imported fish meal (T_2) refuse tea leaf (T_3), heat treated soybean meal (T_4) and formaldehyde treated coconut poonac (T_5) were incorporated in urea molasses bolus at the rate of 30, 30, 150, 50 and 100 g/kg respectively by substituting equal amounts of rice bran in the control formulation. In a total collection experiment test feeds were supplemented at the rate of 0.5 kg/100 kg BW/day to basal feed of ad libitum rice straw. Feed intake, faecal and urinary excretion were measured and the digestibility was calculated for each treatment. The effect of treatment on digestibility, N-balance, blood metabolites and nitrogen status of rumen fluid was studied. Treatment effect was significant on straw DMI and digestibility, but varied with the source of UDP. The inclusion of UDP had no significant effect on rumen parameters and blood metabolites of sheep. A higher weight gain and N retention was obtained by feeding refuse tea leaf. Inclusion of 150 g of refuse tea leaf per kilogram of urea molasses bolus increased the nitrogen retention and body weight gain in sheep.*

INTRODUCTION

Ruminant production systems in most developing countries depend much on agricultural by-products and low quality roughages for feed requirements. These feeds are low in digestibility and protein content, but

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contain highly lignified materials. Consequently, supplementation becomes essential to improve the productivity of animals. Protein supplements are expensive and hardly available for ruminant feeding. Ruminants have the ability to utilize non-protein nitrogen as efficiently as protein (Erb *et al.*, 1976). Considerable amount of non protein nitrogen sources such as urea are added to the concentrate diets of ruminants in order to increase the economic efficiency. Urea molasses based supplements have been tested and successfully used in many countries (Khanal *et al.*, 1997).

Non-protein nitrogen in the feed is converted to valuable microbial protein in the rumen by the microorganisms. However, fast growing animals and lactating cows, producing more than 3 litres of milk per day, require more protein than that can be supplied by rumen microorganisms (Leng and Nolan, 1984). Microbial protein synthesis from straw based diets is low due to energy limitation (Walli *et al.*, 1993). The supply of undegradable dietary protein (UDP), or true protein that escape the rumen degradation, is necessary to provide adequate protein post ruminally, to achieve higher production levels. Many have attempted to increase the supply of amino acids reaching the abomasum by preventing degradation of dietary protein in the rumen.

However, information relating to the effect of UDP on the digestibility and nitrogen metabolism is scanty. The aim of this experiment was to study the effect of adding UDP to urea molasses bolus on the digestibility and nitrogen metabolism in sheep.

MATERIALS AND METHODS

Eighteen castrated male sheep having mean body weight of 18 kg were allotted to six groups. The sheep were housed in metabolic cages with facilities for individual feeding and collection of excreta. Five sources of undegradable dietary protein (UDP) namely; local fish meal (T_1) imported fish meal (T_2), refuse tea leaf (T_3), heat treated soybean meal (T_4) and formaldehyde treated coconut poonac (T_5) were incorporated into urea molasses bolus. The boluses made without a source of UDP were used for control (T_0). Soybean meal was heat treated at 150°C for 2 h duration according to the method described by Hadjipanayiotou (1995). Coconut poonac was treated with formaldehyde (2 g/100 g protein), kept airtight for 2 days and aerated before feeding. Molasses, urea, salt and mineral mixture were used at fixed levels in all formulae while the level of rice bran was altered to permit the addition of UDP supplement. Detailed formulation, proximate composition and energy content of test feeds are presented in Table

1. Feed ingredients were hand mixed thoroughly. The mixture was divided and made into boluses of known weight. The planes of feeding were calculated to meet the nutritional requirements and fed at fixed level of 0.5 kg/100 kg BW/d. Feeds were offered in two equal parts daily at 0800 h and 1600 h. Untreated rice straw was fed *ad libitum* as a basal feed.

Table 1. Ingredients, proximate composition and energy content of test feeds.

Items	T_0	T_1	T_2	T_3	T_4	T_5
	g/kg					
Ingredients						
Molasses	350	350	350	350	350	350
Urea	120	120	120	120	120	120
Rice bran	480	450	450	330	430	380
Salt	30	30	30	30	30	30
Mineral mixture	20	20	20	20	20	20
Fish meal (L)	--	30	--	--	--	--
Fish meal (I)	--	--	30	--	--	--
Refuse tea leaf	--	--	--	150	--	--
Heat treated soybean meal	--	--	--	--	50	--
FTCP	--	--	--	--	--	100
Proximate composition						
Dry matter	877.6	876.2	878.1	880.3	878.9	872.1
* Crude protein	416.2	433.4	441.1	443.3	436.3	439.9
* Crude fibre	52.2	53.4	52.6	46.3	49.5	48.1
* Ether extract	52.4	53.4	52.6	46.3	49.5	48.1
* Total ash	166.6	177.9	167.1	149.2	152.3	152.8

FTCP = Formaldehyde treated coconut poonac
* Dry matter basis

Experimental period consisted of 10 days adaptation period and 7 days collection period. Straw intake was measured for 8 days starting from three days before the commencement of total collection period. Daily total

faecal collection was weighed, thoroughly homogenized and aliquots of 50 g were taken and pooled for each animal. Urine was collected in plastic bottles containing 25 ml of 10% hydrochloric acid. Daily collection was diluted to a known volume, 10% aliquots of this were pooled for individual animal and frozen. Samples of feed, faeces and urine were subjected to proximate analysis (AOAC, 1990). The digestibility and nitrogen balance were calculated.

At the end of the collection period, 2 h post feeding, rumen fluid was collected through stomach tube connected to a suction pump. Total-N, ammonia-N, total volatile fatty acid (Barnet and Reid, 1956), trichloro acetic acid soluble-N and trichloro acetic acid insoluble-N (Cline *et al.*, 1957) contents in the rumen fluid were determined. Parallel to the collection of rumen fluid, blood was withdrawn from the jugular vein to determine blood urea nitrogen, packed cell volume and hemoglobin contents. Data were statistically analysed using ANOVA with the help of SAS software.

RESULTS AND DISCUSSION

Effect of different treatments on feed intake is shown in Table 2. The group fed imported fish meal had the highest straw dry matter intake. But this intake was not significantly different from the intake of the group fed formaldehyde treated coconut poonac ($p<0.05$). The lowest intake was shown by the group fed refuse tea leaf, while no significant difference could be detected among the groups fed without UDP supplement (T_0), local fish meal (T_1), refuse tea leaf (T_3) and heat treated soybean meal (T_4). In all treatment groups all the concentrate was consumed by sheep leaving no refusals. Total dry matter and organic matter intake assumed a trend similar to that of straw dry matter intake. The results of dry matter intake are in agreement with previous findings (Van Houtert and Leng, 1986). Dry matter intake is of interest in animal feeding experiments as it expresses the ability of the fodder to satisfy the animal.

In-vivo dry matter digestibility (DMD) and organic matter digestibility (OMD) values are given in Table 2. Dry matter digestibility was highest in the group fed local fish meal, although no significant difference was found among T_0, T_1, T_3 and T_4. The lowest DMD was found in the group fed formaldehyde treated coconut poonac (T_5), but this difference was significant only compared to T_1. The OMD assumed a trend similar to that of DMD. The results of DMD and OMD suggest the existence of satisfactory rumen environment in all the treatment groups. High level of urea in all treatments

Table 2. Feed intake, DMD, OMD and weight gain of sheep fed undegradable dietary protein from different sources.

Attributes	T_0	T_1	T_2	T_3	T_4	T_5	±SEM
* Intake (kg/100 kg Bw/d)							
Straw	2.37^{abc}	2.26^{bc}	2.76^{a}	2.0^{c}	2.09^{bc}	2.37^{ab}	0.222
Concentrate	0.44	0.438	0.44	0.44	0.44	0.43	0.005
Total DM	2.81^{abc}	2.69^{bc}	3.20^{a}	2.44^{c}	2.53^{bc}	2.93^{ab}	0.228
Total OM	2.49^{abc}	2.39^{bc}	2.84^{a}	2.18^{c}	2.28^{bc}	2.65^{ab}	0.201
Drymatter Digestibility (%)	0.537^{ab}	0.554^{a}	0.496^{ab}	0.471^{ab}	0.486^{ab}	0.442^{b}	0.0339
Organic matter Digestibility (%)	0.537^{ab}	0.554^{a}	0.534^{ab}	0.512^{ab}	0.524^{ab}	0.479^{b}	0.0338
Average Daily Gain (g/head/d)	40.2^{b}	45.3^{ab}	59.1^{ab}	84.3^{ab}	43.9^{ab}	91.6^{a}	18.37

Different superscripts in the same row indicate significant difference ($p<0.05$)
* Drymatter intake is the average of 8 days observation

may mask the changes in rumen environment due to the presence of undegradable dietary protein in the ration. DMD as well as OMD is of interest for its direct effect on the amount of energy that an animal can obtain from the feed (Dixon and Egan, 1987).

The weight gain of sheep is presented in Table 2. All the groups, irrespective of treatments, gained weight while there was a significant ($P<0.05$) difference in gain among treatments. Refuse tea leaf and formaldehyde treated coconut poonac supplementation resulted in higher gain. This may be due to the presence of higher levels of UDP in those feeds. The supply of higher level of UDP to the animal may be necessary to provide adequate protein post ruminally, thus enabling higher production performances.

The results of the N balance experiments are presented in Table 3. In all the treatments animals remained in a positive balance. There was a significant difference ($P<0.05$) in total N intake, with no significant effect on

the intake of concentrate N. Thus, the difference in total N intake was a reflection of straw N intake. There was a significant ($P<0.05$) difference in N excretion among treatments. Faecal N was lowest in the group fed refuse tea leaf and highest in the group fed formaldehyde treated coconut poonac. Faecal N was correlated to the total N intake in all groups except in the group fed heat treated soybean meal in which the faecal N was higher than expected on the basis of N intake. Since the nitrogen found in the straw is not readily digestible, larger proportion of faecal N must have resulted from straw. The difference in total N intake was related with straw N intake. Excretion of nitrogen in faeces correlated to the straw N intake. Higher faecal N than expected in the group fed heat treated soybean meal may be due to the application of excess heat to SBM would have protected even in the lower gut.

Table 3. Nitrogen balance of sheep fed undegradable dietary protein from different sources.

Attributes	T_0	T_1	T_2	T_3	T_4	T_5	±SEM
N intake (g/head/d)							
Straw N	3.73^{bc}	3.38^{c}	4.52^{a}	3.31^{c}	3.31^{c}	4.42^{a}	0.403
Concentrate N	5.49	5.34	6.03	5.95	5.6	6.22	0.446
Total	9.22^{ab}	8.72^{b}	10.46^{a}	9.26^{ab}	8.92^{b}	10.64^{a}	0.734
N excretion (g/head/d)							
Faecal N	4.61^{bc}	4.39^{bc}	5.12^{ab}	4.26^{c}	5.55^{a}	5.69^{a}	0.405
Urinary N	4.51^{a}	3.84^{ab}	4.63^{a}	3.19^{b}	3.24^{b}	3.88^{ab}	0.552
Total	9.12^{ab}	8.23^{bc}	9.75^{a}	7.45^{c}	8.78^{abc}	9.58^{ab}	0.746
N balance (g/head/d)	0.106^{d}	0.491^{cd}	0.705^{bc}	1.812^{a}	0.131^{d}	1.069^{b}	0.246
N retention as % of intake	1.16^{c}	5.95^{b}	6.74^{b}	19.52^{a}	1.52^{c}	9.95^{b}	2.368

Different superscripts in the same row indicate significant difference ($p<0.05$)

Urinary N was highest in the groups fed control diet and imported fish meal, while lowest in the group fed refuse tea leaf. Increased N in urine indicate loss of nitrogen and probably larger proportion of absorbed N was in the form of ammonia. Nitrogen retention was highest in the group fed refuse tea leaf and lowest in the T_0 and T_1 groups fed control and heat treated soybean meal. Refuse tea leaf contain significant amount of tannin

(Subramaniam, 1995). The near neutral pH of the rumen allows dietary tannin to form complexes with dietary protein. The tannin protein complexes are stable in the rumen (Jones and Mangan, 1977) and break up in the abomasum where pH is around 2. The released protein is digested and absorbed by the animal.

Nitrogen retention in the group fed formaldehyde treated coconut poonac was closer to the group fed refuse tea leaf. This indicated the relative efficiency of formaldehyde treatment in protecting protein in the rumen. The expected benefits from feeding fish meal were not achieved. Variable degradability values have been reported for fish meal, depending on the way it is processed. The protein of fish meal used in this experiment might have higher degradability. Low N retention with heat treated soybean meal may be due to the over protection making the protein of soybean meal unavailable even in the lower gut. This was further confirmed by the higher faecal N found in this treatment.

Nitrogen fraction of strained rumen fluid obtained 2 h after feeding is presented in Table 4. Total N, ammonia N, TCA soluble N and TCA insoluble N in the rumen fluid were not affected by the treatments. The rumen ammonia concentrations in this study were in agreement with the results obtained by Van Houtert and Leng (1986). Availability of ammonia in the rumen is an important determinant of microbial protein synthesis, as majority

Table 4. Nitrogen fractions, TVFA and pH of rumen fluid obtained from sheep fed UDP of different sources.

Attributes	T_0	T_1	T_2	T_3	T_4	T_5	±SEM
Total N (mg/100 ml)	64.70	63.00	67.00	82.0	66.00	69.70	13.080
Ammonia N (mg/100 ml)	17.70	13.60	16.30	16.3	13.30	14.30	6.170
TCA soluble N (mg/100 ml)	25.80	22.90	26.60	35.4	23.30	26.20	7.370
TCA insoluble N (mg/100 ml)	34.30	35.60	35.30	41.6	40.30	38.60	9.530
TVFA (mmol/100 ml)	5.85	5.62	6.07	6.3	6.47	6.82	1.490
pH	7.20	7.10	7.20	7.0	7.00	7.20	0.128

of the bacteria in the rumen use ammonia for their nitrogen requirements. The results of TCA insoluble N were in agreement with those of Reddy and Reddy (1985). The amount of TCA insoluble N is a reflection of microbial protein synthesis (Gupta, 1986). The results of this study indicate no significant difference in microbial protein synthesis due to the inclusion of UDP in the diet of sheep.

Total volatile fatty acid (TVFA) concentration and the pH of the rumen fluid are presented in Table 4. The effect of treatment on the concentration of TVFA and pH of rumen fluid was not significant. Similar TVFA values have been reported in previous experiments (Flachowsky and Tiroke, 1993). Church (1976) observed that increasing amounts of soluble carbohydrate in the ration result in higher VFA levels in the rumen. Total volatile fatty acid concentration in the rumen can be considered as an indicator of the rate of digestion.

Optimum rumen pH values were recorded by all groups in this study irrespective of treatments. The rumen pH is of importance to ruminal digestion of cell wall constituents because cellulase activity and hence fermentation of fibre decreases with reduction in pH and becomes negligible when pH is less than 6.

The results of blood urea nitrogen, packed cell volume and haemoglobin contents of the blood samples are presented in Table 5. The effects of treatments on these parameters were not significant.

Table 5. Blood urea nitrogen, packed cell volume and hemoglobin content of blood samples of sheep fed undegradable dietary protein from different sources.

Attributes	T_0	T_1	T_2	T_3	T_4	T_5	±SEM
BUN (mg/100 ml)	24.6	21.7	26.9	25.2	20.4	20.4	3.097
PCV (%)	25.0	23.7	26.3	25.6	24.7	26.0	0.907
Hemoglobin (%)	13.2	13.5	13.8	13.4	13.5	13.2	0.944

Urea nitrogen content of the blood is more closely associated with protein metabolism. There is a relationship between metabolites in the rumen and that in blood. All the blood metabolites studied were in normal range in all the groups irrespective of feeding regime suggesting existence of general health of sheep and also the relative adequacies of ration providing amino acids at tissue level.

CONCLUSIONS

The results suggest that UDP and urea can be incorporated in the ration of sheep efficiently and safely for optimum biological responses. Refuse tea leaf represent a potentially superior source of UDP in the diets of sheep. Inclusion of 150 g of refuse tea leaf per kilogram of urea molasses bolus increased the nitrogen retention and body weight gain in sheep.

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