Rumen degradation and intestinal digestibility of rumen undegraded dietary nitrogen in grass-legume mixed diets estimated by the mobile nylon bag technique.

J.F. Mupangwa[1]*, N.T. Ngongoni[2] and H. Hamudikuwanda[2].
[1]Department of Agriculture, Bindura University of Science Education, P. Bag 1020, Bindura, Zimbabwe.
Corresponding author: tjmupangwa@yahoo.com

[2]Department of Animal Science, University of Zimbabwe, P.O. Box MP 167, Mt. Pleasant, Harare, Zimbabwe.

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Abstract

The rumen degradability and intestinal digestibility of nitrogen (N) in four grass-legume mixed diets were estimated in three rumen and duodenal cannulated non-lactating Holstein/Friesian cows using the mobile nylon bag method. The diets consisted of *Chloris gayana* cv. Katambora that was supplemented (25 %) with tropical herbaceous legumes *Cassia rotundifolia* (Cassia), *Lablab purpureus* (Lablab) and *Macroptilium atropurpureum* (Siratro). The diets were incubated in the rumen for 24 and 48 h. After rumen incubation, the mobile bags were pre-incubated in pepsin-HCl solution before inserting into the duodenum and recovering in the faeces. The supplemented diets after 24 h of rumen incubation had significantly ($P < 0.001$) lower N degradability than the katambora grass hay alone. After 48 h, the four diets did not differ ($P > 0.05$) in their N degradability coefficients. The post-ruminal digestibility of undegraded N was not different between the supplemented diets but was higher ($P < 0.001$) than in the unsupplemented diet. The amounts of UDN digested and absorbed in post-ruminal sites were greater ($P < 0.001$) for the supplemented grass hay than that of katambora hay alone. It is concluded that the inclusion of protein rich forage legumes in low quality diets increased the amount of undegradable dietary nitrogen digested in post-ruminal sites.

Keywords: forage legumes, mobile bag, degradability, intestinal digestibility.

Introduction

Forage legume protein serves as a source of both rumen degradable protein and undegradable protein that escapes rumen degradation and is digested in post-ruminal sites. In tropical regions, protein-rich forage legumes can therefore improve production of ruminants consuming low quality grasses (Coombe, 1981) by alleviating the N deficiency in the grasses. Supplementation with legumes has been shown to improve the balance of nutrients and animal performance (Sierbert and Hunter, 1982).

Although a wealth of information has been published on the effect of supplementing low quality roughages with legumes, few studies have been carried out to measure the post-ruminal digestibility of undegraded nitrogen in the supplemented diets. Knowledge of the quantity of undegradable dietary nitrogen (UDN) and its digestibility is essential for feed evaluation according to the new protein systems (AFRC, 1993; NJK, 1985). Only a little work has been done to compare rumen degradability and post-ruminal digestion of UDN in tropical mixed feeds that are based on forage legumes as protein supplements. The objective of this experiment was to determine rumen degradability and post-ruminal digestion of nitrogen (N) of four different grass-legume mixed diets after two different rumen incubation periods, using the mobile nylon bag technique.

Materials and Methods

Experimental diets

The four experimental diets used in the mobile bag study comprised: 100 % *Chloris gayana* cv. Katambora hay (A), 75 % Katambora hay + 25 % *Cassia rotundifolia* cv. Wynn hay (B), 75 % Katambora hay + 25 % *Lablab purpureus* cv. Highworth hay (C) and 75 % Katambora hay + 25 % *Macroptilium atropurpureum* cv. Siratro (D).

Animals

Three rumen and duodenal-cannulated non-lactating and non-pregnant Friesian cows (body mass 450 ± 20 kg) were used to assess the degradation and intestinal digestibility of N in the four diets. The cows were fed at maintenance, a diet comprising 60:40 Katambora grass hay and siratro hay twice daily at 0800 and 1600 h and dairy meal (5 kg/day/animal). The animals were adapted to the diet for 14 days before the experimental period commenced. Animals were housed in individual pens with clean water always available from automatic drinkers.

Preparation of undegraded dietary protein

The experimental diets (A, B, C and D) were milled through a 2 mm screen and a 5 g sample weighed into bags (60 x 120 mm) made of polyester cloth with a

pore size of 45 μm. The bags were tied on plastic tubing, 30 cm long, which was then suspended in the rumen through the rumen cannula according to the procedure described by Bhargava and rskov (1987) for 24 and 48 h. Each diet had two bags for each of the rumen incubation times per animal. The residues recovered from the bags after rumen incubation were thoroughly washed under running tap water and then dried in an oven at 60 oC for 48 h and weighed. These residues formed the samples for the mobile bag study.

Estimation of intestinal digestibility

The intestinal digestibility of the different diets residues was determined by the mobile bag technique as described by Hvelplund (1985). Approximately 1.0 g of undegraded feed residues from each nylon bag was weighed into two mobile bags measuring 60 x 60 mm and a pore size of 9 m and heat-sealed (Elwis-Pack, Kokendal Industripark, Denmark). The bags were then incubated in pepsin-HCl solution (1g pepsin per litre of 0.01N HCl) in a shaking water bath at 37 oC for 2 h in order to simulate abomasal digestion (Vanhatalo and Ketoja, 1995). After the enzymatic treatment a maximum of 12 bags per cow per day were inserted through the cannula into the proximal duodenum at 5 - 10 min intervals and recovered from the faeces. After recovery from the faeces, by washing the faeces through a sieve, the bags were given a final cold wash in a domestic washing machine (Lamavat 502, AEG, Denmark) for 50 min then dried in an oven at 60 oC for 48 h and weighed before laboratory analysis.

Chemical Analysis

Experimental feeds were analysed for dry matter (DM), Kjeldahl-N (AOAC, 1984), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) and acid detergent insoluble nitrogen (ADIN) as described by Goering and Van Soest (1970). Feed residues after ruminal and intestinal exposure were analysed for Kjeldahl-N (AOAC, 1984).

Calculations and Statistical Analysis

The rumen degradable nitrogen (RDN) coefficients of the dietary nitrogen (DN g/kg DM) were calculated from the rumen undegradable nitrogen (RUN g/kg DM) from the bags after variable incubation times using equation 1. The intestinal nitrogen digestibility (IND) coefficients were estimated from the indigestible nitrogen (IN g/kg DM) remaining in the mobile bags after passage through the intestines using equation 2.

$$RDN = (DN\ RUN)/DN \quad (1)$$

$$IND = (RUN\ IN)/RUD \quad (2)$$

Rumen degradability and true intestinal digestibility data of a randomised complete block design were analysed separately using the General Linear Models Procedures of SAS (SAS, 1990) with the following model:

$$Yijkl = \mu + Di + Tj + Ak + eijkl$$

Where: Yijkl is the dependent variable (e.g. rumen degradability of N), μ is the overall mean, Di (i = 1, 2, 3, 4) is the fixed effect of diet, Tj (j = 1, 2) is the effect of rumen incubation time, Ak (k = 1, 2, 3) is the random effect of animal which was treated as a complete block and eijkl is the random error. Statistical differences between treatment means were assessed using the Tukey Studentised Range test (SAS, 1990).

Results

The chemical composition of the diets that were used is shown in Table 1. The katambora grass hay had a lower CP and higher NDF content than that of the supplemented diets. All the diets contained a relatively large amount of lignin and for each only a very small amount of the N they contained was associated with the ADF.

Table 1: Chemical composition (g/kg DM) of the diets1 used in the mobile bag experiment.

	A	B	C	D
CP	61.6	76.3	76.9	78.5
NDF	829	772	782	783
ADF	540	548	529	532
ADL	138	144	112	134
ADIN(g/kg N)	31.7	25.9	26.5	29.3

1Diets:
A = Katambora grass hay (100 %)
B = Katambora + Cassia (75:25)
C = Katambora + Lablab (75:25)
D = Katambora + Siratro (75:25)

The rumen degradabilities of N in the diets are presented in Table 2. The supplemented diets after 24 h of rumen incubation had significantly ($P < 0.001$) lower N degradability coefficients than that of grass hay alone. However, after 48 h incubation the diets did not differ ($P > 0.05$) in their N degradabilities. There was an increase in N degradation with increasing rumen incubation time for diets B, C and D while that of A was not affected ($P > 0.05$) by increased rumen incubation.

The amount of undegradable dietary N remaining after rumen incubation was greater ($P < 0.001$) in the supplemented diets than that of grass hay alone (Table 2.). There was no significant ($P > 0.05$) difference between the diets B, C and D in their UDN content after similar rumen incubation time. Diets incubated for 24 h had greater amounts ($P < 0.01$) of UDN passing to post-ruminal sites compared to that in 48 h residues. Intestinal digestibilities of UDN in the diet residues and the calculated amounts of UDN digested and absorbed from the post-ruminal sites are summarised in Table 3. The 24 h residues from the supplemented diets (B and D) had a significantly greater ($P < 0.01$) intestinal digestibility of UDN than that from diet A. The intestinal digestibility of UDN in the 24 h residues from diet C was not statistically different ($P > 0.05$) from that of the other diets. In the 48 h residues, diet A had a significantly lower ($P < 0.01$) UDN digestibility compared to that of diets B, C and D.

The amount of UDN digested in the 24 h residues was significantly greater ($P < 0.001$) in diets B, C and D compared to that of diet A (Table 3). A similar difference was observed in the 48 h residues. There was no significant ($P > 0.05$) effect of rumen incubation period on the amount of UDN digested post-ruminally for each diet.

Discussion

The ruminal disappearance of N increased with increasing rumen incubation time. This was due to increased time afforded to the degradation activities of rumen microbes on the diets. This is in agreement with results reported by Moshtaghi- Nia and Ingalls (1995) who observed a decrease in total N for different feedstuffs with increasing rumen incubation. The higher N degradability of katambora grass hay alone compared to the supplemented grass hay was unexpected. In an earlier study (Mupangwa et al, 2003) the legumes on their own were shown to have highly degradable N and this would have been expected to increase N degradability of the grass-legume feedstuffs above that of the grass diet alone.

Most of the grass hay N was degraded by 24 h of incubation resulting in no apparent increase in N degraded in that feedstuff at 48 h of rumen incubation. This lack of increase in N degraded in grass hay may be due to a fraction of the N being closely associated with the fibre fraction. In contrast, the supplemented diets had greater N degraded with increasing incubation time indicating the N contribution of the legume supplement. Similar results have been reported in earlier studies (Kirkpatrick and Kennelly, 1984).

Table 2: Rumen degradation coefficient of dietary N and amount of calculated undegradable dietary N (g/kg DM) of four diets estimated by the mobile bag method.

Diets	Degradability		UDN	
	24 h	48 h	24 h	48 h
A	0.637^{a}	0.646	3.59^{b}	3.50^{b}
B	0.532^{b}	0.606	5.71^{a}	4.81^{a}
C	0.550^{b}	0.616	5.54^{a}	4.73^{a}
D	0.550^{b}	0.617	5.68^{a}	4.83^{a}
SED	0.22	0.22	0.26	0.26

^{ab}Means in the same column with different superscripts are significantly different ($P < 0.001$).

Table 3: Intestinal digestibility coefficients of rumen undegraded nitrogen and estimated UDN digested (g/kg DM) after variable rumen incubations.

Diets	Rumen incubation, h		Digested UDN	
	24 h	48 h	24 h	48 h
A	0.419[b]	0.402[b]	1.51[b]	1.43[b]
B	0.527[a]	0.515[a]	2.99[a]	2.48[a]
C	0.496[ab]	0.555[a]	3.01[a]	2.64[a]
D	0.548[a]	0.557[a]	3.13[a]	2.71[a]
SED	0.044	0.043	0.50	0.50

[ab]Means in the same column with different superscripts are significantly different ($P < 0.001$).

The amount of N degraded in the rumen was greatly increased by legume supplementation. After 24 h of rumen incubation, the amount of N degraded was 6.30 g/kg DM for diet A, 6.50 for B, 6.76 for C and 6.92 gN/kg DM for diet D. With increasing incubation time there was no marked increase in N degraded in the grass hay diet with a mean value of 6.40 g/kg DM while that of legume supplemented diets increased to 7.40, 7.57 and 7.76 gN/kg DM, respectively, for B, C and D. The increased N degradation with legume supplementation could provide nutrients such as ammonia and amino acids required for ruminal microbial growth and therefore improve the rate of microbial attachment and degradation rates of roughages with lower protein content than the grass hay used in this study.

The flow of dietary protein and amino acids to the small intestines in ruminants is related to the extent of degradation of feed protein in the rumen and on the physical and chemical nature of the protein substrate. Supplementation of the grass hay in this study increased the quantities of UDN made available for digestion and absorption in post-ruminal sites. The lower UDN digested post-ruminally for diet A could be due to a high fibre bound N which was not digested by intestinal enzymes. Increased proportion of indigestible fibre bound N has been reported to cause a marked decline in intestinal N digestibility of residual feed N (Varvikko and Vanhatalo, 1991). With the shorter rumen incubation of 24 h, which represents higher outflow rates of 0.04/h, the disappearance of N shifted from the rumen to the intestines especially for the supplemented diets resulting in no statistical differences in the quantities in total tract N digestion for each diet between 24 and 48 h incubations. Post-ruminal digestion has been reported to compensate differences in rumen degradability of a feed resulting in similar total tract N disappearance in a feed irrespective of rumen fermentation time (Moshtangi Nia and Ingalls, 1995). The intestinal digestibility of UDN of the four feeds was considerably lower than the recommended values of 0.82 (NJK, 1985) and 0.85 (ARC, 1984). However, the values for the supplemented diets fell within the range of 0.50 to 0.95 recommended in the French PDI (Verite and Peyrand, 1989) while that of the control diet, A, was outside this range.

The inclusion of the protein-rich forage legumes in low quality diets was effective in increasing UDN made available for digestion in the small intestines. Greater amounts of UDN were digested and probably absorbed from the legume supplemented diets and this could benefit dairy cows and growing ruminants. The compensation effect of post-ruminal digestion could be of great benefit in reducing excessive degradation of the legume supplement in the rumen and increasing supply of dietary amino acids absorbed in the intestines at high levels of feeding.

In this study microbial contamination was assumed to be low when the N disappearance was calculated. However, other studies have reported increased contribution of microbial N in residues of highly fibrous feeds. Further research is required to determine extent of microbial contamination of feeds with high fibre and low protein, as well as the effect of feeding level (bag retention time) on intestinal digestibility as determined by the mobile nylon bag technique for tropical feedstuffs.

Finally, the use of constant digestibility values of UDN such as 0.82 or 0.85 as recommended in some protein systems seems not to apply to tropical forages which tended to have variable UDN digestibility.

Variable digestibility coefficients for legume supplemented poor quality diets, taking into account the feeding levels, outflow rates of particulate matter and extent of degradation need to be used when the new protein systems are applied in diets based on tropical forages.

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