Effects of dietary roughage: concentrate ratio and rumen ammonia concentration on *in situ* feedstuff degradation in the rumen of sheep

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Received 26 June 1991; revised 17 February 1992; accepted 26 August 1992

The aim of this experiment was to distinguish between the effects of dietary roughage: concentrate ratio and rumen ammonia concentration on rumen *in situ* degradation in sheep. Three diets with roughage: concentrate ratios of (*Eragrostis curvula* hay: maize) 75:25, 50:50 or 25:75 were used as basal diets, and three rumen ammonia concentrations (6, 12 or 15 mM) were imposed on each of these. The amounts of supplemental urea required to achieve these rumen ammonia concentrations were determined from regression equations relating urea supplementation level to rumen ammonia concentration for each diet. Rumen ammonia concentration had no effect on nitrogen (N) or dry matter (DM) degradation rates of any of the feedstuffs studied ($P > 0.05$). N degradation rate of *E. curvula* hay was depressed by 48% ($P < 0.05$) by increasing dietary maize content, but the N degradation rates of lucerne hay, maize, sunflower oilcake and cottonseed oilcake were not affected ($P > 0.05$). DM degradation rate of *E. curvula* hay was decreased by 58% by increasing dietary maize content, and that of lucerne hay by 44% ($P < 0.05$). DM degradation rates of maize, sunflower oilcake and cottonseed oilcake were not affected ($P > 0.05$). It was concluded that, although the potential degradative activity of rumen contents is influenced by the ratio of roughage in the diet, the effect of these changes differs between feedstuffs. Expression of basal diet effects appears to be a result of several factors which include the potential rate and extent of degradation of the test feedstuff and the prevailing rate of flow of particulate matter from the rumen.

Die doel van hierdie proef was om te onderskei tussen die invloed van dieetruvoer: konsentraatverhouding en rumen-ammoniakkonsentrasie op rumen-*in situ*-degradeerbaarheid in skape. Drie diëte met ruvoer: konsentraatverhoudings van (*E. curvula*-hooi: mielies) 75:25, 50:50 of 25:75 is as basale diëte gebruik, en vergelykings is teen rumen-ammoniakkonsentrasies van 6, 12 of 15 mM vir elke dieet gemaak. Die hoeveelhede ureum benodig om die verlangde rumenammoniakpeile daar te stel is deur middel van regressievergelykings voorspel wat opgestel is om die verband tussen ureumpeil en ammoniakkonsentrasie vir elke dieet te beraam. Rumenammoniakkonsentrasie het geen invloed op die stikstof (N)- of droëmateriaal (DM)-degradeerbaarheid van enige van die voerkomponente wat ondersoek is, gehad nie ($P > 0.05$). N-afbraaktempo van *E. curvula*-hooi is met 48% onderdruk deur 'n verhoogde mielie-insluitingspeil ($P < 0.05$), maar dié van lusernhooi, mielies, sonneblomoliekoek en katoensaadoliekoek is nie beïnvloed nie ($P > 0.05$). DM-afbraaktempo van *E. curvula*-hooi is met 58% onderdruk deur verhoogde mielie-insluitingspeil, en dié van lusernhooi met 44% ($P < 0.05$). DM-afbraaktempo van mielies, sonneblomoliekoek en katoensaadoliekoek is nie beïnvloed nie ($P > 0.05$). Daar is tot die gevolgtrekking gekom dat terwyl die potensiële afbraakaktiwiteit van rumeninhoud skynbaar deur die verhouding van ruvoer: konsentraat in die dieet beïnvloed word, sal die grootte van hierdie effekte tussen voersoorte verskil. Dit wil voorkom of die uitdrukking van dieet-effekte deur verskeie faktore beïnvloed word, insluitend die potensiële tempo en mate van rumen-degradeerbaarheid en die heersende vloeitempo van partikels vanuit die rumen.

Keywords: Basal diet, degradability, *in situ*, nylon bag, rumen, rumen ammonia, sheep.

Introduction

Estimates of rates of protein degradation in the rumen derived from the *in situ* (nylon bag) technique differ considerably for any one feedstuff. It would appear that the basal diet fed to animals used for incubation of test feedstuffs may be responsible for part of this variation. Unfortunately, the results of studies conducted to compare different basal diets are conflicting and provide little information which could be used to predict basal diet effects. While some authors have shown that protein degradation is increased by the inclusion of roughage in the diet (Schoeman *et al.*, 1972; Ganev *et al.*, 1979; Lindberg, 1981a), others have reported that protein degradation was increased by cereal inclusion in the basal diet (Lindberg, 1981b) or was unaffected by basal diet (Siddons & Paradine, 1981). The effects of basal diet protein content are also conflicting. Kirkpatrick & Kennelly (1987) found that degradability was higher when dietary crude protein content was increased, but Murphy & Kennelly (1987) reported no effect. While Ortega *et al.* (1979) reported no effect of rumen ammonia concentration on degradation, Erdman *et al.* (1986) reported the opposite, and showed that the optimum rumen ammonia concentration for maize (18 mM) differed from that for cottonseed oilcake (12 mM). In addition to this, it has been pointed out (Lindberg, 1985) that several reports indicate the existence of an interaction between level of protein in the diet and the amount of starch that can be added without impairing fibre digestion.

Differences in rumen microbial population size and species composition represent the most probable explanation for these anomalies (Lindberg, 1985). Microbial population dynamics are, in turn, influenced by factors such as rumen pH, ammonia concentration, type of energy substrate (Lindberg, 1987) and, in particular, the balance between rumen ammonia concentration and fermentable carbohydrate supply. For instance, there is evidence that more nitrogen (N) is incorporated into bacteria when rumen fermentation yields a high proportion of propionic acid (Schwartz & Gilchrist, 1975). This implies that N is more likely to become limiting for bacterial growth with high-energy

diets than with high-fibre diets if comparisons of basal diet effects are made on an iso-nitrogenous basis. Moreover, El Shazly (1952) found that deaminative activity was stimulated by increasing intake of soluble protein, and Broderick *et al.* (1981) showed that ruminal amino acid concentrations were increased by rumen ammonia concentrations as high as 16 mM. Deaminative activity may also be influenced by the amount of readily fermentable carbohydrate available for fermentation (Reis & Reid, 1959). While most experiments relating to basal diet effects have been designed to examine either the influence of dietary carbohydrate supply or that of nitrogen supply, there are few studies in which diets have been balanced to ensure that the effects of energy and nitrogen supply are not confounded. The aim of this experiment was to distinguish between the effect of dietary energy substrate and that of non-protein-nitrogen. This was achieved by the addition of urea to three diets of different concentrate:roughage ratios such that three comparable rumen ammonia concentrations were attained for each of the diets.

Materials and Methods

Twenty-seven sheep of mean mass 57 kg (standard deviation 8 kg) were allocated to nine treatments in a completely randomized block design with three periods. The treatments consisted of three main diets differing in the ratio of roughage: concentrate (*E. curvula* hay:maize in the proportions 75:25, 50:50 or 25:75), each supplemented with quantities of urea calculated to result in rumen ammonia (NH_3) concentrations of either 7, 14 or 21 mM. The amounts of urea required to achieve the desired rumen ammonia concentrations were estimated from regression equations developed for each of the three main diets during a pilot trial using the same feeds, feeding schedule and nine of the same sheep as used in the main experiment: Three sheep were allocated to each of the three diets, and urea supplementation was increased from 2 to 30 g/kg feed consumed in 2 g/kg increments over a period of 15 days, during which time rumen NH_3 concentrations were determined from daily samples taken at 1, 2 and 3 h after feeding.

E. curvula hay was hammermilled through a 25-mm sieve, and yellow maize through a 6-mm sieve. These were mixed in the indicated proportions (75:25, 50:50 or 25:75) with the addition of 5% molasses as binding agent, 1% salt and a vitamin-mineral mixture calculated to provide (/kg feed): S, 300 mg; Mn, 20 mg; Cu, 9 mg; Co, 1 mg; Zn, 38 mg; Vitamin A, 6000 IU; Vitamin D_3, 3000 IU and Vitamin E, 2 IU (NRC, 1975). Urea was added to the diets in the required amounts each day in the form of a 20% solution which was sprayed onto the feed with a commercial spraygun to ensure uniformity of application. Diets were fed at a level of *ad libitum* plus 10% (based on a four-day moving average), and daily allotments were offered in six equal portions at 4-h intervals using automated feeders. The sheep were housed indoors in metabolism crates under continuous illumination and were given free access to water.

The effect of the different treatments on the *in situ* degradability of five different feedstuffs, which are known to differ widely with respect to rate and extent of rumen degradation (Cronjé, 1983; Erasmus *et al.*, 1990a; 1990b), was investigated. The feedstuffs were: lucerne hay, *E. curvula* hay, maize, sunflower oilcake and cottonseed oilcake. Polyester bags measuring 160 × 90 mm and of pore size 53 μm were used for *in situ* incubation. Bags were made with double seams sealed with adhesive, and were closed by means of a draw string. A feed sample of mass approximately 5 g, which had been milled to pass through a 5-mm screen, was accurately weighed into each bag. Sheep were fitted with large (8 cm internal diameter) rumen cannulas which facilitated manual placement of bags in the ventral portion of the rumen. Bags were attached to a swivel clip mounted on the inside of the cannula stopper by means of a 250-mm length of nylon line. In order to avoid period effects, all feedstuffs were incubated simultaneously (complete exchange method; Paine *et al.*, 1981) for each of the following durations: 3, 6, 9, 12, 15, 18, 24 and 48 h. Dry matter (DM) and N disappearance were measured in duplicate with each of the three sheep allocated per treatment as recommended by Mehrez & Ørskov (1977), giving a total of six estimates per feedstuff. After removal from the rumen, bags were washed in running water until the fluid squeezed from the bags was clear, and dried for 24 h at 60°C. Degradation rates were calculated from the slope of the regression relating the natural logarithm of the percentage of substrate remaining in the bag to time over the first 12 h of incubation (Miller, 1980), except in the case of *E. curvula* hay where a better fit was obtained by using data for the first 48 h. Effective degradation was calculated according to Miller (1980). This method was used in preference to more complex methods (Ørskov & McDonald, 1979), since previous research in this laboratory has shown no clear advantage for either method (Cronjé, 1983).

Rumen fluid samples for determination of NH_3 concentrations, volatile fatty acid concentrations and pH were taken after the *in situ* incubation trial had been completed. Rumen fluid from two sheep per treatment was collected four times daily for two consecutive days. Following this, the same sheep were used to determine the flow rate of particulate matter from the rumen using chrome-mordanted fish-meal. Chrome mordanting was accomplished by a modification of the process described by Uden *et al.* (1980) in which ^{51}Cr (3 μCi) was also included in the chrome mordant as tracer. The mordanted fish-meal was added to the rumen contents via the rumen cannula and mixed thoroughly, following which faecal samples were collected at 4-h intervals for the first 28 h, and at 8-h intervals until 196 h had elapsed. Relative Cr concentrations were determined using a gamma counter, and ruminal flow rates were calculated by the method of Grovum & Williams (1973).

Estimates of the extent of DM degradation obtained using the *in situ* technique were also compared to those obtained using the first stage of a conventional two-stage *in vitro* technique. Rumen fluid was obtained from one of each of the sheep fed the nine experimental diets, and used as inoculum for the determination of *in vitro* digestibility according to Tilley & Terry (1963). Digestion was allowed to proceed for 48 h (pepsin digestion stage was omitted) and was then compared to that obtained by 48 h *in situ* incubation.

Nitrogen content was determined using an autoanalyser method and ammonia content by Kjeldahl analysis (AOAC, 1980). Dry matter (DM) and organic matter (OM) were analysed by conventional (AOAC, 1980) methods. Neutral detergent fibre (NDF) was determined by the method of Van Soest & Wine (1967) and *in vitro* digestibility by the Tilley & Terry (1963) method. Volatile fatty acids in rumen fluid were analysed by gas chromatography. Rumen contents were mixed thoroughly prior to sampling, and representative samples were strained through nylon gauze. Samples for volatile fatty acid analysis were preserved by the addition of 1 ml of a 10% (*w/v*)

NaOH solution per 10 ml rumen fluid, and those for ammonia analysis by the addition of 1 ml of a 50% (*v*/*v*) sulphuric acid solution.

The statistical significance of differences between treatment effects was determined by two-way analysis of variance and the *F* test.

Results and Discussion

The chemical composition of the feedstuffs used is presented in Table 1. The amounts of urea necessary to achieve the desired rumen ammonia concentrations for each of the main diets were determined from regression equations derived from nine sheep as described previously. Individual regressions were corrected for outlyers by residual analysis with a 95% confidence interval. Rumen NH_3 concentrations below 4 mM were excluded from the regression, as it is to be expected that the relationship will only assume linearity beyond the point of ammonia accumulation (Roffler *et al.*, 1976). As there were no significant differences ($P > 0.05$) between the slopes of regressions for the three main diets (one-way analysis of variance), a mean slope was computed (+1.0132 SEM 0.0832) and used in further calculations. Intercepts differed ($P < 0.05$) between the high-roughage and high-maize diets. The regressions used were:

$Y = 1.655$ (SEM 0.964) + 1.0132 x (75 hay:25 maize diet)
$Y = -1.349$ (SEM 1.28) + 1.0132 x (50 hay:50 maize diet)
$Y = -6.379$ (SEM 1.51) + 1.0132 x (25 hay:75 maize diet)

where Y is the predicted rumen NH_3 concentration and x is the amount of urea (g/kg) added to the diet.

Table 1 Chemical composition of test feedstuffs

Feedstuff	DM (%)	OM[a]	N[a]	NDF[a]
Eragrostis curvula hay	93.0	89.8	0.91	76.2
Lucerne hay	93.6	84.0	2.9	41.4
Maize	91.6	90.2	1.5	7.9
Sunflower oilcake	93.7	86.9	7.5	18.9
Cottonseed oilcake	92.9	86.3	7.5	15.3

[a] % of dry matter.

The amounts of urea added to the diets are shown in Table 2. Although rumen ammonia concentrations were lower than predicted, the regression approach was successful, as there were no significant differences ($P > 0.05$) in rumen ammonia concentration between any of the three roughage:concentrate ratios within the same rumen ammonia level treatment. Overall, the lowest rumen ammonia concentration differed significantly from the two highest concentrations ($P < 0.05$).

Table 2 Urea content of experimental diets (g/kg feed)

Basal diet (hay:maize ratio)	Target rumen ammonia concentration (mM)		
	7	14	21
75 : 25	5.3	12.2	19.1
50 : 50	8.3	15.2	22.1
25 : 75	13.2	20.1	27.0

Measured concentrations were (mean ± standard error) 6.4 ± 0.3; 11.5 ± 0.2 and 14.6 ± 3.8 mM for treatments aimed at achieving concentrations of 7, 14 and 21 mM respectively. Differences between predicted and observed values were greater at the higher concentrations. This may be related to changes in rumen microbial numbers, urea recycling, pH and permeability of the rumen wall. Notwithstanding the inaccuracies of this approach, significant differences were obtained between levels which are close to those (6 and 13 mM) responsible for differences in rate of protein degradation in the trials reported by Wallace (1979). The upper level corresponds with that (13.8 mM) held by Mehrez & Ørskov (1977) to be the optimum for *in situ* OM degradation, while the lower level is closer to that (4 mM) recommended by others (Satter & Roffler, 1975; Roffler *et al.*, 1976) for maximum microbial protein synthesis.

Volatile fatty acid concentrations in rumen fluid were not affected by rumen ammonia concentration ($P > 0.05$), but the concentration of propionic acid increased ($P < 0.05$) as dietary roughage was replaced by concentrate, and acetate concentration decreased ($P < 0.01$) (Table 3). Rumen pH was decreased ($P < 0.05$) by maize inclusion (Table 3). The mean flow rate of particulate matter from the rumen was 0.039/h (standard deviation: 0.009/h). No significant differences could be detected between treatments ($P > 0.05$); this is probably due to the high coefficient of variation associated with this technique and the low numbers of animals used, rather than a true reflection of diet effects.

Table 3 Mean volatile fatty acid concentrations and pH in rumen fluid of animals fed experimental diets (standard errors of the means in parentheses)

Basal diet (hay:maize ratio)	Acetate (mmol/100ml)	Propionate (mmol/100ml)	Butyrate (mmol/100ml)	pH
75 : 25	77.0[a]	14.7[a]	8.3	6.233[a]
	(0.3)	(0.1)	(0.3)	(0.014)
50 : 50	73.0[b]	16.3[ab]	10.7	6.066[a]
	(0.3)	(3.4)	(0.9)	(0.008)
25 : 75	60.3[c]	25.3[b]	14.7	5.767[b]
	(2.8)	(11.4)	(3.9)	(0.004)

[a, b] Means within the same column with different superscripts differ significantly ($P < 0.05$).

Rumen ammonia concentration had no ($P > 0.05$) effect on rate of N or DM disappearance of any of the feedstuffs examined. The data indicate that, in this respect, there would be no advantage to the inclusion of more than 0.5% urea in diets containing 75% *E. curvula* hay:25% maize, or more than 1.3% urea in the case of diets composed of 75% maize:25% *E. curvula* hay (see Table 2). The difference in the amount of urea that had to be added to the different diets in order to achieve similar rumen ammonia concentrations (Table 2), also illustrates the potential dangers of ascribing responses to energy-substrate effects when diets of different energy content are compared on an 'iso-nitrogenous' basis.

The lack of response in degradative activity of the high-roughage basal diet (75 *E. curvula*:25 maize) to urea supplementation may be due to the low potential degradability of *E. curvula* hay. Urea supplementation of low-quality hay will

not increase potential digestibility; it can only assist in realizing potential digestibility. It has been shown (Ørskov, 1982) that responses to urea supplementation of certain roughages will only be obtained if the potential digestibility is first increased by agents such as caustic soda. It is possible that many of the conflicting results reported in the literature may be reconciled by the proposal (Erdman *et al.*, 1986) that maximum fermentation occurs at higher rumen ammonia concentrations as fermentable energy availability is increased. De Faria & Huber (1984) postulated that differences in energy availability between their diets and those of Mehrez & Ørskov (1977) may have been responsible for the dissimilar responses to urea addition. In the case of the high-maize diet, the reason for a lack of response in degradative activity to urea supplementation in this study versus the substantial responses reported for barley-based diets (Mehrez & Ørskov, 1977; Wallace, 1979) may also be due to morphological differences between these two feedstuffs. In the case of barley, the most active portion of the bacterial population is present in microcolonies intimately associated with the starch granules (Allison, 1982). The prevailing NH_3 concentration in such a micro-environment where utilization is fast and the interchange with rumen fluid is limited may be lower than that in the surrounding medium. A higher rumen NH_3 concentration may be required to maintain the estimated minimum concentration of 3.6 mM in the microcolonies (Ørskov, 1982), and thus be responsible for the responses observed by Mehrez & Ørskov (1977) and Wallace (1979).

Both N and DM degradation rates of *E. curvula* hay were depressed ($P < 0.01$) by increasing the proportion of maize in the diet (Table 4). The similar responses for N and DM degradation may indicate that the nitrogenous components of this feedstuff are closely associated with the carbohydrate fractions. A decrease in numbers of cellulolytic bacteria at the expense of amylolytic species may have been responsible for the decrease in *E. curvula* hay degradation observed for the high-maize diet. The fall in pH from 6.2 for the high-roughage diet to 5.8 for the low-roughage diet (Table 3) may have induced changes in the composition of the rumen microbiota. Cellulolytic bacteria, in particular, are known to be sensitive to changes in pH, and growth is reported to be inhibited when pH falls below 6.2 (Ørskov, 1982; Mould & Ørskov, 1984). It is also possible that factors such as frequency of feeding, level of feeding and fermentation rate (see Van Straalen & Tamminga, 1990) could have prevented a fall in pH in other experiments (Lindberg, 1981b) which show that there is little or no depression of fibre digestion with increasing amounts of dietary cereal.

Nitrogen degradation of lucerne hay was not affected by the ratio of *E. curvula* hay : maize in the diet ($P > 0.05$). The mean rate of N degradation was 9.58%/h (standard deviation = 2.86%). The effective percentage degradation at the measured rate constant for flow of particulate matter from the rumen (0.04/h) was 84%. Dry-matter disappearance was depressed at the higest level of maize inclusion (Table 5), as was the case with *E. curvula* hay. The different responses of the N and DM fractions to the treatments may indicate that the degradation of the nitrogenous fraction of lucerne is not limited by its association with the fibrous components of the plant, as was suggested to be the case with *E. curvula* hay.

No treatment effects ($P > 0.05$) were observed for N or DM degradation of maize, cottonseed oilcake or sunflower oilcake. Degradation rates for these feedstuffs are presented in Table 6. The lack of a basal diet effect on maize degradation is in agreement with findings by Siddons & Paradine, 1981. Although basal diet had no effect on the degradation of cottonseed and sunflower oilcake, substantial basal diet effects for these two

Table 4 Effect of *Eragrostis curvula* hay : maize ratio on degradation rate (standard errors of the means in parentheses) of dry matter and nitrogen in *E. curvula* hay and effective degradation calculated from the means using different rate constants for outflow of particulate matter from the rumen (0.04/h = measured fractional outflow rate constant)

			Effective degradation					
			Fractional outflow rate from the rumen					
Diet	Degradation rate		0.02/h		0.04/h		0.06/h	
(hay : maize ratio)	(%/h)	Index	(%)	Index	(%)	Index	(%)	Index
Nitrogen degradation								
75 : 25	1.48[a] (0.19)	100	58.2	100	46.1	100	40.5	100
50 : 50	1.40[a] (0.16)	95	57.1	98	45.2	98	39.8	98
25 : 75	0.77[b] (0.08)	52	47.1	81	37.9	82	34.2	84
Dry matter degradation								
75 : 25	1.03[a] (0.09)	100	41.8	100	29.0	100	23.6	100
50 : 50	0.96[a] (0.08)	93	40.3	96	27.9	96	22.8	97
25 : 75	0.43[b] (0.06)	42	26.9	64	19.2	66	16.4	69

[a, b] Column means with different superscripts differ significantly ($P < 0.05$).

Table 5 Effect of *Eragrostis curvula* hay : maize ratio on degradation rate (standard errors of the means in parentheses) of lucerne hay dry matter and effective degradation calculated from the means using different rate constants for outflow of particulate matter from the rumen (0.04/h = measured fractional outflow rate constant)

Diet (hay : maize ratio)	Degradation rate		Effective degradation					
			Fractional outflow rate from the rumen					
			0.02/h		0.04/h		0.06/h	
	(%/h)	Index	(%)	Index	(%)	Index	(%)	Index
75 : 25	5.27[a] (0.43)	100	80.5	100	68.8	100	61.2	100
50 : 50	4.75[a] (0.54)	90	79.0	98	66.9	97	59.3	97
25 : 75	2.96[b] (0.23)	56	71.3	89	58.3	85	51.1	83

[a, b] Column means with different superscripts differ significantly ($P < 0.05$).

Table 6 Mean degradation rates (standard deviation in parentheses; n = 27) and effective percentage degradation calculated from means using the measured rate constant for outflow of particulate matter from the rumen (0.04/h) for N and DM fractions of maize, sunflower oilcake and cottonseed oilcake

Feedstuff	Nitrogen		Dry matter	
	Degradation rate (%/h)	Effective degradation (%)	Degradation rate (%/h)	Effective degradation (%)
Maize	4.17 (1.15)	66.7	5.02 (1.3)	69.9
Sunflower oilcake	8.03 (2.7)	96.2	4.57 (1.22)	86.9
Cottonseed oilcake	7.21 (4.21)	92.0	2.76 (1.11)	74.8

feedstuffs have been reported by Ganev *et al.* (1979) and Lindberg (1981b). The reason for this apparent anomaly may be related to differences in processing methods employed in the production of oilcake. The sunflower oilcake used in this experiment was much more degradable than that used by Ganev *et al.* (1979); 82% of nitrogen was degraded within 3 h of incubation in this experiment, whereas the sunflower oilcake used by the latter authors took 15 h to reach a comparable extent of degradation. Nitrogen degradation of the cottonseed oilcake used in this study reached 88% within 24 h, but with the sample used by Lindberg (1981b), only 50% N was degraded within 24 h. The influence of rate and extent of degradation on the expression of basal diet effects is well illustrated by the latter experiment in which the effect of basal diet on cottonseed oilcake degradation decreased from 27% after 2 h incubation to 0.6% after 24 h.

The validity of the basal diet effects discussed above was evaluated by comparing estimates of 48 h degradation obtained using the *in situ* technique to those obtained using the first stage of a two-stage *in vitro* method (Tilley & Terry, 1963).

Table 7 Comparison of 48 h *in vitro* DM digestibility with 48 h *in situ* DM degradation (standard errors of the means in parentheses)

Feedstuff	Source of inoculum (dietary hay : maize ratio)		
	75 : 25	50 : 50	25 : 75
Eragrostis curvula hay			
In vitro	54.8[a] (9.1)	51.2[ab] (3.1)	32.5[b] (4.9)
In situ	44.6[a] (2.4)	42.5[a] (2.1)	26.8[b] (1.9)
Lucerne hay			
In vitro	66.2 (0.8)	63.8 (0.3)	58.3 (15.2)
In situ	76.6 (2.4)	76.7 (2.1)	70.4 (1.5)
Maize			
In vitro	84.0 (4.2)	80.2 (0.7)	74.8 (9.6)
In situ	83.1 (1.0)	90.7 (1.2)	85.0 (3.0)
Sunflower oilcake			
In vitro	74.2 (3.1)	73.7 (0.8)	65.7 (16.1)
In situ	84.4 (0.3)	84.7 (0.4)	83.7 (0.9)
Cottonseed oilcake			
In vitro	70.3[a] (0.9)	66.4[b] (0.7)	58.5[ab] (19.1)
In situ	80.8 (0.7)	80.4 (1.1)	74.5 (1.0)

[a, b] Means within the same row with different superscripts differ significantly ($P < 0.05$).

Rumen ammonia concentration had no effect on DM digestion estimates of the feeds examined for either of the techniques ($P > 0.05$), and data were pooled for each ratio of hay : maize (Table 7). Basal diet effects were similar: The depression of *in vitro* DM digestion of *E. curvula* hay with the high-maize diet is similar to the response obtained with the *in situ* technique, and there were no diet effects for either technique with lucerne, maize or sunflower oilcake. Although *in situ* estimates were generally higher than *in vitro* estimates, the similar pattern of response does support the basal diet effects reported using the former technique.

It should, however, be noted that this comparison of 48-h estimates of degradability is only valid in the broadest sense,

as the magnitude of basal diet effects will vary according to the period of exposure to rumen degradation. This is well illustrated by the effect of basal diet on lucerne DM degradation *in situ*, which was progressively diminished as maximum potential degradability was approached. Differences between the the high-roughage and the high-maize diets decreased from 17% after 12-h incubation to 13% after 24 h, and were not significant at 48 h. In an *in vivo* situation, the time of exposure to degradation, and hence the expression of basal diet effects, will be determined by the flow rate of particulate matter from the rumen which is, in itself, a function of degradation rate. Thus the different responses to changes in basal diet observed for different feedstuffs in this study and that of others can be explained by the thesis that the expression of basal diet effects will vary according to the inherent characteristics of the feedstuff in question. This is evident from results in Tables 4 and 5. The relative effect of basal diet on DM degradation was greater for *E. curvula* hay than for lucerne hay. Basal diet effects on *E. curvula* hay degradation were greatest at the slowest flow rate but, in the case of lucerne hay, effects were greatest at the high flow rate. This also indicates that the expression of basal diet effects may be suppressed or potentiated if assumed values for flow rate are used in the calculation of effective percentage degradation. It should also be noted that the relative effect of basal diet on degradation was substantially greater when expressed as a rate (%/h) than when expressed as an amount (%), irrespective of the flow constant used. This may also explain some of the conflicting results in the literature where basal diet effects have been variously expressed as rates or amounts using different methods of calculation, and in most cases using arbitrary figures for flow rate.

Conclusion

It is concluded that rumen ammonia concentration has no effect on *in situ* degradation at concentrations in excess of 6.4 mM with diets based on combinations of *E. curvula* hay and maize. It should be noted that this does not preclude the possibility of rumen ammonia effects in other diets of higher fermentation potential. The potential degradative activity of rumen contents appears to be influenced by the ratio of roughage to concentrate in the diet, possibly via pH-mediated effects on rumen micro-organisms, but the effect of these changes on *in situ* degradation differs between feedstuffs. Expression of basal diet effects appears to be a result of several factors which include the potential rate and extent of degradation of test feedstuff and the prevailing flow rate of particulate matter from the rumen. These results indicate that there is little prospect of predicting basal diet effects with any degree of accuracy. The magnitude (up to 58%) and variability of basal diet effects reported here lend support to the contention (Nocek, 1988; Erasmus, 1990a) that feedstuffs should preferably be evaluated using the basal diet in which the test feedstuff is to be included. The accuracy of estimates of effective degradation may be improved further if the actual flow rate of particulate matter from the rumen is known or can be accurately predicted.

Acknowledgements

The technical assistance of J. Davie, J. Mojela, J. Pienaar and W. Strauss is gratefully acknowledged.

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