Establishment of a ruminal protein degradation data base for dairy cattle using the *in situ* polyester bag technique. 3. Roughages

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The extent of ruminal protein degradation of 13 roughages was determined in the rumen of cannulated lactating dairy cows, and was calculated at three different fractional outflow rates using the *in situ* polyester bag technique. Values ranged from 25,5% for Smuts finger grass hay to 79,5% for maize silage, when calculated at a fractional outflow rate of 0,08/h. Extent of ruminal degradation was higher for silage, lucerne hay, ammoniated roughages and high-quality pastures than for low-quality roughages such as Smuts finger grass hay, teff hay and *Eragrostis curvula* hay.

Die mate van rumenproteïendegradering van 13 ruvoere is met rumengekannuleerde lakterende melkkoeie bepaal. Die effektiewe rumenproteïendegradeerbaarheid is bereken by verskillende fraksionele uitvloeitempo's deur gebruik te maak van die *in situ*-kunsveselsaktegniek. Waardes het gevarieer vanaf 25,5% vir Smutsvingergrashooi tot 79,5% vir mieliekuilvoer wanneer bereken by 'n fraksionele uitvloeitempo van 0,08/h. Die mate van proteïendegradering was aansienlik hoër vir kuilvoer, lusernhooi, geammonifiseerde ruvoere en hoëkwaliteit-weidings as vir laekwaliteit-ruvoere soos Smutsvingergrashooi, tefhooi en *Eragrostis curvula*-hooi.

Keywords: Dairy cattle, *in situ* polyester bag, roughages, ruminal protein degradation.

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During the last decade, several new systems for the evaluation of feed protein and the calculation of protein requirements have been proposed, such as RDP–UDP (ARC, 1984) in England, AAT–PBV (Madsen, 1985) in Nordic countries and the AP-system (NRC, 1985) in the USA. These systems, although different with regard to some coefficient values, are all based on common concepts. Emphasis is placed on the separation of dietary protein into ruminally degraded (RDP) and undegraded (UDP) protein fractions and it is therefore necessary to assess the protein degradation of feed proteins rapidly and accurately (Broderick, Wallace, Ørskov & Hansen, 1988).

For many feedstuffs there are no data available on extent of protein degradation, thereby limiting the widespread application of feed formulation based on RDP and UDP (Kirkpatrick & Kennelly, 1987). This is especially true for basal feedstuffs such as roughages (Negi, Singh & Makkar, 1988a). Diets for dairy cattle generally contain 40—60% roughage (McCullough, 1986) which supplies about 50% of the total dietary protein fed (Waldo & Jorgensen, 1981). This clearly illustrates the urgent need for data on the protein degradation of roughages.

Methods to evaluate feed protein include *in vivo* (sampling of digestive contents throughout the digestive tract), *in situ* (incubation of feeds in bags suspended in the rumen) and *in vitro* (laboratory) techniques (Janicki & Stallings, 1988). Although the *in vivo* method remains the reference technique, it is a complex, expensive and difficult procedure (Owens, 1987).

The *in situ* technique has been criticized (Meyer & Mackie, 1986; Nocek, 1988), but for the time being it appears to be the preferred alternative to *in vivo* experiments (Jarrige, 1987; Lindberg, 1987). At present, the *in situ* technique is used as the standard method for the determination of protein degradation in the protein evaluation systems of the USA, UK and Nordic countries (Van der Honing & Alderman, 1988).

The object of this study was to expand the existing South African ruminal protein degradation data base by determining the extent of ruminal protein degradation of the most common roughages used in the formulation of dairy cattle diets, using the polyester bag (*in situ*) technique. The extent of ruminal protein degradation of various protein and energy sources has been published (Erasmus, Prinsloo & Meissner, 1988; Erasmus, Prinsloo, Botha & Meissner, 1990).

Experimental Procedures

Three samples of each roughage to be evaluated were obtained from various locations and pooled in order to obtain an 'average' sample. The roughages evaluated are presented in Table 1 . Silage and grass were freeze-dried prior to incubation. Nitrogen was determined by the Kjeldahl method and the different fibre contents were analysed by the methods of Goering & Van Soest (1970).

The *in situ* technique previously described (Erasmus *et al.*, 1988; Erasmus *et al.*, 1990), was used. Briefly, three lactating rumen-cannulated Holstein cows with an average DM intake of 20,2 ($\pm$1,9) kg/d were fed a practical complete dairy cattle diet twice daily on an *ad libitum* basis. The basal diet, with a roughage:concentrate ratio of 45:55 (15,2% CP, 10,3 MJ ME/kg, 18,2% CF), contained lucerne hay, wheat straw, *Eragrostis curvula* hay, maize meal, urea, fishmeal, sunflower oilcake, soybean oilcake, cottonseed oilcake, minerals and vitamins. The polyester bags (14 $\times$ 9 cm;

Table 1 Protein and fibre analysis of roughages used for *in situ* incubation

	%DM				
Roughage	CP	CF	ADF	NDF	ADF–N[a]
Eragrostis curvula hay					
(weeping lovegrass)	5,7	38,3	42,6	83,2	11,9
E. curvula hay (ammoniated)	11,5	38,1	41,0	80,6	8,8
Kikuyu pasture					
(*Pennisetum clandestinum*)	21,3	22,1	23,5	53,6	1,6
Lucerne hay	16,1	30,4	39,1	42,8	7,6
Lucerne meal (dehydrated)	22,6	27,6	30,8	45,2	6,6
Maize silage	8,2	27,3	30,1	47,4	6,6
Maize stover	4,8	42,0	49,5	80,9	9,6
Pearl millet hay	5,3	34,8	48,2	68,5	19,2
Ryegrass Midmar	22,3	18,1	20,9	33,1	1,8
Smuts finger grass hay	3,5	40,5	74,6	47,4	19,6
Sorghum hay	5,2	34,4	46,5	67,0	13,1
Teff hay (*Eragrostis tef*)	5,0	33,7	38,6	72,6	14,9
Teff hay (ammoniated)	14,3	31,7	38,3	65,7	7,7

[a] Acid detergent fibre-linked nitrogen expressed as a percentage of total nitrogen.

53 μm pore size) were filled with *ca.* 5 g of test feed (air-dry), which had been milled in a Wiley mill with 2-mm screen. The bags were tied to a stainless steel disc with 10 evenly spaced small holes drilled through the periphery of the disc. Using the complete exchange method (Paine, Crawshaw & Barber, 1981), one bag per test feed was placed in the rumen of each of three cows for every incubation period (0, 1, 2, 4, 6, 8, 12, 18, 24, 36, and 48 h respectively). The procedure was repeated to give a total of six values for N disappearance of each feed per incubation period. After incubation, the bags were rinsed under running tap water (15 s) and washed in a washing machine (cold water) for 10 min, followed by drying (65°C, 48 h) and Kjeldahl N-analyses. The bags used for 0-h values were subjected to the washing procedure only. Cottonseed oilcake was used as a control to monitor day-to-day variation, since it exhibits a characteristically linear rate of DM disappearance over extended time intervals (Nocek, 1985). Incubations were repeated when DM disappearance varied more than 10% from established disappearance curves. However, differences between days and cows were minimal.

The percentage N disappearance at each incubation time was calculated from the proportion remaining after rumen incubation. The degradation rate was fitted to the equation as suggested by Ørskov & McDonald (1979):

$$p = a + b(1 - e^{-ct})$$

where p = proportion degraded at time t, a = an intercept representing soluble protein, b = the insoluble but potentially degradable fraction, and thus $a + b$ present the maximum extent of degradation or the asymptote of the equation. The degradation rate of the b fraction is described by c, the fractional rate constant /h. Non-linear parameters a, b and c were estimated by an iterative least-square procedure (Du Toit & Herbst, 1981). By introducing the fractional outflow rate, k, the effective protein degradation (P) was calculated as follows (Ørskov & McDonald, 1979):

$$P = a + \frac{bc}{c + k}$$

Results and Discussion

The crude protein, fibre fractions and acid detergent fibre-linked nitrogen (ADF–N) contents are presented in Table 1. CP and ADF–N data varied widely, ranging from 3,5—22,6% and 1,6—19,6%, respectively. The ADF–N fraction is insoluble in acid detergent solution, includes lignified nitrogen and Maillard products, and is largely unavailable to the animal (Goering, Gordon, Hemken, Waldo, Van Soest & Smith, 1972). Acid detergent insoluble-nitrogen (ADIN) values, ranging from 3,5—13,6% and 8,3—14% of total N in some roughages, have been reported by Krishnamoorthy, Muscato, Sniffen & van Soest (1982) and Janicki & Stallings (1988), respectively. ADIN and ADF–N refer to the same fraction.

Data on the extent of ruminal degradation of roughage-N at different hours of rumen fermentation are given in Table 2. In general, the extent of N degradation increased with time and, at 48 h, was highest in the Midmar and Kikuyu pastures and lowest in pearl millet hay, *Eragrostis curvula* hay and Smuts finger grass hay. The N disappearance at 0 h (10 min washing in washing machine) was the greatest for maize silage and Midmar ryegrass (74 and 58% of total CP, respectively).

After 1 or 2 h of incubation in the case of maize fodder, pearl millet hay, Midmar ryegrass and Smuts finger grass hay, the percentage N disappearance was less than at 0 h, indicating that some ruminal bacteria had adhered to roughage particles and were not removed by washing and rinsing. The same was found by Janicki & Stallings (1988) when recovery of N from incubation of Orchardgrass hay

Table 2 *In situ* extent of ruminal nitrogen disappearance of roughages in the rumen of lactating dairy cows at some incubation times

	% N disappearance[a] at different incubation times (h)							
Roughage	0	1	2	4	8	12	24	48
E. curvula hay	19,0	22,3	25,6	25,2	26,7	33,2	39,5	49,5
	±1,50	±1,62	±2,17	±0,79	±1,35	±1,68	±2,07	±1,63
E. curvula hay (ammoniated)	46,5	49,2	49,6	53,7	54,6	56,3	60,8	70,5
	±1,36	±1,70	±1,05	±1,67	±0,84	±0,95	±1,70	±0,50
Kikuyu pasture	30,2	41,5	46,0	52,5	58,3	63,5	81,2	91,2
	±0,74	±1,45	±0,58	±0,97	±0,99	±0,81	±0,79	±0,48
Lucerne hay	41,6	51,7	57,6	66,8	78,6	81,3	85,8	89,3
	±0,95	±0,67	±0,80	±0,79	±0,33	±0,49	±0,40	±0,49
Lucerne meal (dehydrated)	20,1	31,5	35,3	43,0	65,3	68,7	84,5	90,3
	±1,19	±0,92	±0,66	±1,89	±1,50	±1,54	±1,12	±0,21
Maize silage	74,0	76,1	76,7	77,0	78,8	80,1	82,8	85,0
	±1,03	±0,77	±0,21	±0,52	±0,48	±0,70	±0,91	±0,44
Maize stover	39,9	38,5	36,4	41,3	42,6	45,8	53,1	58,8
	±4,26	±4,53	±4,03	±3,89	±3,48	±1,90	±3,22	±4,42
Pearl millet hay	36,2	30,7	28,1	30,9	30,4	32,0	41,3	49,6
	±3,69	±1,59	±4,03	±5,31	±3,20	±2,53	±5,10	±3,62
Ryegrass Midmar	58,0	50,6	56,6	65,0	75,2	79,9	91,4	95,4
	±1,61	±1,83	±2,01	±1,00	±2,41	±1,64	±0,88	±0,35
Smuts finger grass hay	13,1	12,5	18,7	17,7	23,1	27,1	36,6	43,5
	±1,09	±1,20	±0,56	±0,42	±2,48	±2,78	±2,28	±2,01
Sorghum hay	40,8	44,0	46,2	46,5	49,3	53,3	60,0	66,0
	±1,01	±1,18	±1,18	±0,80	±2,77	±2,06	±1,44	±1,18
Teff hay	15,6	26,8	23,8	26,1	31,3	37,2	41,6	52,6
	±0,76	±2,59	±1,74	±2,02	±2,12	±2,39	±2,80	±1,22
Teff hay (ammoniated)	53,0	55,1	57,8	60,7	64,8	67,2	73,3	81,5
	±0,82	±0,70	±0,65	±0,49	±0,60	±1,42	±0,95	±0,42

[a] Values are mean ± *SE*; n = 6.

was greater after 2 h of rumen incubation than at 0 h. Negi, Singh & Makkar (1988b) demonstrated that degradability of N remained negative up to 12 h of incubation for wheat straw and up to 24 h for rice straw. Beyond these periods, the extent of negative degradability decreased, suggesting degradation of roughage or adsorbed N. Several researchers (Kennedy, Hazelwood & Milligan, 1984; Nocek, 1985; Nocek & Grant, 1987) demonstrated that bacterial contamination increased curvilinearly with time of rumen incubation. According to Nocek (1988), these data suggested that bacteria easily attach to feed particles up to a particular time of ruminal exposure, after which attachment appears to become a function of attachment-site or substrate availability. Estimation of bacterial N contamination should be considered when establishing ruminal N degradability values for low protein roughages (Nocek & Grant, 1987; Negi *et al.*, 1988b). With protein-rich feeds, however, the microbial error seems small (Varvikko, 1986).

The non-linear parameters estimated by an iterative least-square procedure are presented in Table 3. The soluble fraction *a*, representing the intercept of the degradation curve at time 0 h, ranged from 12,8% for Smuts finger grass hay to 74,8% for maize silage. In general, there was close agreement between the 0 h (wash only) values and the degradation curve intercept values. Janicki & Stallings (1988) reported *a* fractions ranging from 29,4% for lucerne hay to 74,5% for ammonia-treated maize silage. The *a* fractions for maize silage, the ammoniated roughages and the Midmar and Kikuyu pastures were the highest, mainly because of the high

Table 3 Non-linear parameters[a] estimated by an iterative least-squares procedure

	Parameters		
Roughage	a	b	c
Eragrostis curvula hay	21,0	37,7	0,03
E. curvula hay (ammoniated)	48,5	33,3	0,02
Kikuyu pasture	36,1	57,0	0,06
Lucerne hay	42,3	44,7	0,21
Lucerne meal (dehydrated)	21,8	67,8	0,11
Maize silage	74,8	10,8	0,06
Maize stover	37,6	31,1	0,02
Pearl millet hay	29,4	446,5	0,0009
Ryegrass Midmar	52,3	44,7	0,08
Smuts finger grass hay	12,8	33,0	0,05
Sorghum hay	42,1	26,4	0,04
Teff hay	20,5	33,7	0,05
Teff hay (ammoniated)	54,5	29,6	0,05

[a] $P = a + b(1 - e^{-ct})$ (Ørskov & McDonald, 1979).

NPN content in these roughages. Whereas the sum of fractions *a* and *b* should theoretically not exceed 100%, the values observed for pearl millet hay in this study were much higher than 100%. The large *b* value appears to be due to bacterial contamination causing a disproportionate apparent N disappearance at certain time intervals. The same was found by Ha & Kennelly (1984) when evaluating soybean meal. Other researchers also reported *b* values exceeding 100% (Hughes-Jones, 1979, Ørskov, Hovell & Mould, 1980.)

The extent of ruminal protein degradation calculated at three different fractional outflow rates, is presented in Table 4. ARC (1984) recommended that the low outflow rate (0,02/h) should be used for low-level feeding systems, a higher rate (0,05/h) for beef production and low-level milk production systems, whereas the highest value of 0,08/h should be used for high-producing lactating cows. Protein degradation was lowest for the low-quality roughages viz. teff hay, Smuts finger grass hay, pearl millet hay, maize fodder, and *Eragrostis curvula* hay; intermediate for ammoniated teff, ammoniated *E. curvula* hay, sorghum hay and dehydrated lucerne meal; and the highest for lucerne hay, maize silage, Kikuyu pasture and Midmar ryegrass. Wheat and rice straw would also fall in the low degradation category (Negi *et al.*, 1988b). As expected, the extent of ruminal protein degradation was highest for the high-quality pastures and silage, owing to the high NPN content of silages and well fertilized pastures (Bredon & Stewart, 1979). Degradation values similar to the values found in this study have been reported for lucerne hay and maize silage (Satter, 1986; Janicki & Stallings, 1988), *Eragrostis curvula* hay (Cronjé, 1983), maize fodder (Preston, 1988), dehydrated lucerne meal (Owens, 1987) and pasture grass (Van der Honing & Alderman, 1988).

Table 4 Extent of ruminal protein degradation of various roughages calculated at three different fractional outflow rates

Roughage	Fractional rumen outflow rate 0,02/h	0,05/h	0,08/h
Eragrostis curvula hay	42,0	34,1	30,6
E. curvula hay (ammoniated)	66,3	58,6	55,7
Kikuyu pasture	82,3	70,1	62,5
Lucerne hay	83,5	78,8	75,0
Lucerne meal (dehydrated)	79,8	68,9	61,5
Maize silage	83,0	80,8	79,5
Maize stover	54,8	47,9	44,9
Pearl millet hay	49,0	37,5	34,5
Ryegrass Midmar	88,3	80,1	74,9
Smuts finger grass hay	36,2	29,2	25,5
Sorghum hay	61,6	55,1	51,9
Teff hay	44,0	36,9	33,2
Teff hay (ammoniated)	74,2	68,3	65,1

ADF–N contents of feeds have been used to estimate the indigestible undegradable protein fraction (Wilson & Strachan, 1981). The UDP values as estimated, have been corrected for ADF–N to arrive at an estimate of available UDP or digestible undegradable protein (DUP). The DUP values in the roughages are shown in Table 5. The available UDP or DUP is overestimated if not corrected for ADF–N. The UDP values of diets containing high levels of ADF–N may be misleading in terms of diet formulation according to RDP and UDP (Negi *et al.*, 1988b).

Conclusions

Large differences in extent of ruminal protein degradation were found between the roughages, ranging from 25,5% for Smuts finger grass hay to 79,5% for maize silage, calculated at a fractional outflow rate of 0,08/h. The extent of degradation was higher for silage, lucerne hay, ammoniated roughages and high-quality pastures than for low-quality roughages such as Smuts finger grass hay and teff hay.

Table 5 Partitioning of total N between RDN and UDN fractions and UDP and DUP contents of roughages (g/kg DM)

Roughage	Total N	RDN (0,08/h)	UDN (0,08/h)	UDP[a]	ADF–N	DUP[b]
Eragrostis curvula hay	9,12	2,79	6,33	39,6	1,0	33,3
E. curvula hay (ammoniated)	18,40	10,24	8,16	51,0	1,5	41,6
Kikuyu pasture	34,08	21,30	12,78	79,9	0,5	76,7
Lucerne hay	25,76	19,32	6,44	40,3	0,6	36,5
Lucerne meal (dehydrated)	36,16	22,23	13,93	87,1	2,2	73,3
Maize silage	13,12	10,43	2,69	16,8	0,8	11,8
Maize stover	7,68	3,44	4,24	26,5	0,7	22,1
Pearl millet hay	8,48	2,93	5,55	34,7	1,5	25,3
Ryegrass Midmar	35,68	26,72	8,96	56,0	0,6	52,3
Smuts finger grass hay	5,60	1,43	4,17	26,1	1,0	19,8
Sorghum hay	8,32	4,31	4,01	25,1	1,0	18,8
Teff hay	8,00	2,66	5,34	33,4	1,1	26,5
Teff hay (ammoniated)	22,88	14,89	7,99	49,9	1,8	38,7

[a] Undegraded dietary protein = (UDN × 6,25).
[b] Digestible undegraded protein = (UDN – ADF–N) × 6,25.

The degradation figures from this study will be of value in calculating the RDP and UDP composition of the total diet (roughage + concentrate). An important aspect is that the results were obtained from lactating dairy cows.

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