In sacco dry matter disappearance of herbage and maize meal from the rumen of lactating Dorper and Merino ewes supplemented with protein and energy on native pasture

H.O. de Waal

Glen Agricultural Development Institute, Private Bag X01, GLEN, 9360 Republic of South Africa

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Rumen fermentation was studied during three years in lactating Dorper and Merino ewes, supplemented daily with crude protein and/or energy via rumen fistulae on veld. Herbage, collected with oesophageally fistulated wethers, and maize meal were incubated *in sacco* in the rumen of the ewes. Four distinct observations were made in terms of *in sacco* dry matter (DM) disappearance of herbage, namely: (*i*) a significant ($P \leq 0.05$) difference between breeds in winter; (*ii*) an increase of 100% from winter to summer; (*iii*) a significant ($P \leq 0.05$) increase due to protein supplementation in winter; and (*iv*) a non-significant ($P \leq 0.05$) tendency to decrease in summer with increasing levels of energy supplementation. None of these differences or tendencies were evident in the *in sacco* DM disappearance of maize meal during any of the trial periods. Results are discussed in relation to changes in rumen pH and rumen ammonia concentration, as well as feed intake by the ewes, in the same trials.

Rumenfermentasie is gedurende drie jare bestudeer in lakterende Dorper- en Merino-ooie wat daagliks aanvullende energie en/of ruproteïen per rumenfistel op veld ontvang het. Weidingsmateriaal wat met slukdermgefistuleerde hamels versamel is, asook mieliemeel, is *in sacco* in die rumen van ooie geïnkubeer. In terme van *in sacco* droë materiaal (DM) verdwyning van weidingsmateriaal, is die volgende waarnemings gemaak : (*i*) 'n betekenisvolle ($P \leq 0.05$) verskil tussen rasse in die winter; (*ii*) 'n verhoging van bykans 100% vanaf die winter na die somer; (*iii*) 'n betekenisvolle ($P \leq 0.05$) verhoging as gevolg van proteïenaanvulling in die winter; en (*iv*) 'n nie-betekenisvolle ($P \leq 0.05$) tendens om in die somer te verlaag met toenemende peile van aanvullende energie. Nie een van die verskille of tendense was in die *in sacco* DM verdwyning van mieliemeel waarneembaar nie. Resultate word bespreek aan die hand van veranderinge in rumen-pH en rumenammoniakkonsentrasie, asook weidinginname deur die ooie, in dieselfde studies.

Keywords: Artificial fibre bag technique, diet selection, feed intake, *in sacco* rumen fermentation, supplementary feeding, veld.

Introduction

By creating more favourable conditions in the rumen for the micro-organisms, supplements may increase both feed intake and digestibility of low-quality roughages (Louw, 1978). Despite this claim, it was concluded (De Waal *et al.*, 1980; 1981; De Waal *et al.*, 1989a; 1989b) that supplementing crude protein (fish meal or non-protein nitrogen) via rumen fistulae evinced no consistent trends in diet selection, feed intake or body mass changes of Dorper and Merino wethers grazed on native pasture (veld) at Glen.

Subsequently, De Waal & Biel (1989a; 1989b; 1989c) studied the effects of supplementary energy and/or crude protein (CP), also introduced via rumen fistulae, on lactating Dorper and Merino ewes grazed on veld at Glen. When supplied in isolation, neither the supplementary energy nor CP yielded any significant response in body mass changes during winter. However, a combination of energy and CP reduced body mass losses of the ewes by about 50%, at the same time as leading to small increases in the growth rate of their lambs. In contrast, these supplements did not affect the body mass changes of Dorper ewes and their lambs during summer. Also, supplementary CP did not affect the intake of herbage, but a substitution effect on herbage intake by the ewes in response to supplementary energy was present, this effect being greater during summer (De Waal & Biel, 1989b).

In these studies, apparently rumen activity, as influenced by the herbage ingested (De Waal *et al.*, 1980; 1981; De Waal *et al.*, 1989b; De Waal & Biel, 1989b; 1989c), was not sub-optimal and could therefore not have been influenced by supplementary CP. If nitrogen (N) *per se* had been the primary limiting factor, supplementary CP would have stimulated rumen activity and herbage intake, which was not the case (De Waal *et al.*, 1981; De Waal *et al.*, 1989a; De Waal & Biel, 1989a; 1989b). It was not possible to measure the effect of supplementation on *in vivo* digestibility in these studies, but as mentioned, the nutritional advantage of CP supplementation was not reflected positively in animal performance. Since supplementary CP, and CP plus energy, influenced ruminal pH and ammonia (NH_3) to varying degrees (De Waal & Biel, 1989c), the rate of rumen fermentation could also have been influenced by these supplements. Although this objective was not included in the original planning of the trials by De Waal & Biel (1989a; 1989b; 1989c), it was decided to study rumen fermentation (*in sacco* dry matter disappearance) as well during the remaining trial periods.

Procedure

The trials were conducted from 1982 to 1984 on native pasture (veld) at the Glen Agricultural Development Institute. Acocks (1988) described the pasture as a *Themeda* –

Table 1 Experimental design, composition and levels of supplementary feeding provided daily via rumen fistulae to lactating Merino (1982) and Dorper ewes (1982 and 1983)

Treatment	Composition and daily levels of supplementary feeding
E 0	NaCl[a] + Dicalcium phosphate (P)[b] – control
E 100	NaCl + P + 100 g maize meal
E 200	NaCl + P + 200 g maize meal
PE 0	NaCl + P + 60 g HPC 60[c]
PE 100	NaCl + P + 60 g HPC + 100 g maize meal
PE 200	NaCl + P + 60 g HPC + 200 g maize meal

[a] Salt – 8 g NaCl ewe^{-1}
[b] 15 g DiCaP provided 2.5 g phosphorus (P) ewe^{-1}
[c] HPC 60 – High protein concentrate (61.8% of the N derived from urea)

Cymbopogon veld type. The experimental designs, animals used and general procedures have been described by De Waal & Biel (1989a; 1989b; 1989c). In these trials, supplements (Tables 1 & 2) were provided daily via rumen fistulae to lactating Dorper and Merino ewes. Body mass changes of ewes and their lambs, diet quality and feed intake and seasonal and diurnal variation in ruminal pH and ammonia concentration, were studied.

In all trials, feed intake was determined when the ewe flock was, on average, four weeks *post partum* (De Waal & Biel, 1989b). During some of these trial periods, the *in sacco* disappearance of dry matter (DM) was also determined with varying numbers of ewes in each treatment, as indicated in the respective tables. Three oesophageally fistulated (OF) Merino wethers were run with the lactating ewes during each trial to collect herbage samples on three successive days. Pooled samples of the OF extrusa and maize meal were used to determine the *in sacco* DM disappearance. Within seven days after the collection of the herbage, the herbage as well as the maize meal were incubated *in sacco* in the rumen of the supplemented ewes grazed on the veld.

The artificial fibre bags were made according to the dimensions described by Mehrez & Orskov (1977). Nylon material (HS013 Nylon cloth: Simon-Macforman (Pty) Ltd, 2 Purlin Street, Isando, RSA), with the following specifications, was used:

Average diameter of fibres	227 μm
Average distance between fibres	31 μm (25–37 μm)
Number of apertures per unit area	1 502 cm^{-2}

Before stitching the seams (run and fell seams), the material was folded into double layers. The bags had an effective size of 17 × 9 cm. By comparison, the material used by Mehrez & Orskov (1977) had the following specifications:

Average diameter of fibres	180 μm
Average distance between fibres	47 μm
Number of apertures per unit area	1 936 cm^{-2}

After collection of the herbage with OF sheep in the camp where the ewes were grazing (seven days prior to incubation), the extrusa was squeezed through four layers of cheese cloth and dried in a force draught oven at 50°C (Engels *et al.*, 1981). The dry material was ground in a Wiley mill to pass through a 10-mm sieve, mainly to homogenize the herbage before each of the dry bags was accurately filled with 5 g of oven-dried herbage. Since coarsely ground maize meal was used daily to supplement the ewes via rumen fistulae, it was not ground any further before each of the dry bags was accurately filled with 12 g of oven-dried maize meal. Relative to the bag size, the volumes of the 5-g oven-dried herbage and the 12-g oven-dried maize meal, were about the same.

The CP content of the OF extrusa and maize meal was determined as described by De Waal *et al.* (1989a). The *in vitro* digestibility of organic matter (OMD) of the extrusa and maize meal was determined according to the two-stage technique of Tilley & Terry (1963), as modified by Engels & Van der Merwe (1967). The OMD of the herbage (veld) and maize meal was then estimated according to the procedure described by Engels *et al.* (1981). Neutral and acid detergent fibre (NDF and ADF) were determined according to the procedures described by Goering & Van Soest (1970) and Robertson & Van Soest (1981).

For both substrates, incubation in the rumen started between 7:30 and 9:00 and were 24 h for herbage and 24 h or 6 h for maize. Full particulars for the respective incubation periods, number of animals per treatment and number of bags per animal, are given in Tables 5 – 9. At the end of each incubation period, the bags were removed from the rumen, washed under running water until the water was clear and dried at 100°C in a force-draught oven. After cooling in a desiccator, the bags plus their contents were weighed and the percentage *in sacco* DM disappearance was calculated.

Results

The CP and DOM content of the herbage and maize meal, used as substrates in the respective trials, are presented in Table 3.

The CP of the OF-collected herbage (Table 3) was substantially higher in 1983, and especially in 1984 (spring/summer), than in 1982 (autumn/winter). The OMD also tended to be higher during 1983 and 1984, but the differences were of a smaller magnitude than for the CP content. The CP content of the maize meal was substantially lower in 1984 than in 1982 and 1983. The OMD of the maize meal differed between trial periods, but the differences were small.

The NDF and ADF content of the herbage and maize meal, used as substrates in the respective trials, are presented in Table 4.

Table 2 Experimental design, composition and levels of supplementary feeding provided daily via rumen fistulae to lactating Dorper ewes during 1984

Treatment	Composition and daily levels of supplementary feeding
E 0	NaCl[a] + Dicalcium phosphate (P)[b] – control
E 150	NaCl + P + 150 g maize meal
E 300	NaCl + P + 300 g maize meal
E 450	NaCl + P + 450 g maize meal

[a] Salt – 8 g NaCl ewe^{-1}
[b] 15 g DiCaP provided 2.5 g phosphorus (P) ewe^{-1}

Table 3 Crude protein content and digestibility of organic matter of herbage and maize meal incubated *in sacco* in the rumen of grazing ewes to determine dry matter (DM) disappearance on veld in the respective trial periods

	Herbage[a]		Maize meal[b]	
Trial period	CP[c]	DOM[d]	CP[c]	DOM[d]
1982	7.0	57.8	9.9	80.7
1983 Week 1	11.3	59.5	10.4	82.0
1983 Week 2	11.6	63.8	10.8	83.7
1984	18.8	63.5	8.1	82.3

[a] Herbage – collected with oesophageally fistulated (OF) sheep, dried at 50°C and ground through a 10-mm sieve
[b] Maize meal – same as supplemented via rumen fistulae to ewes
[c] CP – crude protein expressed on an organic matter (OM) basis
[d] DOM – estimated from *in vitro* OMD (Engels *et al.*, 1981)

Table 4 Neutral and acid detergent fibre content of herbage and maize meal incubated *in sacco* in the rumen of grazing ewes to determine the dry matter (DM) disappearance on veld in the respective trial periods

	Herbage[a]		Maize meal[b]	
Trial period	NDF[c]	ADF[d]	NDF[c]	ADF[d]
1982	72.1	40.1	24.8	3.3
1983 Week 1	70.8	42.2	27.5	3.4
1983 Week 2	73.9	39.7	24.0	3.8
1984	72.1	37.7	26.7	2.7

[a] Herbage – collected with oesophageally fistulated (OF) sheep, dried at 50°C and ground through a 10-mm sieve
[b] Maize meal – same as supplemented via rumen fistulae to ewes
[c] NDF – Neutral detergent fibre
[d] ADF – Acid detergent fibre

The NDF of the herbage used as the substrate during the different trial periods varied in the relatively narrow range of 70.6% – 73.9%, while the ADF varied between 37.7% and 42.2%. The range of variation for the NDF in the maize was 24.0% – 27.5%, while the ADF varied between 2.7% and 3.8%.

The *in sacco* DM disappearance of herbage and maize from the rumen of lactating Merino and Dorper ewes grazing winter (1982) and summer (1983 and 1984) veld during the respective trial periods, are presented in Tables 5 – 9.

In 1982 (Table 5), the percentage DM which disappeared per 24 h *in sacco* in the rumen on winter veld, was significantly ($P \leq 0.05$; Harvey, 1976) higher for the Merino ewes. After a 24-h incubation, 22% more herbage had disappeared from the bags in the rumen of the Merino ewes (31.8% vs. 26.0%). Supplementary CP significantly ($P \leq 0.05$) increased *in sacco* DM disappearance of herbage in both breeds. In the case of the Merino ewes, supplementary CP increased *in sacco* DM disappearance of herbage by 23.4% (28.5 ± 2.08% vs. 35.1 ± 2.08%), while the increase was 21.0% (23.5 ± 2.08% vs. 28.5 ± 2.24%) for the Dorpers. The differences in *in sacco* DM disappearance of maize meal between treatments and breeds in the winter of 1982 (Table 6), were non-significant ($P \leq 0.05$). The overall mean *in sacco* DM disappearance of maize meal was 36.3 ± 1.09% for a 24-h incubation period.

Table 5 *In sacco* dry matter disappearance of herbage[a] from the rumen of lactating Merino and Dorper ewes grazing winter veld in 1982

Treatment	Merinos	Dorpers
	% ± *SE*	% ± *SE*
E 0	27.2 ± 3.34	22.6 ± 3.34
E 100	26.6 ± 3.34	26.5 ± 3.34
E 200	31.5 ± 3.34	21.5 ± 3.34
PE 0	35.2 ± 3.34	27.1 ± 4.09
PE 100	39.4 ± 3.34	26.7 ± 3.34
PE 200	30.7 ± 3.34	31.3 ± 3.34

[a] 2 Bags/sheep and 3 sheep/breed/treatment, 24-h incubation period
Treatment means in a column with different superscripts differ significantly ($P \leq 0.05$)
Merinos > Dorpers ($P \leq 0.05$)
Within breeds: PE > E ($P \leq 0.05$)

Table 6 *In sacco* dry matter disappearance of maize meal[a] from the rumen of lactating Merino and Dorper ewes grazing winter veld in 1982

Treatment	Merinos	Dorpers
	% ± *SE*	% ± *SE*
E 0	34.8 ± 3.86	38.9 ± 3.86
E 100	31.8 ± 3.35	33.5 ± 3.86
E 200	34.5 ± 3.86	38.0 ± 4.73
PE 0	44.3 ± 3.86	36.8 ± 3.86
PE 100	37.0 ± 3.86	33.9 ± 3.86
PE 200	36.6 ± 3.86	35.0 ± 3.86

[a] 2 Bags/sheep and 3 sheep/breed/treatment
24-h incubation period
Treatment means in a column with different superscripts differ significantly ($P \leq 0.05$)

In 1983 (Table 7), when only Dorper ewes were used, the differences in *in sacco* DM disappearance of herbage in the rumen on summer veld were non-significant ($P \leq 0.05$) for treatments or weeks (overall means for Week 1: 46.6 ± 2.48% and Week 2: 50.8 ± 1.39%). By pooling the data for the two weeks, the differences remained non-significant ($P \leq 0.05$) (overall mean for Week 1 & 2: 48.7 ± 1.33%). However, there was a tendency for *in sacco* DM disappearance of herbage to decrease with an increase in the level of energy supplementation, irrespective of CP supplementation. By pooling the data and testing for main effect tendencies, the level of supplementary energy reduced the rate of *in sacco* DM disappearance of herbage, as described by the following equation:

$$y = 53.319 - 0.03359x \qquad \text{(Equation 1)}$$
$$SE \pm 1.0245 \quad (P = 0.0144) \quad (r = 0.626)$$

where y = % *in sacco* DM disappearance of herbage
and x = g maize meal supplemented day^{-1}.

In the summer of 1983 (Table 8), the differences in *in sacco* DM disappearance of maize meal (6-h incubation period) were non-significantly ($P \leq 0.05$) affected by treatment, either in the individual weeks (overall means for Week 1: 15.1

Table 7 *In sacco* dry matter disappearance of herbage[a] from the rumen of lactating Dorper ewes grazing summer veld in 1983

Treatment	Week 1	Week 2	x
	% ± *SE*	% ± *SE*	% ± *SE*
E 0	54.1 ± 3.44	51.3 ± 3.39	52.5 ± 2.43
E 100	46.5 ± 3.44	53.8 ± 3.39	50.3 ± 2.43
E 200	43.3 ± 2.81	45.2 ± 2.19	44.3 ± 2.19
PE 0	53.2 ± 4.87	54.3 ± 3.39	54.5 ± 2.19
PE 100	47.6 ± 3.44	50.7 ± 3.39	49.0 ± 2.43
PE 200	48.8 ± 3.44	49.6 ± 3.39	49.2 ± 2.43

[a] Week 1 – 2 Bags/sheep and 2 sheep/treatment
Week 2 – 2 Bags/sheep and 3 sheep/treatment
24-h incubation period
Treatment means in a column with different superscripts differ significantly ($P \leq 0.05$)

Table 8 *In sacco* dry matter disappearance of maize meal[a] from the rumen of lactating Dorper ewes grazing summer veld in 1983

Treatment	Week 1	Week 2	x
	% ± *SE*	% ± *SE*	% ± *SE*
E 0	15.8 ± 1.21	18.4 ± 1.92	17.2 ± 1.19
E 100	14.5 ± 1.21	16.4 ± 1.92	15.5 ± 1.19
E 200	15.0 ± 0.98	17.9 ± 1.92	16.4 ± 1.07
PE 0	15.4 ± 1.21	18.2 ± 1.92	16.7 ± 1.19
PE 100	16.1 ± 1.21	18.1 ± 1.92	17.0 ± 1.19
PE 200	14.0 ± 1.21	19.5 ± 1.92	17.7 ± 1.31

[a] Week 1 – 2 Bags/sheep and 2 sheep/treatment
Week 2 – 2 Bags/sheep and 3 sheep/treatment
6-h incubation period
Treatment means in a column with different superscripts differ significantly ($P \leq 0.05$)

± 0.48% and Week 2: 18.1 ± 0.78%) or the pooled data for weeks (overall mean for Week 1 & 2: 16.8 ± 0.49%).

During the summer of 1984 (Table 9), when again only Dorper ewes were used, there was also a tendency, though non-significant ($P \leq 0.05$) (overall mean: 49.0 ± 0.98%), for *in sacco* DM disappearance of herbage to decrease with increasing levels of supplementary energy. By pooling the data and testing for main effect tendencies, the level of supplementary energy again reduced the rate of *in sacco* DM disappearance of herbage, as described by the following equation:

$$y = 51.56325 - 0.01157x \qquad \text{(Equation 2)}$$

$$SE \pm 0.9806 \quad (P = 0.0654) \quad (r = 0.51)$$

where y = % *in sacco* DM disappearance of herbage
and x = g maize meal supplemented day^{-1}.

The level of energy supplementation had a non-significant ($P \leq 0.05$) effect on *in sacco* DM disappearance of maize meal during the summer of 1984 (Table 9) (overall mean: 15.9 ± 0.52%).

Table 9 *In sacco* dry matter disappearance of herbage[a] and maize meal[b] from the rumen of lactating Dorper ewes grazing summer veld in 1984

Treatment	Herbage	Maize meal
	% ± *SE*	% ± *SE*
E 0	52.6 ± 1.96	16.1 ± 1.04
E 150	47.7 ± 1.96	15.8 ± 1.04
E 300	49.2 ± 1.96	15.7 ± 1.04
E 450	46.3 ± 1.96	16.1 ± 1.04

[a] 2 Bags/sheep and 5 sheep/treatment
24-h incubation period
[b] 2 Bags/sheep and 5 sheep/treatment
6-h incubation period
Treatment means in a column with different superscripts differ significantly ($P \leq 0.05$)

Discussion

Activities of the rumen micro-organisms have a major effect on the protein and energy metabolism of ruminants and, consequently, on voluntary feed intake (Mehrez *et al.*, 1977). If activities in the rumen are restricted or altered, it will have a direct influence on animal performance. Supplementation may increase the intake of grazing animals, but in studies by De Waal & Biel (1989a; 1989b), CP supplementation to lactating Dorper and Merino ewes on veld at Glen did not affect herbage intake, while a substitution effect on herbage intake in response to supplementary energy was present, especially during the summer.

In this study, four distinct observations were made in terms of *in sacco* DM disappearance of herbage, namely : (i) a significant ($P \leq 0.05$) difference between breeds in winter (Table 5); (ii) an increase of about 100% from the winter (Table 5) to the summer (Tables 7 & 9); (iii) a significant ($P \leq 0.05$) increase due to CP supplementation in winter (Table 5); and (iv) a non-significant ($P \leq 0.05$) tendency to decrease on summer veld with increasing levels of energy supplementation (Tables 7 & 9). None of these differences or tendencies were evident for the *in sacco* DM disappearance of maize meal during any of the trial periods.

The microbiology of the substrate in the bags was assumed to be similar to that of the surrounding rumen digesta, but Meyer & Mackie (1983) showed that this may not always be the case and suggested that results obtained *in sacco* should be interpreted cautiously. Furthermore, when used to determine rate of degradation, this technique lacks sufficient measures of standardization (Stern & Hoover, 1979; Cronjé, 1982; Udén & Van Soest, 1984), which may result in highly variable estimates of degradation for different feed components (Stern & Satter, 1984). However, in this study the objective with the *in sacco* technique was to determine the effect of CP and energy supplementation on rumen fermentation (DM disappearance) of herbage and maize meal under grazing conditions on veld. It was assumed that factors, which usually influence *in sacco* results, would have been equal or similar between treatments. Furthermore, the herbage used as the substrate (Table 3) was collected within seven days prior to incubation with OF wethers in the camp where the ewes were grazing. Thus, herbage ingested by the ewes and the substrate used *in sacco*, was considered to be similar.

According to Orskov (1982), the rate of cellulose digestion falls rapidly to zero at a rumen pH of < 6.1 – 6.2, but the extent to which cellulose digestion is depressed depends both on the duration and magnitude of the prevailing decrease in ruminal pH. In these studies, the rumen pH of Dorper ewes was consistently lower than for Merino ewes, both for lactating (De Waal *et al*., 1989c) and non-lactating ewes (De Waal, 1986), but the average rumen pH of Dorper ewes was always higher than 6.4 (De Waal & Biel, 1989c). Therefore, the significant ($P \leq 0.05$) breed difference in *in sacco* DM disappearance of herbage (Table 5) may have been partially due to differences in rumen pH between breeds.

The significant ($P \leq 0.05$) increase in *in sacco* DM disappearance of herbage due to CP supplementation in winter (Table 5), suggests that rumen NH_3 concentration was inadequate for optimal rumen activity. During this period the OF samples collected from the veld contained only 7% CP (Table 3). De Waal & Biel (1989c) reported considerable increases in ruminal NH_3 concentrations following CP supplementation, which could have increased cellulolitic activity and, hence, also *in sacco* DM disappearance. A low digestibility is usually associated with an increase in rumen retention time, as well as a decrease in voluntary feed intake. The inability of the ewes in Treatments PE 0, PE 100 and PE 200 to increase feed intake on winter veld (De Waal & Biel, 1989b), in response to an increase in ruminal NH_3 concentration (De Waal & Biel, 1989c), suggests that intake was also influenced by some other factors. They proposed that the selective grazing behaviour of sheep, being a time-consuming process, may have prohibited positive responses in intake during winter under prevailing grazing conditions.

The length of incubation time was chosen arbitrarily for herbage and maize meal, but it was important not to disturb the normal grazing behaviour of the ewes unnecessarily (De Waal *et al*., 1989b). Rumen retention time of herbage and maize meal was not determined, but it may be assumed that herbage is retained longer than maize meal (Orskov *et al*., 1969). The *in sacco* DM disappearance of substrate in this study reflected the situation after 24 h. Because of the possibility of over-estimating *in sacco* DM disappearance of maize meal with a 24-h incubation period, the incubation for maize meal was reduced from 24 h in 1982 (Table 5) to 6 h in 1983 (Table 8) and 1984 (Table 9).

Energy supplementation reduces herbage intake (Langlands, 1969; Milne *et al*., 1981), which is in agreement with the results of this study reported elsewhere (De Waal & Biel, 1989b). In the study by Langlands (1969), the results suggested that, apart from rumen-fill, threshold values and feedback mechanisms for metabolites in blood apparently exerted a major influence on voluntary intake. Similar mechanisms might have been operating in this study. No apparent adverse effects were observed in the ewes, following the sudden introduction and continuous daily administration of up to 450 g maize meal supplement into the rumen.

There were some differences in CP, DOM, NDF and ADF contents of the herbage during the respective trial periods (Tables 3 & 4). However, it seems unlikely that any one of these individually effected the rate of *in sacco* DM disappearance directly or to a great extent. However, collectively, they must have played a major role in altering rumen activity and also, ultimately, been partly responsible for the differences in *in sacco* DM disappearance of the substrate between winter (Tables 5 & 6) and summer (Tables 7, 8 & 9), as well as for some of the differences in intake reported by De Waal & Biel (1989b).

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