The effect of protein degradation products in grass silages on feed intake and intake behaviour in sheep

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The effects of NH_3 and amines on grass-silage intake, intake behaviour and rumen characteristics were studied in sheep. From a single sward, two direct-cut grass silages were prepared, either untreated (WAS) or with 4·5 l formic acid/tonne (FAS). Four experimental diets: WAS, FAS, FAS with addition of 2·9 g NH_3/kg DM (FAS + N) and FAS with 2·8 g amines/kg DM (FAS + A), were offered *ad lib.* once daily to four rumen-cannulated wethers in a 4 × 4 Latin square design. Daily DM intake (DMI) tended to be influenced by dietary treatment ($P = 0·09$). Compared with FAS, DMI was lower for WAS. Addition of NH_3 did not alter DMI, whereas amine addition slightly lowered daily DMI. Reduced DMI resulted from lower intake rates during both the principal meal and the subsequent small meals. Lower initial intake rate during the principal meal suggested reduced palatability of WAS and FAS + A. Amines and NH_3, however, did not influence chewing efficiency. No treatment effects were observed on total rumen pool size, DM and neutral-detergent fibre content. Furthermore, NH_3 and amines did not alter rumen pH, NH_3 and volatile fatty acid concentrations to the extent that they could act on chemostatic intake regulation. Amine addition, however, lowered osmolality of the rumen liquid. No treatment effects on rumen motility were observed. In conclusion, daily DMI was not reduced by the addition of NH_3, suggesting that NH_3 *per se* is not the causal factor in the negative correlations between silage NH_3 content and intake observed by other authors. Amines, however, tended to reduce DMI only by their effect at the oro-pharyngeal level of intake control.

Silage: Feed intake: Ammonia: Amines: Sheep

The generally lower voluntary intake of grass silages compared with hay prepared from the same fresh forage or with the fresh forage itself is mainly attributed to fermentation endproducts present in the silage. This reduction in intake varies widely (ranging from 0 to 64%) and is related to the quantities of the fermentation products in the silage, which vary with the method of preservation (Demarquilly, 1973; Donaldson & Edwards, 1976; Thiago *et al.* 1992*a*). These fermentation products (organic acids, NH_3, amines) may regulate intake by their oro-pharyngeal properties (taste, smell) or metabolically (metabolites in rumen and/or blood; Gill *et al.* 1987). Rumen fill appears to be of minor importance in controlling silage intake, because rumen DM content remains lower when the animals are fed on silages compared with hays prepared from the same original herbage (Thiago & Gill, 1986; Chiofalo *et al.* 1992). Relationships between silage constituents and intake suggest that NH_3 is one of the factors responsible for reduction in intake of poor-quality silages

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(Wilkins *et al.* 1971; Dulphy & Michalet-Doreau, 1981). However, other products of protein degradation (e.g. amines) may also influence silage intake (Buchanan-Smith & Phillip, 1986; Baile & Della-Ferra, 1988). During the fermentation process, cadaverine, histamine, putrescine and tyramine can be formed in considerable quantities (Hole, 1985; McDonald *et al.* 1991). Because of their physiological action when absorbed into the bloodstream (Joosten, 1988), their influence in controlling silage intake is a possibility.

The aim of the present experiment was to investigate the effect of NH_3 and amines on voluntary intake of grass silage, and their possible mode of action in sheep.

MATERIALS AND METHODS

Dietary treatments and feeding

In June 1991 two grass silages were prepared from the first cut of a single sward of cocksfoot (*Dactylis glomerata*) meadow. The grass was harvested directly with a precision-chop forage harvester. One part was ensiled without additive, whereas the other part was ensiled while adding formic acid (4·5 l/tonne fresh material). Four dietary treatments were prepared: the silage without an additive (WAS), the silage with formic acid (FAS), FAS with the addition of 2·9 g NH_3/kg DM (FAS + N), and FAS with 2·8 g amines/kg DM (FAS + A). The quantity of NH_3 and amines added was based on the difference in these fermentation products between WAS and FAS in preliminary samples. The amine addition consisted of a mixture of the four biogenic amines cadaverine (0·6 g), histamine (0·5 g), putrescine (0·7 g) and tyramine (1·0 g). The ratio between amines corresponded to that found in WAS, and values in the literature (Hole, 1985; McDonald *et al.* 1991). The amines, in hydrochloride form and NH_3 solution (Normapur™; 200 ml/l) were purchased from Sigma Chimie, St Quentin Fallavier, France and Prolabo, Paris, France respectively. All quantities of amines provided in the present study reflect the real amines and not their hydrochlorates. Amines were dissolved in water before being added to the silage, whereas NH_3 was added as purchased (200 ml/l solution). After removal of FAS from the silo, FAS + N and FAS + A were prepared by sprinkling these solutions over the silage, and then firmly mixing. Dietary treatments were prepared every 2 d and stored at +4° in portions of 50 kg (fresh material).

After collection of the refusals of the previous day, feed was offered to the sheep *ad lib.* (10% refusals) once daily (09.00 hours).

Animals and management

Four 5-year-old Texel wethers (average live weight 66 kg) fitted with a permanent polyamide rumen cannula (75 mm in diameter) were used. In addition, a group of four non-fistulated 3-year-old Texel wethers (average live weight 61 kg) was used to extend data of daily dry matter (DM) intake (DMI). Throughout the experiment, non-fistulated sheep were kept in individual indoor pens with sawdust for bedding. The fistulated animals were housed in individual pens in an experimental unit where lighting was provided for 11 h daily. During experimentation the fistulated animals were kept in metabolism cages in which they were placed 5 d before. All animals had free access to water and salt blocks.

Experimental design

The animals were randomly assigned to the four dietary treatments in two concurrent 4 × 4 Latin square designs, one with fistulated and one with non-fistulated sheep. Each period lasted 4 weeks and consisted of a 2-week adaptation period followed by 1 week of

measuring intake behaviour and rumen characteristics, and 1 week of measuring rumen fill. The animals were weighed at the beginning of each period.

Measurements and sampling

Silage. During periods of measurements, samples of the offered silage were taken each day and analysed for DM content. A pooled sample of fresh material was stored at −20° until analysed.

Daily intake and intake behaviour. The daily DMI was calculated throughout the experiment as the difference between the amount offered and that refused. Additionally, intake behaviour of the fistulated sheep was monitored during five consecutive days. The pattern of intake was registered by feed dispensers placed on sensors fitted with strain gauges. Weight variations of the feed dispensers were continuously recorded by a microcomputer after digitalization of the signal (Baumont *et al.* 1990). Kinetics of intake during the principal meal (first meal after distribution) were determined by fitting the model: $I_{(t)} = a(1-e^{-b}t)$ to the data, where I is the feed intake (g DM); t is the time after feeding (min); a is the asymptotic intake (g DM), and b is the rate constant of decrease (/h). Initial and final intake rates were calculated as the values of the first derivative of $I_{(t)}$ at $t = 0$ (ab) and $t = T$ (abe^{-bT}, where T is the end of the principal meal) respectively. Jaw movements were recorded simultaneously using a polyurethane-foam-filled balloon placed submandibularly and connected to a microcomputer via a pressure transducer. Eating and ruminating activities were analysed as described by Brun *et al.* (1984).

Rumen motility. Simultaneously with monitoring intake behaviour, rumen motility was recorded over a 48 h period. Contractions were recorded as air-pressure signals from a small foam-filled balloon (length 100 mm, diameter 30 mm) inserted in the dorsal region of the rumen. The signals were converted by a pressure transducer (LCD 86–110; Sélectronic, Paris, France) and registered on paper by a potentiometric recorder (BS 273; Gould Electronics, London).

Rumen fermentation. While recording intake behaviour, rumen fluid samples were withdrawn from the ventral region during two consecutive days, using a peristaltic pump (ISMATEC SA; Laboratoriumstechnik, Bern, Switzerland). From 08.45 to 11.45 hours sampling was carried out continuously (60 ml/h) and samples (15 ml) were collected every 15 min in order to select for analysis the sample corresponding to the end of the principal meal. Two additional samples were withdrawn at 13.30 and 15.30 hours. The pH was measured immediately and samples to be analysed for volatile fatty acids (VFA) and NH_3-N were stored frozen, preserved with orthophosphoric acid (50 ml/l) and NaCl solution (125 g/l) respectively.

Rumen fill. Total rumen contents were determined by manually emptying the rumen before (08.30 hours) and after the principal meal (10.30 hours). Rumen evacuations were carried out with an interval of at least 72 h to ensure normal digestion (Aitchison, 1985). The four animals were emptied simultaneously. After emptying, rumen contents were weighed, homogenized and sampled for DM determination, amine extraction and rumen fluid (30 ml). Osmotic pressure was measured in the fluid within 12 h after sampling. Dried and ground (0·8 mm) rumen contents were preserved for analysis for neutral-detergent fibre (NDF).

Chemical analysis

The DM content of silage and rumen contents were determined by drying samples at 80° for 48 h. The DM content of the silage was corrected for volatile components lost by oven-drying, as recommended by Dulphy *et al.* (1975).

Fermentation characteristics of the silages (pH, VFA, alcohols, lactic acid, NH_3 and soluble N (N_{sol})) were determined in the juice pressed from silage. The pH was measured immediately. Total N and N_{sol} were determined by the Kjeldahl method, NH_3 by the gas-diffusion method of Conway (1957), and lactic acid by the enzymic method described by Noll (1974). VFA and alcohols were analysed by gas chromatography (Jouany, 1981).

In rumen fluid, NH_3-N was determined by the method of Berthelot as modified by Van Eenaeme *et al.* (1969), whereas VFA were analysed as described previously. Osmotic pressure was measured by freezing-point depression (Astor 4000; Humeau, La Chapelle sur Erdre, France). Rumen NDF was determined in the dried material according to Goering & Van Soest (1970).

Amines were extracted from the silages and rumen contents by macerating 20 g of the fresh material in trichloroacetic acid (100 g/l; TCA). After centrifugation (12000 *g*, 10 min), amines were determined in the supernatant fraction by reversed-phase HPLC as orthophthaldehyde derivatives by the method described by Gomez *et al.* (1991). Before injection, sample preparation was carried out automatically by an auto-sampler (AS 3000; Spectra Physics, San José, CA, USA). For adequate separation of the amine peaks the linear gradient of the solvent mixture was sustained at 84–20% for the acetate buffer, 14–73% for methanol and 2–7% for the tetrahydrofuran. Analysis time was prolonged to 28 min.

Statistical analysis

Data for daily DMI for all sheep, fistulated and non-fistulated ($n = 32$), were subjected to analysis of variance, using the general linear model procedure of Statistical Analysis Systems (1987). In the model, effects of silage (3 df), animal (7 df), fistulation (1 df) and period (3 df) were tested. Data concerning measurements on only fistulated animals ($n = 16$) were subjected to analysis of variance using the model, in which effects of period (3 df), silage (3 df) and animal (3 df) were tested. Differences between treatments were compared by the Student's t test. The 2-week adaptation periods were sufficiently long to prevent carry-over effects between periods of measurements.

RESULTS

Silage composition

Composition and fermentation characteristics of the two prepared silages and the principal changes as a result of NH_3 and amine addition to FAS are given in Table 1. The quality of FAS could be considered as good (Dulphy & Demarquilly, 1981), as indicated by low pH and low NH_3, VFA, alcohol and amine contents. The silage WAS underwent a more extensive fermentation, resulting in higher concentrations of organic acids, NH_3, N_{sol} and amines. Despite the increase in fermentation products, WAS could be classified as a medium-quality silage. As expected, NH_3 addition increased NH_3-N concentration in FAS + N, resulting in slightly higher N_{sol} and crude protein (N × 6·25) contents. Amine addition to FAS increased its amine content.

Intake and intake behaviour

The effect of fistulation on daily DMI was not significant. Pooled data for DMI for fistulated and non-fistulated sheep (Table 2) showed that DMI was significantly reduced for WAS compared with FAS. The addition of NH_3 to FAS had no effect on daily DMI, whereas amine addition caused a slight depression. The pattern of effects of dietary silage was reflected in the limited findings for DMI from the 5 d of recording intake behaviour. Dietary treatments did not affect the period of time spent eating each day, which averaged

Table 1. *Chemical composition and fermentation characteristics (g/kg dry matter (DM)) of grass silages preserved without additive (WAS) and with formic acid (FAS) and FAS after addition of ammonia (FAS+N) or amines (FAS+A)*

Dietary treatment...	WAS	FAS	FAS+N	FAS+A
DM (g/kg fresh weight)	199	209	214	213
pH	4·1	4·0	4·3	4·1
Crude protein ($N_{total} \times 6{\cdot}25$)	144	153	167	159
NH_3-N (g/kg N_{total})	120	60	120	60
N_{sol} (g/kg N_{total})	680	400	430	410
Lactic acid	63	21	19	22
VFA	62	19	23	21
Acetic acid	56·6	15·6	19·0	15·8
Propionic acid	4·8	0·6	0·6	0·7
Butyric acid	0·8	2·6	3·4	3·9
Alcohols	19	5	6	6
Amines	6·5	1·1	1·3	3·6
Histamine	0·9	0·1	0·2	0·6
Tyramine	2·0	0·4	0·4	1·3
Putrescine	1·5	0·3	0·3	0·8
Cadaverine	2·1	0·3	0·4	0·8

VFA, volatile fatty acids.

5·8 (SE 0·22) h. Thus, the reduction in intake of WAS and FAS+A was the result of a lower intake rate ($P < 0{\cdot}05$). For the principal meal, intake of the four silages showed the same pattern as the daily intake with a reduced intake ($P < 0{\cdot}05$) for WAS, a slight reduction for FAS+A and no difference between FAS and FAS+N. Sheep consumed WAS for a shorter period of time (not significant) with a reduced intake rate, while the duration of the principal meal for FAS, FAS+N and FAS+A was similar. A slight reduction in intake rate was recorded for FAS+A. Reduction in the overall intake rate of the principal meal for WAS and FAS+A compared with FAS was mainly attributed to the lower intake rate at the beginning of the meal, because final intake rates were the same for the four diets.

Both WAS and FAS+N were eaten in a higher ($P < 0{\cdot}05$) number of smaller meals. In FAS+N this was the only variable of intake behaviour that differed from FAS. Average intake rate during the small meals was lower ($P < 0{\cdot}05$) for FAS+A and tended to be lower for WAS. The period of time spent ruminating on a daily basis was least in sheep fed on WAS, whereas no treatment effects on the lag before the start of rumination were observed. Daily duration of mastication (eating and ruminating) was the lowest ($P < 0{\cdot}05$) for WAS, and was related to daily DMI. Consequently, the efficiency of mastication did not differ among the four diets.

Rumen fill and characteristics of the rumen contents

Rumen fill (Table 3) was not significantly influenced by dietary treatment and averaged 9·2 kg wet weight before the principal meal (at 08.30 hours) and 10·7 kg at the end of the principal meal (10.30 hours). Rumen pool size, however, was greatly influenced by animal effects ($P < 0{\cdot}01$) both before and after the principal meal. Rumen DM content averaged 99·7 g/kg wet weight before the meal and increased slightly with all diets to 116·4 g/kg wet weight after the principal meal. No effects of dietary treatment were observed.

At 08.30 hours, osmolality of the rumen fluid, which was significantly affected by diet, was lowest (249 mosmol/l) for FAS+A and highest for FAS+N (267 mosmol/l). At the

Table 2. *Dry matter (DM) intake (DMI; g), intake rates (g DM/min) and duration (min) of different feed intake activities of sheep offered* ad lib. *grass silage preserved without additive (WAS), with formic acid (FAS) and FAS after addition of ammonia (FAS+N) or amines (FAS+A)*†

Dietary treatment...	WAS	FAS	FAS+N	FAS+A	Statistical significance of the silage effect‡	Pooled SED
Daily DMI§	1371	1477	1486	1437	0·09	40
Daily DMI‖	1249	1475	1475	1308	0·09	87
Intake rate	3·7	4·3	4·3	3·6	**	0·14
Principal meal						
Intake	274	465	452	407	0·06	59
Duration	53	71	67	75	NS	11
Intake rate	5·2	6·7	6·8	5·4	0·07	0·57
Initial rate	10·2	13·9	13·9	11·4	*	1·20
Final rate	3·4	3·4	3·7	3·4	NS	0·64
Small meals						
No.	12·0	9·2	11·3	10·3	*	0·66
Intake rate	3·4	3·6	3·8	3·1	0·06	0·21
Rumination						
Duration	352	471	473	407	*	35
Lag time¶	179	147	163	179	NS	35
Mastication						
Daily duration	687	817	816	774	*	40
Efficiency (g DM/min)	1·84	1·82	1·82	1·69	NS	0·09

SED, standard error of difference 6 df, except for daily DMI for fistulated and non-fistulated sheep (17 df); NS, not significant ($P < 0·10$).

* $P < 0·05$, ** $P < 0·01$.

† For details of treatments and procedures, see Table 1 and pp. 52–53.

‡ For tendencies ($0·05 < P < 0·10$) probabilities are given.

§ Average daily intake of fistulated and non-fistulated sheep during the 2-week period of measurements (days on which rumen emptying was carried out and following day were excluded).

‖ Average daily intake of the fistulated sheep during 5 d of recording intake behaviour.

¶ Start of first rumination after silage distribution (min).

end of the principal meal the average increase in osmolality of the rumen fluid was 13% and did not result in different values across treatments. Likewise, no differences were observed in NDF content at both sampling times. Only traces of amines were recovered in the rumen contents after the principal meal, even on treatments WAS and FAS+A.

The time-course of changes in fermentation products in the rumen fluid is presented in Fig. 1. Generally, rumen pH (Fig. 1(*a*)) decreased on average 0·3 (SE 0·05) pH units during the principal meal and remained relatively constant at approximately 6·6 (SE 0·08) during the day. Before silage distribution, rumen fluid pH was significantly higher in sheep fed on FAS+A compared with the other three silages. Although not significant, the pH remained elevated for FAS and FAS+A at subsequent sampling times.

Rumen NH_3 increased after silage distribution (Fig. 1(*b*)) and showed the highest increase for NH_3-rich silages (WAS and FAS+N). Due to considerable variation in data, average NH_3 concentrations in the rumen fluid of 342 (SE 51) mg/l for WAS and FAS+N and 273 (SE 38) mg/l for FAS and FAS+A at the end of the principal meal were not significantly different. After the principal meal, NH_3 concentrations in the rumen decreased for all silages, except for WAS. Here, the high concentration persisted until 13.30 hours and decreased afterwards but remained higher ($P < 0·05$) than those of the other three diets.

Table 3. *Characteristics of the reticulo-rumen content before (08.30 hours) and after (10.30 hours), the principal meal, in sheep offered* ad lib. *grass silage preserved without additive (WAS), with formic acid (FAS) and FAS after addition of ammonia (FAS+N) or amines (FAS+A)*†

Dietary treatment...	WAS	FAS	FAS+N	FAS+A	Statistical significance of the silage effect	Pooled SED
08.30 hours						
Wet wt (kg)	8·9	8·9	9·7	9·5	NS	0·89
Dry matter (DM g/kg)	97	103	104	96	NS	4·9
Fluid volume (l)	8·0	8·0	8·7	8·6	NS	0·79
Osmolality (mosmol/l)	255	257	267	249	*	4·2
NDF (g/kg DM)	658	684	668	666	NS	7·2
10.30 hours:						
Wet wt (kg)	10·2	10·7	10·9	11·3	NS	0·54
DM (g/kg)	112	118	119	111	NS	4·2
Fluid volume (l)	9·0	9·4	9·6	10·0	NS	0·50
Osmolality (mosmol/l)	295	285	312	291	NS	13·4
NDF (g/kg DM)	650	657	660	648	NS	4·9

SED, standard error of difference (6 df); NDF, neutral-detergent fibre; NS, not significant.
* $P < 0·05$.
† For details of treatments and procedures, see Table 1 and pp. 52–54.

At 15.30 hours, rumen NH_3 nearly approached the average initial concentration of 138 (SE 13) mg/l for FAS and FAS+A.

Patterns of total rumen VFA concentrations were similar for the four treatments, but differed in level (Fig. 1(*c*)). After silage was offered there was an increase in VFA from the average initial concentration of 67 (SE 2·0) mmol/l. After the principal meal, VFA concentrations decreased slightly until 13.30 hours but increased again thereafter. The highest increase during the principal meal was observed with WAS and the lowest with FAS+A. After the principal meal, rumen VFA concentrations tended to be influenced by dietary treatment ($P = 0·08$). At other sampling times, no further effects of dietary treatment on rumen VFA concentrations were observed. Before the meal, molar proportions of acetic acid, propionic acid and valeric plus caproic acid were 72·5 (SE 0·6), 17·8 (SE 1·2) and 1·9 (SE 0·03) mol/100 mol total VFA respectively and did not differ across treatments. The molar proportion of butyric acid was higher in FAS+A compared with FAS (10·1 (SE 0·7) *v.* 8·6 (SE 0·5) mol/100 mol). An important shift in VFA composition was observed at the end of the principal meal. The proportion of acetic acid decreased to 66·0 (SE 1·02) mol/100 mol for FAS, FAS+N and FAS+A, whereas for WAS it remained significantly higher throughout the day (71·1 (SE 0·5) mol/100 mol). With FAS+A the proportion of propionic acid remained lower ($P < 0·05$) and that of butyric acid higher ($P < 0·01$) compared with the other treatments. Average proportions during the rest of the day (13.30 and 15.30 hours), were 17·6 (SE 0·7), 20·2 (SE 2·0), 18·4 (SE 3·1), 15·1 (SE 2·1) for propionic acid and 7·0 (SE 0·7), 9·9 (SE 1·2), 10·4 (SE 1·0) and 13·0 (SE 1·0) for butyric acid for WAS, FAS, FAS+N and FAS+A respectively.

Rumen motility

The number of primary contractions and total number of contractions (primary and secondary) of the reticulo-rumen per min are given in Table 4. These results emphasize the

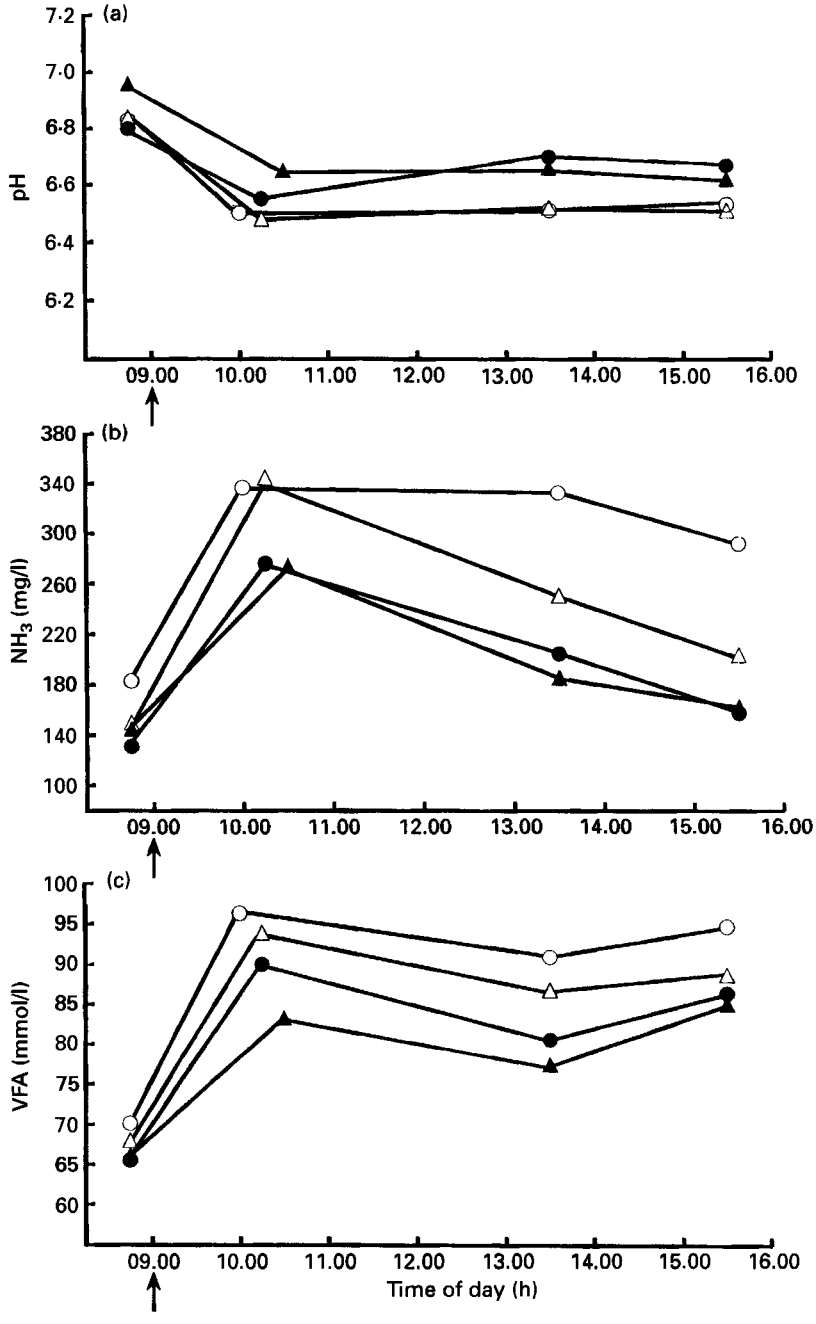

Fig. 1. Diurnal changes in (a) pH, (b) ammonia and (c) volatile fatty acid (VFA) concentrations in rumen fluid from sheep offered *ad lib.* grass silage preserved without additive (WAS; ○), with formic acid (FAS; ●) and FAS after addition of NH_3 (FAS + N; △) or amines (FAS + A; ▲). ↑, Silage distribution. For details of treatments and procedures, see Table 1 and pp. 52–54.

Table 4. *Rumen contractions (/min) during different activities in sheep offered* ad lib. *grass silage preserved without additive (WAS), with formic acid (FAS) and FAS after addition of ammonia (FAS+N) or amines (FAS+A)*†

Dietary treatment...	WAS	FAS	FAS+N	FAS+A	Statistical significance of the silage effect	Pooled SED
Primary contractions						
Principal meal	2·0	2·0	1·9	1·9	NS	0·10
Small meals	1·7	1·6	1·7	1·7	NS	0·10
Rumination	1·3	1·2	1·2	1·4	*	0·04
Rest	1·1	1·0	1·0	1·0	NS	0·04
Primary and secondary contractions						
Principal meal	3·6	3·4	3·4	3·5	NS	0·13
Small meals	3·0	2·8	2·9	2·9	NS	0·18
Rumination	1·9	1·9	2·0	2·1	NS	0·06
Rest	1·8	1·7	1·7	1·7	NS	0·06

SED, standard error of difference (6 df); NS, not significant.
* $P < 0·05$..
† For details of treatments and procedures, see Table 1 and pp. 52–53.

difference in contraction frequencies during the different feed intake activities, rumination and rest. Highest frequencies of primary and total contractions were observed during the principal meal and decreased during the consecutive small meals. A further reduction occurred during rumination and rest.

Considering the effect of treatment on rumen motility, only an increase in primary contraction frequency was observed during rumination in sheep offered FAS+A. Moreover, treatments influenced neither the frequency of total contractions nor total number of contractions per d, calculated as the sum of the duration of the different activities multiplied by their corresponding contraction frequencies. For the silages WAS, FAS, FAS+N and FAS+A, total number of contractions per d were 3061, 2945, 3035 and 3097 respectively.

DISCUSSION

In studies that relate daily intake of grass silages to their quality, NH_3 has been shown to be a protein fermentation product negatively correlated with intake. Amines, endproducts of amino acid decarboxylation, have also been suggested as being responsible for the reduction in intake of poor-quality silages. In the present study the effect of both protein degradation products on silage intake was tested by adding them separately to a grass silage of good quality, with high potential intake.

Silages

The two selected silages ideally suited the purpose of the present experiment. Silages were similar in DM content, pH and crude protein content. Concentrations of fermentation products (NH_3, VFA, alcohols and amines) in the silage conserved with formic acid (FAS) were sufficiently low to ensure a proper intake. This was confirmed by a daily DMI of 65·3 g per kg metabolic weight ($W^{0·75}$) for FAS, which is high compared with other experiments

in which sheep consumed grass silages preserved with formic acid (Wilkins *et al.* 1971; Dulphy *et al.* 1984; Chiofalo *et al.* 1992). The lower quality of the silage WAS was indicated by a considerable quantity of fermentation products (organic acids, NH_3 and amines). According to equations formulated by Dulphy & Michalet-Doreau (1981), predicting silage intake in relation to its composition, the increase in NH_3-N of 6% in WAS compared with FAS could result in an intake reduction of 4·2 g/kg $W^{0.75}$, i.e. 6·4 *v.* 7·2% found in the present study.

The quantities of NH_3 and amines added to FAS were based on their concentrations in WAS in preliminary samples. The NH_3 content of FAS + N was equal to that in WAS, but the amine content in FAS + A was only 55% of that found in WAS samples taken throughout the experiment. The difference in amine concentration in the preliminary and ultimate samples for both FAS and WAS is probably due to unequal distribution of amines in the silos (Tveit *et al.* 1992). Nevertheless, amine concentration in FAS + A reflected the lower part of the range of amine concentrations found in grass silages of medium quality. Likewise, proportions of the different amines used were comparable with those reported in the literature, with the highest quantity being tyramine, followed by putrescine and cadaverine and the lowest histamine (Hole, 1985; Tveit *et al.* 1992). Addition of NH_3 and amines to the diet, rather than infusion, was chosen in order to follow both pre- and post-ingestive effects of these products on daily intake pattern.

Intake and intake behaviour

Values for daily DMI confirmed the potential for decreased intake of the lower-quality silage (WAS) compared with a silage of superior quality (FAS), on the basis of NH_3 content, as would be expected from the equation of Dulphy & Michalet-Doreau (1981) relating silage intake to NH_3 content. A similar decrease in DMI of 4·6 g/kg $W^{0.75}$ was not achieved on the addition of NH_3. In contrast, DMI for FAS + N was equal to that of FAS. The identical intakes of FAS and FAS + N indicate that NH_3 *per se* is not an intake depressant. This is supported by increased intake of hay treated with high levels of NH_3 in sheep (Benahmed & Dulphy, 1987) and the fact that NH_3 addition to silage did not depress intake in dairy cows (Lingaas & Tveit, 1992). The apparent decrease in daily DMI by the limited quantity of amines in FAS + A, however, explained 40% of the difference in daily intake of WAS and FAS. Data in the literature on the influence of amines on silage intake by ruminants are scarce. Intrarumen administration of 0·5 g histamine or 1 g added to the daily silage ration of sheep did not affect daily intake (McDonald *et al.* 1963). Neumark *et al.* (1964) did not find a response in feed intake after infusion of individual amines or a combination of histamine, tyramine and tryptamine (1 g each). Also, in heifers no reduction in intake could be detected either when 3 g histamine was added to the daily ration (Okamoto *et al.* 1964) or when tyramine was infused intraruminally (Thomas *et al.* 1963). These findings may be explained by the use of relatively low doses or the use of individual amines. For instance, Lingaas & Tveit (1992) detected a reduction in intake in silage-fed dairy cows following the introduction of 100 g putrescine into the rumen, whereas intake was reduced in sheep following rumen infusion of a combination of the four amines used in the present study plus γ-aminobutyric acid (Buchanan-Smith & Phillip, 1986).

Silage intake appears to be controlled mainly by oro-pharyngeal factors and chemostatic regulation (Gill *et al.* 1987). In the present study, lower daily DMI for WAS and the trend to lower DMI for FAS + A was related to a decrease in intake rate and not by the shorter period of time spent eating. The decrease in intake rate could be related to both types of intake control. Generally, grass silages are characterized by a considerable soluble fraction and rather high digestibility (McDonald *et al.* 1991; Tamminga *et al.* 1991). Both properties

will lead to a rapid rise in metabolites in the rumen, achieving a state of satiation rapidly (Thiago *et al.* 1992*b*). Moreover, fermentation products do not generally have highly attractive odours and tastes. Silage intake during the day corresponded to intake behaviour reported for silage-fed sheep, by Dulphy (1985) and Chiofalo *et al.* (1992) and silage-fed steers (Thiago *et al.* 1992*b*), i.e. one short principal meal after feed was offered, followed by a relatively high number of discrete meals throughout the day.

From profiles of the principal meals in the present study it seems likely that for WAS both chemostatic regulation and lower palatability were responsible for the reduction in DMI. The lower percentage intake during the principal meal (22% of the daily DMI) and the shorter period of time spent eating suggested faster achievement of satiation. The influence of oro-pharyngeal factors was suggested by the lower initial intake rate. This variable can be considered as an indicator of the perception of the forage, because at the very beginning of a meal the influence of chemostatic regulation can be excluded. The tendency of amines to decrease DMI can only be explained by the lower palatability of FAS+A, because intake during the principal meal, as a percentage of daily DMI, was equal to that of FAS (31% of the daily DMI). The animals ate for the same period of time, but initial intake rate tended to decrease. The addition of NH_3, however, did not result in a change in intake behaviour during the principal meal. Experiments of Buchanan-Smith (1990), with sham-fed sheep, confirm that NH_3 had no negative effect on silage palatability. The addition of a mixture of the amines putrescine, cadaverine and γ-aminobutyric acid to silage (3·5 g/kg DM), however, showed a positive effect on palatability.

Intake-regulating mechanisms seemed to have less effect over a daily period than over the short period of the principal meal (Dulphy, 1985), because the animals partly compensated for the smaller principal meal of WAS by an increased number of small meals during the day. With FAS+A the percentage of DMI during the small meals was equal to that of FAS. The lower average intake rate of FAS+A had to be compensated for by a slight increase in the number of small meals. The increase in the number of small meals with FAS+N is hard to explain in this context. More meals with a shorter duration would suggest a role for NH_3 in regulating intake. This is not supported by the similarity of the other intake-behaviour variables of FAS+N with those of FAS.

The longer duration of rumination (total and per kg DM) with FAS and FAS+N can be considered to be related to particle reduction to compensate for the shorter chewing time per unit DM during eating due to the higher intake rate (Ulyatt *et al.* (1986). The similarities in total chewing time per kg DM indicate that, compared with a good-quality silage, extra fermentation products do not influence chewing efficiency. This finding agrees with the observations of Thiago *et al.* (1992*b*) and Chiofalo *et al.* (1992).

Rumen fill, characteristics and motility

Among the four dietary treatments there were no significant differences in rumen load (on a wet weight basis) or DM and NDF contents before or after the principal meal. The similarity in rumen load after termination of the principal meal indicates the minor importance of rumen fill as a regulating factor of DMI during this meal. Furthermore, Chiofalo *et al.* (1992) did not detect significant differences in rumen fill after termination of the principal meal in low- and high-quality silages. Nevertheless, they found a further increase in the rumen load during the day, which means that termination of the principal meal was not a consequence of limitation of the rumen capacity.

The slightly lower osmolality of rumen fluid observed before the principal meal for FAS+A suggested that amines increased the rumen fluid volume. Although not clearly indicated by calculated values, this diluting effect was also supported by the significantly higher pH for FAS+A before the principal meal and the elevated level during the

subsequent period. Tyramine might be responsible for the increased rumen fluid volume, mediated through increased salivation (Joosten, 1988; Okina *et al.* 1993), or decreased water absorption, caused by deleterious effects on rumen epithelial cells (Kutas *et al.* 1986). An influx of water into the rumen is not likely, because osmolality of rumen fluid was far below the level that would induce the influx of water from the blood into the rumen (Carter & Grovum, 1990). Amines that enter the rumen apparently disappeared rapidly because only traces were found, even in rumen contents after the principal meal of WAS. Kay & Sjaastad (1974) found that histamine was metabolized primarily by rumen microbes and there was little evidence for absorption through the rumen wall. Other amines probably undergo the same fate. The absence of amines in the rumen contents supports the hypothesis that they probably do not act as chemostatic regulators of silage intake.

Values for rumen pH and concentrations of VFA and NH_3 were within the range of those found in silage-fed sheep (Chiofalo *et al.* 1992) and cattle (Thiago *et al.* 1992*a*). In terms of chemostatic regulation of DMI of the principal meal, only the influence of VFA was likely. Similar concentrations for rumen NH_3 for WAS and FAS+N at the end of the principal meal, together with the higher DMI for FAS+N, indicate a minor role for NH_3 in chemostatic regulation. This is supported by the fact that postprandial concentrations of 550 mg/l (cf. 340 mg/l in the present study) were tolerated by sheep without negative effect on intake (Benahmed & Dulphy, 1987). The faster decrease in rumen NH_3 after the principal meal for FAS+N compared with WAS might be explained by a higher rate of NH_3 incorporation in micro-organisms. Generally, silages preserved with formic acid contain higher concentrations of water-soluble carbohydrates (McDonald *et al.* 1991) and the addition of NH_3 to this type of silage probably results in a better equilibrium between energy and N, favourable to microbial growth in the rumen (Russell & Hespell, 1981). However, with all treatments, rumen NH_3 concentrations were well above the minimum value of 50 mg/l for microbial growth (Satter & Slyter, 1974).

Differences in rumen contraction frequencies during the various eating activities and during rest are similar to findings of Ruckebush (1988). Differences in fermentation products in the silages did not affect rumen motility, on a per activity or total contractions per d basis. However, the higher number of primary (mixing) contractions during rumination with FAS+A was probably due to slightly higher rumen distension. The absence of an effect of fermentation products in medium- and good-quality silages on rumen motility patterns was supported by the findings of Thiago *et al.* (1992*b*).

In conclusion, the lower voluntary intake of a medium-quality grass silage (WAS) compared with that of a good-quality grass silage (FAS) could only be explained partly by the fermentation products of proteins (NH_3 and amines). The addition of NH_3 to FAS did not reduce intake, in contrast to the findings of Wilkins *et al.* (1971) and Dulphy & Michalet-Doreau (1981) who suggested that intake was reduced by NH_3 since there was a correlation between silage NH_3 content and silage intake. Amine concentrations in WAS differed from those of FAS+A and there was a tendency towards reduced intake of FAS+A. The present study suggests oro-pharyngeal regulation of intake by amines. The low levels of amines did not permit conclusions to be made relating to the action of amines on chemostatic intake regulation. Therefore, higher quantities of amines need to be tested, as well as the fate of amines in the rumen.

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REFERENCES

Aitchison, E. M. (1985). A study of the removal of fibre from the rumen and voluntary intake of sheep eating hay diets. PhD Thesis, University of Reading.

Baile, C. A. & Della-Ferra, M. A. (1988). Physiology of control of food intake and regulation of energy balance in dairy cows. In *Nutrition and Lactation in the Dairy Cow*, pp. 251–261 [P. C. Garnsworthy, editor]. London: Butterworths.

Baumont, R., Seguier, N. & Dulphy, J. P. (1990). Rumen fill, forage palatability and alimentary behaviour in sheep. *Journal of Agricultural Science, Cambridge* **115**, 277–284.

Benahmed, H. & Dulphy, J. P. (1987). Influence de la complémentation des foins traités à l'ammoniaque sur leur valeur nutritive (Supplementation of ammonia-treated hay: effect on the nutritive value). *Annales de Zootechnie* **36**, 153–170.

Brun, J. P., Prache, S. & Bechet, G. (1984). A portable device for eating behaviour studies. In *5th Meeting of European Grazing Workshop* [R. Armstrong and J. Doney, editors]. Midlothian: Hill Farming Research Organization.

Buchanan-Smith, J. G. (1990). An investigation into palatability as a factor responsible for reduced intake of silage by sheep. *Animal Production* **50**, 253–260.

Buchanan-Smith, J. G. & Phillip, L. E. (1986). Food intake in sheep following intraruminal infusion of extracts from lucerne silage with particular reference to organic acids and products of protein degradation. *Journal of Agricultural Science, Cambridge* **106**, 611–617.

Carter, R. R. & Grovum, W. L. (1990). A review of the physiological significance of hypertonic body fluids on feed intake and ruminal function: salivation, motility and microbes. *Journal of Animal Science* **68**, 2811–2832.

Chiofalo, V., Dulphy, J. P. & Baumont, R. (1992). Influence of the method of forage conservation on feeding behaviour, intake and characteristics of the reticulo-rumen content, in sheep fed *ad libitum*. *Reproduction Nutrition Développement* **32**, 377–392.

Conway, E. J. (1957). *Microdiffusion Analysis and Volumetric Error*, 4th ed., pp. 468. London: Crosby Lockwood & Son Ltd.

Demarquilly, C. (1973). Composition chimique, caractéristiques fermentaires, digestibilité et quantite' ingérée des ensilages de fourrages: modifications par rapport au fourrage vert initial (Chemical composition, fermentation characteristics, digestibility and voluntary intake of forage silages: changes compared to the initial, green forage). *Annales de Zootechnie* **22**, 1–35.

Donaldson, E. & Edwards, R. A. (1976). Feeding value of silage: silages made from freshly cut wilted grass and formic acid treated wilted grass. *Journal of the Science of Food and Agriculture* **27**, 536–544.

Dulphy, J. P. (1985). Etude des quantités ingérées lors des grands repas chez des moutons recevant des fourrages ensilés (Level of dry matter intake per main meal in sheep fed forage silages). *Annales de Zootechnie* **34**, 401–416.

Dulphy, J. P. & Demarquilly, C. (1981). Problèmes particuliers aux ensilages. (Particular problems with silages). In *Prévision de la Valeur Nutritive des Aliments des Ruminants*, pp. 81–104. Versailles: INRA Publications.

Dulphy, J. P., Demarquilly, C. & Henry, M. (1975). Perte de composés volatils lors de la détermination à l'étuve de la teneur en matière seche des ensilages (Determination of silage dry matter content after oven drying and losses of volatile components). *Annales de Zootechnie* **24**, 743–756.

Dulphy, J. P. & Michalet-Doreau, B. (1981). Prévisions de l'ingestibilité des ensilages d'herbe (Intake predictions of grass silages). In *Prévision de la Valeur Nutritive des Aliments des Ruminants*, pp. 169–184. Versailles: INRA Publications.

Dulphy, J. P., Michalet-Doreau, B. & Demarquilly, C. (1984). Etude comparée des quantités ingérées et du comportement alimentaire et mérycique d'ovins et de bovins recevant des ensilages d'herbe réalisés selon différentes techniques (Comparative study of feed intake, feeding and ruminating behaviour in sheep and cattle fed silages subjected to different treatments). *Annales de Zootechnie* **33**, 291–320.

Gill, M., Thiago, L. R. S. & Buchanan-Smith, J. G. (1987). Intake problems associated with ensiled forages. In *Feed Intake by Beef Cattle. Miscellaneous Publication* no. 121, pp. 341–352. Stillwater, Oklahoma: Oklahoma State University.

Goering, H. K. & Van Soest, P. J. *Forage Fiber Analyses (Apparatus, Reagents, Procedures and Some Applications) Agricultural Handbook* no. 379. Washington, DC: Department of Agriculture.

Gomez, L., Bogeart, C., Jouany, J. P. & Lassalas, B. (1991). The influence of lasalocid and cationomycin on nitrogen digestion in sheep. Comparison of methods for estimating microbial nitrogen. *Canadian Journal of Animal Science* **71**, 389–399.

Hole, J. R. (1985). The nutritive value of silage made from *Poa pratensis* spp. *alpigena* and *Phleum pratense*. V. Amines in silage made from the first cut of *Poa pratensis* or *Phleum pratense*. *Scientific Reports of the Agricultural University of Norway* **64**, 1–9.

Joosten, H. M. L. J. (1988). The biogenic amine contents of Dutch cheese and their toxicological significance. *Netherlands Milk and Dairy Journal* **42**, 25–42.

Jouany, J. P. (1981). Dosages des acides gras volatils et des alcools dans les ensilages par chromatography en phase gazeuse (Analysis of volatile fatty acids and alcohols in silages by gas chromatography). *Bulletin Technique CRZV, Theix INRA* **46**, 63–66.

Kay, R. N. B. & Sjaastad, Ø. V. (1974). Absorption and catabolism of histamine in sheep. *Journal of Physiology* **243**, 79–99.

Kutas, F., Gálfi, P. & Neogrády, S. (1986). Deleterious effect of tyramine on ruminal epithelial cells. In *Proceedings of the 6th Conference of Production Diseases in Farm Animals*, pp. 149–153 [C. H. McMurray, D. Rice, S. Kennedy and M. McLoughlin, editors]. Belfast, Northern Ireland: Veterinary Science Division of the Department of Agriculture and Queen's University of Belfast.

Lingaas, F. & Tveit, B. (1992). Etiology of acetonemia in Norwegian cattle. 2. Effect of butyric acid, valeric acid and putrescine. *Journal of Dairy Science* **75**, 2433–2439.

McDonald, P., Henderson, A. R. & Heron, S. J. E. (1991). *The Biochemistry of Silage*, 2nd ed. Aberystwyth: Cambrian Printers Ltd.

McDonald, P., Macpherson, H. T. & Watt, J. A. (1963). The effect of histamine on silage dry matter intake. *Journal of the British Grassland Society* **18**, 230–232.

Neumark, H., Bondi, A. & Volcani, R. (1964). Amines, aldehydes and keto-acids in silages and their effect on food intake by ruminants. *Journal of the Science of Food and Agriculture* **15**, 487–492.

Noll, F. (1974). Determination of lactic acid with LDH, GPT and NAD. In *Methods of Enzymatic Analysis*, pp. 1475–1477 [H. U. Bergmeyer, editor]. New York: Academic Press.

Okamoto, M., Waldo, D. R., Miller, R. W. & Moore, L. A. (1964). Histamine levels in forages and dry matter intake of heifers. *Journal of Dairy Science* **47**, 1231–1236.

Okina, A., Hidaka, S., Tashiro, M. & Abe, K. (1993). The effect of tyramine on salivary flow rate and protein secretion by rat submandibular glands. *Journal of Dental Research* **72**, 879–906.

Ruckebush, Y. (1988). Motility of the gastro-intestinal tract. In *The Ruminant Animal, Digestive Physiology and Nutrition*, pp. 64–107 [D. C. Church, editor]. Englewood Cliffs, NJ: Prentice Hall.

Russell, J. B. & Hespell, R. B. (1981). Microbial rumen fermentation. *Journal of Dairy Science* **64**, 1153–1169.

Satter, L. D. & Slyter, L. L. (1974). Effect of ammonia concentration on rumen microbial protein production *in vitro*. *British Journal of Nutrition* **32**, 199–208.

Statistical Analysis Systems (1987). *SAS/STAT Guide for Personal Computers, Version 6*. Cary, NC: SAS Institute Inc.

Tamminga, S., Ketelaar R. & Van Vuuren, A. M. (1991). Degradation of nitrogenous compounds in conserved forages in the rumen of dairy cows. *Grass and Forage Science* **46**, 427–435.

Thiago, L. R. S. & Gill, M. (1986). The effect of conservation method and frequency of feeding on the removal of digesta from the rumen. *Proceedings of the Nutrition Society* **45**, 97A.

Thiago, L. R. L., Gill, M. & Dhanoa, M. S. (1992*a*). Studies of method of conserving grass herbage and frequency of feeding in cattle. 1. Voluntary feed intake, digestion and rate of passage. *British Journal of Nutrition* **67**, 305–318.

Thiago, L. R. L., Gill, M. & Sissons, J. W. (1992*b*). Studies of the method of conserving grass herbage and frequency of feeding in cattle. 2. Eating behaviour, rumen motility and rumen fill. *British Journal of Nutrition* **67**, 319–336.

Thomas, J. W., Moore, L. A., Okamoto, M. & Sykens, J. F. (1963). A study of factors affecting rate of intake of heifers fed silage. *Journal of Dairy Science* **46**, 1471–1483.

Tveit, B., Lingaas, F., Svendsen, M. & Sjaastad, Ø. V. (1992). Etiology of acetonemia in Norwegian cattle. 1. Effect of ketogenic silage, season, energy level and genetic factors. *Journal of Dairy Science* **75**, 2421–2432.

Ulyatt, M. J., Dellow, D. W., John, A., Reid, C. S. W. & Waghorn, G. C. (1986). Contribution of chewing during eating and rumination to the clearance of digesta from the ruminoreticulum. In *Control of Digestion and Metabolism in Ruminants*, pp. 498–515 [L. P. Milligan, W. L. Grovum and A. Dobson, editors]. Englewood Cliffs, NJ: Prentice-Hall.

Van Eenaeme, C., Bienfait, J. M., Lambot, O. & Pondant, A. (1969). Détermination automatique de l'ammoniaque dans le liquide du rumen par la méthode de Berthelot adapté à l'auto-analyseur. (Automatic determination of ammonia in rumen liquid by the method of Berthelot adapted to an auto-analyser). *Annales de Médecine Vétérinaire* **7**, 419–429.

Wilkins, R. J., Hutchingson, K. J., Wilson, R. F. & Harris, C. E. (1971). The voluntary intake of silage by sheep. I. Interrelationships between silage composition and intake. *Journal of Agricultural Science, Cambridge* **77**, 531–537.

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