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Influence of drastic underfeeding on ruminal digestion in sheep

Brigitte MICHALET-DOREAU*, Michel DOREAU

Institut National de la Recherche Agronomique, Unité de Recherches sur les Herbivores, Theix, 63122 Saint-Genès-Champanelle, France

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Abstract — The effect of severe underfeeding on ruminal digestion was studied in sheep. Six wethers fitted with ruminal cannulae were used in a replicated 3×3 Latin square design. They received the same hay at three intake levels corresponding to energy maintenance requirements (M), 60% (0.6 M) and 20% (0.2 M) of these requirements. Fibre digestibility was higher at 0.2 M than at the other two levels. Ruminal neutral detergent fibre retention time did not vary between M and 0.6 M, but increased at 0.2 M. When intake was low, rumen fill decreased and contents became very liquid. No variation in in situ microbial activity or in enzymatic activities of microbial polysaccharidases or glycosidases was observed. Protozoa concentration decreased with intake, but total bacterial mass did not vary when intake decreased. Adherent bacterial mass expressed as a proportion of solid phase was higher at low intake. Volatile fatty acid pattern showed a shift from butyrate towards acetate when intake decreased. It is concluded that (i) changes in digestion are slight between M and 0.6 M, and (ii) microbial activity is not impaired at very low intake level.

underfeeding / sheep / digestion / rumen

Résumé – Effet d'une très forte sous-alimentation sur la digestion ruminale chez le mouton. Six béliers castrés munis de canules du rumen ont été utilisés dans un double carré latin 3×3. Ils ont reçu le même foin à trois niveaux d'ingestion correspondant aux besoins énergétiques d'entretien (E), à 60 % (0,6 E) et 20 % (0,2 E) de ces besoins. La digestibilité des parois végétales a été plus élevée à 0,2 E qu'aux deux autres niveaux. Le temps de rétention des parois dans le rumen n'a pas varié entre E et 0,6 E, mais a été plus élevé à 0,2 E. Lorsque le niveau d'ingestion était bas, le remplissage du rumen a été plus faible et le contenu plus liquide. Aucune variation de l'activité microbienne in situ ou des activités des polysaccharidases et glycosidases microbiennes n'a été observée. La concentration des protozoaires a diminué avec le niveau d'ingestion, mais pas la masse totale de bactéries. La masse de bactéries adhérentes en proportion de la phase solide a été plus élevée à bas niveau d'ingestion. Le profil des acides gras volatils a montré une réduction du butyrate au profit de l'acétate à bas niveau d'ingestion. En conclusion, les modifications de la digestion ont été faibles entre E et 0,6 E, et l'activité microbienne n'a pas été réduite à très bas niveau d'ingestion.

sous-alimentation / ovin / digestion / rumen

* Correspondence and reprints
e-mail: bdoreau@clermont.inra.fr

1. INTRODUCTION

In temperate countries ruminants are often subject to periodic food restriction. Underfeeding can be due to an economic strategy of the farmer to minimise cost of feeding. It can also be the physiological situation in high-producing dairy cows. In tropical countries underfeeding can be of a high magnitude and is generally not controlled. Lack of forage is the main reason for underfeeding, but other causes may reduce voluntary intake, such as low quality of forages, the depressive effect of high temperatures, water starvation or excessively prolonged search for food. To assess nutritional deficit, irrespective of the cause of food restriction, it is important to study accurately the mechanisms involved in digestibility variations in response to a marked decrease in intake level.

Numerous experiments carried out over more than four decades have clearly shown a negative relationship between level of intake and digestibility of a given diet (reviews of Galyean and Owens [9]; Chilliard et al. [4]). This is explained by a longer retention time of particles in the rumen when intake decreases [9]. However, this trend is not always observed when intake is very low and when feed supply does not cover energy requirements. In these conditions the response of digestibility to a decrease in intake is variable. The general trend is sometimes observed [20]. In other trials, no variation may occur [11] and digestibility may even decrease with diets rich in concentrates [10] or rich in forages [12, 13]. From these experiments it was concluded that at very low intake an increase in retention time of particles in the rumen does not always contribute to an improvement of digestibility. Ruminal digestion of a diet depends both on the time of contact between microorganisms and particles and microbial activity. Thus it can be expected that the decrease in digestibility, when it occurs, is due to a limitation of microbial activity, although it is generally considered to be independent on feed intake for the same diet.

To investigate the modifications of digestion when ruminants are underfed, an experiment was carried out in sheep at three levels of intake, the highest of which corresponded to maintenance energy requirements. Measurements were focused on the determination of microbial activity, and specifically on the variations in mass and enzyme activity of rumen microorganisms. Two levels of underfeeding were studied to determine whether the relationships between digestion and intake depend on the magnitude of the undernutrition.

2. MATERIALS AND METHODS

2.1. Animals, experimental design and diet

Six five-year old wethers, weighing initially 69.7 (S.D. 4.0) kg were used as experimental animals. Each was fitted with a permanent ruminal cannula made of polyamide and polyvinyl chloride (Synthesia, Nogent-sur-Marne, France); surgery was performed more than 12 months before initiation of the experiment, under general anesthesia (Isofluorane, ICIU Pharma-vétérinaire, Paris, France). Animals were housed in individual metabolism pens, and used in a replicated 3×3 Latin square design. The experiment was carried out according to the recommendations of the Guide to the care and use of experimental animals, published by the Canadian Council on Animal Care.

A mountain natural grassland hay, harvested at the first growth, containing 8.8% crude protein and 67.5% neutral detergent fibre (NDF) in the dry matter (DM), was used in this experiment. This hay was offered ad libitum for a one-month adaptation period, and then given in restricted amounts at three intake levels, maintenance (M, corresponding to maintenance requirements for energy), 60% (0.6 M), and 20% (0.2 M) of these requirements. Maintenance

energy requirements were taken according to the Institut National de la Recherche Agronomique [16] as 23 g digestible organic matter (OM)·kg^{-1} of LW$^{0.75}$. Hay OM digestibility was estimated by an enzymatic method [2]. This diet provided 98% of N requirements [16]. As a consequence of the constant composition of the diet, energy and N supplies were decreased in the same proportion. Animals received their daily diet in two equal portions at 09.00 and 21.00 h. Water and salt blocks were available ad libitum for all animals. Each period lasted six weeks, a 28-day adaptation period followed by a 14-day experimental period. At the beginning of each period corresponding to 0.2 M treatment, an intravenous injection of vitamins and trace elements was given (5 mL Fercobsang, Vétoquinol, Lure, France).

2.2. Measurements and chemical analyses

Feed intake was recorded daily throughout the sample collection period. Feed samples were collected and pooled within each period. Total tract digestibility was determined by total collection of faeces for six days (from d 1 to d 6 of the experimental period). After weighing, an aliquot from each daily faecal collection was dried (80 °C for 48 h) and dry samples of faeces were pooled for each animal and each period. Dried hay and faeces samples were analysed for NDF and acid detergent fiber (ADF) [34].

The hay degradation rate was measured with an in situ technique between d 1 and 7 of the experimental period. Dacron bags (Ankom Co. Fairport, NY; pore size: 53 μm; internal dimensions: 5 cm × 10 cm) were dried (80 °C), weighed, filled with 3 g of ground hay (sieve size 0.8 mm), and incubated in the rumen just before the morning feeding for 3, 6, 12, 15 , 24, 48 and 72 h. Two replications were made on each animal for each incubation time. After removal, bags were washed in a washing machine, dried (80 °C for 48 h), and weighed. The residues of the same incubation time and the same animal were pooled before analyses for NDF.

To measure ruminal liquid dilution rate, a pulse dose of Cr-ethylene diamine tetracetic acid solution (50 mL of solution, 1.77 mg Cr·mL^{-1}) was introduced intraruminally on d 10 at 08.00 h. Eight samples were taken via ruminal cannula 2, 4, 6, 8, 11, 15, 23 and 26 h after administration of the marker, and frozen at –20 °C; Cr was determined by atomic absorption spectrophotometry (Model 2380 Spectrophotometer, Perkin-Elmer, Bois-d'Arcy, France) directly on supernatant obtained by centrifugation (5000 × g for 15 min).

Ruminal fluid was collected by suction through a pipe inserted in the ventral sac on d 10 and 11 at 09.00 h just before feeding, 11.00 h and 15.00 h, and immediately strained through a 100-μm nylon filter. The filtrate was maintained under magnetic stirring for pH determination with a digital pH-meter (CG840, Ag/AgCl electrode, Schott Gerate, Hofheim, Germany). The filtrate was preserved with a solution of 5% (v/v) orthophosphoric acid (0.5 mL in 5 mL rumen liquid). On this sample, volatile fatty acid (VFA) concentration and composition were analysed by gas liquid chromatography [17] and NH_3-N concentration was determined according to Conway [5].

Liquid-associated protozoa counts and enzyme activity measurements from solid phase microorganisms (SPM) were carried out on samples obtained just before (09.00 h) and 2 h after morning feeding (11.00 h) on two consecutive days (d 11 and 12). Rumen content samples (200 g) were taken manually from the ventral sac and strained manually through a 100-μm nylon filter to separate a liquid phase (LP) and a solid phase (SP). For protozoa counting, 9 mL of LP was preserved at 4 °C with 1 mL of solution made of 50% glycerol, 48% distilled water, and 2% formaldehyde. Protozoa numbers were determined by using a Dolfuss cell (Elvetec Services, Clermont-Ferrand,

France) [18]. Enzyme extraction from SPM was performed under anaerobic conditions [26]. Samples of SP (70 g) were washed with pre-warmed (40 °C) anaerobic Coleman buffer (350 mL) and recovered by filtration (100 µm). A sample of SP (5 g) containing solid-associated microorganisms was chopped with scissors, suspended in 25 mL of anaerobic buffer containing 2-(N-morpholino) ethane sulphonic acid (MES) buffer (0.025 mol/L, pH 6.5, 4 °C) and stored at –80 °C. The remainder of the washed digesta was used for DM determination (48 h at 80 °C). The microorganisms present in the suspension were then disrupted by defrosting and sonication (Labsonic U, B Braun Biotech Inc., Bethlehem, PA, USA) for four 30-s periods at 4 °C under anaerobic conditions. The remaining solids were removed by centrifugation (15 000 × *g* for 15 min at 4 °C) and the supernatant used as the enzyme preparation. Enzyme activities of SPM were measured using the assay procedures detailed by Martin et al. [27]. Polysaccharidase (xylanase, carboxymethylcellulase (CMCase), avicelase) activities were determined by measuring, using absorption spectrophotometry at 410 nm [25], the amount of reducing sugars released from purified substrates (Birchwood-xylan, Sigma X-0502; carboxymethylcellulose, Sigma C-5678; Avicel, Macherey Nagel 81629) after incubation (60 min at 39 °C) with an enzyme preparation. Similarly, glycosidase activities were determined by measuring the amount of p-nitrophenol released from the appropriate p-nitrophenyl glycoside (Sigma, p-nitrophenyl-b-d-xylopyranoside, N-1232; glucopyranoside, N-7006) after incubation of 1.0 mL substrate with 0.1 mL enzyme preparation at 39 °C for 45 min. Activities were expressed as µmol reducing sugars (polysaccharidases) or µmol of p-nitrophenol (glycosidases) released in 1 h per g DM of solid phase digesta.

Samples of ruminal content were collected (d 13) just before (0 h), 2 h and 6 h after the morning feeding, and pooled to constitute a representative 1-kg sample. This sample was then strained through a 100-µm nylon filter to constitute an SP sample from which the bacterial population was isolated. This sample was processed according to a modified procedure of Legay-Carmier and Bauchart [24] to obtain an SPM pellet. This sample was washed by manual shaking for 5 min with a pre-warmed (40 °C) Coleman buffer (v/v), and strained again through a 100-µm nylon filter. The solid material was suspended in pre-cooled (4 °C) Coleman buffer (v/v), stored for 16 h at 4 °C, and blended three times for 1 min at high speed using a blender. The homogenate containing the adherent population was centrifuged (500 × *g* for 10 min at 4 °C) to discard plant material and the supernatant fraction was re-centrifuged (25 000 × *g* for 30 min at 4 °C). The resulting sediment corresponded to the microbial population adhering to the particles (SPM). Samples of SP and SPM were freeze dried before determination of purine and pyrimidine base content by HPLC [22].

At the end of each period (on d 14 at 15.00 h), the rumen was manually emptied, and the content weighed. After homogenisation, a representative sample was dried (48 h at 80 °C) and then ground at a 0.8-mm grid to determine indigestible NDF as specified below. Another sample was strained through a 100-µm nylon filter to separate LP and SP for weighing and representative sampling. Dry matter and NDF were determined on total contents and SP to estimate total and SP ruminal pool size.

Neutral detergent fibre retention time was determined according to Knowlton et al. [21] as the ratio between ruminal pool size and flux of 120-h indigestible NDF determined by the in situ technique as described above. Dacron bags were prepared with 3 g of hay, or 3 g of rumen contents from each of the six animals. They were incubated in duplicate in the rumen for 120 h. Neutral detergent fibre was then determined on all residues to obtain indigestible NDF in hay and rumen contents.

2.3. Calculations and statistical analyses

Dry matter and NDF degradation kinetics obtained for each animal and each diet were fitted to the exponential model:

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

where D(t) = percentage of disappearance from the bag for time t; a and b rapidly and slowly degradable fractions, respectively; and c = fractional degradation rate of b. The three parameters a, b and c were estimated by an iterative least squares procedure of SAS [33], and best-fit values were chosen using the smallest sum of squares after convergence. Dry matter and NDF effective degradability was calculated using the following equation:

$$\text{Effective degradability} = a + [(b \times c)/(c + k)]$$

where k is a mean particle passage rate taken as $4\%\cdot h^{-1}$.

Liquid dilution rate was calculated from the slope of linear regression of the natural logarithm of CrEDTA concentrations in rumen fluid against time.

The SPM mass (g DM) was calculated by multiplying the pool size of SP obtained by emptying by the ratio of purine base concentrations in SP and SPM.

Data were statistically analysed using the GLM procedure of SAS [33] as replicated ($n = 2$) 3×3 Latin squares with the following model:

$$Y_{ijkl} = \mu + A_i + P_j + L_k + e_{ijkl}$$

in which μ was the overall mean; A, P, and L were the effects of animal, period and intake level, respectively; and e_{ijkl} was the error term (8 df).

3. RESULTS

Apparent DM digestibility did not vary significantly with intake level, although a slight trend was observed at 0.2 M (Tab. I). The effect of intake level on fibre fraction was significant: digestibility increased when intake level decreased from 26.7 to 8.9 $g\cdot kg^{-1}$ $LW^{0.75}$, by 5.2 and 5.1 points for NDF and ADF respectively, whereas DM digestibility increased only by 2.3 points in the same time.

Total and DM ruminal pools were lower at 0.6 M than at M, and lower at 0.2 M than at 0.6 M (Tab. II). Dry matter content did not vary between M and 0.6 M, but dropped at 0.2 M. In the same way, the proportion of SP decreased significantly between 0.6 and 0.2 M. A significant decrease in ruminal liquid dilution rate was observed at 0.2 M,

Table I. Mean daily intake and apparent digestibility of DM and cell wall constituants from hay offered to wethers at three intake levels.

	Intake level (× maintenance)*			
	1.0	0.6	0.2	SEM
DM intake ($g\cdot kg^{-1}$ $LW^{0.75}\cdot d^{-1}$)	44.4[a]	26.7[b]	8.9[c]	7.5
Digestibility (%)				
DM	58.7[a]	59.7[a]	62.0[a]	0.8
NDF	59.4[a]	60.8[a]	66.0[b]	0.9
ADF	53.4[a]	54.1[a]	59.2[b]	1.0

* Different letters in a same row correspond to a significant difference ($P < 0.05$).

compared with the other two intake levels. Mean ruminal NDF retention time was not modified between M and 0.6 M, but a significant increase was observed at 0.2 M.

Parameters of in situ DM or NDF degradation kinetics did not vary with intake (Tab. III). Thus effective degradability was not modified by level of intake.

Protozoa concentration in ruminal liquid was not modified between M and 0.6 M, but decreased significantly at 0.2 M (Tab. IV). These variations were mainly due to variations in the concentration of the entodiniomorphid population. Pool size of bacteria associated with the ruminal SP showed a slight decrease when intake level decreased,

Table II. Effect of intake level on ruminal pool size, liquid dilution rate and NDF retention time.

	Intake level (× maintenance)*			
	1.0	0.6	0.2	SEM
Ruminal pool size				
Fresh matter (kg)	11.35[a]	8.92[b]	6.95[c]	0.29
Solid phase proportion (% fresh matter)	35.1 [a]	33.9 [a]	17.7 [b]	0.5
DM content (%)	9.62[a]	9.00[a]	5.81[b]	0.24
Dry matter (g)	1 084[a]	793[b]	400[c]	41
Ruminal liquid dilution rate (%·h^{-1})	6.8[a]	5.9[a]	4.0[a]	0.4
Ruminal NDF retention time (h)	20.3[a]	22.8[a]	33.0[b]	0.9

* Different letters in a same row correspond to a significant difference ($P < 0.05$).

Table III. Effect of intake level on kinetics of DM and NDF in situ degradation.

	Intake level (× maintenance)*			
	1.0	0.6	0.2	SEM
DM				
Rapidly degradable fraction (%)	26.2[a]	25.1 [a]	25.3 [a]	0.9
Slowly degradable fraction (%)	50.8 [a]	50.3 [a]	52.6 [a]	1.2
Undegradable fraction (%)	23.0 [a]	24.6 [a]	22.1 [a]	1.8
Degradation rate (%·h^{-1})	3.7 [a]	3.5 [a]	3.6 [a]	0.5
Effective degradability (%)	58.4 [a]	55.2 [a]	56.5 [a]	1.5
NDF				
Rapidly degradable fraction (%)	3.8 [a]	2.0 [a]	1.4 [a]	1.3
Slowly degradable fraction (%)	68.0 [a]	68.8 [a]	73.2 [a]	2.4
Undegradable fraction (%)	28.1 [a]	29.1 [a]	25.5 [a]	3.3
Degradation rate (%·h^{-1})	3.2 [a]	3.2 [a]	3.5 [a]	3.3
Effective degradability (%)	34.7 [a]	32.9 [a]	34.5 [a]	1.2

* Different letters in a same row correspond to a significant difference ($P < 0.05$).

Table IV. Effect of intake level on protozoa concentration ($10^3 \cdot mL^{-1}$) in ruminal liquid phase.

	Intake level (× maintenance)*			
	1.0	0.6	0.2	SEM
Total	76.7[a]	72.2 [a]	34.1[b]	5.9
Entodiniomorphids	65.3 [a]	49.3 [a]	27.7 [b]	7.5
Holotrichs	11.4 [a]	13.3 [a]	6.3 [a]	2.9

* Different letters in a same row correspond to a significant difference ($P < 0.05$).

but this variation was not significant (Tab. V). Expressed as a function of total ruminal content, the concentration of these bacteria increased at low intake level, but did not vary when concentration was expressed as a proportion of ruminal SP. Expressed in the same way polysaccharidase and glycosidase activities of this microbial population did not vary with intake level, except for xylanase activity in SPM, which significantly decreased between 0.6 and 0.2 M, 2 h post-feeding (Tab. VI).

After feeding, pH values were higher and concentration of VFA was lower at 0.2 M than at the other two levels (Tab.VII). Undernutrition decreased butyrate proportion and increased acetate proportion, whereas propionate proportion was not modified. These modifications in fermentation patterns were particularly marked after feeding.

4. DISCUSSION

4.1. Dry matter and cell wall digestibility

The increase in digestibility observed when intake level falls to 0.2 M is consistent with the general relationship observed between level of intake and digestibility [4, 9]. In experiments carried out at levels of intake lower than maintenance, different responses in digestibility were observed (review by Doreau et al. [8]). There have been few reported experiments in which intake level was decreased with the same magnitude as in our trial. In these trials, severe underfeeding resulted in a very strong drop in digestibility [1, 10, 13]. This difference in response of digestibility to undernutrition for forage diets cannot be explained either by differences in nutritive value of diets, which at maintenance was similar in the three trials, or on animal species, one

Table V. Effect of intake level on bacterial population associated to ruminal solid phase.

	Intake level (× maintenance)*			
	1.0	0.6	0.2	SEM
Bacterial pool size (g DM)	429.8[a]	314.5 [a]	299.3 [a]	72.2
Bacterial DM / ruminal solid phase ($g \cdot kg^{-1}$)	530.7 [a]	582.8 [a]	586.8 [a]	120.8
Bacterial DM / ruminal DM ($g \cdot kg^{-1}$)	396.4[a]	396.6[a]	748.2[b]	95.2

* Different letters in a same row correspond to a significant difference ($P < 0.05$).

Table VI. Effect of intake level on polysaccharidases and β-D glycosidases activities in the microbial population associated with the ruminal solid phase.

Enzymes	Time after feeding (h)	Intake level (× maintenance)* 1.0	0.6	0.2	SEM
Polysaccharidases (μmol reducing sugars·g^{-1} DM·h^{-1})					
Xylanase	0	297.4 [a]	263.0 [a]	282.8 [a]	25.5
	+ 2	236.4 [a]	221.5 [a]	173.8 [b]	15.8
CMCase	0	33.1[a]	32.9 [a]	29.7 [a]	2.1
	+ 2	20.2 [a]	22.3 [a]	20.8 [a]	1.9
Avicelase	0	15.7 [a]	13.5 [a]	14.7 [a]	0.8
	+ 2	9.4 [a]	8.0 [a]	8.7 [a]	0.8
β – D glycosidases (μmol p-nitrophenol·g^{-1} DM·h^{-1})					
β – D xylosidase	0	87.0 [a]	89.3 [a]	93.6 [a]	3.2
	+ 2	68.2 [a]	67.5 [a]	66.2 [a]	4.9
β – D glucosidase	0	19.6 [a]	18.5 [a]	17.3 [a]	1.1
	+ 2	17.2 [a]	16.9 [a]	16.1 [a]	1.8

* Different letters in a same row correspond to a significant difference ($P < 0.05$).

Table VII. Effect of intake level on ruminal pH, VFA and ammonia N in the ruminal liquid phase.

	Time after feeding (h)	Intake level (× maintenance)* 1.0	0.6	0.2	SEM
pH	0	6.80 [a]	6.81 [a]	6.80 [a]	0.13
	+ 2	6.52 [a]	6.55 [a]	6.73 [b]	0.14
	+ 6	6.60 [a]	6.56 [a]	6.84 [b]	0.14
Total VFA (mmol·L^{-1})	0	65.0 [a]	63.0 [a]	49.9 [b]	6.3
	+ 2	79.2 [a]	69.5 [a]	51.3 [b]	10.2
	+ 6	67.2 [a]	65.8 [a]	44.4 [b]	7.0
Acetate (mol·100 mol^{-1})	0	74.2 [a]	78.1[b]	75.4 [ab]	3.3
	+ 2	65.2 [a]	72.8 [b]	75.8 [b]	4.8
	+ 6	71.7 [a]	73.7 [b]	75.7 [a]	3.9
Propionate (mol·100 mol^{-1})	0	16.8 [a]	16.8 [a]	16.6 [a]	0.4
	+ 2	19.4 [a]	19.3 [a]	18.7 [a]	1.2
	+ 6	18.0 [a]	17.3 [a]	16.9 [a]	1.0
Butyrate (mol·100 mol^{-1})	0	6.6 [a]	5.7 [ab]	4.8 [b]	0.3
	+ 2	6.9 [a]	6.8 [a]	5.3 [b]	0.4
	+ 6	7.4 [a]	6.7 [a]	4.7 [b]	0.3
Ammonia N (mg·L^{-1})	0	124 [a]	141 [a]	141 [a]	33
	+ 2	165 [a]	154 [a]	158 [a]	31
	+ 6	98 [a]	95 [a]	111 [a]	25

* Different letters in a same row correspond to a significant difference ($P < 0.05$).

being carried out with cows [13] and the other two with sheep ([1] and this trial). A possible explanation could lie in the characteristics of forage, a medium-quality hay in our trial, rice straw-based diet in the trial by Grimaud et al. [13] and vetch-oat diet in the trial by Atti [1]. A specific effect of straw may be suspected in the case of negative response in digestibility to decrease of intake level.

A quadratic response of digestibility to undernutrition was observed in this trial, DM and fibre digestibility not varying significantly between M and 0.6 M, but increasing at 0.2 M, particularly for the fibre fraction. In several experiments carried out above maintenance level, linear responses have been observed with concentrate diets [23] or forage diets [30]. It is known that the effect of intake on digestion is lower for forage than for concentrate diets. This may explain why in other experiments with forage diets, no effect of intake was shown both above [15] or below maintenance [14, 29]. A quadratic response as in our trial is thus uncommon.

4.2. Dynamics of particles and water, consequences on ruminal pool size

The relationship between digestibility and intake is a consequence of modifications in the extent of ruminal digestion. Digestibility depends both on microbial activity and time of contact between microorganisms and particles. When intake decreased, ruminal particle retention time increased, especially at 0.2 M. This increase was consistent with the general relationship between the amount of food consumed and ruminal retention time of particles, carried out at levels of intake higher [14] or lower [8] than maintenance. The very low intake at 20% of energy requirements led to a very long stay of feed in the rumen, suggesting a quadratic relationship between intake and retention time. In contrast to the results obtained above maintenance, an increase in particle retention time was sometimes associated with a decrease in digestibility at low intake [12, 13], probably because at maintenance the retention time was long enough to optimise microbial degradation in the rumen. In these cases, it had been concluded that ruminal particle retention time was not involved in digestibility variations.

Total rumen contents decreased with intake, although an increase in water percentage compensated for the strong decrease in DM pool. Between M and 0.6 M, total rumen pool decreased, but water content remained constant, maintaining the fill of the rumen. On the contrary, both in this study and in a study by Atti [1], very severe underfeeding did not prevent a decrease in rumen fill, although the content became very liquid.

4.3. Hydrolytic activity of microbial digestion

Variations in mass and activity of rumen microoorganisms were measured. The decrease in feed intake involved a decrease in protozoa concentration, as previously reported [11, 13]. This decrease in protozoa concentration at very low intake could not result either from a lower availability of energy because no similar effect was observed on SPM concentration, or from modifications of physico-chemical conditions in rumen because post-prandial pH increased when intake decreased. Moreover, the decrease in liquid and solid flows out of the rumen generally leads to an increase in protozoa concentration [6]. This decrease in protozoa concentration could arise from the low DM proportion in ruminal contents, which is observed in all cases of strong undernutrition [8]. It can be hypothesised that the retention and survival of protozoa in the rumen is related to their attachment to particles [36], which is much weaker than for bacteria and could be a purely physical phenomenon [28], contrary to bacteria. In undernutrition, this attachment ability of

protozoa would decrease with low DM proportion in ruminal content.

The most novel results concern the bacterial population, especially the solid-adherent bacterial population, studies on this subject being scant in the literature. The pool size of this population, determined using a microbial marker, did not vary when intake decreased. When expressed as a function of DM present in the rumen, microbial mass was higher at low intake than at high intake, as had been observed in trials where intake levels varied above maintenance [7, 31]. However, when adherent biomass was expressed as a function of ruminal content present in SP, which reflects the colonisation of particulate material by SP-adherent microorganisms, this concentration did not vary. This discrepancy between the two expressions is a consequence of the modifications in ruminal contents. At low intake, ruminal content is much more liquid, and the decrease in protozoa concentration is enhanced by this dilution. On the contrary, concentration of biomass associated with SP varies narrowly. Ruminal bacterial concentration is the balance between bacterial growth and lysis, dilution by feed and turnover of particles. At 0.2 M, particle input and output decreased, but ruminal bacterial concentration was constant, so the balance between bacterial growth and lysis was probably not modified by intake level. Diurnal fluctuations in the energy inputs would involve a hysteresis in bacteria growth rate, less available energy for microbial ecosystem and less of the energy-yielding substrate used for bacterial maintenance [3]. This could lead either to a decrease in maintenance requirements of bacteria, or to changes in bacterial community structure energy requirements for maintenance varies among bacterial species [32]. Lysis is generally described as a feature of cell cultures in starvation, but recently *Fibrobacter succinogenes* appeared to limitate its autolysis when energy was depleted [35].

Polysaccharidase and glycosidase activities of this population firmly attached to rumen particulate material, expressed as a proportion of ruminal SP, did not vary with intake level, and these results agree with previous data concerning ruminal bacterial concentration. In the trial of Kabré et al. [19], underfeeding tended to increase total glycosidase activities, but polysaccharidase activities were not modified. Together, these results of microbial mass and activity show that in the conditions of this experiment, microbial activity is not affected to a large extent by intake level.

In conclusion, this experiment showed a non-linear response of digestibility to a strong decrease in feed intake. At a very low intake, digestibility increased whereas in other studies it decreased. This could be due to the nature of the forage. This increase in digestibility was accompanied by a stasis of ruminal content. The decrease in feed input was compensated for by a marked decrease in liquid and solid output of the rumen, which could explain the increase in fibre digestibility.

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