

## Effect of undernutrition on the ability of the sheep rumen to absorb volatile fatty acids

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**Summary** — The ability of the rumen to absorb the same quantity of VFA with 4 animals previously fed with 2 levels of intake was tested. Animals received maintenance (P1) and half maintenance (P2) energy and nitrogen requirements successively. Absorption was measured with the empty washed rumen technique. Three litres of a solution buffered at pH 6.30 containing VFA (C2:57.1, C3:49.2 and C4:7.4 mM or C2:79.8, C3:23.5 and C4:11.5 mM) and CoEDTA (7.1 mg Co/l) were introduced in the rumen and regularly sampled for 3 h. VFA absorption was linear during the trials. Rates of absorption were expressed as mmol/h or percentage of initial quantity/h for the comparison between VFA. The order of absorption rate (%/h) was C4 > C3 > C2. Water absorption was not significantly different between the periods whereas VFA absorption rates (mmol/h) were significantly reduced after undernutrition. Composition of the solution had no significant effect on VFA absorption rate (%/h).

### VFA / absorption / undernutrition

**Résumé** — Effet de la sous-nutrition sur la capacité du rumen de mouton à absorber les acides gras volatils (AGV). La capacité d'absorber une même quantité d'AGV a été testée dans le rumen de 4 moutons précédemment nourris à 2 niveaux d'ingestion différents. Les animaux ont reçu une ration correspondant au besoin d'entretien (P1) puis à la moitié de leur besoin d'entretien énergétique et azoté (P2). L'absorption des AGV a été mesurée selon la méthode du rumen vidé et lavé. Trois litres d'une solution tamponnée à pH 6,30 et contenant des AGV (C2:57,1, C3:49,2 et C4:7,4 mM ou C2:79,8, C3:23,5 et C4:11,5 mM) et du CoEDTA (7,1 mg Co/l) ont été introduits dans le rumen et régulièrement échantillonnés pendant 3 h. L'absorption des AGV était linéaire pendant la durée des expériences. Les pentes (vitesse d'absorption) ont été exprimées en mmol/h ou en % de la quantité initiale/h pour la comparaison des 3 AGV. L'ordre des vitesses d'absorption (%/h) était C4 > C3 > C2. L'absorption d'eau n'a pas varié significativement entre les périodes mais la vitesse d'absorption des AGV (mmol/h) a été significativement réduite après la période de sous-nutrition. La composition de la solution n'a pas eu d'effet sur la vitesse d'absorption des AGV (%/h).

### AGV / absorption / sous-nutrition

## INTRODUCTION

More than 80% of the VFA produced in the rumen is absorbed through the rumen wall (Bergman, 1990). The rumen epithelium is continuously renewed; dead cells are carried away by the rough feeds and new cells grow to replace them. The characteristics of this epithelium are not constant however; Fell and Weekes (1975) suggested that level of intake may be positively related to the development of the rumen epithelium. Drouillard *et al* (1991) showed that undernutrition reduced liver, stomach and intestine weights of lambs, and Burrin *et al* (1992) indicated that an increase in the level of feeding resulted in a higher hypertrophy of the gut cells. Sakata and Yajima (1984) showed that loads of VFA increased the cell proliferation. When undernourished, an animal develops a new digestive strategy. It is known that the digestibility of a diet increases if the intake is lower, as the feeds remain longer in the digestive tract (Blaxter *et al*, 1956; Doreau *et al*, 1986). As rumen epithelium characteristics vary, it could be of interest to check if its ability to absorb VFA varies during undernutrition. To eliminate the effect of the feed intake (increase of the retention time and VFA concentrations) we have chosen to test the ability of the rumen wall to absorb an equal quantity of VFA with animals previously fed 2 different levels. Two VFA compositions were tested in order to prove a possible interaction between the absorption of the 3 VFA.

## MATERIALS AND METHODS

### *Animals and diet*

Four adult sheep (mean weight:  $64 \pm 6$  kg) were paired into 2 groups of 2 animals. They were fitted with a large ruminal cannula (diameter 75 mm) made of polyamide and polyvinyl chloride

(Synthesia, Nogent-sur-Marne, France) and with a T-shaped cannula made of plastisol (Synthesia) placed at the abomasum. Surgery was performed in a sterile environment under general anaesthesia (Halothane, ICI pharma-Vétérinaire, Paris, France). Sheep received antibiotic treatment during the 4 days following surgery. They were fed a second-cut meadow hay (organic matter (OM) 893; crude protein (CP) 138; acid detergent fibre (ADF) 320; neutral detergent fibre (NDF) 523; crude fibre (CF) 234 g/kg dry matter (DM); digestible energy 3 587 kcal/kg DM). The experiment comprised of 2 successive periods of 4 weeks. During the period P1, the animals received quantities of hay corresponding to maintenance energy and nitrogen requirements (46 g/kg  $W^{0.75}/d$ , level of feeding = 1) then during P2, quantities of hay were abruptly reduced by half (half maintenance energy and nitrogen requirements, 23 g/kg  $W^{0.75}/d$ , level of feeding = 0.5).

### *Absorption trials*

Absorption of VFA was measured on both animals one day a week during P1 and at the end of P2 by a temporarily isolated rumen technique, as described by Gäbel and Martens (1986). On the days of measurement, absorption was measured once or twice when possible. The total number of trials was 6 or 7 for each animal (4 trials during P1; and 2 or 3 trials at the end of P2). A meal was offered once a day at 9 am. On the day of the measurement, a meal was offered at 7 am. The sheep rumens were emptied and washed with a warm saline solution. Rumen content was kept at 39°C and returned to the rumen at the end of the trial. Three litres of a test solution containing VFA and CoEDTA were introduced in the rumen and sampled regularly. The composition of solutions was different for the 2 groups (table 1). Saliva was continuously removed by a saliva collector introduced in the oesophagus and was directed into the abomasum. The solution in the rumen was continuously gassed with CO<sub>2</sub>.

### *Preliminary trial*

A 4-h preliminary trial was performed before P1 with one of the sheep fed *ad libitum*, in order to determine if the VFA concentration decrease was

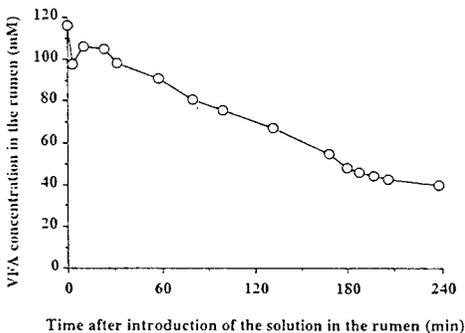
**Table I.** Characteristics of the test solutions.

Component	Mean concentrations	
	Solution 1	Solution 2
Acetic acid (mM)	57.09	79.80
Propionic acid (mM)	49.20	23.50
Butyric acid (mM)	7.43	11.55
CoEDTA (mg Co/l)		7.06
NaHCO <sub>3</sub> (g/l)		8.5
KHCO <sub>3</sub> (g/l)		4.4
NaCl (g/l)		0.8
Mean pH		6.30
Osmotic pressure (mOsm/l)		313

linear. Results showed that the VFA concentration presented an erratic pattern for the first 30 min revealing that equilibrium was not achieved. However after this time, linearity occurred for at least 3 h (fig 1). During the 4th hour, the VFA absorption rate slowly decreased. The next absorption tests were then performed for 3 h only.

### Volume determination

On the hypothesis that the quantity of CoEDTA present at the beginning of the trial in the test solution remained constant throughout the trial,



**Fig 1.** VFA concentration in the rumen showed a decrease after the first few minutes then an increase. A linear decrease then appeared until 3 h after the beginning of the trial.

variations of the CoEDTA concentration gave an estimation of the volume variations due to water movements across rumen epithelium and then of the rumen water volume at each sampling. This calculated volume was a good estimation of the true volume measured by aspiration at the end of the trial if, in addition, the losses of volume due to sampling were taken into account. Water absorption was then calculated as the variation of the liquid volume between 2 sample times.

### Digestibility

The digestibilities of dry matter and organic matter of the hay were measured by total collection of faeces for 5 d in each period. Dry matter and ash content of feed and faeces were determined by weighing a sample residue after 48 h at 80°C and 6 h at 550°C, respectively, in order to calculate dry and organic matter digestibilities.

### Sampling and chemical analyses

The test solution was sampled before its introduction in the rumen, then approximately every 30 min for 2–3 h. The volume of the samples was 15 ml, in which the pH was immediately measured; 5 ml was kept for VFA determination, to which 0.5 ml orthophosphoric acid (5% V/V) was added as a preservative, and 8 ml was kept for CoEDTA determination. Samples were centrifuged at 4 900 g for 15 min. Samples for VFA analysis were filtered then VFA concentrations were determined by gas-liquid chromatography with isocaproic acid used as the internal standard. CoEDTA was analysed by atomic absorption spectrophotometry (Udén *et al*, 1980).

### Calculations

Calculations were performed using the results of the 26 absorption trials. Calculated liquid volume and VFA concentrations in the rumen allowed the amounts of VFA present in the rumen at each sampling time to be quantified. Quantity of VFA absorbed was then calculated as the difference

between the initial VFA quantities in the rumen and the VFA quantities in the rumen at each sampling time.

### **Statistical analysis**

Absorption of acetic, propionic and butyric acids was tested for regression against time for the 26 trials. As equilibrium of the VFA concentration was not reached before the first 30 min, the following values of the absorption were dependent on the amplitude of this phenomenon. The intercept of the regression was then considered as being of questionable interest. The 26 values of the slopes and the VFA concentrations were tested according to a split-plot design using the GLM procedure of SAS (1985), specifying the animal (nested in the composition), the previous level of intake, the composition of the test solution and the interaction between level and composition as the main effects. The effect of the composition of the test solution and the interaction between level and composition were tested specifying the animal effect nested in the composition as an error term. The pH and volume were tested following the same model except that time was added to the parameters specified as main effects.

Rates of absorption (mmol/h) were defined as the slopes of the regressions. Rates of absorption expressed as a percentage of the initial quantity absorbed per min (%/min) were also calculated in order to compare the absorption of the individual VFA.

## **RESULTS**

### **Digestibilities**

The digestibilities of DM during P1 and P2 were 71.8 and 76.3%, respectively, whereas the digestibilities of OM were 76.6 and 77.6% during P1 and P2, respectively.

### **Liquid volume and water absorption**

Liquid volume in the rumen did not vary significantly during the trials. It was not signif-

icantly different between the animals or between the 2 compositions, but was significantly higher with level 1 than with level 0.5 (mean volume 3.11 vs 2.98 l).

Water absorption was not influenced by time, animals, level of feeding or composition.

### **pH of the samples, VFA concentration values**

The mean initial pH was 6.30. The mean increase at the second sample (30 min) was 0.6 pH units. The pH then linearly increased overall by 0.11 pH units per hour. No difference in the pH was found in the compositions.

The concentrations of individual VFA in the rumen varied with the composition of the solution, but total VFA concentration was independent of the composition. Interaction between level of feeding and composition was not significant at any point.

### **VFA absorption**

Rates of absorption (mmol/h) were significantly lower at 0.5 level of feeding in comparison with level 1 for acetic, propionic, butyric acids and total VFA ( $P < 0.01$ ) (table II). Comparisons of the absorption rates as a percentage of the initial quantity absorbed per hour showed that the absorption order was  $C4 > C3 > C2$  ( $P < 0.001$ ) at level 1 (19.93, 18.24 and 16.51%/h for butyric, propionic and acetic acids, respectively) and  $C4 = C3 > C2$  at level 0.5 (14.90, 14.30 and 12.47%/h for butyric, propionic and acetic acids, respectively). The rate of absorption of butyric acid was significantly higher with composition 2 than with composition 1, but only when it is expressed as mmol/h (6.93 vs 3.61 mmol/h,  $P < 0.05$ ) and not when expressed as a percentage of initial/h (18%/h). The

**Table II.** Absorption rates of the 3 VFA.

<i>Solution</i>			<i>Absorption rate (mmol/h)</i>	
			<i>Level 1</i>	<i>Level 0.5</i>
<i>C2</i>				
1	Animal 1	23.90 ± 9.92	8.98 ± 4.92	
1	Animal 2	31.85 ± 3.89	21.42 ± 1.50	
2	Animal 3	42.34 ± 5.75	25.65 ± 9.46	
2	Animal 4	44.40 ± 15.13	40.79 ± 7.68	
<i>C3</i>				
1	Animal 1	22.10 ± 8.45	10.07 ± 3.25	
1	Animal 2	29.47 ± 2.08	19.08 ± 3.32	
2	Animal 3	14.04 ± 1.55	8.66 ± 2.70	
2	Animal 4	14.91 ± 4.83	14.01 ± 1.76	
<i>C4</i>				
1	Animal 1	3.70 ± 1.32	2.14 ± 0.14	
1	Animal 2	4.69 ± 0.04	2.72 ± 1.01	
2	Animal 3	7.74 ± 0.57	4.57 ± 1.16	
2	Animal 4	7.96 ± 2.29	6.85 ± 0.41	
<i>Total VFA</i>				
1	Animal 1	49.70 ± 19.68	21.18 ± 8.03	
1	Animal 2	66.01 ± 5.90	43.22 ± 5.83	
2	Animal 3	64.12 ± 7.78	38.89 ± 13.29	
2	Animal 4	67.28 ± 22.24	61.65 ± 9.84	

interaction between level of feeding and composition was never significant.

## DISCUSSION

The rate of VFA absorption found in P1 represents only 45% of the absorption rate found by Perrier and Doreau (1994) with the same compositions of solution intra-gastrically infused at maintenance level. This difference might be explained by the increase of the pH during the experiment, which does not occur in intra-gastrically fed animals, as the VFA are continuously renewed in the rumen. In intra-gastrically fed animals this difference is probably due to the higher liquid volume, whose contact with the rumen wall resulted in a higher absorption surface than in the present experiment.

The significant effect of the solution composition on the absorption rate of butyric acid can be explained by the difference in concentration of the acid, which was approximately 7% of the total VFA in solution 1 vs 10% in the solution 2. This was confirmed by the non-significant effect of the composition when the absorption rates are expressed as percentages. The non-significant effect of the composition on the absorption rates of acetic and propionic acid (mmol/h) was therefore surprising, but can probably be explained by the response variation between animals. Indeed acetic and propionic acid absorption rates tended to be higher with the compositions in which acetic and propionic acid parts were higher (solution 2 for acetic acid, solution 1 for propionic acid). In intra-gastrically fed animals, Perrier and Doreau (1994) found differences in VFA

absorption rates (%/h) when the composition varied. However this difference was mainly due to variation of the water absorption to which the VFA absorption was related, probably *via* the osmolarity. It is therefore logical not to find different absorption rates (%/h) according to the composition in this experiment, where water absorption was not significantly influenced by the composition. Water absorption did not differ between P1 and P2 either. In the same way, Holtenius and Dahlborn (1990) reported that net water absorption during food deprivation depended on the composition of rumen fluid, particularly the VFA concentration, but that net water absorption was independent of the nutritional status of the animal.

The VFA absorption rates were lower after 4 weeks of undernutrition. As the composition of the test solution was identical irrespective of the previous level of feeding, only the nutritional status of the animal could explain this difference. However, the extent of this result varies between the animals, as total VFA absorption rate after undernutrition was 43, 65, 61 and 92% of the control (level 1) for animals 1, 2, 3 and 4 respectively (table II). Many factors may have an effect on VFA absorption: epithelial cell metabolic activity, number of cells, and epithelial blood flow, *etc.* During undernutrition, portal and hepatic blood flows have been shown to decrease (Ortigue *et al*, 1993) as with fasted animals (Kelly *et al*, 1993). It is therefore likely that during P2, epithelial blood flow was lower than during P1. Blood flow has a positive effect on rumen cell growth (Fell and Weekes, 1975) and absorption (Kohn *et al*, 1993). Sakata and Tamate (1979) reported that mitotic activity of the rumen epithelium was depressed by fasting. Moreover, the decrease in the total metabolic activity of the gut, due to the decrease in the gut tissue weight, has often been reported during undernutrition (Ortigue, 1991). This could

also account for the lowering of the VFA absorption. All of these results may explain a lower VFA absorption capacity during P2.

The order in the rates of absorption expressed as a percentage of the initial quantity during P1 ( $C4 > C3 > C2$ ) was in agreement with the results of different authors, particularly Stevens (1970). The non-significant difference between the absorption rate of propionic and butyric acids during P2 showed that the decrease in absorption rate of butyric acid after undernutrition was greater than for the other acids. It is generally assumed that difference of absorption rates of the 3 VFA may be due to the different metabolisation rates of the VFA by the rumen wall (Stevens and Stettler, 1966). It could then be postulated that undernutrition altered the metabolism of the butyrate more than the metabolism of the other acids.

Using the same technique as in this experiment, Gäbel *et al* (1993) described a 43–56% decrease in the VFA absorption of sheep deprived of food for 2 d. They concluded with the hypothesis that longer undernutrition may stimulate adaptation of the absorption. The present study showed that 1 month undernutrition at half maintenance was not long enough for such adaptative changes to take place, except maybe for one of the animals, whose difference in absorption rates between level 1 and 0.5 was low (animal 4, table II). The next step in this study should therefore be to test VFA absorption throughout the undernutrition period instead of only testing it at the end, and to describe the evolution in VFA absorption ability during an undernutrition period.

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