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Assessment of Rumen Processes by Selected-Ion-Flow-Tube Mass Spectrometric Analysis of Rumen Gases

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ABSTRACT

This work investigated the potential to use measurement of the concentration of certain gases in the rumen headspace to gain information about rumen processes and as a potential diagnostic tool. We used new equipment (selected-ion-flow-tube mass spectrometer) that allows rapid and precise analysis of many of the gases present in a sample. Samples of rumen headspace gas and corresponding samples of rumen liquor were taken from three lactating cows, prepared with rumen fistulae, at intervals after receiving their morning feed allocation (grass silage and concentrates). Hydrogen sulfide, methyl sulfide, and dimethyl sulfide, were the predominant gases that were measured in the rumen headspace by this technique. The concentrations of these sulfur compounds declined over the interval after feeding, mirroring ammonia concentrations measured in rumen liquor, reflecting their common dependence on the fermentation of sulfur amino acids. Ammonia concentrations in rumen headspace gas varied in the opposite direction to the concentration of ammonia in rumen liquor and likely depend more on the pH of rumen liquor. Consideration of the pK_a of ammonia suggests that ammonia concentrations in rumen gas will be very low below pH 6, representing a useful diagnostic for subacute ruminal acidosis. Low concentrations of volatile fatty acids were detected in rumen gas. The molar proportions of volatile fatty acids were similar in gas and liquor samples, with rumen gas containing slightly less acetic acid and disproportionately more valeric and caproic acids.

(**Key words:** dairy cow, rumen, gas analysis, mass spectrometry)

Abbreviation key: DMS = dimethyl sulfide, **SIFT-MS** = selected-ion-flow-tube mass spectrometry (or spectrometer).

INTRODUCTION

A major goal for ruminant nutritionists has been to describe the processes of fermentation and microbial synthesis in the rumen and incorporate this information into improved systems for allocating feeds. Current techniques are often difficult to accomplish, difficult to interpret, or both, and usually involve the use of surgically modified animals. Consequently, results are often subject to large random and between-laboratory variations (Titgemeyer, 1997). The consequent difficulty of identifying consistent effects on rumen processes (e.g., microbial protein synthesis; Agricultural and Food Research Council, 1992) has limited the deployment of this type of knowledge in commercial practice.

Previous attempts to assess rumen function with animals without rumen cannulae have either involved the use of stomach tubes (Dirksen and Smith, 1987) or percutaneous needle aspiration (rumenocentesis; Garrett et al., 1999). Both techniques are stressful to the animal; samples taken by stomach tube are often contaminated with saliva (Dirksen and Smith, 1987), and rumenocentesis has led to infections in some situations (Garrett et al., 1999).

Our laboratory has adopted a different approach of developing less invasive techniques that can be applied to larger groups of experimental animals and also have the potential to spawn diagnostic tests as an inherent technology transfer element. The general approach is outlined by Dewhurst et al. (2000a), in relation to the

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analysis of compounds derived from rumen microbes in urine and milk. The current work developed this concept, utilizing information from gases produced by rumen fermentation with new equipment that allows rapid and precise analysis of most gases present in a sample (Smith and Španěl, 1996a; Španěl and Smith, 1997). Selected-ion-flow-tube mass spectrometry (**SIFT-MS**) has already been applied to the analysis of human breath for clinical diagnosis. Other applications have included therapeutic monitoring (Davies et al., 1997; Smith and Španěl, 1996b), analysis of urine from cancer patients (Smith et al., 1999a), and volatile emissions from food products (Španěl and Smith, 1999a), and from porcine feces and urine (Smith et al., 2000). The application of this work might ultimately involve the incorporation of breath sampling equipment in feeding equipment, as developed by Mottram et al. (1999).

The objective of this work was to identify gases that can be measured in rumen headspace and that indicate changes in the pattern of rumen fermentation. This preliminary experiment used known differences in the pattern of fermentation in relation to feeding times as a test of the approach, and comparisons were made between concentrations of compounds measured in rumen gas and rumen liquor.

MATERIALS AND METHODS

Cows and Their Management

Three multiparous Holstein-Friesian dairy cows, which had previously been prepared with rumen cannulae (Bar-Diamond, Parma, ID) were used for this work. The cows were on average 63 DIM, consumed 16.8 kg of DM per day and were producing 28 kg of milk per day.

The cows were adapted to a diet based on ad libitum consumption of grass silage and concentrates from the time of calving. They were offered fresh grass silage at 0900 h and given their concentrates (8 kg/d) in equal portions at milking times (0800 and 1630 h). The concentrate contained (% of DM): wheat (33), extracted rapeseed meal (16), molassed sugar beet pulp (11), expeller groundnut meal (9.5), soybean meal (7.5), molasses (7.5), palm kernel meal (5.5), extracted sunflower meal (5), vegetable oils (3), and minerals/vitamin (2). A mineral/vitamin mixture (Rumins Cattle High Phosphorus; Rumenco, Burton-on-Trent, UK) was sprinkled on top of the fresh silage allocation (50g/d). Samples of the silage and concentrates were analyzed for a full range of analytes (Dewhurst et al., 2000b). Daily feed intakes (orts) were recorded for each cow over the 5 d before the sampling day. A sample of each feed and the mineral/vitamin mixture were also analyzed for total sulfur (AOAC, 1980).

Sampling of Rumen Liquor and Rumen Headspace Gas

Samples of rumen headspace gas were withdrawn through a needle puncture in the rumen cannulae using a 'Mikrovac 3' high vacuum pump (Edwards, West Sussex, UK), pumping through a liquid trap. The pump and tubes were purged with rumen gas and then gas was pumped into evacuated plastic bottles (2 L) made of food-grade polyethylene terepthalate and closed using silicone rubber stoppers.

Single samples were taken from cows at 0930 h, and duplicate samples were taken at 1130 and 1530 h. Samples were held at ambient temperatures (15 to 20°C) overnight and transported to Keele University for analysis on the next day. At each sampling time, a sample of strained rumen liquor was withdrawn; pH was recorded immediately, and the sample was then acidified (concentrated sulfuric acid) and frozen before analysis for VFA and ammonia concentrations, as described by Dewhurst et al. (2000b).

Analysis of Rumen Gases by SIFT-MS

The SIFT-MS technique utilizes chemical ionization in a flow tube (Smith and Španěl, 1996a, 1996b; Špaňěl and Smith, 1996). Precursor ions of a given mass-to-charge ratio (selected by a quadrupole mass filter) are injected into fast flowing helium carrier gas. There they react during a defined reaction time with the trace gases in a sample of air (in these experiments, rumen gas), breath or liquid headspace, which is introduced into the carrier gas at a known flow rate, and produce characteristic product ions. A downstream quadrupole mass spectrometer is used to detect and count the precursor and product ions. The resultant mass spectrum (over a predetermined mass range) is fed into an on-line computer that immediately identifies and quantifies the trace gases in the sample. H_3O^+ precursor ions are used to detect and quantify most organic vapors and ammonia; they transfer their protons to the different trace gases, M, in the sample producing MH^+ ions [e.g., $(CH_3)_2S.H^+$, protonated dimethyl sulfide; NH_4^+, protonated ammonia]. As a result of the high humidity of rumen gas, a fraction of the product MH^+ ions react with the water molecules to form their monohydrate, $MH^+ \cdot H_2O$, and sometimes their dihydrate, $MH^+ \cdot (H_2O)_2$. These hydrated ions are included in the product ion count for accurate quantification of the individual trace gases in the headspace gas (Španěl and Smith, 1996). Essential for this SIFT-MS analytical method is the kinetics database of the reactions of H_3O^+, NO^+ and O_2^+ constructed from detailed studies of the reactions of these ions with a wide variety of compounds (Španěl and Smith, 1996; Španěl and Smith 1999b). The precur-

sor ions for SIFT-MS are chosen to be unreactive with the major components of air, to make it possible to detect trace gases against the high concentrations of gases such as nitrogen, oxygen, and carbon dioxide.

The caps of the bottles containing the rumen gas were punctured with a needle connected directly to the inlet port of the SIFT-MS. Mass spectra were then recorded with H_3O^+ precursor ions to detect and analyze the composite molecules in the sample. Each mass spectrum was acquired for 30 s in these analyses. During the sampling time, the volume of the plastic bottles reduced, thus maintaining the sample gas at atmospheric pressure. This results in a constant sample flow rate, as is required for simple and accurate analysis. The mean water vapor concentration in the headspace is routinely measured in these analyses. Small variations of the water vapor concentration between the samples were evident due to small variations in the temperatures of the samples. All these analyses were carried out at room temperature.

Statistical Analysis

The effects of time of sampling (0930, 1130, and 1530 h) on the series of measures made using samples of rumen liquor and rumen headspace gas were analyzed using analysis of variance (Genstat 5 for Windows; release 4.1; Lawes Agricultural Trust, 1998). A treatment structure of 'sampling time' and a blocking structure of 'cow' were used. Simple linear regression (Genstat 5 for Windows; release 4.1; Lawes Agricultural Trust, 1998) was used to explore relationships between concentrations of gases in rumen headspace with compounds measured in rumen liquor.

RESULTS

The results of analysis of feed samples are given in Table 1. The grass silage was of relatively poor fermentation characteristics with high levels of ammonia and relatively low concentrations of lactic acid. The statutory declaration for the mineral/vitamin mixture was (per kg): Ca: 180 g; P: 110 g; Mg: 50 g; Na: 80 g; Mn: 5 g; Zn: 4 g; Fe: 2.75 g; Cu: 1.8 g; I: 0.4 g; Co: 0.2 g, Se: 20 mg; vitamin A: 350,000 IU, vitamin B12: 1200 μg; vitamin D3: 60,000 IU, and vitamin E: 1000 IU. The mineral/vitamin mixture contained only low levels of sulfur (0.32% of DM).

Table 2 shows the change in rumen pH, the concentrations and molar proportions of VFA, and the concentration of ammonia in samples of rumen liquor taken just after each collection of rumen gas. There was a tendency for rumen pH to increase and a significant ($P < 0.05$) decline in the concentration (mmol/L) of propionic acid in rumen (24.6, 21.5, and 18.7; SED = 1.52). Figures 1 to 6 show mean concentrations of various gases (with SEM) in rumen headspace gas. The decline in concentrations (mg/kg) of hydrogen sulfide (204, 108, 30; SED = 16.6; $P < 0.001$), methyl sulfide (16.6, 4.0, 0.8; SED = 3.29; $P < 0.05$) and dimethyl sulfide (**DMS**; 11.7, 2.0, 0.5; SED = 2.83; $P < 0.05$) were statistically significant. No other effects of sampling time attained statistical significance. Figure 7 shows the VFA in rumen gas, expressed as molar percentages and with isomers of butyric acid and valeric acid combined.

The relationship between the concentration of ammonia-N (mg/L) in rumen liquor and the concentration (g/kg) of sulfides in rumen headspace gas is shown in equations 1 to 3.

Rumen ammonia-N (mg/L) = 31.4 + 0.355 × hydrogen sulfide in headspace gas (mg/kg)

$$n = 9;\ r^2 = 0.25;\ \text{residual SD} = 40.4;\ P < 0.1. \quad (1)$$

Rumen ammonia-N (mg/L) = 39.3 + 4.54 × methyl sulfide in headspace gas (mg/kg)

Table 1. Chemical analysis of the feeds used in this work (% of DM, unless stated otherwise).

	Grass silage	Concentrates
As-fed DM, %	25.7	88.0
OM	92.2	92.0
CP	13.4	21.9
NDF	58.0	25.1
ADF	35.1	12.2
Starch	ND	29.1
Water-soluble carbohydrates	ND	9.7
(Acid-hydrolysis) ether extract	2.9	(6.1)
Total sulfur	0.18	0.37
pH	4.3	ND
Lactic acid	4.8	ND
Acetic acid	1.9	ND
Butyric acid	0.6	ND
Ammonia-N, % of total-N	17.9	ND

[1]ND, not determined.

Table 2. Effect of time after feeding on rumen pH, and the concentrations of ammonia, and VFA in rumen liquor.

	Sampling time				
	0930 h	1130 h	1530 h	SED	*P*
Rumen pH	6.40	6.50	6.67	0.076	†
Total VFA, mmol/L	93.7	80.6	78.9	6.89	NS
Acetic acid, molar %	53.9	52.1	55.6	1.97	NS
Propionic acid, molar %	26.2	26.7	23.8	1.03	†
Butyric acid, molar %	15.6	16.9	17.1	0.83	NS
Valeric acid, molar %	4.3	4.4	3.5	0.36	NS
Ammonia-N, mg/L	114	57	44	33.7	NS

†$P < 0.1$.

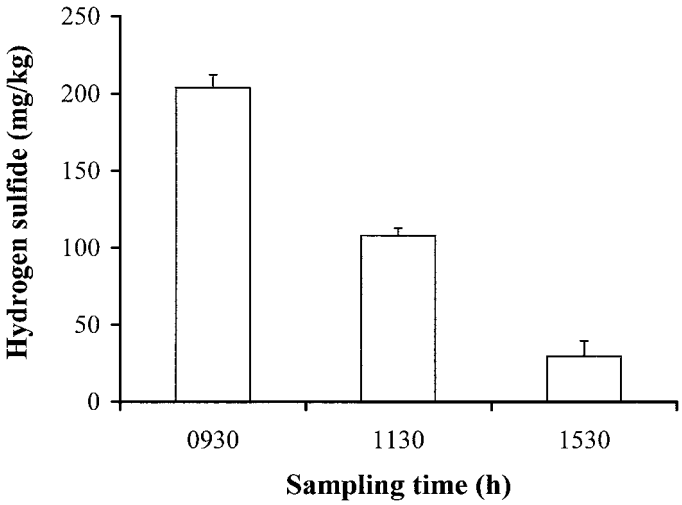

Figure 1. Effect of interval after feeding on the concentration of hydrogen sulfide in rumen headspace gas (means and SEM).

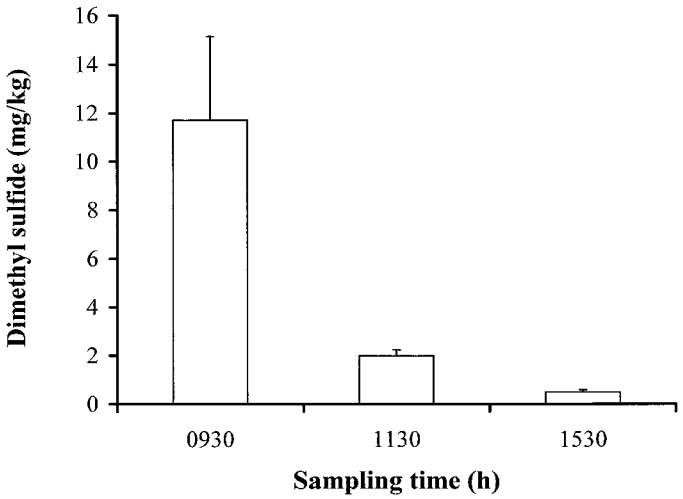

Figure 3. Effect of interval after feeding on the concentration of dimethyl sulfide in rumen headspace gas (means and SEM).

$$n = 9;\ r^2 = 0.54;\ \text{residual SD} = 31.7;\ P < 0.05. \quad (2)$$

Rumen ammonia-N (mg/L) = 45.3 + 5.59 × dimethyl sulfide in headspace gas (mg/kg)

$$n = 9;\ r^2 = 0.46;\ \text{residual SD} = 34.2;\ P < 0.05. \quad (3)$$

DISCUSSION

This approach enabled the simultaneous determination of all of the major components of rumen headspace gas, with the exception of inert gases such as methane, carbon dioxide, and nitrogen. Sulfides were the major constituents of rumen gas (up to 200 mg/kg), and other minor components such as VFA could be measured down to 20 μg/kg.

Duplicate bottles of gas were collected from each cow at 1130 and 1530 h, and there was reasonable agreement between duplicates in the case of hydrogen sulfide (r = 0.92), methyl sulfide (r = 0.79), DMS (r = 0.87), and propionic acid (r = 0.83). The agreement for other gases was poorer, and so further work will need to refine sampling techniques. It is likely that this variation reflects the relatively short sampling interval used in this work and the dynamic nature of the rumen headspace. These problems would be reduced when uti-

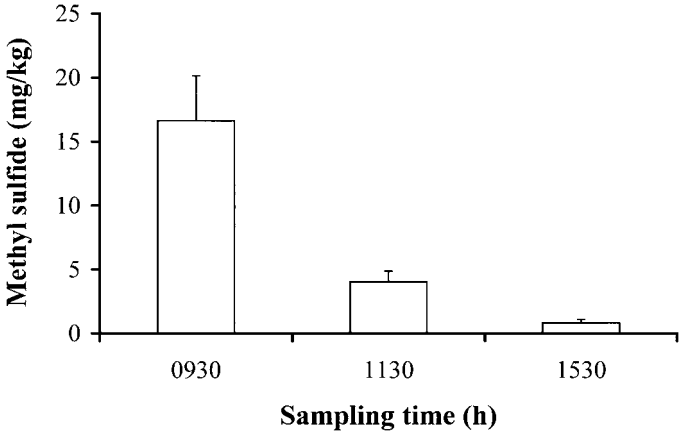

Figure 2. Effect of interval after feeding on the concentration of methyl sulfide in rumen headspace gas (means and SEM).

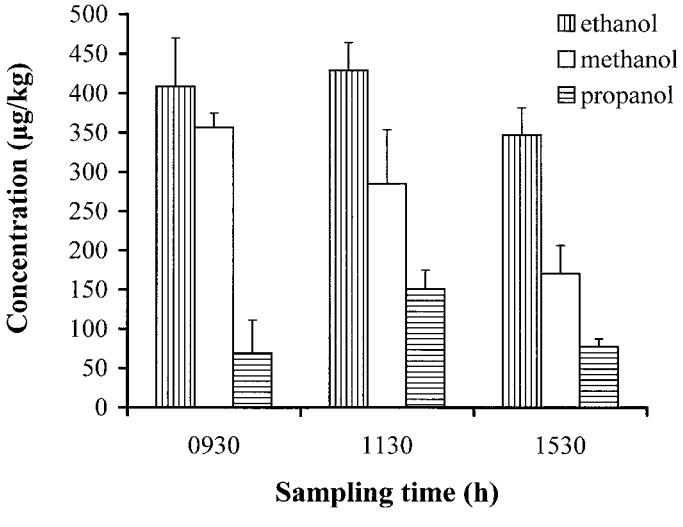

Figure 4. Effect of interval after feeding on the concentrations of methanol, ethanol, and propanol in rumen headspace gas (means and SEM).

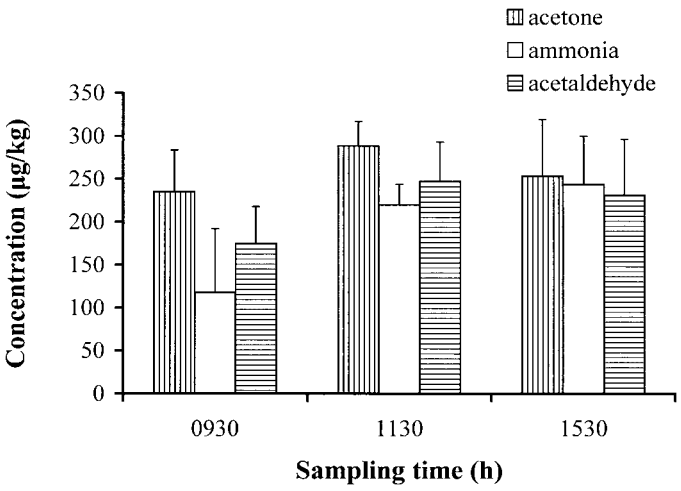

Figure 5. Effect of interval after feeding on the concentrations of acetone, ammonia, and acetaldehyde in rumen headspace gas (means and SEM).

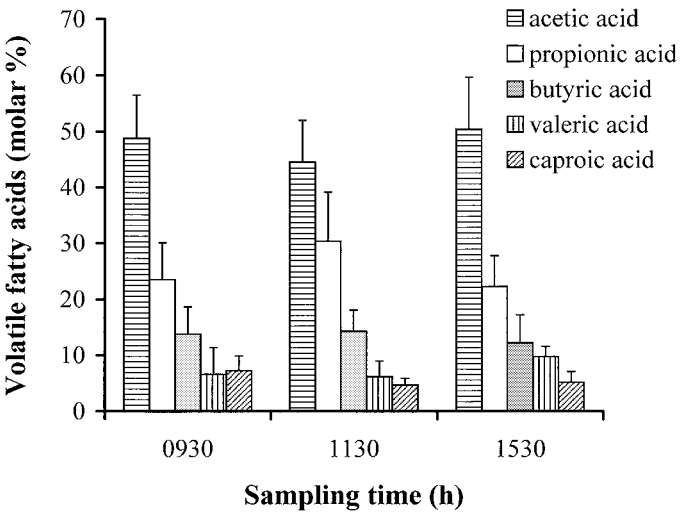

Figure 7. Effect of interval after feeding on the molar % of VFA in rumen headspace gas (means and SEM).

lizing the full potential of this technique for continuous on-line monitoring. Indeed, the fact that rumen gases reflect processes within the entire reticulo-rumen means that this approach might overcome some of the sampling problems relating to compartmentalization and variation across the rumen (Garrett et al., 1999).

Sulfur compounds, hydrogen sulfide, methyl sulfide, and DMS, were the predominant gases in the rumen headspace. The concentrations of each of these sulfur compounds mirrored ammonia concentrations measured in rumen liquor (equations 1 to 3). Sulfur amino acids contain over 90% of the sulfur in plant material

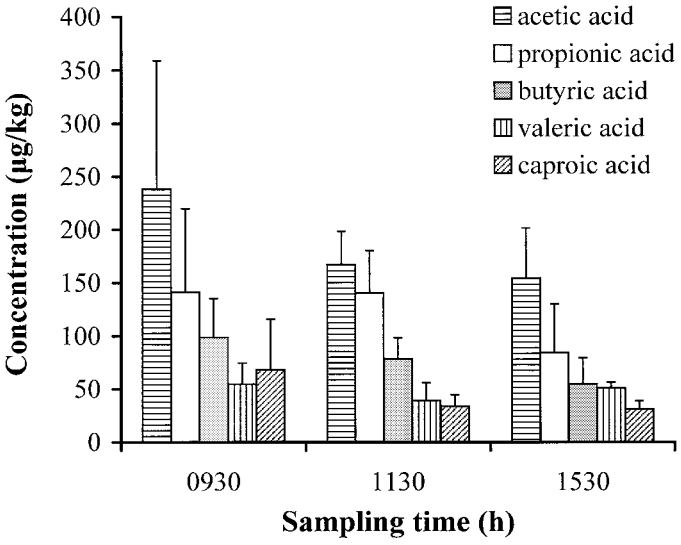

Figure 6. Effect of interval after feeding on the concentrations of VFA in rumen headspace gas (means and SEM).

(Havlin et al., 1999), so it is possible to envisage the development of a new approach to studying the dynamics of protein degradation utilizing this observation.

The concentrations of hydrogen sulfide in rumen gas in the current work were similar to those reported by Gould et al. (1997) for steers fed diets containing normal levels of sulfur. However, neither Elliott-Martin et al. (1997) or Mottram et al. (2000) found hydrogen sulfide above 2 mg/kg (the noise level of the infrared analyzer) in expired breath, confirming that much hydrogen sulfide is absorbed via the lungs and detoxified (Bird, 1972). Dimethyl sulfide is an interesting gas because of its involvement as an inverse greenhouse gas (Bates et al., 1987). These results confirm the significant levels of production of DMS by the rumen (Mottram et al., 2000; Williams et al., 1999), as well as the pattern of higher levels of DMS production immediately after feeding (Williams et al., 1999). Although levels of DMS were 10-fold lower than those of hydrogen sulfide in rumen gas, only DMS was detected in cows' breath (Mottram et al., 2000).

Ammonia concentrations in rumen headspace gas varied in the opposite direction to the concentration of ammonia within rumen liquor ($r = -0.59$; $P < 0.1$). The range of rumen pH encountered in the current work was quite narrow (6.37 to 6.77), and not sufficient to establish statistically significant effects in the regression analysis. However, it seems likely that rumen pH is more important than ammonia concentration in affecting the concentration of ammonia in rumen headspace gas. The pK_a of ammonia is 9.02 at 37°C, which means that there is a 10-fold increase in the proportion of ammonia present as NH_3 as pH moves from 6 to 7

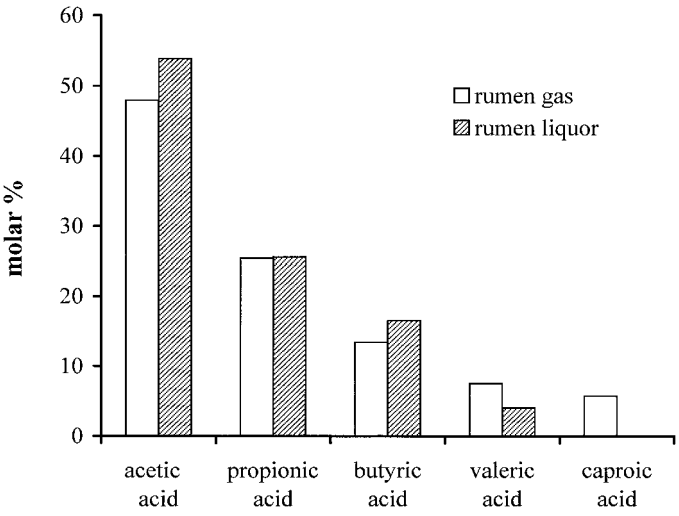

Figure 8. Comparison of mean molar % of VFA in corresponding samples of rumen gas and rumen liquor.

(Lewis and Buttery, 1973). This likely exerts a much greater influence on ammonia concentrations in rumen gas than the variation in ammonia concentration within rumen liquor. This relationship suggests that ammonia concentrations in rumen gas will be very low below pH 6. This observation might be very useful in the diagnosis of subacute rumen acidosis when identification of periods of time when rumen pH falls below 6 is of considerable interest (Mackie and Gilchrist, 1979). This work could be taken forward in practical applications, for example, by studying concentrations of ammonia on cows' breath.

Concentrations of VFA were subject to considerable variability and continuous on-line measurements would be required to give more reliable measurements. Nonetheless, it is interesting that the overall molar proportions of VFA were very similar between rumen liquor and rumen gas. The relative concentrations of VFA in rumen gas and rumen liquor changed with increasing chain length (Figure 8). There was a slightly lower relative concentration of acetic acid in rumen gas, compared with rumen liquor, and rumen gas contained disproportionately more valeric acid. Caproic acid was present in significant amounts in rumen headspace gas, though it was below detection limits in rumen liquor.

This work has shown SIFT-MS to be useful for identifying a wide range of interesting gases in the rumen headspace. It could be used in conjunction with breath sampling (Mottram et al., 2000) to provide noninvasive descriptions that will be useful in evaluating new hypotheses about proteolysis and fermentation in the rumen.

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