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AMMONIA, pH AND VOLATILE FATTY ACIDS IN THE BOVINE RUMEN AFTER FEEDING WITH HAY*

By

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JENSEN, KURT: *Ammonia, pH and volatile fatty acids in the bovine rumen after feeding with hay.* Acta vet. scand. 1975, *16*, 258—268. — The effect of feeding level and interval between feedings on the fermentation pattern in the bovine rumen have been investigated in experiments with hay. The animals were completely adapted heifers fitted with rumen cannulas, and the parameters measured were ammonia, pH, volatile fatty acids (VFA) and non-glucogenic/glucogenic ratio (NGGR) in the VFA mixture.

Increasing feeding levels, ranging from 2/3 to 4/3 of maintenance level, resulted in higher average concentrations of VFA and lower pH in the rumen fluid. Further the highest level of intake caused a considerable diurnal variation in the pH and the concentration of total VFA, and increased the variation in the molar composition of the VFA mixture.

Three feeding intervals ranging from 8 to 16 hrs., with hay administered at maintenance level, caused no changes in the fermentation pattern.

Typical variations in the concentrations of ammonia and valeric acid as related to time after feeding were demonstrated, but the concentrations of the branched-chain fatty acids in the rumen fluid were found to be quite constant.

It may be concluded that a representative mean value of the parameters measured, except for ammonia and valeric acid, may be based on relatively few samples when feed intake does not exceed maintenance level, whereas sampling every hour is required at higher feeding levels.

VFA; pH; ammonia; cows; hay feeding.

During the last decades, dietary physiologists, microbiologists and biochemists have carried out comprehensive research into the fermentation processes in ruminants. The reticulo-ruminal

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flora and fauna are able to break down cellulose and hemicellulose into volatile fatty acids (VFA) and to incorporate non-protein nitrogen (NPN) in the bacterial cell protein, this being the fundamental basis of the symbiosis between microbes and host. In order to obtain maximum food utilization, it is important to be able to manipulate with the fermentation processes.

It appears from the literature, in general, that the pH in the rumen decreases and that the concentration of total VFA increases as the feeding level is raised (e.g. *Bailey & Balch* 1961, *Bath & Rook* 1963, *Rumsey et al.* 1970). It has been found that the distribution of the individual VFA's is also related to the feeding level (*Terry & Tilley* 1963, *Fenner et al.* 1967). However, the results published previously are not in accordance as regards the effect of various feeding levels.

It is usually assumed that hay, as a feedstuff with a high content of cell wall matter, is fermented slowly and has an only slightly varying fermentation curve. However, as the resulting total VFA levels which have been published seem to be rather different, it would appear to be useful to follow the fermentation pattern by means of frequent observations in the period between the feedings.

The aim of the present study is to describe the fermentation pattern in the rumen of hay-fed cattle under the influence of variations of rations and feeding intervals. On the basis of the determination of the diurnal variation, an attempt is made to establish how often samples should be taken for analysis in order to achieve a reasonably certain indication of the daily mean.

MATERIALS AND METHODS

Animals and feeds

The study comprised 3 Jersey heifers (A, B and C) fitted with Balch rumen cannulas. The average weight of the animals was 325 kg. For 6 months previous to the experiment the heifers were fed exclusively with hay (mainly rye-grass hay) of the same lot as that used during the study, in order to ensure complete adaptation. In this period, the daily ration was 6 kg hay supplemented with minerals and vitamins, administered in 2 equal portions, and free access to water was allowed. Analysis of the experimental feed showed a dry matter content of 91.7 %. The

composition of the dry matter was 15.9 % crude protein, 2.6 % crude fat, 32.5 % crude fibre, 42.6 % N-free extracts and 6.4 % crude ash. The gross energy content was 4.423 kcal. per kg dry matter, and the content of structural carbohydrates determined according to the method of *Goering & Van Soest* (1970) was, per kg dry matter: 678 g cell wall matter, consisting of 336 g cellulose, 303 g hemicellulose and 39 g lignin.

Experimental plan

Experiment I was performed in order to elucidate the effect of the feeding interval on the fermentation. The intervals between the experimental feeding and the previous feeding were 8, 12, and 16 hrs., and the daily ration consisted of 6 kg hay plus minerals and vitamins fed in 2 equal portions. The feed intensity corresponded to maintenance level.

The effect of feeding level on the fermentation was examined in experiment II, where the daily intake was 4, 6 and 8 kg, which corresponded to 2/3, 3/3 and 4/3 of maintenance level, respectively. The feeding interval was 12 hrs.

In both experiments, samples of rumen fluid were taken each hour immediately before and until 12 hrs. after feeding, i.e. a total of 13 samples per animal. In the period with feeding at 4/3 of maintenance level, the sampling from heifers A and B was prolonged for a further 12 hrs., while heifer C unfortunately was omitted. In both experiments, no samples were taken until at least 1 week after change in treatment.

Sampling and preparation

Samples of about 200 ml rumen fluid were taken from the middle of the rumen by gentle suction through a perforated tube (*Hungate* 1950). After addition of 10 ppm $HgCl_2$ the samples were cooled in running tap water, to ensure immediate stoppage of the development of gas in the samples.

After centrifugation at 3.000 $\times$ g for 30 min. the supernatant was taken into use or stored at —20°C before analysis.

Hydrogen ion activity

The pH was determined in situ by dipping a glass-calomel combination electrode through the sampling tube immediately before sampling in order to eliminate the uncertainty in meas-

uring on samples outside the rumen because of changes in the CO_2 pressure (*Lampila* 1955). The electrode was rinsed in distilled water and placed in buffer between the determinations. By this procedure the electrode did not show reduced durability, in contrast to permanently fitted electrodes (*Brems* 1962).

Ammonia

The concentration of volatile base was determined titremetrically after distillation of rumen fluid in borate buffer at pH 9.2 (*Petersen* 1965). On account of the rather laborious procedure, the results given are the mean values of pooled samples from the heifers.

Volatile fatty acids

The content of VFA in the rumen fluid, including acetic, propionic, butyric, isobutyric, valeric and isovaleric acids, was determined by gas-solid absorption chromatography, as described previously (*Jensen* 1973, 1974).

Non-glucogenic/glucogenic ratio

The non-glucogenic/glucogenic ratio (NGGR) from the VFA mixture is defined on molar basis as follows:

$$\frac{\text{acetic acid} + 2 \times \text{butyric acid} + \text{valeric acid}}{\text{propionic acid} + \text{valeric acid}}$$

For full validity of the ratio, it may be assumed that the rates of absorption are equal for the individual acids. The formula is identical with the glucogenic ratio proposed by *Ørskov* (1975).

RESULTS

Experiment I

The effect of the feeding interval on the fermentation was assessed at intervals of 8, 12 and 16 hrs. with a feed intake at maintenance level. The results expressed as daily means of each animal are shown in Table 1. The average pH was 6.5 and various feeding intervals did not cause any significant deviations from this, and the treatment did not give any significant differences in the daily mean concentrations of total VFA.

Table 1. Influence of feeding interval on the average pH and on the average concentrations of ammonia and volatile fatty acids in rumen fluid with feeding at maintenance level. (n = 13). Experiment I.

Feeding interval hrs.	Heifer	Ammonia mmol/l (mean of A, B and C)	pH	Total VFA mmol/l	Molar percentage of total VFA						NGGR
					acetic acid	propionic acid	butyric acid	isobutyric acid	valeric acid	isovaleric acid	
	A		6.57	90.2	76.7	16.0	5.7	0.49	0.34	0.77	5.41
8	B	5.73	6.40	102.3	76.1	17.1	5.4	0.39	0.42	0.62	4.98
	C		6.34	88.4	76.9	17.0	5.1	0.32	0.42	0.34	5.02
	A		6.40	96.3	75.8	17.1	5.3	0.42	0.56	0.83	4.92
12	B	6.64	6.70	89.1	74.5	17.6	5.6	0.75	0.52	0.98	4.76
	C		6.42	97.6	75.0	17.6	5.7	0.44	0.68	0.65	4.76
	A		6.54	83.2	75.2	17.4	5.5	0.61	0.66	0.55	4.81
16	B	5.37	6.59	92.9	75.0	16.4	6.3	0.85	0.70	0.85	5.16
	C		6.34	84.1	78.3	16.0	4.9	0.20	0.50	0.14	5.37
Overall mean		5.91	6.47	91.5	75.9	16.9	5.5	0.50	0.53	0.64	5.02
± s.e.m.		0.73	0.014	0.82	0.12	0.06	0.04	0.021	0.032	0.027	0.082

In the experiment, the diurnal variation of pH was small and unsystematic (0.1—0.4 pH units) independent of time after feeding. The concentration of total VFA also varied only slightly and inconstantly during the 3 feeding intervals, with a diurnal variation range of 10—20 mmol/l rumen fluid.

The molar distribution of the VFA mixture is also shown in Table 1. No significant differences were found neither between the treatments nor between the individual animals, and, except for valeric acid, the diurnal variation was very small and unsystematic. Expressed as molar percentage, the range of variations were 1.4 % C_2, 0.6 % C_3, 0.3 % C_4, 0.3 % iC_4, 0.9 % C_5 and 0.4 % iC_5. The valeric acid showed a considerable diurnal pattern of variation, the maximum content of 1.2 molar percentage being found 2—4 hrs. after feeding, followed by a decrease to a level of 0.3 %. Since the molar composition otherwise varied insignificantly, the NGGR was quite constant, and the ratio was in all samples about 5.

The daily mean concentration of ammonia was concordant for all feeding intervals (Table 1). The diurnal pattern of concentration showed a significant change as a function of time with a peak value of 14 mmol/l 1—3 hrs. after feeding, followed

Table 2. Influence of feeding level on the average pH and on the average concentrations of ammonia and volatile fatty acids in rumen fluid with feeding interval of 12 hrs. (n = 13). Experiment II.

Feeding level, part of maintenance	Heifer	Ammonia mmol/l (means of A, B and C)	pH	Total VFA mmol/l	Molar percentage of total VFA						NGGR
					acetic acid	propionic acid	butyric acid	isobutyric acid	valeric acid	isovaleric acid	
	A		6.75	67.8	74.7	16.3	5.7	1.19	0.55	1.60	5.14
2/3	B	5.67	6.79	67.0	74.2	17.0	6.0	1.09	0.48	1.30	4.96
	C		6.60	78.4	74.5	17.4	6.0	0.98	0.41	0.92	4.88
	A		6.40	96.3	75.8	17.1	5.3	0.42	0.56	0.83	4.92
3/3	B	6.64	6.70	89.1	74.5	17.6	5.6	0.75	0.52	0.98	4.76
	C		6.42	97.6	75.0	17.6	5.7	0.44	0.68	0.65	4.76
4/3*	A		6.48	93.6	74.3	17.0	6.1	0.91	0.65	1.13	4.94
	B	5.78	6.51	96.1	74.4	17.4	5.8	0.76	0.83	0.86	4.76

* n = 25.

by a decreased concentration to a low level of about 2 mmol/l rumen fluid.

Since no significant difference could be referred to feeding intervals, all the results were used in the calculation of an overall mean and standard error for each of the actual parameters.

Experiment II

The influence of the feeding level on the fermentation pattern is given in Table 2, which shows the daily means for each animal. A feeding level of 2/3 of maintenance caused a significantly lower concentration of total VFA ($P < 0.001$) and higher pH ($P < 0.001$) than maintenance level, while no differences were found between maintenance level and 4/3 of maintenance level of intake. The daily means of the molar composition of the VFA mixture, the NGGR and the ammonia concentration were unaffected by the experimental treatment.

With the exception of valeric acid and ammonia, the diurnal fermentation pattern for the parameters measured varied only little and inconstantly at the 2 lower feeding levels. Otherwise, the level of intake at 4/3 of maintenance level showed a considerably varied fermentation pattern for all the parameters. This can be seen from Fig. 1, which shows the pH, the total VFA and the ammonia concentration in the rumen fluid throughout

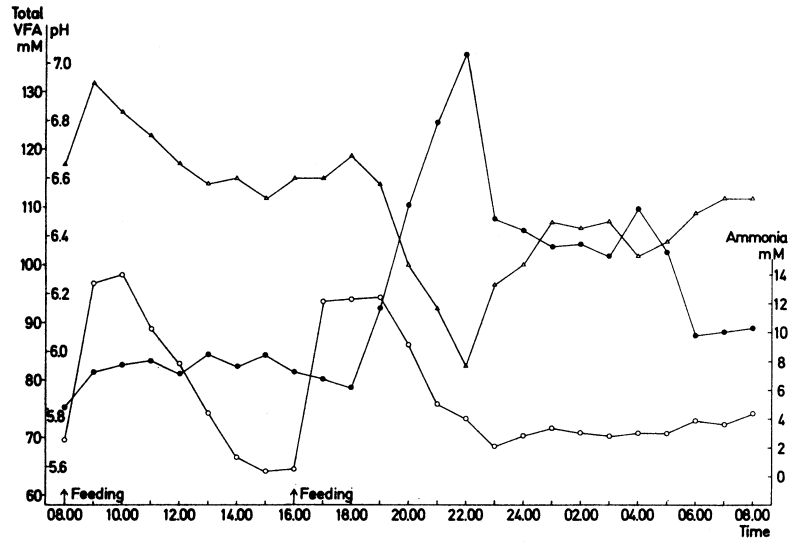


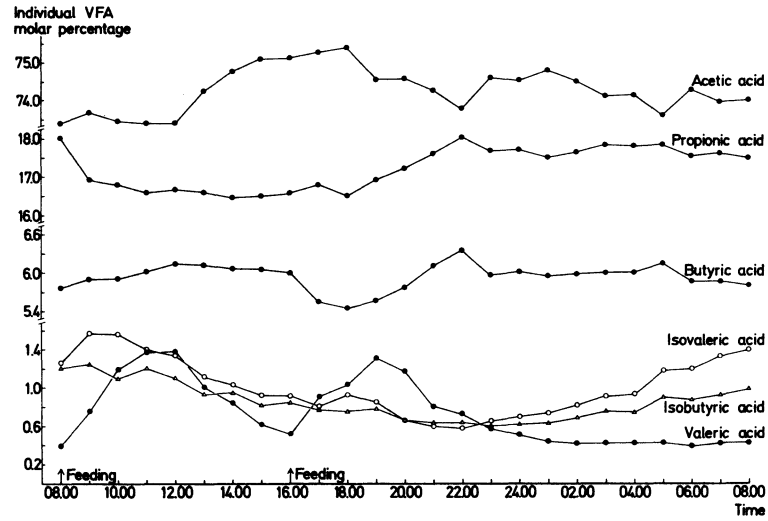
Figure 1. Diurnal variation in rumen pH (△——△), concentration of total volatile fatty acid (●——●) and ammonia (o——o). Experiment II, feeding level 4/3 of maintenance.

24 hrs. The pH was inversely correlated to the concentration of total VFA ($r = -0.91$). Fig. 2 shows the diurnal variation in the molar composition of the VFA mixture. As regards the main acids, viz. acetic, propionic and butyric acids, a remarkable change during the first 6 hrs. after the afternoon feeding was noted when the extent of the fermentation increased. The proportion of acetic acid decreased, while propionic and butyric acids increased. The NGGR varied from 5.13 to 4.64, and thus the hay was strongly non-glucogenic also in the period of increased fermentation rate.

In both experiments, the valeric acid showed identical patterns of variation, and a close correlation with the ammonia concentration was demonstrated ($r = 0.77$). The concentrations of isobutyric and isovaleric acids were remarkably constant, about 0.6 and 0.7 mmol/l rumen fluid, respectively, independent of the level of intake.

DISCUSSION

When hay was fed at maintenance level, differences in feeding intervals caused only inconsiderable changes in the fermentation pattern, and no significant differences could be demon-



F i g u r e 2. Diurnal variation in the molar composition of the volatile fatty acid mixture. Experiment II, feeding level 4/3 of maintenance.

strated between the means obtained. The parameters used, with the exception of valeric acid and ammonia, showed no systematic variations in relation to the feeding time. It is thus clear that hay is fermented slowly and shows a stable fermentation curve.

The overall means obtained cannot be compared directly with figures reported by other workers, since the results are dependent on amounts of feed administered, chemical composition, and sampling frequency. However, in similar experiments *Balch & Rowland* (1957) found a molar composition of the VFA mixture of 68.9 % C_2, 17.3 % C_3 and 9.9 % C_4. *Sutton & Johnson* (1969) found 68.3 % C_2, 17.8 % C_3 and 12.1 % C_4, while the results in the present study were 75.9 % C_2, 16.9 % C_3 and 5.5 % C_4. On the basis of the relations of acetic acid in the total VFA of rumen fluid to the content of structural carbohydrate in the diet, presented by *Bath & Rook* (1963), the batch of hay used should give 71 molar percentage acetic acid. Thus the hay was very acetogenic, whereas the molar percentage of butyric acid was only about half of that usually found, but the proportion of propionic acid was consistent with the results mentioned above.

Using hay as feedstuff the effect of feeding level on pH and on concentrations of VFA has been demonstrated previously by *Bailey & Balch* (1961), *Bath & Rook* and *Fenner et al.*

(1967); they found that increased level of intake resulted in higher mean concentration of VFA and in lower pH. This relationship was confirmed in the present study, but, as in *Bath & Rook's* examinations, a change in the feeding level from maintenance did not cause significant changes in the daily means. The explanation could be that after maximum filling of the rumen, the passage rate increases proportional to the intake of dry matter. Since hay is fermented slowly, the concentration of VFA cannot increase further, in contrast to what takes place in feeding with easily fermentable feedstuffs. The high dependance of the hydrogen ion activity on the total concentration of VFA was apparent from the data shown in Fig. 1. This has not been reported previously in connection with hay-feeding alone.

Bath & Rook found no changes in the daily mean molar composition of the VFA mixture in relation to the feeding level; this has been confirmed by the present study. However, the experiments with feeding at 4/3 of maintenance showed that the diurnal variation in the composition of the VFA mixture increased (Fig. 2), though without causing significant alteration in the mean values. *Terry & Tilley* (1963) showed in an experiment with sheep fed on hay that the feeding level can considerably change the mean molar composition, particularly when hay of poor quality is used. This should be borne in mind when few samples form the basis of a daily mean.

The mean concentration of ammonia was not influenced by the feeding interval or the feeding level. However, the variation pattern showed strong dependence on the feeding time, as also found by *Fenner et al.* The mean molar proportion of valeric acid showed no systematic variation between treatments, whereas in both experiments the concentration changed significantly as a function of time after feeding. The diurnal variation patterns for valeric acid and ammonia were closely correlated, which indicates that similar processes are responsible for the actual concentrations of both of these products.

The concentration of branched-chain volatile acids in the rumen fluid was found to be quite constant and independent of feeding time. This would indicate that these acids result from deamination of amino acids in cell walls and/or decayed bacterial cells and protozoa.

It can be concluded that determination of the daily mean values for the fermentation, with the exception of ammonia and

valeric acid, can be based on a few samples taken from the rumen content, provided that feeding is carried out with a slowly fermentable feedstuff such as hay, and that feeding level does not influence the rate of fermentation. If determination of ammonia and valeric acid is required, or if the feeding level is so high that the concentration of VFA and the molar proportions are changed significantly between the feedings, samples should be taken every hour in order to achieve representative daily means for the composition of the rumen fluid.

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SAMMENDRAG

Ammoniak, pH og flygtige fedtsyrer i vommen hos kvæg fodret med hø.

Fodringsintensitetens og fodringsintervallets indflydelse på fermentationsmønstret i vommen hos kvæg er belyst ved forsøg med rajgræshø som fodermiddel. Der er anvendt fuldt adapterede permanent fistulerede kvier, og de undersøgte parametre har været ammoniak, pH, flygtige fedtsyrer (VFA) samt non-glucogen/glucogen ratio (NGGR) i VFA-blandingen.

Stigende fodringsintensitet, dækkende fra 2/3 til 4/3 af vedligeholdelsesbehovet, har medført højere gennemsnitskoncentration af VFA og lavere pH. Den højeste intensitet har forårsaget en betydelig daglig variation i vomvæskens pH og koncentration af VFA samt øget variationen af VFA-blandingens molære sammensætning.

Tre fodringsintervaller med en fodertildeling svarende til vedligeholdelsesniveauet har ikke medført ændringer af fermentationsmønstret.

Der er påvist et typisk mønster for variationen af ammoniak- og valerianesyrekoncentrationen i relation til fodringstidspunktet, hvorimod koncentrationen af begge de forgrenede fedtsyrer er fundet ret konstant.

Det konkluderes, at selv få prøver udgør et tilstrækkeligt sikkert grundlag for beregning af gennemsnitsværdier for de anvendte parametre bortset fra ammoniak og valerianesyre, når fodringsintensiteten ikke overstiger behovet for vedligehold. Ønskes koncentrationskurverne for ammoniak og valerianesyre bestemt mellem fodringerne, eller fodringsintensiteten overstiger vedligeholdelsesniveauet, bør prøver udtages hver time for at opnå repræsentative gennemsnitsværdier for vomvæskens sammensætning.

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