STUDIES ON THE VOLATILE FATTY ACIDS IN THE RUMEN OF THE GOAT I. INFLUENCE OF FEED INGREDIENTS

STUDIES ON THE VOLATILE FATTY ACIDS IN THE RUMEN OF THE GOAT*

I. INFLUENCE OF FEED INGREDIENTS

By

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Introduction

Large amounts of volatile fatty acids (VFA) are produced by bacterial action in the rumen, and represent an important source of energy to the host. The total concentrations of VFA in the rumen liquor, and the amounts of the individual acids present, are dependent on the composition of the ration and the feeding regime. In previous reports, we confirmed, in general, that the amounts of VFA in the rumen are variable depending on the nature of diet and the time after feeding (1—4). Considerable information on the rumen VFA has accumulated during recent years, but literature on the exact relationship between the composition of rumen VFA and the feed ingredient is scanty. The present study is an attempt to determine the influence of feed ingredients upon the composition of VFA.

Experimental

1. Determination of VFA by paper partition chromatography.

Samples were taken from Saanen goats having permanent rumen fistulae by aspiration through the catheter and centrifuged at 3,000 r.p.m. for 10 min. To 50 *ml* of the supernatant was added 10 *ml* of N/10 – H_2SO_4 and then steam distilated. The distilate was neutralized with aqueous NaOH and evaporated to dryness under reduced pressure. The Na salts were prepared for chromatography and analyses were carried out as described by Jones (5). Spots that appeared on the chromatogram were compared with the spots of authentic formic, acetic, propionic, n-butyric, iso-butyric, n-valeric and iso-valeric acid. Fig. 1 shows the paper chromatogram of the standard solution and the rumen liquor sample.

* The Influence of Feed and Feeding upon the Ruminal Gas Formation. Part XII.

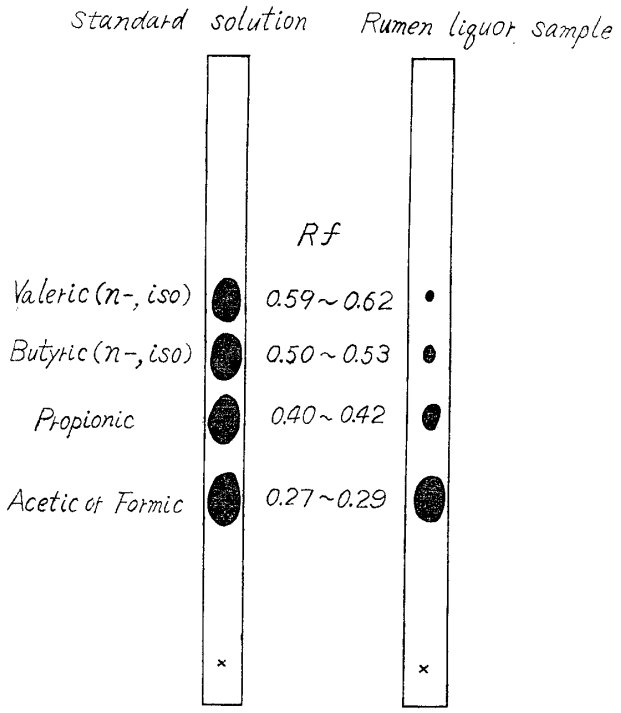

Fig. 1. Paper chromatogram of VFA.

Rf values of both acids formic and acetic, were 0.27—0.29; the mixed solution of formic and acetic acid gave only one spot. Then qualitative analysis by spot test of formic acid was carried out with chromotropic acid (6). Authentic formic acid was detectable by this test even with such a low concentration as 1 : 10,000, but the steam distilate of rumen liquor gave no reaction. On the other hand, Krüger and Tschirch's detective test (6) showed the presence of acetic acid in the distilate of the rumen liquor. *In vitro* experiment, Hungate (7) confirmed that a large quantity of formic acid was produced by rumen bacteria. But, the rapid disappearance of formate in the rumen liquor after the administration of sodium formate to a fistulated goat (8) suggested that although formic acid may arise as an intermediate in carbohydrate fermentation in the rumen, it is unlikely to accumulate to a detectable concentration.

2. Determination of VFA by column partition chromatography.

This was done as described by Belasco (9) using a 40 cm column with an internal diameter of 20 mm. Silica gel from Mallinckrodt's silicic acid served as the partitioning agent. Twenty five grams of freshly retreated silica gel were packed in the column. One hundred and fifty *ml* of benzene, 150 *ml* of 1 per cent n-butanol benzene and 300 *ml* of 5 per cent n-butanol benzene were used as eluting solvents. Standard 0.005 N-NaOH (carbonate free) was used to titrate the amount of acid flowing from the column. Flow rate was regulated at ca. 100 *ml*/hr by pressure controlling. Every 10 *ml* eluate was collected by

a fraction collector and titrated in CO_2 free atmosphere. Fig. 2 shows the typical examples of column partition chromatogram of VFA from the mixed solution of authentic acids, acetic, propionic and butyric, together with VFA from the rumen liquor sample. Table 1 shows the recovery percentage of each authentic acid.

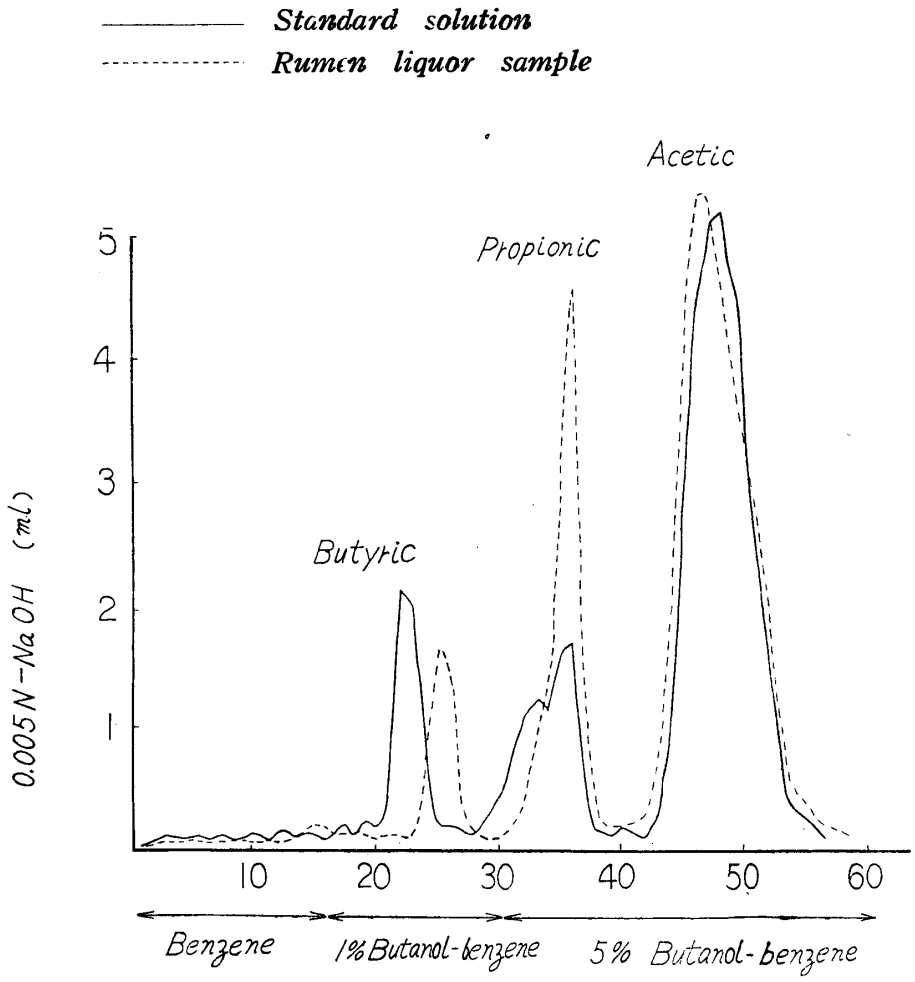

Fig. 2. Silica gel chromatogram of VFA.

Table 1. Recovery test of VFA with silica gel chromatography

VFA	Added (mM)	Recovered (mM)	Recovery (%)
Acetic	0.1445	0.1321 ± 0.0068	94.9 ± 0.1
Propionic	0.0341	0.0352 ± 0.0005	103.2 ± 1.4
Butyric	0.0230	0.0248 ± 0.0001	107.2 ± 0.7

(Mean value and standard deviation)

3. Animals and diets.

Four female goats with rumen fistulae, weighing 32—35 kg, were used in the present work. Each animal had been maintained on a daily ration which consisted of 150 g wheat bran, 150 g barley, 500 g hay at 1 p.m. and 300 g hay at 4 p.m..

One group, goat No. 5 and No. 6, were used from November to December of 1958 (Exp. 1). The other group, goat No. 11 and No. 12, were used from December 1959 to January 1960 (Exp. 2).

The proximate analysis of feeds are given in Table 2. The difference of the crude fiber contents of hay between Exp. 1 and Exp. 2 is remarkable. Calculated amounts of feed ingredients per head per day are given in Table 3.

Table 2. Proximate analysis of feeds used in the experiment (%)

Experiment / Feed / Ingredient	Exp. 1			Exp. 2		
	Nov. 1958~Dec. 1958			Dec. 1959~Jan. 1960		
	Wheat bran	Barley	Hay	Wheat bran	Barley	Hay
Moisture	13.82	15.33	13.74	15.26	14.17	11.50
Crude protein	15.52	11.62	9.55	15.16	11.08	8.21
Crude fat	4.07	1.69	3.74	4.95	2.20	1.59
Nitrogen free extract	54.23	63.44	36.74	52.30	64.67	35.53
Crude fiber	7.72	5.84	26.58	8.41	5.32	36.07
Ash	4.64	2.08	9.65	4.28	2.56	7.10

Table 3. Feed ingredients supplied per head per day (g)

Experiment / Feed ingredient	Exp. 1	Exp. 2
Crude protein	117	105
Crude fat	39	23
Nitrogen free extract	470	460
Crude fiber	233	309
Ash	97	77
Dry matter	946	974

Results and Discussion

Variations of VFA concentration during the day time are shown in Fig. 3. The concentrations of VFA in the rumen liquor were comparatively low in Exp. 2. Molar percentage of VFA is given in Table 4. It seems that the molar percentage of VFA in the rumen liquor suffers little change during the day time. Each individual goat, fed on the same diet, shows almost an equal constitution

Table 4. Percentage composition of VFA found in the rumen liquor of goat at different times after feeding (on a molecular basis)

Experiment 1

Goat / VFA / Time	No. 5			No. 6		
	Acetic	Propionic	Butyric	Acetic	Propionic	Butyric
11 : 30	63.2	15.5	21.3	64.1	21.3	14.6
13 : 00	64.8	22.0	13.3	63.2	21.2	15.6
14 : 30	59.0	26.1	14.9	64.7	21.4	13.9
16 : 00	67.1	20.6	12.3	64.8	23.3	11.9
17 : 30	56.3	21.4	22.2	55.3	31.3	13.4
Mean	62.1	21.1	16.8	62.4	23.7	13.9
S.D.	± 4.4	± 3.8	± 4.6	± 4.0	± 4.3	± 1.4

Experiment 2

Goat / VFA / Time	No. 11			No. 12		
	Acetic	Propionic	Butyric	Acetic	Propionic	Butyric
8 : 30	—	—	—	71.7	18.9	9.4
10 : 00	72.2	19.6	8.2	71.6	19.1	9.4
11 : 30	70.2	20.8	8.9	70.6	19.4	10.0
13 : 00	71.0	21.0	8.0	69.0	18.5	12.5
14 : 30	67.9	23.7	8.5	69.2	20.9	9.9
16 : 00	69.6	22.3	8.1	67.6	23.0	9.5
17 : 30	66.4	25.0	8.7	68.6	23.8	7.6
19 : 00	70.1	22.1	7.9	—	—	—
20 : 30	70.2	21.9	7.8	—	—	—
Mean	69.7	22.1	8.3	69.7	20.5	9.8
S.D.	± 1.8	± 1.9	± 0.4	± 1.6	± 2.1	± 1.5

of VFA. The difference between Exp. 1 and Exp. 2 is remarkable in acetic acid percentage. The mean values of acetic acid percentage are 62.1 ± 4.4 and 62.4 ± 4.0 in the goats No. 5 and No. 6, respectively. In the cases of Exp. 2, the mean values of acetic acid percentage are 69.7 ± 1.8 and 69.7 ± 1.6 in the goats No. 11 and No. 12, respectively.

Molar percentage of propionic acid is nearly equal both in Exp. 1 and Exp. 2. But, the butyric acid percentage in Exp. 1 is larger than that of Exp. 2. Balch and Rowland (10) expressed the view that acetic acid percentage in the rumen liquor increases when the cow is fed on fiber rich hay. The results obtained in the present work may support their opinion. Phillipson (11) showed that starch rich feed lowers the acetic/propionic ratio in the rumen of the lamb, and El-Shazly (12) suggested that protein rich feed increases the molar proportion of butyric and the higher acids in the rumen of the sheep. From the

Table 5. Acetic/Propionic and Butyric/Propionic ratios in the rumen liquor of the goat

Experiment / Goat / Ratio	Exp. 1		Exp. 2	
	No. 5	No. 6	No. 11	No. 12
Acetic/Propionic	3.04±0.69	2.71±0.56	3.18±0.32	3.43±0.39
Butyric/Propionic	0.83±0.38	0.60±0.13	0.38±0.03	0.48±0.11

(Mean value and standard deviation)

data given in Table 4, our results are in agreement with that of Phillipson (11) and El-Shazly (12). Kandatsu *et al.* (13) observed that on feeding twice a day, there was a little variation in the molar percentage of the individual acids. The present study confirms their finding. But, the concentration of a particular acid at any one time is dependent on the rate of production in the rumen, absorption from the rumen, passage from the rumen to omasume, dilution with saliva, utilization by rumen microorganisms, and conversion to other rumen metabolites (14). And, it is conceivable that the compositions of rumen ingesta may vary in the course of time after feeding and the rates of production and absorption of the individual VFA may be different from each other. Although the total concentration of VFA in the rumen and the amounts of the individual acids present are dependent on the diet, the fact that the molar proportion of the rumen VFA does not change appreciably with time after feeding suggests the existence of some other controlling factors.

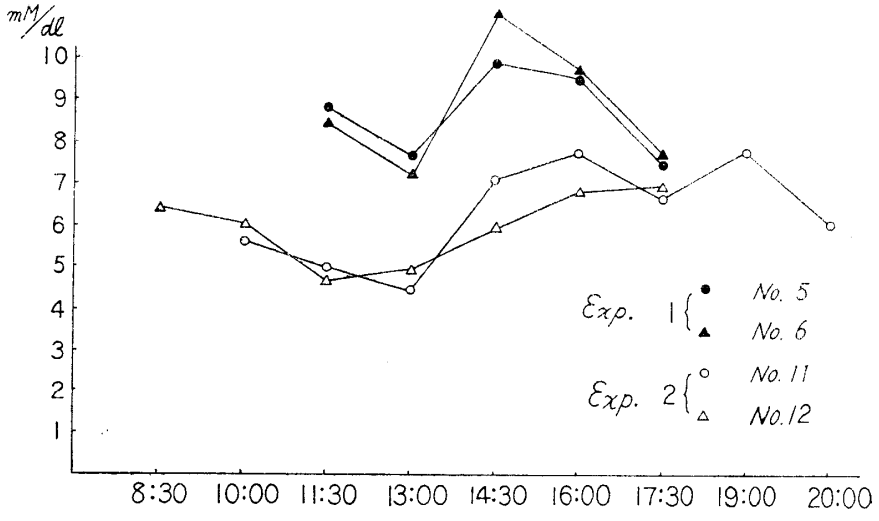

Fig. 3 Variation of volatile fatty acid concentration in the rumen liquor.

Summary

The influences of feed ingredients upon the amounts and the proportion of rumen volatile fatty acids (VFA) were studied. Molar percentages of rumen VFA in each individual goat, fed on the same diet, were almost equal and did not change appreciably with time after feeding. The possibilities that fiber increases the molar percentage of acetic acid in the rumen and protein promotes ruminal production of butyric acid are admitted.

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