CHROM. 5720

Effect of carbon chain length upon extraction of volatile fatty acids from rumen liquor

The accepted method for extraction of volatile fatty acids (VFA) from rumen liquor has been by distillation using a MARKHAM still[1]. This method has been shown by numerous workers to be reasonably efficient, particularly for the lower VFA. LAHOUD[2] showed that recoveries of acetic, propionic and isobutyric acids were in the order of 98–102 %, butyric acid 96–104 %, and 92–96 % for isovaleric and valeric acids. Clearly as the carbon chain length increased the efficiency of the distillations decreased.

In recent years research workers in the field of ruminant nutrition have shown increasing interest in determining levels and proportions of the higher VFA in rumen liquor. There is ample evidence in the literature that the higher acids and particularly isobutyric and isovaleric acids are utilized by the rumen microbial population as carbon skeletons for the production of microbial protein[3–6]. We are at present investigating some aspects of rumen microbial activity and considered it necessary to attempt to obtain absolute recoveries of all VFA produced in the rumen. Acting on the hypothesis that standard techniques of sample preparation according to MARKHAM[1] lead to bias in estimation of higher molecular weight VFA, we explored deletion of the distillation procedure. We deproteinized raw rumen liquor with phosphotungstic acid and then centrifuged. A preliminary trial in which phosphoric acid, tungstic acid and phosphotungstic acid were compared as protein precipitating agents showed the latter to be the most satisfactory agent. The resultant supernatant was then analysed for VFA on a gas chromatograph.

This paper discusses the results obtained with this method and the significance of these results.

Experimental

A comparison of the total VFA and of the individual acid fractions for the distillation method using MARKHAM stills and the centrifuge method were made on rumen liquors collected from 24 wether lambs 40–50 weeks of age. The rumen liquor samples had been neutralized with 10 *N* NaOH and stored at —10°. The lambs had been offered *ad libitum* one of the following five rations: (1) Pelletized lucerne (5 lambs). (2) Pelletized lucerne treated with formaldehyde (4 lambs). (3) Pelletized mixture of 75 % wheat and 25 % lucerne (5 lambs). Pelletized mixture of 75 % wheat and 25 % lucerne, but all components treated with formaldehyde (5 lambs). (5) Whole wheat grain + 1 ½ % ground limestone (5 lambs).

The methods used for the preparation of VFA for analysis on the gas chromatograph are as follows.

(*a*) MARKHAM *still method*. Duplicate 5 ml samples of rumen liquor were buffered with a 1 *M* phosphate buffer to pH 3.0 and immediately quantitatively transferred to a MARKHAM still and 1–2 g $MgSO_4$ added. The mixture was steam distilled until 500 ml of distillate was collected. The collection flask was packed in ice. An excess of 0.1 *N* NaOH was added to the distillate before evaporation in an oven at 100° to approximately 10 ml. This 10 ml was quantitatively transferred to a 15 ml vial and

TABLE I

MEAN CONCENTRATION (mmole/l) OF TOTAL VFA AND INDIVIDUAL ACID FRACTIONS FOR EACH TREATMENT AND EACH METHOD TOGETHER WITH RESULTS OF ANALYSIS

	Total		*Acetic acid*		*Propionic acid*		*Isobutyric acid*		*Butyric acid*		*Isovaleric acid*		*Valeric acid*		*Caproic acid*	
	C[a]	*M*[b]	*C*	*M*	*C*	*M*	*C*	*M*	*C*	*M*	*C*	*M*	*C*	*M*	*C*	*M*
Diet 1	150.51	149.82	114.71	120.35	21.34	22.02	0.37	ND[c]	13.38	6.96	0.37	0.12	0.34	0.36	ND	ND
Diet 2	153.37	107.58	123.35	91.66	17.78	10.77	0.24	1.10	11.61	4.85	0.23	0.04	0.16	0.16	ND	ND
Diet 3	125.69	90.22	82.24	61.35	15.66	14.89	0.56	0.08	25.50	12.54	0.64	0.31	0.85	1.02	0.24	0.03
Diet 4	133.80	94.46	87.59	66.16	16.90	14.70	0.37	0.16	27.44	12.69	0.64	0.28	0.61	0.46	0.25	0.01
Diet 5	48.30	41.98	21.27	29.37	10.91	9.71	0.56	0.16	4.76	2.20	0.64	0.31	0.16	0.23	ND	ND
Analyses																
Methods	$P < 0.01$		$P < 0.05$		NS[d]		$P < 0.05$		$P < 0.001$		$P < 0.01$		NS		$P < 0.001$	
Treatments	$P < 0.001$		$P < 0.01$		$P < 0.05$		NS		$P < 0.01$		NS[d]		$P < 0.001$		$P < 0.001$	

[a] C = Centrifuge method.

[b] M = MARKHAM method.

[c] ND = Not detected.

[d] NS = Not significant.

evaporated to dryness at 50°. The vial was cooled to room temperature, sealed with a rubber septum and stored at —4° until required for analysis. Immediately prior to analysis 0.5 ml of 30 % phosphoric acid was introduced and 0.5 μl injected into the gas chromatograph.

(*b*) *Centrifuge method.* Duplicate 0.5 ml samples of rumen liquor were pipetted into centrifuge tubes and 0.1 ml of a saturated solution of phosphotungstic acid was added. The tubes were stoppered, shaken and allowed to stand for 10 min before centrifuging at 3000 r.p.m. for 5 min. The supernatant liquid was transferred to vials and stored at —4° until required for analyses, when 6.0 μl of the supernatant was injected into the gas chromatograph.

Chromatography. Separation of the VFA in the gas chromatograph was obtained on a 10 ft. × $^1/_8$ in. stainless-steel column packed with 15 % Carbowax 20 M on Chromosorb W acid washed DMCS, 80–100 mesh. The column was maintained at 147° and the injector and detector temperatures were at 180°. Nitrogen and hydrogen flow rates were 25 ml/min. The VFA were detected by flame ionization and the gas chromatograph was operating isothermally.

Efficiency of methods. To determine the efficiency of the MARKHAM still method, standard solutions of VFA were made up and distilled as above. Dry nitrogen was then bubbled through the distillate for 5 min before titration with 0.1 *N* NaOH using phenolphthalein as the indicator.

The efficiency of the centrifuge method was determined by adding known amounts of VFA to the rumen liquor sample prior to centrifugation. Peak heights obtained on the recorder for these samples were then compared with the rumen liquor samples and the standard VFA sample.

Analysis. Analyses of variance were computed for total VFA and for each of the acid fractions[7].

Results and discussion

Table I shows the mean concentration of all VFA for each of the five treatments, and for the two methods, together with the results of statistical analyses. Fig. 1 shows typical gas–liquid chromatograms of acid separation. The table and figure clearly show that significantly more VFA was detected by the centrifuge method and this applied to all individual acids except for valeric acid.

It is of particular interest that the centrifuge method has permitted detection of considerably more of the isobutyric, isovaleric and caproic acids than the distillation method.

The efficiency of recovery by the MARKHAM still method was determined to be 94.0 % for the standard VFA samples tested. For total VFA of the rumen samples the centrifuge method gave a recovery value of 21.0 % higher than the MARKHAM still method suggesting that the MARKHAM still method gave much lower efficiency of recovery values than originally assumed. For individual acids the centrifuge method gave 20.6 % more acetic, 11.5 % more propionic, 76.2 % more isobutyric, 52.4 % more butyric, 58.0 % more isovaleric, 4.8 % more valeric, and 96.1 % more caproic acid than did the MARKHAM still method.

Some of the difference between the two methods would be accounted for by the fact that the analyses were carried out on deproteinized liquor for the centrifuge method and on raw liquor for the MARKHAM still method. In precipitating protein

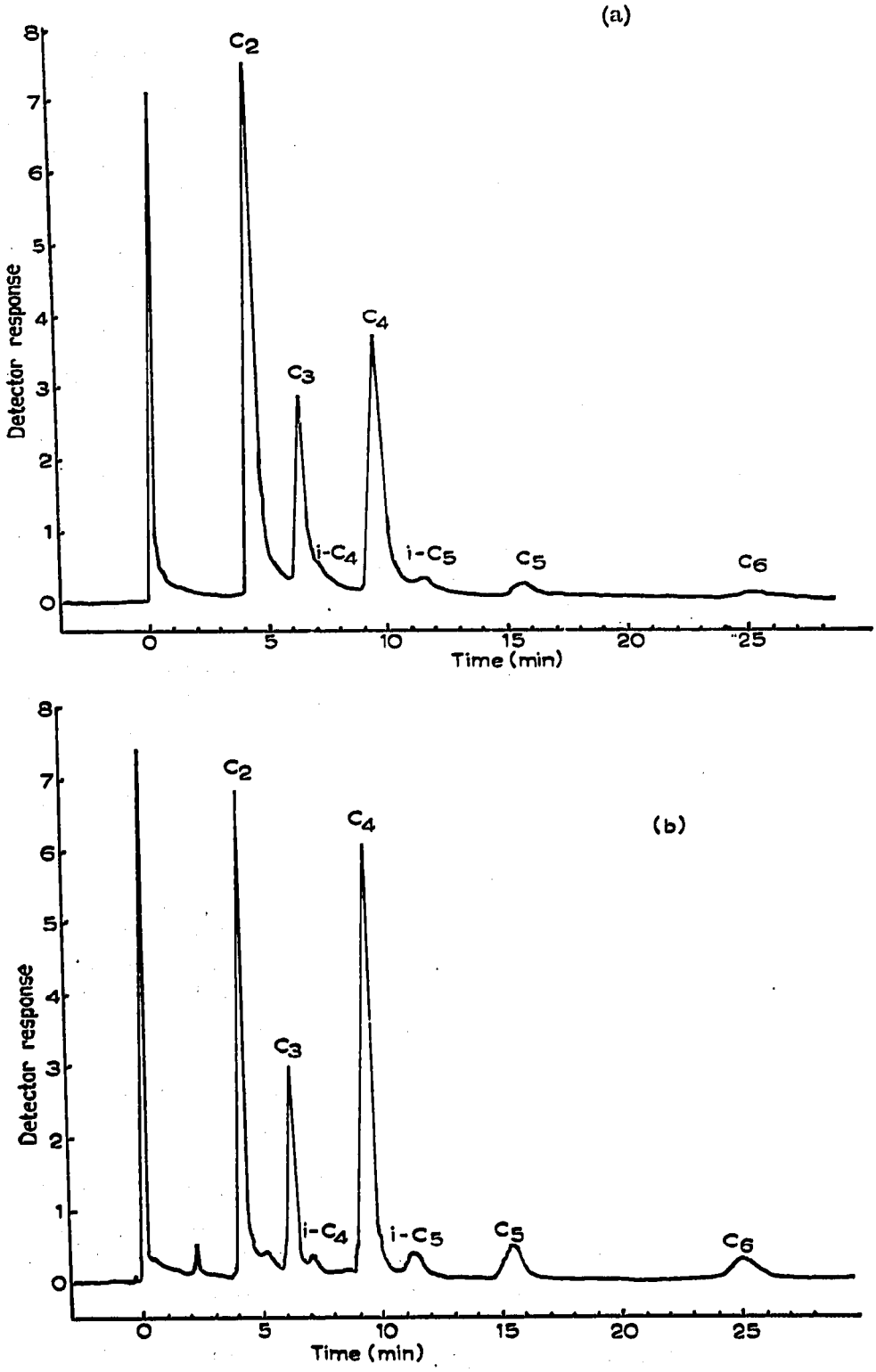

Fig. 1. Gas chromatogram of rumen liquor derived from a mixed diet (TM 5, Ration 4). C_2 = acetic acid, C_3 = propionic acid, i-C_4 = isobutyric acid, C_4 = butyric acid, i-C_5 = isovaleric acid, C_5 = valeric acid, and C_6 = caproic (hexanoic) acid. For particulars of the gas–liquid chromatographic operation see text. (a) Rumen liquor sample distilled with MARKHAM apparatus prior to gas chromatographic separation. (b) Rumen liquor sample centrifuged for 5 min at 3,000 r.p.m. prior to gas chromatographic separation.

and centrifuging to remove all solid material one is, in fact, concentrating the VFA in the sample. However, this could still not account for the large differences observed, particularly for the higher acids.

It is significant that the percentage difference between the two methods increases as carbon chain length increases, except for valeric acid which showed the smallest difference. This is to be expected as the MARKHAM still was originally designed specifically for the extraction of the lower VFA.

Sample preparation by the centrifuge method has two distinct advantages over the MARKHAM still method: (1) The time for analysis is much less than that for the MARKHAM method and is only limited by the time for elution by gas–liquid chromatography, and (2) the analysis of VFA using direct sampling by the centrifuge method gave better recovery of VFA and particularly the higher VFA than the method involving an intermediate steam distillation.

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School of Wool and Pastoral Sciences,
The University of New South Wales,
Kensington, N.S.W. 2033 (Australia)

G. B. EDWARDS
W. R. MCMANUS
M. L. BIGHAM

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