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Brief Communication

GAS-SOLID ADSORPTION CHROMATOGRAPHIC DETERMINATION OF SHORT-CHAIN FATTY ACIDS IN RUMEN FLUID*

Determination of volatile acids (VFA) in rumen fluid, as well as in aqueous solution in general, has always involved the problem of suitable analytical methods to be carried out rapidly and quantitatively. Previously, fractional distillation was generally applied followed by acidimetric determination of the acids. Since the pioneer work by *James & Martin* (1952) on determination of fatty acids by gas-liquid partition chromatography in non-aqueous medium many reports on VFA analysis in rumen fluid have appeared. However, these procedures require extensive sample preparation involving steam distillation, formation of salts, evaporation, and dissolving in organic solvents. Some authors have tried to use the same gas chromatographic determination of VFA in aqueous medium. Among them, *Erwin et al.* (1961) have tried to apply a direct chromatography of rumen fluid on inactive supports coated with phosphoric acid-treated Tween 80. As the column material is unstable and difficult to reproduce, the method has only been applied to a minor extent. Later, *Baker* (1966) increased the stability of the column by using FFAP as liquid phase, but a considerable watertailing spoils the determination of acetic acid, and no information on the separation of the straight and branched short-chain fatty acids was obtained.

The introduction of the porous polymers (*Hollis* 1966) offers new possibilities in separation, as the chromatographic principle is thereby based on differences in adsorption rate. In a comparative work *Dave* (1969) examined the adsorptive properties of some porous polymers against different fatty acids. By the fact that porous polymers are more or less hydrophobe the possibilities for chromatography of aqueous samples seem obvious.

In the present work the author has developed a method on direct analysis of rumen fluid by gas-solid adsorption chromatographic determination on uncoated Chromosorb 101. The analysis is made on a Perkin Elmer 990 gas chromatograph with dual

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flame-ionization detector. Columns of stainless steel, 2 m in length and 2 mm in internal diameter, packed with Chromosorb 101 (60/80 mesh) are used. After conditioning 2—4 days at 225°C with a moderate flow of carrier gas, the columns are ready for use.

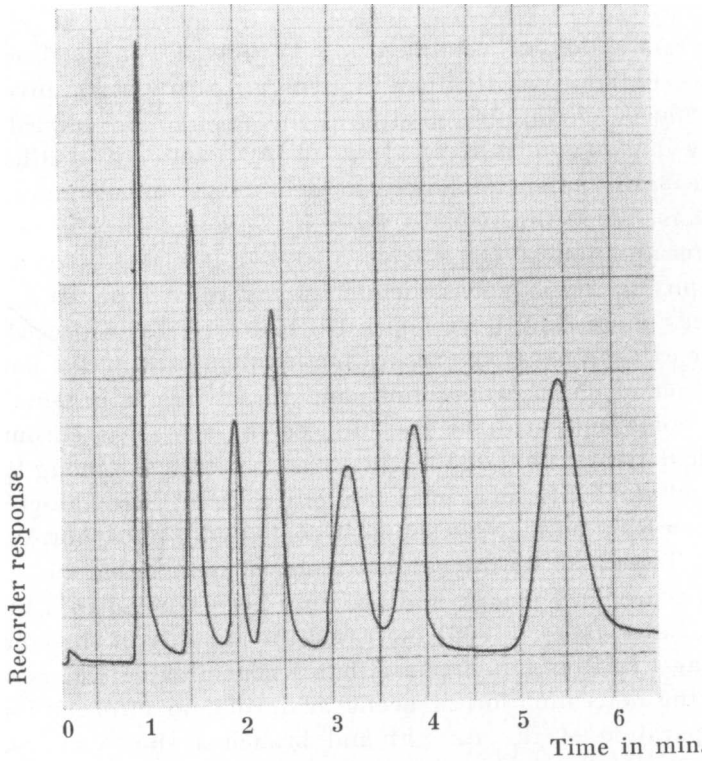

Figure 1. Analysis of rumen fluid. The order of peaks and attenuations is acetic acid x 128, propionic acid x 64, isobutyric acid x 16, butyric acid x 32, isovaleric acid x 16, valeric acid x 16 and isocaproic acid (internal standard) x 64.

A typical chromatogram appears in Fig. 1, which shows an analysis of rumen fluid from a fistulated heifer, taken 6 hrs. after hay-feeding. After sterile filtration, 9 ml rumen fluid is added to 1 ml internal standard solution consisting of 6.5 g isocaproic acid per l 3 M-H_3PO_4. The temperature of the column is 190°C, and a carrier gas flow of 24 ml nitrogen per min. is used. As the time of retention is varying with column temperature and carrier gas flow, a gas chromatographic analysis lasting from a few min. to ½ hr. could be established. The actual chromatogram is obtained in 6 min.

It appears from the chromatogram that the separation between the actual fatty acids is good, and there is no visible inter-

Table 1. VFA in rumen fluid taken 6 hrs. after feeding. Mean of 3 heifers.

	Total VFA mmol/l	Molar percentage of total VFA					
		acetic acid	propionic acid	butyric acid	iso-butyric acid	valeric acid	iso-valeric acid
Oat	89.6	63.9	25.3	7.0	1.2	1.5	1.0
Dried beet pulp with molasses	96.7	71.1	13.4	12.5	0.9	0.7	1.2
Grass pellets	98.5	77.6	13.7	6.8	0.8	0.6	0.5
„ hay	98.5	75.5	14.5	6.5	1.3	1.0	1.1

ference from water or other components in rumen fluid. The material of the column is very steady without alterations after analysis of some hundred samples, furthermore there is found only insignificant ghosting. Several authors apply isovaleric acid as internal standard, which involves that chromatography necessarily should be carried out of samples with as well as without internal standard because of the natural content of isovaleric acid in rumen fluid. Actually, there is found no content of isocaproic acid on examination of rumen fluid from different feeding types. For this reason isocaproic acid is found suitable as standard. The results of the practical applicability of the method appear from Table 1, which shows the content of VFA in rumen fluid taken 6 hrs. after feeding different feeding stuffs.

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