REFERENCES

Andrews, F. N. & Bohren, B. B. (1947). *Poult. Sci.* **26**, 447.
Broadbent, M. & Bean, H. W. (1952). *Poult. Sci.* **31**, 447.
Hammond, J. (1931). *Yearb. nat. Rabbit Coun.* p. 32.
Hankins, C. G., Hiner, R. L., Templeton, G. S. & Kellogg, C. E. (1951). *Physical Composition of Fryer Rabbits of Prime, Choice, and Commercial Grades.* Washington: United States Department of Agriculture.
Hathaway, H. E., Champagne, G. B., Watts, A. B. & Upp, C. W. (1953). *Poult. Sci.* **32**, 968.
Huang, T. C., Ulrich, H. E. & McCay, C. M. (1954). *J. Nutr.* **54**, 621.
Hutchinson, J. C. D. (1947*a*). *Brit. J. Nutr.* **1**, 219.
Hutchinson, J. C. D. (1947*b*). *Brit. J. Nutr.* **1**, 231.
Hutchinson, J. C. D. & Baker, C. J. L. (1949). *Brit. J. Nutr.* **3**, 12.
McNally, E. H. & Spicknall, N. H. (1949). *Poult. Sci.* **28**, 562.
Titus, H. W., Mehring, A. L. Jr. & Brumbaugh, J. H. (1953). *Poult. Sci.* **32**, 1074.

# An estimate of the weights of volatile fatty acids produced in the rumen of lactating cows on a diet of hay and concentrates

BY D. A. BALCH*

*National Institute for Research in Dairying, University of Reading*

(*Received* 28 *February* 1957—*Revised* 13 *August* 1957)

With the increasing appreciation of the nutritional value to the ruminant of the volatile fatty acids produced by fermentation in the reticulo-rumen (hereafter termed rumen for brevity), the need has arisen to assess the amounts of such acids involved. When a cow consumes her diet in two equal meals at 12 h intervals, the mean weight of food digested in the rumen in any 12 h period can be calculated from the rumen-digestibility coefficient. By incubating, under conditions similar to those present in the rumen, a sample of the diet with rumen liquor from the cow receiving that diet, a value for the weight of volatile fatty acids produced per unit of food digested can also be obtained. From these two values an estimate of the weight of volatile fatty acids produced in the rumen per day may be made. This paper presents the results of an attempt to estimate with this procedure the weights of volatile fatty acids produced in the rumen by two lactating cows during the feeding of two diets.

## EXPERIMENTAL

### *Animal management*

The experiment was made with two fistulated, lactating Dairy Shorthorn cows and was divided into two parts. In part 1 each cow received daily 16 lb. hay and cow A 20 lb. and cow B 16 lb. concentrates: in part 2 each received 18 lb. ground hay and

* Present address: Department of Agricultural Chemistry, The University, Reading.

18 lb. concentrates. The hay was good-quality seeds-hay and the concentrate mixture consisted of flaked maize 10, fine wheat offal 7 and groundnut cake 3 parts by weight. Mineral licks and water were always available. Each diet was given in two equal meals at 6 a.m. and 6 p.m. This experiment was part of an investigation of the effect on the fat content of milk of a diet containing all the hay in a finely ground state, details of which will be published elsewhere.

### *Digestion of organic matter in the rumen*

When each cow had been receiving each diet for several weeks, representative samples of each food and of digesta from the region of the reticulo-omasal orifice were collected over a 7-day period and bulked separately. These samples were used for estimating the rumen-digestibility coefficient by means of the lignin-ratio technique. Full details of the technique and of the extent of digestion in the rumen during this experiment are given by Balch (1957).

### *In vitro digestion of organic matter*

Each diet was fermented in triplicate with rumen liquor from each cow on the 1st and 7th days of the period during which the rumen-digestibility coefficients were estimated. The fermentations were made in glass flasks fitted with Bunsen valves. The weight of each diet fermented was adjusted so that the ratio of rumen liquor to diet in the flask was the same as the ratio of rumen liquor to dry matter in the rumen of the cow. In part 1 the weights taken were 28 and 22 g for cows A and B, respectively, and, in part 2, 20 and 18 g, respectively. The concentrate mixture was coarsely milled and, in part 1, the hay was cut into $\frac{1}{2}$ in. lengths. The flasks containing the diet and 600 ml. artificial saliva (Elsden, 1945) were warmed in a constant-temperature water-bath to 39·5°. Just before the 6 a.m. feed a large sample of rumen liquor was withdrawn from each cow. Each sample was strained through cotton gauze into a warm, insulated flask and rapidly taken to the laboratory. The contents of each incubation flask were inoculated with 150 ml. liquor and then saturated with carbon dioxide. The flasks were incubated at 39·5° for 12 h. They were shaken at frequent intervals and resaturated with carbon dioxide after the first 6 h. On the completion of the incubation period the contents of each flask were mixed and a small portion was filtered for the determination of volatile fatty acids. The residue from each filtration was returned to the corresponding flask and 1 ml. 40% formaldehyde was added to prevent further fermentation. The unfermented residue was separated by centrifuging.

### *Analysis*

The concentration of total volatile acids and the proportions of the individual volatile fatty acids in the inocula of rumen liquor and in the fermentation liquors (the filtrates from the completed fermentations) were determined as previously described (Balch & Rowland, 1957). Dry matter and ash contents of the hay, concentrates, inocula and fermentation residues were determined. The method of Armitage, Ashworth & Ferguson (1948) was used for lignin. Samples of inocula and fermentation liquors were preserved with formalin for microscopical examination.

RESULTS

In all flasks gas production, which began shortly after inoculation, continued vigorously throughout the incubation period. Nigrosin-stained slides were examined for the presence of thirteen morphological types of bacteria. The mean ratings of the slides are given in Table 1. No major differences were found between the inocula and the corresponding fermentation liquors. During part 1 total numbers of bacteria increased by a factor of 2·8 for incubations inoculated with rumen liquor from cow A and of 1·4 for cow B. The corresponding increases in part 2 were 3·2 and 4·9, respectively. In vitro the mean digestibility of organic matter was 40·4 (standard deviation 2·0) for

Table 1. *Mean ratings of nigrosin-stained slides of inocula and fermentation liquors*

| | Part 1 | | | | Part 2 | | | |
|---|---|---|---|---|---|---|---|---|
| | Cow A | | Cow B | | Cow A | | Cow B | |
| Type of organism | Inoculum | After fermentation | Inoculum | After fermentation | Inoculum | After fermentation | Inoculum | After fermentation |
| Short fat rods | 8 | 8 | 7 | 7 | 6 | 5 | 6 | 6 |
| Tiny rods and single cocci | 8 | 8 | 7 | 7 | 8 | 8 | 8 | 8 |
| Large and normal diplococci | 6 | 5 | 4 | 5 | 5 | 5 | 5 | 5 |
| Medium-sized rods | 5 | 6 | 5 | 5 | 5 | 5 | 4 | 5 |
| Curved rods | 6 | 5 | 4 | 4 | 5 | 5 | 5 | 5 |
| Chains of cocci | 4 | 4 | 3 | 3 | 0 | 1 | 1 | 1 |
| Spirilla | 4 | 4 | 4 | 3 | 3 | 2 | 3 | 4 |
| Giant cocci and chains of diplococci | 5 | 5 | 6 | 4 | 0 | 0 | 1 | 0 |
| Giant fat rods | 3 | 3 | 3 | 3 | 1 | 2 | 1 | 2 |
| Sarcina | 1 | 2 | 3 | 2 | 0 | 0 | 0 | 0 |
| Actinomyces | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Long slender rods | 3 | 2 | 3 | 3 | 1 | 1 | 1 | 1 |
| Yeasts | 2 | 2 | 0 | 0 | 0 | 0 | 0 | 0 |
| Total numbers of organisms × $10^6$ (calculated/ml. contents of incubation flask) | 3200 | 8801 | 3766 | 5092 | 8159 | 26,218 | 4089 | 19,864 |

The figures denote the following rating: 0, no organisms/slide; 1, 1 organism/slide; 2, 2–4 organisms/slide; 3, 5–10 organisms/slide or 1–2 organisms/field; 4, 3–8 organisms/field; 5, 9–15 organisms/field; 6, 16–30 organisms/field; 7, 31–50 organisms/field; 8, more than 50 organisms/field.

cow A and 37·3 (S.D. 0·9) for cow B, in part 1, compared with estimated rumen digestibility coefficients of 40·0 and 47·7, respectively. In part 2 the corresponding values were, in vitro 48·9 (S.D. 0·8) and 47·1 (S.D. 0·7) and in vivo 44·1 and 47·1 for cows A and B, respectively. The mean weights of organic matter digested daily in the rumens of cows A and B were 4550 and 5550 g, respectively, in part 1 and 5750 and 6250 g, respectively, in part 2.

The mean composition of the mixture of volatile fatty acids in the inocula and fermentation liquors, the mean production of each fatty acid per g organic matter

digested in vitro and the estimated weights and caloric values of each acid produced daily in the rumen are given in Table 2. In calculating the weight of each acid produced per g organic matter digested in vitro the mean of the six replicates for that cow and diet was used. Agreement between replicates was good. In estimating the caloric values the mixture of acids higher than butyric has been calculated as valeric acid. During part 1, on the long-hay diet, both the weight of organic matter digested in the rumen, and the mean production of volatile fatty acids per g organic matter digested, were less for cow A than for cow B. With the ground-hay diet in part 2, both values for both cows were greater than in part 1. The larger weight of volatile fatty acids produced per g organic matter digested was due mainly to an increase in the weight of propionic acid formed.

## DISCUSSION

The reliability of the results obtained with this technique depends on the extent to which the in vitro incubations simulate digestion in the rumen, and the accuracy of the method of estimating the extent of digestion in the rumen. The main criticism of the in vitro incubations is that the end-products of fermentation are not removed. That the technique used gave results that were repeatable is indicated by the good agreement between replicate incubations for a given cow and diet made on different days. The rate of fermentation will decrease during the incubation period (Carrol & Hungate, 1955). However, provided there is not sufficient accumulation of the end-products of digestion to cause the establishment of a new type of flora, the weights of fatty acids produced per g organic matter digested should remain substantially constant. The highest concentration of total volatile acids reached during the incubations was 11·3 m-equiv./100 ml. The range of concentration of total volatile acids in the rumen during a 24 h period varied from 10·0 to 15·7, and from 7·9 to 15·0 m-equiv./100 ml. rumen liquor for cows A and B, respectively, during part 1 of the experiment, and from 7·0 to 20·0 and from 7·7 to 15·2 m-equiv./100 ml., respectively, in part 2 (Balch & Rowland, 1957). Thus, at no time during the incubation period was the flora in the incubation flasks subjected to a concentration of volatile acids greater than was normally present in the rumen during the daily fermentation cycles. The total numbers of bacteria increased during the incubation period. As far as could be estimated microscopically there were no major differences in the relative proportions of the morphological types of bacteria between the inocula and the corresponding fermentation liquors. Gray, Pilgrim & Weller (1951) also obtained satisfactory in vitro fermentation with sheep-rumen liquor when the ratio of dry matter to rumen liquor in the flask was similar to that used here. A critical discussion of the method for estimating the extent of digestion in the rumen and the results for a number of diets are given by Balch (1957).

The estimated values for the weights of volatile fatty acids produced in the rumen of each cow in both parts of the experiment confirm that acetic acid is the acid produced in greatest quantity. In part 1 the weight produced daily was two to four times greater than that of any other acid. Though the relative proportions in which the acids were produced by the two cows were similar, cow B produced appreciably more acids than cow A. In part 2 better agreement was obtained between the two cows. The

Table 2. *Mean composition of the mixture of volatile fatty acids, expressed as molar percentage, in the inocula and fermentation liquors; mean weights of volatile fatty acids produced per g organic matter digested in vitro, and weights and caloric values of the volatile fatty acids produced in the rumen*

| | Present in inoculum (mean molar percentage) | | Present after fermentation (mean molar percentage) | | m-equiv./g organic matter digested in vitro | | Estimated daily production in rumen: Weight (g) | | Estimated daily production in rumen: Energy value (Cal.) | |
|---|---|---|---|---|---|---|---|---|---|---|
| Acid | Cow A | Cow B | Cow A | Cow B | Cow A | Cow B | Cow A | Cow B | Cow A | Cow B |
| | | | | Part 1 | | | | | | |
| Acetic | 68·1 | 66·6 | 58·9 (1·0) | 60·6 (1·2) | 3·2 (0·54) | 4·5 (0·19) | 870 | 1500 | 3020 | 5210 |
| Propionic | 20·3 | 19·9 | 18·4 (0·8) | 17·9 (1·6) | 1·0 (0·21) | 1·3 (0·16) | 340 | 530 | 1660 | 2640 |
| Butyric | 5·5 | 9·0 | 12·5 (2·0) | 14·2 (0·8) | 0·8 (0·24) | 1·1 (0·08) | 320 | 540 | 1890 | 3190 |
| Higher acids | 6·1 | 4·5 | 10·2 (1·5) | 7·3 (1·2) | 0·6 (0·08) | 0·6 (0·09) | 280 | 340 | 1840 | 2260 |
| | | | | Part 2 | | | | | | |
| Acetic | 52·3 | 56·6 | 49·7 (1·2) | 52·1 (1·1) | 3·9 (0·06) | 4·4 (0·12) | 1350 | 1650 | 4680 | 5730 |
| Proprionic | 31·8 | 28·4 | 29·6 (2·1) | 28·4 (0·6) | 2·3 (0·17) | 2·5 (0·10) | 980 | 1160 | 4840 | 5710 |
| Butyric | 9·7 | 9·7 | 13·6 (2·0) | 12·1 (0·4) | 1·2 (0·21) | 1·1 (0·04) | 610 | 610 | 3600 | 3580 |
| Higher acids | 6·2 | 5·3 | 7·1 (1·1) | 7·4 (0·8) | 0·6 (0·10) | 0·7 (0·08) | 350 | 450 | 2340 | 2960 |

Figures in parentheses are standard deviations.

relative weights of acetic, butyric and higher acids produced in part 2 were similar to those in part 1, but more propionic acid was produced. This increase in the proportion of propionic acid formed from a hay–concentrate diet with the hay finely ground, over that from a similar diet with the hay in the long state, confirmed the previous findings of Balch & Rowland (1957).

When a diet is given in two equal meals at 12 h intervals and with water freely available, the fluctuations in both the weight of digesta in the rumen (Balch, 1952) and the concentration of total volatile acids (Balch & Rowland, 1957) follow a regular cycle in each 12 h period. Hence, the weight of fatty acids present in the rumen just before each feed is nearly constant. Since only small quantities of volatile fatty acids reach the abomasum (Elsden, Hitchcock, Marshall & Phillipson, 1946) the estimated values for the weights of volatile fatty acids produced in the rumen will approximate to those absorbed daily by the two cows. The nutritive value of the fatty acids depends not only on their potential value as a source of energy; propionic acid is utilized for gluconeogenesis; in the lactating cow a portion of the acetic acid is used for the synthesis of milk fat and in the fattening animal for body fat. However, if the total volatile fatty acids produced daily in the rumen by the two cows had been completely oxidized, they would have produced 8410 and 13,300 Cal. for cows A and B, respectively, in part 1 and 15,460 and 17,980 Cal., respectively, in part 2. Carrol & Hungate (1955) calculated that oxidation of the volatile fatty acids produced by fermentation in the bovine rumen of diets of hay, grain and pasture would yield 10,100, 16,350 and 6870 Cal., respectively.

SUMMARY

1. Two fistulated lactating Dairy Shorthorn cows were used to estimate the weights of volatile fatty acids produced daily in the reticulo-rumen from hay–concentrate diets. During part 1 of the experiment, each cow received daily 16 lb. long hay and cow A 20 lb. and cow B 16 lb. concentrates. In part 2 each cow received daily 18 lb. ground hay and 18 lb. concentrates.

2. The production of volatile fatty acids per g organic matter was determined by in vitro fermentation of the diet with rumen liquor from each cow. The weight of organic matter digested in the rumen was calculated by means of the lignin-ratio technique.

3. The estimated weights of volatile fatty acids produced in the rumen of cows A and B, respectively, in part 1 were: acetic 870 and 1500 g, propionic 340 and 530 g, butyric 320 and 540 g and higher acids 280 and 340 g. In part 2, the corresponding values were: acetic 1350 and 1650 g, propionic 980 and 1160 g, butyric 610 and 610 g and higher acids 350 and 450 g. These acids, if absorbed and oxidized, would yield a total energy of 8410 and 13,300 Cal. for cows A and B, respectively, in part 1 and of 15,460 and 17,980 Cal., respectively, in part 2.

4. Relatively more propionic acid was produced from the diet containing finely ground hay than from the long-hay diet.

I wish to acknowledge the help and advice of Dr C. C. Balch and Dr S. J. Rowland and to thank Dr L. F. L. Clegg for the bacteriological studies.

REFERENCES

Armitage, E. R., Ashworth, R. de B. & Ferguson, W. S. (1948). *J. Soc. chem. Ind., Lond.*, **67**, 241.
Balch, C. C. (1952). Unpublished.
Balch, C. C. (1957). *Brit. J. Nutr.* **11**, 213.
Balch, D. A. & Rowland, S. J. (1957). *Brit. J. Nutr.* **11**, 288.
Carrol, E. J. & Hungate, R. E. (1955). *Appl. Microbiol.* **2**, 205.
Elsden, S. R. (1945). *J. exp. Biol.* **22**, 151.
Elsden, S. R., Hitchcock, M. W. S., Marshall, R. A. & Phillipson, A. T. (1946). *J. exp. Biol.* **22**, 191.
Gray, F. V., Pilgrim, A. F. & Weller, R. A. (1951). *J. exp. Biol.* **28**, 174.

# The effect of diurnal variations in composition of the faeces of pigs on the determination of digestibility coefficients by the chromium-oxide method

BY J. H. MOORE

*Department of Agricultural Chemistry, University of Reading*

(*Received* 25 *April* 1957)

It has frequently been pointed out that the indicator method of determining digestibility coefficients offers no advantage over the direct or conventional method if a composite sample is taken for analysis from the total faecal production during the whole collection period. The indicator method would, however, possess a distinct advantage if quantitative collection of faeces could be dispensed with and digestibility determined accurately from a partial faecal collection. The feasibility of such a simple collection procedure depends largely on variations in composition of faeces excreted at different times of the day. Diurnal variations in the faecal excretion of chromium oxide have been reported by Kane, Jacobson & Moore (1952), Smith & Reid (1955), Hardison, Engel, Linkous, Sweeney & Graf (1956) working with cows, Raymond & Minson (1955), Pigden & Brisson (1956) with sheep, Clawson, Reid, Sheffy & Willman (1955) with pigs and Dansky & Hill (1952) and Mueller (1956) with poultry. In a number of these papers the influence of variations in the $Cr_2O_3$ content of the excreta on the digestibility of dry matter as determined by the indirect method from faecal samples collected at different times of the day is discussed. Before a simplified $Cr_2O_3$ technique may be applied to determine digestibility coefficients of individual dietary components the nature and extent of the diurnal variations in the faecal excretion of these components must be determined. In experiments with pigs on different feeding treatments Moore (1957) investigated the faecal-excretion patterns of $Cr_2O_3$, crude protein, crude fibre and ash. The form of each excretion curve was found to be related to the feeding system employed, and an explanation based on the relative rates of passage of the different constituents of the diet from the stomach was put forward to account for the observed diurnal variations in faecal composition. In the present