Br. J. Nutr. (1969), **23**, 443

The influence of ruminal infusion of volatile fatty acids on milk yield and composition and on energy utilization by lactating cows

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(*Received* 1 *July* 1968—*Accepted* 18 *April* 1969)

1. In an experiment of 3 × 3 latin square design, four lactating Holstein cows were given a basal ration designed to induce low percentages of milk fat. The treatments were (1) basal ration, a pelleted mixture of lucerne hay (20%) and concentrates (80%), with 40 l. of water infused intraruminally, (2) basal ration with acetic acid substituted for 15·4% of the metabolizable energy (ME) and (3) propionic acid substituted for 15·4% of the ME. In the last 3 weeks of the 6-week experimental period respiration trials were carried out in an open-circuit indirect calorimeter. The levels of feeding offered in the three periods were 325, 275 and 225 kcal ME/kg body-weight $^{0.75}$ in periods 1, 2 and 3 respectively.

2. No differences were detected in the utilization of the energy of acetic and propionic acids, but there were differences in the partition of energy into milk or body tissues; with acetic acid infusion more energy was secreted as milk and with propionic acid infusion more was deposited in body tissue.

3. There was an increase in milk fat percentage with acetic acid infusion, but not complete recovery to normal. The milk fat percentages were 1·96, 2·58 and 1·92 for treatments 1, 2 and 3 respectively. Acetic acid infusion caused increases in the C12, C14 and C16 fatty acids of milk fat and decreased the proportion of C18:1 fatty acids.

4. It is suggested that the low percentages of milk fat found when cows are given concentrates could result from a decreased extent of fermentation in the rumen, allowing a greater proportion of the starch consumed to be absorbed as glucose in the small intestine.

The efficiency of utilization of volatile fatty acids (VFA) for different physiological functions has been studied by several methods. Differences in the proportions of acetic, propionic and butyric acids in VFA mixtures continuously infused intraruminally into fasting sheep had only a small effect on the efficiency of energy utilization (Armstrong, Blaxter & Graham, 1957). For fattening, all acids were utilized less efficiently than for maintenance, particularly acetic acid (Armstrong & Blaxter, 1957) and mixtures containing a high proportion of acetic acid (Armstrong, Blaxter, Graham & Wainman, 1958). With salts of VFA, however, no difference could be found between acetate, propionate and butyrate in the ability to promote growth in young sheep (Ørskov & Allen, 1966*a*, *b*, *c*; Ørskov, Hovell & Allen, 1966). In lactating goats supplementation of a basal ration with a mixture of VFA or with acetic or propionic acid (Armstrong & Blaxter, 1965) had only a minor effect on energy utilization, as judged by the heat increment.

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The effect of VFA on the percentage and composition of milk fat has been extensively studied (Stoddard, Allen & Peterson, 1949; Tyznik & Allen, 1951; Balch, Balch, Bartlett, Cox & Rowland, 1952; Balch & Rowland, 1959; Shaw, Robinson, Senger, Lakshmanan & Lewis, 1959; Rook & Balch, 1961; Stanley, Morita & Ueyama, 1964; Rook, Balch & Johnson, 1965; Jorgensen, Schultz & Barr, 1965; Storry & Rook, 1966) and although the results vary in magnitude it has been established that increases in milk-fat percentage occur when diets which induce low milk-fat percentage are supplemented with acetic acid or sodium acetate (Tyznik & Allen, 1951; Balch & Rowland 1959; Rook & Balch, 1961; Stanley *et al.* 1964; Rook *et al.* 1965; Jorgensen *et al.* 1965; Storry & Rook, 1966). However, a complete recovery of milk-fat percentage on such rations has seldom been accomplished. Recently Storry & Rook (1966) showed that intraruminal infusion of acetic acid caused milk fat to recover to an extent approximating to only about 25% of the decrease, indicating that factors other than acetic acid are involved. The depression of milk-fat percentage generally arose from a greater reduction of the C4–C16 acids (Balch *et al.* 1952; Balch & Rowland, 1959; Shaw *et al.* 1959; Storry & Rook, 1966) than of the C18 acids, and various suggestions have been made to explain the phenomenon.

In the experiment reported here the effects of acetic and propionic acid infusion on milk-fat percentage and composition were studied. In addition calorimetric measurements were obtained which allowed some assessment of the efficiency with which the energy of the infused acids was utilized. While it would have been desirable to have used two different basal rations, the amount of work involved prevented this and it was decided to use a basal ration which was expected to induce low milk-fat percentage to ensure that the milk fat might be influenced by the treatment imposed.

MATERIALS AND METHODS

Animals. Four lactating Holstein cows, in which rumen fistulas had been established 3 months before the experimental period, were used. The calving dates, body-weights, ages and other relevant information are given in Table 1.

Table 1. *Information about the experimental cows*

Cow no.	Date of birth	Last calving date	No. of lactation	Weeks of lactation on experiment	Body-wt (kg)	Breeding date
456	15 Sept. 1962	6 July 1965	1	38–55	571	Not pregnant
459	7 Nov. 1962	24 Dec. 1965	2	14–31	553	1 Sept. 1966
445	17 Mar. 1966	7 Mar. 1966	2	7–23	653	Not pregnant
465	4 Jan. 1963	16 Dec. 1966	2	17–35	584	5 Apr. 1966

Design and treatments. A 3×3 latin square design was used; an additional animal (465) was also used which received the same treatment sequence as one of the cows in the 3×3 design.

To achieve a completely balanced design, the animals should be of the same age and stage of lactation. It was not possible to obtain cows with established rumen fistulas

which conformed to this requirement, but all animals were in a stage of lactation where calving dates or pregnancy were not likely to influence milk composition; three cows were in mid-lactation and cow 456, which was in late lactation, was not pregnant.

The treatments were (1) basal ration plus 40 l. of water, (2) basal ration plus 40 l. of acetic acid solution and (3) basal ration plus 40 l. of propionic acid solution. The water and acid solutions were infused intraruminally. The length of the periods was 6 weeks, of which 3 weeks were preliminary; calorimetric measurements were obtained during the last 3 weeks of each period.

Diets. In period 1 the level of feeding was calculated to supply 325 kcal metabolizable energy/kg body-weight$^{0.75}$ (ME/kg$^{0.75}$), in period 2 this was 275 kcal, and in period 3, 225 kcal ME/kg $^{0.75}$. The basal ration was for this purpose estimated to contain 2·72 kcal ME/g and the ME of the VFA was assumed to be the heat of combustion (Hodgman, 1962) of the acids. In the periods when acids were infused the acids were calculated to replace 15·4% of the ME of the ration. The amounts of acetic and propionic acids infused were for period 1, 1633 and 1149 g/day, for period 2, 1492 and 1149 g/day, and for period 3, 1155 and 901 g/day respectively.

The basal ration was a pelleted mixture of 20% finely ground (1·6 mm screen) lucerne hay (*Medicago sativa*) and 80% concentrate. The ingredients of the concentrate were maize meal 73·75%, soya-bean meal 24·25%, trace-mineralized salt 1·00% and steamed-bone meal 1·00%. The average chemical composition of the pelleted ration was, as percentage of dry matter, nitrogen 3·12, carbon 44·98, ether extract 2·05, ash 5·09, acid detergent fibre (ADF) 12·89, lignin 2·23 and neutral detergent fibre (NDF) 25·20, and its calorific value was 4·45 kcal/g. Acetic acid was found to contain 3·35 and propionic acid 4·87 kcal/g.

Fatty acid infusion. The daily infusion of acids was usually given in 23 h to allow time for the animals to exercise while the chambers were open and while the tanks were refilled. Slow speed pumps (Sigmamotor) were used to regulate the rate of infusion both in the preliminary period and when the cows were in respiration chambers. Acid-resistant Tygon tubing was inserted into the rumen through the cannula with about 60 cm inside positioned so that the acids were not flowing directly on to the rumen wall.

Management of the animals. The cows were milked twice daily. Uneaten food was collected once daily and samples were obtained for determination of dry matter and chemical analysis. During the preliminary periods the animals were allowed 1 h exercise daily in a concrete yard. During the periods in the respiration chambers the animals were given exercise, and body-weights were recorded on days when the chambers were not sealed. Body temperature, heart rate measurements and checks on udder condition and general health were made every day in the energy metabolism laboratory. The animals were given the basal diet in two equal feeds at 07·00 h and 17·00 h.

Collection of foods, faeces, urine, milk, blood and rumen fluid. During each period complete collections and analyses of foods, uneaten foods, faeces, milk and urine were made during two 5-day periods and one 7-day period. The samples were bulked during these periods and preserved for analysis by freezing or drying. On the last day of the 7-day

period, at 11·00 h, samples of rumen fluid were obtained via the fistula and samples of blood from the mammary vein. The rumen samples were preserved with 2% saturated $HgCl_2$ and the blood with NaF.

Respiration trials. Open-circuit respiration chambers were used for collection and determination of carbon dioxide, methane and oxygen as described by Flatt, Van Soest, Sykes & Moore (1958) and Moe & Flatt (1967). The chambers were sealed at the end of the 2nd day after collections of urine and faeces had begun and remained sealed for 5 consecutive days. Measurements in the respiration chamber were then taken for the last 2 days of period 1 and the first 3 days of period 2. After that a third collection period was started within which faeces and urine were collected for 7 days and respiration measurements were made on 5 days, giving a total of 17 days collection of urine and faeces and ten 24 h respiration trials. Small subsamples of incoming air and exhaust gas from the chambers were collected in spirometers continuously over 24 h periods and analysed daily. The results were automatically recorded on punch cards by the system described by Moe & Flatt (1967). Total heat production was calculated from the factors adopted at the Third Symposium on Energy Metabolism (Brouwer, 1965). The chambers were kept at an average temperature of 17·7° and a relative humidity of 58%.

Analytical methods. The methods for analysis for nitrogen, ash, ether extract and dry matter were the standard procedures adopted by the Association of Official Agricultural Chemists (1960). Lignin, ADF and NDF were analysed by the method of Van Soest (1963*a*, 1965). Gross energy was determined in a Parr adiabatic bomb calorimeter with an automatic temperature controller (Arthur H. Thomas Co., Philadelphia, USA). The carbon determinations were made with an induction furnace and gasometric analyser as modified and described by Smith, Flatt, Barnes & Van Soest (1965). Milk yields, corrected for solids content, to achieve equal energy/unit of milk were calculated according to Tyrrell & Reid (1965). Milk fat was determined by the Babcock test, solids-not-fat (SNF) by lactometer, and milk protein by the Orange G dye test (Udy, 1956). Ketone body estimations were made by the method of Behre (1940). For the respiration trials, carbon dioxide and methane were analysed in Beckman Model LB 15 infrared analysers and oxygen in a Beckman Model G2 paramagnetic analyser. Determinations of pH in rumen fluid were made immediately after sampling and straining with a Beckman Zeromatic glass electrode pH meter. VFA determinations were made on strained rumen fluid, after acidification with 25% metaphosphoric acid (5 ml of rumen fluid to 1 ml of acid), by gas–liquid chromatography with a flame ionization detector.

The milk fat was separated by centrifugation. Methyl esters of the fatty acids were prepared by adding approximately 50 mg of fat to 1 ml of 1% (v/v) H_2SO_4 in methanol. Methylene chloride (about 0·5 ml) was then added until the fat was completely dissolved. The container was tightly capped and allowed to stand at room temperature for 14 h. After the addition of 2 ml water, the esters were extracted into two 3 ml portions of hexane. The esters were recovered after evaporating the hexane on a steam bath under a slow stream of nitrogen. The methyl esters were subjected to gas chromatography on a 6 ft × 4 mm stainless steel column packed with diethyleneglycol

succinate polyester (20%) on 80–90 mesh Anakron ABS. The esters were separated at 170° with an inlet pressure of 0·703 kg/cm². The peak areas were measured by triangulation.

Statistical methods. In periods 2 and 3 statistical analyses were performed on the over-all means for the periods totalling 17 days of faeces and urine collection and 10 days of respiration measurements. In period 1 the first 5 days respiration measurements for cows 456 and 459 were discarded because of technical failures; the intake of food and the energy balance were calculated from the last 7-day period of faeces and urine collection and the last 5 days of calorimetric measurements. During period 3, cow 445 became ill and lost appetite, consequently a missing plot had to be calculated which, in the statistical analyses, resulted in only 1 degree of freedom for error. In all the statistical analyses, therefore, the between-cow variability has been pooled into the error term. The standard error of means is the weighted mean standard error for comparing means with two or three observations. The regression analyses (Fig. 1), however, are based on each individual collection period, including those for the cow which was not in the regular design, and totalled twenty-eight collection periods.

RESULTS

In Table 2 the food offered and consumed and the excretion of dry matter and ADF are given. The food left uneaten was slightly greater when VFA was infused, though the differences were small and not significant. During two trials in period 2, cows 459 and 456 were given the uneaten food via their rumen fistulas. This procedure was

Table 2. *Daily food offered and consumed, volatile fatty acids (VFA) infused, faecal excretion of dry matter (DM) and acid detergent fibre (ADF), and daily intake of metabolizable energy (ME)*

(Mean values are given for three cows receiving a pelleted basal ration or having 15·4% of ME replaced by acetic or propionic acid)

Treatment	Basal ration offered (kg)	VFA (g)	Basal ration consumed (kg)	Faecal excretion of DM (kg)	Faecal excretion of ADF (kg)	ME intake (Mcal)
Basal	11·22	0	10·70	2·83	1·03	31·30
Basal + acetic acid	8·90	1427	8·12	2·07	0·72	29·18
Basal + propionic acid	9·12	1043	8·21	1·95	0·65	30·10
SE of means	—	—	0·43	0·22	0·06	0·43

Table 3. *Intake of energy and loss of energy (Mcal/day) in faeces, urine, methane and heat lost in milk*

(Mean values are given for three cows receiving a pelleted basal ration or having 15·4% of metabolizable energy replaced by acetic or propionic acid)

Treatment	Gross energy intake	Faecal output	Urine output	Methane production	Heat production	Milk energy	Body tissue energy balance
Basal	47·63	13·25	1·73	1·33	17·23	10·47	+3·62
Basal + acetic acid	40·85	9·65	1·25	0·83	16·29	9·42	+3·41
Basal + propionic acid	41·82	8·30	1·45	1·10	17·14	7·56	+6·27
SE of means	0·50	0·87	0·54	0·88	0·71	0·71	0·52

discontinued when it was found that progressively more food was left uneaten. The infusion of VFA did not seem to influence the digestibility of the basal ration, as the apparent digestibility of dietary dry matter (excluding infused VFA) was almost the same with each treatment.

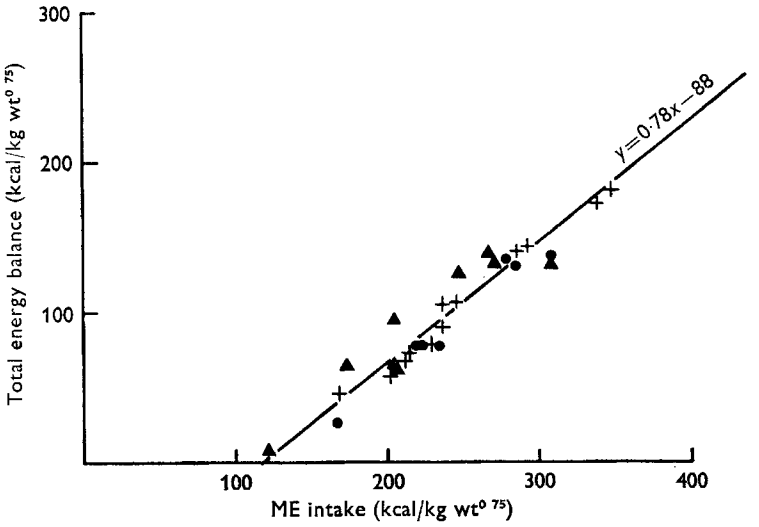

Fig. 1. Relation between energy balance (milk+body tissue) and intake of metabolizable energy (ME) for cows receiving a basal ration (+) or having 15·4% of ME replaced by acetic acid (▲) or propionic acid (●).

Table 4. *Intake of nitrogen and nitrogen loss in faeces, urine and milk (g/day)*

(Mean values are given for three cows receiving a pelleted basal ration or having 15·4% of metabolizable energy replaced by acetic or propionic acid)

Treatment	Nitrogen intake	Faecal nitrogen	Urinary nitrogen	Milk nitrogen	Nitrogen balance
Basal	333	96	116	101	+20
Basal+acetic acid	253	69	91	69	+14
Basal+propionic acid	234	65	65	72	+32
SE of means	11	5	4	14	13

Energy balance. Table 3 summarizes the energy balance of the animals. There were differences in the gross energy intake partly because of uneaten food but mainly because the infused VFA were assumed to be 100% digestible and substituted on a ME basis. The differences in faecal energy were similar in magnitude to the differences in faecal dry-matter excretion. The reduction in energy losses as urine and methane when VFA were infused, although not statistically significant, reflect the decreased intake of fermentable feed. There were no significant differences in heat production. The energy secreted as milk, expressed as a percentage of intake, was greater when acetic acid was infused than with propionic acid infusion, whereas in tissue energy balance the reverse was found. In Fig. 1 the ME intakes expressed as kcal/kg$^{0.75}$ have been plotted against total energy balance (milk+body tissue), also expressed as kcal/kg$^{0.75}$.

Table 5. *Yields of milk and solids-corrected milk (SCM), yields and content of milk fat, solids-not-fat (SNF) and protein and milk energy content*

(Mean values are given for three cows receiving a pelleted basal ration or having 15·4% of metabolizable energy replaced with acetic or propionic acid)

Treatment	Milk yield (kg/day)	SCM (kg/day)	Milk fat		SNF		Protein		Energy (kcal/g)
			g/day	%	kg/day	%	g/day	%	
Basal	19·19	13·91	357	1·96	1·67	8·64	612	3·19	0·55
Basal + acetic acid	15·58	12·18	376	2·58	1·33	8·53	489	3·15	0·61
Basal + propionic acid	14·65	10·13	254	1·92	1·24	8·35	450	3·05	0·54
SE of means	2·58	1·18	21	0·33	0·20	0·23	90	0·09	0·03

Table 6. *Composition of the fatty acid fraction of the milk fat (g/100 g total fatty acids), assuming that the C4 to C10 acids constitute 10% of the total*

(Mean values are given for three cows receiving a pelleted basal ration or having 15·4% of metabolizable energy replaced by acetic or propionic acid)

Treatment	C12:0	C14:0	C14:1 and C15:0	C16:0	C16:1	C18:0	C18:1	C18:2
Basal	1·75	9·83	4·11	26·60	2·63	4·28	35·11	5·54
Basal + acetic acid	2·29	13·13	3·21	34·82	3·61	3·32	25·95	3·68
Basal + propionic acid	3·25	12·37	2·88	28·08	2·42	5·68	30·96	4·33
SE of means	0·17	1·29	0·27	2·14	0·62	0·71	2·32	1·36
Fasting (single value)	0·70	2·50	1·40	24·90	3·40	8·30	46·10	2·70

Table 7. *The pH, total volatile fatty acid (VFA) concentration and molar percentages of VFA in rumen fluid, and the concentration of ketone bodies in blood drawn from the mammary vein*

(Mean values are given for three cows receiving a pelleted basal ration or having 15·4% of metabolizable energy replaced by acetic or propionic acid)

Treatment	Rumen fluid								Blood ketone bodies (mg acetone/100 ml)
	pH	VFA (m-equiv./l.)	Acetic acid	Propionic acid	Isobutyric acid	Butyric acid	Isovaleric acid	Valeric acid	
Basal	5·52	123·2	54·4	28·5	1·6	11·6	1·5	2·3	8·3
Basal + acetic acid	5·67	143·6	70·8	17·4	8·7	0·7	1·5	1·5	12·4
Basal + propionic acid	5·36	88·5	49·6	44·0	1·0	3·0	1·4	1·2	6·6
SE of means	0·41	30·6	4·4	3·2	0·3	2·7	0·7	0·7	1·1

There was a highly significant ($P < 0.001$) linear relationship, $y = 0.78x - 88$, where y = total energy balance, expressed as kcal/kg$^{0.75}$ and x = ME intake in kcal/kg$^{0.75}$. The standard error of the regression coefficient was ± 0.04 and the correlation coefficient $r = 0.96$.

Nitrogen balance. The differences in nitrogen intake (Table 4) reflect differences in intake of the basal ration. The urinary excretion of nitrogen was greater when acetic acid was infused than when propionic acid was infused so more nitrogen was retained in the latter treatment, though not significantly so.

Milk yield and composition. In Table 5 the mean values for milk yield and composition have been summarized. The yield of milk was highest when the basal ration was fed. Between the VFA treatments the yields were slightly, but not significantly, greater when acetic acid was infused. This difference was accentuated when expressed as solids-corrected milk because the milk-fat percentage was highest when acetic acid was infused. There were no differences in milk-fat percentage between the basal ration and the propionic acid treatments. The energy content of the milk reflected differences in the milk-fat percentage.

Milk fat composition. The milk fat composition is given in Table 6. Increases in the C12:0 and C14:0 fractions occurred with both acetic and propionic acid infusions. The most noticeable differences were in the increases in the C16:0 and concomitant decreases in the C18:1 fraction when acetic acid was infused. During period 3, when cow 445 was taken off experiment, a fasting metabolism trial was conducted with her and the milk fat compositions have been included after 5 days of fasting.

VFA composition. Table 7 shows the concentration and proportions of VFA in the rumen liquor and the concentration of ketones in the blood. As would be expected, there were large increases in the proportions and concentrations of the acids that were infused. The proportion of butyric acid on the propionic acid treatment was very low, resulting in the production of a negative value for the missing plot of cow 445 in period 3. Blood ketone concentration was greatest when acetic acid was infused.

DISCUSSION

The experiment reported has obvious shortcomings, mainly due to the small number of animals studied and to the difficulty of constructing a design which would be completely balanced. These limitations were partly due to the amount of work involved in respiration trials and also to the difficulty of having cows successfully fistulated. The results therefore must be considered as a first modest contribution towards solving a very complex problem in the nutrition of the dairy cow; conclusions reached in the following discussion might well be modified when more information of this type becomes available.

Effect of VFA on energy utilization. No difference in energy utilization by the cows could be detected between the treatments, and there was no suggestion of a lower efficiency of utilization of acetic acid than of the metabolizable energy of the basal ration or of propionic acid. This is illustrated in Fig. 1, where the energy balance has been plotted against the metabolizable energy intakes per unit of the body-weight$^{0.75}$.

The figure includes the values for cow 465 and shows the mean values in each of the twenty-eight collection periods. Despite the fact that the molar proportion of acetic acid in the rumen content varied from 50% when propionic acid was infused to 70% when acetic acid was infused, no difference in the slopes was evident. This suggests that differences in heat production between rations for dairy cows were not associated with an inefficient utilization of acetic acid and is in agreement with results obtained with fattening lambs (Ørskov & Allen 1966*a*, *b*, *c*; Ørskov *et al.* 1966). Though there was no difference in energy utilization between the treatments, there were apparent differences in the partition of dietary energy into milk energy or body storage. The ratio of milk energy to ME intake was higher when acetic acid was infused than when propionic acid was infused, 0·33 as opposed to 0·26, a difference which was statistically significant ($P < 0{\cdot}05$). Rook & Balch (1961) found that milk yield increased when acetic acid, but not when propionic acid, was infused.

These trends are similar to those observed in another experiment conducted at Beltsville (Flatt, Moe, Moore, Hooven, Lehman, Hemken & Ørskov, 1967; Flatt, Moe, Munson & Cooper, 1967) which showed that diets giving rise to a high proportion of propionic acid in the rumen resulted in a greater deposition of tissue energy than diets in which a high proportion of acetic acid was found, although intakes of metabolizable energy were similar.

The changes in blood ketone concentrations were in the direction that might be expected if acetic acid was exerting an apparent ketogenic effect. However, no clinical signs of ketosis were noted in this experiment.

Effect of VFA on the fat content of milk. The effects noted on fat content of milk show a similar trend to those observed by other workers (Tyznik & Allen, 1951; Balch & Rowland, 1959; Rook & Balch, 1961; Stanley *et al.* 1964; Jorgensen *et al.* 1965; Rook *et al.* 1965; Storry & Rook, 1966). This demonstrates that if the molar proportions of the VFA in the rumen fluid on a diet inducing milk of low fat content were changed by infusion to attain molar proportions which are not normally associated with such milk, the fat content is still not raised to normal levels (Storry & Rook, 1966). It has been suggested (Van Soest, 1963*b*) that digestion and absorption of starch in the small intestine may be as effective in depressing the fat content of cow's milk as production and absorption of propionic acid in the rumen. If this theory is extended to suggest that it is the gluconeogenic energy ratio (defined as the energy absorbed as acetic acid and butyric acids in relation to that absorbed as propionic acid and glucose) which is most important, an interpretation of the results may be possible. Recently, experiments have shown that substantial quantities of starch can escape rumen fermentation (Wright, Grainger & Marco, 1966; Karr, Little & Mitchell, 1966). In recent experiments at Beltsville we have used a fermentation balance approach to estimate factors influencing extent of fermentation (Ørskov, Flatt & Moe, 1968). This approach requires calculation, from the VFA proportions found in the rumen, of the amount of methane that would be produced if all digestible carbohydrate were fermented. The calculated methane production was then related to the determined methane production and used as a guide to estimate the extent of fermentation. It was then found that increased level of feeding, increased proportions of concentrate and

decreased particle size all tended to decrease the extent of fermentation and that, with the basal ration used here, about 50% of the digestible carbohydrate escaped fermentation; this observation is in agreement with the results of Karr *et al.* (1966).

If this approach is valid, our results suggest that a recovery of milk-fat percentage could not be expected by restoring the molar proportions of VFA to proportions not normally associated with low milk fat content. This is because the molar proportions of VFA do not represent the same proportions of dietary energy as when the proportions of VFA are measured on, for instance, a hay diet; in that instance the calculated extent of fermentation was much greater (Ørskov *et al.* 1968). The results also indicate that the proportion of the diet digested in the small intestine and absorbed, presumably as glucose, could be a very important contributor to the glucose pool. It is thus possible that any factors which reduce the rate of fermentation or increase the rate of passage of concentrate diets (Van Soest, 1963*b*) are likely to decrease the yield of milk fat while factors which have the converse effects will increase it. The effects of dietary supplements of cod-liver oil (Shaw & Ensor, 1959) and bicarbonate (Emery, Brown & Bell, 1965) on milk fat might well be associated with effects on rate of fermentation. Infusion of propionic acid here did not further depress the fat content of the milk, suggesting a limit to which it can be depressed by these means. The mode of action is likely to be associated with the influence of glucogenic materials on the plasma glyceride level as shown by McClymont & Vallance (1962).

Influence on milk-fat composition. The influence of VFA infusion on the composition of milk fat was similar to that which has been reported previously by other workers (Balch *et al.* 1952; Balch & Rowland, 1959; Storry & Rook, 1966). During fasting the milk-fat composition approached that of body fat.

We wish to thank Mr I. McDonald, of the Rowett Research Institute, for advice and assistance in analysing the results, the staff of the Metabolism Laboratory and of the Biometrical Services Unit, USDA, Beltsville, Maryland, for carrying out the balance trials for routine computations, and the farm staff of the University of Maryland for care of the cows. The authors also wish to acknowledge the advice and encouragement received from Dr C. C. Balch of the National Institute for Research in Dairying in carrying out this work. One of use, E.R.Ø., was supported by grants from the Carlsberg Foundation, NATO Foundation and the Rask Ørsted & Lauritz Andersens Fund.

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Printed in Great Britain