

## The metabolism of oleic, linoleic and linolenic acids by sheep with reference to their effects on methane production

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1. Nine experiments, each with one of six sheep with cannulated rumens given a constant diet of dried grass, were made in which oleic, linoleic or linolenic acid was infused into the rumen and energy and lipid metabolism were measured. One experiment was made in which palmitic acid was given. 2. Judged by changes in the composition of isolated fatty acids, the unsaturated fatty acids were hydrogenated in the rumen. An increase in the excretion of lipid in the faeces occurred when the unsaturated acids were given. The heat of combustion of the faeces increased by  $12.6 \pm 3.0$  kcal/100 kcal fatty acid, of which 94% was accounted for by the additional lipid. 3. Methane production fell when the unsaturated fatty acids were infused, the decreases being  $13.8 \pm 1.6$  kcal  $\text{CH}_4$ /100 kcal oleic acid,  $14.2 \pm 1.5$  kcal  $\text{CH}_4$ /100 kcal linoleic acid and  $16.4 \pm 1.3$  kcal  $\text{CH}_4$ /100 kcal linolenic acid. The introduction of a double bond into an *n*-alkyl acid was calculated to reduce methane production by  $0.24 \pm 0.09$  moles/mole double bond. 4. Because the depression of methane production on infusing the fatty acids exceeded the increase in the heat of combustion of the faeces, the metabolizable energy of the fatty acids was  $104.1 \pm 5.3$ % of their heat of combustion. 5. The efficiencies with which the fatty acids were used to promote energy retention were  $74.6 \pm 5.7$ % for oleic acid,  $79.2 \pm 2.0$ % for linoleic acid and  $82.5 \pm 3.0$ % for linolenic acid. These efficiencies agreed with those noted in experiments by others with rats, horses and pigs given glycerides, but were higher than those noted by others when glycerides were added to the diets of ruminants.

A part of the methane produced by micro-organisms in the digestive tract of ruminants arises from the reduction of carbon dioxide. This reduction accompanies the oxidation of formic acid in the rumen, indeed formic acid when added to rumen contents *in vitro*, or given to sheep leads to the production of methane, 1 mole formic acid giving rise to 0.25 moles  $\text{CH}_4$  (Carroll & Hungate, 1955; Vercoe & Blaxter, 1965). Since the  $\text{CO}_2$  reduced is identical with the  $\text{CO}_2$  pool of the rumen (Williams, Hoernicke, Waldo, Flatt & Allison, 1963) it is possible that hydrogen acceptors other than  $\text{CO}_2$  added to the rumen might reduce methane production. Accordingly, linolenic acid was given to a sheep by intraruminal infusion and it was found that the methane production of the sheep fell markedly. The fall in methane production, however, was considerably greater than that expected even assuming that all three double bonds of the linolenic acid had been hydrogenated. This paper deals with the primary observations made with linolenic acid and with similar experiments in which oleic and linoleic acid were given to sheep.

### EXPERIMENTAL

*Animals.* Six castrated male sheep each with a permanent cannula inserted in the rumen were used as experimental animals.

*Food and fatty acids.* Artificially dried grass was given as the only solid food. The amounts given were either 900 or 1000 g daily in two meals and in any one experiment

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the amounts were kept constant. This amount of food was calculated to be enough to maintain body-weight. The fatty acids, which were all given by infusion into the rumen, were not pure. The oleic, linoleic and palmitic acids were obtained from Messrs Hopkins & Williams, Chadwell Heath, Essex. The linolenic acid used in the experiment with sheep R was a preparation of linseed oil fatty acids sold as 'linoleic acid so called' (British Drug Houses Ltd, Poole, Dorset). In the experiments in which linolenic acid was given to sheep D, the linolenic acid was obtained from Messrs Price's (Bromborough) Ltd. Our determinations of the fatty acid compositions of these preparations are given in Table 1. The basal diet of grass also supplied fatty acids. The composition of the fatty acids in the grass changed with time, the percentage of linolenic acid falling from 63 initially to 46 over a period of 5 months, with a compensatory increase in palmitic acid and the appearance of fatty acids which were possibly of bacterial origin (16:0 iso, 15:0 and 17:0 acids). The preparations are referred to in the text and tables as oleic, linoleic and linolenic acids.

Table 1. *Composition (% by weight) of the fatty acid preparations\* infused into the rumens of six sheep (A, C, D, E, F and R)*

Acid infused	Oleic acid (C, F)	Oleic acid (A)	Linoleic acid (F, E)	Linolenic acid (D)	Linolenic acid (linseed oil fatty acids) (R)	Palmitic acid (C)
12:0	—	1.5	—	—	—	—
14:0	3.0	3.9	—	—	—	2.9
16:0	5.0	5.6	5.1	3.2	5.8	87.1
16:1	5.7	6.0	—	—	—	—
18:0	—	—	2.0	—	3.2	10.0
18:1	78.8	71.0	12.7	7.4	16.2	—
18:2	7.5	12.0	80.1	16.4	14.5	—
18:3	—	—	—	68.4	60.3	—
20:4	—	—	—	4.4	—	—
Heat of combustion (kcal/g)	—	9.43	9.31	9.31	9.27	Not determined

\* The preparations are given the names under which they were purchased; they were not pure (see above).

*Experimental design.* Each experiment conformed to the same general plan. The sheep was first given the basal diet until accustomed to it (usually for 14 days) when the preliminary control period of 8–21 days commenced. Infusions of fatty acids were started at the end of this control period and continued for 8–24 days. The sheep then received the control diet alone for periods of up to 21 days in a final control period. Table 2 summarizes the disposition of treatments.

In the first seven experiments listed in Table 2, the sheep were confined in respiration chambers throughout. In the remaining six experiments they were transferred to respiration chambers for the final 6 days of each period.

When the sheep were in the respiration chambers, CH<sub>4</sub> and CO<sub>2</sub> production and O<sub>2</sub> consumption were determined. Faeces and urine were also collected quantitatively

during this time. Faeces were also collected from sheep A, E and D during periods when they were not in the respiration chambers. Samples of rumen liquor were taken before, during and after infusion of the fatty acids. The acids were half neutralized with NaOH, emulsified in water using an homogenizer and the emulsions were infused at constant rate into the rumen with a pump (Distillers Company Ltd, Epsom, Surrey). The fluid volume infused was 2 l./24 h.

Table 2. Design of thirteen experiments in which fatty acids were infused into the rumens of six sheep (A, C, D, E, F and R)

Sheep	Acid infused	Nominal* amount of fatty acid infused (g/24 h)	Preliminary control      Experimental      Final control			Respiration chamber measurements made
			(days)			
R†	Linolenic	44	8	10	10	Throughout
	Linolenic	88	10	10	10	
	Linolenic	128	10	10	10	
F	Oleic	74	14	14	None	
C	Palmitic	51	14	14	None	
F	Oleic	77	12	24	12	
		72	12	24	16	
		(low) (high)		(low) (high)		
A	Oleic	30 60	21	21 21	21	During final 6 days of each period
E	Linoleic	30 60	21	21 21	21	
D	Linolenic	30 60	21	21 21	21	

\* Slight variations from these nominal amounts occurred owing to variations in the outputs of the infusion pumps.

† The final control periods in the first and second experiments with this sheep were the preliminary control periods in the 2nd and 3rd experiments respectively.

*Methods.* The respiration chambers of Wainman & Blaxter (1958) were used. Methane was determined by a thermal conductivity method using a commercial instrument (Cambridge Instrument Co.); otherwise all chemical methods used to determine C, N and heat of combustion of food and excreta were those listed by Armstrong, Blaxter & Graham (1958). Cellulose was determined in food and faeces by the method of Crampton & Maynard (1938). The analysis of rumen liquor for steam-volatile acids was according to James & Martin (1952) following separation of the mixed acids as described by Armstrong, Blaxter & Graham (1957). Total lipids were isolated by extraction from food, rumen liquor and faeces with chloroform and methanol (Folch, Lees & Stanley, 1957). With faeces, a preliminary acid treatment was used to liberate fatty acids from soaps and the crude lipid was extracted with ethyl ether and weighed. The lipids were saponified by the method of Kates (1964). The free fatty acids were estimated gravimetrically and by Duncombe's (1963) method. They were esterified (Kates, 1964) and chromatographed in a Pye Argon gas chromatograph. Acids were identified by the use of reference acids and from published data on their retention volumes.

## RESULTS

*Hydrogenation and fate of fatty acids.* Gas chromatography of the fatty acids showed with little doubt, and in agreement with the results of Garton, Lough & Vioque (1961) and Ward, Scott & Dawson (1964), that the unsaturated fatty acids given were hydrogenated. Details of this work have been discussed elsewhere particularly in relation to *trans-cis* isomerization (Czerkawski & Blaxter, 1965), and Table 3 merely illustrates

Table 3. *Composition of the fatty acids in the diet, or in the diet plus the infusion, in the rumen and in the faeces when 60 g linolenic acid were given to sheep D*

Acid	Preliminary control period			During infusion		
	In diet*	In rumen liquor	In faeces	In diet†	In rumen liquor	In faeces
	(g/100 g total fatty acids)					
	Saturated acids mainly of dietary or tissue origin					
12:0	0.0	0.0	0.0	—	0.0	0.0
14:0	1.7	1.6	2.2	0.3	0.4	1.0
16:0	21.2	12.1	20.1	5.9	9.5	10.4
18:0	2.9	44.7	24.4	0.4	38.2	48.3
20:0	0.0	1.0	1.9	0.0	0.0	1.4
	Unsaturated acids mainly of dietary or tissue origin					
16:1	4.5	Trace	—	0.7	0.0	0.0
18:1	3.7	16.1	8.6	7.0	26.4	13.6
18:2	11.7	5.0	6.3	15.7	8.3	4.4
18:3	46.2	5.1	12.8	65.1	5.5	6.3
20:4	4.0	0.0	0.0	4.3	0.0	0.0
	Acids probably of bacterial origin					
14 iso	0.0	0.0	1.3	0.0	0.0	0.0
15 anteiso	Trace	0.0	3.5	0.0	1.3	1.9
15:0	0.0	1.6	3.4	0.0	1.0	2.7
16 iso	2.0	0.0	0.0	0.3	0.0	0.0
17 anteiso	0.0	0.0	5.7	0.0	0.0	3.2
17:0	2.1	2.3	3.8	0.3	0.7	2.8

\* Dried grass.

† Dried grass + 60 g linolenic acid.

the results of one experiment with sheep D given 60 g linolenic acid. The dietary fatty acids during the control period were unsaturated, the 10.6 g supplied containing 46% of linolenic acid. In the rumen the lipids contained 45% of stearic acid and the linolenic acid fell to 5%. In the faeces appreciable amounts of palmitic acid were present as well as branched-chain and unevenly numbered fatty acids, probably of bacterial origin. In the experimental period the total fatty acid intake was 70.9 g of which 65% was linolenic acid. In the rumen a mono-unsaturated C<sub>18</sub> acid mainly in the *trans* form and stearic acid accumulated, and in the faeces an appreciable amount of stearic acid was present. Though these results are given as concentrations

rather than as absolute amounts they show that considerable hydrogenation of linolenic acid took place. Similar results were obtained with linoleic and oleic acids.

The amounts of crude lipid excreted in the faeces, and the concentrations of crude lipid present in the rumen are summarized in Table 4. Infusion resulted in increases in the concentrations of lipid in the rumen. Even when 128 g linolenic acid were infused each day, that is about ten times the amount given in the basal ration, the concentration in the rumen rose from 4.1 to only 7.2 g/l. In all but one of the experiments in which there were small falls when unsaturated acids were infused, increases in the faecal content of lipids occurred. The largest increase followed the giving of 128 g linolenic acid when an additional 22 g crude lipid were excreted in the faeces each day.

Table 4. *Amounts of crude lipid\* in rumen liquor and in faeces of sheep given fatty acids by intraruminal infusion*

Sheep	Acid infused	Amount of acid infused (g/24 h)	Content of crude lipid* in rumen liquor (g/l.)		Content of crude lipid* in the faeces (g/24 h)	
			During control periods	During infusion	During control periods	During infusion
C	Oleic	77	2.40	5.71	27.1	28.4
F	Oleic	74	3.13	4.45	27.2	39.0
A	Oleic	30	1.92	3.46	33.4	33.2
A	Oleic	60	1.92	3.66	33.4	40.7
F	Linoleic	72	2.71	4.22	26.1	32.4
E	Linoleic	60	1.82	3.11	35.9	48.3
E	Linoleic	30	1.82	1.47	35.9	39.4
R	Linolenic	44	5.03	5.85	26.4	26.6
R	Linolenic	88	4.76	5.60	28.1	37.4
R	Linolenic	128	4.07	7.25	28.0	50.0
D	Linolenic	30	1.71	2.11	31.0	33.1
D	Linolenic	60	1.71	2.68	31.0	42.9
C	Palmitic	51	3.20	6.97	36.1	35.4

\* Crude lipid is defined on p. 351.

*Faecal excretion.* Table 5 summarizes the calorific values of the faeces when the acids were infused. In all but one instance, when the smallest amount of linolenic acid was given, an increase in the calorific value of the faeces occurred. The mean increases were 14.1 kcal/100 kcal oleic acid, 13.8 kcal/100 kcal linoleic acid and 11.2 kcal/100 kcal linolenic acid infused. These increases imply a direct proportionality with the amount of fatty acid given. The table shows that this was not always so. Thus when sheep E was given the larger amount of linoleic acid the increase in faecal energy was smaller than when the smaller amount was given. The mean amounts of crude lipid excreted, expressed in the same way and assuming that the faecal lipids had a calorific value of 9.0 kcal/g, were  $9.2 \pm 4.8$  kcal/100 kcal for oleic acid,  $9.9 \pm 6.7$  kcal/100 kcal for linoleic acid and  $14.1 \pm 2.6$  kcal/100 kcal infused for linolenic acid. For all twelve experiments in which unsaturated acids were given, the mean increase in the heat of combustion of the faeces was  $12.6 \pm 3.0$  kcal/100 kcal fatty acid, and the

increase in faecal lipid (assuming a calorific value of 9.0 kcal/g) was  $11.9 \pm 2.1$  kcal/100 kcal fatty acid, suggesting that the major reason for the increase in the calories excreted in the faeces was an increase in their lipid content. This conclusion is supported by the finding that the heat of combustion per g of the dry faeces increased. Thus when linolenic acid was given to sheep R the heat of combustion of the faeces increased from a control value of 5.02–5.03 kcal/g to 5.28 kcal/g when 88 g were given and to 5.45 kcal/g when 128 g were given. Even when 30 g of fatty acid were given increases occurred. The heat of combustion of the faeces of sheep A given oleic acid increased from 4.83 to 4.91 kcal/g, of sheep E given linoleic acid from 4.80 to 5.02 and of sheep D given linolenic acid from 4.85 to 5.05 kcal/g. The nitrogen content of the faeces also increased slightly, mean increases being 25 mg N/day for oleic acid, 12 mg/day for linoleic acid and 4 mg/day for linolenic acid. In the experiments with sheep F given linoleic acid and with sheep C given oleic acid, cellulose in the faeces was determined. An additional 18 kcal cellulose was excreted by sheep F and 12 kcal by sheep C when these fatty acids were given.

Table 5. *Changes in the heat of combustion of the faeces when fatty acids were infused into the rumens of sheep (kcal/24 h)*

Sheep	Acid infused	Amount of acid infused	Mean faecal energy in control periods*	Mean faecal energy during infusion	Change in faecal energy
C	Oleic	690	$795 \pm 19.5$	859	+64
F	Oleic	699	728	813	+85
A	Oleic	280	1198	1241	+43
A	Oleic	552	1198	1334	+136
Mean faecal loss/100 kcal oleic acid†				$14.2 \pm 3.4$	
F	Linoleic	710	$756 \pm 22.0$	825	+69
E	Linoleic	265	1416	1585	+169
E	Linoleic	555	1416	1477	+61
Mean faecal loss/100 kcal linoleic acid†				$14.5 \pm 10.4$	
R	Linolenic	413	$840 \pm 19.5$	833	-7
R	Linolenic	820	$840 \pm 19.5$	898	+58
R	Linolenic	1166	$840 \pm 19.5$	941	+101
D	Linolenic	257	1138	1191	+53
D	Linolenic	562	1138	1334	+196
Mean faecal loss/100 kcal linolenic acid†				$11.2 \pm 4.8$	
Mean faecal loss/100 kcal all fatty acids				$12.6 \pm 3.0$	

\* Where standard errors are given, more than three separate 4-day determinations of the faecal excretion of energy were made. The statistical errors attached to the remaining values are likely to be higher since they are based on one or two independent estimates of the excretion in 4 days (sheep C, F and R) or in 6 days (sheep A, E and D).

† Obtained by regression of changes in amounts in faeces on amounts infused with zero intercept.

These results suggest that the infusion of the fatty acids resulted in an increased loss of energy in the faeces, of which most could be accounted for by lipid material, and that any fall in the apparent digestibility of the carbohydrate or of the protein of the basal ration was small.

*Methane production.* Fig. 1 shows the results of the first experiments referred to on p. 352 which were made with sheep R. Infusion of linolenic acid reduced  $\text{CH}_4$  production, and the more acid given the greater the reduction. Fig. 2 shows the results obtained with sheep F given 72 g linoleic acid and with sheep C given 77 g oleic acid. The fall in  $\text{CH}_4$  production in each instance was to a value about 40% below those noted in the control periods.

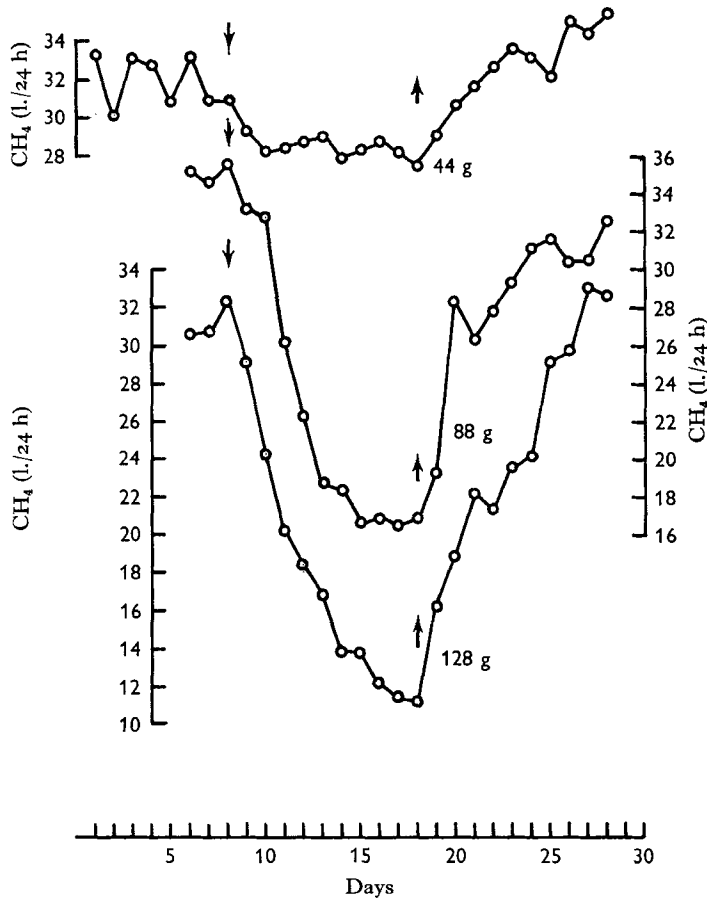


Fig. 1. Effect of infusing three different amounts of linseed oil fatty acids into the rumen on methane production by sheep R. ↓ infusion commenced, ↑ infusion stopped.

It is evident from both Figs. 1 and 2 that the maximal effect on  $\text{CH}_4$  production was not immediately evident and, despite infusion of the acids at a constant rate, at least 8 days elapsed before a stable value was established. In the experiments depicted in Fig. 1, there is a suggestion that the larger the amount of linolenic acid given, the longer it took for  $\text{CH}_4$  production to reach a stable value. Furthermore, on stopping the infusion the return to normal levels of  $\text{CH}_4$  production took a considerable time. With sheep F and C more than 12 days were required to re-establish initial values of  $\text{CH}_4$  production.

Table 6 summarizes the control measurements of  $\text{CH}_4$  production and the final equilibrium values of  $\text{CH}_4$  production after infusion of fatty acids, and in Fig. 3 these values have been plotted against the amount of fatty acid infused. In every experiment  $\text{CH}_4$  production was depressed, in some instances to values as small as a third of the

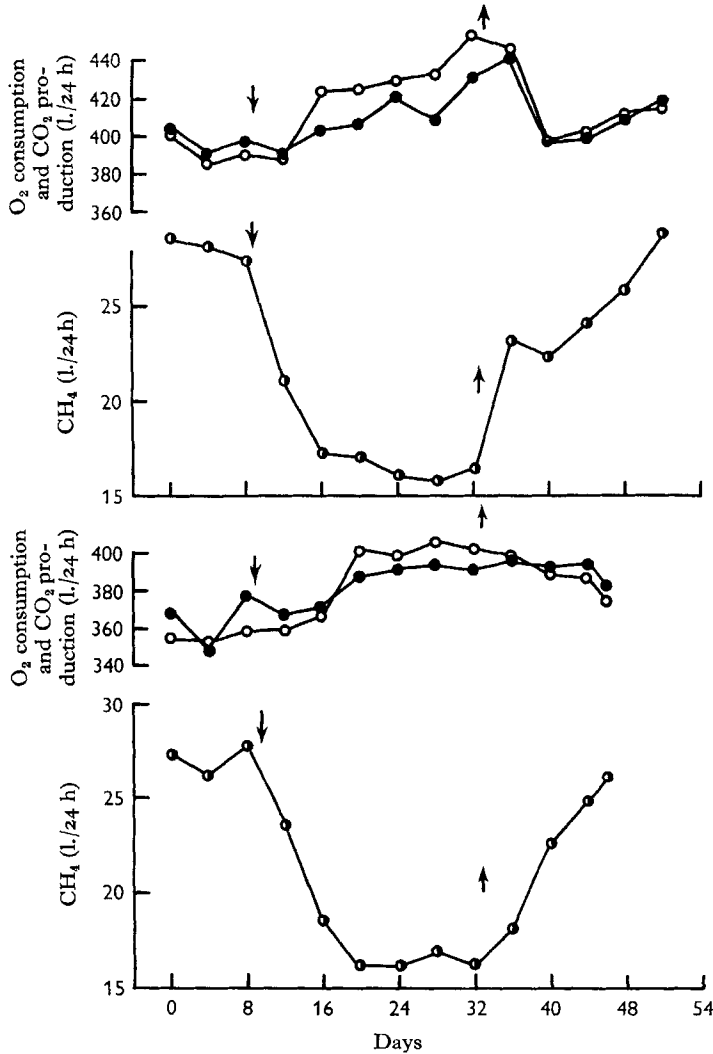


Fig. 2. Effect of infusing linoleic acid or oleic acid into the rumen of sheep on methane production,  $\text{O}_2$  consumption and  $\text{CO}_2$  production. Upper graphs, sheep F given 72 g linoleic acid/day; lower graphs, sheep C given 77 g oleic acid/day;  $\circ-\circ$ ,  $\text{O}_2$  consumption;  $\bullet-\bullet$ ,  $\text{CO}_2$  production;  $\ominus-\ominus$ ,  $\text{CH}_4$  production.  $\downarrow$  infusion commenced,  $\uparrow$  infusion stopped.

original level, and the depressions were broadly proportional to the amounts of acids given. The depressions were on average  $13.8 \pm 1.6$  kcal  $\text{CH}_4$ /100 kcal oleic acid,  $14.2 \pm 1.5$  kcal  $\text{CH}_4$ /100 kcal linoleic acid and  $16.4 \pm 1.3$  kcal  $\text{CH}_4$ /100 kcal linolenic acid. The depression of  $\text{CH}_4$  production tended to increase with increases in the unsaturation



Table 6. Changes in the methane production of sheep when fatty acids were infused into their rumens (kcal/24 h)

Sheep	Acid infused	Amount of acid infused	CH <sub>4</sub> production		
			During control periods	During infusion	Decrease
C	Oleic	690	255.4	155.3	100.1
F	Oleic	699	280.1	183.9	96.2
A	Oleic	280	279.6	271.2	8.4
A	Oleic	552	279.6	193.8	85.8
Mean decrease/100 kcal oleic acid 13.8 ± 1.6					
F	Linoleic	710	266.2	152.7	113.5
E	Linoleic	265	275.4	248.8	26.6
E	Linoleic	555	275.4	208.8	66.6
Mean decrease/100 kcal linoleic acid 14.2 ± 1.5					
R	Linolenic	413	306.0	268.5	37.5
R	Linolenic	820	306.0	159.6	146.4
R	Linolenic	1166	306.0	115.8	190.2
D	Linolenic	257	286.1	267.3	18.8
D	Linolenic	562	286.1	176.5	109.6
Mean decrease/100 kcal linolenic acid 16.4 ± 1.3					
F	Palmitic	479	296.2	183.2	113.0

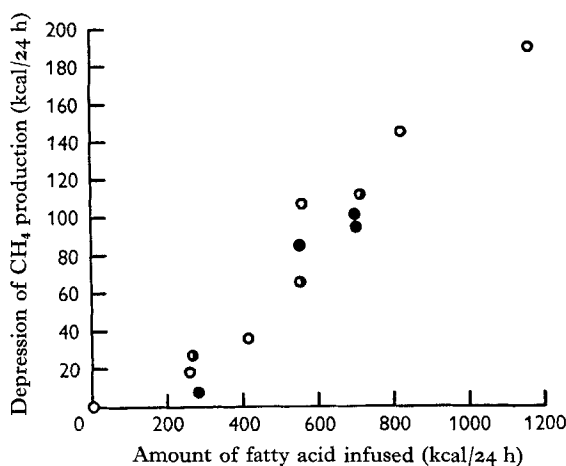


Fig. 3. Relation between the fall in methane production and the amounts of unsaturated fatty acids infused into the rumens of sheep. ●, oleic acid; ●, linoleic acid; ○, linolenic acid.

of the acids infused, but clearly there was no direct proportionality between the number of 'moles of double bond' (sum of products, moles of acid × number of double bonds per acid) given and depression of CH<sub>4</sub> production. When palmitic acid, was infused in one experiment, methane production fell considerably confirming that the effect of long-chain fatty acids on methane production is largely but not entirely independent of their unsaturation.

Table 7. *Changes in energy retention of sheep when fatty acids were infused into their rumens (kcal/24 h)*

	Acid infused	Amount of acid infused	Meta-bolizable energy of infused acid	Energy retained		Increase
				During control periods	During infusion	
C	Oleic	690	723	+238	+77	+533
F	Oleic	699	695	+22	+618	+596
A	Oleic	280	248	-57	+126	+183
A	Oleic	552	536	-57	+254	+311
Mean energy retention/100 kcal metabolizable energy from oleic acid $74.6 \pm 5.7$						
F	Linoleic	710	749	+146	+736	+591
E	Linoleic	265	93	-84	-40	+44
E	Linoleic	555	576	-84	+479	463
Mean energy retention/100 kcal metabolizable energy from linoleic acid $79.2 \pm 2.0$						
R	Linolenic	413	515	+577	1086	+509
R	Linolenic	820	961	+577	1400	+823
R	Linolenic	1166	1292	+577	1631	+1054
D	Linolenic	257	189	+15	136	+121
D	Linolenic	562	469	+15	294	+279
Mean energy retention/100 kcal metabolizable energy from linolenic acid $79.9 \pm 2.7$						

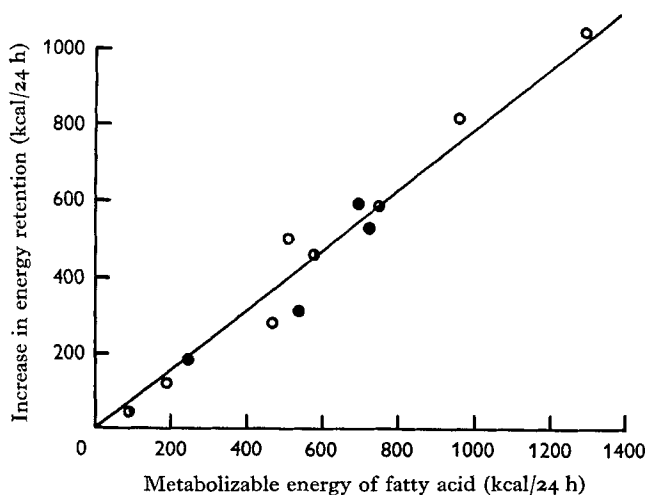


Fig. 4. Relation between increase in energy retention when unsaturated acids were infused into the rumens of sheep and the additional metabolizable energy supplied. ●, oleic acid; ●, linoleic acid; ○, linolenic acid.

*Urine energy and metabolizable energy.* There was no systematic effect of the fatty acids on the heat of combustion of the urine. Small increases were observed in six experiments, small decreases in the remaining seven; mean changes/100 kcal fatty acid infused were all small.

The metabolizable energy of the fatty acids, defined as their heat of combustion less the incremental changes in the heat of combustion of the faeces, urine and CH<sub>4</sub> which occurred when they were given, was  $104.1 \pm 5.3\%$ . No differences between

individual acids were apparent. Clearly the decrease in CH<sub>4</sub> production provoked by the acids compensated for the increase in energy loss in the faeces.

*Heat production and energy retention.* In each experiment heat production calculated from O<sub>2</sub> consumption and CO<sub>2</sub> production using Brouwer's (1965) factors increased when fatty acids were infused, and the increase in O<sub>2</sub> consumption exceeded that in CO<sub>2</sub> production, that is the respiratory quotient fell. The mean of the two energy retentions, one calculated from the heats of combustion of the food and excreta and from the heat production as estimated above and the other from the retentions of C and N by use of the factors of Blaxter & Rook (1953), is given in Table 7. Energy retention increased when the fatty acids were given, the mean increase being  $79.9 \pm 2.7$  kcal/100 kcal metabolizable energy. The mean retentions tended to increase with the unsaturation of the acids from  $74.6 \pm 5.7$  kcal/100 kcal metabolizable energy for oleic acid through  $79.2 \pm 2.0$  for linoleic acid to  $82.5 \pm 3.0$  for linolenic acid. The individual values are shown in Fig. 4 which, as the errors attached to the slopes confirm, show no differences in retention due to unsaturation of the acids.

#### DISCUSSION

Kellner & Köhler (1900) carried out four calorimetric experiments with cattle in which arachis oil was added to a basal ration. In the first two experiments made in 1896-7, the 700 g oil which were added to a basal ration of 9 or 11 kg of mixed feeds were emulsified and no depression of the apparent digestibility of the diet or of CH<sub>4</sub> production was found. In 1897-8 the oil was given to two further oxen in similar amounts without emulsification. Both the apparent digestibility of the diet and CH<sub>4</sub> production fell. With one ox, CH<sub>4</sub> production fell to 53% of the initial values and the heat of combustion of the faeces increased by 52%. Arachis oil was also given to both cattle and sheep by Hoffmann, Schiemann & Nehring (1961) in calorimetric experiments. Methane production increased by 11% with cattle and by 8% with sheep. Losses of energy in faeces also increased and were accounted for by a depression of the digestibility of the basal diet. Fingerling (see Fingerling, Nehring & Franke, 1956) studied the effect of adding 250 g arachis oil to a mixed basal diet of 4.8 kg on the metabolism of a horse and found the energy loss in faeces to increase by 5% and CH<sub>4</sub> production to fall by 15%. The horse produces less CH<sub>4</sub> per unit weight of food than does the ox. Cattle produce about 7 kcal CH<sub>4</sub>/100 kcal feed (Blaxter & Clapperton, 1965) and according to Fingerling's results the horse usually produces less than 3 kcal/100 kcal feed.

The results of the above experiments, with the notable exception of Fingerling's study with the horse (Fingerling *et al.* 1956), are at variance with those we have obtained with long-chain fatty acids. In each of our experiments a decline in CH<sub>4</sub> production occurred, in marked contrast to the increases noted with arachis oil by Hoffmann *et al.* (1961) and the absence of any effect as recorded by Kellner & Köhler (1900) with emulsions of arachis oil. Furthermore, in none of our experiments was a considerable fall in the apparent digestibility of the basal diet observed similar to that noted by Hoffmann *et al.* (1961) with arachis oil and by Franke (1958) with rapeseed

oil. These differences may be related to the mode of administration of the fatty acids compared with the oils or to the possibility that the behaviour of glycerides differs from that of free fatty acids.

The results of the experiment, though they show that the depression of  $\text{CH}_4$  production induced by unsaturated fatty acids is not directly proportional to their unsaturation, nevertheless, suggest that the more unsaturated the acid the greater the depression of  $\text{CH}_4$  production. When the results of the experiments are expressed in terms of molar quantities, they show that the oleic acid preparation given had an unsaturation of 1.00 double bonds/mole and depressed  $\text{CH}_4$  production by 1.70 moles/mole fatty acid. The linoleic acid preparation with a mean unsaturation of 1.72 double bonds/mole depressed methane production by 1.79 moles/mole, and the various linolenic acid preparations with a mean unsaturation of 2.40 double bonds/mole depressed methane production by 2.05 moles/mole. The regression of the depression of methane production on the unsaturation of the acid given suggests that the introduction of a double bond into a fatty acid molecule depresses methane production by  $0.24 \pm 0.09$  moles/mole and that the saturated acid itself would depress it by 1.3 moles/mole. The depression of methane production due to unsaturation is subject to a large error but is consonant with an hypothesis that the double bonds of fatty acids compete with  $\text{CO}_2$  for hydrogen.

Hydrogenation of a single ethylenic bond increases the heat of combustion of a compound with this linkage by 35.6 kcal/mole (Kharasch, 1929). Hydrogenation, if complete, would thus increase the heat of combustion of linolenic acid by 107 kcal or 4%. If the hydrogenation is indeed accompanied by a reduction of  $\text{CH}_4$  production of 0.24 moles/mole double bond when one mole linolenic acid is given,  $\text{CH}_4$  production falls by 156 kcal, whereas the heat of combustion of the stearic acid formed represents a gain of 107 kcal. Nothing, however, is known of the efficiency with which the energy transformations of bacterial hydrogenation and methanogenesis are made.

It is certain that the major reason for the decline in  $\text{CH}_4$  production when unsaturated fatty acids were given was not their unsaturation, an observation borne out by the results of the single experiment with palmitic acid. The depression was clearly not the result of a general depression of fermentation in the gut for cellulose digestion was but little affected even when  $\text{CH}_4$  production was reduced to half the normal amount. This suggests that only the methanogenic flora of the gut is affected. The slow attainment of an equilibrium value of  $\text{CH}_4$  production when fatty acids were infused or when their infusion was stopped further suggests that the effects of the fatty acids were on the growth and survival of the methanogenic organisms. The microbiological aspects of these and later studies will be discussed in a subsequent paper in this Journal.

The efficiency of utilization of the metabolizable energy of the fatty acids for body synthesis was very high, 80% of the metabolizable energy being retained in the body almost entirely, as the N retention values show, as fat. This suggests that the fatty acids once absorbed were not broken down to any appreciable extent but were incorporated directly into the lipids of the tissues.

The efficiency with which the fatty acids were used can be compared with the

results of similar experiments made by others with glycerides. The value of 80% we have found agrees well with values obtained with simple-stomached species. Thus with rats Kriss, Forbes & Miller (1934) found an efficiency of 83.5% and Nehring, Jentsch & Schiemann (1961) one of 83.1%. With pigs, in Fingerling's early work in which arachis oil was given (Fingerling, Köhler & Reinhardt, 1914; Fingerling, Eisenkolbe, Hientsch, Just & Knaut, 1938), a mean value of 84.0% was obtained, and a repetition of these experiments by Schiemann, Hoffmann & Nehring (1961) resulted in a mean efficiency of 85.9%.

Direct determinations of the efficiency of utilization of glycerides for fattening ruminants, however, have given results lower than the value of 80% we have found with fatty acids. Kellner & Köhler (1900), with cattle, found a value of 62.6%, and Hoffmann *et al.* (1961) one of 59.2%. With sheep, Schürch & Jucker (see Heim, 1956) found the efficiency of utilization of the metabolizable energy of arachis oil to be 71.5%, and Hoffmann *et al.* (1961) obtained a value of 57.9%. The experiments of Hoffmann *et al.* (1961) with both sheep and cattle are open to question for the oil caused digestive disturbances. In addition there is indirect evidence to show that the low efficiencies of 60% or so found in all the German studies with arachis oil given to ruminants are underestimates of the efficiency with which lipid is utilized. Thus a regression analysis of Kellner & Köhler's (1900) experiments with cattle given single foods by Hoffmann, Schiemann & Nehring (1960) showed that the energy retained/g lipid (ether extract) apparently digested was 7.40 kcal. A similar analysis of a more recent series of calorimetric trials in which the energy retentions induced by adding cereals or oilseed cakes to the basal diets of sheep and cattle were determined showed that 1 g apparently digested lipid increased energy retention by 7.04 kcal in cattle and by 8.09 kcal in sheep (Nehring, Hoffmann, Schiemann & Jentsch, 1963). Since the metabolizable energy of 1 g digested lipid is about 9.0–9.3 kcal/g, the efficiency of utilization of the metabolizable energy of lipid in these feeds was certainly of the order of 80% and far removed from the values of 57–62% obtained by direct experiment. Further evidence that herbivores utilize the energy of lipids with high efficiency comes from the single experiment made by Fingerling with a horse. The efficiency with which the metabolizable energy of arachis oil was used was 79.5% (Fingerling *et al.* 1956), a value in excellent agreement with our value for fatty acids of 79.9%. Our results and the above interpretation of the experiments in the literature thus suggest that the long-chain fatty acids arising from lipids when absorbed by ruminants are utilized with an efficiency much the same as that found in non-ruminant simple-stomached animals.

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