The effect of linseed oil and of linseed oil fatty acids incorporated in the diet on the metabolism of sheep

By J. W. CZERKAWSKI, K. L. BLAXTER* AND F. W. WAINMAN*

Hannah Dairy Research Institute, Ayr

1. Three sheep were given three diets, a control diet consisting mainly of cereals, a similar diet incorporating linseed oil (mainly glycerides of linolenic acid) and a diet incorporating free fatty acids derived from linseed oil. The diets were given at the maintenance level and at twice this level according to a latin square design. 2. Complete energy balance was obtained and the digestibilities of cellulose and lipid were determined with each sheep receiving each diet. The relative amounts of various fatty acids in the dietary and faecal lipids were also estimated. 3. The inclusion of lipids in the diet resulted in a significant decrease in cellulose digestion, whereas the lipid digestibility increased. 4. The proportion of C_{18} acids increased in the faeces, and the proportion of bacterial fatty acids decreased, but since the total amount of fatty acids excreted increased, the amount of bacterial fatty acids excreted per day did not differ significantly in animals receiving the control diet and the diets containing added lipids. 5. The addition of lipids to the diet resulted in a highly significant drop in methane production, the depression being about 25 % of the control value. The depression (28·9 kcal/100 kcal of fatty acids given) was considerably greater than the depression produced by continuous infusion of the same amount of fatty acids. 6. Because of the depression of CH_4 production and of some reduction in the urinary energy losses, the addition of lipids to the basal diet increased the metabolizable energy by about 9·5 kcal/100 g of the basal diet. 7. The efficiencies of utilization of metabolizable energy were not significantly affected by addition of lipids, being about 82 % for maintenance and 62 % for fattening. 8. In an experiment with one sheep, comparison was made between the effect of slow continous infusion of fatty acids over 24 h and the effect of rapid infusion of the same amount twice daily during feeding. The continuous infusion of linseed oil fatty acids resulted in a drop in CH_4 production of 16·3 kcal/100 kcal of fatty acids given. This was in good agreement with previous findings. Rapid infusion of these fatty acids resulted in a drop in CH_4 production, amounting to 28·0 kcal/100 kcal of fatty acids infused. This is comparable with the reduction of 28·9 kcal/100 kcal fatty acids when the fatty acids were incorporated in the diet.

When fat of plant or animal origin is added to the diets of ruminants, the apparent digestibility of the fibrous components of the diets is usually but not invariably depressed. Relevant experiments have been summarized in papers by Roberts & McKirdy (1964), by Davison & Woods (1960, 1963*a*) and by White, Grainger, Baker & Stroud (1958). Buysse (1962) showed in experiments with sheep that the depression was evident only when more than 30–45 g fat were added to the diet. This represents about 5 % of the diet and it is generally accepted that amounts of fat larger than about 7 % of the diet are not tolerated by ruminants. The effect of fat on cellulose digestion appears most marked with diets of low-quality roughages and the depression is in part reversed by the addition of alfalfa ash or calcium (White *et al.* 1958; Davison & Woods, 1961, 1963*b*; Ward, Tefft, Sirny, Edwards & Tillman, 1957).

When unsaturated long-chain fatty acids were infused at a constant rate into the rumens of sheep given diets of high-quality roughage (Czerkawski, Blaxter & Wain-

man, 1966), it was found that any increases in the energy of the faeces were almost entirely due to increases in their lipid content and that any increase in their cellulose content was very small. In one experiment 128 g linolenic acid were given. This amounted to 14% of the dietary dry matter, and faecal energy increased by only 101 kcal/day, an amount less than that expected from the increase in faecal lipid, and suggesting a slight improvement in the apparent digestibility of the basal diet. Slight improvement of the digestibility of the basal diet by steers had been noted previously by Esplin, Hale, Hubbert & Taylor (1963) when 4% of tallow or of a mixture of vegetable and animal fat was added to the diet.

It has been suggested by Hungate (1962) that CH_4 production is proportional to the total integrated bacterial activity in the rumen. When fatty acids were infused into the rumens of sheep given basal diets of dried grass, there were considerable depressions of CH_4 production with but minor depressions of cellulose digestion (Czerkawski et al. 1966).

The experiments described below were undertaken to find whether reduction in CH_4 output can be produced when lipids are incorporated into entirely different types of diet and the mixture given twice daily. Both glycerides and free fatty acids were used, since it was possible that in some of the feeding trials referred to above, the marked depressions of cellulose digestion might have been due to a slow rate of lipolysis in the rumen, and reflected an effect of glycerides rather than of fatty acids.

EXPERIMENTAL

Animals. Three wether sheep, F, E and B, trained to the respiration chambers, were used to determine the utilization of glycerides and fatty acids incorporated in the diet. A sheep, W, with a rumen fistula was used to study the utilization of linolenic acid given as an infusion.

Table 1. *Chemical composition of the dry matter of the three diets*

Diet	Gross energy (kcal/g)	Crude protein (%)	Carbon (%)	Cellulose (%)	Lipid (%)
Control	4·42	17·8	44·3	13·4	4·0
Containing added glycerides	4·53	16·4	45·2	12·9	8·9
Containing added fatty acids	4·57	17·0	45·8	12·6	8·5

Design. Sheep F, E and B received each of three diets in turn according to a latin square design. Two amounts of the diet were given, one calculated to provide about enough energy to maintain weight and the other twice this amount, the lower amount being given first. The eighteen experimental periods each lasted for 3 weeks. During the last 6 days of each period the sheep were kept in a respiration chamber where faeces and urine were collected and oxygen consumption, carbon dioxide and CH_4 production were determined continuously.

The analyses of the faeces included determination of their dry-matter, carbon, N, cellulose and lipid contents, the individual fatty acids in the lipid and the heat of

combustion of the lipids. Urine was analysed for C and N and its heat of combustion measured. The calorimetric methods and analytical techniques used were those listed by Czerkawski *et al.* (1966).

Diets. A basal mixture of foods was used to make the three diets and consisted of: sugar-beet pulp 30%, decorticated groundnut meal 20%, barley meal 35%, oatmeal 10% and ground oat husk 5%. The high concentration of sugar-beet pulp was included in the basal mixture because, of all the foods examined in calorimetric experiments at this Institute, sugar-beet pulp resulted in the highest production of CH_4 (Blaxter & Clapperton, 1965).

The control diet was this basal mixture to which was added 1·6 kg of a mineral mixture/100 kg, the mineral mixture being 1 kg NaCl, 0·5 kg $CaHPO_4$ and 0·1 kg MgO. The glyceride diet was the basal mixture to which was added 1·6 kg minerals and 5·0 kg linseed oil, and the fatty acid diet was the same as the glyceride diet except that linseed oil fatty acids replaced the linseed oil. All three diets were made into pellets. The composition of the diets and the fatty acid composition of the lipids of the linseed oil and the linseed oil fatty acids and of the lipids of the complete diets are shown in Tables 1 and 2.

Table 2. *Composition of the fatty acids (% by weight) of the linseed oil, the linseed oil fatty acids and of the fatty acids extracted from three diets*

| | | | Fatty acids extracted from: | | |
| | | | | | |
Fatty acid	Linseed oil*	Linseed oil fatty acids†	Control diet	Diet containing added glycerides	Diet containing added fatty acids
14:0	—	—	Trace	0·3	0·2
15:0	—	—	—	Trace	—
16:0	4·5	5·4	11·7	7·4	8·2
17:0	—	—	—	Trace	—
18:0	4·4	4·2	4·0	3·3	2·4
18:1	17·0	17·4	28·8	24·6	18·2
18:2	15·5	13·8	37·8	25·6	25·5
18:3	58·6	59·1	12·7	38·9	34·4
20:0	—	—	Trace	Trace	—
Unidentified‡	—	—	5·0	Trace	11·0

    * Added to the control diet to make the glyceride diet.
    † Added to the control diet to make the fatty acid diet.
    ‡ Probably including some oxidized and polymerized fatty acids.

The amounts of the control diet given were 600 and 1200 g/day and of the diets containing glycerides and fatty acids 630 and 1260 g/day. These last two diets nominally supplied 30 g and 60 g glyceride or fatty acids derived from the additions of linseed oil and linseed oil fatty acids, the amount of basal mixture being the same as for the control diet. The sheep were given the diets in two meals. The pellets, as soon as they were made, were weighed into individual meals, placed in polythene bags and refrigerated at 4°. Samples taken throughout the storage period showed no evidence that any peroxidation of the fatty acids had taken place. When, 3 months after the

completion of the experiment, the analysis of lipid content of the pellets was checked using samples stored under laboratory conditions, the recovery of lipid from the glyceride diet, but not from the fatty acid diet, was found to be about 10 % low. It is thought that some polymerization of the oil had taken place in the interval.

Experiment with infused linseed oil fatty acids. Sheep W was given 900 g dried grass in two meals daily throughout the experiment. After 2 weeks of adjustment to the diet, the sheep was placed in a respiration chamber and metabolism measured daily for 60 consecutive days. Linseed oil fatty acids (60 g/day) half neutralized with NaOH were made into an emulsion in 2 l. water and infused into the rumen during days 9–20 inclusive and from days 37 to 48 inclusive. On the first occasion the fatty acids were infused at the fast rate of 1 l. in 30 min at the same time (05.00 and 16.30 h) that the animal was fed. On the second occasion the fatty acids were infused continuously at a constant rate of 2 l./24 h. The experiment enabled a comparison to be made of the effects of the same amounts of fatty acids given by the same technique either rapidly or slowly. The analytical and calorimetric techniques were those adopted in the experiments with sheep F, E and B.

RESULTS

Apparent digestibility of the diets. Table 3 summarizes the coefficients of apparent digestibility of the dry matter, energy, N, cellulose and lipids of the three diets, the values being means of determinations made at both the low and high levels of feeding. The apparent digestibilities of dry matter, energy and N were very similar for all three diets. With the diets containing added glyceride, however, cellulose was significantly less well digested than cellulose in the control diet, while the digestibility of the total lipid increased. With the fatty acid diet also a fall in cellulose digestion occurred, but addition of fatty acids did not significantly increase the mean apparent digestibility of

Table 3. *Apparent digestibility* (%) *of three diets obtained in experiments with three sheep*

(Each value is the mean of three determinations at a low and three at a high level of feeding)

Diet	Dry matter	Nitrogen	Gross energy	Cellulose	Lipid
Control	82·3	83·9	83·1	63·0	60·2
Containing added glycerides	81·8	81·3	82·2	58·3	75·8
Containing added fatty acids	80·6	82·2	80·0	56·3	62·2
SE of means	±0·68 NS	±1·11 NS	±0·86 NS	±1·82*	±3·38*

NS, not statistically significant.
* Statistically significant from control diet when $0·05 > P > 0·01$.

the total lipid of the diet. This was entirely due to the results obtained with sheep F which apparently digested only 32 % of the total dietary lipid when given the high amount of the fatty acid diet. It digested 65 % of the total lipid when given the low amount of diet.

Composition of the fatty acids from faecal lipid. Table 4 shows the mean percentage composition of the fatty acids isolated from the faecal lipids. There were no systematic

effects of the level of diet and the results are expressed as the means of six experiments. The most obvious change in composition was an increase in the amounts of both saturated and unsaturated C_{18} acids with the diets containing linseed oil glycerides or fatty acids compared with those noted when the control diet was given. In addition, the percentages of uneven numbered and branched-chain acids and of palmitic acid fell with the diets containing added lipids. Since the total amount of fatty acid in the faeces increased with the diets containing added glycerides the amounts of these branched-chain fatty acids which could possibly be of bacterial origin did not change greatly. When the larger amount of the control diet was given, however, 1·4 g of branched-chain and odd carbon number acids were excreted. This amount was considerably greater than the mean of 0·12 ± 0·05 g/day observed when a diet of dried grass was given in previous experiments (Czerkawski *et al.* 1966), a fact which possibly reflects differences in the bacterial flora of the lower gut of animals receiving these very different diets.

Table 4. *Mean percentage composition of the fatty acids isolated from the faecal lipids of three sheep given the three diets*

(The results in each column represent the mean of six experiments*)

Fatty acids	Control diet	Diet containing added glycerides	Diet containing added fatty acids
Saturated acids mainly of dietary or tissue origin†			
14:0	1·6	1·0	2·0
16:0	19·2	15·0	15·2
18:0	32·6	48·3	48·4
20:0	2·8	1·7	1·8
Unsaturated acids mainly of dietary or tissue origin			
18:1	10·9	11·9	14·0
18:2	4·1	5·1	4·4
18:3	0·5	1·3	1·1
20:1	0·0	0·3	0·0
Acids probably of bacterial origin			
14:0 branched	0·2	Trace	0·0
15:0 branched	3·1	1·6	2·2
15:0	5·7	3·3	2·3
16:0 branched	0·9	0·4	0·5
17:0 branched	7·0	3·9	3·4
17:0	4·7	2·6	2·0
Unidentified*	4·1	1·7	1·7

* The sums of the columns do not add to 100 % because very small amounts of C_{12}, C_{13} and acids higher than C_{20} have been ignored.
† Palmitic acid may well be partly of bacterial origin.

Loss of energy as CH_4. Table 5 summarizes the results for the production of CH_4 by the sheep. As expected, the inclusion of sugar-beet pulp in the basal diet resulted in a high production of CH_4. For the control diet at the low level of feeding this was 11·0 kcal CH_4/100 kcal of food given. From the results obtained by Blaxter &

Clapperton (1965) a roughage diet with an apparent digestibility of 82% can be expected to result in production of 8.5 ± 0.8 kcal CH_4/100 kcal food at a maintenance level of feeding, whereas sugar-beet pulp given as the sole constituent of the food can be expected to give a production of 10·8 kcal/100 kcal at the maintenance level of feeding.

The addition of the lipids to the control diet resulted in a highly significant ($P < 0.001$) fall in CH_4 production. The depression was about 25% in both instances. Calculated for 100 kcal added lipid, the depression in CH_4 production was 25·5 kcal/100 kcal with the diet containing added glycerides and 28·9 kcal with the added fatty acids. Both values were considerably greater than the 16 kcal/100 kcal fatty acid noted when linseed oil fatty acids or a similar preparation containing linolenic acid were infused continuously into the rumens of sheep (Czerkawski *et al.* 1966).

Table 5. *Mean amounts of methane produced by three sheep given three diets*

Diet	Level of feeding	CH$_4$ produced (kcal/day)	CH$_4$ produced (kcal/100 kcal food given)
Control	Low	249	11·02
	High	452	10·00
Containing added glycerides	Low	194	7·99
	High	332	6·85
Containing added fatty acids	Low	173	6·99
	High	329	6·65
SE of means	Low	± 5.1**	± 0.22**
	High	± 20.8*	± 0.44*

* Statistically significant when $0.05 > P > 0.01$.
** Statistically significant when $0.01 > P > 0.001$.

Urine energy. The mean heat of combustion of the urine/100 kcal food ingested was 5·51 for the control diet, 4·53 for the diet containing added glycerides and 4·77 kcal/100 kcal food for that containing added fatty acids. The differences between these quantities were statistically significant ($0.05 > P > 0.01$).

Metabolizable energy of the diets. The mean metabolizable energies of the three diets were 67·1, 70·3 and 68·4 kcal/100 kcal food given for the control diet and those containing added glycerides and fatty acids respectively. The metabolizable energy of the total diet was increased by the addition of glycerides but only slightly increased by the addition of fatty acids to the basal diet.

Since each diet contained the same amount of the basal mixture of feeds, that is the difference between them was simply the addition of lipids, the effect of the additions on the loss of energy in faeces, urine and as CH_4 can be calculated directly. This has been done in Table 6 where the determined losses of energy have been expressed per 100 g control diet. Table 6 shows that the faecal loss of energy when the glycerides were added to 100 g basal diet increased by 9·9 kcal. Lipid excretion increased by 4·6 kcal, so it can be concluded that materials with a heat of combustion of 5·3 kcal

were excreted in the faeces as carbohydrate and protein when 5·0 g glycerides were added to 100 g of basal diet. While it is possible that the lipids of the basal diet were less well digested when glycerides were added, it appears probable that the figure of 5·3 kcal/100 g basal diet is a true measure of the depression of the digestibility of the basal diet by the addition of glyceride. Precisely the same depression was noted when fatty acids were added to the diet. Of the increase in the non-lipid energy in the faeces, about 60% was accounted for by cellulose and about 30% by protein. The energy excreted in the urine was depressed by the glyceride and fatty acid additions and so was CH_4 production, the sum of the two depressions being 14·7 kcal/100 g basal diet for the glyceride addition and 14·9 kcal/100 g basal diet for the fatty acid diet. The depression in CH_4 production on adding lipids was considerably greater than the depression of the digestibility of the basal diet, and the addition of the lipids thus increased the metabolizable energy of the basal diet by 9·4–9·6 kcal/100 g.

Table 6. *Mean losses of energy in faeces, urine and as methane per 100 g basal diet given to three sheep in six experiments, and the effects on these losses of adding linseed oil glycerides or fatty acids*

	Diet			Change due to	
Source of energy	Control diet	Diet containing added glycerides	Diet containing added fatty acids	Glycerides (5·0 g)	Fatty acids (5·0 g)
Faeces, total	74·3	84·2	97·7	+ 9·9	+23·4
Lipids in faeces*	14·7	19·3	32·8	+ 4·6	+18·1
Non-lipid in faeces	59·6	64·9	64·9	+ 5·3	+ 5·3
Cellulose in faeces†	20·6	24·2	24·3	+ 3·6	+ 3·7
Protein in faeces‡	13·6	15·1	15·3	+ 1·5	+ 1·7
Other carbohydrates in faeces	25·4	25·6	25·3	+ 0·2	− 0·1
Urine	24·0	20·8	22·1	− 3·2	− 1·9
CH_4	45·7	34·2	32·7	−11·5	−13·0
Metabolizable energy of basal diet	—	—	—	+ 9·4§	+ 9·6§

* 9·0 kcal/g lipid.
† 4·2 kcal/g cellulose.
‡ 30·0 kcal/g N.
§ Algebraic sum of energy losses in non-lipid component in faeces, in urine and as methane, with reversed sign.

Heat production, energy and N retention. Table 7 summarizes the results of the determinations of heat production, energy retention and N retention. Heat production was not significantly affected by the addition of lipids to the diet though at the high level of feeding more heat was produced when lipids were added to the control. Energy retention was significantly greater with the diets containing added lipid than with the control diets. Differences between the diets in the N retention they promoted were not statistically significant. Urinary N excretion was lower in the six determinations made with the diet containing added glycerides and in five of the six determinations with the diet containing added fatty acids than in the six determinations with the control diet; the high errors attached to the retentions of N in Table 7

largely arose from variation in the faecal loss of N on addition of lipids to the control diet.

From the results of the individual determinations of energy metabolism for each diet and determinations of the fasting heat production of the same sheep made on previous occasions, the efficiency of utilization of the metabolizable energy of the diets was calculated. The efficiencies of utilization of energy for maintenance were for the control diet 80·3 %, the diet containing added glycerides 82·9% and the diet containing added fatty acids 82·1 %, each efficiency having a standard error of ± 1·2%. The efficiencies of utilization of metabolizable energy for fattening were 58·5, 62·0 and 63·4% for the control diet and diets containing added glycerides and fatty acids respectively, standard errors of means being ± 3·3%. Differences between the efficiencies of utilization of diets were not statistically significant, but the values were highest with the diets containing added lipids.

Table 7. *Mean heat production, energy and N retentions of three sheep given the three diets, with standard errors of means*

Diet	Level of feeding	Heat produced (kcal/24 h)	Energy retention (kcal/24 h)	N retention (g/24 h)
Control	Low	1793	−300	−1·39
	High	2377	+705	+2·06
Containing added glycerides	Low	1788	−108	−0·26
	High	2416	+1027	+3·53
Containing added fatty acids	Low	1818	−125	−0·21
	High	2487	+985	+4·30
SE of means	Low	±19	±16*	±0·42
	High	±43	±46*	±0·76

* Statistically significant when $0·05 > P > 0·01$.

Rapid infusion and continuous infusion of fatty acids. Fig. 1 shows the daily CH_4 production of sheep W when given the same amount of linseed oil fatty acids either infused with the food or infused continuously into the rumen. Continuous slow infusion resulted in a lower rate of fall in daily CH_4 production than rapid infusion with the food, and the mean amount produced in the period from the 8th to the 12th day of infusion was 20·0 l./24 h, initial values for the 4 days before infusion being 26·6 l./24 h. The fall of 6·6 l. CH_4 or 62·7 kcal from the 384 kcal linseed oil fatty acids given was 16·3 kcal/100 kcal fatty acids infused. Previous work with linolenic acid showed it to depress daily CH_4 production by $16·4 ± 1·3$ kcal/100 kcal linolenic acid (Czerkawski *et al.* 1966). When the linseed oil fatty acids were infused rapidly, at 1 l. in 30 min, CH_4 production fell to the very low level of 10·0 l./24 h, but subsequently recovered to a mean value of 16·1 l./24 h for the last 4 days. The recovery coincided with a change in the sheep's eating behaviour. Individual meals, which normally were quickly consumed within the 30 min during which the rapid infusions were given, were partly refused at this time but were eaten completely later during the day or night. The depression of CH_4 production measured from the last 4 days

of the control period to the last 4 days of the infusion period was 11·37 l./24 h or 107·4 kcal/24 h. The depression was 28·0 kcal/100 kcal fatty acid infused. When linseed oil fatty acids were incorporated in the diet, the experiments with sheep F, E and B showed CH_4 production to be reduced by 28·9 kcal/100 kcal fatty acid.
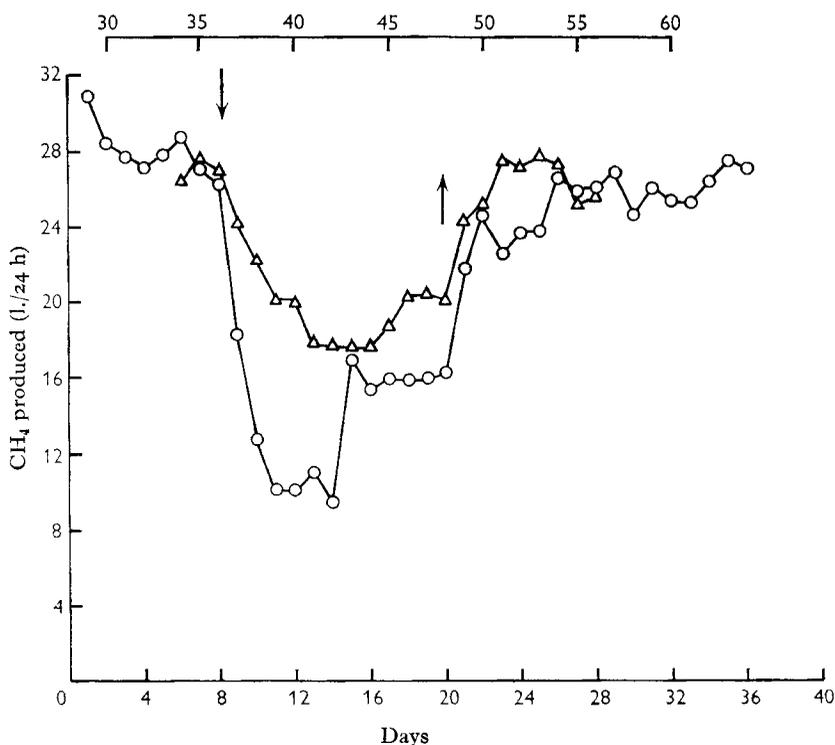

Fig. 1. The effect of infusion of linseed oil fatty acids on methane production in sheep. △—△, slow continuous infusion of 60 g fatty acids/24 h (time scale 30–60 days), ○—○, rapid twice daily infusion of 60 g fatty acids/24 h (time scale 0–40 days). Changes in the sheep's eating habits occurred on the 1st day in the latter experiment.

DISCUSSION

The results of the experiments show that the depression of CH_4 production noted when linseed fatty acids, which largely consist of linolenic acid, were infused into the rumens of sheep receiving diets of dried grass can also be produced by incorporating the acids in a diet consisting mainly of cereals. The depressions of CH_4 production observed when the fatty acids were incorporated in the diet and the diet was given twice daily were much greater than those noted when the fatty acids were infused at constant rates into the rumen of sheep given two meals of dried grass each day. The experiment with sheep W given a diet of dried grass in two meals showed that constant infusion of the fatty acids resulted in a depression of CH_4 production which was indistinguishable from that noted with the same infusion technique and a similar diet in previous experiments (Czerkawski *et al.* 1966), namely 16·0 kcal/100 kcal fatty

acid. Rapid infusion of the same amount of fatty acid at the times the animal was fed, however, resulted in a depression of CH_4 production by 28 kcal/100 kcal fatty acid, a value close to the 28·9 kcal/100 kcal noted when the fatty acids were incorporated in the diet. It thus appears that the depression of CH_4 production is a function of the concentration of fatty acids in the rumen at any one time rather than of the amounts of them that are given over 24 h.

The organism which is of major importance in producing CH_4 in the rumen is a Gram-positive chain-forming short rod *Methanobacterium ruminatium* (Smith & Hungate, 1958; Bryant, 1965). Cellulolytic organisms are, by contrast, either neutral or very weakly positive to Gram's stain (Bryant, 1959). It seems possible that the greater effects of the fatty compounds on CH_4 production than on cellulose digestion reflect differences in the behaviour of Gram-positive and Gram-negative organisms. Reviewing the literature on the effects of fatty acids on the growth of bacteria in vitro, Nieman (1954) concluded that 'generally only Gram-positive organisms are susceptible to the action of fatty acids in minute amounts'. He also concluded that inhibition of growth was caused by both saturated and unsaturated acids, that the effects of un-saturated acids increased with the number of double bonds they contained and that the effects of saturated acids were maximal when there were twelve or fourteen C atoms in the molecule. Such conclusions based on experiments in which the growth of Gram-positive organisms has been measured in vitro, agree with our findings in which the CH_4 production of the sheep has been measured. Direct proof is clearly needed of the hypothesis that the fatty compounds exert their effects by inhibition of growth of Gram-positive methanogenic bacteria while allowing growth of cellulolytic bacteria.

REFERENCES

Blaxter, K. L. & Clapperton, J. L. (1965). *Br. J. Nutr.* **19**, 511.
Bryant, M. P. (1959). *Bact. Rev.* **23**, 125.
Bryant, M. P. (1965). In *Physiology of Digestion in the Ruminant*, p. 311. [R. W. Dougherty, R. S. Allen, W. Burroughs, N. L. Jacobsen and A. D. McGilliard, editors.] London: Butterworths.
Buysse, F. (1962). *Revue Agric., Brux.* **15**, 343.
Czerkawski, J. W., Blaxter, K. L. & Wainman, F. W. (1966). *Br. J. Nutr.* **20**, 349.
Davison, K. L. & Woods, W. (1960). *J. Anim. Sci.* **19**, 54.
Davison, K. L. & Woods, W. (1961). *J. Anim. Sci.* **20**, 532.
Davison, K. L. & Woods, W. (1963a). *J. Anim. Sci.* **22**, 919.
Davison, K. L. & Woods, W. (1963b). *J. Anim. Sci.* **22**, 27.
Esplin, G., Hale, W. H., Hubbert, F. Jr. & Taylor, B. (1963). *J. Anim. Sci.* **22**, 695.
Hungate, R. E. (1962). *The Bacteria.* Vol. 4, Ch. 3. [I. C. Gunsalus and R. Y. Stanier, editors.] London: Academic Press Inc.
Nieman, C. (1954). *Bact. Rev.* **18**, 147.
Roberts, W. K. & McKirdy, J. A. (1964). *J. Anim. Sci.* **23**, 682.
Smith, P. H. & Hungate, R. E. (1958). *J. Bact.* **75**, 713.
Ward, J. K., Tefft, C. W., Sirny, R. J., Edwards, H. N. & Tillman, A. D. (1957). *J. Anim. Sci.* **16**, 633.
White, T. W., Grainger, R. B., Baker, F. H. & Stroud, J. W. (1958). *J. Anim. Sci.* **17**, 797.

Printed in Great Britain