The effect of tertiary branched-chain carboxylic acids on the energy metabolism of sheep

By J. L. CLAPPERTON and J. W. CZERKAWSKI
Hannah Dairy Research Institute, Ayr

(*Received* 29 *December* 1970 – *Accepted* 3 *May* 1971)

1. The introduction of certain tertiary branched-chain carboxylic acids into the rumen of sheep, either by continuous infusion or by direct incorporation of the acids in the concentrate food mixture, had no effect on the oxygen consumption or carbon dioxide production of the sheep. Methane production was depressed, the extent of the depression increasing with the molecular weight of the carboxylic acid.

2. There was a small decrease in the apparent digestibility of the food and a small increase in urinary energy output.

3. There was no significant increase in the metabolizable energy intake of the sheep. There was, however, a small increase in the amount of energy stored in the body. This small increase was about 84% of the gross energy of the branched-chain acids added.

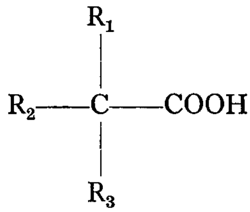
4. It is concluded that any practical advantage to be gained from the addition of branched-chain carboxylic acids to the diet of ruminants remains unproven.

Previous work at this Institute, summarized by Blaxter & Czerkawski (1966), showed that when saturated and unsaturated long-chain fatty acids were added to the diet of sheep, there was a marked reduction in the amount of methane produced by the animals. The extent of this reduction depended on both the chain length and degree of unsaturation of the acids. With saturated C_{18} acids, for example, the dietary addition of 60 g fatty acids/d depressed the methane production by 13 l/d, that is, by 37% of the normal methane production of 35 l/d. The addition to the diet of the same amount of linolenic acid, the most unsaturated C_{18} fatty acid tested, depressed the methane production by 15 l/d. At the same time, the dietary fatty acids had no significant effect on the digestibility of the basal ration and caused only a small increase in the heat production of the sheep. Most of the fat added to the diet was apparently stored in the animal's body. Since branched-chain acids normally occur in the rumen (Garton, 1965), it was decided to study the effects of such acids on methane production. The branched-chain acids that normally occur in the rumen were not available in the quantities required for experiments with sheep, so compounds known as versatic acids were used.

EXPERIMENTAL

Carboxylic acids. The versatic acids used in these experiments were given by Shell Chemicals Ltd. Throughout this paper the acids are referred to by the name versatic and by assigning to each a number equal to the total number of carbon atoms in the molecule. The members of the series investigated were versatic 5, 9, 10, 16 and 15–19.

The acids are synthetic tertiary acids, of the general formula:



The simplest compound in the series is pivalic or trimethylacetic acid. This crystalline solid may be obtained in very pure form (99%). The higher acids in this series are mixtures of isomeric acids in which at least one of the three alkyl groups (R) is a methyl group. For example, the preparation versatic-9 consists of about 56% of 2,2,4,4-tetramethylvaleric acid and 27% 2-isopropyl-2,3-dimethyl butyric acid, the remainder being other isomers. The preparation versatic-10 is a complex mixture of C_{10} isomers (97%), and the preparation versatic-15–19 contains isomers of acids ranging from C_{15} to C_{19}. Versatic-16 is a special preparation consisting mainly of C_{16} carboxylic acids. Because of their structure, the acids are difficult to esterify and, once esterified, are equally difficult to hydrolyse. The above description is based on publications and direct information supplied by Shell Chemicals Ltd.

Table 1. *Heat of combustion of versatic acids* and the corresponding saturated straight-chain fatty acids (kcal/g)*

No. of carbon atoms	Versatic acids	Straight-chain acids	Ratio
5	5·70	6·68	1·17
9	7·79	8·27	1·06
10	8·14	8·48	1·04
15–19	9·18	9·26	1·01
16	9·16	9·36	1·02

* See p. 459.

Since the exact composition of the samples of versatic acids used was not known, the heat of combustion of each sample was determined by igniting it in an adiabatic bomb calorimeter. In Table 1, the values obtained are compared with those for the corresponding fatty acids. Heats of combustion for the latter were obtained from the *Handbook of Chemistry and Physics* (Hodgman, 1962). There were quite large differences between the heats of combustion of the two types of carboxylic acid at low molecular weight, but these differences diminished as the molecular weight of the acids increased. The difference was probably due to the presence of impurities in the versatic acid samples.

Methods. The respiration chamber used was similar to that described by Wainman & Blaxter (1958). The heat production of the animals was calculated from their oxygen consumption, carbon dioxide and methane production and urinary nitrogen output by using the factors of Brouwer (1965). Faeces were collected for 7 consecutive days,

Table 2. *Summary of the experimental arrangements*

Series	Ration	Method of administration of acid	Sheep	Age (years)	Expt no.	Period		Type of carboxylic acid*	Amount of carboxylic acid (g/d)
						No.	Duration (d)		
A	1000 g hay	Infusion into the rumen	2 Down X castrated male	6	1	1	5	—	0
						2	15	Versatic-15–19	6
						3	9	Versatic-15–19	24
						4	13	—	0
					2	1	5	—	0
						2	10	Versatic-15–19	24
						3	12	—	0
B	200 g hay + 800 g cubed mixture	Included in the food	2 Down X castrated male	6	3	1	21	—	0
						2	21	Versatic 15–19	16
					4	1	21	Versatic-15–19	16
						2	21	—	0
C	200 g hay + 800 g High Energy Cubes†	Emulsion added to the food	1 Down X castrated male	6	5	1	21	—	0
						2	21	Versatic-5	10·2
						3	21	Versatic-9	15·8
			1 Cheviot castrated male	5	6	1	21	—	0
						2	21	Versatic-10	17·2
						3	21	Versatic-16	25·6

* See p. 459. † British Oil and Cake Mills Ltd.

including the days in the respiration chamber, and representative samples were subsequently pooled for the determination of dry matter, organic matter, energy and nitrogen. Urine was collected for the whole of the period in the respiration chamber, usually 5 d, and representative samples were combined and analysed for energy and nitrogen. The analytical methods used were those described by Graham, Blaxter & Armstrong (1958).

Plan of experiments. Three series of experiments were carried out. Details of the experimental animals, diets and the method of administering the carboxylic acids are shown in Table 2. In series A, the animals received the diet for 14 d before being put into the chamber. Thereafter, they were in the chamber continuously; in series B and C the duration shown includes the usual preliminary period of 14 d. The acids used in series A were emulsified in water with half their equivalent weight of sodium hydroxide. The emulsion was continuously infused throughout the day into the rumen through a fistula. In series B the carboxylic acids were mixed with the food. The percentage composition of the cubed mixture used in series B was: dried grass meal 48, molassed sugar-beet pulp 20, decorticated groundnut meal 10, oatmeal 10, oat husk 10 and mineral supplement 2. The last contained calcium, phosphorus and magnesium. Part of the mixture was cubed to give the control diet and 2% of versatic-15–19 acids were added to the remainder before cubing to form the carboxylic acid diet. Since the sheep were offered 800 g cubes daily, the intake of additional carboxylic acids was 16 g/d. In series C the animals were given hay and cubes (British Oil and Cake Mills Ltd; High Energy Cubes). The versatic acids were emulsified with half their equivalent weight of sodium hydroxide in water and were added in this form to the cubes shortly before feeding; the cubes were offered to the animals moist. In the periods when no carboxylic acid was given, an equivalent volume of water was added to the cubes. Throughout these experiments, the sheep were fed twice daily at 05.00 hours and 17.00 hours.

Table 3. *Composition of the foods used in the experiments*

			Composition of food (on dry-matter basis)		
Food	Series	Dry matter (%)	Organic matter (%)	Nitrogen (%)	Energy (kcal/g)
Hay	A and B	84·1	90·7	2·03	4·43
Concentrate cube (control)	B	85·5	94·6	2·52	4·47
Concentrate cube containing carboxylic acids	B	85·4	94·9	2·49	4·59
Hay	C	80·9	92·7	1·51	4·49
High Energy Cubes*	C	87·2	88·3	2·49	4·37

* British Oil and Cake Mills Ltd.

The compositions of the foods given in the experiments are shown in Table 3. Judged by their nitrogen contents, the hay samples were of good quality. In series C the addition of versatic acids to the concentrate cube resulted in a small increase in energy content.

RESULTS

Series A. The mean rates of oxygen consumption, of carbon dioxide, methane and heat production and also the calculated respiratory quotients, with their residual standard deviations, for the last 5 d of each period of series A are in Table 4. The infusion of 6 g/d versatic-15–19 had no significant effect upon any of these values. When 24 g of the acids were infused daily there was no effect on the carbon dioxide production, but the oxygen consumption was increased by 3% and the methane production was decreased by 29%. Only the decrease in methane production was statistically significant ($P < 0.01$). The respiratory quotient decreased by 0.05 units, which was consistent with an increased metabolism of carboxylic acids.

Table 4. *Series A: effect of infusion of versatic-15–19 acids* upon the gaseous exchanges, heat production and respiratory quotient* (RQ) *of two sheep*

(Each value is the mean with standard deviation of the results obtained on the last 5 d of each experimental period)

Sheep	Infusion (g carboxylic acid/d)	CO_2 (l/d)	O_2 (l/d)	CH_4 (l/d)	Heat (kcal/d)	RQ
D	0	394.6±9.2	360.5±8.6	33.0±1.5	1834±42	1.095±0.019
	6	398.8±12.9	364.7±6.8	29.6±0.7	1857±41	1.093±0.021
	24	397.0±5.8	373.1±11.8	25.1±0.5	1890±55	1.059±0.018
	0	383.2±7.8	343.3±7.8	31.5±1.0	1759±32	1.117±0.034
E	0	411.3±7.9	396.8±2.7	29.8±0.8	1997±15	1.037±0.021
	24	387.1±9.5	378.6±11.4	18.6±0.7	1904±54	1.022±0.008
	0	382.0±13.9	358.0±11.3	29.6±1.3	1809±58	1.067±0.022
Mean	0	392.8	364.7	31.0	1850	1.079
	24	392.1	375.9	21.9	1897	1.041

* See p. 459.

The detailed results obtained when 24 g versatic-15–19 carboxylic acids were infused into the rumen are shown in Table 5. There was a reduction of 5% in the apparent digestibility of dry matter, organic matter and energy, all these reductions being statistically significant ($P < 0.05$). There were no significant changes in the amounts of nitrogen and energy excreted in the urine. The value for the methane production in the control periods was very close to that predicted by the equation of Blaxter & Clapperton (1965), namely 9.1 kJ/100 kJ food. On this basis, there was a reduction of 33% in the methane production of the sheep when the versatic acids were given. There was a reduction of approximately 1.5% in the metabolizability of the energy of the food when the acids were infused into the rumen. It would appear, therefore, that the increase in faecal energy was very largely compensated for by changes in the methane production and urinary energy. There was an increase of 220 kcal (920 kJ)/d in energy intake, an increase of 179 kcal (748 kJ)/d in faecal energy, a decrease of 31 kcal (129 kJ)/d in urinary energy and, thus, a rise of 97 kcal (405 kJ)/d

https://doi.org/10.1079/BJN19710051 Published online by Cambridge University Press

Table 5. *Series A: effect of an infusion of versatic acids* upon the apparent digestibility of dry matter, organic matter, energy and nitrogen, on urinary nitrogen and on the distribution of energy in urine, methane and the metabolizable energy of the ration*

		Digestibility of					Distribution of energy		
Sheep	Infusion (g carboxylic acid/d)	Dry matter (%)	Organic matter (%)	Energy (%)	Nitrogen (%)	Urine N (g/d)	Urine energy (%)	Methane (%)	Metabolizable energy (%)
E	0	75·7	76·4	72·2	68·6	10·9	6·1	9·2	56·9
	24	71·1	71·7	67·3	61·9	10·4	6·1	5·4	55·8
	0	71·3	72·2	68·2	63·2	12·0	5·7	9·2	53·3
D	0	73·3	74·1	69·6	63·0	10·7	6·4	10·2	53·0
	24	69·7	70·5	65·8	58·8	9·7	6·7	7·3	51·8
	0	74·1	74·3	69·8	66·3	8·3	5·2	9·8	54·8
Mean	0	73·6	74·3	70·0	65·3	10·5	5·9	9·6	54·5
	24	70·4	71·1	66·6	60·4	10·0	6·4	6·4	53·8

* See p. 459.

in metabolizable energy intake when 24 g/d versatic-15–19 acids were infused. Since there was an increase of 47 kcal (196 kJ)/d in the heat production of the sheep, there was, in consequence, an increase of 54 kcal (226 kJ)/d in the energy stored in the body.

Series B. The results obtained in this series of experiments are presented in Table 6. There were no statistically significant effects ($P > 0.05$) upon the apparent digestibility of the organic matter or energy of the food. There was a statistically significant ($P < 0.05$) increase in the urinary energy loss and a statistically significant ($P < 0.01$) fall in methane production when 16 g/d of versatic-15–19 acids were incorporated in the diet. The energy intake increased by 77 kcal (322 kJ)/d, the excretion of faecal

Table 6. *Series B: effect of incorporating versatic*-15–19 *acids* in the food on the proportion of the intake of energy, dry matter and organic matter excreted by various routes* (%)

	Control ration			Carboxylic acid ration		
	Sheep 1	Sheep 2	Mean	Sheep 1	Sheep 2	Mean
Energy lost as:						
Faeces	22.3	23.9	23.1	23.9	24.1	24.0
Urine	3.9	3.8	3.9	5.1	6.1	5.6
Methane	9.7	9.9	9.8	6.4	6.7	6.5
Faecal dry matter	23.0	24.2	23.6	24.1	24.2	24.2
Faecal organic matter	20.2	21.3	20.8	21.9	22.0	21.9

* See p. 459.

energy increased by 53 kcal (222 kJ)/d, methane production fell by 121 kcal (506 kJ)/d, urinary energy increased by 68 kcal (284 kJ)/d, and as a result, metabolizable energy intake increased by 77 kcal (322 kJ)/d. There was a decrease of 4 kcal (17 kJ)/d in the mean heat production and the increase in the amount of energy stored in the body was 65 kcal (272 kJ)/d. This last figure is the average of the energy balance determined directly and that determined from the carbon and nitrogen balances. This increase of 65 kcal (272 kJ)/d in the energy balance is equivalent to 84% of the extra energy that was added to the food in the carboxylic acids. No attempt was made to determine whether, in fact, the acids were absorbed and stored in the body. Czerkawski, Blaxter & Wainman (1966*a*) found a similar value for the retention of energy in the body when straight-chain fatty acids were added to the food.

Series C. The results of the third series of experiments are shown in Tables 7 and 8. The oxygen consumption, carbon dioxide production and methane production (with their standard deviations) are presented in Table 7. Changes in oxygen consumption and carbon dioxide production did not exceed 6 and 7% of the control values respectively. There were, however, no consistent trends in these values with increase in the size of versatic acid molecules and none of these changes was statistically significant ($P > 0.05$). The methane production was inversely related to the molecular weight of the carboxylic acids added to the food. The effects observed with versatic-10 and versatic-16 were statistically significant ($P < 0.05$), but those with versatic-9 just failed to achieve significance at this level.

The values obtained in these experiments for the apparent digestibility of dry matter, organic matter and energy of the food, for the proportion of the energy intake lost as methane and in the urine and for the proportion of the gross energy intake metabolized are presented in Table 8. There were no statistically significant ($P > 0.05$) effects upon apparent digestibility of any components of the food except when versatic-16 was added to the diet, when there was a slight fall in the apparent digestibility of all the components of the ration. As has already been shown above, some of the acids caused a significant fall in methane production but none had any statistically significant effect upon the urinary energy loss or upon the metabolizable energy of the food.

Table 7. *Series C: comparison of the effect of adding emulsified versatic acids* to the diet upon the gaseous exchanges (l/d) of sheep*

(Each value is the mean of results for 5 d with each of two sheep together with its standard error)

	Control	Versatic-5	Versatic-9	Control	Versatic-10	Versatic-16
O_2 consumption	420·0 ±7·5	444·9 ±14·0	431·5 ±10·0	387·9 ±18·4	368·8 ±8·2	405·7 ±19·6
% change from control		+5·9	+2·7		−4·9	+4·6
CO_2 production	426·4 ±5·8	441·6 ±12·0	425·6 ±5·7	397·4 ±14·2	370·2 ±11·0	399·0 ±5·4
% change from control		+3·6	−0·2		−6·8	+0·4
CH_4 production	31·9 ±1·7	33·8 ±1·2	30·6 ±1·2	29·0 ±1·1	24·9 ±1·2	23·1 ±0·5
% change from control		+6·0	−4·1		−14·1	−23·3

* See p. 459.

Table 8. *Series C: effect of adding emulsified versatic acids* to the diet of sheep upon the apparent digestibility of the dry matter, organic matter and energy, upon the losses of energy in urine and as methane and on the metabolizable energy*

(Each value is the mean of two determinations)

	Control	Versatic-5	Versatic-9	Control	Versatic-10	Versatic-16
Organic-matter digestibility (%)	70·5	69·2	72·2	75·8	74·0	72·7
Energy digestibility (%)	68·2	67·4	71·3	74·6	74·2	71·9
Urinary energy (kJ/100 kJ food)	4·3	6·6	5·6	4·9	5·2	5·4
Methane energy (kJ/100 kJ food)	7·9	8·2	7·3	7·4	6·1	5·5
Metabolizable energy (kJ/100 kJ food)	56·0	52·6	58·4	62·3	62·9	61·0

* See p. 459.

Table 9 shows the effect of the different versatic acids used in these experiments on the methane production of sheep (the results being expressed as the fall in the amount of energy lost as methane per 100 kJ of carboxylic acid energy added to the food) together with the corresponding value for stearic and other straight-chain fatty acids obtained by Czerkawski, Blaxter & Wainman (1966*b*). It would appear that the depression in methane tended to increase with the molecular weight of the versatic acid used. The negative value shown for versatic-5 indicates that there was a slight increase in the methane production when this acid was given, but the difference was not statistically significant. The value for stearic acid was 23%, compared with 83% when versatic-15–19 acids were given, indicating that the versatic acids had a much greater effect upon methane production than the corresponding straight-chain fatty acids.

Table 9. *Inhibition of methane production in sheep by versatic acids* and straight-chain acids: a comparison of the effect of chain length and mode of administration*

		Decrease in the energy lost as methane (kJ/100 kJ acid) Method of administration	
Type of fatty acids	Reference	Continuous infusion into the rumen	Incorporation in the food
Versatic-5	Present work	—	−25†
Versatic-9	Present work	—	13
Versatic-10	Present work	—	35
Versatic-16	Present work	—	31
Versatic-15–19	Present work	39	83
Stearic acid	Czerkawski *et al.* (1966*a*)	—	23
Linseed oil fatty acids	Czerkawski *et al.* (1966*b*)	16	29

* See p. 459.
† The negative value indicates that there was a slight increase, but this was not statistically significant.

DISCUSSION

A comparison of the results for the fall in methane production due to the administration of versatic-15–19 acids obtained in series A and B enables a comparison to be made of the effect of infusing the acids and of mixing them with the food. A similar comparison using unsaturated straight-chain fatty acids has been made already by Czerkawski *et al.* (1966*b*). The results obtained by these authors and in the present experiments are included in Table 9. In both experiments the carboxylic acids had a bigger effect upon methane production when mixed directly with the food than when infused continuously.

In the experiments of both series A and B there was an increase in the faecal energy loss when versatic-15–19 acids were added to the rumen. Only in series A was this increase significant statistically ($P < 0.05$). This increase was 81 kJ/100 kJ acid added. Since the rumen contents are in a state of flux it is possible that, when the branched-chain acids are infused slowly, their concentration in the rumen does not increase sufficiently to become toxic to any but the most susceptible micro-organisms. When the fatty acids are ingested with the food, their concentration in the rumen might reach a much higher level, even if only for a short time, and a greater proportion of the micro-organisms might be affected.

There is only one isomer of versatic-5 (trimethylacetic acid), but the number of possible isomers of the other acids increases very rapidly with molecular weight, until, with versatic-16 acids, there are over 2000 possible isomers. Therefore, the chance of the existence of a structure specifically associated with the inhibition of methane production would increase very greatly with the molecular weight of the versatic acids. However, little is known at present about the composition of the complex mixtures of synthetic acids used in these experiments.

The results show that the longer branched-chain carboxylic acids exerted a much larger effect upon the methane production of sheep than saturated straight-chain fatty acids having much the same molecular weight. Like the straight-chain fatty acids, the versatic acids appeared to be more potent inhibitors of methane production when their molecular weight was high. There was some evidence that, unlike the straight-chain acids, the versatic acids tended to depress the apparent digestibility of the food. Thus, there was no significant gain in metabolizable energy intake of the animals following the administration of the versatic acids.

The basal rations used in these experiments were close to maintenance and, since it has already been shown that small amounts of versatic acids added to the food appear to depress the voluntary intake of the animals (Clapperton, 1969), further work will be required before any recommendations can be made with regard to the practical application of these fatty acids in ruminant nutrition.

The authors thank Miss A. T. McKay for skilled technical assistance and Mr D. R. Paterson and Mr J. R. McDill for caring for the animals.

REFERENCES

Blaxter, K. L. & Clapperton, J. L. (1965). *Br. J. Nutr.* **19**, 511.
Blaxter, K. L. & Czerkawski, J. W. (1966). *J. Sci. Fd Agric.* **17**, 417.
Brouwer, E. (1965). *Publs Eur. Ass. Anim. Prod.* no. 11, p. 411.
Clapperton, J. L. (1969). *Proc. Nutr. Soc.* **28**, 57A.
Czerkawski, J. W., Blaxter, K. L. & Wainman, F. W. (1966*a*). *Br. J. Nutr.* **20**, 349.
Czerkawski, J. W., Blaxter, K. L. & Wainman, F. W. (1966*b*). *Br. J. Nutr.* **20**, 485.
Garton, G. A. (1965). In *Physiology of Digestion in the Ruminant* p. 390 [R. W. Dougherty, editor]. Washington: Butterworths.
Graham, N. McC., Blaxter, K. L. & Armstrong, D. G. (1958). *Publs Eur. Ass. Anim. Prod.* no. 8, p. 157.
Hodgman, C. D. (editor) (1962). *Handbook of Chemistry and Physics* 44th ed. p. 1926. Cleveland, Ohio: Chemical Rubber Publishing Co.
Wainman, F. W. & Blaxter, K. L. (1958). *Publs Eur. Ass. Anim. Prod.* no. 8, p. 85.

Printed in Great Britain