Prediction of the amount of methane produced by ruminants

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Metabolizable energy is defined as the heat of combustion of a feed less the heat of combustion of the faeces, urine and gases which are produced when it is eaten. The losses of energy in faeces and urine can be determined easily in sheep and cattle kept in metabolism cages, but to determine the energy they lose as combustible gas, that is as methane, involves quantitative measurement of the gaseous exchange and the use of much more complex and expensive equipment. Understandably, several attempts have been made to predict the losses of energy as methane by cattle and sheep from knowledge of the amount and type of the food they ingest (Kriss, 1930; Bratzler & Forbes, 1940; Swift, Bratzler, James, Tillman & Meek, 1948; Axelsson, 1949). During the course of calorimetric experiments at this Institute many thousands of determinations of methane production by sheep and cattle during 24 h periods have been made. These observations have been used to extend an earlier analysis of the relation between methane production and the type and amount of the diet (Blaxter, 1961).

METHODS

Methane production was determined using closed-circuit respiration equipment (Wainman & Blaxter, 1958*a*, *b*) in which the CO_2 and H_2O produced were absorbed continuously, the O_2 consumed replaced continuously and the CH_4 produced allowed to accumulate. From the volume of the chamber, the estimated volume of the animal, the barometric pressure, and the air temperature and humidity, the total volume of gas in the chamber at s.t.p. was calculated at the beginning and end of each day. CH_4 concentration was determined in samples of gas taken at these times and CH_4 production estimated as the difference between the final and initial volumes of CH_4 at s.t.p. The calorific value of 1 l. CH_4 was taken to be 9·44 kcal throughout. In May 1964 this factor was changed, as the result of a recommendation by Professor Brouwer, to 9·45 kcal (see Brouwer, 1965).

Gas analysis for CH_4 until 1959 was by the Haldane method, in which the gas sample was burned in a gas pipette, and the total contraction in volume and the CO_2 production were determined. After 1959, CH_4 was determined in gas samples, after removal of water vapour and of CO_2, by burning the CH_4 and measuring by thermal conductivity methods the amount of CO_2 formed. The instrument used (Cambridge Instrument Company) was calibrated each day using standard CH_4-free and CH_4-containing gas mixtures, stored in cylinders.

Hydrogen, which on rare occasions arises in metabolic experiments with ruminants

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(Pilgrim, 1948), was not determined routinely. When its presence was thought possible, that is when diets very high in starch content were given, during alimentation, or when an unexpectedly high CH_4 production was noted, gas samples were analysed for H_2 using the Haldane procedure. H_2 was detected in two experiments only and then not on every day of the experiment, nor necessarily in parallel experiments in which other animals were given the same diet. The diets in these two experiments were flaked maize and sugar-beet pulp.

Table 1. *Experiments included in the analysis*

Species	No. of diets*	No. of experiments†	Reference
Sheep	2	12	Blaxter & Graham (1955)
Sheep	3	18	Blaxter & Graham (1956)
Sheep	3	36	K. L. Blaxter & N. McC. Graham (1957) (effects of grinding and pelleting hay, unpublished)
Sheep	1	6	Graham, Wainman, Blaxter & Armstrong (1959)
Sheep	6	64	A. K. Martin (1960) (diets containing groundnut cake, unpublished)
Cattle	1	12	Blaxter & Wainman (1961 b)
Sheep and cattle	1	33	Blaxter & Wainman (1961 a)
Sheep	3	12	Blaxter & Wilson (1963)
Sheep	1	11	J. L. Clapperton (1963) (nutritive value of sugar-beet pulp, unpublished)
Cattle	1	20	K. L. Blaxter & F. W. Wainman (1963) (effects of wind and rain, unpublished)
Sheep	1	8	Clapperton (1964 a)
Sheep	1	10	Clapperton (1964 b)
Cattle	3	32	Blaxter & Wainman (1964 a).
Sheep and cattle	6	72	Blaxter & Wainman (1964 b)
Sheep	12	136	Armstrong (1964)
Sheep	6	37	J. L. Clapperton (1964) (nutritive value of silages, unpublished)
Sheep	1	9	Bateman & Blaxter (1964)
Sheep	1	30	K. L. Blaxter, J. L. Clapperton & F. W. Wainman (1964) (metabolism of different breeds of sheep, unpublished)
Cattle	1	54	K. L. Blaxter, J. L. Clapperton & F. W. Wainman (1964) (effects of age on feed utilization, unpublished)
Sheep	1	3	Vercoe & Blaxter (1965)
Total	55	615	

* With some of these diets one level of feeding only was used so that it was impossible to calculate the regression of CH_4 production on level of feeding.

† An experiment consisted of a continued sojourn of an animal in a respiration chamber. Most of them were of 4–5 days' duration but some were for several weeks.

The routine in most of the experiments was to confine the animal in the respiration apparatus for 4–5 consecutive days, during which time it received a constant amount of food each day. CH_4 production was determined daily. The results of many of these experiments have been published and Table 1 lists the investigations included in the analysis. Experiments in which steam-volatile acids or other compounds were infused into the gut through fistulas were excluded. The number of daily determinations analysed was over 2500.

RESULTS

Analytical accuracy. Analysis of a standard gas mixture containing 4·0% CH_4 by the Haldane method resulted in a standard deviation of $\pm 0.05\%$ or a coefficient of variation of $\pm 1.2\%$. Daily analyses (166) over many weeks of a standard gas mixture using the automatic analyser resulted in a slightly higher standard deviation of $\pm 0.08\%$, corresponding to a coefficient of variation of $\pm 1.7\%$. The error attached to a single determination of CH_4 production involving two analyses is thus about $\pm 1.7 - \pm 2.4\%$, if no account is taken of inaccuracies in measuring the temperature and pressure in the apparatus. It is unlikely that these would ever exceed $\pm 1°$ or ± 1 mm Hg; that is, errors due to these causes would be maximally ± 0.4 and $\pm 0.1\%$ respectively. The total instrumental and analytical error involved in a single 24 h determination of CH_4 was thus less than $\pm 3\%$ of the amount determined.

Table 2. *Day-to-day variation in CH_4 production by sheep and cattle irrespective of type of food**

Range of CH_4 production (l./24 h)	Mean CH_4 production (l./24 h)	No. of experiments	Standard deviation (l./day)	Degrees of freedom	Coefficient of variation (%)
		Sheep			
10–20	16·4	17	±1·59	51	±9·7
20–30	23·7	68	±1·95	199	±8·2
30–40	35·9	44	±2·26	129	±6·3
40–50	42·4	31	±2·73	92	±6·4
Mean	29·9	160	±2·15	471	±7·2
Flaked maize alone	23·0	9	±2·84	27	±12·3
		Cattle			
50–100	73·4	15	±7·72	45	±10·5
100–150	124·6	24	±5·65	46	±4·5
150–200	169·7	33	±13·32	99	±7·9
200–300	248·5	15	±12·75	45	±5·1
Mean	154·2	87	±11·11	235	±7·2

* As discussed below, small systematic effects due to the day of confinement in the chamber have been ignored in this table.

The day-to-day variation in CH_4 production in individual sheep. The results of 210 experiments with sheep and 129 experiments with cattle, involving 989 24 h determinations of CH_4 production, were analysed to find the day-to-day variation in CH_4 production of an individual animal when constant feed was given. The results are shown in Table 2. Analyses of variance showed that CH_4 production was 2·2% lower on the 1st day of confinement than on subsequent days, this effect being statistically significant ($P < 0.05$). The reason for this small effect may lie in the high solubility of CH_4 in body fat and the fact that the concentration of CH_4 in chamber air was higher than in the rooms in which the animals were confined before being admitted to the chamber. Ignoring this small systematic effect, the day-to-day variation in CH_4 production by sheep and cattle was $\pm 7.2\%$ of the amount determined. This value is greater than the instrumental error of $\pm 3\%$. With both cattle and sheep the

absolute error attached to a single estimate of CH_4 production increased with increasing CH_4 production, whereas the relative error declined. High relative errors were encountered when diets consisting entirely of flaked maize were given to sheep (Table 2). The error attached to mean values for the CH_4 produced by an animal given constant feed, calculated over 4 days, can be taken to be $\pm 3.6\%$ of the amount present. This includes instrumental error.

Variation in the CH_4 production by the same animal over long periods of time. In twenty-one investigations with sheep and in five investigations with cattle, constant diets were given for periods ranging from 26 to 46 days, and CH_4 production was determined. CH_4 production was recorded over a total period of 941 days involving 448 determinations of the 24 h production. These experiments were analysed separately from the 4- or 5-day experiments recorded above by determining the regression of CH_4 production on time, and the residual standard deviation. None of the five steers showed a change in CH_4 production with time, the mean daily decline in CH_4 production being 0.16 ± 0.19 l. The residual standard deviation was ± 7.1 l./day, that is lower than that noted in 4-day trials. With sheep, however, eight of the twenty-one regression coefficients were significantly ($P < 0.05$) different from zero, all of them being positive. One of the regression coefficients was greater than 1% of the mean daily CH_4 production, all others being less. When results for all the sheep were combined there was no significant trend in CH_4 production and the residual standard deviation was ± 1.96 l./24 h or $\pm 6.1\%$ of the mean amount found. This last error is also slightly lower than that noted in 4- or 5-day trials. The reason for the slight increases in CH_4 production by some sheep on constant diets with time may reflect a slow adjustment of the flora of their alimentary tracts to their diet. In each of these long-term experiments, however, the animals had been accustomed to the diets for at least 2 weeks before measurement began.

Variation between the CH_4 production of different animals given the same feed. In twenty-three investigations two or more sheep were offered the same amount of the same diet and CH_4 was measured for 4 or 5 days. Small refusals of feed sometimes occurred, however, so that intakes were not precisely the same for each animal. For this reason CH_4 productions were expressed as kcal CH_4/100 kcal feed ingested. These observations permit calculation of whether sheep differ one from another in the amount of CH_4 they produce.

An analysis of variance of these results gave the values shown in Table 3. Differences between the amounts of CH_4 produced by sheep given the same amount of the same feed were highly significant statistically. Part of these differences could be due to size or breed of sheep. In a series of thirty experiments each lasting 5 days five sheep of six different breeds differing markedly in size were all given the same feed in amounts which were constant per kg body-weight raised to the power 0.73. An analysis of variance of the results is also shown in Table 3. Differences in CH_4 production between individuals were still apparent but these were unrelated to breed.

From the analyses of variance in Table 3 it can be computed that the animal-to-animal variation in CH_4 production was ± 0.56 and ± 0.59 kcal/100 kcal feed

consumed in the two series. These values are ± 7.2 and $\pm 8.1 \%$ of their respective means.

Effect of amount and type of diet on CH_4 production by sheep. In all investigations to determine the nutritive value of diets as sources of energy the practice in this laboratory is to give the diet in two or more amounts to each of three or four sheep, and to measure CH_4 production for 4 or 5 days on each occasion. In this way, forty-eight diets have been investigated, involving 391 4- or 5-day measurements of CH_4 production. As the amount of feed given to a ruminant is increased, CH_4 production also increases. To examine the relation between the amount of feed consumed and

Table 3. *Variation in CH_4 production by sheep (kcal CH_4/100 kcal feed)*
determined over 4-day or 5-day periods

Source of variation	Experiments in which sheep were given the same amounts of feed irrespective of their size		Experiments in which thirty sheep of six breeds were given the same amount of feed/kg metabolic size ($W^{0.73}$)	
	Degrees of freedom	Mean square	Degrees of freedom	Mean square
Between individual sheep	34	1·608***	29	1·596***
Between breeds of sheep	—	—	5	1·856 NS
Between individual sheep of the same breed	—	—	24	1·536***
From day to day in the same sheep	171	0·348	120	0·131
Coefficient of variation from day to day (%)		± 7.5		± 5.0

NS, not statistically significant.   *** Statistically significant, $P < 0.001$.

CH_4 production, the relation between the percentage of the dietary energy lost as CH_4 and the feeding level was analysed. Feeding level (L) is defined as the amount of feed consumed divided by the amount required when energy retention is zero, that is the amount required at maintenance. Linear regression was used according to the equation

$$CH_4 \text{ (kcal/100 kcal feed)} = a \pm bL,$$

b being the change in methane production per unit increase in feeding level and the a intercept of the equation.

In all forty-eight trials the regression of CH_4 production on feeding level was negative. There is no doubt, therefore, that though the absolute amount of CH_4 produced increases with increased feeding level the increase is at an ever-decreasing rate. CH_4 production/unit feed consumed has also been found to fall with feeding level in experiments with cows by Coppock, Flatt, Moore & Stewart (1965).

The mean CH_4 production expressed as a percentage of dietary energy at the maintenance level—that is, when the feeding level in the equation above is 1·0—was 8·07 kcal CH_4/100 kcal feed with a range from 6·2 to 10·8 kcal CH_4/100 kcal, the lowest value being for a pelleted meadow-fescue grass and the highest for sugar-beet pulp given as sole feed.

The apparent digestibility of dietary energy was determined in all the trials, and

also decreased with increasing feed intake. Apparent digestibility at the maintenance level of nutrition was determined by regression methods for each of the forty-eight diets, the range being from 54·8 for a poor hay to 87·2 for sugar-beet pulp given as the sole feed. Fig. 1 shows CH_4 production/100 kcal feed at maintenance plotted against apparent digestibility at the maintenance level. The diets used were separated into three classes: roughages, including dried grasses, hays and silages (twenty-nine); mixed diets, for the most part containing some hay (eleven); and diets composed of pelleted or milled materials (eight). These three classes are shown in Fig. 1, and Table 4 summarizes the regression equations for each class. No attempt was made to weight the observations according to number of replications in arriving at these regressions.
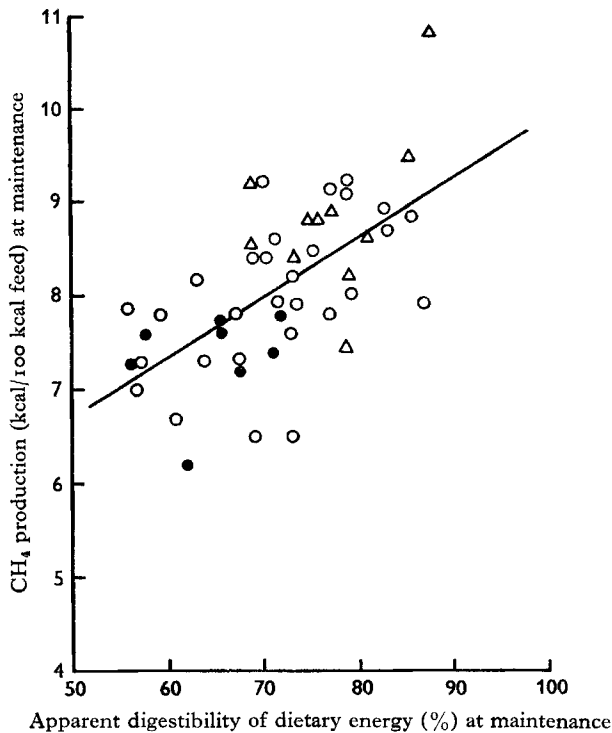

Fig. 1. Relation in sheep and cattle between CH_4 production at maintenance and the apparent digestibility, at maintenance, of dietary energy in: ○, roughages; ●, pelleted or milled feeds; △, mixed diets.

With each class of diet, CH_4 production at the maintenance level (C_m) increased with increased apparent digestibility of the diet (D). The equation for the twenty-nine roughages (Table 4) was not very different from that calculated by Blaxter (1961) from results for twelve of the diets included in this analysis ($C_m = 4\cdot28 + 0\cdot059D$, with a residual standard deviation of $\pm 0\cdot47$). The equation for pelleted diets is that calculated earlier, since no new observations with pellets have been made since 1961).

Analysis of covariance showed that the differences between these regressions were

not statistically significant. Adjusted means obtained by using a common regression coefficient did not differ from class to class of feed. For practical purposes, the equation developed for all diets can therefore be used to predict CH_4 production. The error attached to predicted values is approximately ± 0.7 kcal/100 kcal feed or $\pm 8\%$ of the mean amount of methane produced.

Table 4. *Regressions of CH_4 production (kcal/100 kcal feed) on apparent digestibility of dietary energy (%), both determined at the maintenance level of feeding for different classes of diet*

Diet class	No. of diets	Regression equation*	Residual standard deviation	Statistical significance of regression
Roughages	29	$C_m = 4.67 + 0.047\,D$	± 0.68	$P < 0.01$
Mixed diets	11	$C_m = 3.03 + 0.074\,D$	± 0.84	$P < 0.01$
Pelleted diets	8	$C_m = 6.05 + 0.020\,D$	± 0.53	NS
All diets	48	$C_m = 3.67 + 0.062\,D$	± 0.71	$P < 0.001$

NS, not statistically significant.
* CH_4 at maintenance (kcal/100 kcal) $= C_m$; apparent digestibility (kcal/100 kcal) $= D$.

Table 5. *Regressions of the change in CH_4 production on increasing feeding level by one multiple of maintenance (kcal/100 kcal feed) on apparent digestibility of dietary energy (%) determined at the maintenance level of feeding*

Diet class	No. of diets	Regression equation*	Residual standard deviation	Statistical significance of regression
Roughages	29	$b = 0.028D - 1.03$	± 0.34	$P < 0.01$
Mixed diets	11	$b = 0.054D - 2.26$	± 0.89	NS
Pelleted diets	8	$b = 0.059D - 2.67$	± 0.47	NS
All diets	48	$b = 0.050D - 2.37$	± 0.65	$P < 0.001$

NS, not statistically significant.
* Change in CH_4 production on increasing feeding level by one multiple of maintenance (kcal/100 kcal diet) $= b$; apparent digestibility of dietary energy (%) at maintenance feeding level $= D$.

Table 5 summarizes the results of a similar regression analysis of the change in CH_4 production when feeding level is increased on apparent digestibility at the maintenance level; these results calculated at various values for apparent digestibility have been used to plot Fig. 2. There were no differences between the regressions or adjusted means given in Table 5, and the equation based on all values can be taken to be representative. This equation shows that with poor-quality feeds with a low apparent digestibility, an increase in feeding level has little effect on CH_4 production when it is expressed as a percentage of intake. With high-quality feeds, however, increases in feeding level result in a depression in the amount of CH_4 produced/100 kcal feed consumed. The two equations relating (a) CH_4 production at the maintenance level and (b) the change in CH_4 production with changes in feeding level to apparent digestibility can be combined to give

$$CH_4 = 1.30 + 0.112D - L\,(2.37 - 0.050D),$$

where $D =$ digestibility at the maintenance level of feeding and $L =$ level of feeding
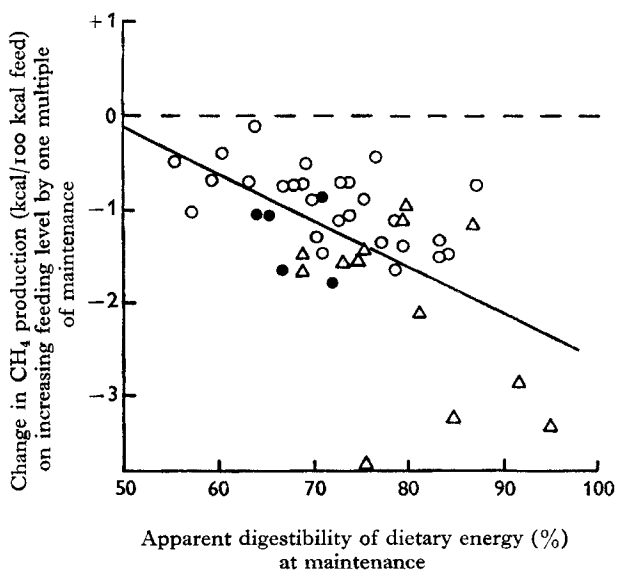
Fig. 2. Relation in sheep and cattle between the change in CH_4 production on increasing the level of feeding by one multiple of maintenance and the apparent digestibility of dietary energy in: ○, roughages; ●, pelleted or milled feeds; △, mixed diets.
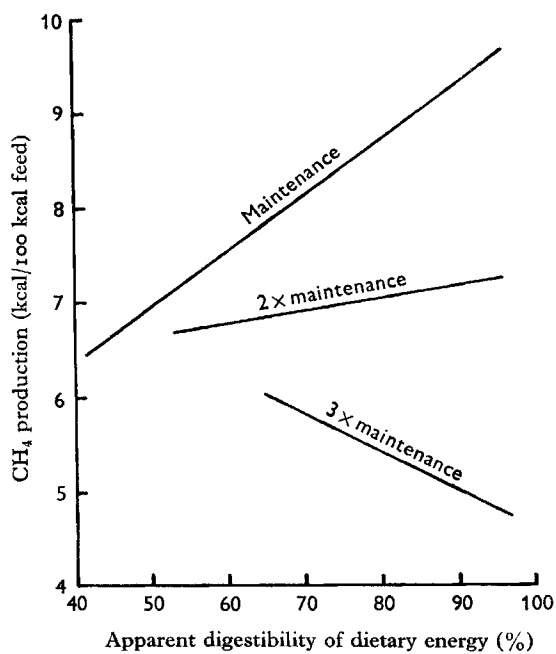

Fig. 3. Estimated CH_4 production by sheep and cattle receiving at different levels feeds of different apparent digestibilities.

as a multiple of the maintenance level. Values obtained with this equation are given in Fig. 3 for three levels of feeding.

Cattle and sheep. When seven different diets were given to both sheep and cattle no significant differences between the two species were found in the amounts of CH_4 produced at the maintenance level (Blaxter & Wainman, 1961 *a*, 1964 *a*).

<div align="center">DISCUSSION</div>

CH_4 is produced by the micro-organisms present in the digestive tracts of ruminants, notably in the rumen, but probably in the caecum also. The day-to-day variation in CH_4 production noted here, which exceeds considerably the analytical error attached to a daily determination, could well arise from changes in the activity of these organisms, but it is more likely to be accounted for by the fact that the greater part of the CH_4 excretion is by eructation (Joyce & Blaxter, 1963; Hoernicke, Williams, Waldo & Flatt, 1965), a process which is not continuous. The evidence for a sequential change in the CH_4 production of some sheep when given constant amounts of a diet of constant composition over long periods suggests, however, that the number or activity of those micro-organisms which produce CH_4 can change. Evidence that CH_4 production in sheep, given either the same absolute amount of feed or the same amount of feed relative to their size, varies significantly from individual to individual also suggests that variations in the methanogenic flora of the rumen can occur. Even so, the extent of these variations is small when expressed as coefficients of variation, for the inter-animal variation and the day-to-day variation each amounts to about $\pm 8\%$ of the amount produced.

There was no doubt that the amount of CH_4 produced was influenced by the diet given. The results show that at the maintenance level of feeding the higher the apparent digestibility of a feed, the greater the CH_4 production/100 kcal feed consumed, and that doubling feed intake depressed CH_4 production more with the high-quality materials than with the low-quality ones. At the maintenance level of nutrition the CH_4 loss was 6·7 and 9·3 % of the energy ingested for feeds with an apparent digestibility of 50 and 90 % respectively. In terms of the apparent digestible energy of feed, CH_4 losses can be calculated to range from 13·4 % for feed with an apparent digestibility of 50 % to 10·3 % for feed with an apparent digestibility of 90 %.

The higher the apparent digestibility of a feed, however, the less of it is required to maintain an animal. An analysis of the effect of feed quality could equally have been based on comparisons made when equal amounts of dry matter were consumed each day; in these circumstances the change in CH_4 production/kg increase in dry-matter consumption could have been the subject of analysis.

The relation between the amount of dry feed, the apparent digestibility of its energy and CH_4 production can, however, be computed from a knowledge of the energy requirements for maintenance (Blaxter, 1964). The results of such a computation are given in Fig. 4. It shows that when the apparent digestibility of feed is low the amount of CH_4 produced, expressed as kcal/100 kcal feed, is virtually independent of the amount of feed, that with small absolute amounts of feed, production of CH_4 rises with

apparent digestibility and with large amounts it tends to fall. The reasons for these relationships probably lie in variations, with amount and type of diet, in the rates of flow of material from the rumen.
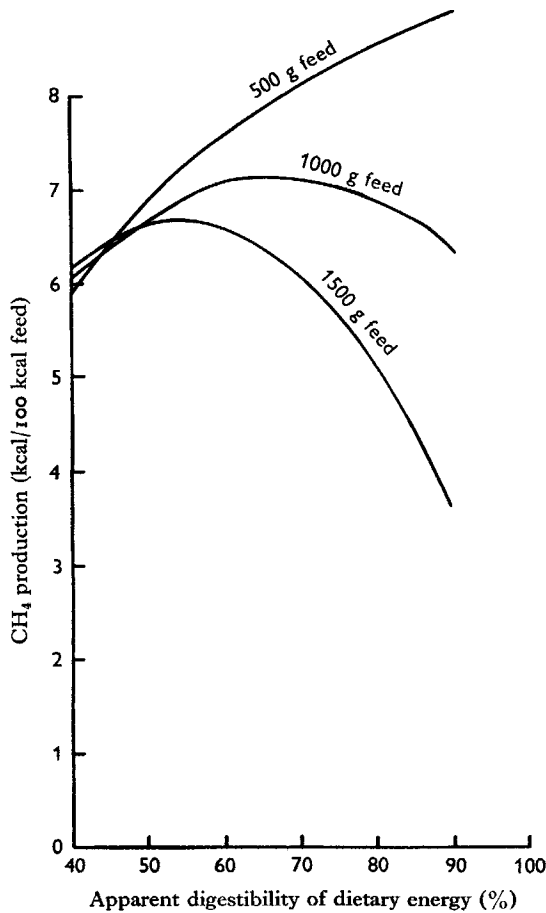

Fig. 4. Estimated CH_4 production by sheep and cattle receiving constant amounts of feed of different apparent digestibilities.

Previous estimates of CH_4 production by ruminants have been based on either the dry matter consumed (Kriss, 1930; Axelsson, 1949) or on the total amount of digested carbohydrate supplied by the diet (Bratzler & Forbes, 1940; Swift *et al.* 1948). The results obtained above suggest that the latter approach is open to error. Thus, since most of the diets given in our experiments varied little in their protein, ash and lipid contents, the amounts of apparently digested carbohydrate at any particular feeding level were much the same for feeds of high and of low digestibility. The CH_4 production with these diets, however, was found to vary considerably.

The same criticism, that no attempt was made to take into account the type of diet, can be levied against those equations based on dry-matter intakes. In addition, there is evidence that the effect of nutritional level on CH_4 production has not been wholly

taken into account. Kriss (1930) showed, for example, that the CH_4 production of cattle was linearly related to dry-matter consumption by the equation

$$CH_4 \text{ (g/day)} = 18 + 22 \cdot 5I,$$

where I is the dry-matter intake (kg/day). On the assumption that the calorific value of the feeds used varied little from 4·4 kcal/g, this equation is reduced to

$$CH_4 \text{ (kcal/100 kcal feed)} = 6 \cdot 8 + (243/x),$$

where x is the number of kcal feed ingested. This equation suggests a much greater constancy of CH_4 production with amount of diet than we have found. Similarly Axelsson (1949) found that with cattle CH_4 production could be predicted from the equation

$$CH_4 \text{ (kcal/day)} = -494 + 0 \cdot 629I - 25 \cdot 0I^2,$$

where I is the dry-matter intake in kg/day. This equation can be reduced to

$$CH_4 \text{ (kcal/100 kcal feed)} = 14 \cdot 3 - (49400/x) - 0 \cdot 0001291x,$$

where x is the number of kcal of feed. This equation suggests that a maximal CH_4 production of 9·2 kcal/100 kcal feed occurs when the animal receives 44 Mcal feed (4 kg), and that it subsequently declines. The range of variation of CH_4 production predicted by this equation is, however, smaller than we have found.

SUMMARY

1. The results of more than 2500 determinations of the 24 h production of methane by sheep and cattle, made incidentally to other experiments, were analysed statistically.

2. Expressed as coefficients of variation, analytical error was less than $\pm 3\%$, day-to-day variation in the same animal $\pm 7\%$ and between-animal variation (sheep) $\pm 7-8\%$.

3. In each of forty-eight trials with different feeds, CH_4 production expressed as kcal CH_4/100 kcal feed fell as the level of feeding increased.

4. At the maintenance level of nutrition, CH_4 production (C_m, kcal/100 kcal feed) varied from 6·2 to 10·8 and was related to the apparent digestibility of the energy of the feed (D) by the equation $C_m = 3 \cdot 67 + 0 \cdot 062D$. The regression was highly significant statistically and the residual standard deviation $\pm 0 \cdot 71$ or $\pm 8\%$ of the mean amounts.

5. The change in CH_4 production when feeding level was increased from maintenance to $2 \times$ maintenance (b, kcal CH_4/100 kcal feed per unit) was also related to the apparent digestibility (D) of the energy of the feed by the equation, $b = 0 \cdot 050D - 2 \cdot 37$. This equation was also highly significant statistically.

6. The two equations show that as the apparent digestibility of feed increases, CH_4 production, expressed as kcal/100 kcal feed, increases markedly at the maintenance level of feeding, but at feeding levels of $3 \times$ maintenance it falls.

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