LXIV. THE FERMENTATION OF CELLULOSE IN THE PAUNCH OF THE OX AND ITS SIGNIFICANCE IN METABOLISM EXPERIMENTS.

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It is well known that in herbivorous animals and especially in ruminants the fermentation processes taking place in the gut are so intensive that they must be taken into account in metabolism experiments if serious errors are to be avoided, but the best methods of measuring the fermentation and of utilising the measurements for correcting the energy balance are still matters of doubt and controversy, though they have been the object of several investigations. We have therefore made a series of experiments which, though they do not solve the problem, will at least make it a little less obscure.

It has long been recognised that the fermentation in the paunch of the ox is mainly a methane fermentation of cellulose. The value of the fermentation from the point of view of the animal's economy lies in the fact, that the wholly indigestible cellulose is converted into substances which can be absorbed and utilised, but this gain is obtained only at the cost of a certain proportion of the energy available in the cellulose. The problem in which we are interested can be formulated as the determination of the loss of energy involved in the fermentation, though the way in which we propose to attack it is an indirect one.

It was shown by Markoff [1913] that in the fermentation cellulose is split up into fatty acids of the average composition of butyric acid, carbon dioxide and methane. From many of Markoff's experiments small quantities of hydrogen are also reported as being present, but their reality appears to us to be extremely doubtful. In many metabolism experiments on cattle [H. Möllgaard and A. C. Andersen, 1917; A. C. Andersen, 1920] and in all our own experiments on the fermentation of material from the paunch no hydrogen was produced, and we must point out that the routine of combustion analysis used in Zuntz's laboratory and adopted by Markoff is very liable to show positive, but spurious hydrogen percentages¹. We are convinced,

¹ The combustion of methane requires a comparatively high temperature, but if the platinum wire in the combustion pipette is too hot methane is dissociated into carbon and hydrogen, and part of the carbon will generally escape combustion. The contraction upon combustion will in such a case be more than double the volume of carbon dioxide formed, and the surplus is set down as hydrogen.

therefore, that hydrogen is not a product of the cellulose fermentation in the paunch.

When the products formed by the fermentation are always the same, viz. CO_2 , CH_4 and $\text{C}_4\text{H}_8\text{O}_2$, there is reason to believe that we have to do with a definite reaction and constant proportions between the products. The reaction might be imagined for instance to take place according to the equation

involving a proportion between the volumes of CO_2 and CH_4 produced as 3/1. The chief object of our experiments has been to study the proportion between the CO_2 and CH_4 formed by the fermentation, to find out whether it is constant or not, and to determine it numerically.

Such a study has been undertaken before by Boycott and Damant [1907] and especially by Markoff [1913], but the results were very disappointing, the relation $\frac{\text{CO}_2}{\text{CH}_4}$ being extremely variable and almost always very high. It can be shown, however, that this is to a great extent due to methodical errors. The fermenting mass in the paunch contains large quantities of carbonates and bicarbonates, especially from the saliva with which the fodder is mixed during rumination. The fatty acids formed will liberate CO_2 from the carbonates, and unless means are found to determine separately the CO_2 formed by the fermentation from organic sources, the ratio of CO_2 to CH_4 will be higher than that demanded by the equation of the reaction. Boycott and Damant made no attempt to distinguish between fermentation CO_2 and carbonate CO_2 . Markoff tried to determine the carbonate separately, but the methods adopted were not sufficiently accurate¹, and nothing definite is known therefore regarding the proportion of CO_2 formed by the fermentation to the CH_4 .

METHODS.

We have used the following technique.

The samples of material were collected by one of us in the Copenhagen public slaughter-houses immediately after the animals had been killed. The cows slaughtered had been fed for one or more days on hay and straw only, and we were likely therefore to find the cellulose fermentation comparatively unmixed with other fermentation processes which might take place in the paunch. In some cases the samples were carried to the laboratory in Dewar flasks, so as to maintain their temperature, but usually they were allowed to cool, as this did not apparently make any difference. In the laboratory the samples were mixed with such an amount either of water or of a suitable buffer mixture ($\frac{1}{4}$ molar phosphates) that it became possible to take out small representative samples. Generally the larger particles of straw had to

¹ In most experiments the samples were sterilised by boiling before the CO_2 was determined. As the contents of the paunch have a high CO_2 tension a very considerable loss could not possibly be avoided.

be removed before taking samples. After thorough stirring, and while stirring was still going on, the samples were taken by means of a small vessel (30 cc. crucible) which was dipped into the mixture. The samples were transferred to fermentation flasks of about 50 cc. capacity, and the exact quantity taken determined by weighing.

The fermentation flasks were provided with a side tube reaching down to the bottom of the vessel and closed by means of a tap. (See Fig. 4, no. 1, in the paper by Krogh [1914, 2].) After weighing, each fermentation flask was connected with a manometer of the type used for microrespiration (Fig. 1, Krogh [1914, 2]), but with somewhat wider tubes, and filled with mercury instead of oil, and put into a water-bath at body temperature, in which arrangements for shaking them vigorously were provided. Before the fermentation experiment could begin, the air in the flasks was washed out by a current of pure nitrogen¹. About 100 cc. of nitrogen were introduced through the side tube, and after bubbling through the mixture taken out through the top of the manometer and collected over mercury in a gas receiver. During this process, which occupied 5–10 minutes, the bottles were shaken vigorously to establish equilibrium between the gas current and the fermenting mixture and especially to drive out all the oxygen. Finally a slight surplus pressure was established, the tap on the side tube closed, the shaking continued for one or two minutes more, a sample of the gas drawn out at the top of the manometer for subsequent micro-analysis [Schmit-Jensen, 1920]², the manometer closed, read and noted together with the barometer, temperature of bath and time of beginning.

The fermentation could now be followed on the manometer, and when a suitable pressure had been reached, the flasks were again vigorously shaken. Immediately afterwards the manometer was read³ and recorded together with the time and the temperature of the water-bath, whereupon a sample of the air in the flask was collected after washing out the manometer tubing by means of part of the excess pressure.

After the sampling of the gas 2 cc. of 20 % hydrochloric acid were introduced through the side tube to stop fermentation and liberate the CO_2 from the mixture, and while a vigorous shaking was kept up, the CO_2 was driven off by a current of air, taken up in baryta solution and determined by titration as described by Krogh [1914, 1]. For the air current the nitrogen (+ CO_2)

¹ We have used nitrogen from air in a gasometer with pyrogallate solution. The generator described by C. van Brunt [1914] is certainly much better.

² This analysis is necessary, because the fermenting mixture always possesses a more or less considerable CO_2 tension. In some cases a trace of oxygen has been left, and in others a slight amount of methane was also present.

³ While the fermentation flask is in a water-bath at 37° moisture is apt to distil up into the narrow tubes of the manometer and collect there in short columns of water, which by their capillary resistance prevent the uniform movement of the column of mercury. To prevent this the fermentation flask was in the later experiments shut off by a clip from the manometer during the whole of the experiment, and connection established only when it was desired to take a reading.

stored since the beginning of the experiment was first used and afterwards supplemented by a suitable amount of CO_2 -free air. In this manner the baryta would absorb all the free and combined CO_2 present in the fermenting mixture before fermentation + the quantity formed during fermentation — the minute quantities present in the gas samples. These were added as a correction.

The quantity of CO_2 present before fermentation was determined in the same manner on separate samples treated just as the others, put into fermentation bottles and placed in the water-bath, but acidified with HCl immediately afterwards.

The gas samples taken at the beginning and end of the fermentation experiment were analysed by the micro-method worked out by Schmit-Jensen [1920] for CO_2 , H_2 , CH_4 and occasionally O_2 , while the remainder was taken to be nitrogen. From the analyses and the pressures and temperatures, the quantity of each gas at the beginning and end of the fermentation was calculated.

We reproduce as an example the protocol and calculation of Exp. 8 B.

Material. From paunch of a single cow, fresh contents of brownish yellow colour, on the whole finely triturated, but containing straw particles of about 3 cm., rather dry, not foaming, alkaline towards litmus, acid to phenolphthalein.

Emulsions for experiments: $\left\{ \begin{array}{l} \text{A. 250 g. material} + 500 \text{ cc. secondary phosphate } (\frac{1}{2} \text{ molar}). \\ \text{B. 250 g.} + 500 \text{ cc. phosphate (7 primary, 3 secondary)}. \end{array} \right.$

Specific gravity of emulsion 1.034.

Sample B. $22.88 \text{ g.} = 22.14 \text{ cc.}$, B^1 (for CO_2 determination) 23.81 g. , B^2 23.81 g.

CO_2 determinations:

B^1 5.02 cc. or 0.211 per g. , B^2 4.76 cc. or 0.200 per g. Average per g. 0.206 cc. B after fermentation 11.83 cc. According to the average of B^1 and B^2 it should have contained 4.72 cc. before fermentation. CO_2 produced by fermentation therefore 7.11 cc.

Volume of fermentation flask B

$59.26 \text{ cc.} + \text{manometer tubing } 0.27 \text{ cc.} - \text{glass beads for shaking } 0.20 \text{ cc.}$

Volume of 1 mm. manometer tubing 0.0009 cc. Volume of compensating bottle 19.95 cc.

Manometer readings.

19'	4 ³⁰ p.m.	138.3	142.8	Diff. $d_4 = 4.5$. T. 37.0° . Barometer, 733 mm.
	8 ⁰⁰ p.m.	122.5	159.0	
	10 ²⁵ p.m.	115.0	166.5	
	11 ⁰⁰ a.m.	101.0	182.0	
20'	1 ³⁰ p.m.	97.5	186.0	Diff. $d_7 = 117.3$. T. 37.5° .
21'	12 ⁰⁰ m.d.	83.2	200.5	

Analyses (corrected for systematic error).

%	Initial sample		Final sample		Average	
	1	2	1	2	Initial	Final
CO ₂	2.0	2.1	5.4	5.5	2.05	5.45
O ₂	0	—	0	—	0	0
H ₂	—	—	—	0	0	0
CH ₄	0	—	8.1	8.4	0	8.25
N ₂	—	—	—	—	97.95	86.3

The total quantity of gas in the apparatus at the beginning and end of the experiments has been calculated as follows: at the beginning we have the gas volume $A = 59.26 + 0.27 - 0.20 - 22.14 = 37.19$ cc. By reducing this to 0° and 760 mm. we get

$$A \frac{273}{273 + t_w} \cdot \frac{P - f_w + d_i}{760} = 37.19 \frac{273}{273 + 37.0} \cdot \frac{733 - 46.5 + 4.5}{760} = 29.75 \text{ cc.}$$

At the end of the experiment we have the total gas volume

$$A_1 = A + \frac{A + C}{2C} v d_f = 37.19 + \frac{37.19 + 19.95}{2 \cdot 19.95} 0.0009 \times 117.3 = 37.19 + 0.15 = 37.34$$

and reduced to 0° and 760 mm.

$$A_1 \frac{273}{273 + t_{w_1}} \cdot \frac{P - f_{w_1} + d_f}{760} = 37.34 \frac{273}{273 + 37.5} \cdot \frac{733 - 48 + 117.3}{760} = 34.67.$$

The initial and final quantities of each gas are found by multiplying the volumes with the corresponding percentages.

cc.	Initial	Final	Produced	Relation $\frac{\text{CO}_2}{\text{CH}_4} = \frac{7.11}{2.86} = 2.49$
CO ₂	(0.61)	(1.89)	7.11	
CH ₄	0	2.86	2.86	
N ₂	29.15	29.9	0.75	

Accuracy and sources of error. The accuracy of our determinations is not very great. It is very difficult to obtain good average samples of materials like the contents of the paunch, and the method finally adopted is the outcome of a number of trials. Even this method, however, is not so accurate as we should desire.

The samples for determining the initial quantity of CO₂ have always been taken and analysed at least in duplicate. Special trials have shown that our CO₂ determinations are not vitiated by fatty acids or HCl being driven over into the baryta. A statistical treatment of the determinations shows that the dispersion of the results from single samples is $\pm 3\%$ with a maximum of 5%. The differences between the initial and final quantities of CO₂, which are affected by these errors, are of the same order of magnitude as the initial quantities themselves, and the mean error on the determination of CO₂ production is in most experiments about $\pm 4\%$.

The gas analyses have been corrected for the systematic errors found by Schmit-Jensen. According to the double determinations made in the course of the present investigation, the mean error of a double determination of CH₄ works out as $\pm 0.12\%$. As the average CH₄ percentage in the samples

is about 7, the percentage error on the quantity of CH_4 works out as 1.7 %. There is a further slight error, due to the determination of total pressure. The mean error on the quantity of CH_4 is therefore about 2 %.

The relation $\frac{\text{CO}_2}{\text{CH}_4}$, which has been the main object of our determinations, is generally found to lie about 2.5. It will of course be influenced by the errors on the CO_2 and CH_4 determinations, and the resulting mean error can be calculated. We have

$$\frac{\text{CO}_2}{\text{CH}_4} = \frac{2.5 \pm 0.1}{1 \pm 0.02}$$

and hence the error

$$\pm \sqrt{2.5^2 \times 0.02^2 + 0.1^2} = \pm \sqrt{0.0125} = \pm 0.11.$$

The determination of total gas pressure has in most of the experiments been rather inaccurate, because drops of condensed water obstructed the tubing between the flasks and the manometer. The error may have amounted to 4 or even 6 mm. or 0.5 to 0.8 % of the total pressure. Such an error has very little influence upon the determination of CH_4 , because the percentage of this gas is always small, but the influence on the nitrogen determination is considerable, and as this determination is further influenced by the sum of the gas analytical errors, the mean error on the difference between the initial and final quantity of nitrogen becomes considerable, viz. about ± 0.25 cc.

EXPERIMENTAL RESULTS.

The results of our experiments are summarised in Table I.

The constancy of the gaseous nitrogen. In Exps. 1, 2, 3, 4, 6 and 7, the gaseous nitrogen remains constant within the limits of error. The average production in one of these experiments is -0.06 ± 0.18 cc. In Exp. 8 A we have no determination of the nitrogen, and the production of 0.75 cc. found in 8 B is probably due to some undetected error. In Exps. 5 A and B we have observed a slight, but probably real, production of gaseous nitrogen. Krogh [1916, p. 53] has found that the presence of nitrates in the fermenting contents of the coecum of rabbits will cause a liberation of free nitrogen. Nitrates or nitrites may, perhaps, have been present in the paunch in this case, though we failed to detect macroscopic remains of beet root, which contains considerable quantities of nitrate¹. In any case we can conclude from our experiments that free nitrogen is neither produced nor absorbed in appreciable amount by the fermentation of cellulose in the paunch.

The production of methane. In column 6 of Table I we have given the average rate at which methane has been produced. This has been figured out per 24 hours and for 100 kg. of undiluted material from the paunch in order to facilitate comparison with the quantities of methane produced in metabolism

¹ According to determinations made in the Danish "Forsøglaboratorium" beet root may contain from 0.05 to 0.15 g. HNO_3 in 100 g. fresh substance. Turnips do not contain any nitrates.

Table I.

No.	Material	Emulsion in	Duration of exp. hours	Nitrogen produced cc.	CH ₄ produced per 100 kg. in 24 h.	Rate of ferment. mm. per hour			CO ₂ CH ₄
						1st day	2nd day	3rd day	
1	Contents of paunch, finely triturated	Secondary phosphate	24	0	54	7, 3.5, 3.5	—	—	2.85
2	Fermentation in paunch very strong. Material moist, fairly well triturated, alkaline to litmus, acid to phenolphthalein	Water	5.5	-0.2	79	18, 19, 17	—	—	2.95
		Secondary phosphate	25	-0.2	19	2.3, 2	—	—	2.7
3	Fermentation not very pronounced. Very finely triturated, alkaline to litmus, acid to p.p. Used after 6 hours	Secondary phosphate	25	0	18	2.3, 2.4	—	—	2.7
4	Fermentation rather weak. Coarse, straw and turnip. Neutral to litmus	7 sec. phosph. + 3 prim. phosph.	14	0	63	10, 17	—	—	2.95
5	Fermentation weak. Finely triturated, very moist. Alkaline to litmus, acid to p.p.	7 sec. phosph. + 3 prim. phosph.	17	-0.2	74	12, 8, 11	—	—	2.2
6	From 5 animals, rich in larger particles, alkaline to litmus, acid to p.p.	7 sec. phosph. + 3 prim. phosph.	15.5	0	75	6, 10, 25	—	—	2.2
		7 sec. phosph. + 3 prim. phosph.	63	+0.6	9	1.9, 2.1	1.1	0.5	(2.8)
		7 sec. phosph. + 3 prim. phosph.	63	+0.6	10	2.7, 2.2	0.8	0.7	(2.2)
		Water	23	+0.1	28	8, 6, 8	—	—	2.4
7	From 13 animals, coarse moist, contains turnips. Turns p.p. slightly red. Used after 6 hours	Water	24	-0.4	28	7, 6, 7	—	—	2.4
		Water + CaCO ₃	65	0	6	2.2	—	1.2	(4.9)
8	From single animal. Fermentation weak. Finely triturated, rather dry. Alkaline to litmus, acid to p.p.	Water + CaCO ₃	66	+0.2	6	2.1	—	1.2	(4.0)
		Secondary phosphate	45	?	17	6, 5, 2	1.4	—	2.65
		7 sec. phosph. + 3 prim. phosph.	43	+0.7	21	8, 6, 2.5	1.2	—	2.5

experiments on cattle. Since a cow, the paunch of which contains between 50 and 100 kg. of food, will produce from 100 to 300 l. methane in 24 hours, it is evident that the intensity of the fermentation has even in the best of our experiments been considerably below that observed in natural conditions, and in most of our determinations it has fallen far short of this ideal. In Markoff's experiments the intensity of fermentation has also, generally, been lower than in the living animal. This is ascribed to the cooling and to the influence of oxygen in the interval between the taking out of the material and removal of the oxygen in the water-bath at 37°. We believe that the contact with oxygen is the factor chiefly responsible.

In column 7 we have given some figures indicating roughly the variation in fermentation rate in the course of each experiment. The figures give the increase in pressure in the fermentation flasks in mm. mercury per hour. They are comparable only within the same experiment and to a certain extent in experiments made with the same buffer mixture. When, for instance, the rate is much higher in Exp. 1 B than in 1 A, this means only that far more CO₂ is evolved as gas in B than in A, owing to the greater acidity of the fermenting mixture.

In Exps. 1 B, 2, 4 A, 5, 6 and 7 the rate remains practically constant during the first 24 hours. In 1 A and 8 it decreases, and in 3 and 4 B it increases materially. In Exps. 5, 7 and 8, which have been continued beyond 24 hours, the rate has decreased on the second day and still more on the third. We are unable to give any explanation of the variations in rate.

The relation $\frac{\text{CO}_2}{\text{CH}_4}$ given in the last column varies between 2.95 and 2.2, except in Exp. 7, in which the fermentation was extremely slow and the CaCO₃ added had probably made it abnormal. Except in this case it does not seem to be in any way influenced by the varying intensity of the fermentation. The double determinations agree very closely, except in the two experiments 5 and 7 lasting over 48 hours and showing the slowest fermentation. The differences between different experiments are so much larger than the mean error of a determination that they must be considered as real, and it must be admitted, therefore, that in the conditions obtaining the relation $\frac{\text{CO}_2}{\text{CH}_4}$ is *not* constant, though the variations found are very much smaller than formerly supposed.

The experiments show that within the range of hydrogen ion concentrations which have been found in the paunches of cows (P_H between 7 and 8) the reaction of the fermenting mixture has no influence upon the character or intensity of the fermentation.

The average for the experiments 1, 3 and 4, which show a fairly rapid rate of fermentation, is $\frac{\text{CO}_2}{\text{CH}_4} = 2.6$, and the average for all the experiments, except the abnormal nos. 5 and 7, is likewise 2.6 ± 0.08 with a dispersion of ± 0.27 .

The application of the result in metabolism experiments on cattle.

The figure 2.6 can be utilised to correct the results directly derived from determinations of the gaseous metabolism. A certain caution is necessary, however, since the relation $\frac{\text{CO}_2}{\text{CH}_4}$ is not quite constant, and may possibly show larger variations in cows fed upon a more varied fodder.

To illustrate the application of our result, we have taken an experiment made by A. C. Andersen [1920], in which both the gas exchange and all solid ingesta and excreta were determined and calculated on a 24 hour basis. The metabolism of nitrogen-free substances was found to require 1451 l. oxygen and produce 1583 l. CO_2 and 127 l. CH_4 . The apparent R.Q. is $\frac{1583}{1451} = 1.091$. According to our result $2.6 \times 127 = 330$ l. CO_2 should be due to fermentation and the true R.Q. for the organism itself should be $\frac{1583 - 330}{1451} = 0.864$.

The energy metabolism is calculated from the oxygen intake by means of Zuntz's formula. We have

$$E = \text{O}_2 \{4.686 + (\text{R.Q.} - 0.707) 1.23\}.$$

In the present instance the uncorrected heat production would be 1451 (4.686 + 0.384 $\times$ 1.23) = 7487 Cal. and the corrected only 7079 Cal.

The summary calculation is evidently inadmissible. Against the application of the correction it might be argued that Zuntz's formula has been elaborated on the assumption of a metabolism of ordinary fats and carbohydrates only, and cannot be used when the lower fatty acids formed by the fermentation are catabolised. If Markoff is right, that the fatty acids correspond on an average to butyric acid, the caloric value of the oxygen used for the catabolisation of these substances will correspond closely to that obtained from the formula. Butyric acid has a respiratory quotient of 0.8 with a caloric value of the oxygen of 4.74 Cal. per l., while the formula gives 4.80.

It is of special interest to compare our calculation with the elegant solution of the problem given by A. C. Andersen himself. Andersen's reasoning is as follows: if the methane produced by the fermentation is burned the total (non-protein) metabolism (methane included) is one of carbohydrate and fat, and Zuntz's formula must be strictly applicable to find a total heat production, and if the heat of combustion of the methane is afterwards subtracted, the rest is the metabolism of the animal.

Methane burns according to the equation $\text{CH}_4 + 2\text{O}_2 = \text{CO}_2 + 2\text{H}_2\text{O} + 213$ Cal. The volume of oxygen used is double that of the methane and carbon dioxide, and we have consequently in the above example

Total $\text{CO}_2 = 1583 + 127 = 1710$ l. Total $\text{O}_2 = 1451 + (2 \times 127) = 1705$ l. R.Q. = 1.003. Hence the total heat production 8610 Cal. and, by subtraction of the heat of combustion of 127 l. methane = 1207 Cal., the metabolism of the animal itself 7403 Cal.

This figure is 324 Cal. or 4.6 % higher than that obtained by our calculation. That it should be higher was to be expected, since in Andersen's calcula-

tion the amount of heat produced by the fermentation in the paunch is included, while our calculation aims at having it excluded. The heat of fermentation cannot strictly be reckoned as due to the metabolism of the animal and is practically lost from the point of view of the animal's economy, even though it is generated inside its body¹.

According to the provisional formula of fermentation given above (p. 687) the heat of fermentation per g.-molecule of methane would be equal to the heat of combustion of 2 g.-molecules of $C_6H_{10}O_5$ (1356 Cal.) minus the heat of combustion of 2 g.-molecules of $C_4H_8O_2$ (1054 Cal.) and 1 of methane (213 Cal.), that is 89 Cal. or 3.97 Cal. per l. methane. In the experiment under discussion the loss by fermentation would therefore, on this assumption, amount to 505 Cal. When Andersen's figure is reduced by this amount it becomes 6898 Cal., 181 Cal. or 2.6 % lower than ours.

Our experiments show that the provisional formula does not represent the fermentation exactly, and it is probable, moreover, that part of the energy liberated by the fermentation is utilised for synthetic processes (building up of organisms?) within the fermenting mixture. The loss is therefore probably somewhat lower than indicated by the formula.

It would be desirable to get direct bio-calorimetric determinations on the fermenting mass combined with determinations of the CH_4 evolved. The making of such determinations has been attempted by Markoff [1913, p. 63], but so far as we are aware the results have not been published. We have also made some attempts, but have not been able to overcome satisfactorily the technical difficulties. So far as they go, our experiments indicate a heat of fermentation of about 50 Cal. per g.-mol. of CH_4 . When corrected by means of this figure Andersen's results would become 7119 Cal., 40 Cal. or 0.6 % higher than ours. The closeness of this agreement has of course no real significance, but it would seem, nevertheless, that our method of calculating the energy expenditure of the ruminating organism itself cannot be very far wrong.

SUMMARY.

Methods are described for measuring the gaseous products of fermentation processes and especially the CO_2 and CH_4 evolved by the cellulose fermentation taking place in the paunch of the ox.

Usually gaseous nitrogen is neither absorbed nor produced by this fermentation.

The relation between the CO_2 and CH_4 formed by the fermentation appears to be independent of the rate of fermentation and, within the limits investigated, of the hydrogen ion concentration. The relation $\frac{CO_2}{CH_4}$ works out

¹ In the calculation made by Andersen it is perfectly legitimate to include this amount of heat, since he compared the heat production found by respiration experiments (+ urine analysis) with the heat computed from weighings and analysis by means of the calorimetric bomb of all the ingesta and excreta of the animal.

as on an average 2.6, but real variations occur. The limits observed are 2.2 and 2.9 respectively.

A method is suggested by which the observed respiratory exchange of cattle can be corrected, when the production of methane is measured.

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