A THEORETICAL RUMEN FERMENTATION BALANCE

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SUMMARY

Certain assumptions are made which permit the calculation of the amounts of CO_2 and CH_4 produced in the rumen which correspond to a given molar distribution of volatile fatty acids present in rumen fluid. This calculation allows the development of a theoretical fermentation balance for a given molar distribution of volatile fatty acids. For a molar distribution of 65 acetate : 20 propionate : 15 butyrate, the following balance is calculated:

$$57.5\ (C_6H_{12}O_6) \rightarrow 65 \text{ acetate} + 20 \text{ propionate} + 15 \text{ butyrate} + 60\ CO_2 + 35\ CH_4 + 25\ H_2O$$

The assumptions upon which the calculations are based are discussed, such as considering volatile fatty acids, CO_2 and CH_4, as sole fermentation products and using the empirical formula $C_6H_{12}O_6$ to represent fermentation substrate. An attempt is made to evaluate the fermentation balance in terms of known experimental data which bear on product-substrate relationships. Values predicted from the theoretical equation are of the same order of magnitude as experimental values.

Application of the balance is made to amplify the interpretation of experimental data concerned with variations in the molar distribution of fatty acids on various dietary regimes. It is proposed that the fermentation balance is useful for interpreting certain quantitative aspects of ruminant nutrition in terms of the quantitative aspects of the rumen microbial fermentation.

Investigations of the microbiology of the rumen and ruminant physiology have provided a qualitative description of the activities of rumen microorganisms and the importance of these activities to the digestion of plant materials by the ruminant. The significance of such microbial activities as plant polysaccharide digestion, volatile fatty acid production, protein digestion and synthesis, and vitamin synthesis is well recognized. Several review articles in this general area are available (3, 6, 15). In the course of preparing some of this material for teaching purposes, the author found it convenient to describe the rumen fermentation in terms of a theoretical fermentation balance. The fermentation balance permits the development of approximations of the quantitative aspects of the rumen fermentation. The theoretical fermentation balance will be the subject of this report.

CALCULATIONS AND DISCUSSION

It should be emphasized that the following analyses are made with idealized systems. Assumptions are made, and a degree of naïveté is introduced, for the sake of achieving coherent, simple descriptions.

Calculation of the balance. For the calculation of the fermentation balance, the following assumptions are made:

(a) The molar proportions of volatile fatty acids found in rumen fluid are 65 acetic : 20 propionic : 15 butyric. These proportions represent the proportions in which these products are produced from fermented substrate.

(b) The only fermentation products produced in addition to the above-mentioned fatty acids are carbon dioxide and methane.

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(c) All fermentation products are formed from plant carbohydrates with the empirical formula $C_6H_{12}O_6$.

Some justification for these assumptions will be presented after the calculation of the model fermentation balance.

An equation for the rumen fermentation can, therefore, be written as:

(1) $X\ (C_6H_{12}O_6) \rightarrow 65\ CH_3CO_2H + 20\ CH_3CH_2CO_2H + 15\ CH_3(CH_2)_2CO_2H + Y\ CO_2 + Z\ CH_4$.

The amounts of CO_2 and CH_4 which would have to be produced to satisfy this equation can be calculated using the standard techniques for calculating fermentation balances (14). The moles of compounds produced containing one carbon atom should be equal to the moles of compounds produced containing two carbon atoms. Butyric acid is assumed to arise from the condensation of two units containing two carbon atoms. Thus:

$$Y\ CO_2 + Z\ CH_4 = 65\ CH_3CO_2H + (2 \times 15\ CH_3(CH_2)_2CO_2H)$$

or (2) $Y + Z = 95$.

Other information exists which will allow the development of an independent equation for the relationship between CO_2 and CH_4. This information arises from the necessity for equivalence between the oxidation level of the fermentation products and the oxidation level of the substrate. The oxidation states are arbitrarily calculated from the following:

$$\text{oxidation state} = \text{number of oxygen atoms per molecule} - \frac{\text{number of hydrogen atoms per molecule}}{2}$$

The oxidation state of the substrate ($C_6H_{12}O_6$) is, therefore, zero and the oxidation states of the products are acetate (0), propionate (−1), butyrate (−2), CO_2 (+2), and CH_4 (−2). The oxidation level is found by multiplying the oxidation state by the moles of product or substrate. The sum of the oxidation levels of the products is equal to zero because the oxidation state of the substrate is zero. Therefore:

$$(65 \times 0) + (20 \times -1) + (15 \times -2) + (Y \times +2) + (Z \times -2) = 0$$

or (3) $Y - Z = 25$.

A simultaneous equation can thus be solved from Equations (2) and (3).

$$\begin{aligned} Y + Z &= 95 \\ Y - Z &= 25 \\ \hline 2Y &= 120,\ Y = 60,\ Z = 35 \end{aligned}$$

Equation (1) can now be written as:

$$X\ (C_6H_{12}O_6) \rightarrow 65 \text{ acetate} + 20 \text{ propionate} + 15 \text{ butyrate} + 60\ CO_2 + 35\ CH_4$$

The number of $C_6H_{12}O_6$ units fermented can be calculated by summing the number of carbon atoms in the products. Equation (1) can now be written as:

(4) $57.5\ (C_6H_{12}O_6) \rightarrow 65$ acetate + 20 propionate + 15 butyrate + 60 CO_2 + 35 CH_4 + 25 H_2O

Discussion of assumptions. The use of the molar proportions of 65 acetic : 20 propionic : 15 butyric for the purposes of calculation is justified on the basis

that these values are not too far astray from many values reported in the literature for the volatile fatty acid composition of rumen fluid. The assumption that the rumen fluid values are indicative of the rates of production of the volatile fatty acids from substrate is supported by the experiments of Carroll and Hungate (4). These investigators followed the rates of production of the acids in rumen fluid immediately after removal of the fluid from the rumen and showed that individual acids were produced in approximately the same proportions as their initial concentrations. Volatile fatty acids, other than those used in these calculations, have been ignored, because they usually do not constitute more than 5% of the volatile fatty acids present. Other products of bacterial fermentations such as hydrogen, ethanol, lactate, succinate, formate, etc. have been ignored, because they usually do not accumulate in significant quantities or they have not been detected in the rumen under normal conditions. Many of these compounds, however, may be intermediates in the formation of the ultimate end products.

The assumption that the fermentation products arise entirely from carbohydrate of empirical formula $C_6H_{12}O_6$ is subject to several criticisms. Certainly, the most important plant carbohydrates digested in the rumen belong to this general class, e.g., cellulose and starch (waters of hydration and dehydration are ignored, since they do not affect the general picture). There are some polysaccharides which contain uronic acids, deoxy sugars, and amino sugars which would alter the calculation of the fermentation balance if they contributed significantly to the fermentation. These carbohydrates, however, are usually only a small part of the ruminant's diet. Noncarbohydrate materials such as proteins and fats may be fermented in the rumen, but fats are not very important in ruminant diets and proteins must, for the most part, be conserved as ingested protein per se, or as microbial protein, in order for the ruminant to be able to survive. There may be certain situations in which the net[1] protein fermentation could be relatively large. It is known that large amounts of NH_3 accumulate in the rumen if the ruminant is fed protein without an adequate supply of carbohydrate (5). It is the author's opinion that in most situations the net protein fermentation is small relative to the carbohydrate fermentation. This is supported by the fact that the ruminant nitrogen requirement is not greater than the nonruminant requirement (13).

Xylans and other pentosans would probably be fermented by a mechanism involving cleavage into a two-carbon and a three-carbon compound. The two-carbon unit would not be accompanied by a one-carbon unit. Thus, the calculations equating one-carbon units with two-carbon units would have to be corrected for the amount of two-carbon units arising from pentase cleavage. These corrections have not been applied to the fermentation balances calcu-

[1] It is conceivable that gross protein fermentation always occurs and is considerable in magnitude. If this gross fermentation is balanced by resynthesis of microbial protein, the contribution to the over-all fermentation would be minor. Such a situation is comparable to ordinary turnover of protein, except that the rumen turnover involves the breakdown of ingested protein and the resynthesis of microbial protein. Net fermentation would occur when the rate of breakdown of protein exceeds the rates of resynthesis.

lated in this paper and it is assumed that all products arise from the cleavage of hexose units.

Evaluation of the balance. One question which is immediately raised about the fermentation balance is whether it is a realistic assessment of the in vivo rumen fermentation. Unfortunately, there are not too many measurements available which will allow a critical evaluation. A few indirect tests can be made, however, which do supply a small degree of encouragement.

One test which can be applied derives from measurements which have been made of the amount of methane produced in the rumen per amount of digestible carbohydrate fed. Several values have appeared in the literature for this relationship and they are approximately in the same range. Kellner (9) reported an increase of 4.29 g. of methane for every 100 g. of digestible carbohydrate fed above the maintenance level. In similar types of experiments, Armsby and Fries (1) reported a figure of 4.8 and 4.7 g. of methane for every 100 g. of digested carbohydrates fed as roughages or a mixture of roughages and concentrates, respectively. For 100 g. of digested carbohydrate in Sudan hay added to a maintenance ration of Sudan hay, 4.4 g. of methane were produced [Kleiber *et al.* (12)]. It can be calculated from the model equation that 6.0 g. of methane would be produced from 100 g. of pure cellulose digested (57.5×162 = grams cellulose). Thus, the calculated value for methane production per 100 g. of carbohydrate digested is anywhere from 25 to 40% higher than the experimentally determined values. Possible explanations for the discrepancy between the theoretical and experimental values will be discussed after further comparisons of experimental results with calculated results.

Another comparison can be made with a value of 3.12 meq. of volatile acid produced from 430 mg. of cellulose digested by rumen fluid, reported by Carroll and Hungate (4). The theoretical equation predicts that the volatile acid produced from 430 mg. of cellulose should amount to 4.62 meq. The calculated value for acid production is 48% higher than the experimental value.

Another comparison with experimental values can be made on the basis of the rates of production of volatile fatty acids found by Carroll and Hungate (4) by in vitro measurements in rumen fluid of steers on different diets. These authors reported figures of 1.42 and 2.35 meq. of volatile acid produced per 100 g. of rumen fluid per hour for hay and grain diets, respectively. By taking the ratio of methane produced to volatile fatty acids produced from the theoretical equation, and extrapolating to a 24-hr. period and 70 kg. of rumen contents, a theoretical yield of methane can be estimated. The calculated values are 187 liters of methane for the hay diet and 312 liters for the grain diet. Kleiber *et al.* (12) experimentally measured a daily rate of methane production of 180 liters per day for dry cows on maintenance rations and 280 liters per day for lactating cows on production diets.

It should also be noted that the molar percentage composition of rumen gas from cows on alfalfa pasture has been reported as 67% CO_2; 26% CH_4; 7% $N_2 + H_2$; 0.1% H_2S, and less than 1% O_2 by Kleiber *et al.* (11). Similar results had been obtained earlier by Tappeiner (16). If we use the figure of 93% from the data above, for the total contribution of CO_2 and CH_4 to the

rumen gas, the percentages of CO_2 and CH_4 predicted from Equation (4) would be 59 and 34%, respectively. Kleiber (10) has pointed out that saliva can contribute to the total rumen CO_2 only when the rumen pH drops below 6.9. Below pH 6.9, the tendency would be to raise the proportion of CO_2 in the rumen gas as a result of the acidification of the bicarbonate in solution by acid fermentation end products.

The results of the comparisons between the experimental and theoretical values indicate that the predictions of the theoretical equation are at least of the same order of magnitude as experimental values. The experiments with which the comparisons are made were obviously not designed to check the fermentation balance, and certain critical information remains unknown for each particular experiment. It is interesting to note that in the two cases where the comparison is made on a product-to-substrate ratio comparison, i.e., CH_4 : carbohydrate or volatile fatty acid : carbohydrate, the amounts of product predicted from the theoretical equation tend to be higher than those amounts actually found. It is not very useful to comment on the comparison of theoretical and experimental total methane production per day, because the theoretical estimation involves assumptions concerning rates of production and volumes of rumen ingesta which are difficult to evaluate.

Applications of the balance. A useful feature of any theory is that it provides a base for the discussion of the various parameters which the theory embodies, whether the theory be correct or incorrect. We might consider, for example, some possible reasons for the discrepancies between the theoretical and experimental values found in the product-substrate ratio comparisons. One possible explanation is that the products considered, i.e., acetate, propionate, butyrate, CH_4, and CO_2, do not represent all of the products formed from ingested carbohydrate. Thus, more carbohydrate should be digested per unit of the above end products formed. Such a situation would arise if a large amount of ingested carbohydrate is incorporated into microbial carbohydrate. Another possibility is the formation of a product which diffuses rapidly through the rumen wall, so that it is not normally found in the rumen, e.g., lactate, ethanol, succinate, and formate. It should be pointed out that, except for lactate, such products would disturb the calculated CO_2 and CH_4 values, because they would affect the predicted amounts of one-carbon compounds and the predicted oxidation-reduction balance of the products. Lactate would have no effect, because its oxidation state is zero and it is a three-carbon compound.

Calculation of a theoretical equation is useful in its emphasis of the interrelationships of end products. It is of interest to note the consequences of a change in the proportion of volatile fatty acids. An increase in the proportions of propionic and butyric acids and a decrease in acetic acid would tend to decrease the methane yield. Calculations of fermentation balances from the data of Elliot and Loosli (7), concerning the molar proportions of volatile fatty acids on different dietary regimes, show a decrease of approximately 9% in the methane produced per mole of carbohydrate digested when the cows were fed 20% of their estimated net energy (ENE) as roughage, as compared to 60%. The calculated values for CH_4 and CO_2 are shown with the experi-

mental values in Table 1, along with the calculated amount of carbohydrate fermented to these products (calculated as starch). Valerate is assumed to be synthesized by a condensation of a two-carbon unit with a three-carbon unit. Calculations of the heats of combustion of the fatty acid products and the substrate fermented (the heat of combustion of starch was used for the calculations) show little difference in the therms of total volatile fatty acids produced per therm of digested carbohydrate for the 20 and 60% ENE as roughage diets. It is interesting to note that the fermentation balance indicates no difference in the available therms of propionic acid per therm of substrate fermented on the two diets, whereas the available therms of butyrate per therm of substrate increases on the low roughage diet. The order of magnitude of the increase, however, can not explain the increased efficiency of the low roughage diet.

TABLE 1
Fermentation end products from data of Elliot and Loosli (7)

Diet	Moles per 100 moles VFA						
	Starch fermented	Acetate	Propionate	Butyrate	Valerate	CH_4	CO_2
High roughage	57.3	66.6	18.7	11.8	2.9	33.9	59.5
Low roughage	59.1	60.5	21.4	15.4	2.7	31.9	62.1

Another point of interest from the above work is that the net efficiency of the fermentation calculated from the balance and represented as therms of available fatty acids to therms of carbohydrate fermented is 0.71 and 0.72 for the high and low roughage diets, respectively. Using the heat of combustion figure of 0.340 therm per pound of fat-corrected milk (2), the efficiencies of the conversion of digestible energy to milk energy, in terms of therms of milk produced per therm of digestible energy consumed over and above maintenance requirements, are 0.53 and 0.66 for the high and low roughage diets, respectively.[2] If it is assumed that the fatty acids derived from the digestible energy are the source of energy for milk production, the efficiency of milk formation can be calculated in terms of the therms of fatty acids produced from the digestible energy utilized. The therms of fatty acids produced per therm of digestible energy consumed can be approximated from the fermentation balances by the previously mentioned values of 0.71 and 0.72 therms of available fatty acid per therm of carbohydrate fermented for the high and low roughage diets, respectively. The efficiency of the conversion of the available fatty acid energy to milk energy would then be 75% for the high roughage diet and 92% for the low roughage diet. In other words, approximately 17% more of the energy available as fatty acids is used for milk production when the low roughage ration is supplied instead of the high roughage ration.

In contrast to the fermentation balances based on the work of Elliot and Loosli, fermentation balances based on the work of Eusebio *et al.* (8) suggest that significantly different energy yields could be obtained on high corn or

[2] Estimated from Figure 2 of Elliot and Loosli (7).

flaked corn diets in contrast to hay-corn rations. These workers measured the volatile fatty acid concentration of rumen fluid on various dietary regimes. If one calculates fermentation balances from the volatile fatty acid concentration[3] for the alfalfa hay-corn meal diet (12.9 lb. U. S. No. 2 alfalfa hay and 5.7 lb. corn meal fed daily) and for the corn meal diet (11.8 lb. corn meal fed daily), the energy yield in therms of fatty acids produced from a therm of substrate is about 13% greater for the corn meal diet than for the hay–corn meal diet. The calculated values for CH_4, CO_2, and starch are shown with the experimental values for volatile fatty acids in Table 2. For the alfalfa hay–corn meal

TABLE 2

Fermentation end products from data of Eusebio *et al.* (8)

Diet	Moles per 100 moles VFA						
	Starch fermented	Acetate	Propionate	Butyrate	Valerate	CH_4	CO_2
Corn meal	54.5	38	43	11	3	13	50
Alfalfa hay–corn meal	57	56	24	14	3	28	59

ration the fatty acids represent 0.75 therm per therm of carbohydrate, whereas 0.85 therm is contained in the fatty acids produced on the corn meal ration.

These calculations serve to emphasize the quantitative relationships between end products and substrate and between end products themselves. The exact numerical values of these relationships are not known, and the calculations are presented as models which may be useful for teaching purposes. Further research in the area of ruminant microbiology and physiology may provide a more accurate picture of the rumen fermentation in quantitative terms. Studies of product-substrates relationships and the rates of production of products from substrates may allow a quantitative description of the nutrition of the ruminant in terms of the quantitative aspects of the microbial fermentation.

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