
VOLUME 62 2002

QUANTITATIVE ASPECTS OF FERMENTATION IN THE GASTRO-INTESTINAL TRACT OF SHEEP FED FRESH HERBAGE

M. J. ULYATT, D. W. DELLOW, A. R. EGAN[1] and D. J. WALKER[2]
Applied Biochemistry Division, DSIR, Palmerston North

SUMMARY

Fermentation balance in the stomach region was studied in sheep prepared with a rumen cannula and a re-entrant duodenal cannula. They were fed fresh perennial ryegrass and white clover.

Energy digested in the stomach was regressed against volatile fatty acid (VFA) production rate (measured by isotope dilution). VFA production accounted for approximately 75% of the energy digested in the stomach.

Fermentation balance (kcal) measured *in vivo* in terms of VFA production, CH_4 production and heat of fermentation was compared with Hungate's (1965) stoichiometric relationship and good agreement was obtained.

Caecal fermentation of fresh white clover was studied using sheep prepared with a rumen cannula and two cannulae in the caecum: one near the blind end and another in a mid-position. The caecum contributed approximately 9% of total VFA production in the gastro-intestinal tract. Approximately 35% of total oxidative metabolism was attributed to acetate produced in the rumen and 4.5% to acetate produced in the caecum, which indicated that 11.5% of all acetate production occurred in the caecum.

Values for the metabolizable energy (ME) content of fresh herbage of high apparent digestibility are presented. The ratio ME/digestible energy was 0.79 at maintenance and 0.83 at 1.5 maintenance. ME (Mcal)/kg DM ranged from 2.7 to 3.0.

INTRODUCTION

Although it has been known for about 100 years that the ruminant is dependent on fermentation for the digestion of structural carbohydrate in its feed, knowledge of the quantitative importance of fermentation to the animal is still fragmentary. Most of the present knowledge of the processes of fermentation is derived from microbiological and biochemical studies conducted *in vitro* (Hungate, 1966). It is only recently that techniques have been developed which enable us to measure, in a quantitative manner, the partition of ruminant digestion between fermentation and host animal enzymic

[1] Present address: Waite Agricultural Research Institute, Glen Osmond, South Australia 5064.
[2] Present address: CSIRO, Meat Research Laboratory, Cannon Hill, Queensland 4170, Australia.

digestion (*e.g.*, Bruce *et al.*, 1966). The importance of obtaining a full knowledge of the digestion processes when the ruminant is fed high-producing pasture cannot be minimized if the animal's ability to digest herbage is to be fully exploited. The current emphasis on manipulation of ruminant digestion (*e.g.*, Phillipson, 1972) makes one aware of this. On the one hand, there is a desire to exploit the system to its fullest, while on the other it is necessary to ensure that a highly efficient system should not be destroyed and replaced with something inferior. In this paper the fermentative digestion of fresh perennial ryegrass and white clover will be considered. The efficiency of fermentation in rumen will be discussed first, and the quantitative importance of caecal fermentation will then be considered.

FERMENTATION BALANCE IN THE RUMEN

METHODS

Romney-Marsh wether sheep aged between 1 and 2 years and weighing between 30 and 44 kg were used in these experiments. They were prepared with a rumen cannula and with a re-entrant cannula in the duodenum, 4 cm from the pylorus. Each sheep had free access to water and was fed fresh herbage from a belt feeder which was timed to step in 1-hourly intervals. All animals were trained to stand or sit quietly, with their heads enclosed in an open circuit Perspex respiration hood (Ulyatt and Webster, 1973).

Two pasture species were used: *Lolium perenne* 'Grasslands Ruanui' perennial ryegrass and *Trifolium repens* 'Grasslands 4700' white clover. The herbage was cut for feeding once daily and was always in a vegetative state between 10 and 15 cm high. The feeding procedures adopted were similar to those of Ulyatt and MacRae (1973).

The experiments with perennial ryegrass were conducted in early spring 1971. They were of a change-over experimental design. Two sheep were fed at approximately maintenance (M) requirements (550 g DM/day) and two were fed approximately 1.5 maintenance (825 g DM/day). At the end of an experimental sequence, intake levels were changed and measurements repeated. Clover was fed from early October until mid-December. In this case four animals were used at each intake level with no change over.

For each sheep the experimental sequence was as follows. A preliminary feeding period of 3 weeks was allowed when diet or intake level was changed. This was followed by a 10-day balance period during which feed intake, and faeces and urine outputs were measured. In a further balance period of 6 to 10 days, each animal spent two periods of 6 to 7 hours with its head in the respiration hood. Oxygen uptake, carbon dioxide output, and methane output were measured during this period. On completion of these metabolism studies, measurements of volatile fatty acid (VFA) production rate and duodenal digesta flow were made. A solution containing Cr-EDTA marker and sodium acetate-2-^{14}C was infused intraruminally for 6 days. The first 3 days were a preliminary period for equilibration of marker and isotope with the digesta. During the last 3 days, the rumen was sampled continuously with a dialysis sampling system (Gray *et al.*, 1967) and twelve 40 ml spot samples of duodenal digesta were collected as described by Ulyatt and Egan (1973). These duodenal digesta samples were bulked and frozen as collected. By reference to the marker, digestion was partitioned between the stomach region and the intestines.

Methods of chemical analysis are the same as those given by Ulyatt and Egan (1973).

RESULTS

The quantities of some of the major feed constituents digested in the stomach region (*i.e.*, predominantly rumen digestion) at M and 1.5M are given in Table 1. The amounts of organic matter (OM) apparently digested in the stomach were the same with both herbages. The percentage of DM digested in the stomach region of sheep fed perennial ryegrass was 54% at M and 56% at 1.5M while the respective figures were 55 and 56% for clover. These figures are approximately 10% lower than in the earlier work of Ulyatt and MacRae (1973).

The amount of energy of perennial ryegrass apparently digested in the stomach was lower than that of clover at both levels of intake. This means that the calorific value of the OM of clover digested in the stomach was higher at 4.2 kcal/g OM than that of perennial ryegrass at 3.9 kcal/g OM. Ulyatt and

TABLE 1: QUANTITIES OF SOME OF THE MAJOR CONSTITUENTS DIGESTED IN THE STOMACH REGION OF SHEEP FED PERENNIAL RYEGRASS AND WHITE CLOVER

	Perennial Ryegrass		*White Clover*	
Energy intake (kcal/day)	2444	3666	2446	3664
OM digested in stomach (g/day)	225	346	225	347
Energy digested in stomach (kcal/day)	873	1345	922	1498
Carbohydrate digested in stomach (g/day)	221	337	182	270
Crude protein digested in stomach (g/day)	—3	—1	8	22
Methane production (l/day)	17.8	22.8	19.6	23.4

MacRae (1973) also observed this effect, which suggests that there is digestion of some high energy-yielding compounds in the rumen of sheep fed white clover. The percentage of digestible energy that was digested in the stomach of sheep fed perennial ryegrass was 44% at M and 46% at 1.5M, while corresponding figures for clover were 47 and 51%. Again, these were in the order of 10% lower than figures found by Ulyatt and MacRae (1973).

The data for carbohydrate and protein digestion show that, with perennial ryegrass, the OM digested in the stomach was mainly carbohydrate. With clover, a small amount of protein was digested and approximately 16% of digestible OM was not accounted for.

The methane production given reflects only the methane produced in the rumen. It was equivalent to 6.4 kcal/100 kcal gross energy intake with perennial ryegrass and 6.9 kcal/100 kcal gross energy with clover.

The main end-products of rumen digestion are VFA and in Fig. 1 measured VFA production rate (Y) is plotted against the amount of energy digested in the stomach region (X). The fitted regression was $Y = 0.59\,X + 195$. ($Sy.x = 193$ kcal/day). Taking mean values, the heat of combustion of the VFA produced in the rumen accounted for 75% of the energy apparently digested in the rumen. The considerable scatter about the regression line was due to error in the measurement of both variables, but particularly the measurement of VFA production rate. It seems that, even when sampling of rumen VFA was continuous over 3 days, mixing was still the major obstacle to obtaining a representative sample from sheep fed fresh herbage. Estimates of duodenal flow of digesta were less variable within experiments.

As shown in Table 1, carbohydrate was the dominant feed constituent digested in the stomach of sheep fed fresh perennial ryegrass and white clover. Hungate (1965) has published the following theoretical stoichiometric relationship to represent the fermentation of carbohydrate in the rumen:

$$58 \text{ Hexose} \rightarrow 66 \text{ Acetic} + 22 \text{ Propionic} + 16 \text{ Butryic} + 60.5\ CO_2 + 33.5\ CH_4 + 27\ H_2O.$$

This equation is expressed in terms of calories below:

$$100 \text{ Hexose} \rightarrow 75.5 \text{ VFA} + 18.0\ CH_4 + 6.5 \text{ Heat of fermentation.}$$

Results from digestion of perennial ryegrass and white clover are expressed in the same way in Table 2. VFA production was calculated from the regression (Fig 1) and heat of fermentation was calculated by difference. Methane production was very consistent and the main source of variability was the

TABLE 2: FERMENTATION BALANCE IN THE RUMEN OF SHEEP FED PERENNIAL RYEGRASS AND WHITE CLOVER COMPARED WITH HUNGATE'S (1965) STOICHIOMETRIC RELATIONSHIP

	Intake (kcal/day)	*Volatile Fatty Acids (kcal/100 kcal)*	*Methane (kcal/100 kcal)*	*Heat of Fermentation (kcal/100 kcal)*
Perennial ryegrass	2444	81.6	19.4	—1.0
	3666	73.7	16.1	10.2
White clover	2446	80.4	20.5	—0.9
	3,664	72.2	15.4	12.4
Pooled mean over feeds and intake levels		74.5	17.9	7.6
Hungate's (1965) equation		75.5	18.0	6.5

calculation of VFA production rate, for clearly, this was over-estimated at M and slightly under-estimated at 1.5M. If the data are pooled over herbages and intake levels, a relationship surprisingly close to Hungate's (1965) equation is obtained.

The stoichiometry of digestion of fresh herbage in the rumen does not therefore appear to be grossly different from

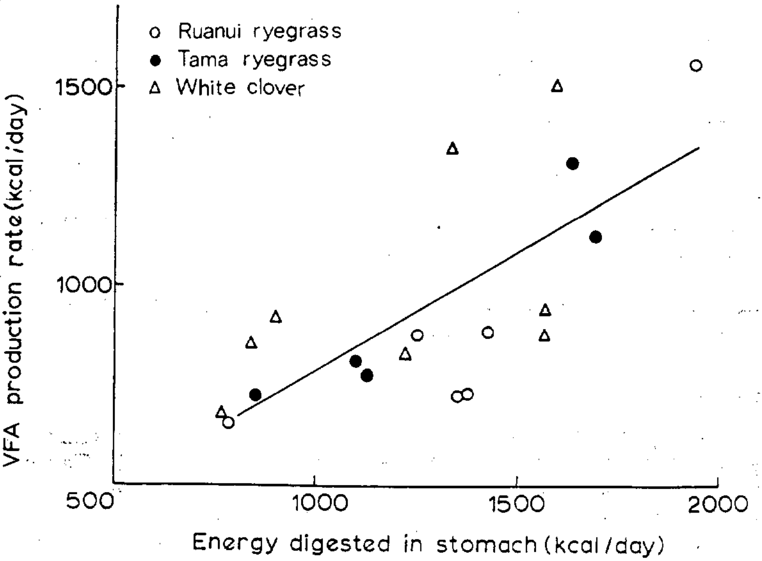

FIG. 1: *The relationship between volatile fatty acid production in the rumen and the amount of energy digested in the stomach region of sheep fed fresh perennial ryegrass, Tama ryegrass and white clover.*

that expected from theoretical and *in vitro* work. While this is not surprising, the confirmation *in vivo* gives confidence that average values used in formulating feeding standards are applicable to fresh pasture.

CAECAL DIGESTION

METHODS

Only white clover was fed in the work to be described on caecal digestion. The sheep were Romney-Marsh wethers weighing 26 to 29 kg. They were prepared with a cannula in the rumen and two in the caecum, one near the blind end and the other in a mid-position (MacRae *et al.*, 1973). Clover preparation, harvesting and feeding procedures were the same as described by Ulyatt and MacRae (1973). The experiment was of changeover design with two sheep fed at M (350 g DM per day) and two at 2M (700 g DM per day). When the first experimental sequence was completed, the intake levels were reversed and the sequence repeated. For each sheep the experimental sequence was as follows. A solution containing Cr-EDTA marker and sodium acetate-2-^{14}C was infused continuously into the blind end of the caecum for 6 days. The first 3 days were a preliminary period for equilibration of marker and during the second 3 days 12 spot samples of caecal digesta were collected from the mid-position cannula. During the latter 3 days each sheep spent 6 hours with its head in the respiration hood and measurements of carbon dioxide output and specific radioactivity were made. The caecal infusion was then stopped for 2 days. Following this break, sodium acetate-2-^{14}C was infused intraruminally for 5 days: the first 2 days were a preliminary period and during the last three days a continuous sample of rumen VFA was obtained with a dialysis probe (Gray *et al.*, 1967). During the last 3 days each animal spent 6 hours with its head in the respiration hood and measurements of carbon dioxide output and specific radioactivity were made.

RESULTS

Measured VFA production in the rumen and caecum of sheep fed white clover at M and 2M are presented in Table 3. In both cases the caecum contributed approximately 9% of total VFA production. An alternative way of assessing caecal fermentation is also shown in Table 3 where the contribution of acetate produced in either the caecum or rumen to oxidative metabolism is given. The percentage CO_2 derived from acetate indicates the contribution of acetate to whole animal

TABLE 3: A COMPARISON OF RUMINAL AND CAECAL PRODUCTION OF VOLATILE FATTY ACIDS TOGETHER WITH A MEASUREMENT OF THEIR IMPORTANCE TO OXIDATIVE METABOLISM IN SHEEP FED WHITE CLOVER

	OM Intake (g/day)	*Rumen*	*Caecum*	*Rumen plus Caecum*	*% Produced in Caecum*
VFA production rate (moles/day)	350	2.13	0.20	2.33	8.6
	700	3.51	0.37	3.92	9.4
% CO_2 derived from acetate	350	33.2	4.3	37.5	11.5
	700	37.1	4.9	42.0	11.7

oxidative metabolism; in this work the contribution of acetate produced in the rumen was 33% at M and 37% at 2M, figures which agree very well with those of Annison *et al.* (1967) with the sheep and Kronfeld (1968) with the cow. Approximately 4.5% of oxidative metabolism could be attributed to acetate produced in the caecum. The data also indicate that approximately 11.5% of acetate production occurred in the caecum. Thus there is good agreement that with white clover about 10% of fermentation occurs in the caecum. This proportion is likely to be higher for other herbages where the proportion of OM digested in the large intestine is higher than with white clover (Ulyatt and MacRae, 1973). This work demonstrates that the caecum contributes a significant proportion of total fermentation in the ruminant.

PRACTICAL CONSIDERATIONS

Metabolizable energy (ME) is currently favoured as the unit of expressing the energy requirements of ruminants (Agricultural Research Council, 1965). Most of the work on the ME system has been done with ruminants fed dried rations and, as Jagusch and Coop (1971) pointed out, very few measurements of the ME content of fresh herbage have been made. Therefore, some pertinent data from the current experiments with perennial ryegrass and white clover are presented in Table 4. Methane production measured in the respiration hood was corrected for caecal methane production. Both pastures were of high nutritive value, having OM digestibilities in the order of 84%. For both feeds the ratio of ME/DE was 0.79 at M and 0.82 to 0.83 at 1.5M. These are close to the standard value of 0.82 recommended by the Agricultural Research Council (1965). Values for ME/kg DM in the region of 2.7 to 3.0 (Mcal) would seem reasonable for high producing

TABLE 4: SOME MEASUREMENTS OF THE METABOLIZABLE ENERGY (ME) CONTENT OF FRESH PERENNIAL RYEGRASS AND WHITE CLOVER FED TO SHEEP AT INTAKES OF MAINTENANCE (M) AND 1.5M

	Perennial Ryegrass		*White Clover*	
	M	*1.5M*	*M*	*1.5M*
OM digestibility (%)	85.5	83.5	84.2	84.0
Energy digestibility (%)	80.7	80.7	80.3	81.0
ME/DE	0.79	0.83	0.79	0.82
Mcal ME/kg DM	2.84	3.00	2.73	2.93
Mcal ME/kg DOM	3.76	3.98	3.65	3.95

pastures in New Zealand during the spring flush of pasture growth. Thus it seems that little error would be incurred in using standard values for calculating the feed requirements of sheep fed fresh herbage.

ACKNOWLEDGEMENTS

One of the authors (A.R.E.) was in receipt of an NRAC Senior Research Fellowship. Another of the authors (D.J.W.) was a guest at Applied Biochemistry Division, DSIR, while on leave from CSIRO, Division of Nutritional Biochemistry, Adelaide, South Australia. The skilled technical assistance of B. S. Henderson, I. D. Shelton, P. J. Boniface, P. Vlieg, Mrs P. Cooper and Mrs B. K. Marsh is gratefully acknowledged.

REFERENCES

Agricultural Research Council, 1965: *The Nutrient Requirements of Livestock. No. 2. Ruminants.* A.R.C., London.
Annison, E. F.; Brown, R. E.; Leng, R. A.; Lindsay, D. B.; West, C. E., 1967: *Biochem. J., 104:* 135.
Bruce, J.; Goodall, E. D.; Kay, R. N. B.; Phillipson, A. T.; Vowles, L. E., 1966: *Proc. roy. Soc. B., 166:* 46.
Gray, F. V.; Weller, R. A.; Pilgrim, A. F.; Jones, G. B., 1967: *Aust. J. agric. Res., 18:* 625.
Hungate, R. E., 1965: In *Physiology of Digestion in the Ruminant* (ed. Dougherty, R. W.), p. 311. Butterworths, London.
——— 1966: *The Rumen and its Microbes.* Academic Press, London.
Jagusch, K. T.; Coop, I. E., 1971: *Proc. N.Z. Soc. Anim. Prod., 31:* 224.
Kronfeld, D. S., 1968: *J. Dairy Sci., 51:* 397.
MacRae, J. C.; Reid, C. S. W.; Dellow, D. W.; Wyburn, R. S., 1973: *Res. vet. Sci., 14:* 78.
Phillipson, A. T., 1972: *Proc. Nutr. Soc., 31:* 159.
Ulyatt, M. J.; Egan, A. R., 1973: *Aust. J. agric. Res.* (in press).
Ulyatt, M. J.; MacRae, J. C., 1973: *J. agric. Sci., Camb.* (in press).
Ulyatt, M. J.; Webster, D. W., 1973: *N.Z. Jl agric. Res.* (in press).