Aust. J. Biol. Sci., 1976, **29**, 209–14

Methane Production and Digestibility Measurements in the Grey Kangaroo and Sheep

T. J. Kempton,[A] *R. M. Murray*[B] *and R. A. Leng*[A]

[A] Department of Biochemistry and Nutrition, University of New England, Armidale, N.S.W. 2351.
[B] Department of Tropical Veterinary Science, James Cook University, Townsville, Qld 4811.

Abstract

Three grey kangaroos and three sheep were given a diet of lucerne chaff and measurements were made of feed intake, digestibility coefficients, methane production rate and volatile fatty acid content of the 'stomach' and caecum for each animal. The kangaroos had lower intakes of digestible dry matter and organic matter than the sheep; this was related to lower intakes of dry matter and lower apparent digestibility coefficients particularly of the crude fibre fraction.

Methane production in the sheep (collected in respired air through a mask) was 0·81 litre/h; no methane was collected in the respired air from kangaroos. Anal release of methane in sheep and kangaroos indicated that some methane was produced in the hind gut of kangaroos and that all of this methane was lost via the anus. This finding was different to the sheep which apparently excreted 80–90% of the hind gut methane via the lungs. Thus in both sites of apparent high microbial growth in the gut of kangaroos methane production is negligible or lower than in the same sites in sheep. Possible explanations for the absence of measurable methane production in the kangaroo fore-stomachs are discussed.

Introduction

The efficiency of utilization of roughage diets by kangaroos has received little attention. Calaby (1958) studied digestion in the quokka *Setonix brachyurus* (Quoy and Gaimard) and concluded that its digestive efficiency was intermediate between that of the ruminant and non-ruminant herbivore. Foot and Romberg (1965), McIntosh (1966) and Forbes and Tribe (1970) compared digestion in the red kangaroo (*Macropus rufus*, Desmarest) with sheep, and found the apparent digestibility of lucerne in these kangaroos to be significantly lower. The rate of passage of digesta was faster in the kangaroo compared with sheep, which appeared to result in a lower digestibility of crude fibre in the kangaroo (McIntosh 1966). Moir (1965) has emphasized the 'ruminant-like' digestive system of the kangaroos, and Moir *et al.* (1956) measured volatile fatty acid (VFA) production in stomach contents *in vitro*. However, there are no results which indicate the extent of fermentation in the stomach of the intact animal.

In ruminal fermentation, hydrogen gas is generated when acetate is produced from pyruvate or when co-enzymes generated in the fermentation pathways are reoxidized (see Baldwin *et al.* 1970). In ruminants this hydrogen is converted to methane and the rate of production [between 10 and 50 litres/day (see Blaxter 1962)] is stoichiometrically related to the fermentation rate (see Leng and Murray 1972). As part of a comparative study of digestion in herbivores, we set out to use methane production as an index of fermentation in the sheep and kangaroo.

Materials and Methods

Animals

Three mature Merino ewes and three mature female eastern grey kangaroos (*Macropus giganteus* Shaw) were housed individually in metabolism cages. Prior to the experiment all animals were given lucerne chaff *ad libitum* and trained to accept the experimental procedures. All animals were weighed at the beginning of the experimental period (see Table 2). The lucerne contained 88% dry matter comprising 36% crude fibre, 2·6% nitrogen and 8·5% ash on a dry matter basis.

Experimental Methods

To facilitate comparisons of the intake and digestibility of lucerne by sheep and kangaroos, all animals were given each day 50 g chopped lucerne hay per unit metabolic body size, i.e. body weight$^{0·75}$. Water was available at all times.

The experimental period consisted of a 10-day pre-experimental period and an 8-day digestibility trial. The faeces and feed refusals were collected daily, a 10% subsample taken, bulked and stored at −20°C for analysis. Urine excretions were collected daily in 20 ml of a mercuric chloride–glacial acetic acid mixture (0·1% w/v). A subsample of 10% by volume was stored at −20°C.

Chemical Methods

The dry matter content of all samples taken each day was determined after heating in a forced air oven at 70°C for 48 h. The bulked subsamples were dried and ground through a 1-mm screen before being analysed.

Feed, faeces and feed refusals were analysed for organic matter and crude fibre (Association of Official Agricultural Chemists 1960). The gross energy content of the dry matter of these materials was estimated using a Gallenkamp Autobomb Automatic Adiabatic Bomb Calorimeter (No. DB110), and nitrogen content was determined by the semi-micro Kjeldahl method of Clare and Stephenson (1964). The concentration and proportions of VFA in the rumen and caecum of sheep and stomach contents of kangaroos were estimated (see Leng and Leonard 1965) on materials obtained following slaughter of two of the kangaroos and two of the sheep.

Measurement of Methane Production in Vivo

All animals were given $\frac{1}{8}$ of their daily ration every 3 h for 2 days prior to and during the methane production rate measurement. Methane production was measured by fitting a mask over the mouth of the animal 1 h after feeding and collecting respired and eructated gases for 1 h. A total of three 1-h collections with a 2-h interval between collections was made for each animal.

The gas handling system was essentially the same as that described by Murray *et al.* (1976). In this system air was drawn across the nose and mouth of the animal at a rate of 50 litres/min and a subsample of 2 litres/min was drawn serially through a freeze-drying unit and a 'Lira' methane analyser (Mine Safety Appliances Co., Pittsburgh, U.S.A.). Methane content in the gases was read directly from a chart recorder attached to the methane analyser which had been previously calibrated with a standard gas mixture.

The release of methane from the anus was measured using the same principle as used for collection of gases from the mouth. A mask designed to allow the free passage of urine and faeces and yet retain any gases produced was used in place of the face mask. Air was drawn across the anus for a single 3-h period per animal and analysed for methane content as described above.

Measurement of Methane Production in Vitro

Mixed digesta from the rumen of a sheep and the upper stomach of a kangaroo were obtained from slaughtered animals. Samples of 100 ml were incubated under nitrogen gas in conical flasks at 39°C and shaken 100 times/min for 1 h. Care was taken to keep the samples under anaerobic conditions at all times. Nitrogen gas was passed through the digesta contents at about 1 ml/min and passed through a $CaCl_2$ drying train and then through the methane analyser.

Results

Feed intake and digestibility coefficients for each animal are given in Table 1. The kangaroo had significantly ($P < 0·01$) lower digestibility coefficients for all feed components than sheep. Methane production rates for individual animals are given

Table 1. Mean apparent digestibility coefficients, nitrogen balance and intake of lucerne chaff by kangaroos and sheep

Values shown are means ± s.e. The significance of the difference between means is also shown

	Intake		Digestibility (%)				
	Dry matter (g/day)	Organic matter (g $W^{-0.75}$ d^{-1})[A]	Dry matter	Organic matter	Crude fibre	Crude protein	Energy
Kangaroo	477	46	55±1·1	56±1·1	36±1·8	73±0·1	56±0·5
Sheep	643	47	62±0·3	63±0·3	48±0·7	76±0·4	61±0·9
P	—	—	**	**	**	**	**

	Nitrogen balance (g nitrogen weight$^{-0.75}$ day^{-1})			
	Feed	Faeces	Urine	Balance
Kangaroo	1·36±0·004	0·37±0·002	0·98±0·049	0·01±0·048
Sheep	1·37±0·002	0·34±0·008	0·81±0·021	0·22±0·014
P	n.s.	n.s.	n.s.	**

[A] Measured as grams per weight$^{0.75}$ per day.
** $P < 0·01$. n.s., Not significant.

Table 2. Methane production measured over a 3-h period in kangaroos and sheep given a lucerne chaff diet, and individual animal weights

Animal	Respired methane (l/h)	Anal methane (l/h)	Total methane production (% DEI)[A]	Weight (kg)
Kangaroo 1	0	0·02	0·40	19·1
Kangaroo 2	0	0·02	0·34	24·1
Kangaroo 3	0	0·02	0·37	22·7
Mean		0·02±0·000	0·37±0·017	
Sheep 1	0·79	0·01	10·60	30·0
Sheep 2	0·75	0·02	9·96	32·8
Sheep 3	0·88	0·01	11·01	34·0
Mean	0·81±0·036	0·01±0·018	10·52±0·305	
Significance of mean differences	**	n.s.	**	

[A] Percentage of digestible energy intake (DEI); 1 litre methane = 39·54 kJ (Brouwer 1965).
** $P < 0·01$. n.s., Not significant.

Table 3. Volatile fatty acid concentrations and proportions in stomach and caecal contents from slaughtered sheep and kangaroos given lucerne chaff

Each value is the mean of two results

Animal	Total VFA concn (mM)	Individual VFA proportions (mol/100 mol)					
		Acetic	Propionic	Butyric	Isobutyric	Valeric	Isovaleric
Sheep							
Rumen fluid	62	68	22	7	1·3	0·6	1·9
Caecum fluid	64	79	14	5	0·4	0·9	0·6
Kangaroo							
Stomach fluid	100	70	17	12	0·2	0·9	0·2
Caecum fluid	68	82	13	4	0·5	0·4	0·3

in Table 2. No measurable amounts of respired or eructated methane were detected in kangaroos, whereas sheep released methane at an average rate of 0·81 litre/h. Kangaroos released methane at a rate of 0·02 litre/h from the anus which was not significantly ($P > 0·05$) different in quantity to the 0·01 litre/h released anally by sheep. The methane production in kangaroos represented 0·4% of the digestible energy intake (DEI) compared to 10·5% of DEI in sheep. No detectable production of methane occurred from 100 g of stomach contents of kangaroos whereas from the same quantity of rumen contents of sheep methane was produced at between 20 and 40 ml/h.

The proportions and concentrations of VFA in the rumen and caecum of sheep and the stomach and caecum of kangaroos are shown in Table 3.

Discussion

Ruminants can effectively utilize a large proportion of the cellulose of their diet because of microbial fermentation and a long retention time of feed particles in the rumen. Moir *et al.* (1956) suggested that because of the kangaroo's 'ruminant-like' digestive tract it could similarly utilize ingested fibre, and consequently most studies on kangaroo digestion have assumed that feed is retained in the forestomach where there is substantial production of VFA and microbial cells.

The intakes and associated digestibility coefficients presented here for kangaroos and sheep are comparable to those found by Calaby (1958), Foot and Romberg (1965), McIntosh (1966) and Forbes and Tribe (1970). Kangaroos had lower digestibility coefficients for all dietary components measured. Even when the effect of the crude fibre fraction was removed during calculation of the organic matter digestibility coefficients, there remained an interspecies difference in 'organic matter' digestibility and therefore the differences were not entirely due to a difference in fibre digestion. Thus the kangaroo does not apparently digest the available dietary components as efficiently as sheep.

In this study it was found that kangaroos produced insignificant quantities of methane during the period of collection in comparison with sheep. Of the methane produced by the kangaroos, none was apparently produced in the forestomach as indicated by mask collections. The methane analyser used could detect methane at production rates as low as 0·5 ml/h (Murray 1974). Incubation *in vitro* of digesta from the forestomach of both species showed methane production for the sheep but none from the kangaroo. Although the mask collections were only for a period of 3 h, Murray *et al.* (1976) have shown that methane production in sheep fed lucerne chaff at hourly intervals is almost constant. The rate of emission of methane from the anus, however, is quite variable and so the values reported here must be considered as being only indicative of relative rates in both species. As sheep excrete a considerable portion (80–90%) of the methane produced in the hindgut via the lungs (Murray *et al.* 1976) this would indicate that the overall production of methane in the hindgut of the kangaroo was less than in the sheep. The absence of methane in respired air in the kangaroos may indicate that methane is produced in the large intestine close to the anus and rapidly excreted.

In these kangaroos the concentration of VFA in mixed stomach contents of slaughtered animals was 85–115 mmol/litre indicating that some fermentation occurred. The extent of fermentation relative to the sheep, however, may not be

indicated by these concentrations since the stomach contents from kangaroos were much drier (15–17% dry matter) than those in the rumen of sheep (12–13%) on the same diet.

A possible explanation for the lack of methane production may be that fermentation occurs in the fundus area of the stomach where oxygen, taken in with the feed, might act as an electron acceptor. Since methanogenic bacteria are obligatory anaerobes (Hungate 1966) the presence of oxygen would prevent their growth thereby inhibiting methane production from hydrogen generated in the production of acetate (see Leng 1970). The simple structure of the kangaroo forestomach may allow entry of oxygen across the wall, and this would result in a lower reducing potential in the whole organ. A low redox potential in the kangaroo's stomach may also explain the low degree of hydrogenation of dietary unsaturated fatty acids in kangaroos (Griffiths *et al.* 1972) as indicated by the high content of these in kangaroo's milk. Such a high concentration of unsaturated fatty acids in milk from kangaroos may indicate that hydrogenation of dietary fatty acids in the stomach is low, which in turn suggests low hydrogen production and a limited rate of fermentation in the forestomach of kangaroos. Conversely, if absorption of VFA was slow the high concentrations of VFA in the deeper fundus area or the area tending towards true gastric function may, through feedback mechanisms, reduce fermentation. Thus the relatively high levels of VFA may be due to a low fermentation rate coupled with a low absorption rate.

Further research is necessary to explain the absence of methane in the forestomach of kangaroos. Since methanogenic bacteria definitely occur in the hind gut of the kangaroo, and unless oxygen is gaining access to the stomach, the lack of methane production appears to be due largely to a low fermentation potential. This is also indicated by the low fibre digestion in these animals. Until more positive measurements are made in intact animals there must be considerable doubt as to whether the kangaroo is 'ruminant-like' and whether methane production can be used as a basis for comparison between the relative efficiencies of digestion in various herbivores.

Acknowledgments

We wish to express our appreciation for the technical assistance of Misses Cynthia Underwood and Clare Allington. Drs J. V. Nolan, B. W. Norton and I. D. Hume are also thanked for considerable discussion, particularly of techniques.

References

Association of Official Agricultural Chemists (1960). 'Official Methods of Analysis'. 9th Edn. (Ed. W. Horowitz.) (Association of Official Agricultural Chemists: Washington.)

Baldwin, R. L., Lucas, H. D., and Cabrera, R. (1970). Energetic relationships in the formation and utilisation of fermentation end products. In 'Physiology of Digestion and Metabolism in the Ruminant'. (Ed. A. T. Phillipson.) pp. 319–34. (Oriel Press: Newcastle-upon-Tyne.)

Blaxter, K. L. (1962). 'The Energy Metabolism of Ruminants'. (Hutchinson and Co.: London.)

Brouwer, E. (1965). Report of sub-committee on constants and factors. In 'Energy Metabolism'. (Ed. K. L. Blaxter.) Proc. 3rd Symp. held at Troon, Scotland, 19–22 May 1964. European Association for Animal Production No. 11. pp. 441. (Academic Press: London.)

Calaby, J. H. (1958). Studies on marsupial nutrition. II. The rate of passage of food residues and digestibility of crude fibre and protein by the quokka, *Setonix brachyurus* (Quoy and Gaimard). *Aust. J. Biol. Sci.* **11**, 571–80.

Clare, N. T., and Stephenson, A. E. (1964). Measurement of feed intake by grazing cattle and sheep. X. Determination of nitrogen in faeces and feeds using an autoanalyser. *N.Z. J. Agric. Res.* **7**, 198.

Foot, J. Z., and Romberg, B. (1965). The utilization of roughage by sheep and the red kangaroo, *Macropus rufus* (Desmarest). *Aust. J. Agric. Res.* **16**, 429–35.

Forbes, D. K., and Tribe, D. E. (1970). The utilization of roughages by sheep and kangaroos. *Aust. J. Zool.* **18**, 247–56.

Griffiths, M., McIntosh, D. L., and Leckie, R. M. C. (1972). The mammary glands of the red kangaroo, with observations on the fatty acid components of the milk triglycerides. *J. Zool.* **166**, 265–75.

Hungate, R. E. (1966). 'The Rumen and its Microbes'. (Academic Press: New York.)

Leng, R. A. (1970). Formation and production of volatile fatty acids in the rumen. In 'Physiology of Digestion and Metabolism in the Ruminant'. (Ed. A. T. Phillipson.) pp. 406–21. (Oriel Press: Newcastle-upon-Tyne.)

Leng, R. A., and Leonard, G. K. (1965). Measurement of the rates of production of acetic, propionic and butyric acids in the rumen of sheep. *Br. J. Nutr.* **19**, 469–83.

Leng, R. A., and Murray, R. M. (1972). Estimation of the fermentation rate in the rumen of sheep using VFA production, carbon dioxide production and methane production. In 'Tracer Studies on Non-protein Nitrogen for Ruminants II'. pp. 21–7. (Int. Atomic Energy Agency, Vienna.)

McIntosh, D. L. (1966). The digestibility of two roughages and the rates of passage of their residues by the red kangaroo, *Megaleia rufa* (Desmarest), and the Merino sheep. *CSIRO Wildl. Res.* **11**, 125–35.

Moir, R. J. (1965). The comparative physiology of ruminant-like animals. In 'Physiology of Digestion in the Ruminant'. (Ed. R. W. Dougherty.) Ch. 1. (Butterworth: Washington.)

Moir, R. J., Somers. M., and Waring, H. (1956). Studies on marsupial nutrition. I. Ruminant-like digestion in a herbivorous marsupial *Setonix brachyurus* (Quoy and Gaimard). *Aust. J. Biol. Sci.* **9**, 293–304.

Murray, R. M. (1974). Nutrition and digestive function of the ruminant. Ph.D. Thesis, University of New England, Armidale, Australia.

Murray, R. M., Bryant, A. M., and Leng, R. A. (1976). Rates of production of methane in the rumen and large intestine of sheep. *Br. J. Nutr.* **36**, 1–14.

Manuscript received 5 November 1975