Gut microbiology and its consequences for the ruminant

S K Baker

CSIRO Division of Animal Production, Floreat Park, WA 6014

Summary

Ruminant herbivores have evolved with a pregastric digestion of the food they ingest. This places them at a lower trophic level than other herbivores, but it gives them greater nutritional flexibility. While a microbial population in the foregut confers upon the host animal a number of advantages, it also has some disadvantages, one of them being the production of methane (CH_4) by the microbial population. This CH_4 production represents a loss of dietary energy for the animal. However if methane production in the rumen is reduced, the animal benefits through a greater supply of glucogenic volatile fatty acids and an enhanced efficiency of microbial protein synthesis from the rumen fermentation. Current understanding of microbial physiology, particularly in continuous mono- and co-cultures fed with particulate cellulose, is valuable in understanding some of the complex interactions amongst the rumen microorganisms.

Introduction

An autochthonous microbial population is ubiquitous in the digestive tract (1). In herbivorous mammals, large complex and stable populations of microorganisms are found either in the hindgut distal to the small intestine, or in both the hindgut and an enlarged region of the digestive tract, the foregut, before the gastric stomach. In the latter group of animals the activity of the microbial population in the foregut tends to predominate over that of the population in the hindgut, and in these animals there is pregastric digestion of the food that they ingest (2). This group includes the bovids (the family to which sheep and cattle belong), the camelids (the camel family) and the macropods (the kangaroo family).

Evolution of pregastric digestion

From paleontological evidence and study of extant herbivores, it appears that foregut fermentation arose in the hindgut fermenters faced with nutritionally inhospitable environments (3). The rapid radiation of both the bovids and the macropods in the Miocene and Pliocene coincided with expansion of the grasslands. A population of fermentative organisms in the foregut could have been important in detoxifying the plant secondary compounds that would have been in the tropical vegetation. The quality and quantity of dietary nutrients in the grassland vegetation fluctuate with the seasons, and as the animals moved into the grasslands they would have relied increasingly on the products of fermentation and growth of a microbial population in the foregut. Thus the association between the animal host and the foregut population has permitted herbivores to survive and persist in such nutritionally inhospitable environments as the grasslands.

Kinnear and Main (4) explained the advantage to the animal of this association in terms of an ecological niche. An ecological niche is an n-dimensional hypervolume or space where each dimension (ni) which defines the niche is an environmental or biological variable that affects the persistence of the animal (5). Kinnear and Main defined a subset of an ecological niche, a nutritional niche, which is defined by nutrient variables essential to the persistence of the species, such as energy-yielding compounds, amino acids, and vitamins. Competition between animals contracts the niche space (a realised niche), but because the association between the microbial population in the foregut and the host animal enables the animal species to survive on a diet that otherwise would be nutritionally inadequate (6), the realised nutritional niche is expanded. Herbivores in which there is pregastric digestion of their diets occupy a lower

trophic level than do other herbivores, because the microbial fermentation is interposed between the host animal and the plant material it eats. This strategy confers on the animal a high degree of nutritional flexibility and allows nutritional specialisation, but it is not a strategy that is restricted to the herbivores. Chitin in the diet of the minke whale, like the celluloses and hemicelluloses in the herbivore's diet, is made up of b-linked sugar moieties and is degraded in a ruminant-like foregut fermentation (7).

The flexibility of the strategy in herbivores is the basis of exploitation of domestic ruminants and camelids for milk, meat, fibre and draft power, but the strategy also has some disadvantages. The microbial population in the foregut can transform dietary constituents into nutrients for survival and growth of the animal, but herbivory appears to be the preserve of adults. Whereas herbivory is adequate for survival of the adult, the immature animal requires a more balanced mix of amino acids than can be supplied from the activities of the microbial population in the foregut (8). The microorganisms also are responsible for transformations of other constituents of the diet, the most common of these being plant secondary compounds which can be toxic to the animal. In some cases these transformations are beneficial and yield compounds that are no longer a threat to the health of the animal, but in other cases the transformations yield compounds that are toxic (9). Another consequence of the association between the microbial population in the pregastric digestive tract and the host animal, and one that is the focus of this paper, is the inevitable losses that are associated with the transfer of energy and material from one trophic level to another, and these are a cost to the host animal.

The microbial population of the rumen comprises a large number of species of eubacteria, archae, ciliate protozoa, flagellates and anaerobic fungi. The organisms range in size by five orders of magnitude, from the very smallest of the eubacteria to the largest of the protozoa (10), analogous to the range in size from the shrew to the elephant. The collective activities of this population yield short-chain volatile fatty acids (VFA, principally acetic, propionic and butyric), microbial cells, carbon dioxide (CO_2) and CH_4. The microbial cells and VFA are important sources of nutrients for the animal, but because CH_4 has no nutritional value for the animal, production of CH_4 represents for the animal a loss of dietary energy. As well, predation by protozoa upon other organisms in the rumen can reduce the supply to the animal of the nutrients in microbial cells.

Methane production in ruminants

Methane produced in the foregut and hindgut accounts for between 2 and 15% of the animal's gross energy intake (11, 12). Fermentation in the hindgut probably accounts for up to 12% of overall CH_4 production (13). Accounting for the loss of energy as CH_4 in ruminants over a wide range of diets has led to derivation of prediction equations, based on dietary factors relating to the composition and intake of the diet (12, 14-6).

Table 1. Estimated CH_4 emissions (Gg/yr) from anthropogenic sources in Australia (18).

Year	1988	1989	1990	1991	1992	1993	1994
Total emissions (net)	5212.6	5063.9	5589.7	5313.9	5306.4	5284.8	5302.3
Emissions from agriculture (net)	3111.5	3161.9	3223.2	3228.3	3159.5	3152.6	3140.8
Emissions from fermentation in the foregut and hindgut of domestic animals	2696.9	2749.2	2814.2	2818.4	2771.7	2762.8	2761.6

In recent years there has been renewed interest in CH_4 production by domestic ruminants as a result of the perceived role of CH_4 as a greenhouse gas involved in potential global warming (17). In Australia the prediction equations of Blaxter and Clapperton (14), Moe and Tyrell (15), and Howden and his colleagues (16) are used to estimate CH_4 emissions by dairy cattle, beef cattle and sheep respectively (18). Current Australian estimates of CH_4 production from anthropogenic sources are listed in Table 1. Net CH_4 production from agriculture accounts for

about 60% of total net CH_4 production, and of this the amount produced in the animal industries accounts for about 87%. Nearly all of the CH_4 produced in the animal industries is from ruminants. Cattle produce an estimated 175 kg CH_4/head/yr and sheep 7 kg CH_4/head/yr.

Production of methane from fermentation in the rumen

Energy for microbial growth in the digestive tract is derived from substrate oxidation and in anaerobic environments like the rumen, metabolic hydrogen produced by oxidation of organic substrates is transferred in the absence of dioxygen (O_2) to alternative electron acceptors ('electron sinks') such as CH_4, propionate, succinate, ethanol and lactate. Exogenous compounds in these environments can act as electron acceptors and include CO_2, unsaturated long-chain fatty acids, sulphates, and some plant secondary compounds. A number of plant secondary compounds are toxic to animals but their hydrogenation in the rumen detoxifies them. Syntrophic and fermentative interactions between organisms that generate hydrogen (hydrogenogens) and organisms that utilise it (hydrogenotrophs) (for examples see 19, 20-2) are likely to significantly influence the outcome of fermentation in the rumen (19, 21). Most (95 to 100%) metabolic hydrogen comes from production of VFA in the rumen (11).

The balance of glucogenic and non-glucogenic VFA from rumen fermentation is important for the animal; propionate is the sole glucogenic substrate for the animal. A strong inverse relationship between the molar proportion of propionate and CH_4 production is predicted from the stoichiometry of rumen fermentation (21, 23), and has been demonstrated repeatedly in vivo and in vitro (see 24, 25). With an improved molar proportion of propionate the molar proportions of acetate and/or butyrate are reduced; that is the pattern of fermentation in the rumen is altered.

As the proportion of roughage in a ruminant's diet increases, CH_4 production increases, and the molar proportion of propionate decreases (26). A consequence of the amount of roughage in the diet is that the animal must expend energy in chewing (27) and there is a strong positive correlation between the number of chews per bolus during rumination and the 'toughness' of the roughage (28). With more rumination, more saliva enters the rumen, and intra-ruminal infusion of 'artificial saliva' into the rumen mimics the effect on the pattern of rumen fermentation of increasing the proportion of roughage in the diet (29, 30). The molar proportion of propionate increases with a decrease in the rumen dilution rate (the fractional rate of clearance of fluid from the rumen) through either a change in rumen volume and/or in the rate of passage of the fluid fraction of the rumen digesta. Under those conditions selenomonads and bacteroides predominate in the microbial population (31), consistent with a fermentation where the molar proportion of propionate is high. The rate of passage of the fluid fraction of the digesta is influenced more by the proportion of hay in the diet than by the proportion of grain or concentrates (32). Interestingly, similar relationships between of rate of passage of digesta, methane production and the pattern of fermentation can be associated with adaptation by the animal to cold (33).

Some of these relationships between the activities of the rumen microbial population and the host ruminant can be explored using data from a recent experiment (34) where CH_4 production was estimated using sulphur hexafluoride (SF_6) as a gaseous phase marker in the rumen (35) of sheep fed diets containing graded amounts of oaten hay and oat grain. The amount of CH_4 produced (g/d) is consistent with the empirical relationship (16) between CH_4 production, and diet composition and intake (Table 2). The loss of energy in CH_4, expressed as a proportion of the estimated energy yield from rumen fermentation is least when the diet contains most grain, and it is associated with an increase in the molar proportion of propionate relative to the other, nonglucogenic, VFA from rumen fermentation (Table 2, 34). This is consistent with estimates based on the stoichiometry of rumen fermentation (23, 36).

Table 2. Methane (CH_4) from fermentation in the rumen

	Oat grain in ration (%)			
	0	17	34	50
CH_4 (g/d), estimated using SF_6 as a gaseous phase marker	21 ± 1.7	18 ± 2.4	20 ± 2.4	16 ± 1.7
Propionate (mmol %)	18 ± 0.08	20 ± 0.9	18 ± 1.2	25 ± 0.9 a[2]
CH_4.(g/d), calculated from (16)	22	20	19	18
CH_4 (MJ/100 MJ in estimated OMADR)[1]	23 ± 1.5	21 ± 2.0	21 ± 1.9	17 ± 1.4 a
CH_4 (MJ/100 MJ hexose fermented), calculated according to (23)	19	18	19	17
Digestibility of dry matter (%)	65.1 ± 0.55a	67.7 ± 0.67	69.7 ± 0.77	69.4 ± 0.61
NI/DDMI3 (g/100 g)	3.3 ± 0.04 a	3.1 ± 0.04	3.0 ± 0.05	3.0 ± 0.03

[1] Organic matter apparently digested in the rumen (OMADR) was estimated using data from sheep fed lucerne hay, and barley and oat grains (37, 38). It was assumed that CH_4 contains 55.27 MJ/Kg and OMADR contains 17.6 MJ/Kg, and the contribution of fermentation in the hindgut to the amount of CH_4 produced was ignored

[2] Differs from other values in the same row ($P<0.05$)

[3] Nitrogen intake (g) per 100 g digestible dry matter intake

Since the composition of the diet determines the substrates available to the rumen population and thus the activities of the rumen organisms, nitrogen intake per unit of digestible dry matter intake (NI/DDMI (g/100 g), Table 2) was used as a covariate in further analysis of the data. Accounting for variation in NI/DDMI in this way accentuated the differences between diets in the amounts of CH_4 (g/d) and propionate (moles/d) produced (Figure). There is no evidence of change in the amount of metabolic hydrogen produced from rumen fermentation. What is puzzling is that while there is an increase in the amount of CH_4 produced with an decreasing grain content of the diet, the size of the population of CH_4-producing organisms (methanogens) (Figure). The likely rate of passage from the rumen of the fluid fraction of the digesta was estimated from the relationship established by Owens and Goetsch (32). These estimates and the increase in the molar proportion of propionate are consistent with the earlier work with sheep fed diets containing graded amounts of roughage and grain (26). An explanation of these observations is that with diets high in grain propionate is used as a 'sink' for metabolic hydrogen by organisms that grow faster under these conditions than do the methanogens. Alternatively acetate synthesis by reduction of CO_2 is a significant contributor to rumen acetate production.

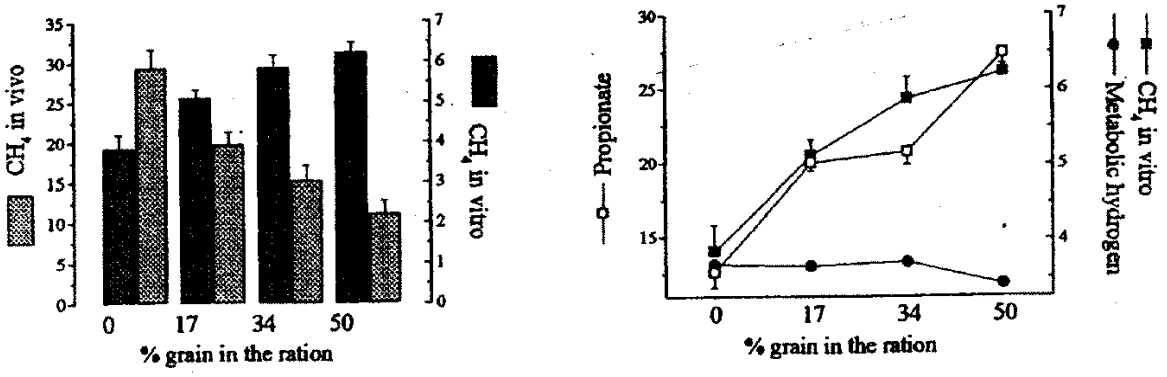

Figure 1. Methane produced in vivo (g/d), sizes of populations of methanogens (indicated by CH_4 produced in vitro (mmol CH_4/mL rumen inoculum) (39)), molar proportion of propionate (mmol%) and estimated production of metabolic hydrogen (mol/mol 'hexose' apparently fermented in the rumen). Data are least squares means (and standard errors) when NI/DDMI (Table 2) is a covariate. Production of metabolic hydrogen was estimated from production of individual VFA (11, 23) and estimated OMADR (Table 2). It was assumed that VFA production was 1.8 mol/mol hexose fermented and molar proportions of the VFA reflect their production rates (40), as this appears to be true for sheep fed diets containing mostly roughage (40, 41).

Some insights into how the metabolic activities of the rumen organisms might influence the pattern of fermentation in the rumen, and thus the supply of nutrients to the animal, can be gained from less complex systems where individual organisms from the digestive tract are grown in vitro. In continuous mono-cultures of some hydrogenogens dilution rate can influence the pattern of fermentation (42-6). These patterns of fermentation can be further modified if the hydrogenogen is grown in co-culture with a hydrogenotroph (47-9). The range in rumen dilution rate with intraruminal infusion of 'artificial saliva' and with altered proportions of grain in the diet (29, 30, 31) was between 0.7 and 2.6 /d. Quite recently cellulose-degrading organisms have been grown in continuous culture systems fed with particulate cellulose, either as mono-cultures or in co-culture with hydrogenotrophs (45, 46, 48, 49). Over a range of dilution rates between 0.32 and 4 /d the proportion of cellulose degraded by the mono-cultures increases as dilution rate decreases (45, 46), even though the proportion of organisms that are not attached to cellulose decreases (46).

The proportion of cellulose degraded is unchanged when the cellulose degrader is grown in co-culture with a methanogen (48), but the amount of acetate produced per mole of cellulose (expressed as hexose) degraded is increased and the amount of metabolic hydrogen generated from cellulose degradation increases. There is no effect of dilution rate on the efficiency of production of either acetate or CH_4 (moles per mole cellulose (as hexose) degraded) in the co-culture. However the amount of CH_4 produced (mmol/d) increases as dilution rate increases, despite a decrease in the size of the methanogen population (number/mL). In methanogens oxidation of hydrogen to produce CH_4 is linked to the maintenance of electrochemical gradients across the cell membrane (50), and thus appears to be obligatory for the organism. Under conditions when hydrogen is limiting, cell yield (g cells produced/ g CH_4 produced) increases, suggesting that CH_4 production might not necessarily be coupled to anabolism (51). In addition there may be poor coupling of CH_4 production and hydrogen utilisation at high dilution rates (48).

When a selenomonad, Selenomonas ruminantium is grown in continuos culture with glucose as the limiting nutrient, the pattern of fermentation changes with dilution rate (42-4). With an increase in dilution rate from 1.25 /d to 1.7/d there is an increase in cell yield (g cells produced/g substrate fermented) that is accompanied by an increase in propionate production relative to acetate in the products of fermentation (44). When the dilution rate is further increased to 3.5 /d but the rate of supply of substrate is unchanged, the increase in cell yield is enhanced but propionate production decreases relative to acetate.

Acetate production by reduction of CO_2 may be more important in rumen fermentation than has been supposed, because in cattle fed either grain or roughage diets the sizes of the rumen populations of acidogenic (presumably acetogenic) and methanogenic organisms that use H_2 and CO_2 are similar (52). Recently Miller and Wolin (49) have shown that at low dilution rates in a continuous co-culture fed particulate cellulose a cellulose-degrader and an acetogen together produce acetate, and about a third of the acetate is produced by reduction of CO_2 by the acetogen.

An improved supply of propionate from fermentation and growth of the rumen population with changes in rumen dilution rate also can be associated with a greater efficiency of microbial protein synthesis and in turn an enhanced supply of protein to the animal (30, 33). This is in agreement with enhanced cell yields in continuous mono- and co-cultures with change in dilution rate. Clearly these techniques can provide insights on the dynamics of rumen organisms and the means to manipulate their activities to enhance productivity in ruminants.

References

1. Savage DC. Interactions between the host and its microbes. In: Clarke RTJ, Bauchop T, eds. Microbial ecology of the gut. London: Academic Press, 1977:278-310.
2. Moir RJ. Ruminant digestion and evolution. In: Code CF, ed. Handbook of physiology, Section 6: Alimentary canal. Washington DC: American Physiological Society, 1968;V:2673-94.
3. Hume ID, Warner ACI. Evolution of microbial digestion in mammals. In: Ruckebusch Y, Thivend P, eds. Digestive physiology and metabolism in ruminants. England: MTP Press, 1979:665-84.
4. Kinnear JE, Main AR. Niche theory and macropod nutrition. J Roy Soc Western Australia 1979;62:65-74.
5. Hutchinson GE. Concluding remarks. Cold Spr Harb Symp Quant Biol 1957;415-27.
6. Hemsley JA. Dietary cellulose content and urea utilisation. Proc Aust Soc Anim Prod 1966;6:384-8.
7. Olsen MA, Aagnes TH, Sørmo W, Mathiesen SD. Chitinolytic bacteria in the minke whale forestomach. Ann Zootech 1996;45:287.
8. Moir RJ. The 'carnivorous' herbivores. In: Chivers DJ and Langer P, eds. The digestive system in mammals. London: Cambridge University Press, 1994:87-102.
9. Dawson KA, Rasmussen MA, Allison MJ. Digestive disorders and nutritional toxicity. In: (53), 1997:633-60.
10. Baker SK. Allometry and implications of cell size in the rumen microbial ecosystem. In: Akin DE, Ljungdahl LG, Wilson JR, Harris PJ, eds. Microbial and plant opportunities to improve lignocellulose utilization by ruminants. New York: Elsevier, 1990:253-64.
11. Czerkawski JW. An introduction to rumen studies. Oxford: Pergamon Press, 1986.
12. Holter JB, Young AJ. Methane prediction in dry and lactating Holstein cows. J Dairy Sci 1992;75:2165-75.
13. Torrent J, Johnson DE. Methane production in the large intestine of sheep. In: Aguilera JF, ed. Energy metabolism of farm animals. EAAP publication 76, 1994:391-4.
14. Blaxter KL, Clapperton JL. Prediction of the amount of methane produced by ruminants. Br J Nutr 1965;19:511-22.
15. Moe PW, Tyrell HF. Methane production in dairy cows. J Dairy Sci 1979;62:1583-6.
16. Howden SM, White DH, McKeon GM, Scanlan JC, Carter JO. Methods for exploring management options to reduce greenhouse emissions from tropical grazing systems. Climatic Change 1994;27:49-70.
17. Crutzen PJ. The role of methane in atmospheric chemistry and climate. In: Engelhardt Wv, Leonhard-Marek S, Breves G, Giesecke D, eds. Ruminant physiology: digestion, metabolism, growth and reproduction. Stuttgart: Ferdinand Enke Verlag, 1995:291-315.
18. National greenhouse gas inventory, 1988 to 1994. Australia: Department of Environment, Sport and Territories. 1996.
19. Wolin MJ. Interactions between the bacterial species of the rumen. In: (54), 1975:134-48.
20. Hino T. Influence of hydrogen on the fermentation in rumen protozoa, Entodinium species. Jpn J Zootech Sci 1983;54:320-8.
21. Wolin MJ and Miller TM. Microbe-microbe interactions. In: (53), 1997:467-91.
22. McAllister TA, Okine EK, Mathison GW, Cheng K-J. Dietary, environmental and microbiological aspects of methane production in ruminants. Can J Anim Sci 1996;76:231-43.
23. Wolin MJ. A theoretical rumen fermentation balance. J Dairy Sci 43:1452-9.
24. Demeyer DI, Van Nevel CJ. Methanogenesis, an integrated part of carbohydrate fermentation, and its control. In: (54), 1975:367-82.
25. Nagaraja TG, Newbold CJ, Van Nevel CJ. Manipulation of rumen fermentation. In: (53), 1997:524-632.
26. Hodgson JC and Thomas PC. A relationship between the molar proportion of propionic acid and the clearance rate of the liquid phase in the rumen of sheep. Br J Nutr 1975;33:447-56.

27. Osuji PO, Gordon JG, Webster AFJ. Energy exchanges associated with eating and rumination in sheep given grass diets of different physical forms. Br J Nutr. 1975;34:59-71.
28. Wang XR, Baker SK, Purser DB. Unpublished data.
29. Harrison DG, Beever DE, Thomson DJ, Osbourn DF. Manipulation of rumen fermentation in sheep by increasing the rate of flow of water from the rumen. J Agric Sci Camb 1975;85:93-101.
30. Harrison DG, Beever DE, Thomson DJ, Osbourn DF. Manipulation of fermentation in the rumen. J Sci Fd Agric 1976;27:617-20.
31. Latham MJ, Sharpe ME. Rumen microbial population of lambs given mineral-supplemented diets. Proc Nutr Soc 1975;34:113A.
32. Owens FN, Goetsch AL. Digesta passage and microbial protein synthesis. In Milligan LP, Grovum WL, Dobson A, eds. Control of digestion and metabolism in ruminants. New Jersey: Prentice-Hall, 1986:196-223.
33. Kennedy PM, Milligan, LP. Effects of cold exposure on digestion, microbial synthesis and nitrogen transformations in sheep. Br J Nutr 1978;39:105.
34. Dynes RA, Edwards NJ, Baker SK. Why does energy partitioning in ruminants differ on isoenergetic herbage or concentrate diets? Proc Nutr Soc Aust 1997;21:
35. Johnson K, Huyler M, Westburg H, Lamb B. Measurement of methane emissions from ruminant livestock using a SF6 tracer technique. Environ Sci Technol 1994;28:359-62.
36. Orskøv ER, Flatt WP, Moe PW. Fermentation balance approach to estimate extent of fermentation and efficiency of volatile fatty acid formation in ruminants. J Dairy Sci 1968;51: 1429-35.
37. Kung L, Tung RS, Carmean BR. Rumen fermentation and nutrient digestion in cattle fed diets varying in forage and energy source. Anim Feed Sci Technol 1992;39:1-12.
38. Archimède H, Sauvant D, Hervieu J, Poncet C, Dorléans M. Digestive interactions in the ruminant: relationships between whole tract and stomach evaluation. Anim Feed Sci Technol 1995;54:327-40.
39. Brass A, Baker SK. Unpublished data.
40. Leng RA. Formation and production of volatile fatty acids in the rumen. In: Phillipson AT, ed. Physiology of digestion and metabolism in the ruminant. England: Oriel Press, 1970: 406-21.
41. Sutton JD. Digestion and absorption of energy substrates in the lactating cow. J Dairy Sci 1985;68:3376-93.
42. Hobson PN. Continuous culture of some anaerobic and facultatively anaerobic rumen bacteria. J Gen Microbiol 1965;38:167-80.
43. Wallace RJ. Control of lactate production by Selenomonas ruminantium: homotropic activation of lactate hydrogenase by pyruvate. J Gen Microbiol 1978;107:45-52.
44. Silley P, Armstrong DG. Changes in metabolism and cell size of the anaerobic bacterium Selenomonas ruminantium 0078A at the onset of growth in continuous culture. J Appl Bacteriol 1985;56:487-92.
45. Pavlostathis SG, Miller TM, Wolin MJ. Fermentation of insoluble cellulose by continuous cultures of Ruminococcus albus. Appl Environ Microbiol 1988;54:2655-9.
46. Shi Y, Weimer PJ. Response surface analysis of the effects of pH and dilution rate on Ruminococcus flavefaciens FD-1 in cellulose-fed continuous culture. Appl Environ Microbiol 1992;58: 2583-2591.
47. Iannotti EL, Kafkewitz D, Wolin MJ, Bryant MP. Glucose fermentation products of Ruminococcus albus grown in continuous culture with Vibrio succinogenes: changes caused by interspecies transfer of H2. J Bact 1973;114:1231-40.
48. Pavlostathis SG, Miller TL, Wolin MJ. Cellulose fermentation by continuous cultures of Ruminococcus albus and Methanobrevibacter smithii. Appl Microbiol Biotechnol 1990;33:109-116.
49. Miller TL, Wolin MJ. Bioconversion of cellulose to acetate with pure cultures of Ruminococcus albus and a hydrogen-using acetogen. Appl Environ Microbiol 1995;61:3832-5.

50. Deppenmeier U, Müller, Gottschalk G. Pathways of energy conservation in methanogenic archae. Arch Microbiol 1996;165:149-63.
51. Morgan RM, Pihl TD, Nölling J, Reeve, JN. Hydrogen regulation of growth, growth yields, and methane gene transcription in Methanobacterium thermoautotrophicum DH. J Bact 1997;179:889-98.
52. Leedle JAZ, Greening RC. Postprandial changes in methanogenic and acidogenic bacteria in the rumens of steers fed high- or low-forage diets once daily. Appl Environ Microbiol 1988;54:502-6.
53. Hobson PN, Stewart CS, eds. The rumen microbial ecosystem. 2nd ed. London: Chapman Hall, 1997.
54. McDonald IW, Warner ACI, eds. Digestion and metabolism in the ruminant. Australia: University of New England Publishing Unit, 1975.