Increasing the Flow of Protein from Ruminal Fermentation* - Review -

R. J. Wallace[1], C. J. Newbold, B. J. Bequette, J. C. MacRae and G. E. Lobley

Rowett Research Institute, Bucksburn, Aberdeen AB21 9SB, United Kingdom

ABSTRACT : This review summarizes some recent research into ways of improving the productivity of ruminal fermentation by increasing protein flow from the rumen and decreasing the breakdown of protein that results from the action of ruminal microorganisms. Proteinases derived from the plant seem to be of importance to the overall process of proteolysis in grazing animals. Thus, altering the expression of proteinases in grasses may be a way of improving their nutritive value for ruminants. Inhibiting rumen microbial activity in ammonia formation remains an important objective: new ways of inhibiting peptide and amino acid breakdown are described. Rumen protozoa cause much of the bacterial protein turnover which occurs in the rumen. The major impact of defaunation on N recycling in the sheep rumen is described. Alternatively, if the efficiency of microbial protein synthesis can be increased by judicious addition of certain individual amino acids, protein flow from ruminal fermentation may be increased. Proline may be a key amino acid for non-cellulolytic bacteria, while phenylalanine is important for cellulolytic species. Inhibiting rumen wall tissue breakdown appears to be an important mechanism by which the antibiotic, flavomycin, improves N retention in ruminants. A role for *Fusobacterium necrophorum* seems likely, and alternative methods for its regulation are required, since growth-promoting antibiotics will soon be banned in many countries. *(Asian-Aust. J. Anim. Sci. 2001. Vol. 14, No. 6 : 885-893)*

Key Words : Amino Acids, Ammonia, Essential Oils, Grass Proteinases, Protein Synthesis, Rumen, Ruminal Bacteria

INTRODUCTION

The quantity of protein flowing from the rumen is a major factor limiting the productivity of ruminant livestock production (Leng and Nolan 1984; Broderick et al., 1991). The protein reaching the abomasum consists of a mixture of dietary and microbial protein and, following digestion and absorption, it provides the amino acids upon which ruminants depend for their amino acid requirements. Rumen wall tissue protein turnover must also be considered as part of the protein drain imposed by ruminal microorganisms, because ruminal bacteria tend to invade and digest ruminal epithelial tissues (Cheng and Costerton 1980). The main considerations dominating the impact of ruminal microorganisms on protein nutrition are thus:

(a) the breakdown of dietary protein in the rumen,
(b) the breakdown of microbial protein in the rumen,
(c) the efficiency of synthesis of microbial protein, and
(d) the breakdown and turnover of rumen wall tissue.

This paper reviews some of our recent findings in these areas.

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[1] Address reprint request to R. J. Wallace. Tel: +44-1224-716656, Fax: +44-1224-716687, E-mail: rjw@RRI.SARI.AC.UK.

BREAKDOWN OF DIETARY PROTEIN

Plant proteinase activity

Virtually all of the early work on proteinase activity in the rumen made a tacit assumption that the proteinase enzymes were derived from the microorganisms. No mention is made in early comprehensive reviews (Blackburn, 1965; Allison, 1970) that the feedstuff could possibly contribute enzymic activity. Subsequently, quite detailed work on where ruminal proteinases were located (Kopecny and Wallace, 1982), which microorganisms were most important (Brock et al., 1982), what sorts of enzyme activities were present (Brock et al., 1982; Kopecny and Wallace, 1982; Prins et al., 1983), and the kinetics of the process whereby proteins adhere to the surfaces of ruminal bacteria where they are converted to smaller peptides, then amino acids, then ammonia (Nugent and Mangan, 1981; Wallace et al., 1999) also paid little heed to the possibility of a contribution from the plant material. Furthermore, the main proteolytic enzymes in the rumen of different animals appeared to be highly variable, so it was suggested that the proteolytic microbial population was also highly variable (Falconer and Wallace, 1998).

The first discussion of a possible contribution by plant proteinases appeared in 1996, when Theodorou et al. proposed that much of the rapid release of ammonia in grazing animals might be initiated by the action of plant, rather than microbial, proteinases. They argued that previous studies had been done in animals receiving dried or conserved rations, which would not possess the enzyme activities of fresh forages; that

grass cells contain vacuoles harbouring broad spectrum proteinases which are known to be responsible for protein breakdown in the silo (Wetherall et al., 1995); that proteins of fresh forage would not be available for microbial attack because they are trapped in plant tissues which are incompletely disintegrated; and finally that, within the microenvironment of the incompletely disintegrated plant tissue, it would be the plant proteinases themselves which would break down the plant proteins.

If one reviews the older papers, it does indeed appear that none of them deals with the digestion of plant proteins *in situ* when the diet is fresh forage. Brock et al. (1982) used ruminal fluid from a Holstein cow receiving alfalfa hay plus corn grain supplement, and azocasein as substrate. Kopecny and Wallace (1982) used a ration consisting of grass hay and barley-based concentrate together with labelled casein as substrate. Nugent and Mangan (1981) employed fraction 1 leaf protein (Rubisco) as the substrate, but it had already been extracted from plant tissues. Prins et al. (1983) and Falconer and Wallace (1998) also used ruminal fluid from animals fed dried rations as their starting material. Therefore, none of these studies could provide a clue as to the validity of the Theodorou et al. (1996) suggestion.

Zhu et al. (1999) set out to assess the importance of plant proteinases in four forages - ryegrass, red clover, white clover, and birds-foot trefoil by following the breakdown pattern of plant proteins by SDS-PAGE in the presence and absence of ruminal fluid *in vitro*. The ruminal fluid was not from a grazing animal, but from a cow receiving grass silage or a sheep receiving hay. The digestion of proteins to peptides clearly occurred with both ruminal fluid in the incubation mixture and without added ruminal fluid after 24 h incubation. The authors concluded that endogenous plant proteinases were responsible for the proteolysis. However, volatile fatty acids accumulated to almost 40 mM, which is about 40% of the total VFA concentration in ruminal fluid, indicating that a very substantial microbial fermentation had occurred. Bacteria at high cell densities in pure culture rarely exceed 10 mM acids produced. Thus, very substantial microbial growth must have occurred in the incubations without added ruminal fluid, which could have contributed in a major way to proteolysis. The inoculum was presumably epiphytic bacteria associated with the plant material.

In order to assess whether plant proteinases were likely to be as significant as had been suggested, an experiment was carried out in our laboratory, in order to evaluate the proteolytic activity of plant proteinases vis-à-vis rumen microbial proteinases and to evaluate the effect on ruminal ammonia production of destroying plant proteinase activity by autoclaving. Fresh ryegrass was chopped into 1-cm lengths and homogenised in a Waring blendor. A portion was autoclaved, and the rate of release of ammonia from homogenised grass and homogenised, autoclaved grass were compared with corresponding rates of ammonia formation from the chopped grass when they were added to ruminal fluid *in vitro*. Ammonia production from chopped grass decreased markedly as the result of autoclaving (figure 1). The rates were 0.38 and 0.13 mmol/L per h for chopped grass and autoclaved grass respectively ($p=0.0002$), indicating that inactivation of the grass proteinases had a major effect on the rate of protein breakdown. Ammonia was released from blended grass only slightly more rapidly than from chopped grass ($p=0.055$), indicating that blending had not altered the susceptibility of plant proteins to proteolysis. Autoclaving also decreased ammonia production substantially (0.45 and 0.24 mmol/L per h for blended and blended, autoclaved grass respectively; $p=0.0016$), which is consistent with a significantly lower rate of hydrolysis when plant proteinases are destroyed. The proteolytic activity of the blended grass preparation was determined using ^{14}C-labelled casein (Wallace, 1983). The proteolytic activity of blended grass (0.2 mg casein hydrolysed/h per g of grass fresh weight), was small in comparison with the proteolytic activity of ruminal fluid, which is

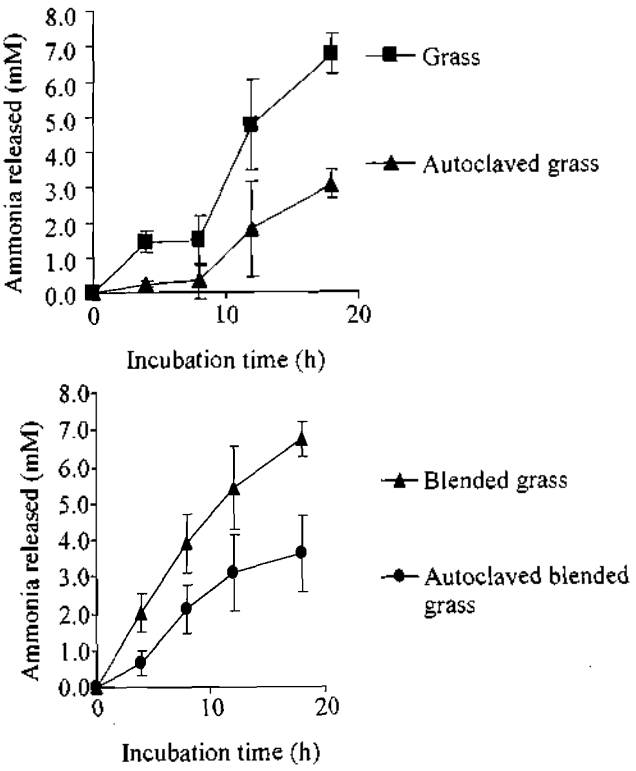


Figure 1. Influence of blending and autoclaving grass on the production of ammonia from grass incubated with ruminal fluid *in vitro*

usually tenfold higher (Wallace 1983). The interpretation of this finding must be that, although plant proteolytic activity is much less than microbial proteoytic activity, the location of the plant proteinases at the same place as the proteins being broken down (figure 2) gives them a significance that outweighs their relatively low proteolytic activity. Thus, altering the proteinase activity of grass by breeding or genetic engineering should produce significant benefits to the protein nutrition of grazing ruminants.

Inhibition of dipeptidyl peptidase activity

The microbiology and biochemistry of protein breakdown has been reviewed extensively (Morrison & Mackie, 1996; Wallace et al., 1997). Proteolysis is a highly variable activity which is carried out by a large number of species. The subsequent breakdown of peptides to amino acids is a two-step process (Wallace and McKain, 1989; Wallace et al., 1990; Depardon et al., 1996). Most peptides are cleaved by the sequential removal of dipeptides from the N-terminus, and the dipeptides which are released are then broken down to amino acids by separate dipeptidases. The former process is carried out predominantly by one of the most numerous bacterial genera, *Prevotella*, (Wallace and McKain, 1991; McKain et al., 1992; Debroas et al., 1998) while the latter activity is associated with several different species of bacteria and ciliate protozoa (Wallace et al., 1997).

Table 1. Inhibition (%) of the different dipeptidyl peptidases of *Prevotella albensis* by synthetic inhibitors

Inhibitor	DPP- I	DPP-II	DPP-IV	Ala-DPP
InhA	76	0	3	48
InhB	50	1	3	60
Diprotin A	3	0	38	0
InhG	63	29	18	9

InhA, Inh B and InhG are synthetic peptide and amino acid analogues.

Table 2. Effect of feeding essential oils to cattle on the subsequent production of ammonia from casein acid hydrolysate in the presence and absence of monensin in rumen fluid *in vitro*

	Ammonia production from amino acids (nmol NH_3 produced/mg protein/h)		
	Control	Essential oils	SED
No monensin	410	372	9.8*
5 μM monensin	280	287	10.9

From Newbold et al. (1999).

A constriction in the flux of nitrogen from protein to ammonia in the rumen therefore occurs at peptide breakdown. This is thus a target for manipulation. At the same time, however, the potential benefit to rumen microorganisms of having pre-formed amino acids available to them cannot be ignored (see below). Significant progress has been made in identifying inhibitors of the peptidases of the ruminal bacterial genus, *Prevotella*. These inhibitors, which are structural analogues of dipeptides, inhibit the main dipeptidyl peptidases of *Prevotella* (table 1). They are potential novel feed additives for improving protein retention in the rumen, and further development work should be done to evaluate their potential.

Inhibition of amino acid deamination

The amino acids released by proteinase followed by peptidase activity are then deaminated, enabling the carbon skeletons to be fermented for energy, and much of the ammonia is lost from the rumen by diffusion across the rumen wall (Leng & Nolan, 1984). Two new opportunities for inhibiting deamination using feed additives have emerged. One is a synthetic amino acid derivative, LY29, the other a group of natural compounds known as essential oils.

LY29 is a diazotization product of proline, with a chemical name of 1-[(E)-2-(2-methyl-4-nitrophenyl) diaz-1-enyl]pyrrolidine-2-carboxylic acid (figure 3). LY29 inhibited the rate of production of ammonia from amino acids and peptides by mixed ruminal microorganisms *in vitro* (figure 3; Floret et al., 1999). Peptidase and proteinase activities were unaffected, and the bacteriolytic activity of ciliate protozoa was unchanged, implying that the site of action was at the deamination of amino acids. The most sensitive ruminal bacteria to LY29 were the ammonia hyper-producing (HAP) species, *Clostridium aminophilum* and *Clostridium sticklandii*, both of which are implicated in ruminal ammonia production (Chen and Russell, 1988, 1989; Russell et al., 1991; Russell et al., 1988). LY29 may therefore have potential as a protein-sparing feed additive for ruminants via its effect on HAP bacteria.

Essential oils are volatile compounds responsible for the characteristic aroma of spices. Essential oils from a variety of sources alter bacterial growth and metabolism in several environments, including the rumen (Oh et al., 1967). Recently, Fernandez et al. (1997) found that a commercial blended essential oil product decreased rumen ammonia concentrations in sheep. It appears that essential oils have a similar mode of action to LY29. A commercial essential oils mixture inhibited the production of ammonia from amino acids (table 2; Newbold et al., 1999). This occurred in the absence but not in the presence of

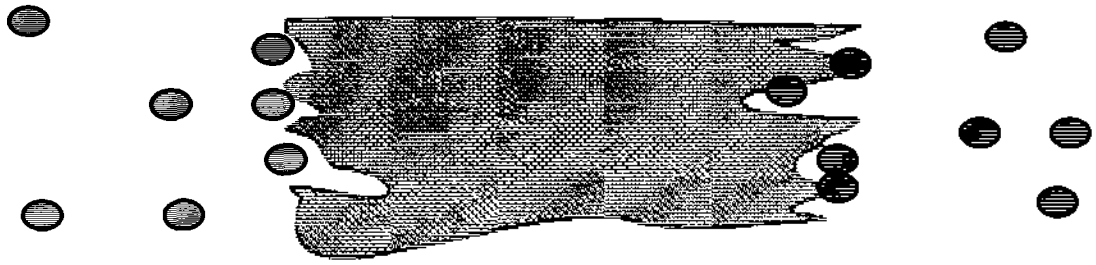


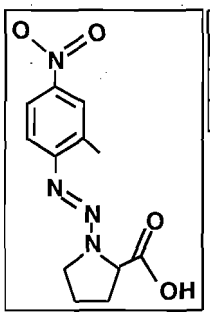
Figure 2. Ruminal bacteria invade plant tissues from damaged ends, rather than through the cuticle. If plant proteinases are present in the plant matrix close to the plant proteinases, it will be plant proteinases rather than bacterial proteinases which will be most effective in breaking down plant protein. Much depends on the degree of comminution of the plant which results from chewing.

monensin, which is known to inhibit HAP bacteria (Chen and Russell, 1989; Russell et al., 1991; Russell et al., 1988). Thus, it is speculated that some essential oils have a useful, specific suppressive effect on HAP bacteria and are therefore potentially useful feed additives for ruminants. Essential oils have the advantage that they are materials which have been used for centuries by man for many purposes, including food flavouring. They are therefore generally regarded as safe, and would consequently not require to pass many of the regulatory hurdles which are required before the introduction of a new, synthetic compound into feedstuffs.

INHIBITING MICROBIAL PROTEIN BREAKDOWN

Protein turnover is an endogenous activity of all microorganisms. In the rumen, however, bacterial protein breakdown is initiated mainly by exogenous factors, principally ciliate protozoa (Wallace and McPherson, 1987; Williams and Coleman, 1992). Arguably, avoiding the engulfment and digestion of bacterial cells by protozoa would be the manipulation strategy which would have the greatest impact on microbial protein flow from the rumen in most ruminants throughout the world. Saponins-containing plants or plant products have a great potential for achieving the suppression of rumen ciliate populations (Navas Camacho et al., 1994; Teferedegne et al., 1999). Until recently, whole-body N flux measurements to support the inferred impact of ruminal protozoa on recycling had been lacking. An experiment which compared normal and ciliate-free sheep, infused with $^{15}NH_3$ in the rumen and ^{15}N-urea in the jugular vein, enabled *in vivo* quantitation. It emerged that the ammonia pool in the rumen decreased in defaunated animals, as did ammonia transfer (table 3). The flux from blood urea to rumen ammonia also declined. The effect on intraruminal N recycling was enormous, decreasing by 88%, and as a consequence microbial protein flow from the rumen increased by almost 50% (table 3). If similar benefits could be obtained in production animals, the need for protein supplementation of ruminants would virtually disappear, with substantial economic and environmental benefits. Establishing methods to control protozoal activity, such as by saponins, remains a priority area of research in ruminant nutrition

1-[(E)-2-(2-methyl-4-nitrophenyl)diaz-1-enyl]pyrrolidine-2-carboxylic acid (LY29)



Inhibitor	Rate of NH_3 production (mol h^{-1} ml rumen fluid^{-1})
None	3.50
10 μM LY29	2.90**

**p<0.05

Figure 3. Influence of LY29 on NH_3 production from Trypticase by mixed ruminal microorganisms

Table 3. Influence of defaunation and refaunation of ciliate-free sheep on N fluxes in the rumen

	Defaunated	Refaunated
Microbial protein flow from rumen (g N d^{-1})	13.3	8.9
Recycling rate (g N d^{-1})	4.1	12.4
Intraruminal recycling (g N d^{-1})	0.8	6.6

ENHANCING MICROBIAL PROTEIN SYNTHESIS

Microbial protein flow depends on the efficiency of microbial protein synthesis as well as the extent to which microbial protein breaks down before it leaves the rumen. Among many other factors (Russell and Wallace, 1997), the yield of microbial protein is affected by the availability of pre-formed amino acids. Baldwin and his colleagues (Maeng and Baldwin 1976;

Maeng et al., 1976; Argyle and Baldwin 1989) attempted to identify whether one or a group of amino acids might limit nutrition of ruminants and factors affecting ruminal fermentation, particularly fiber digestion. The way in which these experiments were done was to determine if single amino acids or subgroups of amino acids could support the stimulation of fermentation rate found with a complete mixture. None of the single amino acids or sub-groups produced the same response as the complete mixture. However, it would be difficult to cover all the combinations of amino acids to be tested if, say, three individual amino acids were co-limiting. An alternative method for identifying which amino acids may be limiting was undertaken using ^{15}N-labelling of ammonia and peptides, in which the effects of adding pre-formed amino acids on de novo synthesis of individual amino acids was investigated. It was hoped that, if the de novo synthesis of certain amino acids was seen to be preferentially switched off when pre-formed amino acids were presented to ruminal microorganisms, these might be the key amino acids upon which to focus.

In mixed ruminal microorganisms fermenting soluble sugars, the amino acid whose synthesis was switched off most was proline (table 4), followed by glycine, methionine, lysine, valine and threonine (Atasoglu et al., 1999). The effect with proline was replicated in pure cultures of non-cellulolytic bacteria (table 4; Atasoglu et al., 1998), presumably the main species growing in the mixed population when soluble sugars are present. However, adding the apparently most-limiting amino acids, including proline, to the mixed population failed to replicate the stimulation given by a complete mixture of amino acids (Atasoglu et al., 1998).

The situation was different with cellulolytic ruminal bacteria. There is a long-held belief that cellulolytic ruminal bacteria use NH_3 as their sole source of N, based on experiments carried out many years ago (Bryant and Robinson, 1961; Bryant and Robinson, 1963; Bryant, 1973). Some recent published results are not consistent with this conclusion, however. The amino acid transport experiments of Ling and Armstead (1995) indicated that *F. succinogenes* accumulated radioactivity from ^{14}C-labelled peptides and amino acids. Pre-formed amino acids stimulate microbial growth and increase fiber digestion *in vivo* and *in vitro* (Merry et al., 1990; Chikunya et al., 1996; Griswold et al., 1996; Carro and Miller, 1999) and pure cellulolytic species grow faster on cellobiose when peptides are added to the medium (Cruz Soto et al., 1994). In addition, bacteria most closely associated with solids derived a substantial proportion of their cell-N from sources other than ammonia (Komisarczuk et al., 1987; Carro and Miller, 1999; Dixon and Chanchai, 2000). All of these observations indicate that amino acids are significant nutrients for cellulolytic bacteria. Our recent experiments with cellulolytic bacteria replicated the earlier studies with non-cellulolytic species, and demonstrated that amino acid-N was incorporated by cellulolytic bacteria. Furthermore, it emerged that the proline response was much less pronounced in these species, but instead phenylalanine synthesis was more sensitive to the presence of pre-formed amino acids, particularly in *Fibrobacter succinogenes* (table 4). The synthesis of both amino acids was affected preferentially to a small extent in *R. flavefaciens* (table 4).

Clearly, much more work on the regulation of amino acid biosynthesis in ruminal microorganisms is called for. As mixed amino acids stimulate microbial synthesis by up to 25% (Cotta and Russell, 1982), judicious amino acid supplementation could achieve important nutritional benefits to ruminants in terms of microbial protein flow.

Table 4. Influence of the addition of pre-formed amino acids (Trypticase, 10 g/L for mixed ruminal microorganisms, 5 g/L for pure cultures) on de novo synthesis of amino acids from ammonia by ruminal microorganisms

	Proportion of total amino acids formed from NH_3	Proportion of proline formed from NH_3	Proportion of phenylalanine formed from NH_3
Mixed ruminal microorganisms[1]	0.29	0.06	0.34
Non-cellulolytic bacteria[2]			
Prevotella bryantii	0.31	0.03	0.09
Selenomonas ruminantium	0.29	0.01	0.22
Streptococcus bovis	0.09	0	0.05
Cellulolytic bacteria[3]			
Fibrobacter succinogenes	0.77	0.71	0.01
Ruminococcus albus	0.89	0.98	0.60
Ruminococcus flavefaciens	0.72	0.51	0.58

[1] From Atasoglu et al. (1999), [2] From Atasoglu et al. (1998), [3] From Atasoglu (2000)

BREAKDOWN AND TURNOVER OF RUMEN WALL TISSUE

The rumen wall has an important function in the absorption of nutrients and metabolites, particularly the volatile fatty acids, urea and ammonia (Hungate, 1966). An equally important function is as a barrier to invasion of the rest of the body by ruminal microorganisms. As a consequence, the rumen wall has evolved to present a tough epithelial layer to the ruminal microorganisms, which resists both physical and microbiological damage. Recent experiments with flavomycin in sheep at the Rowett give us an idea of the metabolic cost of that damage.

Gut tissues in all animals turn over rapidly. In ruminants, although the gut tissues comprise less than 5% of total body protein mass, their rate of protein turnover is equivalent to >40% of total body amino acid flux. Rumen wall tissue turns over at about one-third of the rate of small intestinal tissues. However, because of its large size, the turnover of rumen tissue represents a considerable drain on the availability of amino acid for growth and other functions. Antibiotics have been used for many years at sub-therapeutic concentrations in order to promote the growth efficiency of pigs and poultry (Swick, 1996). The mode of action of these growth-promoting antibiotics (GPA) in improving feed efficiency involves in part a suppression of pathogens, but there is also clear evidence of an anabolic response (Schole et al., 1994). Flavomycin (bambermycin) is one such antibiotic. Flavomycin is not licensed for use in ruminants, however MacRae et al. (1999) found a growth response in lambs equivalent to 22% when they received flavomycin, which appeared to be accompanied by a corresponding decrease in the rate of protein turnover in the gut, as determined by arterio-venous concentration differences. Experiments were undertaken to try to pinpoint the site in the digestive tract where flavomycin had its effect. The methods were based on the uptake of ^{2}H-phenylalanine supplied as a flood-dose, leading to measurements of the fractional protein synthesis rates at different sites sampled immediately post mortem. It emerged that several sites in the tract of lambs receiving flavomycin had lowered fractional synthesis rates, although only two - rumen and duodenum - had differences which reached statistical significance (table 5). The decrease in the fractional synthesis rate in ruminal tissues was 27%.

The sensitivity of different species of ruminal bacteria to flavomycin (table 6) indicated that few were sensitive at a concentration of 2 μg/ml. The incorporation rate of flavomycin in the diet was 0.25 g of a preparation containing 8% active bambermycin. Thus, the likely maximum concentration of antibiotic in the 5-litre rumen of the sheep would be $0.25 \times 0.08/5=0.004$ g/l=4, with the average concentration being much lower. Perhaps the most significant piece of information that might relate to the gut tissue turnover measurements is that the sensitivity of *Fusobacterium necrophorum* to flavomycin *in vitro* was significantly greater than the other species tested. *F. necrophorum* is a Gram-negative species which is known to pass through the rumen wall, eventually being the primary cause of liver abscesses in feedlot cattle (Tan et al., 1996). *Fusobacterium* spp. are occasionally found in the rumen digesta, and have also been isolated from the epimural population (Cheng et al., 1979). They are not typical members of either population. Nevertheless, because of their role in forming liver abscesses in cattle, and their possible degradative role which may lead to damaged rumen wall integrity, together with their sensitivity to flavomycin and the similarity of their morphology to that of bacteria which invade rumen wall tissues (Dinsdale et al., 1980; Rieu et al., 1990), it is reasonable to propose a hypothesis that an important part of the growth-stimulatory properties of flavomycin is to suppress the growth of bacteria such as *F. necrophorum* which invade and digest rumen wall tissue. Thus, controlling the digestive activities of epimural bacteria offers a previously unrecognised scope for improving protein nutrition in ruminants. Further experiments are already under way to determine the impact of flavomycin on the

Table 5. Influence of flavomycin on gut tissue turnover in sheep

Tissue	Fractional synthesis rate (%/d)			
	Control	Flavomycin	SE	P
Rumen	13.9	10.1	1.8	0.075
Abomasum	16.6	15.1	3.2	NS
Duodenum	48.0	36.7	4.1	0.027
Jejunum	42.8	38.3	3.7	NS
Ileum	36.5	34.7	2.3	NS
Caecum	20.2	17.9	2.0	NS
Large intestine	24.5	26.4	5.0	NS
Liver	15.5	28.0	3.0	NS

Table 6. Sensitivity of different strains of ruminal bacteria to flavomycin

Gram-negative species	Strain	MIC_{50} (mg/ml)	Gram-positive species	Strain	MIC_{50} (mg/ml)
Fusobacterium necrophorum	A54, A12	0.25	*Peptostreptococcus anaerobius*	27337	1
Fibrobacter succinogenes	S85	0.5	*Clostridium sticklandii*	12662	2
Ruminobacter amylophilus	WP109	4	*Ruminococcus albus*	SY3	2
Veillonella parvula	L59	4	*Lactobacillus casei*	LB17	8
Prevotella albensis	M384	8	*Butyrivibrio fibrisolvens*	SH13	32
Megasphaera elsdenii	J1	32	*Eubacterium ruminantium*	2388	32
Prevotella bryantii	B14	32	*Eubacterium pyruvovorans*	Isol 6	32
Anaerovibrio lipolytica	5S	>64	*Ruminococcus flavefaciens*	Fd1	32
Mitsuokella multiacidus	46/5	>64	*Clostridium aminophilum*	49906	64
Prevotella brevis	GA33	>64	*Butyrivibrio fibrisolvens*	SH1	>64
Prevotella ruminicola	23	>64	*Lachnospira multipara*	D15d	>64
Selenomonas ruminantium	HD4	>64	*Ruminococcus flavefaciens*	17	>64
Selenomonas ruminantium	Z108	>64	*Streptococcus bovis*	ES1, C277	>64

MIC_{50}-concentration at which growth decreased by 50%. From McKain et al. (2000).

composition of the mixed rumen epimural population.

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