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Influence of flavomycin on microbial numbers, microbial metabolism and gut tissue protein turnover in the digestive tract of sheep

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Flavomycin is an antibiotic that promotes growth in ruminant and non-ruminant livestock. The aim of this study was to determine the mechanism of action of flavomycin in sheep by measuring microbial numbers, microbial metabolism and gut tissue protein turnover at different sites in the digestive tract. Two weight-matched groups (*n* 5) of male castrate lambs (30 kg) received 800 g grass cubes/d for 6 weeks, with one group receiving 20 mg/d flavomycin during the last 2 weeks. Samples of digesta and gut tissue segments were obtained immediately *post mortem*, 90 min after a flood-dose of [ring-D_5]phenylalanine. Viable bacterial counts and volatile fatty acid concentrations were highest in ruminal digesta, followed by the colon and caecum, then the duodenum and ileum. The only effect of flavomycin was an increased bacterial count in the rumen (3·5 *v.* $1{\cdot}2 \times 10^9$ per g; $P=0{\cdot}04$). Acetate was proportionally greater and propionate and butyrate were lower in the caecum and colon than the rumen. Flavomycin had no effect on volatile fatty acid proportions or ammonia concentrations. Bacteria growing on peptides as sole C source were not affected by flavomycin. Proteolytic, peptidolytic and amino acid deamination activities were similar in the rumen, caecum and colon; they tended to be lower in animals receiving flavomycin. Protein turnover in ruminal wall and duodenal tissues, measured by a flood-dose technique, decreased with flavomycin ($P=0{\cdot}075$ and 0·027, respectively). Thus, flavomycin differs from ionophores in its mode of action. It may influence protein metabolism of both digesta and tissue throughout the ruminant digestive tract.

Ammonia formation: Antimicrobial feed additive: 'Hyper-ammonia-producing' bacteria: Protein turnover

Antimicrobial growth promoters will be banned in European livestock production from the end of 2005. It is vital that we understand their mode of action if similar productivity benefits are to be obtained by different means. Flavomycin (bambermycin, flavophospholipol, moenomycin) is a phosphoglycolipid antibiotic that has been used as an antimicrobial growth promoter since its discovery in the mid-1950s (Bauer & Dost, 1971; Wallhausser *et al.* 1965). It has been used most extensively in pig and poultry production systems, but flavomycin also promotes growth in ruminants and a variety of other species (Rebolini *et al.* 1982). Its mode of action in growth promotion is poorly understood. Unlike the ionophores, flavomycin generally stimulates ruminal acetate *in vitro*, with a corresponding decrease in butyrate (Cafantaris, 1981; Masoero *et al.* 1991). *In vivo*, however, the majority of studies, using a variety of different dietary regimens, reported that flavomycin did not alter the concentrations of volatile fatty acids (VFA) or their proportions (Rowe *et al.* 1982; Galbraith *et al.* 1983; Febel *et al.* 1988; Alert *et al.* 1991). The exception was the study by Murray *et al.* (1990), in which the ruminal concentrations of propionate increased with dietary flavomycin.

Flavomycin decreased ammonia production from casein in incubations with ruminal fluid *in vitro* (Cafantaris, 1981; Van Nevel & Demeyer, 1987), and some studies showed that flavomycin decreased ruminal concentrations of ammonia *in vivo* (Febel *et al.* 1988; Murray *et al.* 1990), although others have not (Rowe *et al.* 1982; Galbraith *et al.* 1983; Murray *et al.* 1992). MacRae *et al.* (1999) obtained preliminary evidence to indicate that gut tissue turnover was lower in lambs receiving flavomycin. As gut tissue protein turns over much more rapidly than other body tissues, slowing of this turnover was suggested to be the basis of the effectiveness of flavomycin and possibly other antimicrobial growth promoters. Such a mode of action could be consistent with a similar effectiveness in non-ruminant livestock. Thus, the present study was undertaken to assess the influence of flavomycin on microbial numbers and metabolism and gut tissue turnover throughout the digestive tract of sheep. The results provide useful information about bacteria and their activities throughout the digestive tract, and indicate that flavomycin may have its key mechanisms of action in gut wall tissues and the large intestine as well as ammonia formation throughout the tract. Some of these results have been presented previously in a preliminary form (McKain *et al.* 2000; Wallace *et al.* 2001; Edwards *et al.* 2002).

Methods

Animals

Ten male castrate, 6-month-old, 30 kg lambs were divided into two groups matched for weight. Both groups were fed a control diet of 800 g/d pelleted dried grass for 6 weeks, with the daily ration delivered by automatic feeders as twelve equal meals per d during the last 2 weeks. Also during the last 2 weeks of this period, sheep in one group received a gelatin capsule orally

Abbreviations: APE, atoms % excess; FSR, fractional rate of protein synthesis; HAP, hyper-ammonia-producing bacteria; VFA, volatile fatty acids.

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each day at 08.00 hours that contained 250 mg Flavomycin®80 (equivalent to 20 mg/d flavomycin; SCA Nutrition Ltd, Insch, Aberdeenshire, UK). This frequent meal feeding period was adopted to reduce fluctuations in rates of digestion and absorption, and thus promote quasi-steady-state conditions over the period of protein synthesis measurements by use of the flooding-dose technique. At 09.00 hours on the last day of the 6-week period, fractional rates of protein synthesis along the gastrointestinal tract were measured by slow (over 10 min) administration into a jugular vein of a flooding dose containing 0·6 g [ring-D_5]phenylalanine plus 3·0 g unlabelled phenylalanine. Over the next 30 min, jugular vein blood samples (10 ml) were withdrawn at 5 min intervals (six samples) and placed on ice. At 35 min post-flooding dose, animals were killed by lethal injection, and immediately samples (20–30 g, 20–30 cm lengths) of whole (i.e. serosa plus mucosa) gut tissues were dissected, washed in ice-cold saline and plunged into liquid N. Tissues were subsequently stored at −80°C until processing for [D_5]phenylalanine enrichment. Tissues were dissected from the dorsal rumen (20 × 20 cm), mid-abomasum (10 × 25 cm), proximal duodenum (first 30 cm), mid-jejunum (approximately 60 cm length), ileum (last 25 cm), caecum (whole section) and colon (a 30 cm section between 20 and 50 cm distal of the ileo-caecal junction), and all tissues were cleared of attached fat. From the same sites as the gut tissue dissections, samples of digesta were collected under CO_2. Immediately, digesta samples were processed for microbiological and metabolic analysis. A portion (5 g) of digesta from each gut segment was mixed with 5 ml 10 % TCA and stored frozen for analysis of VFA, L-lactate and ammonia.

Analytical methods

Digesta metabolites. Digesta samples were processed and analysed for VFA by GC (Newbold *et al.* 1995). L-Lactic acid was determined enzymatically (Hochella & Whitehouse, 1965), and ammonia was determined using an adaptation of the phenol–hypochlorite method (Whitehead *et al.* 1967).

Microbiological analysis. Most-probable number methods (Alexander, 1982; Dehority *et al.* 1989) were used for enumeration of total viable anaerobic bacteria in a ruminal fluid-containing general-purpose medium and 'hyper-ammonia producing' bacteria (HAP; Russell *et al.* 1991) in medium containing pancreatic casein hydrolysate (Trypticase® peptone; Becton Dickinson Microbiology Systems, Cockeysville, MD, USA) as sole source of energy (Eschenlauer *et al.* 2002).

Microbial deamination activities. Deamination was measured in digesta incubated with casein (Sigma, Poole, UK), pancreatic casein hydrolysate or casein acid hydrolysate (Gibco casein hydrolysate No. 5; Life Technologies Ltd, Paisley, UK) as substrate. Digesta (0·8 g) were mixed with 0·2 ml 0·1 g/ml substrate and incubated at 39°C under CO_2. Ice-cold 10 % TCA (1·0 ml) was added either at zero time or after 6 h incubation, and ammonia concentrations were determined as before.

[D_5]Phenylalanine enrichment. Fresh blood was centrifuged (2000 ***g***) to obtain plasma, and plasma (0·5 g) was deproteinised by acid-precipitation and processed for GC–MS determination of phenylalanine enrichment as previously described (Calder & Smith, 1988). Frozen whole tissue sections were pulverised under liquid N, and processed as previously described to isolate homogenate free and protein-bound pools (Wester *et al.* 2004). Phenylalanine enrichment in the homogenate free pools was determined as for plasma. Isolated tissue protein was hydrolysed in 4 M-HCl, and phenylalanine converted to β-phenethylamine prior to GC–MS for low enrichment phenylalanine (Wester *et al.* 2004).

Calculations and statistical analyses

Fractional rates of protein synthesis (FSR, i.e. the percentage of the tissue protein pool synthesised per d) in the different segments of the gastrointestinal tract were calculated using the precursor pool estimated as the area under the isotopic enrichment *v.* time curve of plasma free phenylalanine (McNurlan *et al.* 1979). Thus,

$$\mathrm{FSR}(\%/\mathrm{d}) = \frac{E_{\mathrm{pb1}} - E_{\mathrm{pb0}}}{A \times t} \times 100$$

where $E_{\mathrm{pb1}} - E_{\mathrm{pb0}}$ represents the increase in enrichment of protein-bound phenylalanine above background at the end of the incorporation period, A indicates the area under the plasma enrichment curve over the same period of time and t is the time of incorporation (i.e. the time from flooding dose to tissue dissection and removal) in days. Due to tissue-processing difficulties, homogenate free pool isotopic activity could only be determined for eight sheep (five control, three flavomycin), which precluded valid statistical comparisons of treatment. However, for those eight sheep comparisons, differences between the plasma free and homogenate free pool enrichments were small (0–7·7 %, see p. 65). Given these small differences, and the lack of a systematic difference between treatments, use of the plasma precursor pool was deemed appropriate.

Results are recorded as means of values obtained from five sheep per treatment, except where digesta sample volumes were too small to permit analysis. P values for specific comparisons between control and flavomycin treatments, and between different sites, were obtained from unpaired and paired t tests, respectively. Ammonia production rate was analysed by ANOVA using a hierarchical split plot (between and within animal) structure with an additional stratum for between-substrate comparisons included.

Results

In both groups of animals, the total viable count of bacteria in ruminal digesta was higher ($P<0·05$) than in caecal or colonic digesta (Table 1). The counts were $>10^9$ per g ruminal digesta in comparison to numbers of 10^8–10^9 per g in the caecum and colon. Digesta taken from the duodenum and ileum contained bacterial counts two to four orders of magnitude lower ($P<0·05$). The variance was greater in these samples than in samples with more abundant bacteria. The only significant difference caused by flavomycin was a higher ($P=0·04$) count in ruminal digesta. HAP bacteria, which are able to grow on pancreatic casein hydrolysate as C source, comprised 0·05 % of the total viable count in ruminal digesta, 0·08 % in caecal digesta and 0·38 % in the colonic flora. Flavomycin tended to decrease the numbers of HAP bacteria, but the effect was not statistically significant ($P<0·05$).

Digesta concentrations of VFA (Table 1) followed a similar pattern to total bacterial counts. Total VFA concentrations were higher in ruminal, caecal and colonic digesta than in ileal and duodenal digesta ($P<0·05$). The concentration differences between ruminal, caecal and colonic digesta were not significantly different, except between ruminal digesta and colonic digesta in control animals ($P=0·048$). The concentrations in ileal

Table 1. Influence of dietary flavomycin on bacterial numbers and fermentation product concentrations in the digestive tract of sheep receiving dried grass pellets
(Mean values for five sheep per treatment unless otherwise stated)

	Rumen		Duodenum*		Ileum		Caecum†		Colon		SED‡
	Control	Flavomycin	Control	Flavomycin	Control	Flavomycin	Control	Flavomycin	Control	Flavomycin	
Total viable count (per g wet wt)	$1{\cdot}2\times10^{9}$	$3{\cdot}5\times10^{9}$	$1{\cdot}0\times10^{6}$	$0{\cdot}5\times10^{6}$	$8{\cdot}8\times10^{6}$	$3{\cdot}7\times10^{6}$	$0{\cdot}5\times10^{9}$	$0{\cdot}4\times10^{9}$	$0{\cdot}4\times10^{9}$	$0{\cdot}6\times10^{9}$	$0{\cdot}57\times10^{9}$, $3{\cdot}0\times10^{6}$
No. growing on Trypticase (per g wet wt)	$0{\cdot}6\times10^{6}$	$1{\cdot}3\times10^{6}$	$1{\cdot}3\times10^{2}$	$1{\cdot}1\times10^{2}$	$0{\cdot}4\times10^{4}$	$1{\cdot}6\times10^{4}$	$0{\cdot}4\times10^{6}$	$0{\cdot}6\times10^{6}$	$1{\cdot}7\times10^{6}$	$1{\cdot}8\times10^{6}$	$1{\cdot}30\times10^{6}$, $12{\cdot}0\times10^{2}$
NH_3 concentration (μg/g wet wt)	206	187	150	212	160	107	239	324	197	207	55·1
Total VFA (μmol/g wet wt)	97·2	87·8	9·4	15·8	8·2	5·6	82·4	79·5	73·2	72·1	7·05
Molar proportion											
Formate	0·006	0·008	0·33	0·23	0·22	0·21	0·009	0·009	0·017	0·011	0·027
Acetate	0·67	0·63	0·55	0·51	0·51	0·54	0·72	0·73	0·74	0·74	0·069
Propionate	0·19	0·21	0	0·010	0·024	0·015	0·16	0·17	0·15	0·17	0·015
Isobutyrate	0·005	0·003	0	0·025	0·011	0·018	0·010	0·009	0·010	0·010	0·012
Butyrate	0·11	0·14	0·039	0·13	0·16	0·15	0·052	0·054	0·053	0·053	0·064
Isovalerate	0·003	0·002	0·082	0·090	0·076	0·053	0·006	0·006	0·009	0·006	0·018
Valerate	0·009	0·009	0	0	0	0·002	0·016	0·015	0·014	0·014	0·002
Caproate	0·002	0·002	0	0·006	0·003	0·003	0	0	0	0	0·003
L-Lactate (μmol/g wet wt)	1·4	2·2	8·2	11·1	4·5	3·7	2·6	1·3	0·8	1·5	1·76

*Volatile fatty acid (VFA) and L-lactate estimations were carried out using three control and four flavomycin sheep due to small amounts of digesta recovered.
† All caecal analyses were carried out with four animals per treatment due to small amounts of digesta recovered from other sheep.
‡ SED for bacterial counts were calculated separately from variances in ruminal, caecal and colonic digesta and from duodenal and ileal digesta, because the variances were different between these two groups of samples but were similar within groups.

and duodenal digesta were not different ($P>0{\cdot}05$). Flavomycin had no influence on total VFA concentration in digesta from any site ($P>0{\cdot}05$).

The molar proportions of the main VFA were different in digesta taken from the different sites (Table 1). In control animals, acetate was proportionally greater in caecal ($P=0{\cdot}010$) and colonic digesta ($P=0{\cdot}007$) than in samples from the rumen. In the flavomycin group, corresponding differences were also evident, but to a lesser degree ($P=0{\cdot}076$ and 0·028, respectively). The molar proportion of propionate was lower in caecal ($P=0{\cdot}002$) and colonic ($P=0{\cdot}038$) digesta than in ruminal digesta in the control group. The corresponding comparison in the flavomycin group was not significant ($P>0{\cdot}05$). The molar proportion of butyrate was much lower in caecal ($P=0{\cdot}0012$) and colonic ($P<0{\cdot}001$) digesta than in ruminal digesta in the control group. The same comparisons in the flavomycin group revealed significant differences ($P=0{\cdot}057$ and 0·018, respectively), but again differences were less pronounced. L-Lactate concentrations were relatively high in the duodenum and ileum (Table 1; 8·2 and 4·5 μmol/g). Duodenal concentrations were significantly ($P<0{\cdot}05$) higher than in ruminal, caecal or colonic digesta in both treatment groups. Flavomycin had no effect on L-lactate concentrations ($P>0{\cdot}05$). Ammonia concentrations were similar in all samples of digesta and were not affected by flavomycin ($P>0{\cdot}05$; Table 1).

Ammonia formation from pancreatic casein hydrolysate (Trypticase) was greater ($P<0{\cdot}05$) than from casein acid hydrolysate (free amino acids) in digesta from the rumen, caecum and colon (Table 2). Ammonia formation from casein acid hydrolysate was greater than from casein in ruminal digesta from both groups ($P<0{\cdot}05$), but there was no significant difference in samples of caecal or colonic digesta. Volumes of digesta recovered from the duodenum and ileum were too small to carry out similar incubations. Ammonia production rates from all incubations except caecal digesta and casein were numerically lower in animals receiving flavomycin, but variation between animals was high, such that the effects did not reach statistical significance.

The appropriate precursor for protein synthesis is the amino acyl-tRNA pool, but due to the technical challenge to isolate and measure these pools, the more easily accessible plasma free and tissue homogenate free pools are monitored. Herein, measurement of the latter two precursors was attempted, but unfortunately problems (low phenylalanine content) in processing of all tissues for two sheep on the flavomycin treatment were encountered, and for some sheep tissues on the control diet. However, for the eight sheep where both pool measurements were made, the mean plasma phenylalanine enrichment (atoms % excess, APE) prior to killing was 12·57 compared with homogenate free phenylalanine enrichments for rumen of 13·71 ($P<0{\cdot}005$ *v.* plasma), for abomasum of 13·00 ($P=0{\cdot}07$ *v.* plasma), for duodenum of 11·59 ($P<0{\cdot}05$ *v.* plasma), for jejunum of 12·60 (not different *v.* plasma), for ileum of 12·18 (not different *v.* plasma), for caecum of 13·46 ($P<0{\cdot}001$ *v.* plasma) and for colon of 13·35 ($P<0{\cdot}005$ *v.* plasma). The higher phenylalanine enrichments in the homogenate free pool of the rumen, caecum, abomasum and colon compared with plasma values prior to killing probably relates to the slow rates of protein turnover in these tissues, compared with the rapid clearance rate of the vascular plasma pool. The relatively small differences (0–7·7 %) observed in plasma and homogenate free pool activities suggests that effective flooding conditions were attained in the sheep in the present study, and

Table 2. Influence of dietary flavomycin on ammonia production from protein, peptides and amino acids in digesta samples taken from the digestive tract of sheep receiving dried grass pellets

(Mean values for control and flavomycin treatments for five sheep per treatment unless otherwise stated)

	Ammonia production rate (μmol NH_3 released/h per g wet wt)*					
	Rumen		Caecum†		Colon	
	Control	Flavomycin	Control	Flavomycin	Control	Flavomycin
Casein acid hydrolysate	9·0	7·1	6·8	5·6	8·0	6·7
Trypticase	12·3	10·1	13·6	13·4	15·3	14·8
Casein	4·0	3·8	5·3	6·5	6·1	3·9

* SED values were as follows: for comparing treatments control with flavomycin, 2·16; for comparing sites within treatments, 1·93; for comparing substrates within sites, 1·41.

† All caecal analyses were carried out with four animals per treatment due to small amounts of digesta recovered from other sheep.

that the results based on plasma precursor activity provide a close, if not relative, measure of the true activity within the tissues.

Herein, based on the plasma precursor area, comparison of the FSR in the different gut tissue segments followed similar trends as previously reported. Thus, protein turnover was highest in small intestinal compared with ruminal tissues, and there was a trend for FSR tissue protein to decrease between the duodenum and ileum (Fig. 1). Sheep fed flavomycin had lower FSR of protein in the duodenal ($P<0·027$) and ruminal tissues ($P=0·075$). And, although inter-animal variation was high in this study, in general, FSR of protein were lower in the flavomycin group for all gut segments, with the apparent exception of the colon.

Discussion

In order to form a basis for replacing growth-promoting antimicrobials in ruminants, our aim was to evaluate the mode of action of flavomycin and determine whether flavomycin acts through influences on ruminant gut microbiology and the metabolic activities of the gut tissues. The results also provided a comparative evaluation of the microbial numbers and activities in different parts of the digestive tract of sheep that were not available previously. Much research has been carried out on ruminal micro-organisms and on the intestinal flora of non-ruminant farm animals. In comparison, studies that deal with the intestinal microbial population in ruminants are relatively few, and recently have focused mainly on the proliferation of *Escherichia coli* O157 rather than the predominant anaerobic populations (Russell *et al.* 2000). Only Michalet-Doreau *et al.* (2002) have used molecular methods to analyse the caecal population, concentrating on cellulolytic bacteria.

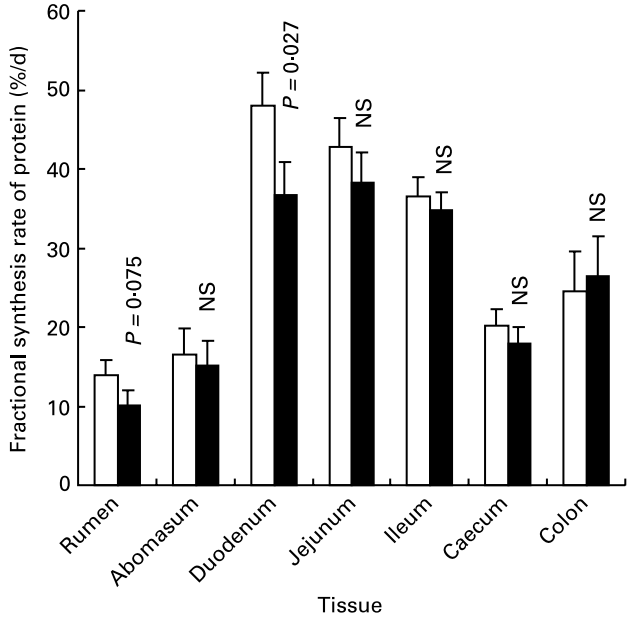

Fig. 1. Influence of dietary flavomycin (□; ■, control) on fractional rates of protein synthesis (%/d) by gut tissue segments taken from sheep receiving a pelleted dried grass diet. See Materials and Methods for anatomical descriptions of the gut segments dissected. Values are means with their stanard errors depicted by vertical bars. NS, $P>0·20$.

Flavomycin had no effect on VFA concentrations at any point in the digestive tract. This is consistent with most previous reports, as described earlier. Concentrations of VFA were very low in the duodenum and ileum, and highest in the rumen, in a manner very similar to that found by others in sheep (Packett *et al.* 1966) and cattle (Ward *et al.* 1961). VFA from caecal fermentation contribute significantly to the energy metabolism of sheep consuming dried grass cubes (Faichney, 1969). The molar proportions of VFA differed between the rumen and the hindgut digesta in a manner that has been noted by others (Packett *et al.* 1966; Arieli & Sklan, 1985; Michalet-Doreau *et al.* 2002). Butyrate and to a lesser extent propionate concentrations were lower in the caecum and colon than in the rumen. Flavomycin tended to shift the molar proportions in ruminal digesta to a pattern more similar to those found in caecal and colonic digesta. Although the difference between ruminal and large intestinal digesta may reflect differences in substrates available for fermentation in the different segments of the digestive tract, a microbiological explanation is not obvious and would require detailed phylogenetic analysis to establish how the rumen and large intestinal bacterial communities differ. L-Lactate concentrations were higher in duodenal and ileal digesta than elsewhere. This may originate from a *Lactobacillus* fermentation in the abomasum.

Bacterial counts paralleled VFA concentrations throughout the gut, in the sense that lower bacterial counts were found in the duodenum and ileum than at the other sites (Table 1). Total bacterial numbers in the rumen increased in a statistically significant way with flavomycin, but, because flavomycin would be expected to have changed the composition of the flora, too much biological significance should not be attached to this finding. Different bacterial species can form clumps to differing extents, for

example, leading to misleading viable counts. The magnitude of the bacterial counts at the different sites found here is consistent with those found by Ullyatt *et al.* (1975). The viable counts in caecal digesta were similar in magnitude to those found by Mann & Ørskov (1973) in lambs.

Characterisation of specific bacteria was restricted to the so-called 'hyper-ammonia-producing' bacteria, which are able to grow using a pancreatic casein hydrolysate as C and N source (Russell *et al.* 1991; Attwood *et al.* 1998; Eschenlauer *et al.* 2002). Numbers of HAP bacteria were not affected by flavomycin. These bacteria, which are detrimental to N retention in the rumen because they deaminate amino acids rapidly (Russell *et al.* 1991), were present at much lower numbers in the rumen (0·05 % of the total viable count) of these grass-fed animals than has been found in sheep and cattle receiving higher-concentrate diets (>1 %; Chen & Russell, 1988; Russell *et al.* 1991; Eschenlauer *et al.* 2002). Numbers were much lower than the 1·3–11·6 % obtained with a different growth medium, and using roll tubes rather than MPN, obtained with grazing ruminants by Attwood *et al.* (1998). Here, for the first time, the numbers of HAP bacteria were recorded in parts of the digestive tract other than the rumen. Numbers in colonic digesta (0·38 % of the total) were higher than in the rumen. This may reflect the relatively lower importance of carbohydrate fermentation in the colon, particularly of soluble sugars, and a correspondingly greater reliance on protein hydrolysis products for energy. The results suggest that HAP bacteria might have a significant role in the intestine of non-ruminants as well.

Proteolysis and deamination have been investigated most thoroughly in the rumen (Morrison & Mackie, 1996; Wallace 1996), with the exception of Hecker (1971), who found that proteolytic activity in the sheep large intestine was greater than in the rumen, and the reverse was true for deamination. Here, rates of ammonia formation were similar in ruminal, caecal and colonic digesta. Flavomycin tended to cause rates to be lower, but the effect was not statistically significant. The rate of ammonia formation from pancreatic casein hydrolysate, which contains predominantly peptides, was greater than from amino acids and from casein, where proteolysis would be the rate-limiting step (Eschenlauer *et al.* 2002). A similar higher deaminative rate with peptides has been observed previously in ruminal digesta (Armstead & Ling, 1993), reflecting a preference of the main bacteria involved in deamination for peptides rather than for free amino acids (Ling & Armstead, 1995).

In vivo studies with germ-free animals (Reidel *et al.* 1974) indicate that the feeding of flavomycin fails to promote growth, thus eliminating the possibility that flavomycin may also promote growth through direct effects on tissue metabolism. In this connection, our observations of the general trend towards lower rates of gut tissue protein turnover in ruminant sheep receiving flavomycin points to a potential mode of action of flavomycin via influences on microbial–gut tissue interactions. The lower rates of protein synthesis by ruminal and duodenal tissues of sheep fed flavomycin would be expected to reduce not only energy demands of the gut, but also reduce endogenous losses of protein from the gastrointestinal tract with the net effect being an increase in amino acid absorption. Indeed, when flavomycin was fed to lambs given grass pellets (MacRae *et al.* 1999), net absorption of amino acids into the portal vein (i.e. venous drainage from the whole gut) increased by approximately 20 %.

Invasion of ruminal wall tissues by bacteria is well known (Cheng & Costerton, 1980; Dinsdale *et al.* 1980). Our observations, when combined with the observation that the tissue-invasive bacterium, *Fusobacterium necrophorum*, has a high sensitivity to flavomycin (Edwards *et al.* 2005), suggests that growth-promoting antibiotics such as flavomycin may act by suppressing the invasion of the host gut wall tissues by *Fusobacterium* spp. Such a mechanism would be consistent with the growth-promoting benefits of antimicrobials observed in non-ruminant animals (Tan *et al.* 1996). If similar breakdown of wall tissues is caused by bacteria in the lower gut, then flavomycin and other antimicrobial growth promotants may decrease wall tissue turnover in general throughout the gut, as was the general trend observed herein for gut protein turnover. In ruminants fed flavomycin, favourable effects on N retention have been observed (Galbraith *et al.* 1983; Alert *et al.* 1991), consistent with the mode of action proposed herein. This mechanism also offers a potential explanation for the ability of flavomycin to increase wool growth and the protein content of milk (Hamann, 1983; Murray *et al.* 1992).

The effects of flavomycin reported herein, for the most part, differ from the effects of ionophores, which are in common use in ruminants. Ionophores affect a wide range of Gram-positive bacteria (Nagaraja *et al.* 1997), in contrast to the narrower range found with flavomycin (McKain *et al.* 2000; Edwards *et al.* 2005). The most consistent effect of ionophores is an increased proportion of propionate in ruminal digesta (Nagaraja *et al.* 1997), which does not occur with flavomycin. Both ionophores and flavomycin appeared to decrease deamination, but by different mechanisms. The effect was not statistically significant here because of variation between animals. However, Van Nevel & Demeyer (1987) reported that flavomycin decreased ammonia production by ruminal digesta incubated with casein, without affecting degradation of casein itself, which suggests an influence on metabolism of hydrolysis products, either peptides or amino acids. Herein, numbers of HAP bacteria were not affected by flavomycin, which is in contrast to observations with the ionophore, monensin (Russell *et al.* 1991; Krause & Russell, 1996). Thus, the effect of flavomycin on ammonia production may originate from effects on Gram-negative species such as *Prevotella* (Wallace, 1996). *Fusobacterium* spp. are also Gram-negative bacteria (Garcia *et al.* 1992) and, although they are sensitive to many antibiotics (Mateos *et al.* 1997; Jimenez *et al.* 2004), these bacteria are relatively insensitive to ionophores (Lechtenberg *et al.* 1998; Nagaraja *et al.* 1999). The evidence, therefore, suggests that growth-promoting antibiotics such as flavomycin exert their effects via different mechanisms compared with ionophores, and that a mechanism not shared by ionophores is the suppression of invasive *Fusobacterium* spp. in host gut wall tissues by flavomycin.

The present experiments had a number of limitations. With large variations in microbial populations between animals on this diet (Edwards, 2003), the five animals per group proved to limit detection of microbially initiated differences caused by flavomycin. In addition, the sheep received flavomycin for only 2 weeks. This might be considered as a relatively short time period for the gut flora to adapt. Nevertheless, in separate experiments conducted by our group (Edwards, 2003), ruminal fermentation parameters were stable by day 7, indicating that the rumen bacterial flora had probably adapted to the presence of flavomycin during the 2 weeks of feeding in the present study. Further,

molecular analysis of the rumen bacterial population confirmed that ruminal bacterial populations remained similar after 4 d of flavomycin supplementation (Edwards, 2003).

In conclusion, the present study showed that feeding flavomycin has a general effect of decreasing tissue protein metabolism throughout the digestive tract of sheep. An indirect effect on gut tissue turnover is implied, by suppressing invasive bacteria, particularly *Fusobacterium* spp. In view of the impending ban on antimicrobial growth promoters in Europe by the end of 2005, it will be important to identify and find alternative means of controlling the growth or activity of those bacteria that are responsible for interacting with and eliciting responses by the gastrointestinal tract.

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