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Altair V. Brondani

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Robert N. Cook
Major professor

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A STUDY ON THE ACTION OF TEICHOMYCIN A2, AVOPARCIN, AND ISOACIDS IN SHEEP

By

Altair V. Brondani

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ABSTRACT

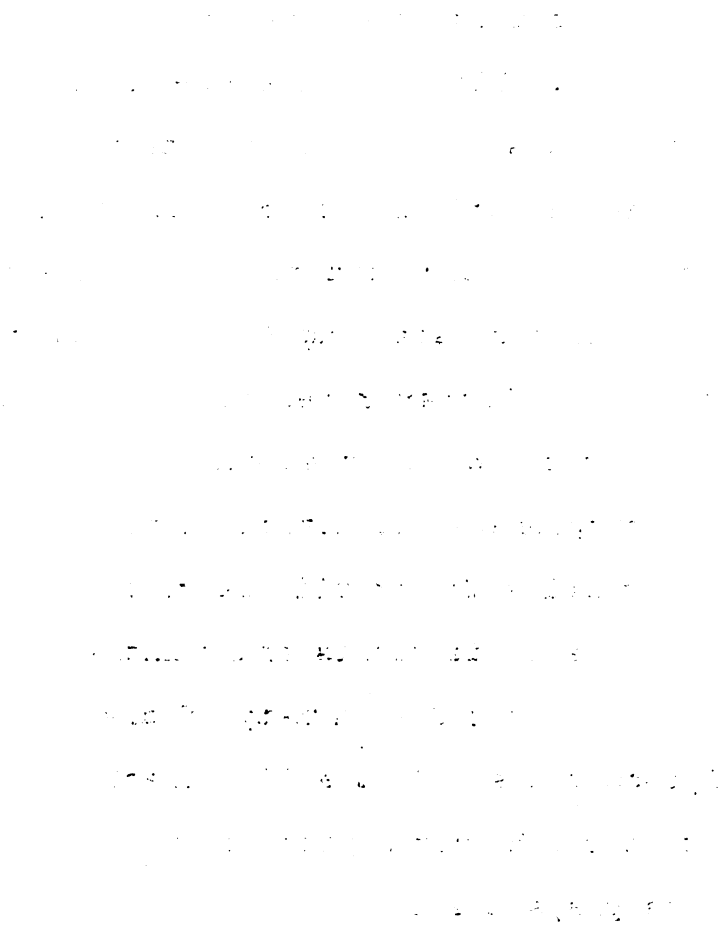
A STUDY ON THE ACTION OF TEICHOMYCIN A2, AVOPARCIN, AND ISOACIDS IN SHEEP

By

Altair V. Brondani

The objective of this study was to investigate the action of glycopeptides and isoacids in the rumen in an attempt to further explain how those compounds affect productive performance in ruminants. Production rates of rumen VFA were measured by a single injection radioisotope technique. In the first experiment, twelve rumen-cannulated crossbred ewes were fed either high roughage (HR) or low roughage (LR) diets supplemented with 0 (CONTROL), 30 ppm TE-A2, or 30 ppm AVO. In both diets, TE-A2 and AVO increased propionate ($p<.10$) without significantly affecting acetate ($p>.10$) and butyrate ($p>10$) production. Protozoal, bacterial, and soluble protein fractions were not altered by glycopeptides in either diet. Concentrations of ammonia-N were decreased (HR and LR, $p<.05$) and of alpha-amino-N were increased (HR, $p<.05$; LR, $p<.10$) by TE-A2 and AVO.

The effects of isoacids, urea, and sulfur on rumen fermentation rates in sheep fed high fiber rations were studied in two trials. Rates of acetate production were taken as a measure of rumen fermentation. Overall, feeding



isoacids at 0.2 g/kg bw/day increased ($p<.05$) production rates of acetate in both trials. This effect was dependent on urea and sulfur supplementation.

Six rumen-cannulated crossbred ewes (BW=40 kg) were fed 1200 g alfalfa hay/day supplemented with 0 (CONTROL), 0.1 (ISO1), or 0.2 (ISO2) g of isoacids/kg bw/day. In each of three 15-day periods, one level of isoacids was administered to all animals. Blood samples (30-min intervals for 12 hr) and rumen fluid samples (hourly intervals for 8 hr) were collected at the end of each period. Plasma concentrations of growth hormone and cortisol were not affected ($p>.10$) by isoacids. Animals receiving ISO2 had lower mean plasma insulin ($p<.10$), higher rumen acetate ($p<.10$) and lower rumen propionate ($p<.10$) than controls. It is proposed that decreased propionate production in the ISO2 group resulted in lower stimulus for insulin secretion. These results may explain why isoacids consistently increase milk production in lactating cows fed a variety of diets. They also suggest that glycopeptides and isoacids affect performance of ruminants through overlapping mechanisms, in which propionate plays the central role.

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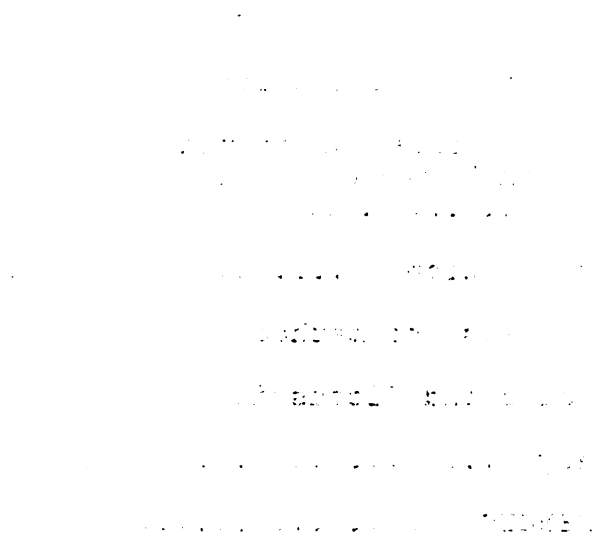
TABLE OF CONTENTS



LIST OF TABLES

LIST OF FIGURES

I. INTRODUCTION

The potential for chemical manipulation as a means of improving productive performance in ruminants is enormous. Currently, the direct effect of hormonal growth promotants and antibiotics accounts for 18% of meat production per unit of input in the United States (Rumsey, 1983).

Chemical agents known to be effective in ruminants can be divided into two broad classes according to their mode of action (Cook, 1985). One class of compounds acts to increase the quantity or quality of nutrients made available for absorption. Examples of this type of products are ionophores (monensin, lasalocid, narasin), glycopeptides (avoparcin, teichomycin A2), and isoacids. The second group increases the efficiency with which the absorbed nutrients are utilized for productive purposes. These include hormones (growth hormone, diethylstilbestrol), beta-agonists (clenbuterol, cimaterol), and anabolic agents such as zeranol (Cook, 1985).

Because of the complexities associated with every metabolic reaction, in which a single change may result in a cascade of interrelated responses, the two mechanisms may not be completely independent. For instance, recent reports indicate that isoacids, in addition to their known effect on the rumen fermentation, can also affect the plasma endocrine

profile of lactating cows (Towns and Cook, 1984; Fieo et al., 1984). In the studies reported here, some of the aspects involved in the action of rumen additives are addressed. In Chapter III, the effects of the glycopeptides teichomycin A2 and avoparcin on the rate of production of volatile fatty acids in vivo are reported. Chapters IV and V describe the effects of isoacids on rumen fermentation and on plasma hormone hormone concentrations in sheep. Results are discussed in terms of the relationship between final products of fermentation and regulation of nutrient utilization by the host animal.

II. LITERATURE REVIEW

IONOPHORES

Ionophores constitute a group of compounds that have the ability to mediate the transport of cations across biological membranes. Several of these compounds, including monensin, lasalocid, narasin and salinomycin have been used successfully as feed additives for ruminants. In this review, monensin, the most widely used ionophore, is used in the discussion of the possible mechanisms of action of this class of compounds in the rumen. The basic mode of action of ionophores in the rumen, including their effects on microbial metabolism has been reviewed (Bergen and Bates, 1984).

Monensin is the major component in a complex of four closely related, biologically active compounds produced by a strain of Streptomyces cinnamonensis (Haney and Hoehn, 1968). Preliminary studies indicated the ability of monensin to alter the normal patterns of rumen fermentation under both in vitro and in vivo conditions (Richardson et al., 1976). Results of subsequent feeding trials, in which monensin was fed to cattle under a variety of environmental conditions, clearly demonstrated its positive effects on cattle performance (Goodrich et al., 1976; Potter et al., 1976; Raun et al., 1976). Monensin has been shown to improve feed efficiency either by reducing intake,

increasing average daily gain, or both, depending on the feeding regimen (Short, 1978). In animals on a roughage diet, where rumen size limits intake, monensin increases rate of gain without altering feed intake. In concentrate-fed animals, where intake is controlled by chemostatic mechanisms (Conrad, 1966), monensin decreases feed intake without altering the rate of gain (Short, 1978).

Studies on the mechanisms of action of monensin have been intense during the past few years. Ruminal changes observed in monensin-treated animals include a shift in the VFA molar proportions favoring propionate, a decrease in methane production and a decrease in rumen protein breakdown and deamination, resulting in lower levels of ammonia-N (Bergen and Bates, 1984). Additionally, changes in digestibility, rate of protein utilization, rumen fill and rate of passage have also been reported (Bergen and Bates, 1984; Schelling, 1984).

The information currently available indicates that monensin causes important changes in the distribution of bacterial species in the rumen. The ultimate result is the generation of final products of fermentation which are highly favorable to the host animal. In the following review, some of these changes are discussed.

Effects of monensin on rumen volatile fatty acids

The effect of monensin on rumen fermentation is well established. Its most consistent effect is the increase in

the molar proportion of propionic acid with a concomitant decline in the molar proportion of acetate and butyrate, but with minor effects upon total VFA in the rumen (Richardson et al, 1976; Chalupa, 1980; Bergen and Bates, 1984). Usually, this shift in the VFA molar proportion is associated with a decrease in methane production without accumulation of gaseous hydrogen (Van Nevel and Demeyer, 1977; Chalupa, 1980).

Increased propionate production at the expense of acetate and methane should improve the efficiency of rumen fermentation due to improved retention of carbon and energy during the fermentative process. However, it is unlikely that this effect accounts for all the improvement in performance measured in monensin-treated animals. Assuming that all VFA arise from hexose (672 kcal/mole), Hungate (1966) has estimated the efficiency of conversion of hexose to acetate, propionate, and butyrate. One mole of hexose yields 2 moles of acetate (420 kcal), with an efficiency of 62%. One mole of butyrate (524 kcal) is produced per mole of hexose, with an efficiency of 78%. In propionate synthesis, however, all the energy of the hexose plus the energy corresponding to the two electrons required for the conversion of pyruvate to propionate, is retained in the product. It is evident, therefore, that a high-propionate type of fermentation is energetically more efficient than one yielding more acetate or more butyrate.

Based on the shift in the molar proportion of VFA associated with monensin (from 60:30:10 to 52:40:8 for

acetic, propionic and butyric, respectively), Richardson et al. (1976) calculated a theoretical energy savings to the animal of 5.6%. Therefore, fermentation efficiency was improved by monensin because of increased recovery of metabolic hydrogen in the rumen. The figures obtained by Richardson et al. (1976) are slightly higher than the 3% increase in efficiency estimated by Prange et al. (1978).

The theoretical increase in energy savings by monensin via the increase in propionate production evidently assumes the capacity of the host to use efficiently the extra propionate available. Results of short term studies involving intraruminal infusions of VFA (Armstrong et al., 1958; Armstrong and Blaxter, 1961) indicated a higher efficiency of utilization for propionic acid than for acetic acid. Blaxter and Wainman (1964) gave further support to this hypothesis by relating the higher molar proportion of propionate in concentrate diets to the increase in efficiency of metabolizable energy utilization by the animal. This evidence has been used frequently to explain part of the improvement in animal performance obtained with monensin. However, the hypothesis of higher efficiency of utilization for propionate has been challenged. Results obtained in long-term infusion studies (Bull et. al, 1970; Poole and Allen, 1970) have indicated that the major VFA are used with equal efficiencies. The main reason for the discrepancies between results seems to be the length of time involved in different experiments. Longer periods would be

required for the animals to adapt to the sudden increase in acetic acid in studies involving acetate infusion (Bull et al., 1970). This adaptation would involve the increase in the synthesis of pentosephosphate pathway enzymes responsible for NADPH generation. In short-term studies, the shortage of NADPH molecules would channel acetate to oxidation rather than to fatty acid synthesis, resulting in a higher heat increment (Bull et al., 1970).

Raun et al. (1976) have indicated that a shift toward more ruminal propionate production at the expense of acetate and methane could not account for all the improvement in performance obtained in feedlot studies. This observation led them to suggest that monensin is responsible for energy savings beyond those accounted for by the shift in VFA. These other potential savings would include a lower heat increment (Smith, 1971), a protein sparing effect involving amino acids normally used for gluconeogenesis (Leng et al., 1967; Reilly and Ford, 1971) and stimulation of protein synthesis in the host (Eskeland et al., 1974).

Effect of Monensin on Nitrogen Metabolism in the Rumen

Results of several experiments have indicated that at least part of the increase in efficiency of growth caused by monensin is associated with a decrease in ruminal losses of nitrogen. In in vitro studies in which casein was used as sole substrate, monensin depressed protein degradation (Van

Nevel and Demeyer, 1977). In the same study, monensin resulted in a slightly higher accumulation of alpha-amino nitrogen and lower levels of ammonia-N.

In vivo studies have confirmed the above observations. Dinius et al. (1976) reported significantly lower rumen ammonia-N levels in monensin-treated animals. These values were in line with the higher nitrogen retention measured in monensin-treated animals.

Poos et al. (1979) fed lambs diets containing either brewers dried grain (BDG) or urea. Addition of monensin decreased rumen ammonia-N regardless of the nitrogen source. When the same diet was fed to steers, monensin increased total-N and non-ammonia-N (NAN) flow to the lower intestine. Also, monensin supplementation increased plant nitrogen passage by 55% and 37%, for the grain- and urea-supplemented diets, respectively. The amounts of both essential and non-essential amino acids reaching the duodenun were higher for the combination BDG-monensin relative to control.

The above results clearly indicate an effect of monensin in depressing protein degradation in the rumen. The resulting increase in ruminal escape of dietary protein should constitute an additional factor contributing to the increase in performance of monensin-treated animals. However, several studies have indicated that this process may be coupled with a decrease in efficiency of rumen microbial growth (Van Nevel and Demeyer, 1977; Poos et al., 1979; Isichei, 1980; Bergen and Bates, 1984).

Van Nevel and Demeyer (1977) reported a severe depression of total and net microbial growth yields by monensin under *in vitro* conditions. *In vivo* studies have shown that monensin causes a significant decrease in the turnover of rumen contents (Dinius *et al.*, 1976; Lamenager et al, 1978). Based on these observations, Bergen and Bates (1984) have suggested that the lower efficiency of microbial growth caused by monensin might be attributed to an increase in maintenance requirements of the rumen microorganisms. Similar observations were reported by Allen and Harrison (1979). In this study, lambs were fed a diet based on grass hay and ground maize, supplemented with monensin. The Y_{ATP} values were 14.9 and 11.8 respectively for control and monensin-treated animals. The authors suggested that these values might be associated with the lower dilution rate measured in the treated group (0.071 vs. 0.941/h). This lower turnover rate might have resulted in a higher energy requirement for maintenance of the microbes (Stouthamer and Bettenhousen, 1973; Isaacson *et al*, 1975; Hespell and Bryant, 1979).

Effects of monensin on rumen microorganisms

Although considerable research has been devoted to determine how rumen fermentation is affected by monensin, the mechanism by which the microbes and their activities are controlled by the antibiotic has not been elucidated. The

following review will cover the work on species sensitivity to ionophores.

Monensin is a member of the carboxylic class of ionophore antibiotics (Short, 1978). These compounds have the ability to form complexes with certain monovalent cations, rendering them permeable in biological membranes (Pressman, 1976). Therefore, dissipation of the normal cation or proton gradients across bacterial cell membranes seem to be the central event in monensin action on rumen microorganisms (Romatowski, 1979; Bergen and Bates, 1984).

The effect of monensin on specific rumen bacteria species has been examined in only a few studies. The observation that lower methane production is obtained with monensin treatment has raised the question as to whether this might be due to a direct effect of the antibiotic upon methanogens. In vitro studies carried out by Van Nevel and Demeyer (1977) discarded this possibility. Methane formation from H_2 and CO_2 by washed-cell suspensions of mixed rumen bacteria was not inhibit by monensin. However, when formate was used as the sole substrate, considerable inhibition was observed. These results led the authors to suggest that the methane-depressing property of monensin is due to an inhibition of organisms decomposing formate into CO_2 and H_2, rather than to a direct toxic effect on the microbes.

Additional studies have confirmed the hypothesis that methanogens may not be the primary site of monensin action. Chen and Wolin (1979) reported that while the sensitivity

of methanogens to monensin is dependent upon the specific organism, none of the species tested were completely inhibited. Similar results were reported by Henderson et al. (1981).

The effect of monensin on carbohydrate-fermenting rumen bacteria have also been assessed. Chen and Wolin (1979) found that Ruminococcus albus, Ruminococcus flavefaciens and Butyrivibrio fibrisolvens, are highly sensitive to monensin. Succinate-producing bacteria, such as B. succinogenes and B. ruminicola were less sensitive to the antibiotic. Selenomonas ruminantium, which decarboxylates succinate to propionate, was found to be highly resistant to monensin.

Similar results were observed in studies conducted by Dennis et al. (1981). Major succinate producers (Bacteroides, Selenomonas, Succinivibrio) were not inhibited by monensin. Most of the lactate-producing species were inhibited by levels of the antibiotic ranging from 0.38 to 3.0 ug/ml. However, major lactate fermenters were not sensitive at these levels. The authors proposed that monensin could be effective in preventing lactic acidosis in ruminants, which was subsequently confirmed in studies by Nagaraja et al (1981).

Attempts have been made to explain the changes in the final products of rumen fermentation of monensin-treated animals based on the sensitivity of important rumen bacteria species to the antibiotic (Chen and Wolin, 1979; Henderson et al., 1981). These changes are likely to result from the

selective inhibition of bacteria species which do not contribute significantly to the propionate pool in the rumen. Consequently, growth of organisms such as Selenomonas ruminantium and Bacteroides ruminicola are favored. Inhibition of growth of major acetate and hydrogen producers in the rumen (Ruminococci and B. fibrisolvens) would result in a decrease in those end-products. This would therefore contribute to the decrease in the acetate to propionate ratio and to a reduced availability of hydrogen to the methanogens (Chen and Wolin, 1979; Henderson et al., 1981).

GLYCOPEPTIDES

Avoparcin

Avoparcin, a biologically active compound produced by a strain of Streptomyces candidus (Kunstmann et al., 1968), has both in vivo and in vitro activity against Gram positive bacteria (Redin and Dornbush, 1969). Its structure is similar to those of vancomycin and ristocentin, although substituents on the phenolic rings of the seven amino acids are slightly different (Hlavka et al., 1974).

Most of the preliminary work with avoparcin in animal nutrition was conducted in Europe, where it has been marketed largely as growth promoter for poultry and swine. Studies with ruminants carried out in the United States have shown promising results. Johnson et al. (1979) conducted a

112-day trial to evaluate the effect of 0, 16.5, 33, and 66 ppm avoparcin and 33 ppm monensin on the performance of steers fed a high-barley ration. Avoparcin-treated animals showed improved feed efficiency and higher weight gain relative to controls or monensin-treated steers. Both avoparcin and monensin caused a shift in the VFA molar proportions towards propionate. Essentially the same results were obtained with finishing heifers (Dyer et al, 1980). All avoparcin-treated animals consumed less feed per unit gain than controls. Carcass characteristics were not affected by avoparcin.

DeLay et al. (1978) fed 0, 16.5, 33 or 66 ppm avoparcin to steers. They reported 1.5, 2.7, and 4.9% reduction in average daily feed intake and 1.9, 5.7, and 11.6% improvement in feed efficiency for the three avoparcin levels relative to control.

The mechanisms by which avoparcin exerts its positive effects on the performance of ruminants still remain to be completely elucidated. As in the case of monensin, improved efficiency in treated animals has been associated with the action of avoparcin in the gastrointestinal tract. Chalupa et al. (1981), measured the effect of avoparcin on rumen environment and fermentation. Two rumen-cannulated heifers were maintained on a 25% corn silage plus 75% concentrate diet and supplemented with 0 or 75 ppm avoparcin in a reversal experiment. Quantities of rumen ingesta (47.1 vs 31.6 kg), dry matter (3.8 vs 2.8 kg) and liquid (43.3 vs

28.8 kg) were increased by avoparcin. However, slower rates (%V/h) of dry matter disappearence (7.6 vs 10.5) and of liquid turnover (4.8 vs 6.7) resulted in similar values of dry matter disappearence (6.92 vs 6.93 kg/day) and flow of liquid (49.8 vs 46.5 kg/day). Molar percent of both propionate and butyrate were increased by avoparcin, whereas acetate was decreased. Total VFA concentrations were not affected by the antibiotic. Higher rumen ammonia and lower alpha-amino nitrogen levels were also reported for the avoparcin-treated group. In vitro incubation of ingesta from the treated group generated less methane. When corrected for the larger ingesta volume, however, production was the same (Chalupa etal. 1981).

Froetschel et al. (1983) fed sheep either high or low fiber diets with or without addition of 50 ppm avoparcin. Compared to control, avoparcin decreased total VFA concentration (100 mM vs. 118 mM) and increased the molar percent of propionate (23.5% vs. 19.3%). Avoparcin significantly increased propionate production relative to control (2.07 vs. 1.68 moles/day). In the low fiber diet, avoparcin decreased ammonia concentration in vivo as well as amino acid degradation in vitro.

As in the case of monensin, attempts have been made to relate the changes in final products of fermentation to the sensitivity of important rumen bacteria to the antibiotic. Stewart et al. (1983) tested the effect of avoparcin on pure and mixed cultures of rumen bacteria. Of the Gram negative species tested, all except B. succinogenes were

able to grow in the presence of 200 ug avoparcin/ml. Conversely, most Gram positive bacteria could not grow in the presence of 8 ug avoparcin/ml, even after adaptation. Among the cellulolytic species, R. flavefaciens was most vulnerable to growth inhibition by avoparcin. Incubation of rumen contents in vitro with the addition of 5 and 10 ug avoparcin/ml had virtually no effect on the digestion of dried grass and straw. It appears, therefore, that avoparcin is able to inhibit the growth of many Gram positive bacteria, with little undesirable effects on the Gram negative species (Stewart et al., 1983). This might explain the similarity between avoparcin and monensin in terms of changes in final products of fermentation.

Teichomycin A2

Teichomycin A2 is one of the major components of a complex of antibiotics produced by Actinoplanes teichomyceticus nov. sp. ATCC 31121. Properties such as antibacterial spectrum, absence of activity on bacteria lacking cell wall, and chemical structure qualify teichomycin A2 as a member of the glycopeptide class of antibiotics. This class also include vancomycin and the ristocetins (Bardone et al., 1977). The main features distinguishing teichomycin A2 from the other glycopeptide antibiotics are the ocurrence of glycosamine as the basic sugar and the presence of aliphatic acid residues (Somma et al, 1984).

Teichomycin A2 is active *in vitro* and *in vivo* against medically important Gram positive bacteria such as *Streptococci*, *Staphylococci*, and *Diplococci*. Its antimicrobial properties are derived from its ability to interfere with cell wall biosynthesis by inhibiting polymerization of peptidoglycan (Parenti *et al.*, 1978).

In vitro and *in vivo* studies with teichomycin A2 (Phillips and Tadman, 1980; Brondani, 1983) have shown that the antibiotic was able to alter the normal patterns of rumen fermentation. Most importantly, those changes were of the same magnitude of those reported for avoparcin. Decreased methane production, shift in the molar proportion of volatile fatty acids towards propionate, and decrease in ammonia production were among the changes caused by teichomycin A2 in the rumen.

ISOACIDS

The branched-chain fatty acids (isobutyric acid, 2-methylbutyric acid, and isovaleric acid), and the straight chain 5-carbon valeric acid are important growth factors for certain cellulolytic bacteria (Bryant and Doetsch, 1955; Allison *et al.*, 1962; Allison, 1969; Dehority *et al.*, 1967) In the rumen, these acids are primarily derived from degradation of dietary proteins, but can also come from the recycling of bacterial protein (Annison, 1954; Pittnann and Bryant, 1964). Low availability of isoacids limits rumen

fermentation, especially with high roughage diets, or in situations of high feed intake and high energy demand as in lactation (Cook, 1985).

In vivo studies have demonstrated that C4 and C5 VFA can positively affect growth rates (Lassiter et al., 1958; Felix et al., 1980a), feed intake (Hemsley and Moir, 1963), nitrogen retention (Cline et al, 1966; Umunna et al., 1975; Felix, 1976), and milk production (Felix, 1976; Felix et al., 1980b; Papas et al., 1984).

The mechanisms by which isoacids exert their positive effects on animal performance have not been completely elucidated. However, several changes have been observed in animals fed isoacids. These include increased microbial protein synthesis, increase dry matter digestibility, increased nitrogen retention, as well as changes in the plasma concentration of growth hormone and insulin. In this review, the possible mechanisms of action of isoacids in ruminants are addressed. Aspects of the involvement of branched-chain VFA on amino acid synthesis by rumen bacteria have been reviewed (Allison, 1969, 1970; Bryant, 1973; Felix, 1976).

Sources of branched-chain VFA in the rumen

The requirement of branched-chain VFA by rumen microorganisms is well established. Although recycling of bacterial protein in the rumen may produce some C4 and C5 acids (Annison, 1954; Miura et al., 1980), they are

primarily derived from the deamination of amino acids from dietary protein. Hydrolysis of dietary proteins by mixed rumen microorganisms is quite rapid. The rate of hydrolysis appears to be directly related to the degree of solubility of the protein (Smith, 1975). The intermediate products of protein hydrolysis, that is amino acids and peptides, remain in the medium for a short period of time after feeding, indicating rapid degradation into ammonia and carbon skeletons or rapid fixation into microbial cells (Bryant, 1973).

Amino acid degradation in the rumen is accomplished primarily via oxidative decarboxylation. Final products include ammonia, carbon dioxide, and the carbon skeleton corresponding to the parent amino acid. The carbon skeletons can be further metabolized, giving rise to acetic, propionic, butyric, and valeric acids (Bryant, 1973). Additionally, isobutyric, isovaleric,and 2-methylbutyric acids (El-Shazly, 1952; Annison, 1954) can be derived from protein catabolism by mixed rumen bacteria. Final products of aromatic amino acid degradation include phenylacetic, phenylpropionic, and indole acetic acids (Scott et al., 1964). Proline undergoes reductive ring cleavage and deamination generating valeric acid (Dehority et al., 1958).

The mechanism of synthesis of several amino acids by rumen microorganisms involve the reversal of the oxidative decarboxylation pathway previously described. Isobutyrate, 2-methylbutyrate, isovalerate, acetate, and phenylacetate

can be carboxylated into their corresponding keto acid and then aminated to produce valine, isoleucine, leucine, alanine, and phenylalanine, respectively. Although the majority of branched-chain VFA in the rumen originates from deamination of amino acids, alternative sources have been reported (Annison, 1954; Miura et al., 1980). Several groups of bacteria are able to synthesize the carbon blocks required for amino acids synthesis. Precursors include intermediates of carbohydrate fermentation (mainly phosphoenolpyruvate), as well as fermentation end products, such as acetate, propionate, butyrate, valerate, and carbon dioxide (Otagaki et al., 1955; Kay and Hobson, 1963; Allison, 1969; 1970; Dehority, 1971). Lysis of those bacteria in the rumen can therefore provide extra sources of branched-chain VFA. Quantification of branched-chain VFA generated either from amino acids or from de novo synthesis is difficult, because of the dynamic nature of protein metabolism in the rumen. However, the available information seems to indicate that the vast majoriy of the branched-chain VFA is in fact derived from dietary protein (Allison, 1969; 1970).

Effects of isoacids on rumen microbial protein synthesis

The main function of branched-chain fatty acids in the rumen is to serve as carbon skeletons for the synthesis of amino acids by the bacteria. In addition, they can be utilized in the biosynthesis of long-chain fatty acids and

aldehydes (Allison et al., 1961; Dehority et al., 1967). It would be expected, therefore, that isoacid supplementation would result in increased bacterial protein synthesis. This has in fact been confirmed. Russell and Sniffen (1983) studied the effect of isoacids on growth of mixed rumen bacteria mantained on different substrates. Supplementation of isobutyrate, 2-methylbutyrate, isovalerate, and valerate when bacteria were grown on mixed carbohydrates had little effect on synthesis of bacterial dry weight, DNA, RNA, or carbohydrates. When timothy hay was used, isovalerate and 2-methylbutyrate increased protein synthesis by 11.2 and 16.4 % respectively. Isobutyrate and valerate alone had no effect. Combination of the four acids increased bacterial protein synthesis by 18.7%. Responses to an inoculum of 60% concentrate and timothy hay were not significant.

Russell (1984), incubated mixed rumen bacteria in artificial media with mixed carbohydrates as substrate to evaluate the effect of isoacids on bacterial protein synthesis. When all four acids were present at 2 mM each, bacterial protein synthesis was increased by 22%. Tests with individual acids indicated that only isovalerate and 2-methylbutyrate were effective.

Hemsley and Moir (1963) tested the influence of isoacids on microbial protein synthesis in sheep. The addition of 0.56% of a mixture of isobutyrate, isovalerate, and n-valerate to a diet of milled oaten hay increased total rumen microbial protein by 70 percent. When sheep being fed low-protein teff hay were supplemented with urea plus

isoacids, total concentration of cellulolytic bacteria doubled relative to control, and feed intake increased significantly (Van Gylswyk, 1970).

Hume (1970) fed sheep a low protein purified diet supplemented with a mixture of isobutyric acid (27.0%), 2-methylbutyric acid (26.3%), isovaleric acid (29.9%), and n-valeric acid (16.8%). Rumen microbial protein production was significantly higher in the treated group. Also, the amounts of total nitrogen and TCA-nitrogen flowing out of the rumen were greater in those sheep receiving the VFA mixture than in those on the basal diet.

Similar results were reported by Kay and Phillipson (1964). Sheep receiving a poor quality hay and infused with a urea plus isoacids supplement showed a 10 to 15% increase in the flow of nitrogen to the duodenun. A similar infusion of the straight-chain VFA had little effect on nitrogen flow rate.

Effects of isoacids on dry matter digestibility

There is an enormous amount of information indicating that the predominant rumen cellulolytic bacteria, as well as certain noncellulolytic bacteria, either require or are stimulated by branched-chain fatty acids (Bryant and Doetsch, 1955; Robinson and Allison, 1967; Allison, 1969, 1970; Slyter and Weaver, 1971). Allison *et al.* (1958) reported an absolute requirement of branched-chain VFA by several strains of *Ruminococci*. Bentley *et al.* (1954),

Dehority et al. (1957; 1967) and Allison and Bryant (1963) reported that isoacids are required for the growth of several rumen cellulolytic species, including strains of Ruminococci, Butyrivibrio and Bacteroides. These results coupled with the increase in bacterial protein synthesis discussed previously would suggest that isoacids would invariably cause improvement in dry matter digestibility. Studies conducted in vitro have in fact confirmed that prediction. However, in vivo results have not been as consistent. Burroughs et al. (1951) reported that cellulose digestion by mixed cultures of rumen bacteria occurs even when urea or ammonia are used as the sole nitrogen source. Bentley et al. (1955) reported that rumen microorganisms grown under in vitro conditions had a higher rate of cellulose digestion and of urea nitrogen incorporation into protein when isoacids or their amino acid precursors were added to the medium.

Soofi et al. (1982) reported an increase in digestibility of soybean stover when isoacids were supplemented to an artificial medium. Addition of starch to the medium had a depressing effect on digestibility, indicating either a negative action of starch on isoacid utilization or a higher utilization of isoacids and starch at the expense of fiber.

Gorosito et al. (1985) incubated mixed ruminal bacteria in an artificial medium to which an equimolar mix (<.30 mM) of C4 and C5 acids was added. Cell wall digestion of both

isolated cell walls and of intact forages was increased by isobutyric, 2-methylbutyric, and isovaleric acids, but not by n-valeric.

An increase in cellulose digestion by lambs fed urea and isoacids has been reported by Cline et al. (1966). Hungate and Dyer (1966) reported that steers fed wheat straw and urea supplemented with valeric and isovaleric acid had higher intake, probably due to higher fiber digestion.

Hefner et al. (1985) conducted extensive studies on isoacids supplementation of corn crop residue diets. In situ cotton thread disappearence was increased by isoacids. However, results were dependent on the source of dietary nitrogen. Lambs fed natural protein supplements had higher dry matter and fiber digestibilities than isoacids and urea supplements. These results give support to the findings of Hemsley and Moir (1963) and Hume (1970). It is worth pointing out that despite the lack of evident changes in digestibility, isoacids increased nitrogen retention and decreased urinary nitrogen loss in the aforementioned experiments.

Effects of isoacids on nitrogen retention

One of the most consistent effects observed when isoacids are fed to ruminants is increased nitrogen retention. Oltjen et al. (1971) fed steers either a protein-free or a protein-containing diet supplemented with 0.27% 2-methylbutyrate, 0.31% isovalerate, 0.25%

isobutyrate, and 0.20% phenylacetate. Sources of nitrogen were either urea or isolated soy protein. Isoacid supplementation resulted in higher nitrogen retention and lower urinary nitrogen loss. Most of these changes were found in steers fed isolated soy protein. Oltjen et al. (1970) reported similar results.

Felix (1976) supplemented two mixtures of isoacids to lactating cows fed a corn silage plus concentrate diet. Percent of absorbed nitrogen retained was higher and loss of urinary nitrogen was lower in cows fed isoacids. No differences between the two isoacid mixtures were shown.

Umuna et al. (1970) fed lambs a high roughage diet supplemented with urea plus isobutyric and isovaleric acids. Treated animals had higher nitrogen retention and lower urinary nitrogen loss. Neither rumen ammonia nor plasma urea were affected by isoacid supplementation. Similarly, Cline et al. (1966) reported a significant increase in nitrogen retention by lambs supplemented with 4.18 g isobutyrate, 1.18 g n-valerate, and 5.9 g isovalerate per lamb per day.

Effects of isoacids on plasma hormone concentrations

Recent reports indicate that in addition to their effect in the rumen, branched-chain VFA alter plasma concentrations of growth hormone and insulin in lactating cows. Towns and Cook (1984) were the first investigators to report such an effect by isoacids. Cows fed 80 g/day of an

isoacid mixture (equal amounts of isobutyric, 2-methylbutyric, isovaleric, and valeric acids) had higher plasma levels of growth hormone but lower levels of insulin and glucose relative to controls. Milk production was higher in the treated group (Towns and Cook, 1984).

Fieo et al. (1984) investigated the effect of ammonium salts of VFA (AS-VFA) on plasma growth hormone levels in lactating cows. Treatments consisted of 0 and 120 g AS-VFA per cow/day added to a total mixed ration. Cows fed AS-VFA had higher mean growth hormone concentrations than control cows (8.42 vs 5.68 ng/ml). Unlike the Towns and Cook (1984) experiment, however, milk production was not increased. The authors (Fieo et al., 1984) suggested that the short duration of the experiment was the probable reason for the lack of difference in milk production.

The mechanisms by which changes in plasma hormones take place in isoacid-treated animals are obscure at this point. It is not known if the changes were caused by isoacids directly or if they were a product of a change in the balance of other rumen metabolites caused by isoacids. In this section, the possible influences of products of rumen fermentation on the host animal endocrine profile are addressed.

Manns and Boda (1967) reported that intravenous injection of propionate and butyrate caused an augmentation in the plasma insulin levels in sheep. These findings raised the possibility that production of VFA in the rumen

might be directly linked to the control of intermediary metabolism in ruminants. Since then, the effect of VFA on insulin secretion has been the subject of intense investigation. Most of the available information seems to indicate that butyrate and propionate (Horino et al., 1968; Trenkle, 1970; Basset, 1972), isobutyrate (Ross and Kitts, 1973) and valerate (Hertelendy et al., 1969) have a stimulatory effect on insulin secretion when injected intravenously.

The physiological importance of VFA on insulin secretion has been questioned, however. Ordinarily, part of the propionate and butyrate produced in the rumen is metabolized in the rumen epithelium during absorption (Stevens, 1970). The fraction actually reaching the circulation is promptly removed by the liver (Ricks and Cook, 1981). Therefore, little propionate and butyrate would actually reach the pancreas to have any effect on insulin secretion (Stern et al. 1970). Acetate, the only VFA occurring in peripheral plasma in substantial quantities, has no effect on insulin secretion (Horino et al., 1968). The main reason for the dispute, however, resides on the fact that in the majority of the studies linking propionate, butyrate and valerate to insulin secretion, the acids were injected or infused intravascularly, sometimes in concentrations too high to be considered physiological (Manns and Boda, 1967; Manns et al., 1967; Horino et al., 1968; Stern et al., 1970; Trenkle, 1970; Basset, 1971).

Intraruminal injections of .1 mole of propionate or .1 mole of butyrate increased the ruminal concentrations of those acids by two to five times in goats fed ad libitum, but did not change plasma insulin levels (Stern et al., 1970). Similar results were obtained by Basset (1972). Injection or infusion of VFA into the rumen which elicited changes in plasma VFA similar to those after feeding, failed to cause any significant changes in plasma insulin concentrations.

Trenkle (1978) reported an increase in plasma concentration of insulin following a 4-hour infusion of physiological concentrations of acetate, propionate, and butyrate in the rumen of sheep fasted for 36 hours. Increasing butyrate concentration in the mixture increased insulin concentrations more than did propionate. Acetate consistently decreased plasma insulin levels. The author concluded that production of fatty acids in the rumen has some effect in the regulation of plasma insulin. The discrepancy between results of this study and those reported by Stern et al. (1970) might be explained by differences in feeding regime. Animals in the latter study (Stern et al., 1970) had free access to feed during the experiments and the higher concentrations of insulin in ad libitum fed goats may have prevented any additional response to the treatments (Trenkle, 1978).

Bines and Hart (1984) infused a complete mixture of VFA (10.8 moles acetic acid, 3.9 moles propionic acid, 2.2

moles n-butyric acid, and .5 mole isovaleric acid) into the rumen of fistulated, nonlactating Friesian cows over a 210-min period. Following feeding of 3 kg of hay, solutions were infused at a rate that resulted in VFA concentrations in peripheral blood similar to those elicited by the feeding of 7 kg concentrate. Infusion of the complete mixture resulted in an insulin response similar to feeding concentrate. Omission of butyrate or isovalerate had no effect on plasma insulin levels. Ommission of propionate from the mixture (equivalent to 27% of the energy in the complete mixture), decreased the insulin response by 70%. The authors concluded that when diets that elicit a high-propionate type of fermentation are fed to cows, propionate is a major factor governing insulin secretion. Since no other hormone or metabolite changed during the infusions, it appears that propionate is the only major stimulant of insulin secretion in the bovine (Bines and Hart, 1984). Similar results have been reported by Istasse and Orskov, (1984) and by Emmanuel and Kennelly (1984).

The mechanisms by which propionate stimulates insulin secretion in ruminants is not known. Since the propionate fraction that actually reaches the circulation is promptly removed by the liver (Ricks and Cook, 1981), it is very unlikely that a direct interaction of propionate with the pancreatic beta-cells is involved. Therefore, a system in charge of sending anticipatory signals to the pancreas should exist. Such a mechanism is in fact operative for glucose in non-ruminants (For review, see Sawchenko et al.,

1979; Miller, 1981). The system involves a complex network of receptors and nerve branches located in the portal vein (Russek, 1963; Niijima, 1969; Niijima, 1981) as well as in the walls of the duodenum (Mei, 1978). All branches are tributaries of the abdominal vagus, 90% of which are composed of afferent fibers (Niijima, 1981). Although other mechanisms exist for the control of insulin by glucose, it appears that glucose-sensitive receptors in the liver serve as monitors of portal blood glucose and can trigger an anticipatory response for the regulation of blood glucose levels (Niijima, 1981). Increases in glucose concentration in the portal venous blood results in a decrease in the rate of firing from the liver to the hypothalamus through the vagus nerve. Efferent signals to the pancreas are intensified while signals to the adrenals are diminished, resulting ultimately in increased insulin secretion and decreased secretion of epinephrine and norepinephrine.

There is evidence that a reflex mechanism sensitive to propionate is involved in the control of insulin secretion in ruminants (Anil and Forbes, 1980; Elliot, 1980). Most of the evidence for such a system has been derived from studies on mechanisms regulating feed intake (Forbes, 1980). The route by which the liver transmits information about its uptake of propionate to the central nervous system involve afferent fibers of the hepatic plexus, a major tributary of the vagus nerve (Anil and Forbes, 1980). Of course, the

participation of a reflex mechanism would necessarily require the existence of efferent pathways to the pancreas. Evidence for such a pathway has been provided by the observation that insulin secretion is decreased in sheep chronically exposed to cold (Sasaki and Takahashi, 1980; Sasaki et al., 1982). The decrease in insulin is mediated by adrenergic alpha-receptors in the pancreatic beta-cells. These receptors are activated both directly, via decreased rate of firing of efferent fibers to the pancreas, and indirectly, as a result of higher sympatho-adrenomedulary activity. The final result is a decrease in insulin secretion which persists until the cold stimulus is terminated (Sasaki and Weekes, 1986).

III. Effects of teichomycin A2 and avoparcin on *in vivo* production rates of rumen volatile fatty acids in sheep fed high and low roughage diets.

Introduction

Teichomycin A2 is a biologically active compound produced by *Actinoplanes teichomyceticus*. Avoparcin is an antibiotic produced by a strain of *Streptomyces candidus* (Kunstmann *et al.*, 1968). Properties such as antibacterial spectrum, absence of activity in bacteria lacking cell wall, and chemical composition, suggest that both avoparcin and teichomycin A2 belong to the vancomycin group of glycopeptide antibiotics. Both compounds are active *in vitro* and *in vivo* against Gram positive bacteria. Their antimicrobial properties stem from their ability to interfere with cell wall synthesis (Redin and Dornbush, 1969; Parenti *et al.*,1978).

Several reports indicate that cattle fed avoparcin have lower feed intake and improved daily gains and feed efficiency (DeLay et. al., 1978; Johnson *et al.*, 1979). Changes in the rumen include increase in propionate with a concomitant decrease in acetate (Chalupa et. al., 1981; Froetschel *et al.*, 1983) and butyrate (Froetschel *et al.*, 1983) molar percent. Rumen nitrogen metabolism is also affected. Avoparcin-supplemented animals have shown lower ammonia and alpha-amino nitrogen concentrations in the rumen (Chalupa, 1981; Froetschel *et al.*, 1983).

In vitro studies with teichomycin A2 have indicated that this glycopeptide alter rumen fermentation in a fashion similar to that of avoparcin (Phillips and Tadman, 1980; Brondani, 1983). However, studies directly comparing the two antibiotics under *in vivo* conditions have not been conducted. Additionaly, the majority of the studies on the effect of rumen additives on VFA production rates has been restricted to measurements of propionate, with no attention given to acetate and butyrate. Therefore, the present study was conducted to determine the effects of teichomycin A2 and avoparcin on rates of production of acetate, propionate, and butyrate *in vivo*. The effects of those glycopeptides on several rumen nitrogen variables were also measured.

Materials and methods

Two trials were carried out to evaluate the effect of teichomycin A2 and avoparcin on rumen function. In each trial, six rumen-cannulated crossbred ewes were housed in an environmentally controlled room and kept in individual pens. Average body weight for animals in trials 1 and 2 were 40.6 kg and 39.2 kg, respectively. In trial 1 (high roughage diet), animals were fed 1000 g of alfalfa hay per day and had free access to trace mineral salt. In trial 2 (low roughage diet), animals received 900 g per day of a ration composed of 43% alfalfa meal, 10% ground corn cobs, 20% ground shelled corn, 20% ground oats, 5% H_2O, 1% trace mineralized salt, 0.5% dicalcium phospate, and 0.5% a ADE

vitamin premix (vit. A, 4,400 IU/g; vit D, 500 IU/g; vit E, 44 IU/g). Daily feed was provided in two portions, at 0800 hr and 1600 hr, and free access to water was allowed.

In each trial, animals were divided in three groups and randomly allocated to one of the treatments: control, 30 ppm teichomycin A2, or 30 ppm avoparcin (dry mater basis). The antibiotics were injected directly into the rumen through the cannula immediately following feeding. On days 28 and 29, samples for the determination of rumen VFA and rumen nitrogen constituents were collected. Rumen fluid samples were collected prior to morning feeding and then at hourly intervals for the next eight hours. Individual samples were divided into aliquots as follows: 3 ml were acidified with 150 ul of 2N H_2SO_4 and saved for ammonia determination; 10 ml were transferred to a vial for subsequent VFA analysis; and 15 ml were transferred directly to centrifuge tubes for further fractionation and subsequent determination of nitrogen components.

Measurements of rates of acetate production and liquid flow were performed on days 31 and 37. Three hours after the morning feeding, 100 ml of water containing 10 g of polyethylene glycol (PEG, M.W. 4000) and 100 uCi of Na-[1-C14]acetate were added to the rumen. To facilitate mixing, the solution was infused in different locations within the rumen. This was performed with the aid of a dosing syringe attached to a perforated plastic tube (15 mm i.d. x 30 cm). A similar device was used for the collection

of rumen fluid samples. Serial rumen fluid samples were collected every 20 minutes for the next four hours. Samples were strained through surgical gauze, frozen in a dry ice-acetone bath and stored at -20°C until further analysis. The rates of production of propionate and butyrate were measured on days 32 and 39, and days 33 and 40, respectively. The procedure was essentially the same as that used for acetate (Cook, 1976; Knox et al, 1967).

Sample analyses

Volatile fatty acids

Volatile fatty acid concentrations in rumen fluid were determined by gas liquid chromatography according to the procedure of Ottenstein and Bartley (1971a, 1971b), with minor modifications. One 1-ml aliquot was obtained from the rumen fluid supernatant and mixed with 50 ul of 9N H_3PO_4. Samples were stored at 4°C for 60 minutes, then centrifuged at 40,000 x g for 20 minutes to remove protein-like material. Supernatant fractions were transferred to the automatic sampler vials for analysis. Standards were prepared similarly, except for the centrifugation step. Analyses were performed on a Hewlett-Packard gas chromatograph, model 5030A, equipped with a 7671A automatic sampler and a 3880A integrator-recorder (Hewltt-Packard Co., Avondale, Pennsylvania). The all-glass column was packed with 10% SP-1200/1% H_3PO_4 on 80/100 Chromosorb W AW (Supelco, Inc., Bellefonte, Pennsylvania). Nitrogen was the

carrier gas, with flow rate mantained at 20 ml/min. Temperatures of 200°C, 150°C, and 250°C were used for injection port, column, and flame ionization detector, respectively. Attenuation and range were mantained at 64 and 1 settings, respectively.

Protein Fractions

Separation of rumen fluid for the determination of nitrogen components was performed via differential centrifugation (Bergen et al., 1968; Isichei, 1980). Samples were first centrifuged at 150 x g for 5 minutes. Pellets were resuspended in 5 ml of 0.9% NaCl and centrifuged again at 150 x g for 5 minutes. This procedure was repeated three times and the resulting pellet saved for protozoa protein analysis. The initial 150 x g supernatant was centrifuged at 500 x g for 10 minutes to remove feed particles, and then centrifuged again at 34,000 x g for 15 minutes. The resulting supernatant was analyzed for soluble protein. The pellet (bacterial cells) was suspended in 3.5 ml of 0.9% NaCl and then centrifuged at 34,000 x g for 15 minutes. The resulting pellet was analyzed for bacterial protein. All samples were stored at -20°C until analyzed. Protein concentrations in the different fractions were determined in the Technicon Auto-Analyzer System (Technicon Instrument Corporation, Tarrytown, New York). The method of Lowry et al. (1951), as modified by Phillips and Tadman

(1980) was used. Reagent A (2% Na_2CO_3 in 0.1N NaOH), reagent B (0.5% $CuSO_4$.5H20 in 1% $C_4H_4NaO_6$), and reagent C (50 volumes of reagent A plus 1 volume of reagent B) were prepared fresh for each analysis.

Soluble Protein and Alpha-Amino Nitrogen

Three milliliters of strained rumen fluid were mixed with 0.7 ml of 3M trichloroacetic acid (TCA) and stored at 4°C for 45 minutes to allow complete protein precipitation. Next, samples were centrifuged at 28,000 x g for 20 minutes. Supernatant fractions were saved for alpha-amino nitrogen analysis. Pellets were resuspended in reagent C and immediately centrifuged to remove any material that failed to go into solution. Samples were then transferred to vials for soluble protein determination.

Insoluble protein

Pellets containing either protozoal or bacterial protein fractions were suspended in 5 ml of reagent C and treated in a sonifier cell disruptor (HET Systems Ultrasonic, Inc., Long Island, New York). Sonication time was for 15 seconds at a setting of 3. Samples were then stored at 40°C for 15 minutes to allow protein solubilization and then centrifuged at 28,000 x g. Reagent C was used to bring the concentration within the range of 200-800 ug/ml. A standard curve based on the average of the

recorder readings was obtained on each analysis. Protein concentrations were estimated from the standard curve, then corrected for dilutions.

Ammonia nitrogen

Preparation of samples for ammonia-N analysis was according to the method of Okuda et al. (1965), as modified by Kulasek (1972). Ammonia-N concentrations were determined with the aid of a Lazar GS-136 electrode-potentiometer (Lazar Research Laboratories, Los Angeles, CA).

Rumen volume and rumen fluid turnover rate

Rumen fluid samples were centrifuged at 10,000 x g for 15 minutes to separate food particles. Polyethylene glycol concentrations in the supernatant were determined by the method of Hyden (1955), as modified by Smith (1959). Rumen volume and fractional turnover rate were calculated by using the slope and intercept of the best fit line obtained by regressing PEG concentrations against time (Bauman et al., 1969).

Production rates for acetate, propionate and butyrate

Preparation of the samples for the determination of the specific activity of acetate, propionate and butyrate was conducted as follows. Initially, samples were centrifuged

at 15,000 x g for 15 minutes to remove food particles. The resulting supernatant was then steam distilled. Four milliliters of supernatant were placed in the still along with four drops of 50% H_2SO_4. Aproximately 30 ml of distillate were collected in 100 ml Erlenmeyer flask. Isolation of the acids from the distillate was performed by ion exchange chromatography. Samples were passed through a glass collum packed with 0.5 g Bio-Rex 5 anion exchange resin (Bio-Rad Laboratories, Anaheim, CA). Columns were eluted twice with 0.5 ml of 5% H_2SO_4. The second eluent fraction, which contained the VFA, was stored at -20°C until further analysis. Efficiency of VFA recovery by this procedure was always above 90%. The ion exchange resin was discarded after each sample.

Separation of the individual acids was performed by high performance liquid chromatography . The system consisted of a 300 mm x 7.8 mm analytical column packed with Aminex ion exclusion HPX-87H (Bio-Rad Laboratories, Anaheim, CA) and a 4.6 nm x 70 mm Perisorb RP-8 guard column. The mobile phase was 1% H_2SO_4 in deionized, degassed water. Flow rate was set at 2.0 ml/min, which required a pressure of 1500 to 2000 psi. Following each injection, the guard column was thoroughly washed to prevent carryover to the analytical column. In this procedure, 10 ml of 65% aqueous acetonitrile and 10 ml of deionized water were used. Standard curves were made for each acid. Concentration of acids in the standard solutions were of the same order of

magnitude as those found in the samples. The amount of sample injected into the system was 500 ul. Eluent collection started immediately following the detection of the colum residue peak. For each sample, twenty 1-ml fractions were collected into 10-ml scintillation vials using a Isco 328 fraction collector. To each vial, 5 ml of Safety-Solve aqueous scintilation counting cocktail (Research Products International, Mount Prospect, Illinois) were added. Fractions were counted for 2 minutes in a ISOCAP/300, Model 68708, liquid scintillation spectrophotometer (Searle Analytic Inc.). No appreciable radioactivity was detected after chromatographing non-radioactive samples following a radioactive sample, indicating that recovery of radioactivity from the column was 100%.

Acetate, propionate, and butyrate specific activities (SA) were determined for all samples. The SA varied considerably in the early samples, probably due to incomplete mixing of the radioactive material in the rumen (Cook, 1966; Knox et al., 1967). Therefore, the values corresponding to the two initial sampling points were eliminated. Acetate pool fractional turnover time was derived by regressing the ln SA of acetate against time. The pool size was obtained by multiplying the rumen volume in liters by the average acetate concentration. Daily ruminal acetate production was obtained by dividing the acetate pool size by the acetate fractional turnover time (Cook, 1966; Knox et al., 1967; Froetschel et al., 1983).

Rumen propionate and butyrate data were subjected to the same procedure.

Statistical analysis

Overall significance of treatment effects was determined by analysis of variance (Gill, 1978a; 1978b; 1978c) according to the model in (I):

(I) $Y_{ij} = u + A_i + E_{(i)j}$

where,

Y_{ij} is the observed response of jth sheep to the ith treatment;

u is the overall mean;

A_i is the fixed effect of the ith treatment, i=1,2,3;

$E(i)j$ is the random residual error.

For each variable, Bonferroni-t tests (Gill, 1978a) were used to test specific differences between treatment means.

Results and discussion

The effects of teichomycin A2 and avoparcin on concentrations of rumen VFA in sheep fed high and low roughage diets are summarized in Table 1. Volatile fatty acids expressed as a percent of total moles are shown in Table 2. In the high roughage ration, supplementation with glycopeptides did not change the concentrations of acetate

(p>.10) but increased propionate concentrations. Increases relative to control were 26.0% and 30.8% respectively for teichomycin A2 and avoparcin (p<.10). There was a tendency for lower butyrate concentrations with glycopeptide supplementation, but differences were not significant (p>.10). Total VFA concentrations were not significantly changed by the treatments (p>.10). In general, concentrations of isoacids tended to be lower in teichomycin A2 and avoparcin treated animals. Teichomycin A2 decreased the concentrations of isobutyrate and isovalerate (p<.05), whereas avoparcin decreased isovalerate concentrations relative to control (p<.05). Consequently, total isoacids were lower in both teichomycin A2 and avoparcin groups (p<.10). The molar percent of acetate and butyrate tended to be lower for the glycopeptide-treated groups in the high roughage diet (Table 2). However, differences relative to control were not significant (p>.10). Propionate molar percent was increased by the glycopeptides relative to control (p<.05). As a result, the acetate to propionate ratio was lower for the treated groups in the high roughage diet (p<.05).

In the low roughage ration, the trends for VFA concentrations were similar to those in the high roughage ration. Propionate concentrations increased (p<.10) and butyrate concentrations decreased (p<.10) due to glycopeptide supplementation. Concentrations of acetate and total VFA were not affected by treatments (p>.10). The lower concentrations of isoacids caused by the glycopeptides

TABLE 1: Effects of teichomycin A2 and avoparcin on concentrations of rumen VFA in sheep fed high and low roughage diets (mmoles/dl)[a]

Variable	High Roughage				Low Roughage			
	Control	TE-A2	AVO	SEMg	Control	TE-A2	AVO	SEMg
Acetate	6.94	6.75	6.51	0.57	6.31	5.89	6.12	0.63
Propionate	1.77[e]	2.23[f]	2.31[f]	0.12	1.74[e]	2.38[f]	2.75[f]	0.13
Isobutyrate	0.19[c]	0.11[d]	0.14[cd]	0.02	0.21	0.19	0.17	0.03
Butyrate	0.81	0.73	0.65	0.14	0.97[e]	0.63[f]	0.71[f]	0.07
2-M-Butyrate	0.16	0.11	0.13	0.03	0.18	0.14	0.19	0.04
Isovalerate	0.14[c]	0.09[d]	0.07[d]	0.01	0.16	0.15	0.13	0.03
Valerate	0.17	0.14	0.16	0.04	0.19	0.21	0.18	0.04
Total VFA	10.26	10.16	9.97	0.96	9.76	9.59	9.75	1.13
Total Isoacids	0.66[e]	0.45[f]	0.50[f]	0.04	0.74	0.69	0.67	0.06

[a] 36 observations per mean.

[b] Means in a row within a ration without a common superscript differ: cd($p<.05$); ef($p<.10$).

[g] Standard error of each mean in a row within a ration.

in the high roughage ration were not detected in the low roughage ration. Teichomycin A2 and avoparcin increased propionate ($p<.01$) and decreased butyrate ($p<.05$) molar percent with respect to control. Acetate molar percent was slightly lower in the treated groups but differences were not significant. This ultimately resulted in lower acetate to propionate ratios in the treated groups than in the control group ($p<.01$).

The effect of teichomycin A2 and avoparcin on the rates of production of acetate, propionate and butyrate in sheep fed high and low fiber diets are shown in Tables 3, 4, and 5, respectively. Since measurements for each acid were carried out on separate days, rumen fluid volumes and dilution rates corresponding to each acid are also provided.

Pool fractional turnover time for each acid was derived by regressing the ln SA of the respective acid against time. Pool size was obtained by multiplying the rumen volume in liters by the average concentration of the acid on testing day. Daily production was obtained by dividing the pool size of the acid by its fractional turnover time (Cook, 1966; Knox et al, 1967; Froetschel et al., 1983). Rumen volume and fractional turnover rate of rumen fluid were obtained by using the slope and intercept of the best fit line generated by regressing PEG concentrations against time (Bauman et al., 1969). The average correlation coefficients for the line of best fit for each acid and its corresponding PEG data were as follows: acetate, 0.969 and

TABLE 2. Effects of teichomycin A2 and avoparcin on the molar percent distribution of rumen VFA in sheep fed high or low roughage dietsab

Variable	High Roughage				Low Roughage			
	Control	TE-A2	AVO	SEMe	Control	TE-A2	AVO	SEMe
Acetate	68.0	66.3	65.2	0.89	64.6	61.4	62.8	0.82
Propionate	17.3c	22.0d	23.2d	0.64	17.8c	24.8d	23.1d	0.59
Isobutyrate	1.9	1.1	1.5	0.11	2.1	2.0	1.7	0.16
Butyrate	7.9	7.2	6.5	0.49	9.9e	6.6f	7.3f	0.51
2-M-Butyrate	1.6	1.1	1.3	0.13	1.8	1.5	1.9	0.09
Isovalerate	1.4	0.9	0.7	0.15	1.6	1.6	1.3	0.12
Valerate	1.9	1.4	1.7	0.11	1.9	2.2	1.8	0.13
A:P ratio	3.9e	3.0f	2.8f	0.13	3.6c	2.5d	2.7d	0.17

[a]36 observations per mean.

[b]Means in a row within a ration without a common superscript differ: cd($p<.01$); ef($p<.05$).

[e]Standard error of each mean in a row within a ration.

0.912; propionate, 0.964 and 0.891; and butyrate, 0.977 and 0.883. These high correlation coefficients indicate that first order kinetics were maintained during the experiments (Cook and Ross, 1964; Cook, 1966; Knox et al., 1967; Rogers, 1981).

Rumen fluid volume and rumen fluid dilution rates corresponding to each individual acid are reported in Tables 3, 4, and 5. Overall, animals fed the high roughage diet had a higher rumen fluid turnover rate (and consequently a lower turnover time) than animals fed low roughage. These results are in line with those reported for sheep (Froetschel et al., 1983) and cattle (Bauman et al., 1969). It appears that this difference is due to higher salivary flow in animals fed high roughage diets (Bauman et al., 1969). Froetschel et al. (1983) reported an increase in rumen volume in animals fed avoparcin. In the present study such a trend was not detected for either the avoparcin or the teichomycin A2 groups.

In the high roughage ration, there was a trend for lower acetate production in the treated groups (Table 3). However, differences were not significant ($p>.10$). Although acetate pools were not affected by the treatments ($p>.10$), the lower rate of acetate production due to avoparcin resulted in significantly higher acetate pool turnover time relative to control ($p<.10$). In the low roughage ration, trends were basically the same as those found in the high roughage ration. Acetate production and acetate pool sizes tended to be lower in the treated groups, but differences

TABLE 3. Effects of teichomycin A2 and avoparcin on acetate production and rumen fluid variables in sheep fed high and low roughage diets[a]

Variable	High Roughage				Low Roughage			
	Control	TE-A2	AVO	SEM[e]	Control	TE-A2	AVO	SEM[e]
Acetate								
Pool size, moles	0.36	0.34	0.39	0.02	0.31	0.27	0.29	0.03
Turnover time, min	110.3[c]	112.4[c]	134.1[d]	6.9	103.5	97.6	102.9	9.1
Production, moles/day	4.74	4.41	4.12	0.27	4.39	3.89	4.18	0.36
Rumen fluid								
Volume, liters	5.1	4.4	4.6	0.47	4.4	3.8	4.0	0.31
Turnover time, hr	8.7	9.2	8.5	1.39	14.3	16.1	13.1	0.98
Turnover rate, %/hr	11.1	10.5	11.9	1.03	6.7	6.3	7.7	0.62

[a]4 observations per mean.

[cd]Means in a row within a ration without a common superscript differ ($p<.10$).

[e]Standard error of each mean in a row within a ration.

were not significant ($p>.10$).

Propionate production rates were significantly increased by glycopeptide supplementation, in both rations (Table 6). In the high roughage diet, increases in propionate production relative to control were 48% ($p<.05$) and 38.7% ($p<.05$) respectively for teichomycin A2 and avoparcin. In the low-roughage ration, increases in propionate production due to teichomycin A2 and avoparcin were 34.8% ($p<.10$) and 36.8% ($p<.10$), respectively. The increase in propionate production resulted in higher pool sizes for both antibiotics in both rations. However, significant differences from control were detected only for avoparcin in the low roughage diet ($p<.10$).

Butyrate production (Table 7) was not affected by treatments in either ration ($p>.10$). The small numerical decrease in butyrate production measured for the treated groups in the high roughage ration was also detected in the butyrate pool sizes within that ration. However, differences were not significant ($p>.10$).

In general, the changes in the rate of production were in good agreement with the changes in VFA concentrations. The only exception was butyrate, in which there was a trend for decrease in the concentration despite the lack of change in the production rates. The reasons for the results for butyrate are unclear. One possibility would be a change in rumen volume. If the rate of production of butyrate is unchanged but the rumen volume is increased by the

TABLE 4. Effects of teichomycin A2 and avoparcin on propionate production, rumen volume, and rumen fluid turnover rate in sheep fed high and low roughage diets[a]

Variable	High Roughage				Low Roughage			
	Control	TE-A2	AVO	SEM[d]	Control	TE-A2	AVO	SEM[d]
Propionate								
Pool size, moles	0.12	0.16	0.14	0.02	0.13[b]	0.17[bc]	0.21[c]	0.03
Turnover time, min	136.8	123.5	115.2	9.73	121.7[b]	119.8[b]	114.4[c]	7.12
Production,moles/day	1.24[b]	1.84[c]	1.72[c]	0.12	1.55[b]	2.09[c]	2.12[c]	0.14
Rumen fluid								
Volume, liters	4.4	5.1	4.7	0.36	3.5	3.9	4.4	0.42
Turnover time, hr	9.3	8.4	10.1	1.24	15.2	13.4	14.3	0.86
Turnover rate, %/hr	10.8	12.1	9.1	0.97	6.4	7.3	6.8	0.74

[a]4 observations per mean.

[bc]Means in a row within a ration without a common superscript differ ($p<.10$).

[d]Standard error of each mean in a row within a ration.

treatments, a tendency would exist for lower concentrations of butyrate in the rumen. Increases in rumen volume have in fact been reported for avoparcin (Froetschel et al., 1983) and for monensin (Lamenager et al., 1978; Prange et al., 1978). However, such an effect would necessarily result in decreased concentrations for the other acids as well, and this was not observed in the present study.

One of the main objectives of the present study was to determine if the changes in rumen VFA concentrations elicited by teichomycin A2 (Phillips and Tadman, 1980; Brondani, 1983) and by avoparcin (Johnson et al., 1979; Chalupa et al., 1981; Froetschel et al., 1983) could be explained on the basis of changes in production rates. Changes in VFA found in the present study followed similar trends for both glycopeptides. In general, supplementation with teichomycin A2 and avoparcin resulted in significant increases in propionate concentrations and production rates. These results are in line with previous reports for teichomycin A2 (Phillips and Tadman, 1980; Brondani, 1983) and for avoparcin (Johnson et al., 1979; Chalupa et al., 1981; Froetschel et al., 1983; Lindsey et al., 1985). Acetate and butyrate, on the other hand, failed to show significant changes despite the trends toward lower concentrations and production rates. This is in sharp contrast with results of rumen metabolism studies in both sheep (Froetschel et al., 1983) and cattle (Chalupa et al., 1981). Froetschel et al. (1983) reported higher propionate but lower acetate, butyrate, and total VFA concentrations in

TABLE 5. Effects of teichomycin A2 and avoparcin on butyrate production, rumen volume, and rumen fluid turnover rate in sheep fed high and low roughage diets[ab]

Variable	High Roughage				Low Roughage			
	Control	TE-A2	AVO	SEM[c]	Control	TE-A2	AVO	SEM[c]
Butyrate								
Pool size, moles	0.051	0.044	0.047	0.004	0.067	0.071	0.059	0.003
Turnover time, min	150.6	138.9	143.8	13.1	213.2	214.6	199.3	17.5
Production, moles/day	0.475	0.453	0.461	0.06	0.443	0.469	0.429	0.09
Rumen fluid								
Volume, liters	5.4	4.8	5.1	0.49	4.3	5.1	3.8	0.39
Turnover time, hr	8.1	8.5	9.2	1.19	12.2	14.4	12.9	0.96
Turnover rate, %/hr	12.1	11.4	10.2	0.97	8.5	6.8	7.5	0.71

[a]4 observations per mean.

[b]Effects of treatment are not significant in either ration ($p>.10$).

[c]Standard error of each mean in the same row within a ration.

sheep fed high and low fiber diets supplemented with avoparcin. Chalupa et al. (1981) reported that avoparcin supplementation to cattle increased propionate and butyrate and decreased acetate. Total VFA concentrations were not affected by the antibiotic. The changes in VFA patterns found in the present study, however, are in line with those obtained in feedlot trials (Johnson et al., 1979; Dyer et al., 1980). Under those conditions, avoparcin consistently increased propionate without affecting significantly acetate or butyrate concentrations.

The reasons for such discrepancies are unclear. Evidently the conditions under which each experiment was carried out may have affected the results. Differences in diet composition, time of exposure to the antibiotic, and type of animal all must have contributed to the differences. However, based on the mode of action of glycopeptides on rumen microorganisms (Stewart et al., 1983) it would be expected that such differences for acetate and butyrate would be in magnitude rather than in trend. One point to be considered is the time required for the patterns of rumen VFA to return to baseline values when feeding of antibiotics is dicontinued. While return to baseline following withdrawal seems to be almost instantaneous when ionophores are fed (W.G. Bergen, personal communication), the effects of glycopeptides seem to be more persistent (Phillips and Tadman, 1980; Brondani, 1983). In the present study, the persistency of glycopeptide effects on rumen VFA following

withdrawal were checked. Ten days after glycopeptide supplementation had been discontinued, rumen VFA were measured in animals fed the high roughage diet. Molar percent distribution of rumen VFA in animals previously belonging to control, teichomycin A2 and avoparcin groups were as follows: acetate, 69.2, 64.0, and 66.4 ($p>.10$); propionate 18.9, 23.7, and 22.5 ($p<.10$); butyrate, 7.9, 7.3, and 7.1 ($p>.10$). These results question the validity of using experimental designs such as latin squares or reversal designs to study the effects of glycopeptides on rumen metabolism. If these designs are to be used, it is absolutely crucial that long adaptation periods to the new treatment be observed to avoid carryover of residual effects from one period to the next.

It is possible, therefore, that occurrence of carryover could explain the differences in results between the present study and those of Froetschel et al. (1983). In the study by Froetschel et al. (1983), the design used was a 4x4 latin square, with sheep and period serving as blocking criteria. Despite the fact that each treatment period lasted for 28 days, measurements started on the 10th day of each period. This might have affected the VFA concentration results. In the same study (Froetschel et al., 1983), propionate production rates were measured at the end of each period (days 21 and 25). The authors reported an increase in propionate production due to avoparcin. This is in line with the results of the present study.

Table 6 summarizes the effects of teichomycin A2 and avoparcin on rumen nitrogen constituents in sheep fed high and low fiber diets. Soluble protein, bacterial protein, and protozoa protein fractions were not affected by the treatments ($p>.10$). Values for the protozoal protein fraction were markedly different between the two rations. Mean protozoal protein concentrations were 66.2 and 250.1 mg/dl respectively for high and low roughage diets. Several factors may explain this difference (Brondani, 1983). The first possibility involves the rumen fluid fractionation process. Since the protozoa remain primarily associated with coarse feed particles, a possibility exists that most of the protozoa would be discarded with the feed residue. The resulting smaller pellet would therefore underestimate the protozoa protein fraction for the high roughage ration.

The second possibility is related to the fact that the proportion of protozoa to bacteria in the rumen fluid is a function of the composition of the diet (Coleman, 1975). Eadie et al. (1970) reported a protozoa to bacteria ratio of 4:1, on a volume basis, for animals on a high grain ration. In animals receiving a high roughage diet, however, this ratio was only 1:1. In the present study, differences in protozoal protein concentration between the two diets are in good agreement with the four-fold difference in protozoa numbers favoring the low roughage diet reported by Eadie et al. (1970). A third possibility is related to the mechanism of protein degradation by protozoa. In these organisms,

TABLE 6: Effects of teichomycin A2 and avoparcin on rumen nitrogen constituents in sheep fed high and low roughage diets (mg/dl)[a]

Variable	High Roughage				Low Roughage			
	Control	TE-A2	AVO	SEM[f]	Control	TE-A2	AVO	SEM[f]
Soluble protein	46.19	52.1	39.43	4.76	37.09	44.03	49.12	4.11
Bacterial protein	57.29	64.8	61.20	5.12	63.14	54.28	63.19	4.63
Protozoal protein	61.34	72.13	65.23	6.17	265.19	234.29	251.12	19.25
Alpha-amino-N	2.94[b]	5.34[c]	6.41[c]	0.48	3.19[d]	4.81[e]	4.74[e]	0.42
Ammonia-N	16.59[b]	13.21[bc]	11.19[c]	1.19	17.35[b]	10.24[c]	11.86[c]	1.28

[a] 36 observations per mean.

[bc] Means in a row within a ration without a common superscript differ ($p<.05$).

[de] Means in a row within a ration without a common superscript differ ($p<.05$).

[f] Standard error of each mean in a row within a ration.

protein degradation takes place within the cell, requiring that small feed particles be absorbed before protein degradation can occur (Coleman, 1975). Since the particle size of the low roughage ration was much smaller it is possible that the protozoal protein fraction from animals on that diet included both protozoal and feed protein.

The alpha-amino-N and ammonia-N fractions were significantly affected by glycopeptide supplementation in both diets. In the high roughage ration, increases in alpha-amino-N relative to control were 81.6% ($p<.05$) and 118% ($p<.05$) respectively for teichomycin A2 and avoparcin. In the low roughage diet, teichomycin A2 and avoparcin increased alpha-amino-N concentrations by 50.1% ($p<.05$) and 48.6% ($p<.05$), respectively. Ammonia-N in the high roughage diet was decreased by 10% ($p>.05$) and 23.8% ($p<.05$) by teichomycin A2 and avoparcin, respectively. In the low roughage diet, decreases in ammonia-N due to teichomycin A2 and avoparcin were 33.2% and 22.7%, respectively (Table 6).

The overall results for ammonia-N and alpha-amino-N indicate that teichomycin A2 and avoparcin exert a marked effect in the nitrogen metabolism in the rumen. These results are in good agreement with *in vitro* results for teichomycin A2 (Brondani, 1983) and *in vivo* results for avoparcin (Chalupa *et al.*, 1981; Froetschel *et al.*, 1983). The decrease in ammonia-N concentration concomitantly with the increase in the alpha-amino nitrogen suggest that teichomycin A2 and avoparcin decrease deamination.

Additional evidence is provided by the decline in the concentration of isoacids in treated animals (Table 1). Although some rumen bacterial species are able to synthesize carbon skeletons de novo (Bryant and Doestch, 1955; Miura et al., 1980), the majority of isoacids in the rumen come from the degradation of dietary protein. Therefore, the decline in concentration of isoacids suggests that amino acid breakdown was depressed by the glycopeptides. The mechanism by which this is accomplished is not clear. Because utilization of most amino acids occurs intracellularly, it has been proposed that prevention of transport into bacterial cells might be the process involved (Stuart et al., 1977). This mechanism has in fact been associated with the action of diaryliodonium chemicals, which also suppress ruminal degradation of amino acids (Chalupa, 1976; Broderick and Balthrop, 1979). However, because of the mode of action of glycopeptides on bacterial cells, a mechanism involving a selective effect against specific microbial species (Stewart et al., 1983) would appear more plausible.

Results of this study have confirmed in vitro results indicating the striking similarity between avoparcin and teichomycin A2 in their ability to alter the metabolism of carbon and nitrogen in the rumen. Both compounds increase propionate production but do not have a major effect on acetate and butyrate production rates. Additionally, both glycopeptides increase alpha-amino-N and decrease ammonia-N concentrations in rumen fluid, suggesting a depressing

effect of these compounds on protein and/or amino acid degradation in the rumen. Effects of glycopeptides were more pronounced in the high roughage ration.

IV. Effects of isoacids, urea, and sulfur on rumen fermentation in sheep fed high fiber diets.

Introduction

The ruminal fermentation is a coupled process between carbohydrate degradation and microbial cell synthesis (Bergen, 1979). Ammonia-N and other factors such as carbon skeletons and sulfur are required for this process. A deficiency in any of these substrates will decrease the efficiency of microbial growth and consequently reduce the availability of volatile fatty acids and microbial protein to the host animal (Bergen and Yokoyama, 1977). Therefore, a major concern in ruminant nutrition is to define the nutrients required by rumen microorganisms for maximum fermentation of feedstuffs, particularly of low protein, highly fibrous plant material (Cook, 1985).

There is a considerable amount of information in the literature indicating the advantages of supplementing isoacids, urea, and sulfur to ruminants (for reviews, see Bray and Till, 1975; Huber and Kung, 1981; Orskov, 1982; Cook, 1985). However, very little information is available concerning the interaction among those supplements, particularly when high fiber diets are fed.

The present study was designed to evaluate the effects isoacids, urea, and sulfur, fed alone or in combination, on the rate of rumen fermentation in sheep fed high fiber diets. By varying the level of each supplement in the diet, it was possible to obtain different concentrations of

isoacids, ammonia-N, and hydrogen sulfide in the rumen fluid. The rate of acetate production, measured by a radioisotope technique, was taken as a measure of the rate of rumen fermentation (Cook, 1966; Davis, 1967; Rogers and Davis, 1981). The changes in acetate production rates as affected by the interaction between the three main factors are discussed.

Materials and methods

Two trials were carried out. The experimental design for both trials consisted of a 2x2x2 factorial crossover conducted in two 4x4 quasi-latin squares (Gill, 1978b). Double blocking criteria were animals and time (non-random repeated measurements were obtained from each subject assigned to a sequence of treatment combinations). In each trial, 8 rumen-cannulated adult sheep were used. Animals were divided in two groups of four animals according to body weight, and randomly assigned to rows of one square, corresponding to a predetermined sequence of tratment combinations (Gill, 1978b).

Composition of the basal diets for trials 1 and 2 is given in Table 7. In each trial, eight different rations were prepared from the basal diet, to provide combinations of isoacids, nitrogen, and sulfur, each at two levels.

TABLE 7. Composition and analysis of basal diets[a]

Item	Trial 1	Trial 2
Ingredient, %		
Corn stover (IFN 1-02-776)	----	25.0
Corn cobs (IFN 1-02-782)	----	25.0
Sugarcane bagasse (IFN 1-04-686)	44.0	----
Sorghum grain (IFN 4-08-139)	55.0	49.0
Bone meal (IFN 6-00-400)	0.5	0.5
Trace mineral salt[b]	0.5	0.5
Analysis[c]		
Crude protein, %	7.22	7.71
Crude fiber, %	22.0	18.2
Digestible energy, Mcal/kg	2.57	2.89
Total nitrogen, %	1.155	1.233
Total sulphur, %	0.144	0.181
N/S	8.0	6.81

[a]Dry basis.
[b]TMS containing a guaranteed minimun of: 0.35% Zn, 0.20% Mn, 0.20% Fe, 0.03% Cu, 0.005% Co, 0.007% I, and 96% NaCl.
[c]Calculated NRC values.

Based on results of previous experiments (Felix, 1976; Quispe-Salas, 1982), isoacids were administered at 0.1 (ISO1) and 0.2 (ISO2) g/kg bw/day. In order to achieve two levels of ammonia in the rumen (about 5 and 15 mg/dl), the basal ration was fed either alone or supplemented with urea at 1.5% of the dry matter. Sulfur supplementation was designed to provide four different nitrogen to sulfur ratios (Bray and Hemsley, 1969, Bray and Till, 1975; Orskov, 1982). Elemental sulfur at 0.2% of the dry matter was used as supplement. Final N:S ratios were aproximately 3:1, 5:1, 8:1, and 12:1 (Quispe-Salas, 1982). Isoacids, urea and sulfur were first pre-mixed with part of the sorghun and

then incorporated into the totally mixed ration. Daily ration was offered at 0700 hours and feed intake was recorded daily for each animal. Water was provided ad libitum. Animals were adapted to a given diet for 14 days prior to measurements.

In vivo production rates of acetate were measured by a single injection radioisotope technique. On day 15, three hours after the morning feeding, each animal received an intraruminal injection of 100 uCi of Na-[1-C14]acetate, along with 100 ml of a 10% polyethylene glycol solution (PEG, M.W. 4000, Sigma Chemical Company). To ensure a more homogeneous distribution, solutions were infused at several different locations in the rumen. This was performed with the aid of a dosing syringe attached to a perforated plastic tube (15 mm dia.x 30.5 cm long). A similar device was used for collection of rumen fluid samples.

Following infusion, rumen fluid samples were collected every 20 minutes for the next three hours. Samples were strained through four layers of surgical gauze, immediately acidified with 50% sulfuric acid, frozen at -20^{o}C and stored for subsequent analyses. Concentrations of hydrogen sulfide in the rumen were determined as described by Quispe-Salas (1982). Procedures for the determination of acetate production, rumen fluid dilution rates, rumen ammonia-N and concentrations of rumen VFA were described in chapter III.

Overall significance of treatment effects was determined by analysis of variance (Gill, 1978a; 1978b; 1978c) according to the model in (II):

(II) $Y_{ijklmn} = u + O_i + P_{(i)j} + G_{(i)k} + A_l + B_m + (AB)_{lm} +$

$+ C_n + (AC)_{ln} + (BC)_{mn} + (ABC)_{lmn} + E_{(ijklmn)}$

where,

Y_{ijklmn} is the observed value for the jth sheep within the ith square under the lth level of urea, the mth level of isoacids, and the nth level of sulfur during the kth period;

u is the overall mean;

O_i is the fixed effect of the ith square;

$P_{(i)j}$ is the random effect of the jth sheep within the ith square;

$G_{(i)k}$ is the fixed effect of the kth period within the ith square;

A_l is the fixed effect of the lth level of urea;

B_m is the fixed effect of the mth level of isoacids;

C_n is the fixed effect of the nth level of sulfur;

$(AB)_{lm}$ is the fixed effect of the interaction between level of urea and level of isoacids;

$(AC)_{ln}$ is the fixed effect of the interaction between level of urea and level of sulfur;

$(BC)_{mn}$ is the fixed effect of the interaction between level of isoacids and level of sulfur;

$(ABC)_{lmn}$ is the fixed effect of the interaction among level of urea, level of isoacids, and level of sulfur; in this case, non-separable from the effect of squares;

$E_{(ijklmn)}$ is the random residual error.

Specific differences between treatment means within each two-way interaction were determined by Bonferroni-t tests (Gill, 1978a; 1978b).

Results and discussion

The objective of these studies was to evaluate the effects of isoacids, urea, and sulfur on rumen fermentation in sheep fed high fiber diets. Results are presented according to the effect of the three two-way interactions (Gill, 1978b), i.e, the interactions between urea and sulfur, urea and isoacids, and between isoacids and sulfur.

Rumen volumes and rumen fluid turnover rates were not affected by treatments in either trial. Mean rumen fluid volumes averaged across treatments and respective standard errors were 3.2 (0.29) and 3.8 (0.26) liters, respectively for trials 1 and 2. Mean rumen fluid turnover rates for trials 1 and 2 were 13.1 (0.74) and 11.8 (0.81) percent/hour, respectively.

The effects of urea and sulfur on feed intake, rumen ammonia-N, rumen sulfide-S and acetate production rates are presented in Table 8. Average daily dry matter intake did not differ among treatment groups in either trial ($p>.10$). Addition of urea increased the concentrations of rumen ammonia-N in both trials ($p<.01$). Similarly, sulfur supplementation resulted in higher levels of sulfide sulfur in rumen fluid for both trials ($p<.01$). This was expected, since urea is readily converted to ammonia and inorganic sulfur to sulfide by the bacterial enzyme systems (Bray and Hemsley, 1969). In trial 1, the rate of acetate production was not changed by either urea or sulfur when the two

TABLE 8. Effects of urea and sulfur on feed intake and rumen fermentation variables in sheep fed high fiber diets

Variable	Trial 1					Trial 2				
	Control	Urea (U)	Sulfur (S)	U + S	SEM[g]	Control	Urea (U)	Sulfur (S)	U + S	SEM[g]
Dry matter intake, g/day[a]	387	454	442	462	16.5	430	412	461	442	14.9
Nitrogen intake, g/day[ac]	4.47	8.23	5.11	8.38	0.49	5.44	7.92	5.83	8.50	0.33
Sulfur intake, g/day[ad]	0.55	0.65	1.63	1.66	0.13	0.77	0.74	1.83	1.80	0.11
N/S	8.1	12.7	3.1	5.0	----	7.0	10.7	3.2	4.7	----
Ammonia-N[bc]	6.11	12.19	5.14	13.11	0.84	5.28	14.19	6.93	13.11	0.71
Sulfide-S[bd]	2.43	2.16	5.84	5.51	0.33	2.97	3.34	5.26	4.81	0.28
Acetate[a]										
Pool size, moles	0.24	0.22	0.25	0.31	0.02	0.27	0.29	0.26	0.35	0.03
Turnover time, min	156.4	161.2	164.2	144.7	6.1	169.4	151.2	166.8	141.6	8.7
Production, moles/day	2.18[e]	1.94[e]	2.25[e]	3.16[f]	0.24	2.24[e]	2.82[e]	2.31[e]	3.67[f]	0.21

[a]8 observations per mean.
[b]64 observations per mean.
[c]Significant effect of nitrogen ($p<.01$).
[d]Significant effect of sulfur ($p<.01$).
[ef]Means in a row within a trial without a common superscript differ ($p<.10$).
[g]Standard error of each mean in a row within a trial.

factors were fed separately. Supplementation of the two factors combined, however, resulted in 44% increase in the rate of acetate production relative to the control group ($p<.10$). In trial 2, there was a tendency for increased rates of acetate production by the addition of either urea or sulfur. However differences relative to control were not significant ($p>.10$). When the two factors were fed in combination, acetate production rates relative to the other groups increased ($p<.10$).

While the lack of response in the sulfur group can be explained on the basis of the low level of rumen ammonia-N (Table 8), the results in the urea group were somewhat unexpected. The nitrogen to sulfur ratio (Table 8) and the percentage of sulfur in the basal diets (Table 7) were within the values commonly recommended for adult sheep (Bray and Hemsley, 1969; Bray and Till, 1975; Orskov, 1982). It seems that the total sulfur intake for the animals in the urea group may have been limiting. Hume and Bird (1969) reported that a total sulphur intake of 1.95 g/day supported maximum rumen microbial protein synthesis in sheep. In the present study, intake of total sulfur in the urea group averaged 0.65 g/day and 0.74 g/day, respectively for trials 1 and 2. For the Urea + Sulfur group, however, average daily intake of total sulfur for trials 1 and 2 were 1.66 and 1.80, respectively. Apparently the sulfide-S concentrations elicited by the low sulfur intake were limiting. The precise level at which rumen sulfide concentration limits microbial growth or fermentation has

not been clearly defined (Bray and Till, 1975), but a limiting level of 1 ug sulfide-S/ml of rumen fluid has been suggested (Harrison and McAllan, 1980). In the present study, sulfide-S concentrations in the urea group were at least twice that value, but evidently they were not high enough to allow for maximun fermentation. These results clearly indicate that sulfur supplementation based solely on the nitrogen to sulfur ratio or on the percentage of sulfur in dry matter is not adequate when high fiber diets containing urea are fed. The total amount of sulfur that would allow for maximum microbial protein synthesis should be considered first. Once that is provided, supplementing urea to attain a N/S ratio of about 10 (Orskov, 1981) should result in maximun fermentation efficiency.

Effects of urea and isoacids on feed intake, ammonia-N and total isoacid concentrations, and on the rate of acetate production are shown in Table 9. Concentrations of ammonia-N were significantly increased by the addition of urea in trial 1 ($p<.01$) and trial 2 ($p<.01$). Similarly, increasing the level of isoacids in the diet resulted in higher concentration of these acids in the rumen in both trials ($p<.01$).

In trial 1, the rate of acetate production was higher when urea was supplemented along with isoacids. Increasing the amount of supplemental isoacids in the diet (ISO1 vs ISO2) did not affect the rate of acetate production ($p>.10$). However, urea supplementation to the low level of isoacids

TABLE 9. Effects of isoacids and urea on feed intake and on rumen fermentation variables in sheep fed high fiber diets

Variable	Trial 1					Trial 2				
	ISO1	ISO1-U	ISO2	ISO2-U	SEM[h]	ISO1	ISO1-U	ISO2	ISO2-U	SEM[h]
Dry matter intake, g/day[a]	384	421	438	417	16.2	431	456	471	448	14.9
Ammonia-N[bc]	6.82	15.1	7.14	14.36	0.64	8.31	17.11	7.34	15.21	0.71
Total isoacids[bd]	0.28	0.29	0.49	0.53	0.03	0.26	0.28	0.47	0.48	0.02
Acetate[a]										
Pool size, moles	0.18[e]	0.24[e]	0.19[e]	0.29[f]	0.01	0.24	0.27	0.26	0.33	0.03
Turnover time, min	137.9	123.5	133.8	114.7	6.12	127.8	129.1	133.4	120.6	7.65
Production, moles/day	1.91[e]	2.86[f]	1.97[e]	3.74[g]	0.17	2.64[e]	2.95[e]	2.88[e]	3.87[f]	0.21

[a]8 observations per mean.
[b]64 observations per mean.
[c]Significant effect of urea ($p<.01$).
[d]Significant effect of isoacids ($p<.01$).
[efg]Means in a row within a trial without a common superscript differ ($p<.05$).
[h]Standard error of each mean in a row within a trial.

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(ISO1-U) resulted in 49% increase in the rate of acetate production relative to ISO1 ($p<.05$). When both factors were present in the higher level (ISO2-U), acetate production was increased by 30% relative to the ISO1-U group ($p<.05$). This increase in acetate production rate was responsible for the increase in both concentration (Table 10) and pool size (Table 9) of acetate in the ISO2-U group. These results clearly illustrate the fact that rates of fermentation in the rumen can be only as high as the availability of the most limiting nutrient. In trial 1, ammonia-N provided by the basal diet was more limiting than isoacids. Once nitrogen supply was corrected by the addition of urea to the diet, availability of carbon skeletons for amino acid synthesis became limiting. Evidence for this assertion is given by the further increase in acetate production in the ISO2-U group relative to the ISO1-U group.

Trends in acetate production found in trial 2 were basicaly the same as in trial 1. However, significant increases in acetate production were found only in the ISO2-U treatment ($p<.05$). Apparently the levels of ammonia-N provided by the basal diet in trial 2 (Table) was sufficient to support microbial growth as efficiently as the one elicited by urea (ISO1-U) supplementation (Huber and Kung, 1981; Orskov, 1982). It was not sufficiently high, however, to allow the utilization of the higher amounts of isoacids supplied by the ISO2 treatment.

The increases in the rate of acetate production were accompanied by changes in concentrations of individual VFA

TABLE 10. Effects of isoacids and urea on concentrations of rumen volatile fatty acids in sheep fed high fiber diets (mmoles/dl)[a]

Variable	Trial 1					Trial 2				
	ISO1	ISO1-U	ISO2	ISO2-U	SEM[e]	ISO1	ISO1-U	ISO2	ISO2-U	SEM[e]
Acetate	4.51[c]	5.43[c]	4.79[c]	6.83[d]	0.28	5.48[c]	5.92[cd]	6.07[cd]	7.12[d]	0.31
Propionate	1.16	1.36	1.22	1.55	0.12	1.41	1.53	1.51	1.61	0.11
Isobutyrate[b]	0.08	0.09	0.14	0.15	0.006	0.06	0.09	0.12	0.14	0.002
Butyrate	0.43	0.47	0.44	0.51	0.04	0.51	0.50	0.52	0.54	0.05
2-M-Butyrate[b]	0.06	0.05	0.10	0.09	0.005	0.07	0.05	0.08	0.10	0.004
Isovalerate[b]	0.06	0.07	0.11	0.13	0.006	0.06	0.07	0.12	0.10	0.005
Valerate[b]	0.08	0.07	0.14	0.16	0.008	0.08	0.07	0.15	0.14	0.006
Total VFA	6.38[c]	7.54[c]	6.94[c]	9.42[d]	0.54	7.68[c]	8.24[cd]	8.58[cd]	9.75[d]	0.41
Total isoacids[b]	0.28	0.29	0.49	0.53	0.03	0.26	0.28	0.47	0.48	0.02

[a]64 observations per mean.
[cd]Means in a row within a trial without a common superscript differ ($p<.05$).
[b]Significant effect of isoacids ($p<.01$).
[e]Standard error of each mean in a row within a trial.

TABLE 11. Effects of isoacids and urea on the molar percent distribution of rumen volatile fatty acids in sheep fed high fiber diets[a]

Variable	Trial 1					Trial 2				
	ISO1	ISO1-U	ISO2	ISO2-U	SEM[f]	ISO1	ISO1-U	ISO2	ISO2-U	SEM[f]
Acetate	70.6[b]	72.0[bc]	69.1[b]	72.6[c]	0.51	71.3[b]	71.8[b]	70.6[b]	73.0[c]	0.41
Propionate	18.6[d]	18.9[d]	17.6[e]	16.4[e]	0.28	18.4[d]	18.6[d]	17.6[e]	16.7[e]	0.29
Isobutyrate[b]	1.2	1.2	2.1	1.8	0.02	0.8	1.1	1.5	1.6	0.03
Butyrate	6.6	6.3	6.3	5.4	0.10	6.7	6.0	6.2	5.5	0.13
2-M-Butyrate[b]	1.0	0.7	1.5	1.2	0.03	0.9	0.6	1.0	1.1	0.02
Isovalerate[b]	1.0	0.9	1.8	1.6	0.04	0.8	0.9	1.5	1.1	0.03
Valerate[b]	1.2	1.0	2.1	2.0	0.05	1.0	0.9	1.8	1.5	0.04
A:P ratio	3.9	4.0	3.9	4.4	0.16	3.9	3.9	4.0	4.4	0.14

[a]64 observations per mean.
[b]Significant effect of isoacids ($p<.05$).
[cd]Means in a row within a trial without a common superscript differ ($p<.10$).
[de]Means in a row within a trial without a common superscript differ ($p<.05$).
[f]Standard error of each mean in a row within a trial.

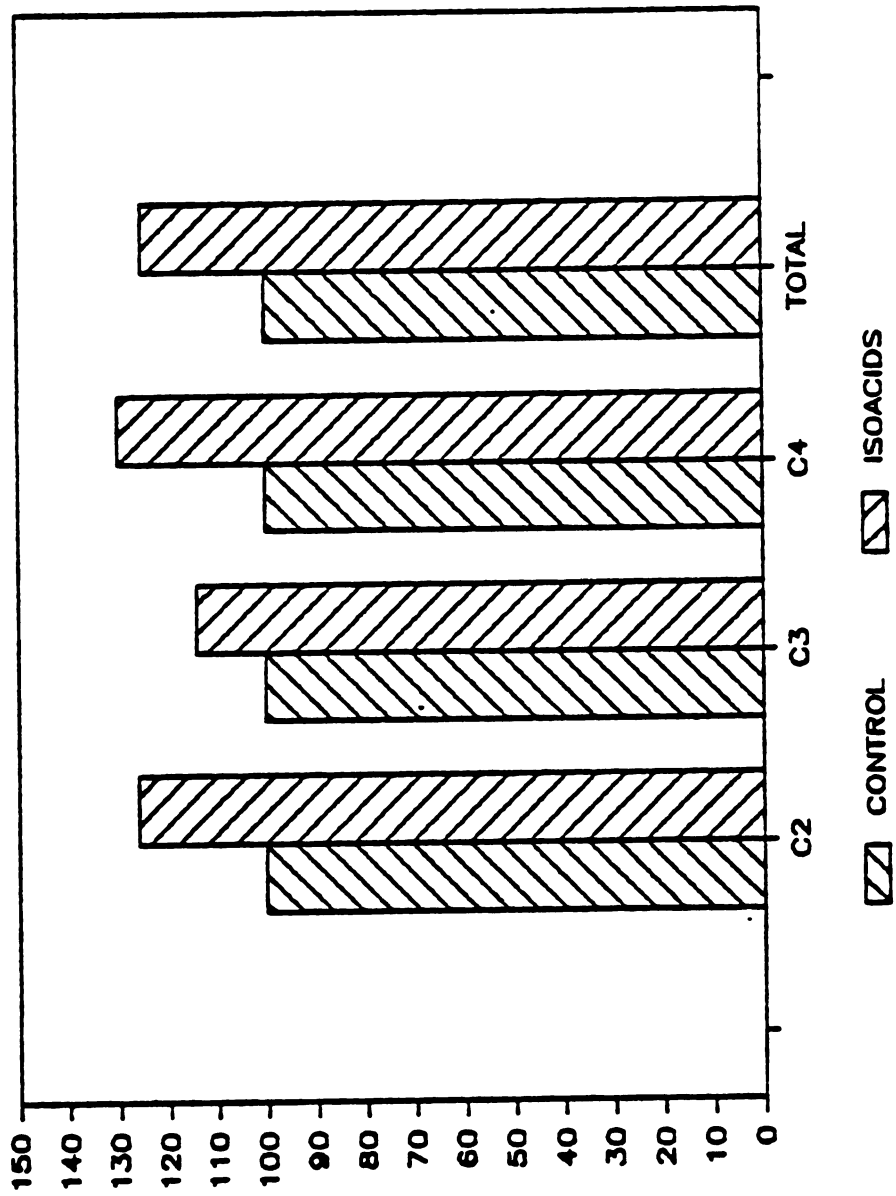

Figure 1. Effects of isoacids on rumen VFA in sheep fed high fiber, high NPN diets. Values are relative concentrations with respect to control (control=100).

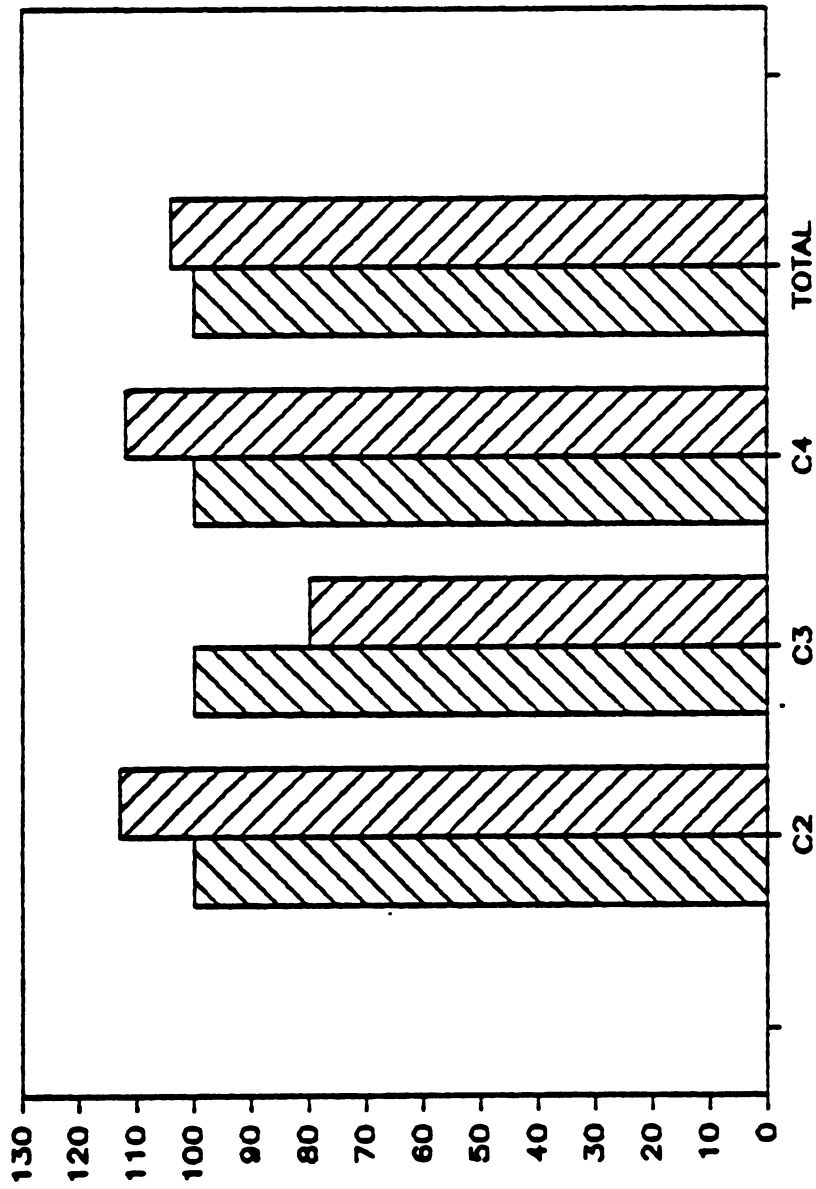

Figure 2. Effects of isoacids on rumen VFA in sheep fed diets containing preformed protein. Values are relative concentrations with respect to control (control=100).

in the rumen (Table 10). The overall increase in fermentation in the ISO2-U group in both trials resulted in higher concentrations for both acetate and propionate. On a molar percent basis, however, this increase favored acetate (Table 11). These results plus the results reported in Chapter V (Table 13) indicate that the effects of isoacids on VFA production has two components. In diets low in preformed protein, where the contribution by the diet to the ruminal isoacid pool is small, addition of isoacids will result in an increase in the production of all major VFA and consequently of total VFA (Table 10). In this situation, changes in the acetate to propionate ratios are less pronounced (Table 11). Similar results have been obtained *in vivo* (Quispe-Salas, 1982) and *in vitro* (Machado *et al.*, 1985). When preformed protein is fed, on the other hand, the increases in total VFA due to isoacid supplementation are usually small (Hume, 1970; Oltjen et al, 1971; Felix, 1976). However, because isoacids select for cellulolytic species of bacteria (Van Gylswyk, 1969; Oltjen *et al.*, 1971), addition of these acids to the diet would tend to divert the flux of carbon towards acetate production, decreasing production of propionate. This is evident from the results shown in Table 13. Addition of isoacids increased acetate and decreased propionate but did not change significantly the concentrations of total VFA in sheep fed an all-alfalfa diet. Similar shifts have been reported when isoacids were added to diets with low fiber

TABLE 12. Effects of isoacids and sulfur on feed intake and on rumen fermentation variables in sheep fed high fiber diets

Variable	Trial 1					Trial 2				
	ISO1	ISO1-S	ISO2	ISO2-S	SEM[e]	ISO1	ISO1-S	ISO2	ISO2-S	SEM[e]
Dry matter intake, g/day[a]	454	471	419	429	19.2	412	426	452	464	18.3
Sulfide-S[bc]	4.13	7.84	3.19	6.31	0.43	3.29	5.63	4.09	6.51	0.35
Total isoacids[bd]	0.31	0.27	0.39	0.42	0.06	0.33	0.34	0.43	0.45	0.04
Acetate[a]										
Pool size,moles	0.21	0.18	0.23	0.25	0.03	0.24	0.27	0.28	0.26	0.02
Turnover time,min	123.1	115.3	126.9	128.4	6.17	131.7	137.4	128.1	124.2	8.12
Production, moles/day	2.43	2.27	2.58	2.73	0.19	2.64	2.86	3.11	3.06	0.24

[a]8 observations per mean.

[b]64 observations per mean.

[c]Significant effect of sulfur ($p<.01$).

[d]Significant effect of isoacids ($p<.01$).

[e]Standard error of each mean in a row within a trial.

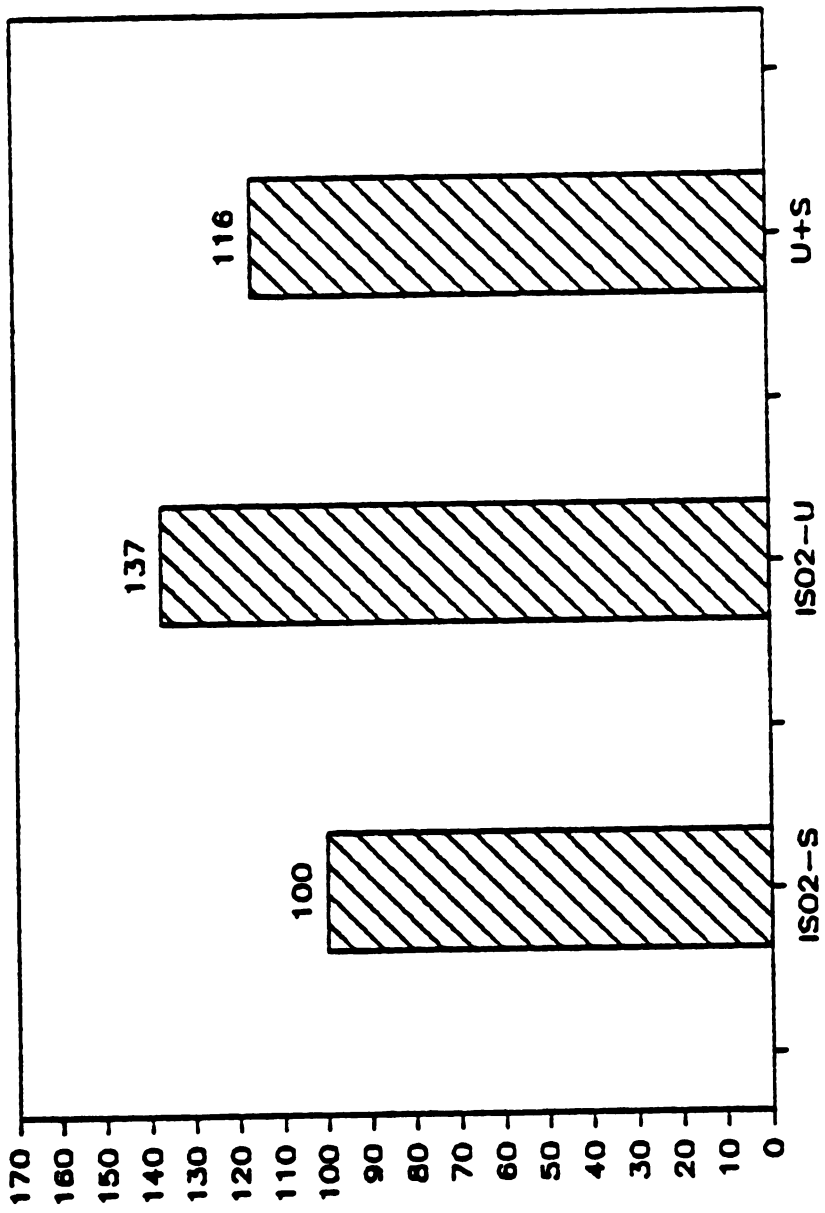

Figure 3. Acetate production rates as affected by isoacids + sulfur (ISO2-S), isoacids + urea (ISO2-U), or urea + sulfur (U + S) in sheep fed high fiber diets (Trial 1). Values are relative rates of production (ISO2-S=100).

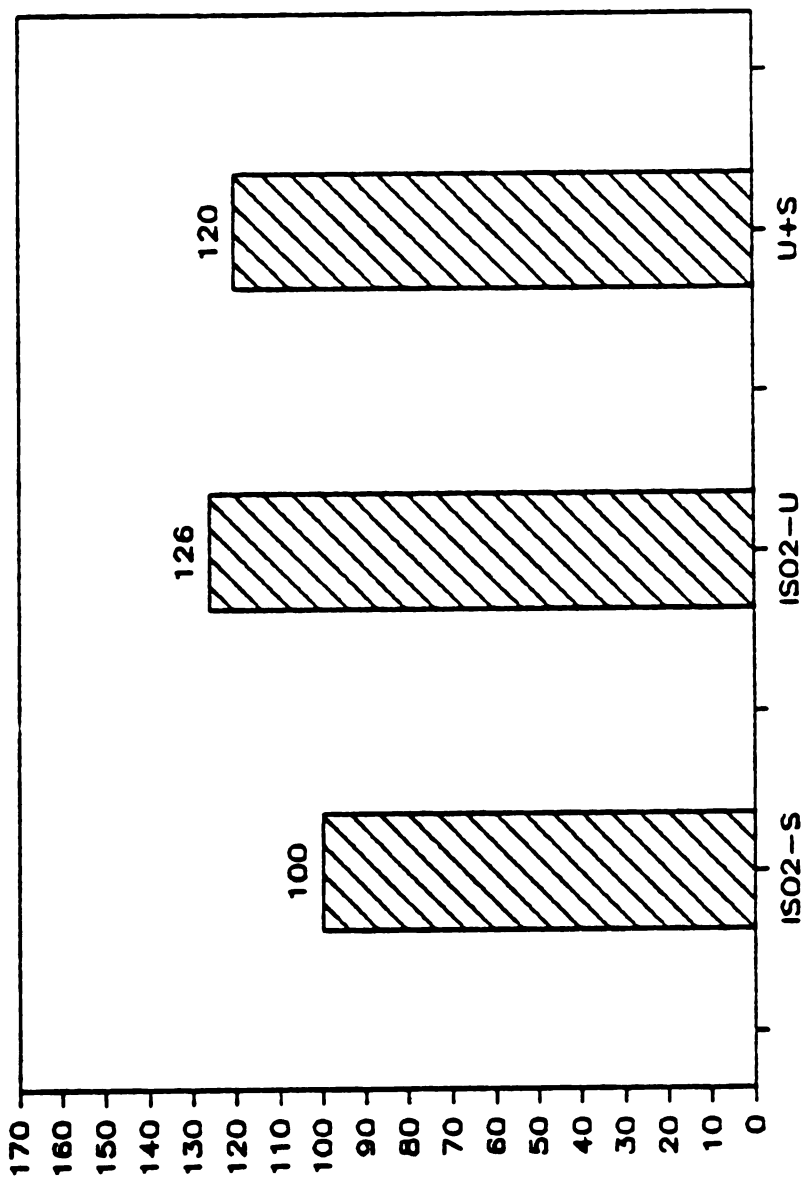

Figure 4. Acetate production rates as affected by isoacids + sulfur (ISO2-S), isoacids + urea (ISO2-U), or urea + sulfur (U + S) in sheep fed high fiber diets (Trial 2). Values are relative rates of production (ISO2-S=100).

(Oltjen et al., 1971; Felix, 1976) and high fiber (Hume, 1970). The overall effects of isoacids on rumen VFA as related to the source of dietary nitrogen is summarized in Figure 1 (NPN) and Figure 2 (preformed protein). The possible implications of the shifts in rumen VFA with respect to the intermediary metabolism of the host animal are adressed in Chapter V.

Effects of isoacids and sulfur on feed intake, total isoacids, sulfide-S concentrations and rate of acetate production are summarized in Table 12. Feed intake was not affected by treatments in either trial ($p>.10$). Increasing the level of isoacids in the diet resulted in higher concentrations of these acids in the rumen ($p<.01$). Similarly, sulfide-S was higher in the treatment groups receiving elemental sulfur in the diet ($p<.01$). The rate of acetate production did not differ among treatments in either trial ($p>.10$). On the average, therefore, providing supplemental sulfur to diets containing two levels of isoacids did not improve feed utilization relative to isoacids fed alone. It should be noted, however, that acetate production values in the ISO2-S group (Table 12) were lower than those obtained with the ISO2-U group (Table 9) and Urea+Sulfur group (Table 8) in both trials. This suggests that in the ISO2-S group fermentation was being limited by the level of ammonia-N in the rumen. A summary of these results is presented in Figures 3 and 4.

As pointed out by Bergen and Yokoyama (1977), the production of VFA from carbohydrates in the rumen is coupled

with microbial growth. Maximum microbial yield can be attained only if precursors for protein synthesis are made available to the microbiota simultaneously and in adequate quantities. In this study it has been demonstrated that high fiber diets low in nitrogen are not well utilized unless urea, isoacids and sulfur are supplemented. Addition of these factors to high fiber diets should improve performance of ruminants.

V. Effects of isoacids on plasma concentrations of growth hormone, insulin, and cortisol in sheep.

Introduction

Branched-chain volatile fatty acids (isobutyric, 2-methylbutyric, isovaleric) and the straight-chain valeric acid are either required by or enhance the growth of major rumen cellulolytic bacteria (Bryant and Robinson, 1962; Dehority et al., 1967; Slyter and Weaver, 1969; Bryant, 1973). In vivo studies have demonstrated that supplementation of isoacids to ruminants positively affects growth rates (Lassiter et al., 1958; Felix et al., 1980a), and milk production (Felix et al., 1980b; Papas et al., 1984).

The mechanisms by which isoacids improve performance of ruminants are not completely understood. Changes observed in animals fed isoacids include increases in microbial protein synthesis (Hume, 1970; Russel, 1984), in dry matter digestibility (Cline et al., 1966; Soofi et al., 1962), and in nitrogen retention (Cline et al., 1966; Umunna et al., 1975; Felix, 1976).

Recent reports have indicated that lactating cows fed isoacids have lower plasma concentrations of insulin (Towns and Cook, 1984) and higher plasma concentrations of growth hormone (Towns and Cook, 1984; Fieo et al., 1984). Since low insulin and high growth hormone concentrations in plasma

have been associated with higher milk production (Hart, 1983; McDowell, 1983), it has been proposed that the changes in plasma hormone profile might further explain the positive effects of isoacids in lactating cows (Towns and Cook, 1984; Fieo et al., 1984; Cook, 1985). However, it is unclear whether the changes in plasma hormone found in lactating cows were caused by isoacids directly or were a consequence of variations in metabolic demands in the treated cows (Hart, 1983; Bauman and McCutcheon, 1986). The objective of the present study was to determine the effect of isoacids on plasma hormone concentrations in adult sheep fed at maintenance level. Changes in rumen volatile fatty acid and in plasma concentrations of growth hormone, insulin, and cortisol due to treatments are discussed.

Materials and Methods

Six crossbred ewes (average BW= 41.2 kg) were fitted with rumen cannulas and placed in individual pens in an environmentally controlled room for the entire experiment. Animals were fed 1200 g of alfalfa hay per day offered in equal portions, at 0800 and 1600 hr. The experiment was divided into three periods of two weeks each. In each period, one of the treatments was administered to all six animals. Treatments consisted of 0 (CONTROL), 0.1 (ISO1), and 0.2 (ISO2) gram of isoacids/kg bw/day. The isoacid mixtures containing equal amounts of isobutyric, 2-methylbutyric, isovaleric and valeric acids were injected

directly into the rumen through the cannulas.

At the end of each period, measurements were performed. In order to prevent stress due to excessive manipulation of the animals, rumen and blood samples were collected in separate days. On day 14 of each period, rumen fluid samples were collected at 0800 hr and then at hourly intervals for the next eight hours. After the last rumen sample, animals were fitted with jugular catheters and prepared for the next day bleeding. Blood samples were collected 30 minutes prior to morning feeding and then every 30 minutes for the next 12 hours. Preparation of rumen samples and VFA determination were performed as described in ~~in~~ Chapter III. Hormone concentrations in plasma were analized by radioimunoassay procedures. Insulin and cortisol were determined using commercial kits (Micromedic System, Inc., Horsham, PA). Determination of growth hormone was according to the procedure validated by the National Hormone and Pituitary Program. Plasma glucose was determined by the coupled system of glucose oxidase and peroxidase (Sigma Chemical Company, St. Louis, MO).

Concentrations of rumen VFA and plasma variables were averaged across the 12-hr sampling period. Means were submitted to anlysis of variance (Gill, 1978a) for the determination of overall significance. Specific differences among treatment means were tested by Bonferroni t-tests (Gill, 1978a; 1978c).

Results and discussion

Concentrations and molar percent distributions of rumen volatile fatty acids are presented in Table 13. Concentrations of isoacids in rumen fluid were higher in the isoacid-treated groups. Increases in mean total isoacid concentrations relative to control group 55.9% ($p<.001$) and 108% ($p<.001$) respectively for ISO1 and ISO2 groups (Table 13). Animals in the ISO2 group had higher acetate and lower propionate concentrations relative to control animals ($p<.10$). Similar trends were observed in animals receiving the lower levels of isoacids (ISO1). However, differences from control were not significant ($p>.10$). Aspects of the effect of isoacids on rumen VFA have been adressed in Chapter IV.

Plasma concentrations of growth hormone, insulin, and cortisol as affected by isoacids are depicted in Figures 5, 6, and 7, respectively. Results averaged across the 12-hr sampling period are summarized in Table 14. Growth hormone, cortisol, and glucose concentrations were not affected by the treatments ($p>.10$). Mean concentration of plasma insulin was 26.4% lower in animals of the ISO2 group than in the control animals ($p<.10$).

One of the most consistent responses obtained in animals treated with isoacids is increased nitrogen retention. Similar responses have been found in animals

TABLE 13. Effects of isoacids on rumen volatile fatty acids in sheep fed a high roughage diet[a]

Variable	Isoacids			
	CONTROL	ISO1	ISO2	SEM[m]
VFA, mmoles/dl				
Acetate	6.73[i]	7.04[ij]	7.55[j]	0.24
Propionate	1.95[i]	1.80[ij]	1.56[j]	0.11
Butyrate	0.75	0.71	0.84	0.08
Total VFA[b]	9.43	9.55	9.95	0.41
Total isoacids	0.34[c]	0.53[d]	0.71[e]	0.03
C2/C3	3.44[f]	3.92[fg]	4.84[g]	0.21
VFA, molar %				
Acetate	71.3[f]	73.6[fg]	75.8[g]	1.25
Propionate	20.5[f]	18.9[fg]	15.8[g]	0.92
Butyrate	7.9	7.4	8.3	0.54

[a]Animals in each group (n=6) were sampled at 0800 hr and then at hourly intervals for the next 8 hours.
[b]Isoacids are not included.
[cde]Means in a row without common superscripts differ($p<.001$)
[fg]Means in a row without common superscripts differ($p<.05$)
[ij]Means in a row without common superscripts differ($p<.10$)
[m]Standard error of each mean in the same row.

treated with the anabolic agents trenbolone (Sharpe et al., 1984) and zeranol (Sinnet-Smith et al., 1983). The increase in nitrogen retention by the anabolic agents has been associated with changes in the circulating levels of cortisol (Sharpe et al., 1984). The fact that supplementation with isoacids in the present study had no effect on plasma cortisol concentrations (Table 14, Figure 7) suggests that this hormone is not involved in the mechanism of action of isoacids in ruminants.

Plasma concentrations of growth hormone were not affected by isoacid supplementation (Figure 5, Table 14). Towns and Cook (1984) and Fieo et al. (1984) reported that lactating cows fed isoacids had higher plasma levels of growth hormone. The reasons for those changes are unclear. The primary role of growth hormone is to preserve body protein, particularly during periods of energy deficit. This is accomplished by diverting glucose and fatty acids away from tissue deposition while inhibiting proteolysis and stimulating protein incorporation into muscle (Hart, 1983; McDowell, 1983; Bauman and McCutcheon, 1986). During lactation, growth hormone plays an important role in partitioning nutrients towards milk production and away from tissue deposition (Bauman and McCutcheon, 1986). Therefore, it is difficult to establish if the changes in plasma growth hormone measured in lactating cows fed isoacids were caused by the acids directly or were a consequence of increased metabolic demands. The inverse relationship

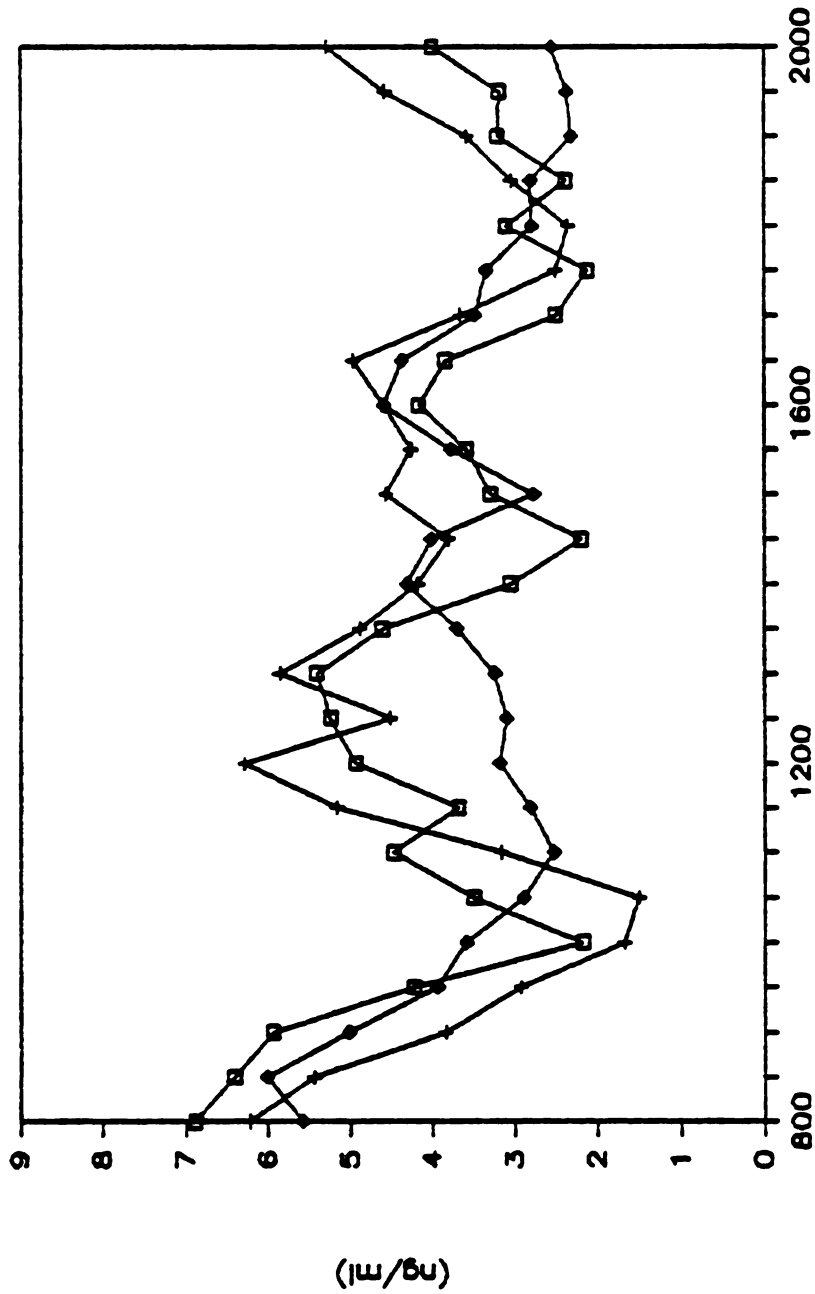

Figure 5. Effect of isoacids on plasma concentrations of growth hormone in sheep fed a high roughage diet (n=6).

between plasma levels of growth hormone and nutrient availability is well established (Hertelendy and Kipnis, 1973; Hart, 1983; McDowell, 1983). Since isoacid supplementation increases milk production in lactating cows, the higher demand for nutrients in the isoacid-treated group might have triggered a higher response in growth hormone release (Hart, 1983; McDowell, 1983). However, in one of the experiments (Fieo et al., 1984), changes in hormone concentration were found despite the lack of response in milk production.

The possibility that isoacids affected growth hormone secretion directly in the studies with lactating cows cannot be ruled out. However, it should be kept in mind that because of the almost complete removal of VFA with more than three carbons from the blood by the liver (Young, 1977; Ricks and Cook, 1981), the concentration of isoacids in peripheral blood is usually low. Therefore, a direct interaction of these acids with the pituitary would be rather unlikely. Towns and Cook (1984) suggested that the increase in plasma levels of growth hormone might be governed by a reflex mechanism mediated by chemoreceptors specific for isoacids in the rumen wall. A similar mechanism, mediated by stretch receptors, is known to participate in the inhibitory action of feeding on growth hormone secretion in goats (Tindall, 1982). In the present experiment, changes in plasma growth hormone due to feeding were detected (Figure 5), but they did not differ among treatments. It would be expected that if isoacids actually

TABLE 14. Effects of isoacids on plasma concentrations of insulin, growth hormone, cortisol, and glucose in sheep fed a high roughage diet[a]

Variable	Isoacids			SEM[d]
	CONTROL	ISO1	ISO2	
Insulin, uU/ml	24.9[b]	23.1[bc]	19.7[c]	1.36
Growth hormone, ng/ml	3.84	4.11	3.51	0.23
Cortisol, ng/ml	5.42	4.71	5.12	0.44
Glucose, mg/dl	52.6	47.8	49.3	2.11

[a]Animals in each group (n=6) were sampled at 0730 hr and then at 30-minute intervals for the next 12 hours.
[bc]Means in a row without a common superscript differ ($p<.10$)
[d]Standard error of each mean in the same row.

had a direct effect on growth hormone secretion through a reflex mechanism, changes in pattern should have been apparent.

Another point to be considered is the relationship between growth hormone, insulin, and glucose concentrations in plasma. High levels of growth hormone tend to inhibit the effect of insulin in promoting peripheral glucose utilization (McDowell, 1983) resulting in augmentation of plasma levels of insulin and glucose. In cows fed isoacids, however, this relashionship was not apparent. Towns and Cook (1984) reported that during the eight-hour sampling period, isoacid-treated cows had higher growth hormone but lower glucose and lower insulin than control cows.

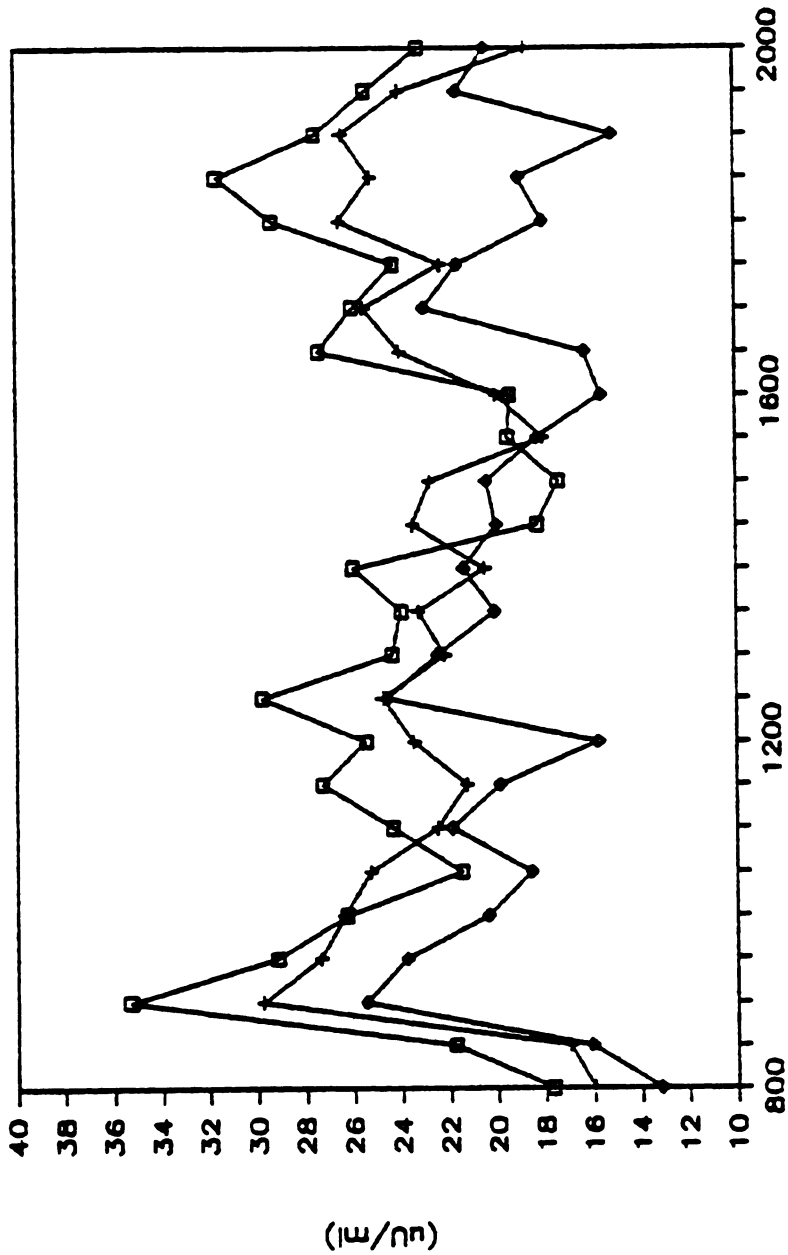

Figure 6. Effect of isoacids on plasma concentrations of insulin in sheep fed a high roughage diet (n=6).

Based on the above considerations and on the results of the present experiment, it seems that isoacids do not affect the plasma levels of growth hormone directly. The reasons for the differences in result from those of Towns and Cook (1984) and Fieo et al. (1984) cannot be elucidated at this point, indicating that further research in this area is necessary.

In additions to the changes in growth hormone, Towns and Cook (1984) reported lower levels of plasma insulin in lactating cows fed isoacids. In the present study, supplementation of 0.2 g of isoacids/kg bw/day to sheep fed an all-alfalfa ration also resulted in lower levels of plasma insulin (Table 14, Figure 6). The mechanism by which such a decrease took place is unclear. As in the case of growth hormone, the possibility that isoacids absorbed from the rumen might have a direct effect on the beta-cells of pancreas should be considered. However, because most of the VFA with more than three carbons are removed from the blood by the liver (Young, 1977; Ricks and Cook, 1981), a direct effect of isoacids is very unlikely. Arguably, a direct interaction with the islets would not be a requirement for the action of isoacids on insulin secretion. As discussed previously, the presence of specific chemoreceptors in the rumen wall or in the portal system (Leek, 1986) could allow isoacids to decrease insulin secretion through reflex mechanisms. From a physiologycal standpoint, however, the necessity for such an intervention is difficult to justify. Although there are differences in emphasis to accomodate the

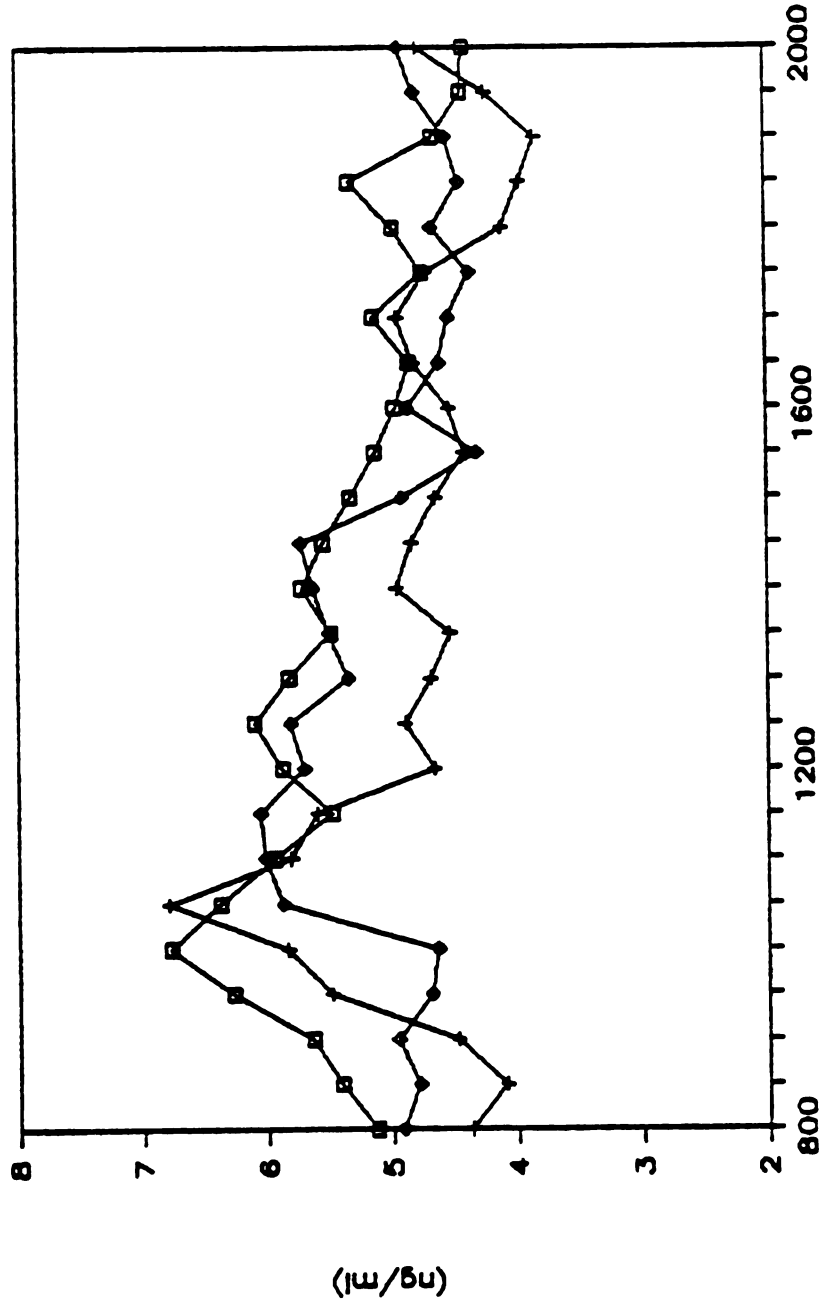

Figure 7. Effect of isoacids on plasma concentrations of cortisol in sheep fed a high roughage diet (n=6).

ruminant mode of digestion and intermediary metabolism (Basset, 1978; Hart, 1983; Brockman, 1986), insulin performs the same anabolic actions in ruminants as in nonruminants, i.e., it stimulates allocation of substrates such as fatty acids, amino acids and glucose into body tissues (Basset, 1978; Hart, 1983). The four components of the isoacid mixture used in the present study are final products of microbial fermentation in the rumen. Their concentration in rumen fluid and in portal blood are higher in the hours following a meal, coinciding with the peak in substrate availability in plasma (Huntington, 1983). It is reasonable to expect, therefore, that if isoacids were to participate in the mechanisms governing insulin secretion in ruminants, their action would be stimulatory rather than inhibitory. Support for this contention is given by studies in which isobutyrate (Ross and Kitts, 1972) or valerate (Hertelendy et al., 1968) were injected intravenously in sheep. The increase of these acids in peripheral circulation resulted in higher plasma level of insulin.

Several final products of fermentation have been proposed to increase insulin secretion in ruminants (Brockman, 1978; Trenkle, 1978; Basset, 1980). However, considerable evidence suggests that propionate is the only major metabolite involved directly in the process (Emmanuel and Kennelly, 1984; Bines and Hart, 1984; Istasse and Orskov, 1984). In the present experiment, isoacid supplementation significantly decreased the concentrations

of propionate in the rumen (Table 13). It is proposed, therefore, that the lower plasma levels of insulin found in this study were due to the decrease in propionate production in the rumen, which resulted in lower stimulus for insulin secretion (Emmanuel and Kennelly, 1984; Bines and Hart, 1984; Istasse and Orskov, 1984).

As indicated previously, Towns and Cook (1984) reported that lactating cows fed high grain diets supplemented with isoacids have lower concentrations of plasma insulin. Despite the fact that in that study measurements of VFA concentrations were not made, results reported by others (Hume, 1970; Oltjen et al., 1971; Felix, 1976; Hefner et al., 1985) clearly indicate that supplementation of isoacids to ruminants fed high concentrate diets results in a shift in the molar proportion of rumen VFA favoring acetate. Therefore, it is very likely that the decrease in plasma insulin found by Towns and Cook (1984) were also caused by lower propionate production in the rumen.

In the present study it has been demonstrated that isoacids decrease insulin but do not change concentrations of growth hormone and cortisol in plasma. It has also been proposed that the decrease in insulin in ruminants fed isoacids is due to lower propionate production in the rumen. The significance of these results with respect to changes in animal productivity is addressed in the following section.

VI. SUMMARY

Attempts to explain the mechanisms by which isoacids improve performance in ruminants have consistently implicated the increases in digestion and in microbial yield as the major factors. Towns and Cook (1984) were the first to report that isoacids alter the plasma endocrine profile in lactating cows. Cook (1985) suggested that those changes would be an additional factor to explain the positive effects of isoacids on milk production. In the present study it has been proposed that the decrease in plasma insulin caused by isoacids is due to lower propionate production in the rumen. Based on the results reported in this dissertation and on literature data, it is possible to predict that the efficiency with which isoacids improve performance in ruminants will vary according to the physiological stage of the animal (i.e., lactation or growth) as well as on the type of diet animals are fed (Figure 8).

In lactating cows, where low levels of plasma insulin are necessary if adequate supply of substrates to the mammary gland are to be mantained (Hart, 1983; McDowell, 1983), isoacid supplementation should increase milk production regardless of the diet (Figures 8a and 8b). This

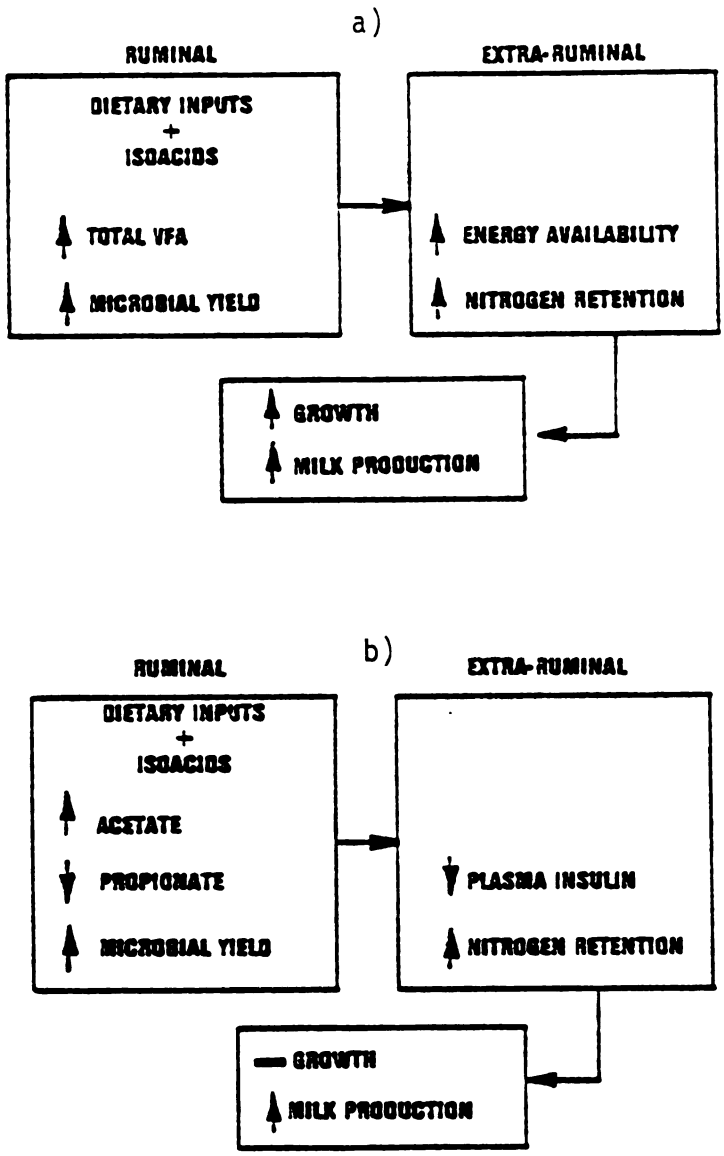

Figure 8. Summary of the effects of isoacids in ruminants fed a) high fiber, high NPN diets or b) diets containing preformed protein.

explains why milk production in cows fed either low quality, high NPN diets (Felix, 1976; Felix et al., 1980) or high quality diets containing preformed protein (Felix, 1976; Papas et al., 1984) is consistently increased by isoacids.

In growing animals, however, the degree of efficacy of isoacids is diet-dependent. Ideally, animals in the growing stage should have high levels of plasma insulin so that partition of nutrients towards deposition is maximized (McDowell, 1983). Since plasma insulin is lower when isoacids are fed, isoacid supplementation to growing animals fed high quality diets should be less effective or even non-existent. Any possible positive effect that isoacids might have in the rumen would be offset by the decrease in insulin secretion, resulting in lower rates of uptake of amino acids (Bergen, 1978) and other substrates by peripheral tissues (Figure 8b). This may explain why feedlot cattle have shown little (Deetz et al., 1985) or no response (Owens et al., 1983) to isoacid supplementation.

In growing animals fed high roughage, high NPN diets (Figure 8a) the increase in feed utilization (See Chapter IV; Cline, 1966; Soofi et al., 1982) and in microbial cell yield (Chalupa and Bloch, 1983; Russel, 1983) by isoacids should increase the rate of growth and feed efficiency (Felix et al., 1981; Cook and Barradas, 1985).

This discussion has centered on the proposition that part of the beneficial effects of isoacids can be explained on the basis of the shift in rumen fermentation towards the

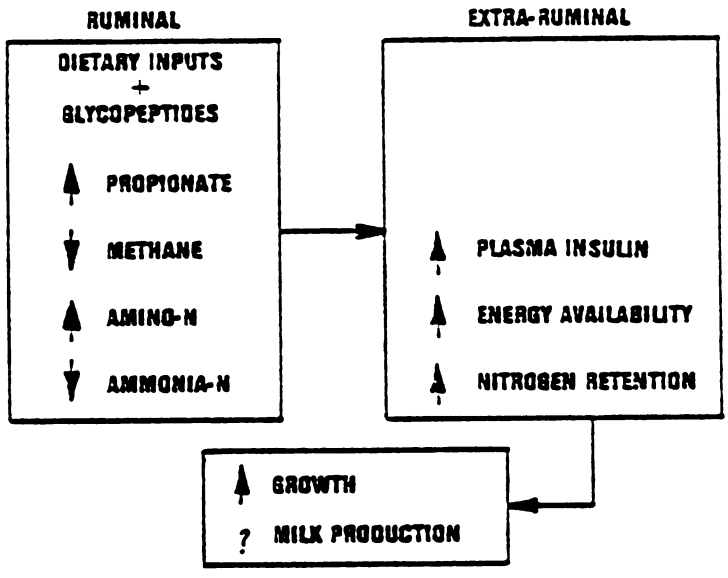

Figure 9. Summary of the effects of glycopeptides (teichomycin A2 and avoparcin) in ruminants.

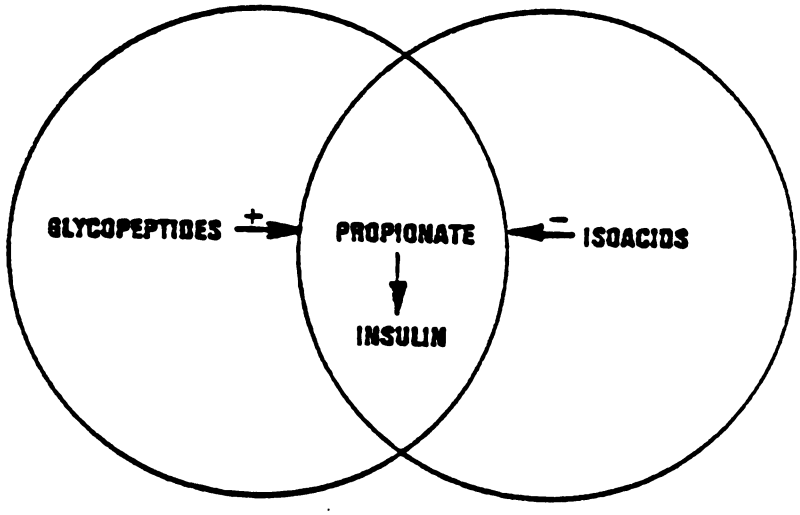

Figure 10. Action of isoacids and glycopeptides in ruminants.

production of more acetate and less propionate. The similarity of this concept to the concept proposed to explain the effect of glycopeptides and ionophores on animal growth is readily apparent. As demonstrated in Chapter III, glycopeptides increase propionate production and decrease amino acid degradation in the rumen. Therefore, in addition to the savings in energy during fermentation, the extra propionate would also promote, via stimulation of insulin secretion, the utilization by the host animal of amino acids spared in the rumen. These actions in combination should account for the positive effects of glycopeptides on animal growth. The effects of these compounds on milk production are yet to be determined (Figure 9).

The present study is the first to offer an explanation as to why isoacids consistently increase milk production in lactating cows fed a variety of diets. Also, it is the first study to demonstrate that isoacids and glycopeptides affect animal performance through overlapping mechanisms, in which propionate plays the central role (Figure 10). The possible interactions between these two classes of compounds should receive further attention.

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