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THE EFFECT OF IONOPHORES, GLYCOPEPTIDES AND THEIR COMBINATION ON CULTURABLE RUMINAL BACTERIA AND VARIOUS RUMINAL PARAMETERS

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ABSTRACT

THE EFFECT OF IONOPHORES, GLYCOPEPTIDES AND THEIR COMBINATION ON CULTURABLE RUMINAL BACTERIA AND VARIOUS RUMINAL PARAMETERS

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Ionophores and glycopeptides were investigated in vitro and in vivo to determine their effects on bacterial growth, fermentation patterns and protein degradation. In the in vitro study pure cultures of ruminal bacteria were utilized to determine the minimum concentration of ionophores and an ionophore-glycopeptide combination necessary to alter growth of these organisms. Of the compounds examined monensin and lasalocid were the most effective in inhibiting bacterial growth. In the in vivo study four cannulated steers were used to investigate fermentation shifts, alterations in protease and deaminase activity and bacterial growth rates in the rumen due to addition of an ionophore, a glycopeptide or a combination to the diet. Of the compounds examined, only the narasin-actaplanin combination significantly altered the volatile fatty acid profile. With respect to alterations in bacterial protease and deaminase activity and bacterial growth, the compounds examined were ineffective in significantly changing any of these parameters.

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INTRODUCTION

With monensin's overwhelming success in the feedlot industry, other fermentation manipulators have been developed to compete with monensin. To date the only other semi-successful feed additive of this type is lasalocid but it falls a distant second to monensin in the market place. Along with the continual introduction of new compounds an increasing interest towards understanding these compunds action in the rumen has arisen. Although it is well recognized that monensin alter the rumen bacterial population which shifts the volatile fatty acid profile and leads to an increase in feed efficiency in the animal, there is mounting evidence that there are other underlining effects of ionophores. Raun et al. (1976) spectulated that these compounds have a protein sparing effect in the rumen which probably contributes to the increased efficiency. With this baseline understanding there is a need to investigate these new compounds to determine their effects in the rumen. Thus, the following investigations were designed to examine the effects both ionophores and glycopeptides have on ruminal bacteria and to investigate their action in the rumen.

LITERATURE REVIEW

DIET AND RUMEN ECOSYSTEM INTERACTION

The microbial population inhabiting the rumen is influenced in large measure, by the dietary constituents with the carbohydrate source being the most influential. Hemicellulose, cellulose, pectin, starch and sugars are the carbohydrates which are typically present in the rumen. Hemicellulose, cellulose and pectin are cell wall constituents and starch and sugars are associated with the cell contents. Typical roughage-type diets fed to ruminants contain large amounts of cell wall constituents in comparison to soluble carbohydrates while grain or concentrate diets are the reverse. Under roughage feeding regime then, the predominant bacterial strains inhabiting the rumen are the fiber digesters (e.g. Ruminococcus albus, Ruminococcus flavefaciens, Butyrivibrio fibrisolvens and Bacteroides succinogenes (Hungate, 1966; Schwartz and Gilchirst, 1975). These ruminal organisms are closely associated with the solid fraction of the digesta (Hobson and Wallace, 1982). Forsberg and Lam (1977) showed that seventy-seven percent of the diaminopimelic acid (DAPA), a compound found exclusively in procaryotic organisms, is associated with the particulate matter. Using radioactive labeled cellulose, Rasmussen et al. (1983) determined that

the extent of attachment is regulated by the substrate supply. Therefore, on a diet consisting mainly of roughages the feed particles are saturated with adherent bacteria. Cheng and Costerton (1980) postulated that the attachment is a specific and rapid process. Under high roughage feeding conditions the cellulolytic bacteria are not, however, the only bacteria present in the rumen. A secondary class of organisms exist which survive and proliferate through the utilization of intermediates and/or end-products produced by the primary population (Schwartz and Gilchrist, 1975).

With the shift to high concentrate diets (e.g. alpha linked carbohydrates) the rumen ecosystem shifts from a cellulolytic dominated population to a amylolytic population. A large proportion of high concentrate diets consists of readily fermentable carbohydrates (starch and sugars) which support amylolytic organisms like Streptococcus bovis, Bacteroides amylophilus, Bacteroides ruminicola and Selenomonas ruminantium (Hungate, 1966). Cellulolytic bacteria are characteristic of slow growing organisms while the amylolytic bacteria are generally rapid growing organisms. With the availability of readily fermentable carbohydrates the cellulolytics can not compete with the rapid growing organism and thus, they lose their dominating position in the rumen ecosystem under these conditions (Hungate, 1966).

Overall fermentation on high concentrate diet in comparison to the fermentation of high roughage diet is

illustrated in Table 1. Concentrate diet supports a larger population of organisms than a roughage diet (Ogimoto and Imai, 1981). The pH range for a high concentrate diet is 5.75-5.80 while a roughage diet range is 6.0-7.0 (Ogimaoto and Imai, 1981). Total volatile fatty acid (VFA) production increases with the increase of cereal grains in the diet (Orskov, 1982). In fact, the proportions of the VFAs shift towards a higher propionate production. This primarily results from a higher concentration of lactic acid utilizers which convert lactate to propionate (Mackie and Gilchrist, 1979). The protozoal population of the rumen, also, differs with alternate substrates. Purser (1959) showed protozoa numbers are considerably lower in animals fed a high concentrate diet as compared to a high roughage diet. This difference is primarily due to the lower ruminal pH which accompanies the feeding of readily fermentable carbohydrates (Purser, 1959).

With respect to the rumen fermentation there seems to be a clear divison between high roughage diets and high concentrate diets. Corn silage, a prevalent feedstuff in Michigan, is a mixture on a dry matter basis of approximately fifty percent forage and fifty percent cereal grain (Goodrich and Plegge, 1984). It seems reasonable then, to rank corn silage as an intermediate feedstuff between high roughage and cereal grain diets in respect to the overall microbial population inhabiting the rumen. Therefore, it appears that a corn silage diet should

TABLE 1. FERMENTATION PATTERNS OF CONCENTRATE AND ROUGHAGE DIETS.

	Concentrate		Roughage	
	Maize	Barley	Timothy	Lucerne
pH	5.76	5.96	6.61	6.81
ammonia (mg/dliter)	3.2	28.7	8.2	16.2
acetate (mM)	34.8	42.8	53.8	64.3
propionate (mM)	28.7	23.7	12.3	17.7
butyrate (mM)	3.8	11.1	4.7	5.3

support both cellulolytic and amylolytic bacteria in relatively equal proportions and all ruminal parameters should be between the aforementioned extremes.

NITROGEN METABOLISM

Overview

Ruminal nitrogen metabolism is a series of complex reactions which are conducted by the microbiota inhabiting the system. Dietary proteins, as well as endogenous proteins, are degraded first to amino acids and short peptides in the rumen. Through further catabolism the amino acid are deaminated and/or decarboxylated to form branched-chain volatile fatty acids, carbon dioxide and ammonia. These products are then, utilized by the microorganisms as growth factors in respect to the two former and as a nitrogen sources in the case of the latter. This entire process of protein degradation and metabolism is carried out by a wide range of organisms interacting with one another. Examination of ruminal nitrogen metabolism is as complex as the reactions and organisms involved. Therefore, to facilitate the discussion the subject shall be subdivided into three major categories; 1) protein hydrolysis, 2) intermediate catabolism and 3) microbial protein synthesis or cell growth.

Protein Hydrolysis

Protein hydrolysis is the first step in the overall processing of dietary protein. The hydrolysis, according to Nugent and Mangan (1973), is the rate limiting step in ruminal protein degradation. Through the use of uniformally radioactive labelled leaf fraction I protein these workers showed proteolysis exhibits first order kinetics. Earlier work conducted by Henderickx and Martin (1969) and Hobson and Wallace (1982) indicated that the rate of proteolysis is dependent on the proteins solubility. Recent research, however, supports a more complex viewpoint. Although many proteins, like bovine serum albumin and ovalbumin, are soluble in water their rate of degradation in the rumen as compared to casein, a relatively insoluble protein, is much slower (Hobson and Wallace, 1982). According to Mangan (1972) the rate of proteolysis of casein is approximately 4.6 pecent per hour while the rate of degradation of ovalbumin is considerably slower. Mangan (1972) attributed this to the cyclic structure of the ovalbumin. This molecule lacks both the terminal amino and carboxyl ends which inhibits the attachment of the exoproteases, thus, decreasing the rate of proteolysis. Nugent and Mangan (1978) showed that the disruption of disulfide bridges present in bovine albumin by the addition of dithiothreitol increases albumin proteolysis several fold. By comparing the degradation of a variety of soluble and insoluble proteins

by the ninhydrin method (which measures liberated amino acids), Mahadevan and coworkers (1980) provided further evidence that solubility alone is not a strong enough criteria for the rate of protein degradation and that structural characteristics like disulfide bonds are important. Wallace and Kopecney (1983) indicated through the use of azocasein that secondary and tertiary structures influence the overall rate of protein hydrolysis. The rate of proteolysis, therefore, is determined by many factors other than solubility (i.e. disulfide bridges, tertiary structures and availability of end terminal groups of the molecule) (Bergen and Yokoyama, 1977; Hobson and Wallace, 1982).

Factors other than the molecular characteristics of a protein determine the extent to which the protein is degraded in the rumen. Dietary regime plays an important role in the overall hydrolysis of a protein. Nugent and others (1983) demonstrated when changing from a hay-concentrate diet to a lucerne diet the rate of proteolysis of casein, leaf fraction I protein and bovine serum albumin increases. Earlier reports by Blackburn and Hobson (1960) showed proteolytic activity varies with diet but the change is not apparent immediately after switching the diets. The change is, therefore, an adaptive response to the availability of protein as a substrate. Through the isolation of strong proteolytic bacteria Hazlewood and coworkers (1983) confirmed that an increase in ruminal

proteolysis occurs when fresh fodder diets are substituted for dry diets. In washed cell preparations, however, Annison (1956) and Warner (1956) reported activity is independent of the diet. Recently, Siddon and Paradine (1983) when comparing cereal and forage diets reported higher activity is associated with cereal diets when casein is used as the substrate. Compiling this information with the earlier studies Siddon and Paradine (1983) concluded that the higher activity is probably a function of microbial numbers rather than an increase in the activity of proteolysis which suggests that bacterial protease activity is constitutive (Hungate, 1966).

The rumen microbial system is mainly a sacchrolytic population. Few, if any, true proteolytic bacteria have been isolated from the rumen. The majority of the proteolytic activity is linked to organisms which have been already classified as major ruminal inhabitants. Primarily then, ruminal bacteria ferment dietary carbohydrates for energy and utilize proteolytic activity to sequester nitrogen for use in microbial protein production or growth. Brock et al. (1982) reported that the proteolytic activity in the rumen is associated twenty-five percent with the fluid fraction and seventy-five percent with the particulate fraction, indicating protease activity is possessed by all ruminal inhabitants. While attempting to isolate and characterize proteolytic ruminal bacteria Fulghum and Moore (1963) discovered that the majority of organisms isolated from

ruminal ingesta tested positive for proteolytic activity. Blackburn and Hobson (1960) showed all fractions of rumen microbial population, large and small bacteria as well as protozoa, possess proteolytic activity. The relative amounts of activity, however, varies between the bacterial fractions and protozoa fraction. Blackburn and Hobson (1960) expressed their data on activity per weight bases which illustrates that the large bacteria have the highest activity between the separated fractions. The specific activity is six to ten times higher in bacteria fraction when compared to the protozoa fraction (Brock et al., 1982).

The organisms which are highly proteolytic are primarily gram negative (Kopecny and Wallace, 1982) like Butyrivibrio sp., Succinivibrio spp., Selenomonas ruminantium var. lactilytica, Lachnospira multiparus, Bacteroides ruminicola, Borrelia spp., and Bacteroides amylophilus. Streptococcus bovis, although a gram positive organisms is also highly proteolytic (Fulghum and Moore, 1963; Brock et al., 1982). The proteolytic activity of the rumen is then, primarily due to bacterial species which are known to already occur in large numbers in the rumen. Therefore, it seems that this process as a whole is a secondary mechanism in the major species of the ruminal bacteria and suggests that the importance of proteolysis is more related to the cell growth rather than the survival of the organisms.

The process of protein hydrolysis is carried out by

proteolytic enzymes which are associated with the cell wall (Nugent and Mangan, 1978). With the aid of nonionic detergents and density gradient centrifugation of the bacterial membranes, Kopencny and Wallace (1982) determined that the largest proteolytic activity is associated with the cell coat or capsular material of the bacteria. Some activity is contained in the intracellular material, however, these protease enzymes are primarily important in turnover of cellular protein rather than dietary proteins. The only instance these endoenzymes become involved in the degradation of dietary proteins is when cell lysis occurs (Goldberg and St. John, 1976; Kopecny and Wallace, 1982). Further investigation showed that the predominant proteolytic enzymes of the rumen bacteria are serine-type proteases (Kopecny and Wallace, 1982; Forsberg et al., 1984). Forsberg et al. (1984) reported that approximately sixty to seventy percent of the proteolytic enzymes are serine type but this probably changes with diet.

Deamination

Ruminal intermediates of total protein hydrolysis, amino acids and small peptides, are found at very low levels in rumen fluid. These intermediate metabolites, therefore, must be rapidly catabolized to other constituents (McDonald, 1952; Lewis, 1955; Annison, 1956). Pilgrim et al. (1970), through the uses of N-15, showed amino acids are directly

incorporated into the bacteria and the proportion of direct microbial utilization of the amino acids is diet dependent. Feeding a low energy:low nitrogen diet the bacteria incorportate eighty percent of their nitrogen in the form of ammonia. The ammonia available to the organisms arises from degradation of dietary proteins and the ammonia recycling process. When high energy:high nitrogen diets are fed the percentage of ammonia utilized for microbial protein synthesis declines to less than sixty-five percent. This suggests that amino acids are directly used in the production of microbial protein when high amounts of both energy and nitrogen are available. Nolan and Leng (1972) further investigated amino acids utiliztion by ruminal bacteria and revealed similar information. For diets containing twenty to twenty-six percent crude protein, twenty percent of the microbial protein is derived directly from the incorporation of dietary amino acids. Similar findings have been reported by other as well (McMeniman et al., 1976; Salter et al., 1979; Armstrong and Weekes, 1983).

Under typical feeding practices where neither energy nor nitrogen is limiting only twenty percent or less of microbial protein is derived from amino acids directly and since eighty-two percent of ruminal microorganisms require ammonia for growth (Bryant and Robinson, 1962) the majority of the amino acids formed through hydrolysis of dietary protein must be further degraded. As Mathison and Milligan reported, Portugal and Sutherland (1963) used of carbon

labelled amino acids to show that ten percent of the amino acids in microbial protein arose from direct incorporation while Weller et al. (1962) reported up to eighty percent of dietary plant nitrogen is found in microbial protein. Again the above findings illustrate carbon and nitrogen of dietary proteins are separated during degradation. Therefore, the primary nitrogen involved in de novo synthesis of microbial protein is in the form of ammonia. Under normal feeding regimes ammonia nitrogen is not present in the diet, unless urea is fed, therefore, the ammonia must come from further catabolism of the amino acids.

The precise amino acid degradation pathway predominating in the rumen has not been fully determined. Two possible pathways for amino acid catabolism by microorganisms have been elaborated. First, a non-oxidative deamination reaction resulting in the formation of volatile fatty acids and ammonia has been reported in Megasphaera elsdenii (Prins, 1977). It appears that a single enzyme is responsible for this non-oxidative reaction (Lewis and Elsdens, 1955; Walker, 1958; van den Hende et al., 1963). The Stickland reaction, a coupled oxidation-reduction reaction between two amino acids which produces carbon dioxide, ammonia and volatile fatty acids (Prins, 1977) is the second proposed pathway. Only certain amino acids have been found to be involved in this reaction. According to Barker (1961) the most activity involved amino acids are alanine, leucine, isoleucine, glycine, proline,

hydroxyproline and ornithine. Other amino acids have been implicated in the Stickland reaction but the relative rates of their utilization are not as high as for the above mentioned amino acids. It should be noted that the majority of the work in this area has been conducted with clostridia and this pathway has not been investigated in ruminal organisms. More importantly, the Stickland reaction has a pH optimum of about 6.0 while the non-oxidative deaminase pathway required pH is around neutrality (Prins, 1977). Under normal conditions the pH in the rumen ranges from 6.7 to 7.0 (near neutrality), therefore, the primary route for amino acid degradation must be via the non-oxidative deamination pathway.

Ruminal microorganisms in an overall sense have been studied in respect to the effect diet has on the deamination process. The information, however, is quite limited. Basically, it has been shown that ruminal deaminase activity varies with diet. El-Shazly (1952) reported that in washed suspensions of rumen microorganisms deamination depends strongly on the diet. This dependence might be linked to types of amino acids found in the dietary proteins or to the type of bacteria population inhabiting the rumen at that time. Mangan (1972) demonstrated that different amino acids are degraded at different rates. Lewis and Emery (1962) reported that amino acids such as serine , cysteine, aspartic acid, arginine and threonine are dissimilated when added to either strained rumen fluid or washed cell

suspensions. The percent degraded is lower in the washed cell suspensions than the strained rumen fluid. This is probabily due to the lack of important co-factors which absent in the cell preparation. Chalupa (1976) showed that with incubations of mixed rumen microbial populations methionine and valine are degraded slowly while arginine and threonine are degraded much more rapidly and other essential amino acids fall into an intermediate group between these two extremes rates. Chalupa (1976) indicated that in vivo rates of degradation are much more rapid than in vitro rates, again implying the absence of important co-factors. Siddon and Paradine (1981) compared deaminase activity between cereal and forage diets. Cereal diets exhibited a two-fold higher activity than the forage diets. These results may be related to the types of amino acids found in each diet or to the increased number of bacteria found with cereal diets or to the predominating species present under the feeding conditions.

Since the information on the overall deamination process in anaerobic organisms and the rumen environment is limited it is necessary for futher investigation of this area before any firm conclusions may be reached. Therefore, experiments, in vitro as well as in vivo, need to be conducted to determine the overall existence and importance of the individual deaminase pathways in the rumen. Once a full understanding of this process from a bacterial stand point the manipulation of this process may be investigated.

Protein Synthesis

Through the utilization of protein breakdown intermediates, as well as the final products, the rumen microbiota synthesizes cellular protein. To date there seems to be a general consensus that the primary nitrogen source incorporated into the cell is ammonia as mentioned previously. However, there is evidence that bacteria in the rumen can and do utilize free amino acids and small peptides in synthesizing protein.

Bryant and Robinson (1962) using freshly isolated rumen bacteria demonstrated that eighty-two percent of isolates could survive on media containing ammonia as the sole protein source while fifty-six percent of the isolates could use either ammonia or casein hydrolysate. They also established that twenty-five percent of the isolates have an obligate requirement for ammonia and only six percent require amino acids. Similar results have been reported by Stevenson (1978). Earlier work by Bryant and Robinson (1961) showed that Ruminococcus flavefaciens and Ruminococcus albus require ammonia as a sole nitrogen source regardless of the presence or absence of amino acids. Bacteroides succinogenes also requires ammonia to achieve maximal growth rate but can utilize amino acids to some extent. Maeng and coworkers (1976) determined that for maximal microbial cell yield and volatile fatty acid

production with mixed rumen bacteria grown in batch culture a seventy-five percent urea and twenty-five percent preformed protein on a nitrogen basis must be present. These studies suggest that some bacteria may incorporate preformed nitrogen compounds into their cellular protein. Teather et al. (1984) illustrated that the bacteria population increases seventy percent when a combination of urea-silage or soybean meal alone is fed as compared to urea alone. The primary species enhanced under these dietary conditions are Bacteroides ruminicola, Lactobacillus, Bifidobacterium spp., Fusobacterium spp., Butyrivibrio fibrisolvens, Megasphera elsdenii and Lactobacillus spp. which have been shown to have a essential requirement for amino acids (Allison, 1970).

Maeng and coworkers (1976) provided information that the amount of amino acid incorporation into microbial cells is highest immediately after feeding and then rapidly declines. Pittman and Bryant (1967) determined that Bacteroides ruminicola possesses a general system for the uptake of peptides with these peptides are rapidly hydrolyzed during and after uptake. The primary function of these peptides appears to supply amino acids for the production of microbial protein.

It appears that within the vast microbial population present in the rumen ammonia, amino acids and even peptides are incorporated into microbial protein. In fact it may be that the slower growing adherent bacteria (i.e.

cellulolytics) (Hungate,1966) use primarily ammonia as a nitrogen source while the free fraction incorporates either ammonia or the other intermediates depending on the availability of the compounds (Hungate, 1966). Microbial protein synthesis and efficiency values for a typical dilution rate during the day for both the free and adherent population have been reviewed by Bergen and coworkers (1982). Depending on the diet, the dilution of the liquid phase of the rumen may vary from .04-.12 per hour while the solid phase exhibits dilution values from .02-.09 per hour which suggests that the corresponding specific growth rates for specific phase associated bacteria must be greater than .1 and .05 per hour, respectively. Since protein metabolites are quickly degraded to ammonia it seems likely that only the bacteria with a high growth rate will be able to use these compounds. Nolan and others (1976) estimated that thirty to eighty percent of the dietary nitrogen passes through the ammonia pool before its incorporation into microbial protein. They inferred that carbohydrate availability might be partially responsible for this variation. Different carbohydrate are degraded at different rates in the rumen, thus, the energy microorganisms derive from the breakdown of these compounds is staggered. In other words, the bacteria which obtain energy from readily available carbohydates can use this energy to synthesize protein. Since at this point, the dietary protein has not been totally reduced to ammonia, the incorporation of

peptides and amino acids seems likely for these species. The carbohydrates which exhibit a lag in digestion become an available energy source much later in the fermentation process. At this point the dietary protein is strictly in the form of ammonia except that which can be considered as escape protein and the organisms which derive energy from these carbohydrates primarily incorporate ammonia into their cellular protein.

The process by which the rumen bacteria transport amino acids and peptides is not well understood but the transport process of ammonia incorporation is well defined. Assimilation of ammonia into microbial protein can follow two distinct processes dependent on the ammonia concentration present. When ammonia concentration is low the high affinity enzyme glutamine synthase comes into play, however, at high concentrations of ammonia a low affinity enzyme dominates. Smith and Bryant (1979) used Selenomonas ruminantium to investigate ammonia transport and reported glutamine synthase pathway recycles glutamate via glutamine which eventally leads to the formation of two glutamates. This is at the expense of four moles of ATP. The glutamate dehydrogenase pathway consumes one less ATP per mole of ammonia assmilated in glutamate through the use of alpha-ketoglutarate. Since these two pathway exist the ability of the rumen microbiota to assimilate ammonia into microbial protein can occur irrespective of ammonia concentration present in the rumen.

IONOPHORES

Feedlot Performance

Generally, the effects of different ionophores on feedlot cattle performance are comparable. Many researchers have characterized the effects of these feed additives with the most investigated ionophore being monensin (Perry et al., 1976; Raun et al., 1976; Steen et al., 1978; Hanson and Klopfenstein, 1979; Perry et al. 1979; Perry et al., 1983). Feedlot performance of cattle supplemented with lasalocid has been outlined by Bartley and coworkers (1979). Salinomycin, narasin and laidlomycin butyrate, all experimental compounds, have also been tested in the feedlot (McClure et al., 1980; Potter et al. 1976; Spires and Algeo, 1983, respectively).

The overall effect of ionophores in feedlot cattle has been summarized by Owens (1980). When carbohydrates are highly available in the diet ionophores depress feed intake without an accompanied decline in body weight gain, therefore, overall feed efficiency (e.g. feed/gain ratio) is improved. Dyer and coworkers (1980) as well as Ferrell and others (1982) evaluated monensin and lasalocid when fed in conjunction with high concentrate diets and reported similar results. Feed efficiency of the animals increased in both of these experiments primarily because dry matter intake was

reduced. Including an ionophore in a high roughage diet yields a similar endpoint as with the high concentrate diet (e.g. enhances feed conversion), however, under these conditions average daily gains are increased without alteration in feed consumptions (Johnson et al. 1979; Owens, 1980; Brown et al., 1982). With corn silage diets supplemented with ionophores there seems to be a discrepancy in the literature to which parameter(s) are really altered. Perry et al. (1983) illustrated with corn silage diets that monensin supplementation decreased dry matter intake without any apparent effect on average daily gains. Brown and coworkers (1982) observed increased average daily gains and stationary feed intakes when corn silage diets are supplemented with lasalocid.

Ionophore supplemented cattle are the same as non-supplemented animals in respect to carcass characteristics (Dyer et al., 1980; Johnson et al., 1979; Thompson and Riley 1980; Perry et al. 1983; Rioni and Bittante, 1983). The cattle receiving feed additives, therefore, grade similarly to the non-supplemented cattle and bring a similar market price. The overall feed input per pound of gain or lean tissue, however, is considerably reduced with supplementation. This translates into an overall savings to the feedlot owner since feed consumption in the feedlot is less over the entire process with the supplemented animals.

Lactic Acidosis

Besides their ability to increase feed conversion ionophores decrease the incidence of lactic acidosis in feedlot cattle. Lactic acidosis can be described as a fermentation disorder which occurs as a result of grain engorgement. The basic symptoms of lactic acidosis are lacticacidemia, acid-base imbalance, rumen stasis, diarrhea, dehydration, sytemic acidosis and, in acute forms of the disease, cardiovascular and respiratory failure (Huber, 1976; Dennis et al., 1981a & b). These symptoms are attributed to the increase in lactate producing bacteria in the rumen (e.g. Streptococcus bovis) and the subsequent drop in ruminal pH (Dennis et al., 1981a & b). When readily fermentable carbohydrates become abruptly abundant the rapidly growing bacteria, generally lactic acid producers, become the predominating organisms and alter the fermentation process which results in a subsequent pH decline. Coinciding with this rise in lactate producers and decreased pH, the lactic acid utilizing bacteria disappear which further contributes to the accumulation of lactic acid (Counottee, 1978/1979). As the pH approaches 5.5 other gram negative organisms, as well as the protozoa, decrease in number while Steptococcus species and other lactate producers numbers continue to increase. Eventually the pH drops below 5.0 and the predominant organism becomes a Lactobacillus spp.. At this point the metabolic

alterations caused by excess lactic acid are apparent in the host.

With the feeding of ionophores in feedlot diets the problem of lactic acidosis is greatly depressed. Primarily, these feed additives inhibit the growth of lactic acid producers in the rumen without altering the lactate utilizing populations, therefore, preventing lactic acid accumulation. Nagaraja et al. (1981) illustrated the ability of ionophores to prevent lactic acidosis with the use of intraruminal administration of glucose. Nagaraja et al. (1981) concluded from this experiment that addition of ionophores to rations during the switch over help prevent lactic acidosis.

Bacterial Population

Ionophores like other antibiotics inhibit the growth of certain microoganisms. These compounds when feed to cattle alter the ruminal bacteria population which ultimately leads to a shift in the ruminal fermentation end-products. Extensive pure culture research has been conducted in order to determine the antibiotic sensitive strains in the rumen. Chen and Wolin (1979) determined that the ruminal microbes sensitive to antibiotics, monensin and lasalocid, are primarily the gram positive microoganisms (e.g. Ruminococcus albus and Ruminococcus flavefaciens). Chen and Wolin (1979) observed that gram negative species

(e.g. Bacteriodes succinogenes, Bacteroides ruminicola and Selenomonas ruminantium) are relatively resistant to these compounds. In general they concluded that gram positive organisms illustrate a high sensitivity to ionophores while gram negative bacteria are resistant to the compound. They reported, however, that Butyrivibrio fibrisolvens, a gram negative bacteria which possesses a gram-positive like cell-wall structure (Cheng and Costerton, 1977), is suppressed by the antibiotics. The microorganisms for which ionophores select for are succinate producers and succinate utilizers (e.g. propionate producers). The inhibited organisms are the carbohydrate fermenters which produce formate, acetate, butyrate and hydrogen as their end-products. With this reduction of these specific end-products the survival of bacteria dependent upon these compounds is reduced. Chen and Wolin (1979) suggested that the reason methanogenesis is reduced with the presence of monensin is due to a reduction in the availability of substrate (e.g. hydrogen) rather than a specific toxic affect on the cells.

Another group of feed additives similar to the ionophores are the glycopeptide antibiotics. One of the more investigated glycopeptide antibiotics is avoparcin, a growth promotant for broiler chickens (Lesson et al., 1984), and ruminants (Chalupa et al., 1980). Froetshel and coworkers (1983) demonstrated that avoparcin causes similar shifts in the rumen population as monensin. Specifically, avoparcin inhibits many gram positive bacteria directly

while indirectly, through reduced hydrogen availability, decreased methane producers. Gram negative flora, like with monensin, are virtually unaffected by avoparcins presence (Stewart et al., 1983). Stewart et al. (1983) suggested that avoparcin alters the ruminal microbial population in a similar manner to monensin but is required in higher concentration than monensin to yield the similar results.

Fermentation

It has been demonstrated that ionophores increase the molar proportion of propionic acid while decreasing acetate and butyrate proportions but does not influence the total volatile fatty acid (VFA) production (Thornton et al., 1976; Chalupa et al., 1980; Richardson et al., 1976). As stated before methane production is reported to be reduced by an indirect effect on the methanogenic bacteria (Hungate et al., 1966; Chalupa et al., 1980; Richardson et al., 1976; Chen and Wolin, 1979). Glycopeptides, on the other hand, cause a similar shift in VFA concentration but these compounds also reduce total VFA concentration (Froetschel et al., 1983).

The mechanism controlling the VFA shift has been attributed primarily to the selection for succinate-forming organisms (e.g. Bacteroides succinogenes and Bacteroides ruminicola) and propionate-producer (e.g. Selenomonas ruminantium) which decarboxylates succinate to propionate

(Chen and Wolin, 1979). Romatowski and coworkers (1979) illustrated in batch culture that monensin increases the succinate decarboxylating capacity of mixed rumen bacteria. Chalupa and others (1980) reported that the increase in propionate production is due to increased activity through the acrylate pathway. These findings, according to Chalupa et al. (1980), suggest that the enhanced propionate production seen when monensin and other ionophores are present is probably a result of both population shift and increased enzyme activity.

Methanogenesis is reduced in cultures exposed to ionophores. The alteration in methane production is a result of the selection by the ionophore against hydrogen producing organisms (e.g. Ruminococcus albus) (Chen and Wolin, 1979; Van Nevel and Demeyer, 1977). Therefore, the ionophore effect on methane production is a secondary characteristic and not a direct metabolic inhibitions of these organisms by the ionophore (Van Nevel and Demeyer, 1977).

Blaxter and Waiman (1964) suggested that propionate can be more efficiently utilized by the ruminant. The primary reason is propionate has a lower heat increment than acetate. As reported by Rowe et al. (1981) twenty percent more metabolizible energy is available when the shift in VFA production has been accomplished. Rowe et al. (1981) and Richardson et al. (1979) supported the idea that a diet containing monensin is more efficiently utilized than an

unsupplemented diet. Bull et al. (1970), Johnson et al. (1972), Orskov et al. (1979) and Byers (1980), however, demonstrated that acetate and propionate are energetically the same and are utilized with similar efficiency for growth. The increase in performance, therefore, may not be totally due to the shift in VFA production. Raun and cowokers (1976) agreed that propionate fermentation is more favorable than an acetic and butyric acid fermentation from a energy stand point because carbon conservation. Still this phenomenon cannot account for all of the increase performance which occurs when monensin is fed to feedlot cattle. Raun et al. (1976) suggests that a suppression of deaminase and protease activity might contribute to the enhanced performance. Poos et al. (1979), Owens et al. (1980), Isichei (1980) and Van Nevel and Demeyer (1977) reported increased non-ammonia nitrogen reaching the lower gut with monensin supplementation which further supports Raun et al. (1976) hypothesis that ionophores are protein sparing. The increase in dietary protein reaching the lower digestive tract observed in the above studies ranged form twenty-two to fifty-five percent (Bergen and Bates, 1984). Since the range of "by-pass" protein is quite large this might suggest that the process is governed by diet. With ruminal protein and amino acid degradation inhibited or reduced the major site of protein digestion is shifted to the lower gut which might account for some of the increased performance seen in feedlot cattle. One must remember,

however, that this shift could be detrimental if the dietary protein is of low quality. Since microbial protein is reduced and protein by-pass elevated with monensin the dietary protein should be of higher quality than need be in a diet without monensin.

Van Nevel and Demeyer (1977) and Bartley et al. (1979) used in vitro techniques in determining that monensin and lasalocid decrease bacterial growth in cultures not previously exposed to either of the compounds. Herod et al. (1979) showed that bacterial growth is not depressed with monensin feeding. In mixed cultures, total and net growth, is reduced with supplementation of monensin while the amount of substrate fermented is approximately the same. Thus the microbial growth efficiency is reduced (Van Nevel and Demeyer, 1977). These workers incubated unadapted mixed cultures in casein and monensin and observed a lower protein degradation and ammonia production as compared to the control. This suggests that monensin's effect in vivo may extend far beyond the simple population and VFA shift. However, as stated all these experiments were conducted using unadapted mixed ruminal bacteria and therefore the results may all indicate the effects of monensin when first added to the animals diet. Further investigation of the area is necessary.

Mode of Action

Monensin and other carboxylic polyether ionophores have been described as cation-H ion antiporters (Harold et al., 1972). The direction of the tranport and degree of cation exchange is determined by the chemical gradients which exist (Bergen and Bates, 1984). The affinity of the particular cation varies between ionophores. Monensin is a sodium/hydrogen transporter (Pressman et al., 1976) while lasalocid prefers potassium, calcium and sodium.

The chemiosmotic hypothesis, postulated by Peter Mitchell, states that metabolic energy is conserved at the level of the membrane as an electrochemical gradient of hydrogen ions (Harold, 1972). This gradient can be established through the passing of an electron down the electron transport chain or by the extrusion of a hydrogen ion during the hydrolysis of ATP (e.g. ATPase). In the case of hydrogen extrusion, the established gradient exerts a force on the electron and pulls it back into the cell. This causes a dissipation of the chemical gradient and may be coupled with movement of important metabolic substrates or ATP production (Bergen and Bates, 1984). Both bacterial and mammalian cells have been shown to utilize this electrosmotic energy to transport amino acids, sugars and other ions (Eddy, 1978; Rosen and Kashket, 1978; Booth and Hamilton, 1980).

Rumen bacteria are obligate anaerobes dependent primarily on substrate phosphorylation for ATP synthesis

(Hungate, 1966). Some ruminal bacteria have a partial cytochrome system which contributes to the ATP supply (Prins, 1977). The ATP produced in bacteria is primarily used toward the production of the electrochemical gradient which in turn is used for secondary transport.

As stated before, monensin effects are dictated by the concentration gradients of hydrogen and sodium ions. The ion in the highest concentration will be dissipated while the other, whether it be against its gradient, will be driven in the opposite direction. In the rumen environment the hydrogen gradient is greater than the sodium gradient. Thus the hydrogen electrochemical gradient which the cells have established will be dissipated. The cell then will be required to increase its ATP utilization to correct this problem. It is believed that organisms with electron transport chains can more easily adjust to this phenomenon because they can extrude hydrogen electrons via the electron transport chain. These organisms, therefore, are not required to use their ATP reserves to create a new proton gradient. In other words, monensin places an addition stress upon the microbiota inhabiting the rumen. Those organisms capable of adjusting to the increased ATP demand will survive while those unable to change will perish. Also, the organisms which survive may be unable to reach their maximum growth potential since energy usually partitioned towards growth, protein synthesis, will be shunted towards maintenance. Although it seems apparant that ionophores as

well as other similar compounds cause this response in ruminal bacteria the specific metabolic changes in the bacteria have not been fully explained. Therefore, a great need for further investigation into this area is needed.

MATERIALS AND METHODS

In Vitro Study Overview

Eight pure cultures of rumen microorganisms were utilized in an experiment to determine the effects of four different fermentation manipulators (e.g. monensin, narasin, lasalocid and actaplanin) on microbial growth. This compounds were a gift from Elanco. The rumen bacteria involved in this study were Selenomonas ruminantium (Sr), Megasphera elsdenii (ATCC19169), Bacteroides ruminicola (B4), Bacteroides succinogenes (S85), Butyrivibrio fibrisolvens (D1), Ruminococcus flavefaciens (C94), Ruminococcus albus (7), and Streptococcus bovis (24). These ruminal organisms were grown in batch culture in a complex-carbohydrate rumen fluid media (Table 2) which contained either 0.0, 0.5, 2.5, 5.0, 10.0, or 20.0 ppm of the respective compound. On two consecutive weekends four of the eight microorganisms were carried through the following experiment to determine the minimal inhibitory concentration (MIC) of the compounds. Some ruminal bacteria are readily resistant to these compounds while others are either adaptively resistant or totally non-resistant an adaptation period seemed necessary to study these organisms in a restricted time period. One week prior to the commencement of the experiment, the organisms were adapted to each of the compounds at the 0.5 ppm level. Following the adaption period, fresh 0.5 ppm batch culture tube were inoculated

TABLE 2. PURE CULTURE RUMINAL BACTERIA.

ORGANISM	GRAM STAIN	TYPE
Selenomonas ruminantium (Sr)	negative	Not inhibited
Megasphera elsdenii (ATCC19167)	negative	Not inhibited
Bacteroides ruminicola (B4)	negative	Adapts
Bacteroides succinogenes (S85)	negative	Adapts
Butyrivibrio fibrisolvens (D1)	negative	Inhibited
Ruminococcus flavefaciens (C94)	positive	Inhibited
Ruminococcus albus (7)	positive	Inhibited
Streptococcus bovis (24)	positive	Slowed growth

with the organism and incubated at 39 C overnight for subsequent use the following day in the actual trial. The organisms which were unadaptable to the compounds were simply obtained from a fresh control batch cuture. At the onset of the trial the fresh cultures were transferred to the experimental test tube and incubated at 39 C in an incubation room. The incubation period lasted approximately forty hours during which optical density (OD) reading were taken every two hours for the first eighteen hours and at hours nine, fourteen and twenty-one for the remaining twenty-one hours. Optical densities were read from a Spectrophotometer 80 at a wave length of 660 nm.

Media Preparation

An anaerobically prepared complex-carbohydrate rumen fluid medium was used in this experiment (Table 3). Seventy-five milliliters (ml) of deionized water were added to a mixture containing twenty-five ml of clarified rumen fluid, 3.75 ml of both Mineral I and Mineral II, 0.1 ml of resazurin solution, 0.1 ml hemin solution, 0.1 ml volatile fatty acid solution, 0.05 gram of cellobiose, dextrose, maltose and yeast extract and 0.2 gram of trypticase. The resulting mixture was adjusted to pH 6.7 with 6 N NaOH, followed by the addition of five ml of an eight percent sodium carbonate solution and the appropriate amount of

TABLE 3. COMPLEX CARBOHYDRATE RUMEN FLUID MEDIA.

CONSTITUENTS	ml/100 ml
Deionized water	75.0
Clarified rumen fluid	25.0
Mineral I	3.75
Mineral II	3.75
Resazurin solution	0.10
Hemin solution	0.10
Volatile Fatty Acid mixture	0.10
Cellobiose	0.05 g
Dextrose	0.05 g
Maltose	0.05 g
Yeast extract	0.05 g
Trypticase	0.20 g
pH to 6.7 with 6 N sodium hydroxide and bubbled under oxygen-free carbon dioxide	
8% sodium carbonate solution	5.00
Cysteine chloride	2.00
stoppered, wired and autoclaved for fifteen minutes	

For more complete details consult Holdeman et al., 1977.

antibacterial compound. The medium was placed in round bottom flask and brought to a boil under oxygen-free carbon dioxide. Following boiling the medium was allowed to equilibrate under oxygen-free carbon dioxide for an additional fifteen minutes before the aseptic addition of two ml of cysteine chloride solution. Then nine ml of the reduced medium were transfered anaerobically to a test tube (13 x 150 mm), stoppered and autoclaved at 121 C with 15 lb pressure for fifteen minutes to insure the media were free of unwanted bacterial contaminates. Upon commencement of the trial one ml of adapted culture was added to the each broth. All transfers were conducted according to the Hungate technique (Hungate, 1950) for culturing rumen anaerobes.

In Vivo Study Overview

Four ruminal cannulated Holstein steers weighing approximately 500 kg were used in a single 4 x 4 changeover repeat measure Latin square design to determine the effects of specific feed additives on the major ruminal fermentation processes. Steers were individually housed in pens approximately fifteen by twenty meter in the metabolism room at Michigan State Beef Cattle Research Center. During the twenty-one day adaption and five day collection period the steers received a diet composed of corn silage and soybean meal supplement (Table 4). The supplement was also

used as the treatment vehicle. The steers, during each block, received either: 1) soy, 2) soy plus 12.5 ppm monensin, 3) soy plus 33 ppm actaplanin and 4) soy plus 36 ppm narasin-actaplanin combination. Upon termination of each block the steers were given a three day rest period during which they received the control diet.

Ten days prior to and during the collection periods the steers were fed at twelve hour intervals. Samples were collected over five consecutive days (one per day) to construct a 0, 2, 4, 7, 10 hours post feeding cycle. At each sampling period whole rumen contents were placed in a thermos and transported to the laboratory for further analysis. At the same time nineteen ml of strained rumen fluid was collected, fixed with one ml of saturated mercuric chloride and stored at 4 C for use later in the volatile fatty acid and ammonia nitrogen determination.

Laboratory Preparation

Freshly obtained whole rumen contents were squeezed through two layers of cheesecloth to separate the liquid and solid fractions (Figure 1). The strained rumen fluid (SRF) was saved for further fractionation to obtain free floating rumen bacteria. The solid residue remaining was resuspended in an equal volume of cold anaerobic dilution solution (Bucholtz, 1972) to SRF. The resulting combination was mixed and strained through two layers of cheesecloth to

TABLE 4. COMPOSITION OF BASAL DIETS

INGREDIENTS	% DIET DM
Corn, aerial pt, w-ears, w-husk, ensiled, well eared mx 50% mn 30% dry matter	88.7
Soybean, seeds, meal solv-exted	11.3

Crude protein content of diet equalled 12%.

Adequate amount of vitamins and minerals were added.

Supplement was used as carrier of experimental feed additives.

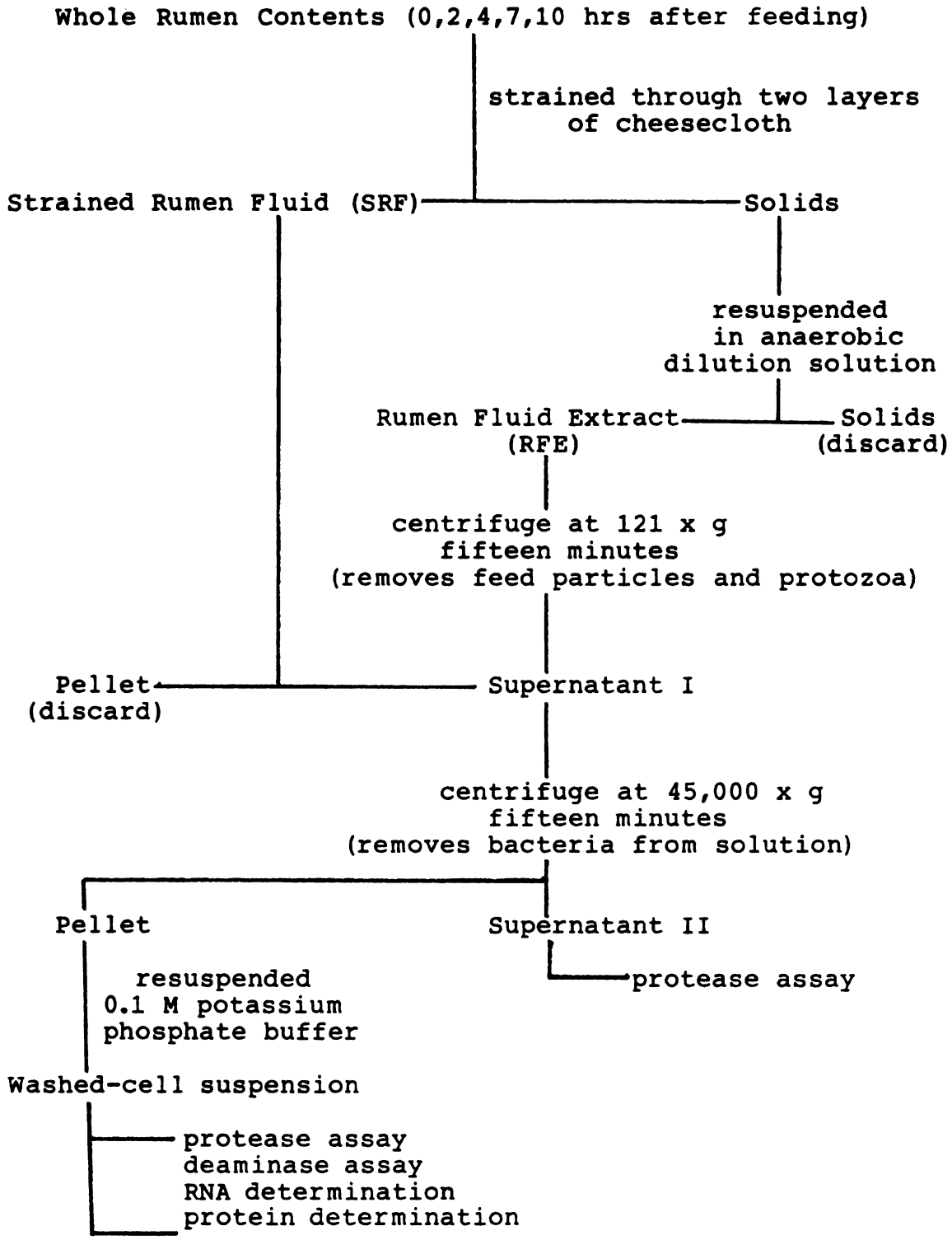

FIGURE 1. SCHEMATIC OF SEPARATION OF WHOLE RUMEN CONTENTS.

separate the liquid and solid fractions. The fluid obtained was classified as rumen fluid extract (RFE) and was retained for further processing to obtain adherent ruminal bacteria.

After separation the SRF and RFE were processed by differential centrifugation. The first spin (121 x g) removed feed particles and protozoa while the second spin (45,000 x g) fractionated the first supernatant into a supernatant and a bacterial pellet. The final supernatant was retained for protease activity determination and the pellet was resuspended in ten ml of 0.1 M potassium phosphate buffer and vortexed until a homogeneous washed cell supension (WCS) was produced. The WCS was analysized for protease and deaminase activity, as well as, RNA and protein content.

Protease Analysis

Protease activity was determined on the free floating and adherent bacterial fractions by the method of Brock et al. (1982). Two ml of the WCS were added to one ml of a three percent azocasein solution. The sample was incubated in a 39 C water bath for ninty minutes. The incubation period was completed by the addition of 0.3 ml of fifty percent TCA. Immediately following the addition of the precipitant the sample was placed on ice for approximately thirty minutes, and then centrifuged at 27,000 x g for fifteen minutes. Two ml of the resulting supernatant were

added to two ml of an 1 N NaOH solution, vortexed for approximately thirty seconds and allowed to sit at room temperature for fifteen minutes prior to reading at 440 nm on the Gilford spectrophotometer. Cell-free supernatant was handled similarly to the WCS in determining the protease activity. With the supernatant, however, one ml of sample was combined with one ml of 0.1 molar potassium phosphate buffer and one ml of the three percent azocasein solution. Blanks were prepared in a similar manner to the experimental samples except TCA additions preceded the addition of the WCS and cell-free supernatant. For each set of samples a standard curve was constructed using final concentrations of .006, .012, .018, and .024 units of (Sigma Type P5380 Bacillus subtilis alkaline protease, crystallized and lypholized, 12 units/mg) protease enzyme activity per ml to calculate the proteolytic activity present in the three fractions.

Deaminase Analysis

A modified version of the Broderick and Balthorp (1979) procedure was used in the determination of deaminase activity. The original assay system required strained rumen fluid while the modified version replaced SRF with WCS. Two ml of the suspension were added to a solution composed of 0.4 ml of an 1.8 percent casein hydrolysate and 1.6 ml of a 0.1 M potassium phosphate buffer. Blanks were prepared with

two ml of the potassium phosphate buffer rather than two ml of WCS. The sample was incubated for three hours. One ml of a fifty percent TCA solution was added to terminate the reaction before the sample was placed on ice. After thirty minutes the sample was centrifuged at 27,000 x g for fifteen minutes with the resulting supernatant being assessed for total amino acid remaining by the alpha amino nitrogen analysis (Palmer and Peters, 1969). Thus, 0.2 ml of the supernatant was added to a solution containing 1.6 ml of 0.05 M sodium borate and 0.2 ml of twenty-five percent solution of fresh trinitrobenzene sulfonic acid. The combination was incubated at 39 C for twenty minutes followed by addition of two ml of 1 N hydrochloric acid. The absorbance was read on the Gilford spectrophotometer at 420 nm. Citrulline was used to constructed a standard curve.

RNA Determination

Two ml of WCS and two ml of ten percent TCA were combined in a twevle ml centrifuge tube and placed on ice for thirty minutes. The bacterial pellet was harvested via centrifugation (27,000 X g) for fifteen minutes. The WCS pellet remaining was frozen and used later for RNA and protein determination.

RNA was determined in WCS pellets according to Schneider (1957). Ribonucleic acids were extracted by

hydrolyzing the pellet in six ml of five percent TCA for thirty minutes in a 97 C water bath. The sample was allowed to cool prior to centrifugation at 45,000 x g for twenty minutes. The supernatant was carefully decanted and placed into an appropriately labeled test tube. Two ml of the supernatant was added to two ml of the orcinol reagent (one ml of orcinol per 100 ml of hydrochloric acid containing 0.5 g of ferric chloride). The sample was covered heated for twenty minutes in a 97 C water bath, allowed to cool to room temperature and then, read at 660 nm on the Gilford spectrophotometer. Standards were prepared in a similar manner from a stock solution composed of Sigma ribonucleic acid prepared from Torula Yeast. Concentrations of the standards utilized in the construction of the standard curve were .008, .010, .040, .100, and .200 mg/ml.

Protein Determination

The pellet remaining after the RNA extraction was completely hydrolyzed in three ml of an 1 N NaOH by placing them in a 97 C boiling water bath for five minutes to extract the protein. Hartree (1972) modification of the Lowry procedure (1951) was used to determine the amount of protein present in the bacterial fractions. Since the Lowry procedure is only linear within a certain range of protein concentration 0.1 ml of the extract was mixed with 0.9 ml of water to insure protein concentration was within

the designated range. The diluted extract was then combined with 0.9 ml of reagent A (Table 5) and incubated for ten minutes in a 50 C water bath. Following the incubation the sample was cooled and 0.1 ml of reagent B (Table 5) was added. The sample was vortexed and placed at room temperature for approximately ten minutes. Finally, three ml of fresh Folin-phenol reagent (Table 5) were added and the final combination was vortexed and placed in a 50 C water bath for ten more minutes. Following a cooling period the sample was read on the Gilford spectrophotometer at 650 nm. Bovine serum albumin was used to create a standard curve.

Volatile Fatty Acid Analysis

Five ml of strained rumen fluid were placed in a twelve ml centrifuge tube containing one ml of fresh twenty-five percent meta-phosphoric acid, mixed and placed at room temperature for thirty minutes. This step deproteinized the sample. The sample was vortexed for thirty seconds and centrifuged at 15,000 x g for fifteen minutes. The supernatant was transferred to injection vials and capped. Two microliters of the extract was analyzed by a Hewlett-Packard gas-liquid chromatograph (Model 5840A). A stainless steel (183 cm by .32 cm) Chromosorb column (10% SP 1200 and 1% phosphoric acid 80/100 WAW mesh) (Supelco Inc., Bellefonte, PA) was used. The following conditions were

TABLE 5. LOWRY REAGENTS.

Reagent A:
Two g potassium sodium tartrate (sodium potassium tartrate) and 100 g sodium carbonate are dissolved in 500 ml of 1 N sodium hydroxide and diluted with water to 1 liter.
Reagent B:
Two g potassium sodium tartrate and one g copper sulfate are dissolved in ninety ml water and ten ml 1 N sodium hydroxide is added.
Phenol Reagent:
Use commercially prepared Harleco Item No. 2690. (Phenol Reagent, Folin and Ciocalteu). Dilute one part plus ten part water. Diluted solution will be stable for several weeks.

programed into the GC: column temperature, 125 C; injection temperature, 170 C; flame ionization detector temperature, 175 C and carrier gas flow (helium) 25 ml/min. A Supelco VFA standard was used to determine the concentration of acetate, propionate and butyrate present in the rumen fluid sample. Total run time for each sample was approximately 20 minutes.

Ammonia Nitrogen Analysis

Five ml of the mercuric chloride fixed rumen fluid were placed in a twelve ml centrifuge tube containing one ml of nine normal sulfuric acid. The resulting mixture was vortexed for thirty seconds and centrifuged at 45,000 x g for fifteen minutes. The supernatant was transfered to a suitable vial and analyzed on a Technicon Auto Analyzer II. A stock solution (one mg N/ml) of ammonia sulfate was prepared and used to construct a standard curve.

Statistical Analysis

The vivo study was statistical analyzed by Genstat analysis for a repeat measure Latin square design. Bonferroni T statistic was used for the treatment comparisons.

RESULTS

In Vitro Experiment

Growth curves, OD vs time, were constructed for each organism with respect to the various antibacterial agents and their varying concentrations. The curves for an organism within a particular treatment were compared to determine the minimum concentration (i.e. .5, 2.5, 5, 10, or 20 ug/ml) required to inhibit the organisms growth. This concentration will be referred to as the minimum inhibitory concentration or MIC.

The antibacterial agents (Table 6) used in the experiment were ineffective against S. ruminantium, M. elsdenii, and B. ruminicola, three gram negative organisms. Lasalocid, however, did increase S. ruminantium's lag phase at all the examined concentrations. A similar shift in the lag phase was observed when B. ruminicola was exposed to the full spectrum of antibacterial agents. B. succinogenes and B. fibrisolvens, two other gram negative organisms, were sensitive to the antibiotics. Both monensin and narasin completely inhibited B. succinogenes at the .5 ug/ml level while 2.5 ug/ml of lasalocid was required for similar results. Narasin-actaplanin combination did not change the growth rate of B. succinogenes. As for B. fibrisolvens, monensin and lasalocid inhibited growth at .5

TABLE 6. MINIMAL INHIBITORY CONCENTRATIONS FOR RUMINAL BACTERIA

BACTERIA	GRAM STAIN	MON-ENSIN	LASA-LOCID	NARASIN	COMBIN-ATION
S. rumintium	-	NI	NI	NI	NI
M. elsdenii	-	NI	NI	NI	NI
B. ruminicola	-	NI	NI	NI	NI
B. succinogenes	-	I(.5)	I(2.5)	I(.5)	NI
B. fibrisolvens	-	I(.5)	I(.5)	I(2.5)	I(5)
R. flavefaciens	+	I(2.5)	I(.5)	I(20)	I(2.5)
R. albus	+	I(2.5)	I(.5)	I(2.5)	I(5)
S. bovis	+	I(.5)	I(.5)	I(5)	I(5)

Calculated from growth curves (OD vs. time).

NI means not inhibited.

I means inhibited.

ug/ml while narasin and the combination caused growth inhibition at the 2.5 and 5.0 ug/ml respectively, These concentrations were 5 and 10 fold higher than the effective concentrations for monensin and lasalocid.

The gram positive organisms examined, contrary to many of the gram negative organisms, showed sensitivity to the antibacterial agents. R. flavefaciens demonstrated sensitivity to lasalocid at .5 ug/ml while monensin and the combination caused inhibition at a concentration five times (2.5 ug/ml) that of lasalocid. Narasin was relativily ineffective at low concentrations but caused complete growth inhibition of R. flavefaciens at 20 ug/ml. R. albus was susceptible to lasalocid at .5 ug/ml concentration while monensin and narasin concentrations had to be elevated to 2.5 ug/ml before growth inhibition occurred. Narasin-actaplanin combination, the least effective against R. albus, inhibited its growth at 5 ug/ml. Sensitivity of S. bovis to monensin and lasalocid was observed at .5 ug/ml and complete growth inhibition was detected at a 10 fold higher concentration when narasin and the combination were present.

In summary under these experimental conditions the gram negative species, except for B. succinogenes and B. fibrisolvens, were resistant to the antibacterial agents while the gram positive species showed a level dependent sensitivity to the compounds. It appeared that within the sensitive species monensin and lasalocid were generally more

effective inhibitors of growth than either narasin or the narasin-actaplanin combination.

In Vivo Experiment

Volatile Fatty Acid Concentrations

Ruminal volatile fatty acid concentrations (mM) were measured to examine feed additive effects on ruminal fermentation end-products.

Ruminal acetate concentrations (mM) for the four treatments with time after feeding are presented in Table 7. For all treatments only the narasin-actaplanin combination (combination) significantly differed ($P<.05$) from the other treatments. At all sampling times the acetate concentration in the combination fed animals was significantly lower ($P<.05$) than in the other treatment groups. The basic pattern for acetate concentration as well as the other volatile fatty acids, was a cyclic pattern based upon the feeding regime. Initially, the concentration would start at a prefeeding level, increase and plateau at a peak level and then decline to the prefeeding level just prior to refeeding. In general across the four treatments the acetate concentration followed this cyclic pattern. For both the control and actaplanin diets acetate concentration significantly increased ($P<.05$) between the prefeeding and two hours postfeeding concentration. This upper concentration was maintained through the 7 hour sample and

TABLE 7. RUMINAL ACETATE CONCENTRATIONS ACROSS TREATMENT WITH TIME AFTER FEEDING

TIME	TO	T2	T4	T7	T10
TREATMENT	----------mM----------				
Control	52.3	65.4	60.4	64.7	56.9
Monensin	52.3	65.2	65.1	58.2	54.7
Actaplanin	44.7	55.7	55.4	56.3	45.0
Combination	38.9	50.7	51.4	45.3	41.0
SEM	3.0				

Means of four animal.

Overall treatment means differ (P<.05).

Row means with different superscripts differ (P<.05).

by 10 hours after feeding the acetate concentration had significantly declined (P<.05) to the prefeeding value. The monensin and combination treatments followed a similar pattern except the plateau stretched only until the four hour sample. By the seven hour sampling period a significant (P<.05) decrease in ruminal acetate concentration was observed with both the monensin and combination treatments.

Propionate concentrations (mM) are represented in Table 8. The combination had a significantly higher (P<.05) ruminal propionate concentration across all sampling times in comparison to the other treatments. The general trend within treatments was similar to that observed with ruminal acetate concentration. For the control, monensin and actaplanin treated animals the propionate concentration significantly increased (P<.05) between the prefeeding and two hours postfeeding values. This increase was followed by a gradual, although nonsignificant, decline over the next ten hour period. As for the combination treated animals the ruminal propionate concentration significantly increased (P<.05) initially, plateaued and declined significantly (P<.05) between seven and ten hours after feeding.

The four feed additives contained in this experiment did not significantly alter the butyric acid concentrations (Table 9). The treatment means, therefore, were pooled and examined with respect to changes in ruminal butyrate concentrations over the feeding cycle. Ruminal butyrate mean concentrations significantly changed over time (P<.05)

TABLE 8. RUMINAL PROPIONATE CONCENTRATIONS ACROSS TREATMENT WITH TIME AFTER FEEDING.

TIME	TO	T2	T4	T7	T10
TREATMENT	----------	----------	mM ----------	----------	----------
Control	18.0	24.4	21.6	22.0	18.8
Monensin	18.3	24.1	23.4	21.6	19.5
Actaplanin	18.9	25.0	24.7	24.5	22.0
Combination	28.4	34.0	36.1	34.9	29.5
SEM	1.4				

Means of four animal.

Overall treatment mean differs from other treatment ($P<.05$).

Row meanswithdifferent superscriptsdiffer ($P<.05$).

TABLE 9. OVERALL TREATMENT MEANS FOR BUTYRATE CONCENTRATION ACROSS TREATMENT

TREATMENT	mM
Control	12.0
Monensin	11.9
Actaplanin	11.8
Combination	8.4
SEM	1.3

Means for four animals

(Table 10). The butyric acid concentration increased significantly ($P<.05$) between prefeeding and two hours postfeeding, then remained constant for the next five hours at which point it significantly declined ($P<.05$) to the prefeeding level by ten hours after feeding.

Protease Activity of Adherent and Free Floating Bacteria

The adherent and free floating bacteria were examined to ascertain antibacterial treatment effects and feeding effects on bacterial protease activity.

Adherent bacterial protease activity exhibited no significant treatment effects (Table 11). The antibacterial compounds were ineffective in altering proteolytic enzyme activity of the adherent bacterial population. Treatment means were pooled to examine time after feeding effects on protease activity (Table 12). Significant time effects were not evident under these experimental conditions, as protease activity (u units of protease activity/ mg protein) remained constant over the feeding cycle. Although there seemed to be a slight numerical decline in proteolyic activity of the adherent bacteria at the four hour sample, this fluctuation was not significant.

Similarly, significant treatment differences in protease activity were not observered in the free floating bacterial fraction (Table 13),thus, the pooled treatment means were used as before to probe for significant time

TABLE 10. POOLED TREATMENT MEANS FOR BUTYRATE CONCENTRATION WITH TIME AFTER FEEDING

TIME	mM
0	8.8
2	12.5
4	12.5
7	11.6
10	9.7
SEM	.6

Means for four animals.

Means with different superscripts differ (P<.05).

TABLE 11. OVERALL TREATMENT MEANS FOR PROTEASE ACTIVITY FOR ADHERENT RUMEN BACTERIA

TREATMENT	u units/mg protein
Control	7.35
Monensin	7.65
Actaplanin	6.25
Combination	5.45
SEM	1.30

Means for four animals.

TABLE 12. POOLED TREATMENT MEANS FOR PROTEASE ACTIVITY FOR ADHERENT BACTERIA WITH TIME AFTER FEEDING

TIME	----------u units/mg protein----------
0	6.75
2	6.81
4	5.25
7	7.81
10	6.75
SEM	.96

Means for four animals.

TABLE 13. OVERALL TREATMENT MEANS FOR PROTEASE ACTIVITY FOR FREE FLOATING RUMEN BACTERIA

TREATMENT	----------u units/mg protein----------
Control	8.55
Monensin	8.35
Actaplanin	6.10
Combination	6.85
SEM	.91

Means for four animals.

after feeding effects. Unlike the adherent bacteria significant time effects ($P<.05$) were demonstrated by the free floating bacterial fraction. Protease activity significantly increased ($P<.05$) between the initial sampling period and four hours postfeeding and then decreased significant ($P<.05$) between four and ten hours after feeding (Table 14).

Cell Free Supernatant Proteolytic Activity

Overall treatment means for the four treatments are presented in Table 15. The compounds examined were unable to change assayable protease activity of the cell free supernatant. The means were again pooled to evaluate the changes in supernatant protease activity in the rumen over the daily feeding cycle. Pooled means showed no significant time differences (Table 16) in proteolytic activity in this fraction. Although minor numerical fluctuations were observed, the proteolytic capacity of the cell free supernatant remained constant over the feeding cycle under these experimental conditions.

Deaminase Activity of Adherent and Free Floating Bacteria

Values reported for adherent and free floating bacterial deaminase activity have been expressed as umoles of amino acids remaining per milligram of bacterial protein

TABLE 14. POOLED TREATMENT MEANS FOR PROTEASE ACTIVITY FOR FREE FLOATING BACTERIA WITH TIME AFTER FEEDING

TIME	----------u units/mg protein----------
0	5.94
2	8.50
4	10.69
7	7.38
10	4.81
SEM	.95

Means for four animals.

Means with different superscripts differ (P<.05).

TABLE 15. OVERALL TREATMENT MEANS FOR PROTEASE ACTIVITY FOR CELL FREE SUPERNATANT

TREATMENT	u units/mg protein
Control	8.80
Monensin	8.60
Actaplanin	8.85
Combination	10.00
SEM	1.50

Means for four animals.

TABLE 16. POOLED TREATMENT MEANS FOR PROTEASE ACTIVITY FOR CELL FREE SUPERNATANT WITH TIME AFTER FEEDING

TIME	----------u units/mg protein----------
0	8.86
2	10.25
4	9.63
7	10.13
10	6.69
SEM	1.70

Means for four animals.

per incubation. When comparing values between treatments or across time lower levels of amino acids relate to higher deaminase activity and visa versa.

Deaminase activity of adherent ruminal bacteria was not significantly affected by the antibacterial agents (Table 17). Pooled treatment means showed significant ($P<.05$) time after feeding effects with the four hour sample being significantly lower ($P<.05$) than the two hour sample (Table 18).

Deaminase activity of free floating bacteria was found to violate one of the primary assumptions of statistics, homogeneous variance (Gill,1978). Neither the simple square root transformation nor the log transformation resolved the problem of heterogenous variance. Hence, it was impossible to conduct a proper statistical analyses on this data and, therefore, this data can not be discussed further.

RNA/Protein Ratios for Adherent and Free Floating Bacteria

RNA to protein ratios (R/P) for adherent bacteria are shown on Table 19. The treatment mean for narasin-actaplanin combination was significantly lower than the treatment mean for monensin treated animals. Comparisons between corresponding times across treatments, however, did not reveal any significant differences (Table 19). The significant differences were probably blanketed by the negative correlation between the R/P ratios and time. R/P

TABLE 17. OVERALL TREATMENT MEANS FOR DEAMINASE ACTIVITY FOR ADHERENT RUMEN BACTERIA

TREATMENT	-------umoles of amino acids/mg protein--------
Control	1.56
Monensin	1.83
Actaplanin	1.48
Combination	1.48
SEM	.12

Means for four animals.

TABLE 18. POOLED TREATMENT MEANS FOR DEAMINASE ACTIVITY FOR ADHERENT BACTERIA WITH TIME AFTER FEEDING

TIME	----------umoles amino acid/mg protein----------
0	1.75
2	2.09
4	1.42
7	1.42
10	1.27
SEM	.21

Means for four animals.

Means with different superscripts differ (P<.05).

TABLE 19. RNA/PROTEIN MEANS FOR ADHERENT BACTERIA ACROSS TREATMENT WITH TIME AFTER FEEDING

TIME	TO	T2	T4	T7	T10
TREATMENT					
Control	.297	.336	.275	.433	.428
Monensin	.282	.233	.399	.449	.459
Actaplanin	.247	.269	.278	.505	.422
Combination	.220	.261	.239	.485	.359
SEM	.070				

Means of four animal.

Overall treatment means differ ($P<.05$).

ratios did not change over time in either the control or monensin treated animals while R/P ratios for the combination and the actaplanin treatments were significantly ($P<.05$) higher at seven hours after feeding than initially. All other points did not significantly differ from one another.

The antibacterial agents did not alter R/P ratios for the free floating rumen bacteria (Table 20), however, pooled treatment means showed significant ($P<.05$) change over the daily feeding cycle in this fraction. The R/P ratio at seven hours after feeding was higher than the initial and two hours post feeding ratio (Table 21).

Rumen Ammonia Levels

Rumen ammonia levels (mM) were not significantly effected by the antibacterial agents (Table 22). The pooled treatment means, however, showed significant ($P<.05$) time after feeding effects. Ruminal ammonia nitrogen concentration increased significantly ($P<.05$) over the first two hours after feeding (Table 23). This increase was followed by a persistant decline in ruminal ammonia nitrogen concentration which occurred over the next five hours.

TABLE 20. OVERALL TREATMENT MEANS FOR RNA/PROTEIN FOR FREE FLOATING RUMEN BACTERIA

TREATMENT	
Control	.381
Monensin	.366
Actaplanin	.329
Combination	.336
SEM	.030

Means for four animals.

TABLE 21. POOLED TREATMENT MEANS FOR RNA/PROTEIN FOR FREE FLOATING RUMEN BACTERIA WITH TIME AFTER FEEDING

TIME	
0	.278
2	.289
4	.381
7	.435
10	.382
SEM	.030

Means for four animals.

Means with different superscripts differ (P<.05).

TABLE 22. OVERALL TREATMENT MEANS FOR RUMINAL AMMONIA NITROGEN CONCENTRATION

TREATMENT	mM
Control	10.1
Monensin	10.6
Actaplanin	9.5
Combination	8.8
SEM	.4

Means for four animals.

TABLE 23. POOLED TREATMENT MEANS FOR RUMEN AMMONIA CONCENTRATION WITH TIME AFTER FEEDING

TIME	----------mM----------
0	8.1
2	17.6
4	10.5
7	6.2
10	6.3
SEM	.7

Means for four animals.

Means with different superscripts differ (P<.05).

DISCUSSION

In Vitro Experiment

Both Chen and Wolin (1979) and Henderson (1981) while studying pure cultures of ruminal bacteria illustrated that ionophoric antibiotics, monensin and lasalocid, were less effective in inhibiting the growth of gram negative organisms than gram positive organisms. Among the gram negatives S. ruminantium proved to be the most resistant while B. fibrisolvens and B. succinogenes showed the highest sensitivity. B. fibrisolvens, although it stains as a gram negative, possesses a gram positive like cell wall structure (Cheng and Costerton, 1977) which may account for the organisms high suspectibility to ionophores. B. succinogenes may adapt to the antimicrobial agents over time but initially this organism shows sensitivity. R. albus, R. flavefaciens and S. bovis, gram postive organisms, were reported to be inhibited by both monensin and lasalocid (Chen and Wolin, 1979; Henderson, 1981). In the present investigation results similar to reports by Chen and Wolin (1979), Henderson, (1981) were observed for all organisms except B. succinogenes. B. succinogenes in the present study showed high sensitivity to monensin, lasalocid and

naracin, however, previous experiments (Chen and Wolin, 1979) revealed that B. succinogenes adapted to concentrations in excess of 10 ug/ml. The differences between experiments may be attributed to differences in concentration exposure time. Chen and Wolin (1979) used inoculum from cultures grown in the presence of 5 ug/ml of antibiotic and observed growth approximately seventy-two hours later in both monensin (20 ug/ml) and lasalocid (10 ug/ml). In the current investigation pretreated cultures (.5 ug/ml) were exposed to the new concentration for only forty hours. Adaption may not have been completed before termination of the experiment.

The effect of an ionophore-glycopeptide combination on pure culture of ruminal bacteria growth has not been examined previously. From studies reported by Stewart et al. (1983) and the present study some conclusions may be drawn about ionophore-glycopeptide combinations. Stewart et al. (1983) reported that ruminal microogranisms displayed similar resistance and sensitivity when exposed to the glycopeptide, avoparcin, as when exposed to ionophoric compounds. Avoparcin, however, seemed to be required in higher concentrations than ionophores to cause the same extent of inhibition. The present results showed that narasin-actaplanin combination was slightly less effective than narasin alone but more effective than avoparcin (derived from Stewart et al., 1983). Since both the ionophore (narasin) and glycopeptide (actaplanin) were

present in equal proportions in the combination and assuming that actaplanin yields antibacterial effects similar to avoparcin, then the growth inhibition caused by the combination in the present experiment may be solely due to narasin as the actaplanin concentration in all cases was below it probable MIC (Stewart et al., 1983). B. fibrisolvens and R. albus results support this concept since the MIC concentration for the combination was twice that for narasin. The results for R. flavefaciens and S. bovis appear to conflict with the hypothesis. In these cases there may have been a synergistic effect between the ionophore and glycopeptide. To be certain of these hypotheses, however, actaplanin, narasin and the narasin-actaplanin combination minimium inhibitory concentrations should be examined. Unfortunately the pure glycopeptide was not available for this study.

In general the in vitro data seemed to infer that monensin and lasalocid were more potent inhibitors of ruminal microorganisms than either narasin or the narasin-actaplanin combination. Taylor and Nagaraja (1983) reported that narasin was a more effective inhibitor of lactate-producers than either monensin or lasalocid. No direct conclusions about actaplanin's ability to inhibit microbial growth can be discussed, however, Stewart et al. (1983) suggested glycopeptides potency may have been less than the ionophores.

The results described here, as previous studies (Chen

and Wolin, 1979; Henderson et al., 1981), support the current hypothesis that the growth promoting effect of ionophores and glycopeptides in feedlot cattle is related to a shift in ruminal bacteria towards succinate and propionate producers. Raun et al. (1976), however, reported that the shift in volatile fatty acid concentration towards propionate could not account for all the improvement in performance. Therfore, although it appears that VFA shift is the main contributor to antibiotics effects many other factors may also be important contributors and thus warrant investigation (ie proteolysis and lower gut metabolism).

In Vivo Experiment

An in vivo study was designed to investigate the effects of glycopeptides and ionophores on rumen fermentation when feeding an all corn silage diet. More specifically the experiment examined the effects antibacterial agents have on molar proportions of VFAs, bacterial protease and deaminase activity, bacterial RNA to protein ratios and ruminal ammonia-N concentration. The following sections shall discuss the experimental results.

Volatile Fatty Acids

Dinius et al. (1976), Richardson et al. (1976), Potter et al. (1976) and Raun et al. (1976) reported that total volatile fatty acids concentrations were not altered by the addition of monensin to the diet of feedlot cattle from 22 to 33 ppm while molar proportions of VFAs were shifted towards propionate and away from acetate. Richardson et al. (1976) also observed a decline in butyrate level while Dinius et al. (1976) found no change in butyric acid. At low levels of monensin (11 ppm) Dinius et al. (1976) observed no changes in total VFA or individual VFA concentrations. Froetshel et al. (1983) examined ruminal fermentation changes upon feeding avoparcin and reported the glycopeptide significantly decreased total ruminal VFA concentration by reduction of acetate, butyrate and isovalerate. Propionate concentration when expressed as mM was not affected by avoparcin but when expressed as molar percent of total VFA the proportion of propionate was significantly increased (Froetshel et al., 1983). In the present experiment monensin was fed at 12.5 ppm and alteration in VFA concentrations were not observed which agrees with data reported by Dinius et al. (1976). Actaplanin at 30 ppm did not alter ruminal VFA concentrations in the present study, but Froetschel et al. (1983) observed that avoparcin, at 50 ppm, only decreased ruminal acetate conentration but had no effect on ruminal propionate. Assumming that glycopeptides affect rumen

fermentation similarly to ionophores then actaplanin concentration used in the present in vivo experiment was probably below the dietary level needed to affect ruminal VFA concentrations. The narasin-actaplanin combination (16 and 10 ppm, respectively) increased ruminal propionate molar concentration while significantly decreasing the molar concentrations of acetate. This glycopeptide-ionophore combination may, under feedlot conditions, have feed efficiency enhancing abilities and thus, may be a viable alternative to monensin.

Volatile fatty acid concentrations with respect to time after feeding have a cyclic pattern as reported by both Dinius et al. (1976) and Siddons and Paradine (1983). Shortly after a meal ruminal concentration of VFA increase, peak between 2 to 4 hours after feeding and decline to prefeeding concentrations about 8 to 10 hours after feeding. The characteristic increase of VFA concentrations is due to the fermentation of incoming substate by ruminal microoganisms while the subsequent decline may be attributed to end-product removal via the rumen wall, liquid absorption, substrate depletion or bacterial uptake of these compounds. In this experiment the cyclic pattern of VFA concentrations with time after feeding was also observed.

Protease and Deaminase Activity in the Rumen

Poos et al. (1979), Isichei (1980) and Muntifering et al. (1981) reported an increase in dietary nitrogen passage to the abomasum upon addition of monensin in the diet of cattle. This suggested that ruminal proteolysis was decreased during monensin feeding. Van Nevel and Demeyer (1977) and Whetstone et al. (1983) showed that for mixed ruminal cultures the addition of monensin decreased protein degradation. Dinius et al. (1976) and Barao et al. (1983), in agreement with the present investigation, found that proteolysis activity, measured in vivo, was not significantly affected by monensin at any level tested. Bates and Bergen (1983) reported Bacteroides ruminicola GA33 did not show a significant inhibition of proteolysis, however, they did report a significant sugar-monensin interaction suggesting that substrate availability may play an important role in the monensin effect on bacterial proteolytic activity. It should be noted that methods for assaying proteolysis in these experiments differed and this may account for different results. Bates and Bergen (1983), Barao et al. (1983) and the present investigation assayed enzyme activity and reported a rate for protein degradation while the others (Poos et al., 1979; Isichei, 1980; Muntifering et al., 1981) reported gross amounts of protein disappearance or appearance over a longer time interval. Enzyme rates of protein degradation are not always synonymous with the gross amount of protein

disappearence. The fact that workers such as Poos et al. (1979), Isichei (1980) and Muntifering et al. (1981) observed decreased ruminal feed protein digestion with monensin may be related to the change in total production of protease enzymes in the rumen rather than a change in the rate of proteolysis per mg of bacterial cell. The rate of protein hydrolysis can not be inferred as a measurement of total protease activity under these conditions.

The reports on glycopeptides have been very limited, thus far and for ruminal feed protein degradation little research has been conducted with these compounds. Froetshel et al. (1983), however, did observe a decrease in ruminal ammonia nitrogen concentration with the feeding of avoparcin which may be due to a decrease in ruminal proteolysis and/or ruminal deamination. The present investigation did not confirm this idea.

In this experiment as well as by Barao et al. (1983), protease activity of the free floating bacteria changed over the daily feeding cycle. For the adherent population examined under these experimental conditions their protease activity remained constant over the sampling period. Although no direct evidence exist to confirm or dispute this observation results reported by Gillett et al. (1982) on RNA to protein ratios implied adherent bacterial exist as a stationary phase population in the rumen. If protease activity were assumed to be linked to growth rate then a slow growing or stationary population would have constant

protease activity. However, data to be discussed in a later section suggested that under these experimental conditions the harvested adherent population was not in a stationary situation. The interpretation of these results is unclear, therefore, no definitive conclusions can be drawn from this data.

Tobert et al. (1977) and Schelling et al. (1978) suggested that monensin may have a protein-sparing effect which may be mediated in part through inhibition of amino acid deamination. Chalupa et al. (1980) evaluated the effects of monensin and amicloral upon batch culture deaminase activity, and reported both compounds were effective in decreasing utilization of exogeneous amino acids by ruminal bacteria. Barao et al. (1983) reported that in free floating bacteria deaminase activity was decreased when monensin was added to a soy-urea supplemented diet. In the present investigation ruminal adherent bacteria deaminase activity was not altered by the presence of ionophores, glycopeptides or the drug combination. With the discrepancy between the former and latter results there seems to be a need for further investigation of the effects of antibacterial compounds on free-floating ruminal bacteria deaminase activity.

With time after feeding, deaminase activity in the adherent population changed significantly. As with other parameters, deaminase activity appeared to peak (2 to 4 hours after feeding) during the time of maximal fermentation

activity and maximal growth of the adherent bacteria. The present investigation suggests then that deaminase activity of the adherent bacteria may be closely related to bacterial fermentation and metabolism. Barao et al. (1983) reported similar findings with the free floating bacterial population in the rumen.

RNA to Protein Ratios

Bergen and his associates (1982) reported RNA to Protein ratios fluctuate in the rumen and concluded these changes were highly correlated with bacterial growth rate. High RNA to Protein ratio (R/P) (.3-.5) represent an actively growing bacterial population while R/P ranging from .2 to .3 suggested stationary phase populations (Bates et al., 1985). Therefore, changes in R/P ratios may be used to characterize bacterial growth in the rumen. In the in vivo experiment R/P ratios were determined with respect to both treatment and time effects in adherent and free-floating bacteria. In general ionophores , glycopeptides and a combination were not effective in altering R/P ratios (growth rates) in comparison to the control. The measurements were taken from animals adapted to the diets for twenty-one days and thus the bacteria present in the rumen upon sampling was representive of a resistant population. Therefore, the ecosystem in the rumen would be expected to react to substrate addition as would a "typical"

rumen population. Changes in R/P ratio may be apparent during the adaptation time since organisms are adjusting there metabolic processes to cope with the compound. Unfortunately, this has yet to be addressed.

Monensin and the combination treatment means were significantly different ($P<.05$), however, the differences were not expressed with the treatment by time comparison. The negative correlation which existed between the R/P ratios and time may have been responsible for these results. This correlation is explained by the graph of R/P over time (Figure 2). The area of concern are where the lines cross one another. Since the curves cross the a negative correlation is the result. This may be caused by either sampling problems which result in outlying values. If the outlying values were to be removed, then the results seem to imply that the narasin-actaplanin combination depress the growth rate of the ruminal bacteria in comparison to the control.

Observation of R/P ratios for both the adherent and free floating bacterial fractions illustrated that R/P significantly change over the feeding cycle. Gillett et al. (1982) showed that R/P ratios in adherent bacteria do not change with respect to time after feeding while ratios for the free-floating bacteria change over time. The conflicting results may be resolved by the fact in the present study cold anaerobic dilution solution and gentle stirring were the method used to remove the adherent bactera

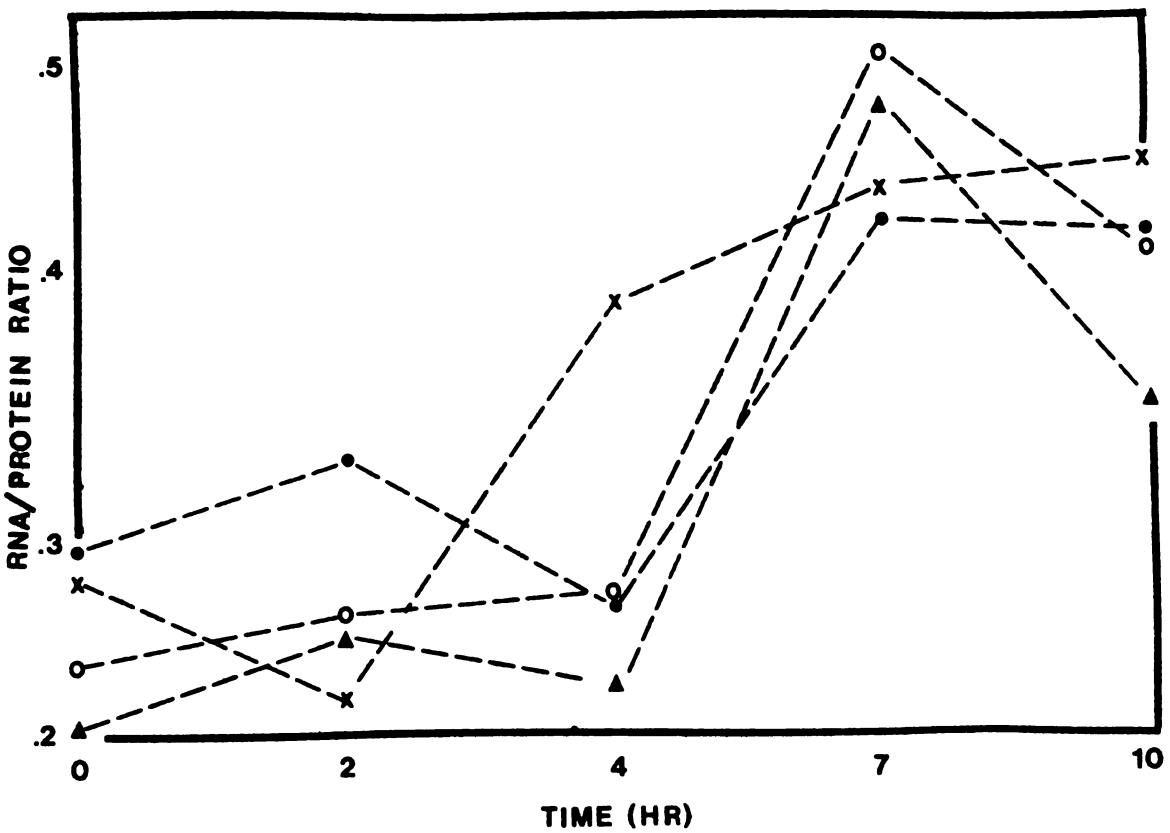

FIGURE 2. RNA/PROTEIN RATIOS FOR ADHERENT BACTERIA ACROSS TREATMENTS WITH TIME AFTER FEEDING.

x CONTROL
• MONENSIN
o ACTAPLANIN
▲ COMBINATION

from the feed particles while Gillett et al. (1982) homogenized the sample in a Waring blender. Merry and McAllan (1983) reported using 4 C Tween buffer without homogenization lead to recovery of 49 % of the adherent bacterial population while homogenization increased recovery to 65 %. Therefore, in the present experiment loosely bound bacteria or free-floating bacteria simply trapped during the squeezing process were probably harvested while Gillett et al. (1982) extracted the more tightly adhering bacteria. At present this hypothesis of an artifact adherent bacteria population is being investigated.

In the present study the RNA/Protein ratios for adherent and free-floating bacteria ranged between .31 to .36 and .32 to .38, respectively. Bates et al. (1985) reported ratios of .23 and .29 for adherent and free floating bacterial populations harvested from sheep feed a high corn silage diet. The latter experiment suggests in high corn silage diets the adherent bacteria are in a stationary phase of growth while the free-floating bacteria approach exponential growth rates. The former illustrates high corn silage diets support exponential growth in both bacterial fractions. These differences may be attributed to availability of carbohydrates in the diets. Unfortunately, only book values were reported for both diets, therefore, it is impossible to determine if any differences truly existed. However, if the corn silage diet fed the steers provided more fermentable carbohydrates to the rumen bacterial than

the diet fed to the sheep (Bates et al., 1985) then these differences may be a result of energy supply. Another more feasible explanation may be that the differences in the extraction procedures between the experiments may have harvested two distinctly different populations of bacteria.

Rumen Ammonia Nitrogen

Ruminal ammonia nitrogen can be classed as the end-product of ruminal protein degradation. General pattern of ammonia nitrogen concentration in the rumen have been reported by Dinius et al. (1976) and Siddons and Paradine (1983). Both groups reported cyclic patterns of ammonia nitrogen concentration with time after feeding. Similar results were observed in the present experiment. Thus, like VFA concentration, ruminal ammonia nitrogen concentration appears to be hinged on the feeding cycle.

Dinius et al. (1976) besides reporting on the cyclic nature of ruminal ammonia concentrations stated that monensin addition to cattle diets did not significantly alter the ammonia concentration. Hanson and Klopfenstein (1979), Thompson and Riley (1980) showed similar results in vivo while Van Nevel and Demeyer (1977) illustrated a reduction in ammonia concentration in vitro with monensin. Once again the reports on monensin conflict. For glycopeptides ruminal ammonia-N and other parameters

investigated in the present experiment have not been extensively researched. Froetshel et al. (1983) observed upon addition of 50 ppm avoparcin to a low fiber diet a significant decrease in rumen ammonia-N was apparent, however, these changes were not observed in high fiber diets. Under the present experimental conditions significant effects of glycopeptides were not observed with any treatment. As suggested by Froetshel et al. (1983) with high concentrate diets these feed additives alter ammonia nitrogen in the rumen but Barao et al. (1983) disputes this claim. With corn silage diets, as in the current experiment, the feed additives may not alter ammonia concentration.

Relationship of VFA and Ammonia Concentration and Protein Degradation and Bacterial Growth in the Rumen

Bates and Bergen (1983) suggested that RNA to protein ratios were indicators of microbial growth. High ratios imply growing population while low ratios suggest a more stationary phase population. In the present investigation, as mentioned before, the free-floating bacterial population R/P ratio changed with respect to time after feeding. Since the adherent bacteria data violated the assumption of homogeneous variance it will not be included in the present discussion. If the other parameters measured in the experiment with respect to time after

feeding were superimposed over one another it becomes quite appearant that the general fluctuations in each parameter coincide with one another except for the rumen ammonia concentration which seemed to peak earlier than the others. In other words, the rumen system fluctuates and the changes observed in bacterial growth or protease activity parallel one another thus illustrating the interdepedence of the total cyclic pattern of the rumen.

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