AN ABSTRACT OF THE THESIS OF

TIMOTHY DELCURTO for the degree of MASTER OF SCIENCE in Animal Science presented on August 12, 1986.

Title: SUPPLEMENTATION WITH LASALOCID THREE TIMES WEEKLY TO STOCKER CATTLE ON PASTURE

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Two experiments were conducted to study the efficacy of feeding lasalocid to cattle on pasture on a three times weekly basis. In experiment 1, 72 crossbred yearling steers were stratified across a randomized block design of 12 pens including three replications of four treatments. Treatments consisted of: (1) .45 kg ground corn per head per day; (2) .45 kg ground corn per head three times weekly on Monday, Wednesday, and Friday; (3) .45 kg ground corn with 200 mg lasalocid per head per day; (4) .45 kg ground corn with 467 mg lasalocid per head three times weekly. Lasalocid fed everyday increased ($P<.05$) average daily gains (ADG) by 10%. Lasalocid fed three times weekly did not have a significant effect on ADG.

In experiment 2, six rumen fistulated heifers were allotted randomly to two 3x3 Latin Squares. Heifers consumed a diet of mid-bloom alfalfa hay ad libitum plus the treatment supplements. Treatments consisted of: (1) .45 kg ground corn per head per day; (2) .45 kg ground corn with 200 mg lasalocid per head per day; (3) .45 kg

ground corn with 467 mg lasalocid per head three times weekly. Gas chromatographic analysis indicated that lasalocid fed daily or on a three times weekly basis did not change the acetate to propionate ratio ($P>.10$). Both lasalocid treatments increased ($P<.05$) the butyrate to propionate ratio. Analysis of all C2-C5 rumen metabolites indicate that only butyrate was significantly affected by lasalocid treatments. The addition of lasalocid on a daily basis decreased ($P<.05$) the pH within the rumen. After centrifugation, high performance liquid chromatographic (HPLC) analysis failed to detect lasalocid in the rumen fluid.

Feeding lasalocid on a three times weekly basis did not appear practical based on weight gain responses. Measurement of ruminal VFA and pH indicate that while slight differences exist between the two lasalocid treatments, they do not correlate with the lack of weight gain response with animals fed three times weekly.

SUPPLEMENTATION WITH LASALOCID THREE TIMES WEEKLY TO STOCKER CATTLE ON PASTURE

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

SUPPLEMENTATION WITH LASALOCID THREE TIMES WEEKLY TO STOCKER CATTLE ON PASTURE

REVIEW OF LITERATURE

Introduction

Ruminant animals are unique in respect to their symbiotic relationships with digestive bacteria and protozoa. The host animal and rumen microbe interaction allows for utilization of low quality feedstuffs not suitable for human and nonruminant animals. While fermentation in the rumen is a definite advantage to the host animal, it has been the desire of ruminant nutrition researchers to manipulate rumen metabolism. An increase in propionate at the expense of acetate, a decrease in methane production, and a decrease in ruminal protein catabolism have all been targeted as specific areas of potential benefit to the animal. It has only been within the past ten years that chemical agents have been utilized for desirable manipulation of rumen function. Among these chemical agents are carboxylic polyether ionophore antibiotics (henceforth referred to as ionophores) that were originally used as anticoccidial feed additives for poultry. These ionophores are produced by various strains of streptomyces and include monensin, lasalocid, salinomycin, and narasin. The results of utilizing these drugs in livestock feeding regimes include increased average daily gain (ADG) and improved feed utilization.

What follows is a review of the current literature relevant to the effects of ionophores on the ruminal-reticular environment.

Modification of the Ruminal-Reticular Environment

Mode of Action

Ionophore Function. It is generally accepted that the effects of lasalocid and monensin on ruminal fermentation are due to changes in the microbial ecology of the rumen (Chen and Wolin, 1979; Wallace et al., 1981). Gram positive bacteria that are the primary acetate, butyrate, hydrogen, and formate producers are inhibited by the ionophores. Gram negative strains, many of which produce succinate, are not as sensitive. Methanogenic bacteria are not directly inhibited by ionophores; however, methanogenisis decreases due to the decrease in hydrogen and formate substrate availability (Van Nevel and Demeyer, 1977).

In very simple terms, ionophores facilitate the transport of ions across biological membranes. In figure 1, the structure of lasalocid indicates that ionophores have a hydrophobic and hydrophilic side. The transport cycle begins with the anionic form of the ionophore on the external surface of the bimolecular lipid membrane. Here the molecule is stabilized by the polar environment characteristic to the surface of the membrane and rumen fluid (Orchinnikov, 1979). As an anion, the ionophore is capable of binding to various cations present in the ruminal-reticular fluid. The ionophore-cation bonding occurs at the carboxylic acid end or at other internal

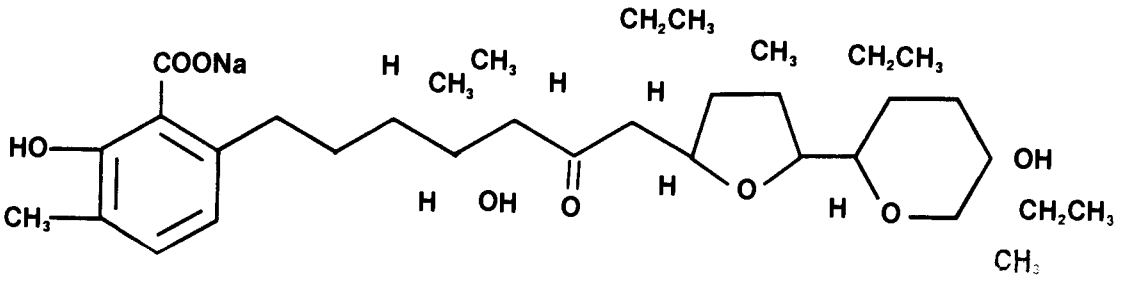

Figure 1. Chemical structure of lasalocid-sodium, active ingredient of Bovatec, the commercial product produced and marketed by Hoffmann-La Roche Inc.

hydroxyl sites. The binding of the cation results in the formation of a cyclic lipophilic complex that can diffuse through the interior of the phospholipid bilayer of the microbial membrane (Bergen and Bates, 1984). Ultimately, the complex reaches the internal side of the membrane where it is again subject to a polar environment. The electrostatic forces that had stabilized the complex are no longer greater than the unfavorable ΔG° (Gibbs free energy change) of cyclization and the ionophore releases its enclosed cation and reverts to the low energy acyclic conformation. The next step of the transport cycle is the movement of the ionophore back across the lipid membrane. Both monensin and lasalocid enter microbial membranes in this fashion (Pressman, 1976). A salient feature of this model is that the ionophore must be in the anionic form before it is capable of binding to a metal cation (M^+) (see figure 2; Painter et al., 1982).

Ionophores do not display the same affinity for all cations. Monensin facilitates primarily $Na^+ - H^+$ exchange because the affinity of monensin for Na^+ is ten times that for K^+, its nearest competitor (Pressman, 1976). Experimental results indicate that monensin crosses the membrane in a 1:1 complex with Na^+; the return passage of the ionophore takes place in only the protonated form (Sandeaux et al., 1982). A study by Harold (1972) revealed that protons were obligatorily coupled to Na^+

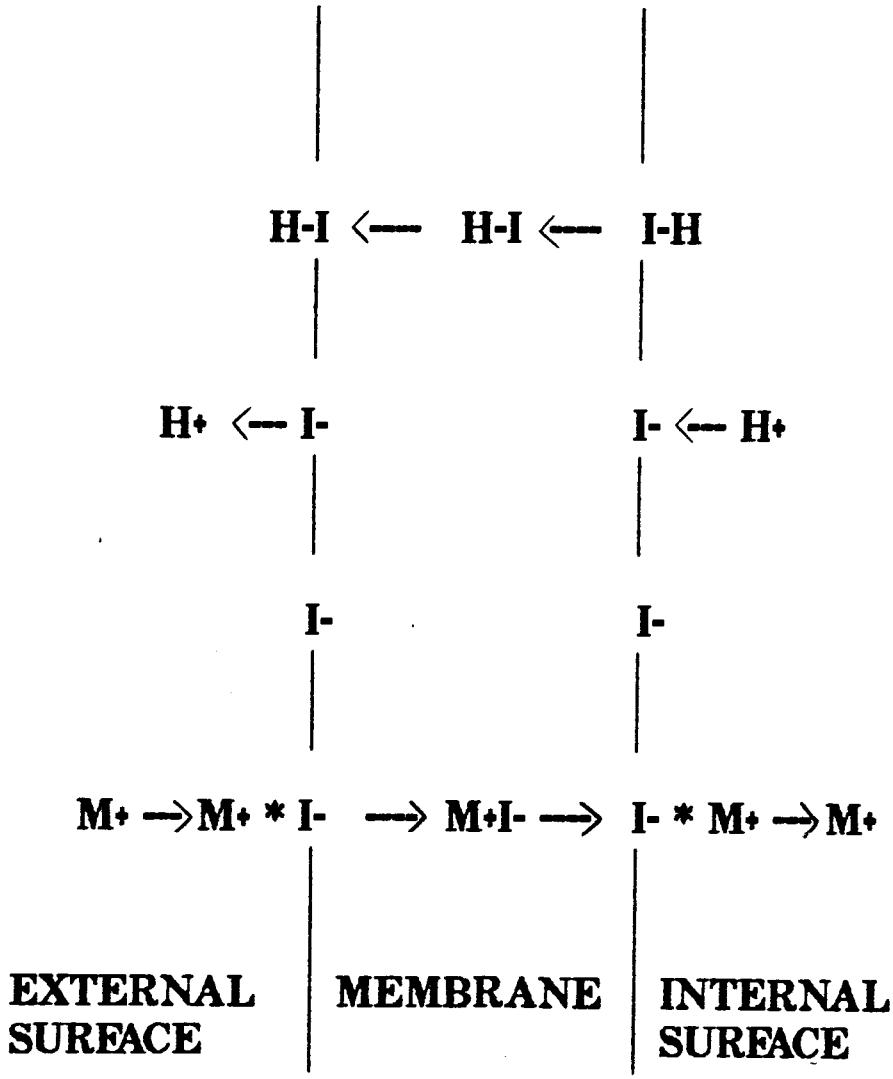

Figure 2. Carboxylic ionophore mediated cation transfer across a bimolecular lipid membrane. M^+=metal cation; M^+I=zwitterion of metal cation and anion form of ionophore (Pressman, 1976).

transport and moved from high potential to low potential energy areas.

Lasalocid does not display an obligatory cation-proton antiport mechanism. Instead, an electroneutral Ca^{2+} for $2K^{+}$ exchange has been identified (Haynes, et al., 1980). The affinity constant of lasalocid's carboxylic acid end for monovalent cations ranges from $10^{1.3}$ to $10^{2.0}$, with the Km for Na^{+} around $10^{2.0}$ and for K^{+} about $10^{1.7}$. Unlike monensin, lasalocid is capable of transporting divalent cations. The Km of lasalocid for divalent cations range from $10^{1.9}$ to $10^{3.2}$ (Haynes and Pressman, 1974). In general terms, lasalocid displays a higher affinity for K^{+} and an equal affinity for Ca^{++} and Na^{+}. The rate and degree with which a cation will be transported by lasalocid will depend upon the affinity constant for the cation and the relative concentration gradient of the ion across the membrane (Bergen and Bates, 1984). Breakdown or decline of several ion gradients can occur simultaneously with lasalocid-cation transport.

To understand the mode of action for ionophores, the significance of biological membranes must be appreciated. Membranes act as barriers to free diffusion. The high resistance to diffusion enables membranes, with the aid of active and active-coupled transport, to regulate the movement and concentration of substances in the intracellular and extracellular environment. The existence of

electrochemical concentration gradients across the membrane implies that the membrane actively participates in the transport and regulation into and out of the cell. In the bacteria cell, the membrane precisely regulates the intracellular concentration of ions and molecules, establishing an environment favorable for balanced metabolic and synthetic activities of the cell.

Both monensin and lasalocid dissipate electrical and chemical gradients between external and internal portions of the membrane. The cation "leakage" associated with ionophores disrupts the intricate system described above and leads to cell disfunction. The onset of cation "leakage," causes the cell to expend ATP to reestablish electrical and chemical gradients (Jolliffe et al., 1981). Eventually, the microbe is inhibited due to a lack of ATP necessary for metabolic functions. The destruction of gradients limits the cell in metabolic maintenance and functions. Several experiments have shown this effect on microbes relative to Na^+-dependent active transport (Andu et al., 1982; Kitado and Houkashi, 1982). Ionophores, bacteriostatic in action, cause a deficiency in cellular energy and, thus, the microbe is severely inhibited in normal functions.

Microbial Ecology. The ability of lasalocid to disrupt the cation balance in cells is associated with its mechanism of action on biological membrane systems. In

terms of benefit to ruminal-reticular fermentation, however, the selective inhibition of bacteria is of greater significance.

The introduction of ionophores to the rumen causes a shift in microbial populations. Several factors may be involved in the selection process. First, the cell envelope of gram negative bacteria consists of a multilayered complex of two distinct membranes separated by a rigid peptidoglycan layer. The most likely function of this outer membrane is to serve as a penetration barrier (Kadner and Bassford, 1978). It is generally believed that gram negative bacteria are resistant to ionophores because the ionophore cannot traverse the outer membrane.

The selective inhibition of gram positive bacteria results in a decrease of lactate, formate, butyrate, and hydrogen producers (Chen and Wolin, 1979; Bartley and Nagaraja, 1982). The gram negative bacteria resistant to ionophores are succinate producers, lactate fermentors, and methane producers. While gram positive bacteria are inhibited, total concentration of ruminal-reticular VFA are unchanged (Rogers and Davis, 1982; Shell et al., 1983). It can be inferred that the decreased competition for digestive substrate leads to an increase in gram negative bacteria.

It appears that membrane differences are not the only

explanation for ionophore selectivity. The gram negative bacterial species Bacterides fibrisolvens, Ruminococcus albus, and Ruminococcus flavefaciens are also sensitive to lasalocid and monensin. This may partially be due to gram-positive-like cell wall structure (Patterson et al., 1975; Cheng and Costerton, 1977).

Many ruminal strains of bacteria have linked the extrusion of H^+ ions to the process of electron transport allowing them to benefit from ionophore presence (Bergen and Bates, 1984). These bacteria are able to derive energy from the extrusion of H^+ ions. In essence, these bacterial strains are unaffected and/or possibly enhanced by ionophore presence in the ruminal-reticular environment. As a result, these bacteria have an obvious advantage over those that depend exclusively on the utilization of ATP to maintain cellular proton gradients (Bergen and Bates, 1984).

Modification of Ruminal-Reticular Acids

Volatile Fatty Acid Production

One of the most often cited benefits derived from ionophores is a favorable shift in proportions of acetate, propionate, and butyrate. The largest end products of microbial digestion are volatile fatty acids (VFA) and these in turn represent the largest quantity of nutrients absorbed by the host ruminant. With a roughage diet, relative VFA ratios in the rumen are typically 60:30:10, while with a high concentrate diet, the ratios are closer to 45:45:10 for acetate, propionate, and butyrate, respectively. The lower acetate to propionate ratio is due partly to the pathways used by microbes to digest starch versus fiber. When large quantities of starch become available in the rumen, bacteria ferment it rapidly to lactate and propionate. With slower fermentation, as obtained with frequent feeding, limited feed intake, or roughage diets, fermentation is more constant, resulting in increased acetate production. The VFA are largely absorbed in the rumen, with only ten percent flowing out of the rumen to be absorbed in the omasum.

The concept that propionate is more efficiently utilized by the host than acetate or acetate precursors is based on two factors. First, propionate production by rumen fermentation appears to be more efficient than that

of acetate (Hungate, 1966; Chalupa, 1977). The second factor, and much more controversial, is that there is evidence for propionate being utilized more efficiently in the tissues (Smith, 1971). An obvious advantage of propionate is that it is gluconeogenic, in addition to being able to enter the citric acid cycle. Having more substrate for glycolysis may provide significant energetic advantages to the ruminant at specific times by generating more reduced coenzyme A outside the mitochondrial membrane (Schelling, 1984). Researchers have also reported that the increased ruminal propionate results in an increased glucose metabolism rate with a subsequent sparing effect on protein that might have otherwise been metabolized to form gluconeogenic precursors (Van Maanan et al., 1978; Armentano and Young, 1983). These results tend to support the theory that propionate is the most efficiently utilized ruminal VFA.

Feeding trials have shown that monensin and lasalocid can significantly alter ruminal proportions of VFA. The addition of monensin to high concentrate diets has been shown to decrease ruminal acetate and butyrate concentrations, with a concurrent increase in propionate (Van Nevel and Demeyer, 1977; Prange et al., 1978; Rogers and Davis, 1982; Shell et al., 1983). While the relative levels of individual VFA are altered by feeding ionophores, they do not appear to affect total

concentrations of VFA (Richardson et al., 1976; Shell et al., 1983). Trials conducted with lasalocid report similar effects on individual and total ruminal VFA concentrations and ratios (Fuller and Johnson, 1981; Thonney et al., 1981).

On high concentrate diets, the effects of ionophore on ruminal VFA production is well documented. On roughage diets, however, changes in VFA concentrations are less obvious. Fuller and Johnson (1981) conducted an in vitro study comparing rumen fermentation as affected by lasalocid in roughage and concentrate diets. Results from their study indicate that lasalocid, when fed with a high roughage diet, had no significant effects on ruminal VFA patterns. In a similar study with high roughage diets conducted by Spears and Harvey (1984), acetate levels were unchanged while propionate levels increased and butyrate and valerate levels decreased. To further complicate the picture, Armentano and Young (1984) found a significant increase in butyrate and propionate and a decrease in acetate production. While these studies seem to contradict each other, additional studies seem to indicate that the effect of ionophores in roughage diets on ruminal VFA concentrations are marginal and varies according to the type of roughage fed.

While ionophores don't significantly affect ruminal VFA patterns in roughage diets, significant weight gain

responses in roughage fed animals have been reported (Thonney et al., 1981; Spears and Harvey, 1984; DelCurto and Weber, 1985). Thus, it appears unlikely that the effect of ionophores on VFA patterns account for a significant portion of the weight gain response with cattle on roughage or concentrate diets. The increased fermentation efficiency responses reported in some work (Owens, 1980) could account for a benefit equivalent only to a 1.6% increase in digestible energy (Schelling, 1984). These results tend to suggest that other ionophore-fermentation modifications are taking place.

Branch Chain Fatty Acids

The effect of ionophores on ruminal branch chain fatty acid (BCFA) production has not been well researched. With recent research revealing the positive benefits of supplementing BCFA to dairy cattle, it is obvious that these digestive end-products could play an active role in rumen fermentation. Research trials have demonstrated that BCFA are utilized by bacteria for biosynthesis of amino acids (Bryant, 1973). Muira et al. (1983) reported BCFA-dependent bacteria do not require preformed BCFA for growth, but growth and cellulose digestion were enhanced with BCFA additions.

While it is generally accepted that BCFA additions improve rumen fermentation, research recently has

indicated that increased dietary BCFA lead to increases in growth hormone (GH) (Fieo et al., 1984; Towns et al., 1984). Towns et al. (1984) also found that feeding BCFA caused a decrease in insulin and blood glucose. It is probably safe to conclude that if lasalocid or monensin enhances BCFA production in the rumen, the animal will display more efficient digestion and possibly an increase in GH.

Studies with ionophores have not shown a significant effect on ruminal BCFA concentrations. Fuller and Johnson (1981) found that lasalocid, but not monensin, decreased ruminal isovalerate production, while isobutyrate and valerate levels remained unchanged. Other studies with monensin and lasalocid have reported no significant effect on BCFA (Horn et al., 1981; Spears and Harvey, 1984).

While research indicates that ionophores have no appreciable effects on BCFA levels, the fact that BCFA are not decreased warrants merit. As will be discussed later, ionophores tend to reduce deamination of amino acids in the rumen (Dinius et al., 1976; Schelling et al., 1977). BCFA are derived from the oxidative deamination of the corresponding branch chain amino acids in the diet. Therefore, if BCFA levels are unchanged by ionophore supplementation, efficient ruminal-reticular fermentation will be maintained.

Lactic Acid

Ionophores have been found to inhibit bacteria that produce lactate as a major end product. In particular, Eubacterium, Lochnospira, Lactobacillus, Ruminococcus, and Streptococcus are inhibited by both monensin and lasalocid (Bartley and Nagaraja, 1982). In addition, many of the major lactate fermentors in the rumen are unaffected by lasalocid or monensin (Chen and Wolin, 1979; Bartley and Nagaraja, 1982). Studies by Dennis et al. (1981) indicate that lasalocid inhibits all strains of Streptococcus while monensin inhibits most strains of Streptococcus with the exception of Streptococcus bovis 124 strain. While there appears to be a difference between the two ionophores, both are effective in reducing ruminal-reticular lactic acid levels (Dennis et al., 1981; Nagaraja et al., 1981).

While the above observations probably have little to do with the feed efficiency and weight gain responses observed with ionophore-fed cattle, the work indicates some advantages in feeding ionophores to aid in the prevention of lactic acidosis. Lactic acidosis is a common feedlot digestive disorder associated with the rapid conversion of cattle from a high roughage to high concentrate diet. Lactic acid accumulates in the rumen following large carbohydrate (starch) intake. Ultimately, the high levels of lactic acid in the rumen lead to acidotic conditions in the blood stream of the ruminant

animal. Initial signs of acidosis include depressed feed intake and performance commonly associated with mild digestive disturbances. In more severe states, blood pH declines to the point of coma and death. Studies with induced-acidotic cattle show that ionophores decrease the severity and occurrence of lactic acidosis (Dennis et al., 1981; Nagaraja et al., 1981).

Changes in Gas Production

Eructation of methane gas represents 5 to 11% of the total gross energy of the diet (Hungate, 1966). Ionophores have been shown to decrease methane production in ruminants with both concentrate and roughage diets (Bartley et al., 1979; Joyner et al., 1979; Chalupa et al., 1980; Thornton and Owens, 1981).

Ionophores have no effect on gram negative bacteria that produce methane as a major end product. However, bacteria that produce hydrogen and formate, substrates for methanogenesis, are inhibited by ionophores (Van Nevel and Demeyer, 1977; Chen and Wolin, 1979). The reduced methane, hydrogen, and formate production in the rumen represent a more efficient energy system in terms of microbial end products available for host digestion.

While reported studies indicate that ionophores do reduce methane production, it is only a partial inhibition. Many chemical agents are much more effective

at inhibiting methane production; however, most result in an increase in hydrogen gas production, in contrast to ionophores (Chalupa et al., 1980). The effect of ionophores on reducing methane production and the benefit derived by the host animal are not entirely understood. Researchers report a decrease in methane between 13 to 31% (Schelling, 1984). The reduction of carbons lost as methane, CO_2, and formate represent additional energy available to the host animal.

Changes in Protein Utilization

Metabolic changes in ruminal, postruminal and tissue nitrogen (N) metabolism with ionophore supplementation have been implicated (Bergen and Bates, 1984; Schelling, 1984). In the presence of ionophores, ruminal NH_3-N production is decreased (Chalupa, 1980). This finding implies that ionophores have either an overall depressing effect on total ruminal bacteria cells resulting in a decrease in proteolytic and deaminating enzymes, or a direct effect on both protease and deaminase activity (Van Nevel and Demeyer, 1977). Regardless of the exact mode, ionophores do improve the utilization of high quality feed proteins by changing their site of degradation from the rumen to the abomasum and small intestine.

The escape from ruminal degradation (protein bypass) of preformed dietary protein in the presence of ionophores has been studied in vivo. In studies by Poos et al. (1979) and Owens (1980) ionophores increased protein bypass by 22 to 55%. In addition, reduction in ruminal N, as correlated to a decrease in deamination of amino acids, has been cited (Schelling et al., 1977; Hansen and Klopfenstein, 1979). In a recent study by Beede et al. (1986), monensin supplementation with steers on a low or marginal protein diet increased N digestibility and N retention. A subsequent decrease in urine and fecal N was also identified (Beede et al., 1986).

The ultimate fate of dietary N in ruminal-reticular fermentation has many possible routes. Frequently, dietary nitrogen is broken down into various amino acids by various proteolytic enzymes. From here, the amino acids can be either passed to the abomasum or small intestine for absorption, or they can be further degraded by deaminase enzymes. If they are deaminated in the rumen, they are either resynthesized by bacteria to another amino acid or they are utilized by the host animal as a C2-C5 fatty acid. This step often involves the loss of dietary protein and subsequent increase in ruminal ammonia or urea. It is clear from the previous literature cited that ionophores decrease ruminal ammonia and increase the proportion of dietary proteins reaching the abomasum and small intestine. This, in turn, allows for more efficient protein utilization and benefits the ruminant in both quantity and quality of dietary protein absorbed.

Other Ionophore Related Benefits

Many of the indirect benefits resulting from ionophore supplementation and corresponding modification of rumen ecology have been discussed in previous sections. There are other benefits, however, that are derived from ionophore-related changes in ruminal-reticular ecology. It is safe to assume that while many benefits have been cited, there are still many others that remain unknown. Because most of the beneficial changes associated with ionophore supplementation are associated with metabolic end products of the ruminal-reticular microflora, it is very difficult to quantitatively estimate their significance to the host animal. What follows is a brief discussion of the less cited benefits derived, yet important in a holistic approach, from ionophore supplementation.

3-Methylindole (3MI) is a fermentation product of L-tryptophan (TRP) that causes acute bovine pulmonary edema and emphysema (ABPE) (Carlson et al., 1972; Carlson et al., 1975; Yokoyama et al., 1975). This naturally occurring disease is associated with an abrupt transition from dry, low-quality forage to lush pasture and can be induced experimentally by oral TRP administration (Selman et al., 1974; Yokoyama et al., 1975). Recent research has shown that various antibiotics can reduce the formation of 3MI in the rumen (Hammond et al., 1980). Potchoiba and

coworkers (1984) found that monensin supplementation lowered the rate of 3MI production as well as total production over time. In vitro and in vivo studies with lasalocid (Nocerini et al., 1985) noted similar declines in 3MI production and subsequent prevention of ABPE. The results of these studies indicate that ionophores can be useful in preventing a nutritional disease associated with stocker/commercial cattle.

Research has also indicated that feeding ionophores to heifers results in earlier puberty (McCartor et al., 1979). The problem in validating this statement involves proving that the early puberty response is independent of the weight gain increase associated with ionophores. Studies with propionate infusion to prepuberal heifers, however, have indicated an increased release of luteinizing hormone resulting from a gonadotropin releasing hormone change (Rutter et al., 1981). Thus, the propionate increase associated with ionophores can also have beneficial hormonal effects with prepuberal heifers.

An oftentimes overlooked benefit of ionophores is the reduced occurrence or prevention of coccidiosis. As stated earlier, lasalocid was originally developed as a coccidiostat used in poultry feeds. Lasalocid has been found to decrease the occurrence of coccidia in both feedlot cattle and lambs (Fitzgerald and Mansfield, 1970; Horton and Stockdale, 1981).

The supplementation of ionophores to mature cattle have revealed marginal benefits. Turner et al. (1977) reported monensin-fed cows outgained control cows on less intake. Feed efficiency was, as a result, improved with the monensin-fed cattle. Walker and coworkers (1980) also reported decreased intake and improved feed efficiency; however, no change was observed in cow weights, condition score, calf birth weights, 205-day adjusted weaning weights, or first service conception rates. In a similar study with lasalocid, Hopman et al. (1985) found no difference between lasalocid-treated and control cows in terms of cow weights, body condition score, twelve hour milk production, percent milk fat and protein, average days open, calf weights, and 205-day adjusted weaning weights. These results suggest that the primary benefit of feeding ionophores to mature cattle is seen primarily in improved feed efficiency.

Toxicity of Ionophores

Lasalocid and monensin are safe to use in beef cattle at the recommended levels of 10 to 30 grams per ton. At this level, most of the drug is confined to the digestive system. The small quantities of drug that do reach the blood stream are metabolized by the liver and excreted via the kidneys (Donoho, 1984). Monensin does not accumulate in the tissues of orally dosed animals. When fed to cattle and chickens according to recommended practices, monensin was not detected (less than .05 ppm) in edible tissues (Donoho, 1984). Donoho (1984) also found both lasalocid and monensin to be biodegradable in manure and soil.

Improper feed preparations and drug additions can lead to toxicity problems with cattle. In a study by Potter et al. (1984), toxicity associated with extreme monensin supplementation occurred only when the cattle first received the drug. After initial consumption, cattle would no longer consume the high concentrated monensin supplement. For this reason, acute LD_{50} is the best and most meaningful measurement of ionophore toxicity. With cattle, the acute LD_{50} for monensin is 26.4 mg/kg of body weight (Potter et al., 1984). It is interesting to note that the consumption of lasalocid at over five times the recommended level (30 g/ton) produced no clinical toxicological effects (Messersmith and Hanson,

1982).

The location of target organs and clinical signs of ionophore toxicity have been determined (Todd et al., 1984). Animals given toxic doses of ionophores develop anorexia, hypoactivity, weakness, ataxia, diarrhea, and body weight loss. These signs are usually delayed for one to several days after drug intake, depending on dosage, and are reversible even when animals are maintained on an ionophore supplement (Todd et al., 1984). The target organs most extensively damaged by toxic doses of monensin were skeletal and cardiac muscle. The muscles most severely and extensively damaged are those that maintain the highest level of activity, such as the diaphragm, abdominal, and heart muscles. As expected, the effect of ionophores on the tissue of the host animal is directly related to disruption of electrochemical gradients across bimolecular lipid membranes. More specifically, ionophores produced ultrastructural changes in muscles consisting of dilated sarcoplasmic reticulum, misshaped mitochondria, and lysis of myofibrils (Griffing et al., 1971). The functional disturbances observed in animals given toxic doses of monensin can be explained by the structural changes in muscles due to the altered transport of cations (Todd et al., 1984).

While the clinical signs of ionophore toxicity are similar for all mammals, the reported level of drug

required for toxicity (LD_{50}) is variable. The LD_{50} estimates for monensin are 2 to 3 mg/kg body weight for the horse, 16.8 mg/kg body weight for swine, 11.9 mg/kg body weight for sheep, and 26.4 mg/kg body weight for goats (Elanco Products Company, 1978b). LD_{50} estimates for lasalocid are 21.5 mg/kg body weight for the horse and 28.7 mg/kg body weight for swine (Messersmith and Hanson, 1982). It should be obvious from these figures that horses are very susceptible to ionophore toxicity, especially that associated with monensin.

STATEMENT OF THE PRESENT PROBLEM

The most important aspect of any feed additive or any change in management is the return of income to the producer. Ionophores have been found to increase feed efficiency (Raun et al., 1976; Boling et al., 1977; Hanson and Klopfenstein, 1979) and improve weight gains with feedlot cattle (Davis, 1978; Brethour, 1979; Brown and Davidovich, 1979). Goodrich and coworkers (1984) summarized 228 trials with monensin-fed feedlot cattle and found that on the average they gained 1.6% faster and consumed 6.4% less feed than control cattle. Cattle fed lasalocid have been shown to gain faster (Brethour, 1979) and more efficiently (Brethour, 1979; Brown and Davidovich, 1979) when fed moderate to high energy diets than did monensin-fed cattle. Monensin, however, has the advantage of being cleared for use with tylosin phosphate (Heinemann et al., 1978). Tylosin fed continuously at 50 mg, 75 mg, and 100 mg daily increases average daily gains, improves feed efficiency, and reduces the occurrence of liver abscesses (Brown et al., 1973).

Supplementation of ionophores to cattle on pasture has also been shown to be beneficial. Both lasalocid and monensin have been found to increase ADG with cattle on pasture (Horn et al., 1981; Spears and Harvey, 1984; DelCurto and Weber, 1985). In all of these trials, the ionophore was hand-fed in a ground grain carrier.

Studies with self-fed supplements have yielded mixed results. In a study conducted by Males and coworkers (1979), monensin hand-fed in a ground grain supplement had a significant effect on ADG, while monensin fed in a liquid supplement had no effect on ADG. Likewise, DelCurto and Weber (1985) reported that lasalocid fed in a self-fed mineral did not increase ADG. Both of these studies indicate that variable consumption patterns over time and between animals tend to limit the potential of self-fed supplements.

Although the cost of ionophores is low, the cost of supplementation can be expensive. The actual cost of lasalocid per head per day is a little more than a penny per day (W. E. Brandt, personal communication). In feedlot situations, the drug can be easily added to rations with little labor and no added supplemental cost. With cattle on pasture, however, often the producer has to either buy the supplement or add the drug to a supplement already in use. As stated above, self-fed supplements yield variable results and may not prove to be an effective means of providing the drug to the animal. Hand-fed grain supplements seem to be a very effective carrier, yet, in most cases the producer has to buy the carrier and feed it on a daily basis. The cost of the grain carrier and the labor cost associated with daily feeding may prove to outweigh the benefit derived from the

drug.

Most of the ionophore research in the past five years has been directed toward stocker and commercial cattle supplementation techniques. Manipulation of salt content in self-fed mineral supplements has helped to assure adequate drug consumption (Muller et al., 1986). Recently, interest has been aroused in feeding ionophores in hand-fed supplements on an alternate day basis. Muller and coworkers (1986) summarized five research trials feeding monensin on an alternate day basis. In their study, monensin supplemented in a hand-fed carrier on an alternate day basis increased daily gains over controls and was no different than monensin supplement hand-fed on a daily basis.

The report that follows is a similar study in which lasalocid supplementation is evaluated on a three times weekly basis.

EXPERIMENT 1 AND 2: SUPPLEMENTATION WITH LASALOCID THREE TIMES WEEKLY TO STOCKER CATTLE ON PASTURE

Introduction

Lasalocid, a polyether antibiotic ionophore, favorably modifies ruminal-reticular fermentation. One of the most often cited benefits is the molar increase of propionate at the expense of acetate (Chalupa, 1977; Van Maanen et al., 1978; Armentano and Young, 1983). Lasalocid also decreases the catabolism of proteins and deamination of amino acids in the rumen allowing for by-pass of proteins to the abomasum and small intestine where they are more efficiently utilized (Van Nevel and Demeyer, 1977; Chalupa, 1980). Other ionophore mediated benefits include: reduction of methane (Bartley et al., 1979), reduction of lactate (Dennis et al., 1981), reduced occurrence of acute bovine pulmonary emphysema (Nocerini et al., 1985), and modified feed intake (Schelling, 1984).

Both monensin and lasalocid have been found to increase feed efficiency (Raun et al., 1976; Boling et al., 1977; Brethour, 1979) with feedlot cattle. Lasalocid has also improved weight gains with feedlot cattle (Davis, 1978; Brethour, 1979; Brown and Davidovich, 1979). In cattle grazing pasture or fed forage in confinement, average daily gain has been improved when ionophores were fed at 200 mg/day (Horn et al., 1981; Spears and Harvey,

1984; DelCurto and Weber, 1985). In all of these trials, the ionophore was hand-fed in a ground grain carrier.

Studies incorporating self-fed supplements as ionophore carriers have yielded mixed results. In a study by Males et al. (1979), monensin hand-fed in a ground grain carrier increased ADG, while monensin fed in a liquid supplement had no effect on ADG. Likewise, DelCurto and Weber (1985) reported that lasalocid fed in a self-fed mineral carrier had no effect on weight gains for test animals. Both of these studies seem to indicate that variable consumption patterns between animals and over time tend to limit the potential use of self-fed supplements.

Ionophores are extensively used in feedlot production schemes. The application of ionophore supplementation to stocker and commercial cow-calf production has yet to reach its full potential. In many cases the producer has the added expense of buying a supplement to act as a carrier for the ionophore. As stated earlier, the use of self-fed supplements has yielded variable results. Hand-fed supplements seem to offer the most potential, yet the cost of the grain carrier, in addition to the labor associated with daily feeding, may outweigh the possible benefits.

The objective of this research trial was to evaluate the efficacy of feeding lasalocid in a hand-fed grain

carrier on a three times weekly basis. In Experiment 1, stocker cattle were used to measure weight gain responses associated with control, lasalocid daily, and lasalocid 3X weekly feeding. In Experiment 2, the objective was to make possible correlations between rumen metabolite changes and observed differences in weight gain response in Experiment 1.

Materials and Methods

Experiment 1. Seventy-two head of exotic-British crossed yearling steers were stratified by weight across three replications of four treatments. The average weight of steers within pens was 267 ± 3 kg. All of the animals were from the same management background. All steers were wormed one week prior to the study and growth implants were not used.

Treatments consisted of: (1) .45 kg ground corn per head per day; (2) .45 kg ground corn per head three times weekly; (3) .45 kg ground corn with 200 mg lasalocid per head per day; (4) .45 kg ground corn with 467 mg lasalocid per head three times weekly. The treatments were hand-fed in feedbunks associated with each paddock of animals. As a result, consumption of the treatment supplements was on a pen basis and not on an individual animal basis.

Each replication of the four treatments also corresponded to a different location. All paddocks or

pens of cattle were similar in size and forage available. The average paddock size was 2.5 HA and each had a plentiful water source. The forage species consisted of tall fescue, perennial ryegrass, meadow foxtail, and various bromes and clovers. All paddocks had a source of cover and a mineral feeder.

Pens of cattle were allotted to treatment and location at random. The pens were rotated in a random manner every 14 days to reduce any variation due to pasture differences. Steer weights were taken every 28 days; however, only initial and final shrunken weights (held off feed and water for 18 hours) were used in the analysis. Shrunken weights were used to reduce variation due to fill. The trial began April 22 and concluded July 29, for a total of 98 days.

Statistical Analysis. Daily gains were analyzed by analysis of variance. Specifically, a randomized block design, with location being the block effect, was utilized to evaluate treatment effects (Steele and Torrie, 1960). Treatment means were compared using least significant differences analysis (LSD).

Experiment 2. Six rumen-canulated heifers were allotted at random to two 3x3 latin squares (see figure 3). Heifers were of mixed exotic-British crosses and averaged 340 kg at the beginning of the trial. All the

Square	1			2		
Heifer	1	2	3	4	5	6
Period 01	A*	B	C	A	B	C
Period 02	B	C	A	C	A	B
Period 03	C	A	B	B	C	A

** Letters A, B and C denote control, lasalocid and lasalocid 3X weekly treatments respectively.*

Figure 3. 3x3 Dual Latin Square Design of Experiment 2. Heifers were allotted at random to initial treatments of the first crossover.

heifers had similar prior management before the initiation of the experiment.

Each animal was housed in an individual pen, with an adequate supply of water and minerals. The pens were identical; therefore, rotating animals between pens was not carried out. Heifer diets consisted of mid-bloom alfalfa hay fed ad libitum at 6:00 morning and night.

Treatments consisted of: (1) .45 kg ground corn per head per day; (2) .45 kg ground corn with 200 mg lasalocid per head per day; (3) .45 kg ground corn with 467 mg lasalocid per head three times weekly. The treatments were hand-fed to each animal following procurement of the rumen samples.

The experimental protocol for each crossover of animals to treatments involved three distinct periods (see figure 4). First, the animals were placed in an equilibration period for the first 11 days. During this period, the animals were allowed to readjust to the alfalfa diet without treatment supplements. In effect, the time was used for rumen metabolism/fermentation to re-equilibrate prior to initiation of the next treatment. The second period involved the initiation of the treatment supplements, which lasted five days. The final period was the sampling period. Samples were taken daily for ten days and analyzed. The three periods were repeated three times to complete the crossover design.

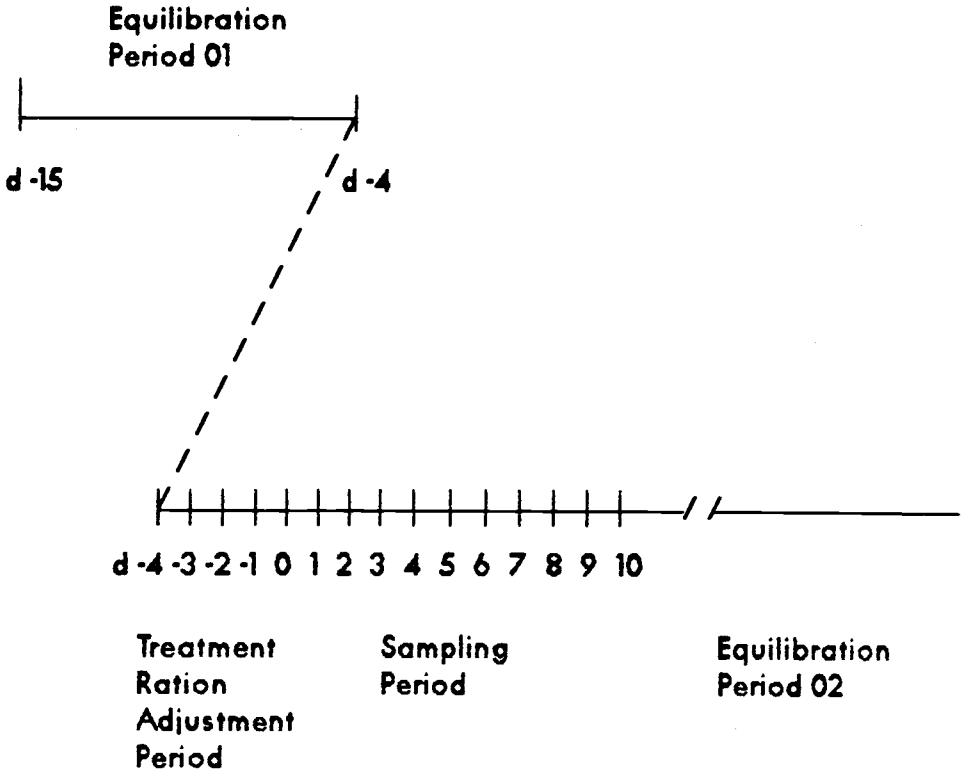

Figure 4. Sampling and experimental protocol of Experiment 2. Samples were taken on day 1-10 of Sampling Period. Schedule was repeated for period 02 and 03 of three treatment crossover design.

Rumen samples were taken just below the feed layer of rumen contents. This layer was usually the midsection between the dorsal sac and ventral sac of the rumen. After the procurement of the rumen fluid sample, pH measurements were taken and recorded.

The rumen fluid samples were placed in thermoses for subsequent transport to the laboratory. At the laboratory, five ml of strained rumen fluid was pipetted into a centrifuge tube. One ml of 25% metaphosphoric acid was added, mixed thoroughly, and allowed to stand for 30 minutes. After this time, the samples were centrifuged at 2000 rpm (International Centrifuge, size 2, model head 250A) for ten minutes. The supernatant liquid was poured off and prepared for gas chromatography analysis or frozen at -3 C until later analysis. These sample preparation procedures are outlined by Supelco, Inc. (1975).

Gas Chromatography Analysis. One ml of the supernatant was added with one ml of .2% T-butyl acetic acid, the internal standard. After thorough mixing, the two ml sample was centrifuged again at 2000 rpm for 10 minutes. At this time, the supernatant remaining was placed in a chromatography vial for analysis, using a Perkin-Elmer Sigma 2000 gas chromatograph. Use specifications called for a flame ionization detector (FID) used in conjunction with a SP-1200/H_3PO_4 column.

Two chromatography vials were made for each daily

sample to reduce variation caused by faulty pipetting of internal standard. Two injections were taken from each vial to reduce possible variation in the C2-C5 analysis itself. Thus, four analyses were conducted for each sample taken. The mean of the four analyses was used as an individual ruminal observation per day.

The following analysis has been found to be accurate with an error of only .05% associated (Supelco, Inc., 1975). Peaks were integrated according to retention time and relative area. Integrated peaks included acetate, propionate, isobutyrate, N-butyrate, isovalerate, and valerate.

HPLC Analysis. High performance liquid chromatography (HPLC) was conducted to measure lasalocid levels in the rumen fluid. The remainder of rumen fluid samples not used in gas chromatography analysis were used for HPLC analysis. The chromatography technique was the same as the one recommended by Osadca and Araujo (1978). A silica column (altex altrasphere -SI, 5u particle size) was used with a fluorescence detector. Peak sensitivity with rumen fluid samples was found to be as low as .05 ng/ml rumen fluid.

Statistical Analysis. Analysis of variance for C2-C5 acids and pH was performed as outlined by Steele and Torrie (1960). A 3x3 dual latin square design was

utilized with sources of variation for squares, sample period, heifers within square, and treatments (see figure 3). If there was a difference among treatments, least significant difference (LSD) comparisons were made with the treatment means.

Results

Experiment 1. The treatment means of steer ADG are listed in table 1. Consistent with previous studies, lasalocid hand-fed daily increased ADG by more than 10% ($P<.01$) (Horn et al., 1981; Spears and Harvey, 1984; DelCurto and Weber, 1985). Lasalocid hand-fed on a three times weekly basis had no effect on ADG ($P>.10$).

Experiment 2. Treatment effects of lasalocid fed daily and three times weekly appear to be moderate (table 2). Both lasalocid treatments caused a numerical decrease in acetate to propionate ratio; however, this was not significant ($P>.10$). Research with cattle fed high roughage diets often display a similar lack of response (Fuller and Johnson, 1981). Butyrate to propionate ratios were found to increase with both lasalocid treatments ($P<.05$). Individual acetate and propionate levels were unchanged ($P>.10$), while butyrate was increased with lasalocid treatment groups ($P<.05$). Obviously, the change in butyrate to propionate ratio was due primarily to an increase in butyrate relative to propionate. Armentano

treatment	ADG (kg)
control daily	1.12
control 3X weekly	1.06
lasalocid daily	1.25***
lasalocid 3X weekly	1.11

*** significant (P<.01)

Table 1. ADG of yearling steers in Experiment 1. ADG was based on initial and final shrunken weights over the 98 day trial.

Parameter measured	control	lasalocid daily	lasalocid 3X weekly
acetate/prop.	3.058	3.031	2.998
butyrate/prop.	.424	.478**	.473**
acetate	.197	.214	.197
propionate	.066	.071	.067
butyric	.028	.034**	.033**

** Different from control (P<.05)

Table 2. Results of VFA concentrations (mg/ml fluid) in experiment 2. Fluid analyzed consisted of: 50% internal standard; 42% rumen fluid, and 8% metaphosphoric acid.

and Young (1984) reported a similar increase in butyrate with lasalocid supplementation; however, other studies have noticed decreased butyrate levels (Spears and Harvey, 1984).

Branch chain acids (isobutyric, isovaleric, N-valeric) were numerically increased with cattle receiving lasalocid supplements daily; however, this was not significant ($P>.10$) (table 3). Lasalocid fed on a daily basis caused a decrease in ruminal-reticular pH ($P<.05$).

HPLC analysis of rumen fluid contents failed to detect lasalocid. It is generally believed that the particulate matter that was centrifuged-off for GC analysis contained most of the lasalocid drug (Craig and coworkers, unpublished data). It appears the drug is tightly bound to the feed contents and microbial contents in the rumen. As a result, HPLC chromatographs did not detect lasalocid in either lasalocid daily or lasalocid 3X weekly samples.

Discussion

Experiment 1. Based on ADG, supplementation of lasalocid 3X weekly does not appear to be as effective as lasalocid supplemented on an everyday basis. Lasalocid fed daily caused an increase in ADG of over 10% ($P<.01$), while lasalocid fed 3X weekly had no effect on weight

Parameter measured	control	lasalocid daily	lasalocid 3X weekly
isobutyric	.0055	.0060	.0057
isovaleric	.0078	.0089	.0084
valeric	.0036	.0037	.0034
pH	6.47	6.35**	6.39

** Different from control (P<.05)

Table 3. Concentrations of branch chain fatty acids and pH as affected by treatments, Experiment 2. Concentrations expressed in mg/ml fluid analyzed.

gain response.

There are many potential explanations for lack of weight gain response with cattle fed 3X weekly. Studies with lasalocid and monensin indicate that ionophores fed at recommended levels on a daily basis do not reduce roughage intake (Baile et al., 1979; Horn et al., 1981). The high concentrations of ionophore fed 3X weekly, however, might reduce feed intake. If intake was reduced, the benefit of ionophore mediated rumen fermentation modification would be decreased.

The frequency of administration of the ionophore might also explain the lack of weight gain response with 3X weekly supplementation. The function of ionophores is to selectively modify ruminal-reticular microflora. This mode of action will be dependent on amount of drug in the rumen and population dynamics of the bacteria and protozoa present. Most of the benefits derived from ionophores are related to changes in ruminal-reticular ecology. It is possible that administration of lasalocid 3X weekly does not produce the desirable shifts in ruminal-reticular microflora associated with daily lasalocid intake.

Experiment 2. Measurements of VFA, BCFA, and pH do not support or explain the lack of weight gain response with 3X weekly lasalocid supplementation. Butyrate was the only VFA affected by lasalocid treatments. Both lasalocid treatments caused an increase in ruminal

butyrate concentrations. Increased production of butyrate might benefit rumen fermentation efficiency. Butyrate serves as an energy source of the rumen wall. If butyrate is deficient in the ruminal-reticular environment, ruminal wall problems, such as ruminal abscesses and papillary clumping, might develop. In this physiological state (butyrate deficiency), an increase in butyrate can have a very large and beneficial impact on digestive efficiency.

While an increase in ruminal-reticular butyrate levels might be beneficial to the host, it is unlikely to be a significant factor in experiment 1 weight gain response data. Butyrate is largely converted and absorbed as beta-hydroxy butyrate. This compound cannot be used for glucose synthesis, but instead is used primarily for fat synthesis. Butyrate appears to be lower in energy value as compared to propionate (Hungate, 1966; Smith, 1971; Chalupa, 1977). It is also unlikely that butyrate levels were deficient with animals fed ab libitum mid-bloom alfalfa. For these reasons, the change in butyrate production as associated with lasalocid supplementation is probably of little biological significance.

Both lasalocid treatments had no effect on ruminal BCFA (isobutyrate, isovalerate, and valerate) concentrations. Lasalocid supplemented daily tended to cause a numerical increase in ruminal BCFA; however, the increases

were not significant (P>.10). An increase in BCFA could benefit rumen fermentation. Increases in BCFA have been reported to enhance bacteria growth and cellulose digestion (Muira et al., 1983). In addition, increases in BCFA lead to elevated blood growth hormone levels (GH) (Towns et al., 1984). Regardless of the biological significance of BCFA in the ruminal-reticular environment, the lack of response of BCFA to lasalocid supplementation precludes their relative importance.

Lasalocid fed on a daily basis caused a decrease in ruminal-reticular pH. The effect of pH probably has little effect on digestion, absorption, and production of digestive nutrients. The pK of acetate, propionate, and butyrate range from 4.7 to 4.9. Therefore, the modest change in pH caused by daily lasalocid supplementation is also of little biological significance.

Results of experiment 2 indicate that lasalocid supplementation alters ruminal-reticular fermentation. However, the parameters measured do not clearly separate the difference between daily and 3X weekly supplementation. Other ionophore mediated benefits, such as more efficient protein utilization, could account for the advantage of daily versus 3X weekly feeding. As indicated in many previous studies, the effects of ionophores on rumen fermentation are diverse and difficult to quantify. While results of experiment 2 indicate that

the addition of lasalocid on a daily and 3X weekly basis caused changes in rumen fermentation, they do not provide a significant biological difference between daily and 3X weekly supplementation schemes.

In summary, lasalocid hand-fed 3X weekly did not appear as a practical supplementation scheme in this trial. Efforts to isolate rumen fermentation parameters correlated to the performance data were unsuccessful.

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