Use of Ionophores in Grazing Ruminants

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Introduction

The use of ionophores in ruminant diets is one of the success stories of application of technology to livestock production. They have been fed for more than 20 years with positive and repeatable responses across a wide range of production systems. Ionophores are antibiotics that affect certain bacteria, protozoa and fungi in the rumen. Three polyether ionophores (ionophores with carboxylic terminal groups) available for ruminants today are monensin (Rumensin[®]), lasalocid (Bovatec[®]) and laidlomycin (Cattlyst[®]). When added to the diet per manufacturer's recommendations, they change the profile of fermentation products and the degradation of dietary protein in the rumen, thereby improving the efficiency of nutrient use by ruminants. The fermentative processes that are affected by ionophores production of ammonia and volatile fatty acids (VFA) in the rumen - are key processes because they affect directly nitrogen and energy metabolism in the host ruminant. This review will emphasize the results of studies with grazing cattle, with data and information from other reviews^{30,48,62,65} as well as data from recent publications.

Biological and Chemical Characteristics of Ionophores

There are data available for various ionophores, but the majority of data for ruminants (cattle and sheep) come from studies of monensin or lasalocid. However, there are some generalities in terms of their modes of action. Results of *in vitro*, *in situ* and *in vivo* studies indicate that ionophores in general cause three changes in the ruminal fermentation:⁴⁸

• increased production of propionic acid and decreased production of methane;

• decreased protein degradation and less deamination of amino acids;

• decreased production of lactic acid and froth.

They are called ionophores because they have an affinity for ions, sometimes specific ions and other times

a more general affinity (Table 1). Organisms of the genus *streptomyces* produce ionophores, ostensibly as weapons in their microbial "war" for sources of food. They are relatively small molecules, with molecular weights less than 2,000 Daltons. The polyether ionophores, with carboxylic end groups, form lipophilic complexes with ions that enable them to cross living as well as synthetic membranes. Other ionophores, called "the valinomycin group", form neutral complexes and do not aid transport of cations across cellular membranes. Studies with isotopically labelled monensin and lasalocid indicate that essentially all ionophores in the rumen are bound to bacteria, protozoa or feed particles, and that there is little free ionophore in ruminal fluid.¹³

Table 1.Characteristics of ionophores^a

| Ionophore | Molecular weight | Cation preference ^b |
|-------------|------------------|--|
| Lasalocid | 591 | $Ba^{**} \approx K^* > Rb^{**} > Na^* > Cs^* > Li^*$ |
| Laidlomycin | 721 | not determined |
| Lysocellin | 660 | $Na^+ > K^+ \approx Ca^{++} \approx Mg^{++}$ |
| Monensin | 671 | $Na^+ > K^+ \approx Li^+ > Rb^+ > Cs^+$ |
| Narasin | 765 | $Na^+ > K^+ \approx Rb^+ \approx Cs^+ \approx Li^+$ |
| Salinomycin | 751 | $Rb^{+} \approx Na^{+} > K^{+} >> Cs^{+} \approx Sr^{+} \approx Ca^{++} \approx Mg^{++}$ |
| Tetronasin | 628 | $Ca^{**} > Mg^{**} > Na^* \approx K^* > Rb^*$ |

^a From the review of Nagaraja.⁴⁷

^b Preference priority for a bilayer membrane.

A large part of cellular energy (ATP) is spent maintaining appropriate ionic concentrations inside the cell, because these ionic concentrations are basic to cell viability, or to life in general. Ionophores are toxic because they interrupt the normal interchange of ions in monocellular organisms as well as in cells of animal tissues. Because of differences in the composition of their cell membranes, gram negative bacteria are less susceptible to the toxic effects of ionophores than are gram positive bacteria.47 This means ionophores negatively affect populations of ruminal bacteria that produce lactic acid, butyric acid, ammonia and hydrogen (e.g., Ruminoccus albus, R. flavefaciens, Butyrivibrio fibrisolvens, Streptococcus bovis, and Peptostreptococcus anaerobius). The ruminal populations of protozoa and fungi also are negatively affected by ionophores. In vitro incubations of ruminal fluid from sheep indicate that ionophores reduce endotoxin production. 68

It is important to remember that there is appreciable variation in the ruminal microbial profile, even among animals at the same location and eating similar diets. Therefore, it is possible to observe variation in response to feeding ionophores; sometimes they cause changes in the relative numbers of bacteria⁴¹ and protozoa,⁵³ and other times they do not.^{9,18} Although it is difficult to measure it independently of other factors, this variation in microbial profile has much to do with the variation associated with nutrient digestion, weight gain, and feed efficiency.

Some of the responses to ionophores are direct, and others are indirect. For example, methanogenic bacteria are not affected by ionophores; decreased production of methane results from lack of availability of precursors, formic acid and hydrogen.⁴⁷ Increased ruminal production of propionic acid in response to monensin was associated with decreased production of acetic acid^{2,55,72} or a subtle increase in acetic acid production.⁵⁸ Similarly, feeding ionophores decreased VFA concentrations in ruminal fluid in some studies^{26,62} but not in others.^{3,9,10,17,18,34,37,65} It is logical to assume that differences in response among studies reflect the amount of ionophores actually consumed as well as differences among ionophores in potency; therefore, it is worthwhile to adjust for those factors in direct comparisons of ionophores. In any event, almost all studies in vivo and in vitro report a shift in the acetate:propionate ratio in rumen fluid, which means relatively more propionate in response to feeding ionophores. Production of more propionic acid and less methane means improved retention of reduced carbon in a form that is useful to the host ruminant, which in turn means improved energetic efficiency. Armentano and Young² estimated a 6% improvement in ruminal fermentation in response to feeding ionophores.

Less ruminal degradation of protein in effect means less degradation of peptides, because ionophores do not inhibit (or have little effect) on proteolysis in the rumen.47There is decreased production of ammonia in the rumen and increased escape of dietary protein from the rumen (ostensibly as peptides, but usually measured as non-ammonia, non-bacterial nitrogen). Ammonia is readily absorbed and presents an immediate metabolic chore to the liver, removal of potentially toxic ammonia from blood. In addition to being relatively expensive, many high quality proteins are extensively degraded in the rumen, thereby minimizing or negating any nutritional benefit in improved amino acid supply for absorption from the small intestine. Therefore, producers could expect better feed efficiency from use of products that diminish protein degradation and ammonia production in the rumen.

Generally, production of lactic acid presents prob-

lems with high grain diets, or diets that permit rapid fermentation of carbohydrates in the rumen. Froth production could be a contributing factor to the development of bloat, again generally in the feedlot with cattle fed high grain diets. However, monensin supplementation to cattle grazing wheat pasture decreased the incidence of bloat.¹⁰

Nutrient Digestibility

There are several factors such as forage quality. associative effects among dietary ingredients, and rates of ruminal degradation that can shift the site of digestion in the digestive tract of grazing ruminants.⁴⁰ Ellis et al.²⁰ created ruminal models for ruminants grazing bermudagrass (Dactylis glomerata) or ryegrass (Lolium perenne) that were supplemented with monensin. The ionophore increased digestibility of organic matter 4% relative to control. The authors attributed this increased digestibility to decreased rate of passage of organic matter from the rumen when the grass contained from 65 to 75% neutral detergent fiber. They also observed increased intake when organic matter digestibility ranged from 45 to 65%; however, daily forage intake with monensin was less than that of control when organic matter digestibility was less than 45%. The addition of ionophores to high-fiber diets did not change digestibility of neutral detergent fiber in the rumen of steers²¹ or sheep,⁹ but did decrease ruminal digestibility of fiber in nonlactating cows³¹ and *in vitro* dry matter digestibility of praire hav.¹⁹

The bulk of available data for forage-based diets show no statistical effects of ionophores on *in vivo* digestibility of dry matter, organic matter, or fiber components.^{6,9,17,18,25,28,31,63} Generally, these data were collected in digestion crates; therefore, it is possible that there was less opportunity for animals to select high quality forage as they might do in a pasture situation. It is also possible that the effects of ionophores were too subtle to survive statistical scrutiny. Steers and cows on pasture (6% crude protein and 83% neutral detergent fiber) were supplemented with several levels of lasalocid.³⁴ Intermediate levels (100 or 200 mg per head daily) of lasalocid decreased organic matter digestibility (for example, from 41.3 to 37.8%), but 300 mg lasalocid per head daily did not affect digestibility.

In general, supplementation with ionophores increased nitrogen (crude protein) digestibility in the studies cited above. There was an interaction in the results of Galloway *et al.*²⁵ that indicates greater nitrogen digestibility in response to lasalocid or monensin when forage digestibility was lower. There was also an interaction between ionophores and herbage species, perhaps because bermudagrass (*Cynodon dactylon*) contained more nitrogen than bromegrass (*Bromus spp.*). Of course, all these herbages were high quality relative to herbages or grasses that normally are found in praire or high plains regions of North America.

Studies of nutrient digestibility in lactating dairy cows³⁶ indicate no effect of lasalocid on the digestibility of dry matter, neutral detergent fiber, or starch. Other than the recent report of increased ruminal and total tract digestibility of starch in response to dietary laidlomycin,⁷⁹ studies with cattle and sheep fed high grain diets indicate that ionophores do not affect digestibility of dry matter, neutral detergent fiber, acid detergent fiber, or starch.^{44,77,78} However, in these studies, the ionophore salinomycin or laidlomycin increased nitrogen digestibility. In his review, Spears⁶¹ indicated that lasalocid or monensin increased energy digestibility by two percentage units, and shifted the site of starch digestion from the rumen to the small intestine.

As predicted by the effects on ruminal microbes previously discussed, ionophores decreased ruminal degradation of protein and passage of microbial protein from the rumen, but increased appearance of non-ammonia nitrogen in the duodenum.^{21,53,78} Ruminal digestion of dietary protein decreased in cows fed high-forage diets supplemented with monensin.³¹ These research results suggest a potential benefit from combining ionophores with dietary sources of high quality protein in terms of improving rates or levels of production, especially with grazing ruminants. Unfortunately, there does not appear to be a big effect of ionophores on relative amounts of amino acids entering the duodenum.^{21,28} It is logical to expect effects of ionophores on mineral digestibility, and they do increase apparent absorption of magnesium, phosphorus, zinc, and selenium. Effects on apparent absorption of calcium, potassium or sodium are not as clearly defined.35,61,79

Postabsorptive Metabolism

It appears that ionophores (when fed at recommended levels) at best have subtle effects on intermediary metabolism. Monensin and lasalocid increased concentrations of intermediary metabolites and enzymes in the liver⁷. In spite of these effects on the liver and the effects of producing an important glucose precursor (propionic acid in the rumen), ionophores (or at least monensin) have little effect on the production of glucose by the liver or other tissues in ruminants^{2,72}. The ionophore lysocellin increased plasma concentrations of glucose and decreased slightly concentrations of zinc in cattle.⁶³ After 79 days of supplementation, tetronasin had not affected plasma concentrations of calcium, magnesium, or potassium.⁶⁵ Plasma concentration of urea decreased in sheep in response to lasalocid^{29,69} but urea did not change in response to tetronasin in cattle.⁶⁵ Plasma concentrations of glucose,

insulin, somatotropin, and prolactin,⁴⁶ or of amino acids⁵³ were not changed by supplementation with lasalocid or monensin. Serum concentrations of nonesterified fatty acids, β -hydroxybutyrate, triglycerides, glucose, and insulin were not changed by adding lasalocid to the diet of lactating cows.³⁶ This lack of effect on plasma or serum concentrations and the lack of effect on carcass traits³⁰ indicate that ionophores are not working as partitioning agents such as hormone implants or β -agonists. Their biological effects are focused on fermentative changes in the rumen, **and the advantages of supplementation with ionophores lies in better capture of potential nutrients and energy in the diet.**

Toxicity with ionophores

Ionophores are in part absorbed and metabolized by the liver. Products of this metabolism are returned to the digestive tract in the bile.¹⁶ It is possible to kill ruminants^{23,24,52,74} as well as other animals^{48,59,67,71,73} with excessive dietary levels of ionophores. For example, grazing cattle suffered myopathy and cardiac arrests after eating approximately 10 kg daily of poultry litter that contained an ionophore.⁵² There are many research reports available as well as FDA-approved levels of ionophores that provide optimum responses in terms of health or weight gain, so producers are well advised to follow manufacturers' recommendations for products containing ionophores.

Grazing Ruminants

Weight gain, feed consumption, and feed efficiency

As do feedlot cattle, grazing ruminants benefit from supplementation with ionophores. The review of Goodrich et al.³⁰ contains data from almost 1,000 grazing cattle in Minnesota that indicate on average a 13% improvement in weight gain in response to monensin. The data of Potter et al.⁵⁴ in Table 2 represent more than 30 studies with more than 2,000 cattle and indicate on average a 16% improvement in weight gain for cattle supplemented with monensin. Similarly, there was an improvement in daily gain that ranged from 2 to 13% in 28 studies with more than 2,200 steers and heifers that had a slow-release, monensin device placed in the rumen.⁵⁰ In studies appropriately designed to study it, the response to ionophores was independent of the level of nutrition (energy or protein) in the supplement.^{54,76} I made a linear regression of weight gain with ionophores (Y) as a function of weight gain for controls (X) with data from grazing cattle (mainly with growing steers) that were grazing a large variety of forages (in terms of quality or digestibility), and that were gaining between .35 and 1.58 kg daily (Table 2). The result - Y, kg/d = $0.046 (\pm 0.024) + 1.046 (\pm 0.030) \bullet X, R^2 = 0.984 - indi-$

| Time, days | Live weight, kg | Ionophore | | n, kg/d Ionophore | Reference |
|------------|-----------------|-------------|------|----------------------|-----------|
| 140 | 537 | lasalocid | 0.09 | 0.09 | 12 |
| 90 | 472 | lasalocid | 0.09 | 0.16 | 35 |
| 70 | 200 | monensin | 0.26 | 0.28 | 22 |
| 80 | 355 | lasalocid | 0.35 | 0.40 | 57 |
| 105 | 210 | monensin | 0.44 | 0.50 | 51 |
| 161 | 180 | salinomycin | 0.49 | 0.73 | 33 |
| 120 | 225 | monensin | 0.56 | 0.65 | 54 |
| 100 | 225 | monensin | 0.59 | 0.68 | 54 |
| 150 | 210 | monensin | 0.60 | 0.64 | 50 |
| 80 | 355 | lasalocid | 0.60 | 0.62 | 57 |
| 116 | 228 | monensin | 0.61 | 0.69 | 50 |
| 113 | 278 | monensin | 0.61 | 0.69 | 60 |
| 113 | 278 | lasalocid | 0.61 | 0.72 | 60 |
| 118 | 245 | monensin | 0.86 | 0.88 | 50 |
| 140 | 224 | monensin | 0.97 | 1.02 | 50 |
| 112 | 256 | tetronasin | 1.03 | 1.12 | 26 |
| 100 | 216 | lasalocid | 1.03 | 1.14 | 1 |
| 107 | 249 | lysocellin | 1.15 | 1.23 | 65 |
| 107 | 249 | tetronasin | 1.15 | 1.26 | 65 |
| 80 | 229 | monensin | 1.16 | 1.25 | 50 |
| 80 | 250 | lasalocid | 1.44 | 1.58 | 76 |

| Table 2. | Weight | gain | for | grazing | ruminants | supple- |
|----------|--------|------|-----|---------|-----------|---------|
| | mented | with | ion | ophores | | |

cates the possibility (P < .07) of a constant positive effect of 46 g/d across the range of weight gains (Y = 0.047 kg/d when X = zero) and predicts an improvement (P < .01) of approximately 5% (1.046 times X) in weight gain across the range of animals and conditions on the studies listed in Table 2. The sum of the constant and proportional effects would equate to approximately a 10% increase in weight gain in cattle gaining 1 kg/d.

The models of Ellis et al.²⁰ previously mentioned predict a 10% increase in intake for grazing cattle in response to supplementation with an ionophore (monensin). They attributed this response to the combined effects of decreased rate of passage of digesta from the rumen and an increased retention time of organic matter in the rumen. Responses of grazing sheep in Texas³³ confirmed a positive relationship between the level of monensin or lasalocid in a supplement and retention of digesta in the rumen, voluntary intake of forage, and a negative relationship between the level of ionophore and rate of digesta passage from the rumen. However, Huston et al.³³ found little response (nonsignificant statistically) directly attributable to ionophores in terms of intake or digestibility of feed by grazing sheep or goats. Feed intake by cattle eating fresh forage or hay,^{17,50,63,70} corn silage or grass silage,^{4,8,45} alfalfa cubes,⁵¹ or ensiled corn stover²¹ either was not affected or was decreased by supplementation with monensin or lysocellin, was decreased by supplementation with tetronasin, and either was not affected or was increased by supplementation with lasalocid or laidlomycin. Generally speaking, efficiency (live weight gain divided by dry matter consumed) was improved by supplementation with ionophores.

Direct comparison of ionophores among themselves

is complicated by differences among studies in feed intake and potency of ionophores studied. Goodrich *et al.*³⁰ analyzed data from 228 studies of monensin (11,274 head of feedlot cattle total) and concluded that local conditions explained the major portion of variation in response - or lack of response - to supplementation with ionophores. They also noted that the effects of monensin and growth-promoting implants to enhance weight gain were additive.

$Reproductive\ performance$

Ionophores do not affect criteria used to evaluate reproductive performance of grazing ruminants. As with the feedlot data, there is a great deal of variation among published data, ostensibly reflecting local conditions. In his review, Sprott et al.⁶⁴ mentioned this variation with respect to supplementation with ionophores, body condition, feed quality, and the responses such as milk production, weight gain of nursing calves, days between parturition and first estrus for cows, and days between birth and first estrus for heifers. Addition of lasalocid to two levels of energy supplementation for first-calf heifers indicated that there was more response to the ionophore with the lower energy level in terms of milk production and weight gain of the calves.²⁷ Weight gain, feed efficiency, and age at puberty were greater for growing heifers fed ionophores, but these advantages were not reflected in greater overall pregnancy rate relative to control heifers.⁵⁶ There are no apparent effects of ionophores on length of gestation, dystocia, birth weight, or fertility of bulls.⁶⁴

Delivery of ionophores to grazing cattle

The main challenge for managers of grazing ruminants is to implement a suitable supplementation system that can provide daily amounts of appropriate levels of ionophores for all animals.¹⁵ Presently, it appears that the most likely system will use mineral or protein supplement blocks; however, there are data available for a slow-release pellet that can be placed in the rumen.⁵⁰ Supplementation with 200 mg of monensin every day did not affect the intake of steers eating corn stalks, but supplementing every other day reduced dry matter intake and tended to decrease weight gain.¹⁴ The economics of supplementation of grazing ruminants vary with the market value of cattle and costs of feed; however the advantages in weight gain and stocking density attained by supplements³² of 150 g of weight per steer daily and approximately a one-third increase in stocking density of steers grazing wheat pasture were in part attributable to the ionophore in the supplement.

Other Effects of Ionophores

In addition to the effects on ruminal lactic acid production previously described, supplementation with ionophores may ameliorate symptoms of acidosis by way of their effects on voluntary intake of readily fermentable carbohydrates^{11,66} or on the reduction of ruminal concentrations of lactate.⁵ Dietary supplementation of ionophores may ameliorate the deleterious effects of coccidiosis, acute pulmonary emphysema, acute sarcocystosis, horn flies, face flies, and bloat in ruminants.^{30,38,49} Use of ionophores as coccidiostats usually results in decreased fecal egg counts, increased feed consumption, and increased rate of gain.^{43,75} In addition to ruminants, ionophores have been used successfully (mainly as coccidiostats) in a variety of domesticated species, including poultry and swine. A national U.S. survery of dairy herds reported an average increase of 329 kg of milk per cow yearly in herds that fed ionophores from birth to first calving of replacement heifers.³⁹ Ostensibly, more milk results from healthier, thriftier heifers entering the milking herd. There is also interest in the approval of monensin for use in supplements for lactating dairy cows in the U.S., because of the previously described benefits of the improved capture of dietary protein and energy.^{35,36,42}

Conclusions

Ionophores - antibiotics of bacterial origin - affect the ruminal microbial population, and thereby affect the dynamics of feedstuff fermentation in the rumen of cattle. Ionophores have neutral or positive effects on ruminal digestion of dietary fiber and ruminal escape of dietary protein in ruminants consuming high- or allforage diets. Changes in blood plasma or serum concentrations of metabolites, minerals or hormones are subtle or nondetectable. There are many production and environmental factors that will affect intake and weight gain of grazing ruminants; therefore, there is a great deal of variation among responses to ionophores. Across a variety of conditions, results of grazing studies indicate that ionophores have little effect on voluntary intake. Regression of average daily weight gain of grazing cattle receiving ionophores on average daily weight gain of control cattle within the same experiment predicts a 5% improvement in weight gain with ionophores. Ionophores are used successfully as cocciodostats in cattle as well as in other species.

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Abstract

Bovine immunodeficiency-like virus: inactivation in milk by pasteurisation

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Bioassay was used to determine whether bovine immuno-deficiency-like virus (BIV) in milk was inactivated by pasteurisation. Three groups of three calves were inoculated with virus (BIV isolate FL112), milk seeded with virus and milk seeded with virus that had been pasteurised before inoculation, respectively. Seroconversion to BIV was monitored for 12 months by an indirect immunofluorescence assay. The presence of BIV proviral DNA in peripheral blood was determined by a nested polymerase chain reaction (PCR). The animals were euthanased and virus isolation and PCR were attempted on peripheral blood mononuclear cells, prescapular lymph node and spleen. Transmission of BIV was confirmed in the groups that were inoculated with the virus and with the virus in milk, but no evidence of its transmission was demonstrated in the group that received the pasteurised inoculum.