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EFFECTS OF LASALOCID AND CORN GRAIN PARTICLE SIZE ON PERFORMANCE, RUMEN PARAMETERS AND FEEDING BEHAVIOR OF EARLY LACTATION DAIRY CATTLE

By

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ABSTRACT

EFFECT OF LASALOCID AND CORN GRAIN PARTICLE SIZE ON PERFORMANCE, RUMEN PARAMETERS AND FEEDING BEHAVIOR IN EARLY LACTATION DAIRY CATTLE

BY

KATHARINE FROST KNOWLTON

The effect of lasalocid and corn grain particle size on performance, rumen parameters and feeding behavior of early lactation dairy cattle was examined. Eight multiparous and four primiparous cows were fed diets with cracked or ground corn grain, with or without the ionophore lasalocid in 3, 4x4 Latin squares with 21 d periods. Lasalocid tended ($P<.11$) to improve dry matter and water intake, and ground corn increased water intake. Lasalocid and ground corn decreased body condition loss, and milk fat percent, and increased milk protein percent. Primiparous cows increased milk yield with lasalocid, but across all cows, neither treatment affected milk yield, 4% fat-corrected-milk, or body weight. Ground corn increased starch digestibility and decreased NDF digestibility and starch ruminal turnover time. Lasalocid increased lactate concentrations, and did not affect acetate to propionate ratio. Ground corn did not affect lactate but increased propionate and range in ruminal pH.

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KEY TO ABBREVIATIONS

A:P	Acetate to Propionate ratio
ADF	Acid detergent fiber
ATP	Adenosine triphosphate
ß-HBA	ß-hydroxybutyrate
BCFA	Branch chain fatty acids
BCS	Body condition score
BW	Body weight
CP	Crude protein
DIP	Ruminally degraded intake protein
DM	Dry matter
DMD	Dry matter digestibility
DMI	Dry matter intake
FCM	4% Fat corrected milk
NDF	Neutral detergent fiber
NEFA	Non-esterified fatty acids
NSC	Non-structural carbohydrates
PS	particle size
TG	Triglycerides
TMR	Total mixed ration
UIP	Undegraded intake protein
VFA	Volatile fatty acid

INTRODUCTION

Meeting the early lactation cow's demand for energy to support milk production is a challenge. Cows in negative energy balance, unable to consume energy sufficient to meet these demands, draw on their reserves of adipose tissue. Excessive loss of body condition has been shown to lead to higher incidence of metabolic disease and poor reproductive performance (Emery et al., 1992). Maximizing dry matter intake so as to minimize body condition loss while achieving peak milk production is important for maintaining cow health.

Increasing energy density of the dairy cattle rations may be done by adding fat to the diet, or by increasing the quantity of fermentable carbohydrate in the diet. Adding fat to the diet is expensive, and may impair rumen function and dry matter intake at higher levels (Dinius et al., 1974; Bines et al., 1978). Adding fermentable carbohydrate to the diet is a less expensive alternative that will increase the energy available to the cow and may increase microbial protein yield. However cows on diets with high levels of ruminally degraded carbohydrate may decrease dry matter intake under some conditions (McCarthy et al., 1989; Casper et al., 1990b; Oliveira et al., 1990; Moore et al., 1992; Aldrich et al., 1993; Dhiman and Satter, 1993; Seymour et al., 1993; Varela et al., 1993). High starch diets leading to increased concentrations of lactic acid in the rumen have been shown to play a key role in the development of sub-acute and acute

acidosis in feedlot cattle, reducing dry matter intake and subsequent performance (Slyter, 1976; Russell and Hino, 1985). A similar mechanism may cause reduced DMI in early lactation cows on high starch diets. The rapid fermentation of large quantities of starch in the rumen increases the production of lactate (Slyter, 1976; Russell and Hino, 1985). Increased concentrations of lactate in the rumen may cause a decrease in mean or minimum ruminal pH, causing a reduction in dry matter intake.

The rate of ruminal starch degradation in cereal grains can be manipulated through type of grain and grain processing (Ørskov, 1986; Theurer, 1986; Nocek and Tamminga, 1991). The production of lactic acid has been shown to be inhibited by ionophores (Dennis et al., 1981a; Dennis et al., 1981b; Nagaraja et al., 1981; Nagaraja et al., 1982; Nagaraja et al., 1985). Reducing the level of ruminally degraded starch, or inhibiting lactate producers with the ionophore lasalocid may allow the inclusion of increased levels of starch in the rations of early lactation dairy cows.

The objective of this project was to examine the mechanism of the observed reduction in dry matter intake with increased level of ruminally degraded starch in early lactation dairy cattle by manipulating rumen fermentation and lactic acid production with corn grain particle size and the ionophore lasalocid.

CHAPTER 1

REVIEW OF LITERATURE

1.1 REGULATION OF DRY MATTER INTAKE IN RUMINANTS

Feed intake determines the nutrients available to the animal, and is regulated by the requirements of the animal's physiology and metabolism. Dry matter intake directly affects milk production, and typically is the first limiting factor when formulating rations for high producing dairy cows. Maximizing feed intake, therefore, is an important component of maximizing profitable milk production.

Control of feed intake is thought to be through physical limits on gastrointestinal fill and through physiological mechanisms reflecting satiety. Conrad et al. (1963) observed that physical and physiological factors regulating dry matter intake change in importance with increasing digestibility of the feed. He suggested that 66% dry matter digestibility in the diet is the breakpoint between intake limitation by physical fill in diets (< 66% DMD) and limitation by metabolic controls diets (> 66% DMD). This distinction between physical and physiological regulation likely differs among animals in different physiological states. Alternatively, Grovum (1986) suggests that it is the critical, intake-regulating level of fill within the reticulo-rumen that varies with physiological state.

The basic concept of the gut fill model of dry matter intake control is that intake is limited by dietary bulk and subsequent distention of the digestive tract. The feeding response to rumen fill is probably through tension receptors in the

rumen (Baile and Della-Fera, 1981), or the reticulum (Grovum, 1986). Intake control can be thought of as a long-term regulation, or as the result of individual meal patterns and feeding behavior within a day. In cows with intakes limited by physical fill, for example, meal cessation may occur when digesta volume in the rumen has reached some maximal volume, and meal initiation may occur when digesta is removed from the rumen through nutrient passage, digestion and absorption.

The limitation on dry matter intake due to physical distention appears to be primarily a reticulo-rumen volume limitation, rather than a lower tract limit, as fecal output has been shown to increase with fine grinding (Mertens, 1985). Infusion of methyl cellulose to the abomasum of sheep also increased fecal output without affecting intake (Grovum, 1986). Limitations on intake due to fill have been demonstrated on high fiber diets, and with the addition of rumen inert bulk such as water filled bladders (Grovum, 1986; Johnson and Combs, 1992) or plastic ribbons (Welch, 1967). The addition of plastic ribbons 30 cm long to the rumen of sheep reduced dry matter intake by nearly 50% immediately following treatment (Welch, 1967). With time (~ 15 days), intake recovered somewhat, indicating some ability of the rumen to expand, but intake did not return to pre-treatment levels.

Mertens (1983, 1985) proposed that the neutral detergent fiber (NDF) content of the diet be used as a primary predictor of dry matter intake, as it is closely associated with bulk density of forages. With 187 forages, including 61 legumes and 126 grasses, fed to sheep, the correlation between cell wall content and voluntary intake (g/kg $bw^{0.75}$) was -.76. Osbourn et al (1974) showed that expressing this relationship relative to intake of a standard forage removed a large component of the animal variation, and demonstrated a strong relationship between cell wall content and intake.

Particle size of the forage also likely plays a role in the control of dry matter intake, as grinding and pelleting feed increases dry matter intake (Van Soest, 1982). Increased rate of fiber and dry matter digestion should allow increased intake if intake is controlled by fill, as gut fill is relieved by removal of digesta from the rumen through digestion or passage. Lambs fed the brown midrib-3 mutant corn silage (a low lignin mutant, with increased fiber and DM digestibility) increased voluntary dry matter intake by 29% over controls (Muller et al., 1972). This suggests that in these lambs, intake was limited by physical distention. Other workers saw no effect of forage fiber digestibility on intake of midlactation dairy cows (Robinson and McQueen, 1992). These cows were gaining weight throughout the trial, indicating positive energy balance. This may indicate that intake in these mid-lactation cows was not limited by rumen capacity, but rather by metabolic controls.

The satiety or chemostatic model of control of dry matter intake accounts for limits on intake observed with diets with higher caloric density. Again, intake control can be thought of as a long-term regulation, or as the result of control of individual meal patterns and feeding behavior within a day. With the classic chemostatic model of intake control, the animal eats to meet its long term metabolic requirements. This would not appear to be the primary factor regulating feed intake in early lactation dairy cattle, as these animals are usually in negative energy balance. Alternatively, intake of an individual meal ceases in response to some factor triggered by excess nutrients in the blood stream as a result of feed intake. This factor may be an absorbed nutrient or some hormone released in response (Seoane et al., 1972).

When blood from fasted and fed sheep was trans-circulated, intake of the fasted sheep was lowered and intake of the fed sheep increased 48% (Seoane et al., 1972). The previously satiated sheep began to eat again 10 minutes after the

initiation of cross-circulation and stopped 15 minutes after cross-circulation ended. This suggests the presence of some substance that triggers meal cessation. In monogastrics, one likely key is the post-prandial increase in blood glucose. Ruminants, however, do not show a post meal increase in blood glucose, because most of the sugar and starch in feed are fermented to volatile fatty acids in the rumen. Significant quantities of starch may pass to the small intestine on some diets (Owens et al., 1986; Nocek and Tamminga, 1991), but blood glucose does not increase with increasing starch disappearance from the small intestine (Nocek and Tamminga, 1991), implying that gut tissue metabolism increases with increasing intestinal starch digestion.

There is some evidence that volatile fatty acids play a role in limiting intake in ruminants. Intra-ruminal infusions of acetic, propionic, or a mixture of acetic, propionic and butyric acids decreased dry matter intake in sheep on hay diets (Weston, 1966). Egan (1966) observed a decrease in voluntary feed intake with infusion of volatile fatty acids that was only partly related to the energy content of the infused acids. The effect was most pronounced on the day following infusion. Shinozaki (1959) found that short term (10 minutes) infusion of a .6 M solution of propionate and a .5 M solution of butyrate inhibited rumen motility and appetite of goats, but a .5 M solution of acetate had no effect under these conditions. These infusion levels were approximately equivalent to ruminal VFA concentrations of 180 mM.

Receptors sensitive to the concentration of acetate are apparently found on the lumen side of the rumen wall, as infusion of acetate to the rumen had a greater effect on dry matter intake than injection of the same amount to the jugular vein (Baile and Della-Fera, 1981). Infusion of acetate to the dorsal area of the rumen had greater effect on intake than infusion to the ventral rumen, reticulum or abomasum (Baile and Della-Fera, 1981). Effect of infused propionate

on intake is apparently through different receptors than is the effect of acetate. Infused propionate depressed intake regardless of site of ruminal infusion, and propionate injected to the ruminal vein depressed intake to a greater extent than that infused to the rumen, or injected to the mesenteric vein, portal vein, or carotid artery.

Large quantities of fat added to the diet of ruminants also have been shown to decrease feed intake (Dinius et al., 1974; Bines et al., 1978). This may be through effects on rumen fermentation, through satiety control of intake, or through the release of hormones that may limit intake.

Osbourn et al. (1974) demonstrated that intake of high quality immature forages was limited under certain conditions, and suggested that this was due to their high level of soluble carbohydrate. The rapid fermentation of these soluble carbohydrates would be expected to cause a rapid accumulation of acids, reducing rumen pH. They suggested that the effect of ruminal pH on intake was through the inhibition of cellulolytics, slowing forage cell wall digestion. Delayed cell wall digestion would increase rumen pool size of NDF, and the limit on intake could therefore be attributed to a fill effect. Intake limits due to rumen fill seem unlikely with immature forages. The alternative possibility is that the rapid accumulation of acid, *per se*, decreased intake. Treatment of grass silage with sodium bicarbonate increased silage pH from 4.0 to 5.4, and increased subsequent DMI of sheep and cattle by 9.7 to 20.7% (McLeod et al., 1970). Addition of lactic acid to grass silage reduced pH from 5.4 to 3.8, and reduced subsequent DMI of sheep by 22%. This suggests that intake of silage may be affected by acid content of the silage. The effect of acid on intake in these studies may be due to either an effect on palatability or a ruminal effect.

Evidence exists that level of ruminally degraded starch may affect dry matter intake in early lactation cows, possibly through a short term inhibition of

intake by lowered mean or minimum pH. Increased levels of ruminally degraded starch decreased intake in several studies (McCarthy et al., 1989; Oliveira et al., 1990; Casper et al., 1990b; Moore et al., 1992; Aldrich et al., 1993; Dhiman and Satter, 1993; Seymour et al., 1993; Varela et al., 1993), but had no effect in others (Veira and MacLeod, 1980; Herrera-Saldena and Huber, 1989; Poore et al., 1989; Casper et al., 1990a; Grings et al., 1992). The inconsistency in results among trials may be due to differences in fiber levels, fiber composition, starch source, rumen size or physiological state of the animal. The role of ruminally degraded starch in regulating dry matter intake is discussed more thoroughly in section 1.4.

The effect of VFA infusion, acid content of silages and ruminally degraded starch on feed intake may be through ruminal pH. According to Baile and Della-Ferra (1981) receptors sensitive to changes in pH have been isolated in the rumen epithelium. They suggest that intake is depressed as ruminal pH falls below 5 or 5.5 because of ruminal stasis. They assert that pH is not likely a physiological controller of intake, but rather a primary cause of reduced intake in pathological situations.

1.2 ASPECTS OF ENERGY METABOLISM IN EARLY LACTATION DAIRY COWS

Cows in negative energy balance draw on their tissue reserves to meet the energy demands of peak milk production. Lipolysis in adipose tissue is controlled by the enzyme hormone-sensitive triacylglycerol lipase, which converts triglycerides to monoglycerides and non-esterifed fatty acids (NEFA). Monoglycerides are then hydrolyzed to glycerol and NEFA. NEFA, complexed with bovine serum albumin, are transported to the liver for oxidation, ketogenesis, or re-esterification to triglycerides. Increased lipolysis increases serum

concentrations of NEFA and subsequent uptake by the liver, as the liver takes up a fixed percentage of the NEFA presented (15-20%) (Emery et al., 1992). Normal concentrations of NEFA are in the range of .3 mM, or 300 μeq/l, while cows in severe negative energy balance have serum NEFA concentrations in the range of .6 - 2 mM (600 to 2,000 μeq/l) (Bergman, 1971). Excessive hydrolysis of triglycerides from the adipose tissue may lead to increased incidence of fatty livers, metabolic disease and poor reproductive performance (Emery et al., 1992).

Dietary triglycerides and those formed by the liver are transported in the blood as lipoproteins (chylomicrons and VLDL respectively), although secretion of endogenous triglycerides from the liver is limited in ruminants (Zammit, 1990; Emery et al., 1992). Serum triglyceride levels are depressed in early lactation cows, elevated on high fat diets and elevated during fasting. Normal range of serum triglycerides in ruminants is 10 - 20 mg/dl.

Ketones (acetone, acetoacetate and ß-hydroxybutyrate) are important metabolic fuels for the heart, skeletal tissue and kidney. In ruminants, fatty acids, oxidized to acetyl CoA in the mitochondria of liver cells, are converted to ketones through ß-Hydroxy-ß-methylglutaryl-CoA (HMG CoA) in the cytosol. An alternative source is the conversion of butyrate to ß-hydroxybutyrate in the rumen wall. This source declines in importance as intake declines during severe ketosis (Bergman, 1971). Ketones can be converted to acetyl CoA which can enter the TCA cycle to be used as an energy source, or be used for fat synthesis. Ketogenesis appears to be regulated by the supply of fatty acids to the liver and by the metabolic state of the liver, as influenced by levels of insulin and glucagon (Zammit, 1990). Excessive triglyceride hydrolysis, fatty acid oxidation, gluconeogenesis and ketogenesis can lead to ketosis, a condition characterized by abnormally high levels of ketones in the blood. The availability of carbohydrate is thought to play a key role in the development of ketosis

(Bergman, 1971). The critical point in the development of ketosis may be insufficient precursors for oxaloacetate production and subsequent glucose synthesis, although this theory is controversial (Kronfeld, 1969; Bergman, 1971). According to this theory, excessive ketogenesis in early lactation dairy cattle may occur when the demand by the mammary gland for glucose exceeds the capacity for gluconeogenesis. Insufficient hepatic oxaloacetate diverts acetyl CoA to the production of ketones. The normal range of ketone concentrations in the blood of ruminants is below 10 mg/dl, while ketotic cows will have ketone levels in excess of 30 mg/dl (Bergman, 1971). Ketone bodies are acids and excessive concentrations put a strain on the buffering capacity of the blood. Ketosis causes a marked decline in feed intake and milk yield (Bergman, 1971).

Lactating dairy cows must synthesize large quantities of glucose to support milk lactose production. Most of the sugar and starch in feed are fermented to volatile fatty acids in the rumen, so the major source of glucose for the ruminant is gluconeogenesis, with propionate as the primary precursor (Bines and Hart, 1982). Some amino acids are gluconeogenic as well. Elevated serum glucose levels stimulate the ß cells of the pancreas to secrete insulin. Insulin is an anabolic hormone, directly and indirectly stimulating glycogen synthesis, fatty acid synthesis and uptake of glucose by peripheral tissues. Gluconeogenesis and glucose release from the liver are inhibited by insulin (Bines and Hart, 1982). Insulin concentration is negatively correlated with milk yield (Walsh et al., 1980). Glucose challenge (Cummins and Sartin, 1987) or pulse infusion of a VFA mixture high in propionate to the rumen of early- and mid-lactation cows (Istasse and Ørskov, 1984) increased serum insulin concentrations, while continuous infusion of the VFA mixture did not affect insulin concentration (Istasse and Ørskov, 1984). Mid-lactation cows on 85% grain diets increased serum insulin concentration relative to cows fed 35% grain, but there was no difference in

serum insulin concentration between high and low grain diets in early lactation cows (Jenny et al., 1974). Increases in insulin concentration are more likely, therefore, with meal fed animals or those fed grain and forage separately, than those fed a total mixed ration (TMR). With TMR feeding, large differences in ruminal propionate production or glucose absorption from the intestine are apparently necessary to see an insulin response (Bines and Hart, 1982).

1.3 ROLE OF RUMINALLY DEGRADED STARCH AND LACTATE IN THE ETIOLOGY OF ACIDOSIS

Diets consumed by early lactation dairy cows often contain high levels of ruminally degraded starch. Lactate is a major fermentation end product of starch under certain rumen conditions. There is evidence that in early lactation cows both enhanced lactate production and impaired lactate utilization may cause sub-acute acidosis conditions similar to that commonly observed in feedlot cattle. Lactate has a pKa of 3.86 while acetate, propionate and butyrate have pKa's around 4.8. When rumen pH declines, lactate may cause a more dramatic decline in pH than the VFA's, due to this lower pKa.

Cattle abruptly switched to high concentrate diets often accumulate lactate in the rumen, which decreases ruminal pH and causes acute or sub-acute acidosis (Slyter, 1976). Lactate production increases because large quantities of readily fermentable carbohydrate are available. Normally, lactate production is not advantageous in the rumen, because each sugar fermented to lactate yields only 2 ATP. When carbohydrate is limiting, production of other VFA's is more efficient. When carbohydrate is plentiful, however, microbes capable of rapid growth, which often produce lactate, are favored. *Streptococcus bovis* is a gram positive starch fermenter that produces a mix of formate, acetate, and ethanol

when growing slowly, but shifts to L-lactate production when liquid dilution rate increases (Russell et al., 1981). *In vitro*, *S. bovis* has a fast maximum growth rate and has been shown to increase lactate production per gram of maltose utilized by tenfold when it is growing quickly as compared to slower growth (Russell et al., 1981; Russell and Allen, 1983). Overgrowth of this microorganism, and accumulation of lactate is often the involved in the development of acidosis. The decline in rumen pH to below 5 caused by these factors then creates a niche for *Lactobacillus* species, lactate producers which are resistant to low ruminal pH (Slyter, 1976; Russell and Allen, 1983; Russell and Hino, 1985)

The displacement of the rumen by the reproductive tract during the preceding dry period causes early lactation cows to have a smaller rumen volume than do cows in later lactation (Forbes, 1983; Grovum, 1986). Primiparous cows also generally have a smaller rumen volume than do older cows. These smaller rumen volumes likely increase liquid dilution rates. Rapid turnover of liquid favors rapid bacterial growth, as slow growing species are more at risk of being washed out of the rumen before they can reproduce. Thus, small rumen volumes favor the rapid growth of *S. bovis*, increasing lactate production still further. *S. bovis* also shifts to lactate production as rumen pH declines. In cows with a fast ruminal dilution rate on high starch diets, therefore, *S. bovis* producing lactate will yield more ATP per unit time than other bacteria, despite low ATP yield per unit substrate (Slyter, 1976; Dennis et al., 1981b; Russell et al., 1981; Russell and Hino, 1985).

Slyter (1976) observed that when pH is in the range of 5-6, free amylase increases and microbial glucose utilization decreases, causing an accumulation of glucose. This may also encourage the accumulation of lactate as lactic acid production is more stimulated by mono- and disaccharides than by starch (Malestein et al., 1981).

The second half of the lactate accumulation equation is lactate utilization. Lactate utilizers may take time to adjust to changes in diet, and become less competitive as pH declines. *Megasphaera elsdenii* and *Selenomonas ruminantium* are the most important lactate fermenters in the rumen (Slyter, 1976; Russell and Allen, 1983). Inoculation of acidotic beef cattle with *M. elsdenii* at the induction of acidosis or 2 hours later decreased lactate, increased ruminal pH, and increased total VFA concentrations as compared to controls (Robinson et al., 1991). Some lactate accumulation is required for lactate utilizers to adjust, as lactate infusion and concentrate feeding increased the rate of lactate utilization (Kunkle et al., 1976). This is supported by feedlot data that show cattle developing mild acidosis and going off feed before adapting to high concentrate diet (Kunkle et al., 1976). A decline in rumen pH to below 5 makes most lactate utilizers uncompetitive. *M. elsdenii* becomes less competitive as pH declines; its maintenance energy expenditure is higher than that of *S. bovis*, and it was washed out of a chemostat (dilution rate exceeded growth rate) at pH 4.8 (Russell et al., 1981). Lactate utilization by *S. ruminantium* is also inhibited by the accumulation of free glucose in pH range 5-6 (Slyter, 1976).

In summary, cows in early lactation on high starch diets may be prone to lactate accumulation. A decline in rumen pH to below 5, or an increase in liquid dilution rate makes most lactate utilizers uncompetitive, causes *S. bovis* to produce still more lactate and creates a niche for *Lactobacillus* (Slyter, 1976; Dennis et al., 1981; Russell et al., 1981; Russell and Hino, 1985). Lactate, at pKa 3.86, is likely to affect rumen pH when it accumulates. This spiraling effect of increasing lactate production and decreasing pH contributes to the development of acute or sub-acute acidosis.

Symptoms and consequences of acute acidosis may include dullness, diarrhea, hyperventilation, dehydration, inhibited muscular activity of the rumen

wall, parakeratosis (a thickening of the rumen wall which decreases VFA absorption), reduced salivation, reduced intestinal motility, damage to the rumen, damage to the liver and death. Consequences of sub-acute acidosis are reduced feed intake and performance (Slyter, 1976; Nagaraja et al., 1985).

1.4 EFFECT AND FUNCTION OF IONOPHORES

The Na^+ and K^+ ionophores monensin and lasalocid are used extensively in the cattle industry to improve feed efficiency. Monensin was approved for use in feedlot cattle in 1976 and lasalocid was approved in 1982. Neither of these ionophores is currently approved for use in lactating dairy cattle. Effects of ionophores include decreased methane production (Bartley et al., 1979; Chen and Wolin, 1979; Fuller and Johnson, 1981; Russell and Strobel, 1988), decreased acetate to propionate ratio (Dinius et al., 1976; Bartley et al., 1979; Poos et al., 1979; Nagaraja et al., 1982; Rogers and Davis, 1982; Burrin and Britton, 1986; Katz et al., 1986; Russell and Strobel, 1988), and decreased ruminal protein degradation (Bartley et al., 1979; Poos et al., 1979; Fuller and Johnson, 1981; Muntifering et al., 1981; Russell and Strobel, 1988). Ionophores have been shown to decrease lactate production *in vitro* (Dennis et al., 1981a; Dennis et al., 1981b) and *in vivo* (Nagaraja et al., 1981; Nagaraja et al., 1982; Nagaraja et al., 1985) and may allow greater levels of starch to be included in diets for cows in early lactation without adverse effect on rumen function. Increased starch in the diet will allow more energy dense diets for early lactation cows without the addition of fat supplements.

1.4.1 MODE OF ACTION OF IONOPHORES

Ionophores act as antibiotics which primarily affect gram positive microorganisms by dissipating chemical and electrical gradients across cell membranes (Russell, 1987; Russell and Strobel, 1989). Bacteria use membrane bound ATPases to move protons across cell membranes. Removing protons from the cell creates chemical and electrical gradients. Energy stored in these gradients can be used to drive ATP synthesis. Normally, the intracellular environment is more negative and alkaline than the exterior environment. Intracellular concentrations of K^+ are high and concentrations of Na^+ and H^+ are low. Ionophores are highly lipophilic substances with a hydrophobic exterior which allows them to pass through membranes, and a hydrophilic interior which can bind ions. They are thus able to 'shield' ions, helping move K^+ ions out of the cell and H^+ ions in, creating 'leaky' membranes. Pumping H^+ ions out to counteract ionophore action is energetically costly. This increases maintenance costs, depletes intracellular ATP, and inhibits the growth of bacteria (Russell, 1987; Russell and Strobel, 1989).

Other possible explanations for inhibition of bacterial growth by ionophores include a decline in intracellular pH, change of intracellular ions or the loss of active transport activity with dissipation of the gradient and the ATP pool (Russell, 1987; Russell and Strobel, 1989). Ionophore efficiency may vary by mineral concentrations in the diet, if extracellular ion concentration is altered, as high external concentration of K^+ ions decreases the effect of ionophores (Dawson and Boling, 1987). Lasalocid has higher affinity for K^+ than for Na^+, while monensin has the reverse affinity, but the magnitude of the ion gradient is quantitatively more important than the affinity of the ionophore for individual ions.

Ionophore resistance is related to microbial cell wall structure (Russell and Strobel, 1989). The outer membrane of gram negative bacteria is impermeable to many macromolecules, and solute movement is by porins or hydrophilic channels. Ionophores are large, hydrophobic molecules with molecular weights between 500 and 2,000. This membrane structure appears to provide protection from ionophores as gram negative bacteria are generally ionophore resistant, and gram positive organisms are generally ionophore sensitive. Protozoa and fungi lack an outer membrane, and are generally, but not always, sensitive *in vitro* (Dinius et al., 1976; Poos et al., 1979; Hino, 1981; Cumming et al., 1984; Dennis et al., 1986; Katz et al., 1986). Some microbes which stain gram negative have gram positive cell structure and are ionophore-sensitive (i.e. *Butyrovibrio fibrisolvens* and *Ruminococcus flavefaciens*). However not all true gram negatives are resistant to high concentrations of ionophores, sensitive gram negatives can adapt and become resistant, and some gram positives can become resistant (Dennis et al., 1981b; Russell and Strobel, 1989).

Mutants that have adapted to resist ionophore action have been observed (Chen and Wolin, 1979). Antibiotic resistance in bacteria develops by three general schemes (Russell and Strobel, 1989). These include the synthesis of enzymes to degrade antibiotic, the alteration of the cellular target of the antibiotic, and a change in cellular permeability. Another possible resistance mechanism is increased ion pump activity, but this is energetically costly and may decrease chances of survival *in vivo*. Russell and Strobel (1989) observed that after ten years of widespread use, ionophores still improve beef cattle performance, and concluded that sensitivity of rumen bacteria is relatively stable.

1.4.2 EFFECT OF IONOPHORES ON RUMINAL FERMENTATION

Microbes sensitive to ionophore action generally are those that produce more hydrogen, ammonia and lactate than do resistant bacteria (Fuller and Johnson, 1981). The reduction in hydrogen production decreases methanogenesis by up to 30% (Chen and Wolin, 1979). In beef cattle, methane production can be up to 12 liters h^{-1} (Russell and Strobel, 1989). Methane is a high energy compound, and its loss through eructation represents a loss of up to 12% of feed energy. Therefore, reducing methanogenesis may significantly improve energetic efficiency.

Ionophores increase production of the gluconeogenic precursor propionate, by selecting for succinate producers (*Bacteroides*) or propionate producers (*S. ruminantium*) to dispose of reducing equivalents (Chen and Wolin, 1979; Dennis et al., 1981b). Succinate can be de-carboxylated to propionate. *In vitro*, reduced acetate, increased propionate and decreased acetate to propionate ratios have been observed with ionophore treatment (Bartley et al., 1979; Katz et al., 1986; Russell and Strobel, 1988).

In vivo, however, effects of ionophore on acetate and propionate vary. Acetate to propionate ratios decreased with ionophore treatment in steers on a 90% forage diet (Dinius et al., 1976), in lambs fed ionophores for 6 weeks (Poos et al., 1979), in feedlot steers (Owens, 1980), in steers on a 50% forage and 50% concentrate diet (Rogers and Davis, 1982), in short term studies with beef cattle dosed ruminally with high starch mixtures or glucose to induce acidosis (Nagaraja et al., 1981; Nagaraja et al., 1982; Nagaraja et al., 1985), in beef cattle switched from a high forage diet to a high grain diet (Burrin and Britton, 1986), and in early lactation cows from 0 - 3 weeks postpartum (Sauer et al., 1989). In early lactation

cows, acetate and total VFA concentrations decreased while propionate concentrations, and acetate to propionate ratio were unaffected by lasalocid.

In steers fed 100% concentrate diets consisting of whole corn, or a mix of varying proportions of whole corn and whole wheat fed for 50 and 56 days monensin had no effect on acetate or propionate concentrations (Lyle et al., 1981). In midlactation cows, acetate to propionate ratios were unaffected by lasalocid in one study (Beede et al., 1986), while in two others, initial decreases in A:P were observed but the ionophore's effects diminished with time (Johnson et al., 1988; Weiss and Amiet, 1990). Adaptation of the microbial population to lasalocid was suggested as one explanation for these observations. The time period for adaptation was greater than 35 days in one trial (Johnson et al., 1988), and less than 28 days in the other (Weiss and Amiet, 1990). Russell and Strobel (1988) suggested that the effect of ionophore on acetate and propionate concentration may vary by diet, as the decreased A:P in *in vitro* incubations with hay as substrate was due to a decrease in acetate, while with corn as substrate, it was due to an increase in propionate.

Ionophores have been shown to decrease concentrations of both D- and L+ lactate *in vitro* (Dennis et al., 1981a; Dennis et al., 1981b) and *in vivo* (Nagaraja et al., 1981; Nagaraja et al., 1982; Nagaraja et al., 1985), slow *in vitro* fermentation of various carbohydrates (Dennis et al., 1981a) and raise *in vitro* or ruminal pH (Dennis et al., 1981a; Dennis et al., 1981b; Nagaraja et al., 1981; Nagaraja et al., 1982; Nagaraja et al., 1985). Most of the major lactate producers, including *S. bovis* and *Lactobacillus* species, are inhibited by lasalocid (Dennis et al., 1981b; Nagaraja et al., 1982). The lactate fermenters (*Megasphaera, Selenomonas, Anaerovibrio, Veillonella*) are resistant to ionophores (Dennis et al., 1981b; Nagaraja et al., 1982). The decline in lactate concentration with ionophore treatment is attributed to both decreased production and enhanced

utilization. Minimum inhibitory concentrations of lasalocid on *S. bovis in vitro* range from .38 - .75 μg/ml.

In two other *in vitro* studies, however, lactate production was increased by ionophores (Beede and Farlin, 1977; Bartley et al., 1979). This may be due to the fact that some lactate producers (*Bacteroides*, *Selenomonas*, *Succinimonas* and *Succinivibrio*) are not inhibited by ionophores (Dennis et al., 1981b). Schelling (1984) suggests that ionophores increase lactate and do not affect ruminal pH when animals are not 'stressed with carbohydrate', but that with carbohydrate stress, ionophores increase ruminal pH and decrease lactate concentrations.

Ionophores have been shown to help control acute acidosis in beef cattle (Nagaraja et al., 1981; Nagaraja et al., 1982; Nagaraja et al., 1985). Nagaraja et al. (1985) induced acidosis with an intra-ruminal dose of a ground corn-corn starch mixture. Ionophores were administered with the corn mixture. Steers on the lasalocid treatment resisted acidosis (pH > 4.5) for up to 78 hours after dosing, while control animals became acidotic within 54 hours. Treated cattle had higher pH and lower lactate concentrations vs. control. In lasalocid treated animals, rumen pH did eventually drop below 5, but this decline was due to an increase in volatile fatty acid concentration, not to lactate accumulation (Nagaraja et al., 1985). In a similar study, (Nagaraja et al., 1981), intraruminal infusion of lasalocid and monensin prior to dosing the rumen with glucose or corn decreased lactate concentrations and increased ruminal pH, preventing acidosis in beef cattle.

Monensin increased rumen pH, decreased total VFA concentrations, but tended to increase lactate in beef cattle with sub-acute acidosis, induced by abrupt change from high forage to high concentrate diets (Burrin and Britton, 1986). In these steers, VFA concentrations increased nearly twofold, and rumen pH declined to around 5.5 in the first twelve hours post feeding. Lactate

concentrations were low across all treatments (<2.0 mM), and poorly correlated with ruminal pH (r=-.14, P<.10). Ruminal pH was more highly correlated with total VFA concentrations than with lactate (r=-.69, P<.01).

Ionophores have also been shown to inhibit proteolytic bacteria, reducing ruminal nitrogen digestibility and ammonia concentrations *in vitro* (Bartley et al., 1979; Katz et al., 1986; Russell and Strobel, 1988) and *in vivo* (Poos et al., 1979; Fuller and Johnson, 1981; Muntifering et al., 1981; Yang and Russell, 1993). Increased retained nitrogen was observed in sheep fed monensin (Poos et al., 1979). Reduced ruminal degradation of protein would increase passage of undegraded intake protein from the rumen to the small intestine. This factor may affect response to ionophores of lactating cows. With diets high in ruminally degraded protein and deficient in total protein or specific amino acids presented to the intestine, ionophores may improve performance by increasing the escape of protein from the rumen. With diets high in ruminally undegraded protein, or diets low or deficient in ruminally degraded protein, however, ionophores may not improve performance.

Effects of ionophores on dry matter, energy and non-structural carbohydrate (NSC) digestibility vary. Some of this variation is likely due to differences between trials on such parameters as diet, intake and passage of feed from the rumen, species and experimental design. In midlactation cows, lasalocid did not affect apparent whole tract digestibility of NSC or ether extract (Johnson et al., 1988). In early lactation cows (Dye et al., 1988), lasalocid did not affect apparent whole tract dry matter digestibility. Ionophores had no effect on energy digestibility in continuous culture (Fuller and Johnson, 1981). In steers (Muntifering et al., 1981), monensin decreased ruminal digestion of organic matter and starch without affecting total tract digestion. In sheep, whole tract DM and

fiber digestibility were decreased with monensin, although this effect diminished by 46 days on treatment (Poos et al., 1979).

In vitro, ionophores have been shown to decrease cellulose digestion (Russell and Strobel, 1988), a potentially negative consequence, particularly in dairy cattle. *In vivo* results, however, suggest that ionophores do not impair fiber digestion (Dinius et al., 1976; Johnson et al., 1988; Russell and Strobel, 1989), perhaps because ionophore treatment generally increases rumen pH and also decreases feed intake, which may increase retention time of fiber in the rumen, increasing the probability of degradation (Russell and Strobel, 1989). Another possible explanation of the difference between *in vitro* and *in vivo* data is that with time, resistant species in the rumen that have similar function as the sensitive bacteria may grow to fill the niche. Ionophores inhibit *Ruminocci*, for example, but another cellulolytic bacteria, *Bacteroides succinogenes*, is resistant and may eventually replace *Ruminocci in vivo* (Russell and Strobel, 1988).

Protozoa lack an outer membrane to protect them from ionophores, but reports in the literature are mixed on whether ionophores inhibit protozoa. Ionophores decreased protozoal concentrations in some studies (Poos et al., 1979; Hino, 1981; Katz et al., 1986; Dennis et al., 1986) but not in others (Dinius et al., 1976; Cumming et al., 1984). *In vitro*, ionophores are reported to be toxic to *Diplodinium*, *Ophryoscolex* and *Holotrich* protozoa at concentrations greater than 8 μg/ml (Hino, 1981) and to *Entodinium* above 4 μg/ml (Hino, 1981; Dennis et al., 1986), although in another study *Holotrich* protozoa were unaffected by either lasalocid or monensin, even at concentrations of 48 μg/ml (Dennis et al., 1986). The observed inhibition of some protozoa by ionophores above 6 μg/ml appears to be short term, as protozoa in rumen fluid from cattle fed ionophore for 19 weeks were not ionophore sensitive (Dennis et al., 1986). Ionophore feeding

may select for ionophore resistant protozoa (Nagaraja et al., 1986; Dennis et al., 1986).

Protozoa play a "buffering" role in the rumen of grain fed cattle, slowing down the rate of starch digestion by sequestering starch and starch-fermenting bacteria (Hungate, 1966; Slyter, 1976; Russell and Hespell, 1981; Jouany et al., 1988). The quantitative importance of this ruminal protection of starch by protozoa is not known. *Entodinium* protozoa have also been shown to consume significant quantities of lactate *in vitro* (Newbold et al., 1987), although the *Holotrich* protozoa produce lactic acid. Fermentation of starch in rumen fluid without protozoa increased lactate concentration when compared to fermentation with protozoa (Nagaraja et al., 1986). Defaunated animals usually have higher levels of lactic acid (Johnson et al., 1986). Ionophores increased lactate concentrations versus controls in one study (Nagaraja et al., 1986), possibly because ionophores inhibited protozoal engulfment of starch or fermentation of lactate.

Overall, then, in most cases ionophores decrease concentrations of acetate and butyrate, increase concentrations of propionate, reduce methanogenesis by up to 30%, decrease lactate concentration and decrease ruminal proteolysis. Ionophores may reduce fiber digestion, and may inhibit protozoa.

1.4.3 ANIMAL PERFORMANCE EFFECTS OF IONOPHORES

In beef cattle on high grain diets, monensin generally decreases intake immediately upon addition to the diet without affecting rate of gain, particularly on very low quality (low fiber digestibility) forages (Baile et al., 1979; Owens, 1980). This increases energetic efficiency. Lasalocid has also been reported to reduce feed intake, but not to the same extent as monensin (Delfino et al., 1988). Lasalocid either causes no change in, or an increase in growth rate to improve

feed conversion. The combination of decreased methanogenesis and increased propionate production with ionophore treatment is believed responsible for the improvement in feed efficiency generally observed in feedlot cattle.

Interestingly, in cattle on high fiber diets with medium to good quality forages (high fiber digestibility), the decrease in intake with monensin is minimized, and rate of gain increases by an average of 14.1% (Owens, 1980). The difference in intake response with fiber quality may be related to the reported effects of ionophores on fiber digestion. Decreasing fiber digestion on poor quality high fiber forages would be expected to decrease intake in animals limited by physical distention. Animals fed the higher quality forages are less likely to be limited by bulk. Intake would thus be less affected by any decrease in fiber digestibility.

Ionophores are not approved for use in lactating dairy cattle, and few studies have been reported. In midlactation cows, there was no effect of lasalocid on milk yield, fat corrected milk (FCM) or milk composition (Beede et al., 1986; Weiss and Amiet, 1990). Milk yield, FCM, milk fat and milk protein were decreased by high levels of lasalocid (36.7 mg/kg DMI or ~ 450 g/d) in another study (Johnson et al., 1988). Dry matter intake was decreased with lasalocid in two studies (Johnson et al., 1988; Weiss and Amiet, 1990). A third study showed decreased intake only at the highest level of addition of lasalocid (36 mg/kg DMI) (Beede et al., 1986). Lasalocid increased body weight gain in two studies (Beede et al., 1986; Weiss and Amiet, 1990). Ionophores did not affect serum glucose in midlactation cows (Johnson et al., 1988).

These results with mid-lactation cows may not reflect conditions in early lactation cows as rumen volume, passage rate of feed from the rumen as well as nutrient requirements and energy status are likely to be different between early and mid-lactation cows.

In early lactation cows, monensin at two levels (Sauer et al., 1989) and lasalocid (Dye et al., 1988) had no effect on milk yield, milk protein or lactose, but decreased milk fat (Dye et al., 1988; Sauer et al., 1989). Lasalocid (Dye et al., 1988) did not affect dry matter intake. Intake was not affected by monensin at 15 mg/kg (~ 200 mg/d), but was depressed at 30 mg/kg (~ 400 mg/d) (Sauer et al., 1989).

Monensin decreased serum ß-hydroxybutyrate concentrations and incidence of sub-clinical ketosis in the first three weeks post-partum (Sauer et al., 1989). No effect of ionophore on serum glucose was seen in early lactation cows. Monensin had no effect on plasma lipid concentrations, including free fatty acids and triglycerides (Sauer et al., 1989).

Feeding behavior of beef cattle treated with ionophore was examined in one study (Baile et al., 1979). They tested the effects of both the active ingredient, monensin sodium, and the commercial product, Rumensin®, on feed intake and intake patterns. Cattle on a roughage diet fed Rumensin® decreased meal size and eating rate intake/eating time, resulting in lower feed intake compared to control animals. Cattle on a concentrate diet fed Rumensin® decreased number of meals, meal size and eating rate while decreasing feed intake relative to control. Infusion of pure monensin into the rumen of concentrate fed animals did not affect eating parameters measured, while roughage fed animals ate more slowly, but for a longer period of time with no net effect on intake. Animals previously fed Rumensin® and offered monensin sodium in the feed ate as much feed as the animals on the control diet. This suggests that cattle were able to detect, and developed an aversion to, the Rumensin® flavor, rather than to the active ingredient, monensin sodium.

1.5 EFFECT OF SITE OF STARCH DIGESTION ON PERFORMANCE

Site and extent of starch digestion by ruminants vary by species and physiological status of animal, grain type, genotype and growing conditions, and physical and chemical processing method (Owens et al., 1986; Nocek and Tamminga, 1991). In typical diets for beef cattle and high producing dairy cattle, large quantities of dietary starch may escape ruminal fermentation and become available for degradation, fermentation and absorption from the hindgut. Owens et al., (1986), observed that in 40 experiments with cattle, between 18 and 42% of dietary starch from corn and sorghum passed from the rumen intact. Nocek and Tamminga (1991), found that site of starch digestion is an important factor in predicting dry matter intake. Regression analysis showed that net energy intake accounted for 48% of the variation in milk yield. Adding level of ruminally digested starch and level of intestinally digested starch to the regression equation increased the r^2 to .58. Additional parameters (i.e. ruminally degraded NDF) did not improve the prediction. They concluded that maximum milk yield was associated with maximum intake of both ruminally digested starch and intestinally digested starch (Nocek and Tamminga, 1991).

Ruminal digestion of any nutrient depends on the competition between its rate of digestion and rate of passage from the rumen. A treatment that increases rate of digestion in the rumen may have no effect on ruminal starch digestion if rate of passage increases as well. Small and large intestine digestibility are also important factors in overall starch utilization. Starch that escapes the rumen must be digested and absorbed in the lower tract to be of benefit to the cow.

Physical processing methods to alter starch digestion include breaking, cracking, grinding, rolling or pelleting grain. Physical processing breaks the outer

coat of the grain to allow access of rumen microorganisms and enzymes. Chemical processing includes the application of heat and moisture, such as steam flaking or ensiling of the grain. Interactions of heat, moisture and pressure increase susceptibility of the starch to enzymatic attack and degradation. In steam flaked cereal grains, decreased density of the flake increases rate of starch degradation (Nocek and Tamminga, 1991). Disrupting the protein matrix around starch may be another mechanism by which processing improves digestion of grains (Theurer, 1986). Most processing methods, therefore, increase ruminal and post ruminal starch digestion (Theurer, 1986; Campling, 1991; Nocek and Tamminga, 1991). High moisture treatments improve extent of ruminal digestion over dry forms, and grinding increased extent of digestion for all forms, although decreasing corn grain particle size has been shown to increase passage from the rumen (Ewing et al., 1986). Other treatments that decrease the rate of ruminal rate of degradation have been suggested. These include NaOH treatment (Ørskov et al., 1978), infra-red heat treatment (Ørskov et al., 1978), and ammoniation (Robinson and Kennelly, 1988).

The 'bypass' of starch to the small intestine may be desirable for a number of reasons. It has been suggested that the metabolism of starch through glucose absorbed from the small intestine may be energetically more efficient than metabolism through fermentation to propionate in the rumen and subsequent gluconeogenesis in the liver (Owens et al., 1986). Additionally, excess fermentation of starch to volatile fatty acids in the rumen may lead to fluctuations in rumen pH that may decrease dry matter intake in the high producing dairy cow (Robinson and Kennelly, 1988; McCarthy et al., 1989).

Diets higher in ruminally degraded starch result in increased ruminal propionate concentrations and decreased acetate to propionate ratios (Ørskov et al., 1970; Ørskov, 1986; McCarthy et al., 1989; Moore et al., 1992; Aldrich et al.,

1993). Barley diets increased VFA concentrations relative to corn diets (McCarthy et al., 1989). Mean rumen pH in this trial was low, and not different for corn and barley diets, but no measure was made of pH range or fluctuation. Ruminal pH in sheep and cattle was lower with ground and pelleted barley treatment than with whole barley (Ørskov and Fraser, 1975; Ørskov et al., 1978) or NaOH treated barley (Ørskov et al., 1978). Treatment of high moisture barley with increasing levels of ammonia, which decreased *in situ* ruminal dry matter degradation rate relative to whole high moisture barley (Robinson and Kennelly, 1988), caused slower rate of ruminal pH decline and butyrate accumulation post feeding in mid to late lactation dairy cows. Feed intake patterns did not differ with ammoniation, although a trend existed for increased rate of consumption with increased level of ammoniation (Robinson and Kennelly, 1989). They suggested that less rapid accumulation of acid post-feeding allowed consumption to occur for a longer period of time before it was inhibited by feedback mechanisms.

Decreased dry matter intake in early lactation cows with more rapidly available starch sources have been observed with steam rolled barley as compared to ground shelled corn (McCarthy et al., 1989; Casper et al., 1990b), and with diets formulated to be high in ruminally available non-structural carbohydrate (RANSC) (Aldrich et al., 1993). In these studies (McCarthy et al., 1989; Casper et al., 1990b; Aldrich et al., 1993), differences in diet composition (i.e. starch source, forage source and/or protein source) made the direct cause of these results difficult to assess. Decreased dry matter intake was observed with ground or rolled barley compared to NaOH treated or whole barley with sheep (Ørskov and Fraser, 1975) and cattle (Ørskov et al., 1978). Decreased DMI was also seen in early lactation cows with steam flaked sorghum relative to a mix of dry rolled and steam flaked sorghum (Oliveira et al., 1990), with dry rolled

compared to steam flaked sorghum (Moore et al., 1992), and with finely flaked corn relative to corn flaked to medium density (Varela et al., 1993). Intake decreased in mid-lactation cows with partially steam-flaked, partially pelleted corn relative to a less fermentable, pelleted corn (Seymour et al., 1993) and with high moisture ear corn or ground corn relative to dry shell corn (Dhiman and Satter, 1993). Other studies, however, showed no change in intake with increased ruminal degradation of starch (Herrera-Saldena and Huber, 1989; Poore et al., 1989; Casper et al., 1990a; Grings et al., 1992). The increased dry matter intake with decreased ruminally degraded starch observed (McCarthy et al., 1989; Casper et al., 1990b; Oliveira et al., 1990; Aldrich et al., 1993; Dhiman and Satter, 1993; Seymour et al., 1993; Varela et al., 1993) may have been due to a minimization of ruminal pH fluctuation and less rapid accumulation of ruminal acids post-meal.

Effect of level of ruminally degraded starch on milk yield is less clear. Decreased milk yield or FCM yield with increased ruminally available starch was observed in early lactation cows fed barley compared to corn (McCarthy et al., 1989; Casper et al., 1990b), high RANSC diets (Aldrich et al., 1993) and with partially pelleted steam flaked grain relative to pelleted (Seymour et al., 1993). Midlactation cows fed ammoniated (less ruminally degraded) barley increased milk yield, but the effect of ruminally degraded starch in this trial was confounded with the difference in nitrogen level in the diet (Robinson and Kennelly, 1989). Increased level of ruminally degraded starch did not affect milk yield or FCM in early lactation cows in three studies (Oliveira et al., 1990; Grings et al., 1992; Dhiman and Satter, 1993) and increased milk yield in early lactation cows in four others (Herrera-Saldena and Huber, 1989; Poore et al., 1989; Casper et al., 1990a; Moore et al., 1992).

The improvement in milk yield and/or FCM observed in some studies with diets lower in ruminally degraded starch (McCarthy et al., 1989; Robinson and Kennelly, 1989; Casper et al., 1990b; Aldrich et al., 1993; Seymour et al., 1993) may be due to a number of factors. Two possible mechanisms are a minimization of rumen pH fluctuation and less rapid accumulation of acid post-meal in the rumen or more efficient use of the glucose absorbed from the small intestine for milk with the more slowly available starch source than the propionate from rumen degradation of barley. The relative importance of these potential mechanisms, the 'ruminal pH effect' or the glucose/propionate efficiency effect certainly would vary. This could explain the variation in response of lactating cows to increased level of ruminally degraded starch in the literature.

Milk protein % is usually unaffected by level of ruminally degraded starch (Herrera-Saldena and Huber, 1989; McCarthy et al., 1989; Casper et al., 1990a; Casper et al., 1990b; Grings et al., 1992; Moore et al., 1992; Varela et al., 1993) However increased milk protein with increased ruminally available starch has been observed (Oliveira et al., 1990; Aldrich et al., 1993). This increase in milk protein may be due to an increase in microbial protein production with increased level of starch fermented in the rumen. Supporting this second proposed mechanism, lactating cows iso-calorically infused with glucose in the rumen or propionate in the duodenum suggest that the increase in milk protein observed with increased rumen starch degradability is due to altered ruminal metabolism rather than increased propionate absorption (Wu et al., 1993). Again, however, the relative importance of different mechanisms would vary. Milk fat is often decreased with increased ruminally degraded starch (Herrera-Saldena and Huber, 1989; Poore et al., 1989; Casper et al., 1990b; Moore et al., 1992; Aldrich et al., 1993) although other trials have observed no effect (McCarthy et al., 1989; Casper et al., 1990a; Oliveira et al., 1990; Grings et al., 1992).

1.6 INTESTINAL STARCH DIGESTION AND GLUCOSE ABSORPTION IN RUMINANTS

The potential for digestion of starch and absorption of glucose in the small and large intestine is an important factor in diets with potential for significant quantities of starch escaping the rumen. Owens et al. (1986) observed that 47 to 88% of the starch presented to the small intestine disappears. Plotting supply of starch to the small intestine against starch disappearance from the small intestine, they observed no plateau of starch disappearance and concluded that enzymatic hydrolysis of starch is not a limiting factor. Nocek and Tamminga (1991) observed an increase in intestinal small and large starch digestion as ruminal escape starch increased in 14 production studies. As starch delivery to the intestine increased, however, starch digestion as a percent of that entering decreased.

1.6.1 SMALL INTESTINE

Most infusion studies show a quantitative limit to starch disappearance from the small intestine in ruminants (Huntington and Reynolds, 1986; Kreikemeier et al., 1991). This limit may be due to limitations on starch hydrolysis in the small intestine, or to limits on glucose absorption from the small intestine, as starch disappearance from the small intestine does not necessarily indicate that glucose is available for use by the animal.

In studies with dairy cows where portal concentration of glucose is measured simultaneously to starch disappearance from the lumen of the small intestine, net glucose absorption (portal blood flow times the concentration difference between portal and arterial blood) is usually negative (Huntington,

1982; Lomax and Baird, 1983), indicating glucose utilization by the gut tissue. Glucose absorption in the small intestine may be limited by residence time or abundance of glucose transport proteins. Also, metabolic requirements of the gut tissue may increase with increases in starch digestion and glucose absorption in the small intestine (Harmon, 1991).

1.6.2 LARGE INTESTINE

Large intestinal fermentation of starch yields similar end-products to ruminal fermentation (Ørskov et al., 1970). Continuous infusion of starch to the terminal ileum of sheep (Ørskov et al., 1970) showed that up to 138 g of starch per day may be fermented in the cecum and colon. Incidence of scouring, indicated by decreased dry matter of the feces, increased as starch fermented in the cecum increased.

1.7 CONCLUSIONS

Diets with high levels of ruminally degraded starch may increase lactate concentrations in the rumen. This effect occurs through both increased lactate production and decreased consumption by different populations of rumen microbes. This effect may be pronounced in early lactation cows, and in first calf heifers due to their small rumen size and rapid liquid dilution rate, as these conditions favor rapidly growing lactate producers. This increase in lactate, and subsequent decrease in mean or minimum rumen pH may be a mechanism by which lactating dairy cows on high starch diets decrease dry matter intake.

The use of ionophores may be one method for controlling lactate accumulation as ionophores have been shown to decrease lactate concentrations *in vitro* and *in vivo*. Another method for controlling this may be to alter the site

of starch digestion, by selection of grain type or processing method. If one attempts to increase passage of starch from the rumen, the digestibility of starch in the small intestine becomes important. The literature suggest that there may be some limit to both starch digestion and glucose absorption in the ruminant small intestine. If quantity of starch passed to the intestine exceeds these limits, decreasing the ruminal availability of starch may decrease total tract starch digestibility and decrease performance.

The objective of this project was to examine the mechanism of the observed reduction in dry matter intake with increased level of ruminally degraded starch in early lactation dairy cattle. Our hypothesis is that finely ground corn will increase level of ruminally degraded starch and increase lactate concentration relative to coarsely cracked corn, decreasing mean or minimum ruminal pH, decreasing dry matter intake and impairing subsequent performance of early lactation dairy cows. We hypothesize that the ionophore lasalocid will alleviate these effects by decreasing lactate concentration. Further, these effects may be more pronounced in primiparous cows than in the multiparous cows, due to differences in rumen dilution rate.

CHAPTER 2

EFFECT OF LASALOCID AND CORN GRAIN PARTICLE SIZE ON RUMEN PARAMETERS AND FEEDING BEHAVIOR IN EARLY LACTATION DAIRY CATTLE

ABSTRACT

The effect of corn grain particle size and the ionophore lasalocid on rumen fermentation and feeding behavior was examined to elucidate the mechanism of reduced feed intake with increased ruminally degraded starch. Eight multiparous and four primiparous early lactation cows were fed diets with cracked or ground corn grain, with or without lasalocid in 3, 4x4 Latin squares with 21 d periods. Ground corn decreased starch ruminal turnover time and increased rate of ruminal starch degradation. Lasalocid increased lactate concentrations, and did not affect acetate to propionate ratio (A:P). Ground corn did not affect ruminal lactate concentrations, increased propionate, decreased A:P, and decreased branch chain fatty acids (BCFA). Range in ruminal pH within a day increased with ground corn, while mean pH was unaffected by treatment. Lasalocid increased total ruminating time, and ground corn decreased rumen contractions. Lasalocid tended ($P<.11$) to increase water intake, and ground corn increased water intake.

Interactions between lasalocid and corn grain particle size were not observed for most effects.

2.1 INTRODUCTION

Diets consumed by early lactation dairy cows often contain high levels of ruminally degraded starch. The literature suggests that level of starch degraded in the rumen may significantly affect ruminal fermentation. Increased VFA concentrations (McCarthy et al., 1989) and a more rapid decline in ruminal pH post-meal (Robinson and Kennelly, 1989) have been observed in lactating cows on diets with more rapidly available starch sources (i.e. barley) compared with more slowly available starch sources (i.e. corn). Ruminal pH was lower in sheep and cattle fed ground and pelleted barley relative to whole barley, and intakes were lower on these diets as well (Ørskov and Fraser, 1975; Ørskov et al., 1978). The effect of level of ruminally degraded starch on acid production in the rumen may explain the decrease in dry matter intake observed on diets with more rapidly available starch sources (McCarthy et al., 1989; Casper et al., 1990b; Oliveira et al., 1990; Moore et al., 1992; Aldrich et al., 1993; Dhiman and Satter, 1993; Seymour et al., 1993; Varela et al., 1993).

Accumulation of lactic acid may cause more dramatic declines in rumen pH than acetate, propionate and butyrate, as the pKa of lactate is lower (3.86 vs. ~ 4.8). Lactic acid accumulation is associated with the onset of acute and sub-acute acidosis in beef cattle on high starch diets (Slyter, 1976; Russell and Hino, 1985). Lactate concentration has not usually been measured in lactating cow trials, but can be a key factor in regulation of ruminal pH and dry matter intake in beef cattle (Slyter, 1976; Russell and Hino, 1985). Ruminal pH may be an important factor regulating feed intake in early lactation cows as well.

Streptococcus bovis is a gram positive, ionophore sensitive starch fermenter that produces a mix of formate, acetate, and ethanol when growing slowly, but shifts to lactate production when liquid dilution rate increases (Russell et al., 1981). Lactate production by *S. bovis* per gram of maltose utilized can increase tenfold when it is growing quickly as compared to slower growth, particularly at a low pH (Russell et al., 1981). Early lactation cows probably have faster liquid dilution rates than do cows in later lactation due to smaller rumen size (Forbes, 1983; Grovum, 1986), and so may be prone to lactate accumulation on high starch diets.

The ionophore lasalocid has been shown to decrease lactate production *in vitro* (Dennis et al., 1981a; Dennis et al., 1981b) and *in vivo* (Nagaraja et al., 1981; Nagaraja et al., 1982; Nagaraja et al., 1985), primarily by inhibition of *S. bovis* (Dennis et al., 1981b; Nagaraja et al., 1982), and may allow greater levels of starch to be included in rations for cows in early lactation without adverse effect on rumen function. Cracked corn and ground corn are chemically identical sources of starch differing only by ruminal availability of starch. Slowing down ruminal starch fermentation, shifting the site of starch digestion to the small intestine, or otherwise controlling lactate production from starch may allow increased starch in the ration without causing lactate accumulation. Increased starch in the ration increases energy density of diets, to better meet the early lactation cow's energy demands without the use of expensive fat supplements.

Our objective was to examine the effect of level of rumen degradable starch and the ionophore lasalocid on rumen fermentation and feeding behavior of cows in early lactation. We hypothesized that diets with ground corn would have higher levels of ruminally degradable starch than cracked corn diets, and that ground corn diets and diets without the ionophore would increase lactic acid and VFA concentrations in the rumen, increase ruminal pH fluctuations, decrease mean ruminal pH and affect feeding behavior of early lactation dairy cows.

2.2 MATERIALS AND METHODS

Twelve ruminally cannulated Holstein cows (eight multiparous and four primiparous) averaging 16 days in milk (DIM) were grouped by parity and calving date and assigned to one of three Latin squares. Squares were staggered to minimize average DIM. Two squares were composed of multiparous cows and one square of primiparous cows. Within a square, each cow was randomly assigned to one of four treatment combinations.

A 2x2 factorial arrangement of treatments was utilized. Dietary treatments were particle size of corn grain, (ground or cracked) and the inclusion or absence of lasalocid (0 vs. 360 mg/cow per d). The data were analyzed as replicated (n=3) 4x4 Latin squares balanced for carryover effects.

Lasalocid premix (Bovatec®) and ground corn were mixed in bulk, and then 114 g of the mix, containing 180 mg of lasalocid, was weighed into individual envelopes in the laboratory. One envelope was hand mixed into the feed for cows on the lasalocid treatment at each feeding to assure even intake throughout the day. Cows on the no lasalocid treatment had 114 g of ground corn carrier without lasalocid hand mixed into their feed at each feeding.

Rations consisted of alfalfa silage, corn silage, dried shelled corn, soybean meal, blood meal, and minerals and vitamins. Corn silage was ensiled in a bag (100 T) to minimize variation, and reduce spoilage at feed out. Each load of corn silage was sampled at filling and analyzed prior to the initiation of the experiment to assess variation in composition. Dried shelled corn in quantity sufficient for the duration of the experiment was thoroughly mixed and divided in half. Half of the corn was then coarsely cracked and half was finely ground. Mixing was done to minimize variation in starch digestibility due to source.

Experimental treatment periods were 21 days, with 14 days for diet adjustment followed by seven days of data collection. Throughout the experiment, cows were fed twice daily at 0700 and 1900 h, at 110% of expected intake.

Rumens were evacuated at 1300 h on day 7 of the collection period, immediately prior to the introduction of the new diets. Volume and weight of rumen digesta was recorded, and digesta was subsampled. Rumen fluid was subsampled from the rumen contents for VFA and lactate analysis. Samples were frozen immediately. Rumen contents were exchanged among cows according to the schedule of treatments to decrease carryover effects and hasten adjustment to the new treatment. Rumen pool size (moles) of VFA and lactic acid were determined by multiplying the concentrations of each acid by rumen digesta volume (liters). Rumen pool sizes (kg) of dry matter, liquid, NDF, starch and indigestible fiber were calculated by multiplying the concentrations of each by the rumen digesta dry matter weight (kg).

Rumen fluid samples were taken every three hours beginning at the morning feeding of day 6 of the collection period and continued for 24 hours for measurement of lactate and VFA concentrations. Samples of the liquid from five to six sites in the rumen were combined and subsampled at each time point. Rumen fluid samples were frozen immediately. In preparation for analysis, rumen fluid samples were thawed and centrifuged at 13,000 * g for thirty minutes. The supernatant was centrifuged at 26,000 * g for thirty minutes immediately prior to analysis. VFA and lactate concentrations were determined via high pressure liquid chromatography (Bio-Rad 87-H column, Bio-Rad Laboratories, Life Science Group, Melville, NY; Waters 410 Refractive Index Detector, Waters Chromatography, Milford, MA). Column temperature was 50°C and flow rate was .6 ml min^{-1}.

Ration, orts, and silage samples were dried in a 55° C forced air oven and analyzed for dry matter. Water concentration in the rumen contents was determined by toluene distillation (A.O.A.C., 1984). All samples were ground through a 1 mm screen in a Wiley mill. Neutral detergent fiber was analyzed according to Van Soest et al. (1991). Samples were soaked in 8M urea and α-amylase overnight prior to analysis to facilitate complete removal of starch and protein (Van Soest et al., 1991). Samples were analyzed for ash, and NDF was analyzed sequentially for acid detergent fiber and acid detergent sulfuric acid lignin with A.O.A.C. (1984) methods. Crude protein was analyzed according to Hach et al. (1987). Undegraded intake protein (UIP) and degraded intake protein (DIP) were estimated from NRC book values (National Research Council, 1989). Starch was analyzed by the two stage enzymatic method of method of Aman and Hesselman (1984), with a NaOH gelatinization step according to O'Neil et al. (1993), and analysis of glucose by Karkalas (1985). Diet ingredients and composition are listed in Table 1. Alfalfa silage and corn silage were analyzed weekly throughout the trial for dry matter, neutral detergent fiber and crude protein, and diets were re-balanced accordingly.

Rumen liquid dilution rate was determined by dosing the rumen with cobalt-EDTA in a pulse dose at the morning feeding of day 6 of the collection period, and collecting rumen fluid at 0, 1.5, 3, 6, 9, 12, 15, 18, and 21 hours following dosing. Samples of liquid from five to six sites in the rumen were combined and subsampled at each time point. Samples were frozen immediately. Samples were thawed, filtered through 8 layers of cheesecloth, digested with sulfuric acid and hydrogen peroxide (Hach et al., 1987) and diluted to 100 ml with dH2O. Cobalt concentration in each sample was assayed with atomic absorption spectrometry (IL951 AA/AE Spectrophotometer, Thermo Jarrell Ash) following the manufacturer's guidelines. Cobalt standard curves were created in

a rumen fluid matrix to correct for mineral interactions in the rumen fluid. The rumen fluid for the curve was a composite of 5 ml subsamples of the 0 h rumen fluid samples from all cows, all periods. Liquid dilution rate (k), original concentration of cobalt in the rumen immediately post-dosing (Ao), and background concentration of cobalt in the rumen (BG) were estimated from the disappearance of cobalt over time, using non-linear regression (JMP, version 2.05, SAS Inst. Inc., Cary, NC., 1991) with the equation:

$A = Ao * e^{-kt} + BG$

A = cobalt remaining, ppm

Ao = Initial cobalt concentration, ppm, in the rumen post dosing

k = rate, % h^{-1}

t = time post dosing, h

BG = background levels of cobalt in the rumen, ppm

Ruminal starch turnover time in hours (the sum of starch removal by passage and digestion in the rumen) was determined by the equation:

Turnover time (h) = (kg starch in the rumen) / (kg starch fed h^{-1})

Kg starch in rumen = kg rumen contents DM * % starch in rumen contents

Kg starch fed h^{-1} = ((DM offered, kg/d * % starch in feed) - (DM orts, kg/d * % starch in orts))/24 h

Indigestible residue was determined on feed samples, rumen contents, and orts for the estimation of rates of passage and digestion of feed by the *in vitro* degradation of samples for 115 hours with the method of Goering and Van Soest (1970). Duplicate samples of TMR, rumen contents, and feces, and single samples of orts were incubated *in vitro* with media, reducing solution and rumen fluid inocula for 115 h, and the remaining sample was analyzed for neutral detergent

fiber as above with the exclusion of urea and α-amylase. Inocula was from an alfalfa hay-fed, non-pregnant, non-lactating Holstein cow, and was collected two hours post-feeding. The indigestible fraction of NDF (fi) was calculated as (NDF remaining after 115 hr incubation) / (Original NDF). The digestible fraction of the NDF (fd) was calculated as (1-fi).

Total rate of escape of fiber from the rumen (passage + digestion) was calculated as the intake of digestible fiber in kg per hour divided by the pool size of digestible fiber in the rumen in kg to yield total rate of escape, h^{-1}. Estimated rate of fiber passage was calculated as the intake of indigestible fiber in kg per hour divided by the pool size of indigestible fiber in the rumen in kg to yield rate of passage, h^{-1}. Rate of digestion of the fiber was calculated as the difference between total rate of escape and rate of passage. Assumptions in rates calculations were that the indigestible fraction of fiber (fi) disappears only by passage, that the digestible fraction of fiber (fd) disappears by both passage and digestion, that indigestible and digestible NDF pass at the same rate, that intake of NDF is constant throughout the day, and that the rumen is in steady state (Q feed in = Q feed out). Equations used were:

kp + kd = (fd of TMR * dintake/dt) / D

kp = (fi of TMR * dintake/dt) / I

kd = (kp + kd) - kp

dintake/dt = kg NDF consumed h^{-1}

fi = Indigestible fraction of the NDF

fd = Digestible fraction of the NDF = 1 - fi

I = pool size (kg) of indigestible NDF in rumen contents

= rumen contents kg DM * rumen contents % NDF * fi of rumen contents

D = Pool size of digestible NDF in rumen contents

= rumen contents kg DM * rumen contents % NDF * fd of rumen contents

Rate of ruminal starch degradation was measured by *in vitro* degradation with the method of Goering and Van Soest (1970). Samples with approximately .4 grams of starch were incubated with media, reducing solution and rumen inocula collected from an alfalfa hay-fed non-pregnant, non-lactating Holstein cow, two hours post-feeding. Samples were analyzed for residual starch at 0, 3, 6, 9, 12, 18, 24, 40 and 48 hours. Rates of degradation of starch (k) and lag time for cracked and ground corn were estimated by non-linear regression with the software package JMP, version 2.05 (SAS Inst. Inc., Cary, NC., 1991) with the equation:

$A = 100 * e^{-k(t-lag)}$

A = residual starch, % of original

k = rate, % h^{-1}

t = time of incubation, h

lag = discrete lag, h

Rate of starch passage from the rumen was estimated by the difference between total rate of removal of starch (kg starch fed h^{-1}/kg starch in the rumen) and estimated rate of degradation.

Corn grain particle size was measured on cracked and ground corn samples taken weekly throughout the trial, by dry sieving through 8 pans (Pan #'s 4, 8, 16, 30, 50, 100, 200 and bottom pan) for about 15 minutes, or until the bottom pan weight was constant (Sieve Shaker Model RX-86, W. S. Tyler Inc., Gastonia, NC) (ASAE 1968). Mean particle size of corn was calculated and variance was determined by fitting the data to the Gamma distribution (Allen et al. 1983).

Ruminal pH, reticular contractions, chewing activity, and feed and water consumption were recorded at five second intervals on days 1 through 5 of the collection period by a computerized data acquisition system (Dado and Allen, 1993). A pH electrode was partially encased in Tygon tubing sealed with black rubber cement to prevent rumen fluid accumulation and placed in the rumen. The electrode was surrounded by a wire cage to keep it away from the rumen wall and weighted to keep it suspended in the ventral rumen. Electrodes were removed from the rumen and calibrated every two days during collection periods. Electrodes generally held their calibration within .05 pH units. Electrodes outside of this range were recalibrated or replaced. Severe calibration problems, and occasional problems with wiring or connectors were recorded for each cow, and pH data from that cow for the entire day was removed from the data set prior to analysis.

Means were calculated for meal size (kg meal^{-1}), number of meals (day^{-1}), minutes between meals, eating time in minutes (meal^{-1} and day^{-1}), eating chews (meal^{-1} and day^{-1}), eating chew rate (chews minute^{-1}), number of ruminating bouts (day^{-1}), minutes between ruminating bouts, ruminating time in minutes (bout^{-1} and day^{-1}), ruminating chews (bout^{-1} and day^{-1}) and ruminating chew rate. Drinking bouts, water consumption, drinking rate and reticular contractions per day, mean pH, and fraction of time below pH 5.5, 5.75, and 6 were calculated. Range in rumen pH was calculated as the 95% confidence interval around the mean, to prevent undue effect of an aberrant observation. All parameters were summarized for each day, and days were averaged within cow.

Statistical analyses for all parameters were done with the statistical package JMP, version 2.05 (SAS Inst. Inc., Cary, NC., 1991). Factors included in the model were square, period(square), cow(square), and treatment. Square by treatment interaction was tested for VFA concentrations and ruminal pH, and

were included in the model if significant. Orthogonal contrasts of square (primiparous vs. multiparous; square 1 multiparous vs. square 3 multiparous) and treatment (lasalocid vs. no lasalocid, cracked vs. ground and the particle size by lasalocid interaction) were analyzed. Significance indicates a *P* value of less than .05, unless otherwise noted.

2.3 RESULTS AND DISCUSSION

2.3.1 CORN GRAIN PARTICLE SIZE

Mean and standard deviation of the particle size of the ground corn grain was 827 +/- 152 μ, and for the cracked corn was 3265 +/- 139 μ. Particle size differed ($P < .01$).

2.3.2 RUMEN NUTRIENT POOL SIZES, AND CALCULATED RATES OF DIGESTION AND PASSAGE

Effect of lasalocid:

Lasalocid had no effect on starch turnover time, liquid dilution rates or estimated rates of passage and digestion of fiber (Table 2). Ionophores have been shown to decrease fiber digestibility *in vitro* (Russell and Strobel, 1988), but this data and that of others (Dinius et al., 1976; Johnson et al., 1988; Russell and Strobel, 1989) would suggest that this does not occur *in vivo*. The difference between *in vitro* and *in vivo* data may be due in some cases to decreased intakes with ionophore increasing retention time to maximize chance for fiber digestion, or due to growth of ionophore resistant cellulolytics to fill the niche left by ionophore sensitive fiber fermenting bacteria (Russell and Strobel, 1988).

Effect of lasalocid on rate of starch digestion and passage could not be estimated using these *in vitro* techniques. Lasalocid did not affect rumen pool size of dry matter, starch, fiber, indigestible fiber or liquid.

Effect of corn grain particle size

Grinding corn increased *in vitro* rate of starch degradation (6.56 h^{-1} vs. 30.2 h^{-1}) (Figure 1), and did not alter calculated rate of starch passage (24.5 h^{-1} vs. 25.3 h^{-1}). Total starch turnover time (h) was 52% shorter with ground corn (Table 2). Ruminal degradation of starch, calculated from *in vitro* rate of starch degradation and rumen turnover time, increased with ground corn treatment. This calculation assumes that the *in vivo* rate of fermentation is approximated by *in vitro* results. The dramatic decrease in starch turnover time with ground corn was apparently entirely due to the increased rate of ruminal starch degradation.

Corn grain particle size had no effect on rate of digestion of fiber, but ground corn increased rate of fiber passage (Table 2). Ground corn decreased rumen pool size of dry matter, indigestible fiber and starch, and tended to decrease pool size of NDF.

Effect of lasalocid and corn grain particle size interaction

There was a significant interaction of corn grain particle size and lasalocid on starch turnover time and starch pool size (Table 2). Lasalocid increased starch turnover time, increasing starch pool size with ground corn, but had the opposite effect with cracked corn diets. One possible explanation is that *S. bovis* may have been a primary starch fermenter of the ground corn diets, with their rapidly available source of starch. The expected (although not measured) inhibition of *S. bovis* with lasalocid would therefore decrease rate of starch degradation of the ground corn diets. The more slowly available starch in the cracked corn diets

may have favored other, ionophore-resistant starch fermenters, such as *Bacteroides amylophilus*, *Selenomonas ruminantium* or *Bacteroides ruminocula*.

2.3.3 VOLATILE FATTY ACIDS, LACTATE, AND RUMINAL PH

Effect of lasalocid

Lasalocid increased lactate concentration, and had no effect on concentrations of acetate, propionate, butyrate, valerate, formate, or the BCFA (Table 3). Acetate to propionate ratio was not affected by lasalocid. Lasalocid had no significant effect on pool size of lactate or any of the individual volatile fatty acids (Table 4).

This increase in lactate concentrations contradicts *in vitro* data (Dennis et al., 1981; Dennis et al., 1981) and *in vivo* data from beef cattle with experimentally induced acidosis (Nagaraja et al., 1981; Nagaraja et al., 1982; Nagaraja et al., 1985). One possible mechanism for this increase in lactate concentration is an inhibition of protozoa by ionophores. Protozoa lack an outer membrane to protect them from ionophore, but the literature is mixed on whether ionophores inhibit protozoa. Hungate, (1966) refers to protozoa as playing a buffering role in the rumen, consuming both starch and starch-fermenting, lactate-producing bacteria on high starch diets. Protozoa would thus decrease the rate of starch degradation and lactate accumulation. If lasalocid inhibited protozoa in this trial, the net effect could be an increase in the amount of starch available for hydrolysis and fermentation to lactate with lasalocid treatment. Another possible explanation of the increase in lactate with ionophore treatment is that the expected (not measured) inhibition of *S. bovis* may have allowed more slowly growing, but ionophore resistant, lactate producers to proliferate.

The similarity of acetate and propionate concentrations between control and lasalocid treatment is in contrast to previous findings (Owens, 1980; Nagaraja et al., 1985; Burrin and Britton, 1986; Sauer et al., 1989). One possible explanation is an adaptation of the microbial population to lasalocid during the course of the experiment. Apparent adaptation to ionophore treatment has been observed *in vitro* and *in vivo* (Johnson et al., 1988; Weiss and Amiet, 1990). As rumen contents were exchanged among cows between periods to facilitate adjustment to the new treatment, this is a plausible explanation. However, the increase in lactate concentration with lasalocid treatment in this study indicates that there was some sustained effect of the ionophore on rumen fermentation.

Exchanging rumen contents among cows was used as a technique to decrease carryover effects and hasten adjustment to diet. Ionophore treatment may be expected to affect both the microbial population in the rumen and the cow. Pre-adapting the rumen contents will therefore shorten the diet adjustment period, although the cow must still adjust to treatment. It is important to recognize, however, that exchanging contents affects the analysis of results. The net effect of exchanging contents may be that we are evaluating the long term (4*21d, or 84 d), rather than the short term (21 d) effects of ionophore treatment on rumen fermentation. Exchanging rumen contents, therefore, may improve our ability to truly evaluate the effects of ionophore in the early lactation cow. Had a similar experiment been run without exchanging contents, the results would have reflected the short term (21 d) effects of treatment. These results could not truly have been applied to a typical group of early lactation cows on the farm, which may range from 0-100 DIM.

Lasalocid had no effect on mean pH, or fraction of time below pH 5.5, 5.75, or 6 (Table 5). Lasalocid tended ($P = .14$) to decrease range in pH. The similarity of mean pH with or without ionophore treatment is despite the observed increase

in lactate. Others have observed that ruminal pH is more closely correlated with total VFA concentration than with lactate (Nagaraja et al., 1985). These results would support that finding, at least within the pH range observed. However, the rate of absorption of lactate and the various VFA's may change as ruminal pH declines, changing the relationship among lactate, VFA concentrations, and ruminal pH in some situations.

Effect of corn grain particle size

Corn grain particle size had no effect on total VFA concentration, concentration of lactate, acetate, butyrate, valerate or formate. Ground corn increased propionate concentration, decreased acetate to propionate ratio and decreased concentration of the BCFA when compared to cracked corn (Table 3). When compared to cracked corn, ground corn tended to decrease acetate pool size, decreased butyrate and BCFA pool size, and had no effect on pool size of total volatile fatty acids, lactate, propionate, valerate or formate (Table 4).

The increase in propionate, and resulting decrease in the A:P ratio with ground corn likely is a result of the increase in ruminal starch degradation (Hungate 1966, Van Soest 1982) and is in agreement with others (Ørskov et al., 1970; Ørskov, 1986; McCarthy et al., 1989; Moore et al., 1992; Aldrich et al., 1993). Decreased concentration of BCFA may indicate a decrease in ruminal protein degradation with ground corn, as the major source of BCFA in the rumen is deamination of the branched chain amino acids, leucine, iso-leucine and valine (Allison, 1969). Decreased BCFA, therefore, indicates a decrease in deamination of these amino acids. Ruminal protein degradation and ruminal ammonia concentrations were not measured, but the increase in estimated passage of fiber from the rumen with ground corn could also indicate that any protein associated

with fiber may have passed from the rumen more quickly on the ground corn diets.

Corn grain particle size had no effect on mean pH, or fraction of time below pH 5.5, 5.75 or 6 (Table 5). Ground corn increased the range in rumen pH. The increase in pH range is despite no observed increase in total VFA or lactate concentration. Concentrations do not measure production directly, however, and varying absorption rates may mask varying rates of production. That ruminal pH range increased with no effect on mean ruminal pH implies that cows were able to regulate rumen pH under these dietary and management conditions.

Effect of interactions

Interaction of square and treatment was not significant for ruminal VFA and lactate concentrations or for ruminal pH (Tables 3 and 5). An interaction between corn grain particle size and lasalocid existed for pool size of acetate, where lasalocid increased acetate on the ground corn diet, but decreased it on the cracked corn diet (Table 4). A trend ($P < .1$) toward the same interaction was seen for pool size of total volatile fatty acids. Again, the difference in availability of starch would be expected to favor different species of bacteria. These different species would likely vary in their sensitivity to ionophore.

2.3.4 FEEDING BEHAVIOR

Effect of lasalocid

Lasalocid (as Bovatec®) did not affect meal number, size, or frequency, chewing behavior during meals, or number of rumen contractions per day (Table 6). This is in contrast with the decrease in feed intake, meal size and eating rate (intake/eating time) of beef steers fed Rumensin® (monensin sodium) in roughage or concentrate diets (Baile et al., 1979). Lasalocid and monensin are, however,

chemically different compounds, and the carriers of the commercial products, Bovatec® and Rumensin®, differ as well. The altered feeding behavior with monensin was apparently due to the flavor of Rumensin®, rather than to the ionophore, monensin sodium, as animals previously fed Rumensin® and offered monensin sodium in the feed ate as much feed as the animals on the control diet (Baile et al., 1979).

Number of chews or chewing rate during rumination were not affected by lasalocid. Total chewing time (eating + ruminating) tended ($P < .13$) to increase with lasalocid. Lasalocid tended ($P < .10$) to increase number of ruminating bouts per day and length of individual ruminating bouts, and tended ($P < .12$) to reduce time between ruminating bouts. Lasalocid significantly increased total ruminating time per day (Table 6). The increase in ruminating time with ionophore treatment could have important implications. Increased rumination increases saliva production and may help buffer the rumen. The trend ($P < .16$) for lasalocid to minimize ruminal pH range despite increased ruminal lactate concentration may be related to this increase in rumination time.

Water intake tended ($P < .10$) to increase with lasalocid (by 4.2 liters, 5.1%) (Table 6). The tendency for increased water intake with lasalocid treatment is in contrast to a trial with steers on a 50% forage, 50% concentrate diet in which there was no significant difference in water intake with monensin (Rogers and Davis, 1982). As has been noted before, however, lasalocid and monensin are chemically different compounds, and the ingredients of the commercial products, Bovatec® and Rumensin®, differ as well.

Effect of corn grain particle size

Corn grain particle size did not affect meal number, size, frequency or chewing during meals. Corn grain particle size had no effect on ruminating time

or chewing during ruminating. Ground corn decreased number of rumen contractions per day (Table 6). The decrease in rumen contractions with the ground corn diet may be due to the increase in pH fluctuation or the increase in propionate concentration in the rumen, as infusion of various VFA's, including propionate, has been shown to inhibit rumen motility (Ash, 1959; Shinozaki., 1959). Water intake increased on the ground corn diet. This may help explain the increased rate of fiber passage, and decreased pool size of dry matter and indigestible fiber.

2.4 CONCLUSIONS

The data suggest that the originally proposed mechanism was not appropriate under the conditions of this trial, as lasalocid increased lactate concentration and ground corn did not affect it, and neither treatment affected mean ruminal pH. Ruminal pH was not abnormally low under the conditions of this trial. Examination of this hypothesis under different conditions may be warranted. Situations where this hypothesis could be further explored include rations with lower fiber concentrations or fluctuating silage dry matter levels.

Also, examination of this hypothesis in very early lactation cows, (i.e. from 0 to 30 DIM), may be warranted. These cows are the most at risk of metabolic problems, and the most negatively affected by reduced dry matter intakes. Exchanging rumen contents among cows between 21 day periods of a Latin square meant that in effect, we evaluated the effects of continuous treatment on cows from 16 to 100 DIM. That the proposed mechanism does not apply to this relatively long period of treatment is important to know, but these results do not rule out this mechanism as a factor in the impaired intakes and performance observed in the short term immediately post calving.

Further investigation of the biological effect and importance to the animal of the increase in water intake and decrease in rumen contractions with ground corn, and the increased water intake and increased rumination time with lasalocid is warranted. The possible inter-relationships among water intake, regulation of ruminal pH and passage of liquids and solids from the rumen are worthy of further investigation.

TABLES

Table 1. Diet ingredients and composition, % of DM

	Cracked		Ground	
	0[1]	+[2]	0	+
Ingredient				
Alfalfa silage	15.9	15.9	15.9	15.9
Corn silage	31.9	31.9	31.9	31.9
Dry rolled corn:				
Cracked	32.8	32.8		
Ground			32.8	32.8
Soybean meal	15.7	15.7	15.7	15.7
Blood meal	1.91	1.91	1.91	1.91
Lasalocid		.016		.016
Mineral/Vitamin mix	1.82	1.82	1.82	1.82
Composition				
NDF	27.1	27.1	27.2	27.2
ADF[3]	15.0	15.0	15.0	15.0
Lignin[3]	2.46	2.46	2.48	2.48
Ash	2.28	2.28	2.27	2.27
Crude protein	18.2	18.2	18.4	18.4
UIP, % CP[4]	39	39	39	39
DIP, %CP[4]	61	61	61	61
Starch	34.8	34.8	34.6	34.6

[1] 0 = without lasalocid

[2] + = with lasalocid

[3] ADF and lignin determined sequentially

[4] UIP and DIP determined from book values

Table 2. Effect of lasalocid (L) and corn grain particle size (PS) on ruminal nutrient passage, digestion and pool sizes.

	Cracked		Ground		*P* value		
	0	+	0	+	L	PS	LxPS
Rumen parameters							
Fiber digestion, %/h	8.03	7.88	6.77	7.73	NS	NS	NS
Fiber passage %/h	4.58	4.68	5.80	5.78	NS	**	NS
Liquid dilution, %/h	14.9	15.2	15.4	15.6	NS	NS	NS
Starch turnover time, h	3.99	3.27	2.07	2.70	NS	**	*
Fiber turnover time, h	26.9	30.9	28.6	27.1	NS	NS	†
Ruminally dig. starch, %	26.4	21.6	62.6	81.5	NS	**	†
Digesta volume, l	75.1	74.8	71.3	73.6	NS	NS	NS
Digesta wt, kg	63.9	63.7	62.2	61.5	NS	NS	NS
Rumen pool sizes, kg							
Liquid	53.5	53.4	53.0	51.9	NS	NS	NS
DM	10.4	10.4	9.2	9.7	NS	*	NS
Fiber	6.08	6.15	5.69	5.77	NS	†	NS
Indigestible NDF	3.85	3.86	3.35	3.50	NS	**	NS
Starch	.91	.79	.49	.70	NS	**	*

** $P < .01$; * $P < .05$; NS = $P > .10$

Table 3. Effect of lasalocid, corn grain particle size and square by treatment interaction (SqxT) on concentrations of rumen fluid volatile fatty acids and lactate.

	Cracked		Ground		*P* value			
	0	+	0	+	L	PS	LxPS	SqxT
Total VFA, mM	115	115	108	118	NS	NS	NS	NS
Lactate mol/100 mol VFA+lactate	5.14	7.56	6.05	7.18	*	NS	NS	NS
mol/100 mol VFA								
Acetate	56.7	56.3	54.3	55.2	NS	NS	NS	NS
Propionate	24.4	25.0	26.9	27.6	NS	**	NS	NS
Butyrate	12.8	13.8	14.5	13.2	NS	NS	NS	NS
Valerate	1.75	1.67	1.77	1.68	NS	NS	NS	NS
Formate	.23	.03	.14	0	NS	NS	NS	NS
BCFA	2.89	3.24	2.49	2.23	NS	**	NS	NS
Acetate:Propionate	2.36	2.3	2.08	2.09	NS	*	NS	NS

** $P < .01$; * $P < .05$; NS = $P > .10$

Table 4. Effect of lasalocid and corn grain particle size on rumen digesta volume and weight, and rumen pool sizes of various components

	Cracked		Ground		*P* value		
	0	+	0	+	L	PS	LxPS
VFA's and lactate, moles							
Total VFA	9.68	9.27	8.57	9.54	NS	NS	†
Lactate	.02	.15	.12	.07	NS	NS	NS
Acetate	5.82	5.53	5.08	5.58	NS	†	*
Propionate	2.34	2.26	2.17	2.54	NS	NS	NS
Butyrate	1.12	1.1	.94	1.05	NS	*	NS
Valerate	.15	.14	.13	.15	NS	NS	NS
Formate	0	0	.067	0	NS	NS	NS
BCFA	.25	.25	.18	.23	NS	*	NS

** $P < .01$; * $P < .05$; † $P < .10$

Table 5. Effect of lasalocid and corn grain particle size on rumen pH.

	Cracked		Ground		*P* value			
	0	+	0	+	L	PS	LxPS	SqxT
Daily mean pH	6.34	6.39	6.37	6.31	NS	NS	NS	NS
Fraction < pH 5.5	.009	.015	.041	.015	NS	NS	NS	NS
Fraction < pH 5.75	.06	.05	.10	.07	NS	NS	NS	NS
Fraction < pH 6	.154	.136	.187	.19	NS	NS	NS	NS
Range (95% CI)	.94	.91	1.13	1.01	NS	**	NS	NS

** $P < .01$; NS = $P > .10$

Table 6. Effect of lasalocid and corn grain particle size on feeding behavior

	Cracked		Ground		*P* value		
	0	+	0	+	L	PS	LxPS
Eating (LSM):							
Meals, # /d	12.4	12.33	12.14	12.18	NS	NS	NS
Meal size, kg	2.43	2.64	2.59	2.73	NS	NS	NS
Meal length, min	14.6	15.1	14.9	15.8	NS	NS	NS
Eating time, min/d	176	179	174	189	NS	NS	NS
Min. between meals	101	100	102	105	NS	NS	NS
Chews, /meal	826	819	824	906	NS	NS	NS
Chews, /d	9946	9668	9573	10800	NS	NS	NS
Chew rate, chews/min	54.7	51.7	53.4	55.5	NS	NS	NS
Ruminating:							
Bouts, # /d	11.6	12.9	11.1	12.4	†	NS	NS
Bout length, min	27.3	29.1	26.8	29.5	†	NS	NS
Ruminating time min/d	322	373	310	369	*	NS	NS
Min. between bouts	107	86.8	125	95.9	.11	NS	NS
Chews, /bout	1500	1590	1390	1550	NS	NS	NS
Chews, /d	17980	20530	16510	19480	NS	NS	NS
Chew rate, chews/min	53.6	53.1	49.7	51.4	NS	NS	NS
Total chewing, min/d	498	552	501	558	.12	NS	NS
Total chews, /d	27930	30190	26970	30280	NS	NS	NS
Rumen contractions, /d	2110	2090	2010	2000	NS	**	NS
Drinking:							
Bouts, # /d	20.6	20.6	21.1	23.0	NS	NS	NS
Bout size, l	4.50	4.50	4.58	4.41	NS	NS	NS
Min. between bouts	78.7	74.1	71.8	66.7	NS	NS	NS
Total water intake, l	78.3	83.12	86.42	89.95	†	**	NS
Drinking time, min/d	20.0	20.0	19.7	21.6	NS	NS	NS
Drinking rate, l/min	4.55	4.64	4.65	4.83	NS	NS	NS

** $P < .01$; * $P < .05$; † $P < .10$

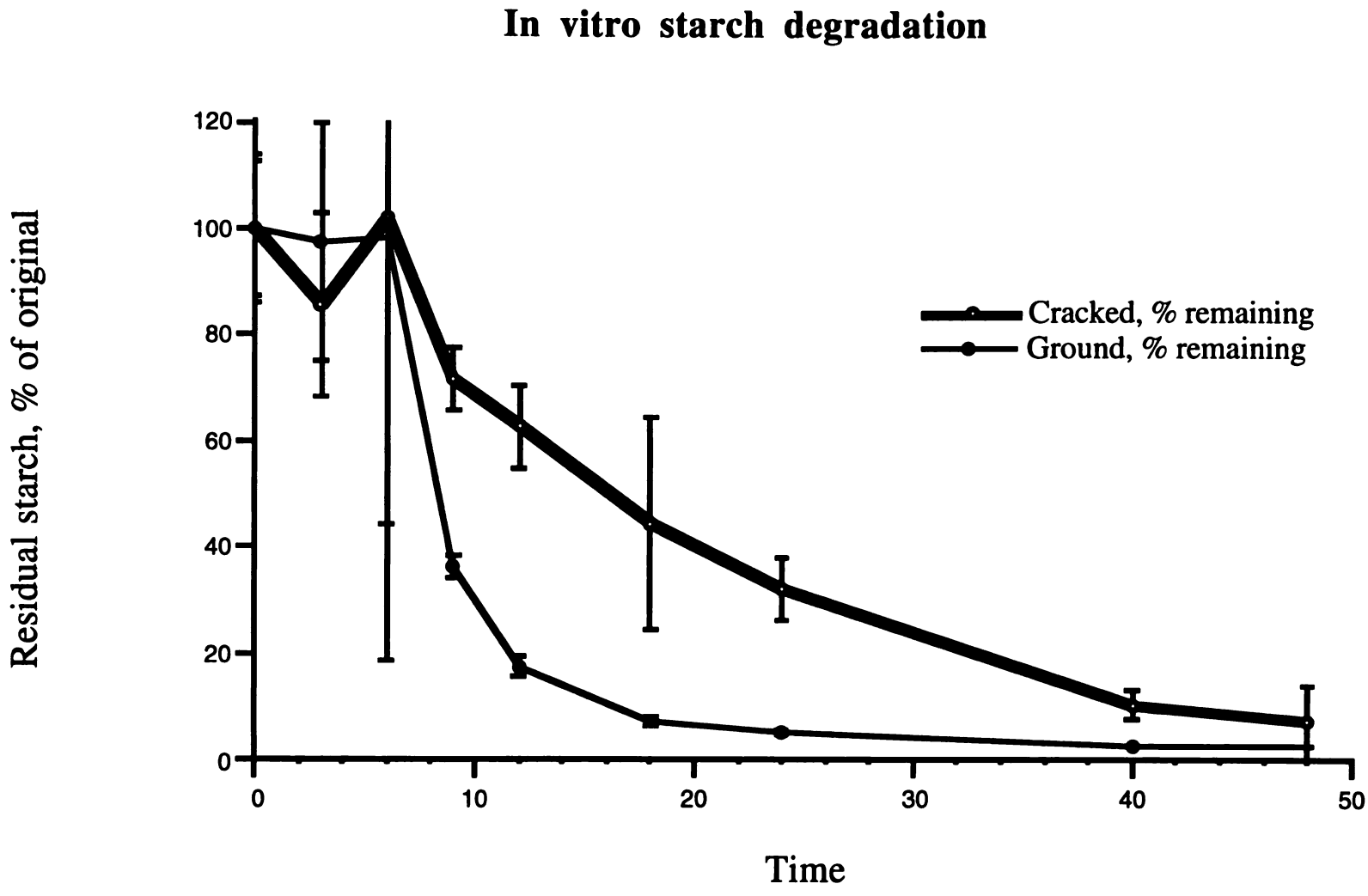

Figure 1. *In vitro* rates of starch degradation of cracked and ground corn.

CHAPTER 3

EFFECT OF LASALOCID AND CORN GRAIN PARTICLE SIZE ON PERFORMANCE, BLOOD PARAMETERS AND NUTRIENT DIGESTIBILITY IN EARLY LACTATION DAIRY CATTLE

ABSTRACT

The effect of corn grain particle size and the ionophore lasalocid on performance and blood parameters, and nutrient digestibility were examined to determine the effect of ruminally degraded starch on performance of early lactation cows and elucidate the mechanism of action. Eight multiparous and four primiparous early lactation cows were fed diets with cracked or ground corn grain, with or without lasalocid in 3, 4x4 Latin squares with 21 d periods. Lasalocid tended (P<.11) to improve dry matter intake (DMI). Lasalocid and ground corn decreased body condition loss and milk fat, and increased milk protein. For all cows, milk yield, 4% FCM, lactose, and body weight were not affected by treatment, however primiparous cows increased milk yield with lasalocid. Ground corn increased total tract starch digestibility, decreased neutral detergent fiber digestibility, and decreased ruminal turnover time for starch. No interactions between lasalocid and corn grain particle size were observed for most effects.

3.1 INTRODUCTION

Diets consumed by early lactation dairy cows often contain high levels of ruminally degraded starch. Level of ruminally degraded starch may significantly affect dry matter intake and subsequent performance in early lactation cows. Improved milk yield (McCarthy et al., 1989; Robinson and Kennelly, 1989; Seymour et al., 1993), FCM (Robinson and Kennelly, 1989; Casper et al., 1990b; Aldrich et al., 1993; Seymour et al., 1993), and dry matter intake (McCarthy et al., 1989; Casper et al., 1990b; Oliveira et al., 1990; Aldrich et al., 1993; Dhiman and Satter, 1993; Seymour et al., 1993; Varela et al., 1993) have been observed with diets replacing sources of rapidly available non-structural carbohydrate with more slowly available NSC sources. However the specific cause of the results of many of these trials is difficult to assess, as the diets also varied in starch source, fiber level and source and/or protein level and source.

Physically manipulating corn grain particle size by grinding or cracking may be a feasible method of obtaining chemically identical diets that differ in the ruminal availability of starch (Theurer, 1986; Nocek and Tamminga, 1991). Physical processing breaks the outer coat of the grain to allow access of rumen microorganisms and enzymes. Disrupting the protein matrix around starch may be another mechanism by which processing improves digestion of grains (Theurer, 1986). Grinding increased rate of digestion of starch (Theurer, 1986; Campling, 1991; Nocek and Tamminga, 1991).

Ionophores have been shown to prevent acidosis and maintain ruminal pH in beef cattle on high starch diets or with high starch or glucose mixtures infused to the rumen (Nagaraja et al., 1981; Nagaraja et al., 1982; Nagaraja et al., 1985; Burrin and Britton, 1986). Consequences of sub-acute acidosis are reduced feed intake and performance (Slyter, 1976; Nagaraja et al., 1985). The effect of

ruminally degraded starch on dry matter intake in early lactation cows may be through a similar mechanism observed in beef cattle on high starch diets.

Ionophores have not been shown to improve milk yield in mid-lactation cows (Beede et al., 1986; Weiss and Amiet, 1990), and reduced dry matter intake in two studies (Johnson et al., 1988; Weiss and Amiet, 1990). A third study showed decreased intake only at the highest level of addition of lasalocid (36 mg/kg, twice the levels in our study) (Beede et al., 1986). Lasalocid increased body weight gain in two studies (Beede et al., 1986; Weiss and Amiet, 1990). These observation with mid-lactation cows may not reflect conditions in early lactation cows as rumen volume, passage rate of feed from the rumen as well as nutrient requirements and energy status are likely to be different between early and mid-lactation cows. Ionophores did not improve milk yield or DMI in early lactation cows (Beede et al., 1986; Sauer et al., 1989). Recently, rations fed in the field to high producing, early lactation cows include very high starch levels (> 35%) and low levels of fiber (~ 25% NDF). The effectiveness of ionophores in improving performance in early lactation, high producing cows needs to be re-examined in these conditions.

Slowing down ruminal starch fermentation, shifting the site of starch digestion to the small intestine, or controlling acid production with lasalocid may allow increased starch in the ration without impaired rumen function. Increased starch in the ration will allow more energy dense diets without expensive fat supplements.

Our objective was to examine the effect of level of rumen degradable starch on energy intake and performance of dairy cows in early lactation. We hypothesized that diets with ground corn would have higher levels of ruminally degraded starch and that these diets, and those without the ionophore would

depress dry matter intake and milk yield, and affect milk composition and energy metabolism in early lactation dairy cows.

3.2 MATERIALS AND METHODS

Twelve ruminally cannulated Holstein cows (eight multiparous and four primiparous) averaging 16 days in milk (DIM) were grouped by parity and calving date and assigned to one of three Latin squares. Squares were staggered to minimize average DIM. Two squares were composed of multiparous cows and one square of primiparous cows. Within a square, each cow was randomly assigned to one of four treatment combinations.

A 2x2 factorial arrangement of treatments was utilized. Dietary treatments were particle size of corn grain; ground = 827 μ, cracked = 3265μ, and the inclusion or absence of lasalocid (0 vs. 360 mg/cow per d). The data were analyzed as replicated (n=3) 4x4 Latin squares balanced for carryover effects.

Lasalocid premix (Bovatec®) and ground corn were mixed in bulk, and then 114 g of the mix, containing 180 mg of lasalocid, was weighed into individual envelopes in the laboratory. One envelope was hand mixed into the feed for cows on the lasalocid treatment at each feeding to assure even intake throughout the day. Cows on the no lasalocid treatment had 114 g of ground corn carrier without lasalocid hand mixed into their feed at each feeding.

Rations consisted of alfalfa silage, corn silage, dried shelled corn, soybean meal, blood meal, and minerals and vitamins. Corn silage was ensiled in a bag (100 T) to minimize variation, and reduce spoilage at feed out. Each load of corn silage was sampled at filling and analyzed prior to the initiation of the experiment to assess variation in composition. Dried shelled corn in quantity sufficient for the duration of the experiment was thoroughly mixed and divided in half. Half

of the corn was then coarsely cracked and half was finely ground. Mixing was done to minimize variation in starch digestibility due to source.

Experimental treatment periods were 21 days, with 14 days for diet adjustment followed by seven days of data collection. Throughout the experiment, cows were fed twice daily at 0700 and 1900 h at 110% of expected intake. Dry matter intake was measured for each cow daily, and averaged by collection period. Daily feed consumption and refusals were recorded at the morning feeding. Samples of both rations (.5 kg) and orts (10%) were collected daily during the collection period and composited to one weekly sample per cow.

Cows were milked twice per day in their stalls at 0700 and 1900 h, and milk was sampled at both milkings on days 3, 4, and 5 of the collection period. Milk samples were analyzed for fat, protein and lactose by Michigan DHIA.

Body weight was measured prior to the start of the first period and on days 6 and 7 of the collection period. Body condition score on a scale of 1 to 5, (1 = thin, 5 = fat) was analyzed by four trained investigators prior to the start of the first period, and on day 7 of the collection period

Rumens were evacuated on day 7 of the collection period and rumen contents were exchanged among cows according to the schedule of treatments to decrease carryover effects and hasten adjustment to the new treatment.

Ration, orts, silage, and fecal samples were dried in a 55° C forced air oven and analyzed for dry matter. Fecal samples were subsampled and composited, and all samples were ground through a 1 mm screen in a Wiley mill. Neutral detergent fiber was analyzed according to Van Soest et al. (1991). Samples were soaked in 8M urea and α-amylase overnight prior to analysis to facilitate complete removal of starch and protein (Van Soest et al., 1991). Samples were analyzed for ash, and NDF was analyzed sequentially for acid detergent fiber and acid detergent sulfuric acid lignin with AOAC methods. Crude protein was

analyzed according to Hach et al (1987). Undegraded intake protein (UIP) and degraded intake protein (DIP) were estimated from NRC book values (National Research Council, 1989). Starch was analyzed by the two stage enzymatic method of Aman and Hesselman (1984), with a NaOH gelatinization step according to O'Neil et al. (1993) and analysis of glucose by Karkalas (1985). Diet ingredients and composition are listed in Table 1. Alfalfa silage and corn silage were analyzed weekly for dry matter (DM), neutral detergent fiber (NDF) and crude protein (CP), and diets were re-balanced accordingly.

For determination of apparent total tract nutrient digestibility, fecal samples were taken every fifteen hours on days 2, 3, 4 and 5 of the collection period to account for diurnal variation in fecal composition. Eight samples for each cow representing every three hours of a 24 hour period were collected. Digestibility of dry matter, fiber, and starch was determined using acid detergent lignin as an indigestible marker. Equations (for nutrient X) are as follows:

$$\text{Digestibility} = (X_{in} - X_{out}) / X_{in}$$

$$X_{in} = (\text{DM offered, kg} * \%X \text{ in feed}) - (\text{DM orts, kg} * \%X \text{ in orts})$$

$$X_{out} = \text{kg feces} * \%\ X \text{ in feces}$$

$$\text{Kg feces} = (\text{kg DMI} * \%\ \text{lignin in feed})/\%\ \text{lignin in feces}$$

Blood was sampled from the tail vessel one hour prior to the morning feeding on days 6 and 7 of the collection period. Blood samples were stored overnight in the cooler to allow clotting, centrifuged at 750 x g, and the serum was collected and frozen. Serum was analyzed for glucose with an *in vitro* enzymatic colorimetric method utilizing glucose oxidase and peroxidase (Sigma Chemical Co., Glucose oxidase kit #510). Serum non-esterified fatty acids (NEFA) were analyzed by an enzymatic method using acyl-CoA synthetase, acyl-CoA oxidase and peroxidase (Wako Chemicals USA, Inc., NEFA C kit) with

modifications to reduce sample and reagent volume according to McCutcheon and Bauman (1986). Serum ß-hydroxybutyrate (ß-HBA) was analyzed enzymatically using ß-hydroxybutyrate dehydrogenase (Sigma Chemical Co., ß-HBA kit #310-UV). Serum triglycerides (TG) were analyzed enzymatically using lipoprotein lipase, glycerol kinase, glycerol phosphate oxidase and peroxidase (Sigma Chemical Co., TG (GPO-Trinder) kit #337). The assay was modified to allow more accurate reading of low serum triglyceride levels by using higher concentrations of serum (40 µl vs. 10 µl) .

Blood samples for insulin determination were drawn from jugular catheters every thirty minutes over a six hour period, beginning two hours before the morning feeding on day 7 of the collection period. Serum was collected and frozen. Insulin was measured by radio-immuno assay according to Villa-Godoy et al. (1990).

All parameters were analyzed using the statistical package JMP, version 2.05 (SAS Inst., Inc., Cary, NC. 1991). Factors included in the model were square, period(square), cow(square), and treatment. Square by treatment interaction was tested for most parameters and included in the model if significant. Orthogonal contrasts of square (primiparous vs. multiparous; square 1 multiparous vs. square 3 multiparous) and treatment (lasalocid vs. no lasalocid, cracked vs. ground and the particle size by lasalocid interaction) were analyzed. Significance indicates a *P* value of less than .05, unless otherwise noted .

3.3 RESULTS AND DISCUSSION

3.3.1 MILK YIELD, MILK COMPOSITION, DRY MATTER INTAKE, BODY WEIGHT AND BODY CONDITION

Effect of lasalocid

Lasalocid had no effect on milk yield, 4% FCM yield, milk fat yield, milk protein yield or milk lactose % or yield (Table 2). Milk fat % was decreased by 4.5% (.16 units) and milk protein % was increased by 2.3% (.08 units) with lasalocid treatment. Recently, others have reported no effect of lasalocid or monensin on milk yield or composition in early lactation cows (Murphy et al., 1993, Thomas et al., 1993).

Others have observed a decrease in milk fat (Dye et al., 1988; Johnson et al., 1988; Sauer et al., 1989) with ionophore treatment. The decrease in milk fat is commonly explained by a decrease in the acetate to propionate ratio often observed with ionophores. We did not see this alteration in this trial (chapter 2). Ionophores decrease ruminal proteolysis (Bartley et al., 1979; Poos et al., 1979; Fuller and Johnson, 1981; Muntifering et al., 1981; Katz et al., 1986; Russell and Strobel, 1988; Yang and Russell, 1993), which may increase escape of undegraded intake protein to the lower tract. This is one possible mechanism for the increase in milk protein observed with lasalocid. We did not measure ruminal protein degradation or ammonia concentrations.

Dry matter intake tended to be greater on the lasalocid diet ($P = .11$), by a magnitude of .9 kg (5%). Others have reported no effect of similar dosages of ionophore on DMI, (Dye et al., 1988; Murphy et al., 1993; Thomas et al., 1993), although decreased intake in early lactation cows has been observed with monensin (Sauer et al., 1989). Lasalocid had no effect on body weight change,

but body condition loss was decreased by .15 units in 21 days with lasalocid treatment (Table 2). The decreased loss of body condition with lasalocid treatment can at least partially be attributed to the trend toward increased dry matter intake with lasalocid treatment. The magnitude of the advantage in body condition status with lasalocid treatment, an average of .15 units over four twenty-one day periods, can be extrapolated to .6 units in eighty four days in early lactation. This is a potentially valuable benefit of feeding lasalocid.

The treatment by square interaction was significant for milk and milk component yield. Primiparous cows (Table 3) increased milk yield, milk protein yield and milk lactose yield, and tended ($P < .10$) to increase 4% FCM yield in response to lasalocid. One possibility is that the original hypothesis of small rumen sizes favoring lactate production was a factor in the heifers. We did not directly measure rumen volume, but the rumen digesta volumes of the primiparous cows were smaller than those of the older cows (64.8 l vs. 78.2 l). However (chapter 2) ruminal pH and lactate concentrations were not affected by the interaction of square and treatment. For the third square only, lasalocid decreased milk yield, FCM, milk fat yield, and milk lactose yield of multiparous cows (Table 3). This observation is different from the other square of multiparous cows and difficult to explain.

Effect of corn grain particle size

Corn grain particle size had no effect on milk yield, 4% FCM yield, milk fat yield or milk lactose % or yield (Table 2). Ground corn decreased milk fat % by 5.4% (.19 units) and increased milk protein % and yield by 3.4% and 7.3% respectively (.10 units and .07 kg). Others have reported decreased milk yield (McCarthy et al., 1989; Robinson and Kennelly, 1989; Seymour et al., 1993) or

FCM (Casper et al., 1990b; Aldrich et al., 1993) with increased levels of ruminally degraded starch.

The decrease in milk fat with ground corn treatment agrees with other studies (Herrera-Saldena and Huber, 1989; Poore et al., 1989; Casper et al., 1990b; Moore et al., 1992; Aldrich et al., 1993), and is commonly explained by a decrease in the acetate to propionate ratio often observed with an increase in ruminally fermented starch. We did see an increase in propionate with ground corn treatment in this trial, although acetate concentrations were not affected (chapter 2).

The increase in milk protein observed with ground corn agrees with other studies (Oliveira et al., 1990; Aldrich et al., 1993). One possible mechanism is that increased propionate may spare amino acids from gluconeogenesis (Dye et al., 1988), but lactating cows iso-calorically infused with glucose in the rumen or propionate in the duodenum suggest that the increase in milk protein observed with increased rumen starch degradability is due to altered rumen metabolism of glucose rather than increased propionate absorption (Wu et al., 1993). Other possibilities are increased microbial yield with diets higher in ruminally degraded starch or increased escape of protein from the rumen to the small intestine due to increased rate of nutrient passage.

The multiparous cows in square one only (Table 3) increased milk yield, FCM, milk fat yield, milk protein yield and milk lactose yield with the ground corn treatment. Primiparous cows (Table 3) decreased FCM and milk fat yield with ground corn treatment. The decreased FCM with the increased level of ruminally degraded starch in first calf heifers again may be related to rumen digesta volume, which was smaller in the primiparous cows than in older cows (64.8 l vs. 78.2 l). However ruminal pH and lactate concentrations were not affected by the interaction of square and treatment.

Corn grain particle size did not affect DMI (Table 2). Others have observed impaired feed intake (McCarthy et al., 1989; Casper et al., 1990b; Oliveira et al., 1990; Moore et al., 1992; Aldrich et al., 1993; Dhiman and Satter, 1993; Seymour et al., 1993; Varela et al., 1993) with increased levels of ruminally degraded starch. Three possible explanations are that grinding corn did not affect level of ruminally degraded starch, that increased ruminally degraded starch did not affect lactate or ruminal pH enough to affect DMI, or that lactate concentrations or ruminal pH do not affect DMI. Determination of ruminally degraded starch using ruminal turnover time and *in vitro* rates of digestion (chapter 2) would seem to contradict the first possibility.

Corn grain particle size had no effect on body weight change, but ground corn decreased body condition loss by .2 units in 21 days (Table 2). The magnitude of decrease in body condition loss with ground corn treatment can be extrapolated to .8 units in eighty-four days during early lactation. This may be a valuable benefit to fine grinding corn for cows under these conditions.

3.3.2 TOTAL TRACT DIGESTIBILITIES OF DRY MATTER, FIBER AND STARCH

Effect of lasalocid:

Lasalocid had no effect on total tract starch digestibility, NDF digestibility, or dry matter digestibility (Table 4). Ionophores have been shown to decrease fiber digestibility *in vitro* (Russell and Strobel, 1988). These data, and that of others (Dinius et al., 1976; Johnson et al., 1988; Russell and Strobel, 1989) would suggest that this does not occur *in vivo*. The contradiction between *in vitro* data and *in vivo* observation of fiber digestibility has been explained in some cases by a decrease in dry matter intake. Decreased dry matter intake would increase rumen retention time, allowing more time for fiber digestion. In this study, however, dry matter intake tended to increase with lasalocid and yet total tract

fiber digestion was not affected by ionophore. Another possibility is that with time, resistant microbial species in the rumen that have similar function may grow to fill the niche left by the ionophore-sensitive bacteria. For example, ionophores inhibit *Ruminocci*, but *Bacteroides succinogenes*, another cellulolytic bacteria, is resistant and may eventually replace *Ruminocci in vivo* (Russell and Strobel, 1988).

Effect of corn grain particle size

Ground corn increased total tract starch digestibility , decreased total tract fiber digestibility and had no effect on total tract dry matter digestibility (Table 4). This increase in starch digestibility may explain the decreased body condition loss on ground corn diets. The effect of particle size on total tract starch digestibility implies that significant quantities of starch were passed to the lower gut with the cracked corn diets, and that either intestinal starch digestion was limiting, glucose absorption was limiting, or glucose metabolism by the intestinal mucosa increased with increased starch digestion (Harmon, 1991). While Owens et al (1986) concluded that starch digestion was not limiting in the ruminant small intestine, infusion studies (Kreikemeier et al., 1991) suggest that starch digestion and glucose absorption may be incomplete when large quantities of starch escape the rumen, and many studies show negative net absorption of glucose from the small intestine despite measured starch disappearance (Huntington, 1982; Lomax and Baird, 1983).

3.3.3 BLOOD METABOLITES

Effect of lasalocid

Lasalocid had no effect on serum parameters (Table 5). Serum concentrations of NEFA, TG, ß-hydroxybutyrate, glucose and insulin were all

within the normal range on this trial. As body condition loss decreased with ionophore treatment, serum NEFA concentrations might have been expected to decline, but no effect was seen. In one recent study, reduced serum NEFA concentrations were observed with ionophore treatment in multiparous cows in the first 28 days postpartum (Thomas et al., 1993), but no effect was seen in primiparous cows (Thomas et al., 1993) or in another study (Sauer et al., 1989). Ionophores have been shown to decrease serum ß-hydroxybutyrate concentrations (Sauer et al., 1989; Thomas et al., 1993), but concentrations in this trial were relatively low and unaffected by treatment. In agreement with these results, ionophores did not affect serum glucose in early lactation cows (Sauer et al., 1989; Thomas et al., 1993), or in midlactation cows (Johnson et al., 1988).

Effect of corn grain particle size

Ground corn decreased serum non-esterified fatty acids (Table 5). The decrease in serum NEFA concentrations with ground corn is likely correlated with the decrease in body condition loss. Other serum parameters were not affected by corn grain particle size. Although total tract starch digestion was higher with the ground corn, serum levels of glucose and insulin were not affected by corn grain particle size. This is not surprising, as a VFA mixture with high levels of propionate infused continuously, to simulate ad libitum feeding with a TMR, did not affect serum insulin concentrations (Istasse and Ørskov, 1984). Pulse dosing of the VFA mixture did increase serum insulin concentration, implying that changes in insulin are more likely with meal fed animals, or those fed grain and forage separately.

Effect of interactions

Square by treatment interactions had no significant effect on concentration of serum parameters in this study. There was a significant lasalocid by corn grain particle size interaction for change in serum insulin concentration. Lasalocid decreased change in serum insulin on the ground corn diet, whereas lasalocid increased change in serum insulin on the cracked corn diet.

3.4 CONCLUSIONS

Lasalocid may reduce body condition loss in early lactation dairy cows due in part to increased dry matter intake. Assuming a decrease in body condition loss of .6 units over 21 days, that one unit of body condition is equivalent to 80 kg of body weight (Ferguson, 1991), and that one kg body weight lost is worth 4.92 Mcal of NEl (National Research Council, 1989), the advantage in body condition with lasalocid is equivalent to 2.81 Mcal NEl per day (.6 units/84d * 80 kg BW/unit * 4.92 Mcal/kg BW). The numerical increase in dry matter intake with lasalocid is equivalent to 1.47 Mcal NEl per day (.85 kg * 1.73 Mcal NEl/kg).

The increase in dry matter intake accounts for only 50% of the decreased body condition loss with lasalocid, and nutrient digestibility was unaffected by lasalocid. As concentrations of VFA were not altered with lasalocid treatment (chapter 2), it is unclear what was responsible for the remainder of the reduction in body condition loss.

Ground corn may also reduce body condition loss and serum NEFA concentrations relative to cracked corn in diets for dairy cattle in early lactation, likely due to increased total tract starch digestibility. Further investigation of the parity by treatment interactions is warranted.

TABLES

Table 1. Diet ingredients and composition, % of DM

	Cracked		Ground	
	0[1]	+[2]	0	+
Ingredient				
Alfalfa silage	15.9	15.9	15.9	15.9
Corn silage	31.9	31.9	31.9	31.9
Dry rolled corn:				
Cracked	32.8	32.8		
Ground			32.8	32.8
Soybean meal	15.7	15.7	15.7	15.7
Blood meal	1.91	1.91	1.91	1.91
Lasalocid		.016		.016
Mineral/Vitamin mix	1.82	1.82	1.82	1.82
Composition				
NDF	27.1	27.1	27.2	27.2
ADF[3]	15.0	15.0	15.0	15.0
Lignin[3]	2.46	2.46	2.48	2.48
Ash	2.28	2.28	2.27	2.27
Crude protein	18.2	18.2	18.4	18.4
UIP, % CP[4]	39	39	39	39
DIP, % CP[4]	61	61	61	61
Starch	34.8	34.8	34.6	34.6

[1] 0 = Without lasalocid

[2] + = With lasalocid

[3] ADF and lignin determined sequentially

[4] UIP and DIP determined from book values

Table 2. Effect of lasalocid (L), corn grain particle size (PS) and square by treatment interaction (SqxT) on dry matter intake, milk yield and composition.

	Cracked		Ground		*P* value			
	0	+	0	+	L	PS	LxPS	SqxT
DMI, kg/d	18.3	19.2	19.2	20	.11	NS	NS	NS
Milk yield, kg/d	34.7	34.7	35.7	36.1	NS	†	NS	*
4% FCM, kg/d	33.3	32.8	33.6	32.7	NS	NS	NS	*
Milk fat ,%	3.79	3.67	3.64	3.44	*	**	NS	NS
Milk fat, kg/d	1.30	1.26	1.29	1.23	NS	NS	NS	*
Milk protein, %	2.75	2.80	2.82	2.92	*	**	NS	NS
Milk protein, kg/d	.95	.97	1.01	1.05	NS	**	NS	*
Milk lactose, %	4.82	4.81	4.83	4.78	NS	NS	NS	NS
Milk lactose, kg/d	1.67	1.68	1.73	1.73	NS	†	NS	*
BW Change, kg/21d	-8.9	-1.9	-3.2	2.7	NS	NS	NS	NS
BCS Change/21d	-.24	-.17	-.13	.1	*	**	NS	NS

** $P < .01$; * $P < .05$; † $P < .1$

Table 3. Effect of lasalocid and corn grain particle size on milk yield and milk composition within square.

	Cracked		Ground		*P* value		
	0	+	0	+	L	PS	LxPS
Square 1, Multiparous							
Milk yield, kg/d	36.7	38.0	40.8	40.8	NS	**	NS
4% FCM, kg/d	34.3	35.0	38.4	36.2	NS	**	NS
Milk fat, kg/d	1.31	1.32	1.47	1.37	NS	*	NS
Milk protein, kg/d	.99	1.04	1.15	1.19	NS	**	NS
Milk lactose, kg/d	1.76	1.83	1.96	1.95	NS	**	NS
Square 2, Primiparous							
Milk yield, kg/d	28.6	30.1	26.6	30.6	*	NS	NS
4% FCM, kg/d	29.2	29.8	25.8	28.6	†	*	NS
Milk fat, kg/d	1.18	1.19	1.01	1.09	NS	**	NS
Milk protein, kg/d	.81	.86	.74	.89	*	NS	NS
Milk lactose, kg/d	1.41	1.49	1.32	1.48	*	NS	NS
Square 3, Multiparous							
Milk yield, kg/d	38.6	36.0	39.5	37.0	*	NS	NS
4% FCM, kg/d	36.4	33.6	36.8	33.3	**	NS	NS
Milk fat, kg/d	1.39	1.28	1.40	1.23	**	NS	NS
Milk protein, kg/d	1.05	1.01	1.13	1.06	NS	NS	NS
Milk lactose, kg/d	1.82	1.70	1.89	1.76	*	NS	NS

** $P < .01$; * $P < .05$; † $P < .1$

Table 4. Effect of lasalocid and corn grain particle size on total tract nutrient digestibility

	Cracked		Ground		*P* value		
	0	+	0	+	L	PS	LxPS
Digestibility, % intake							
Starch	82.8	83.8	91.2	91.2	NS	**	NS
NDF	45.0	43.8	41.0	41.9	NS	*	NS
Dry matter	66.4	66.4	67.8	66.9	NS	NS	NS

** $P < .01$; * $P < .05$; NS = $P > .1$

Table 5. Effect of lasalocid and corn grain particle size on serum metabolites.

	Cracked		Ground		*P* value			
	0	+	0	+	L	PS	LxPS	SqxT
Serum NEFA, ueq/l	323	334	261	235	NS	**	NS	NS
Serum B-HBA, mg/dl	6.44	6.66	6.30	6.14	NS	NS	NS	NS
Serum TG, mg/dl	14.0	14.4	13.5	14.8	NS	NS	NS	NS
Serum glucose, mg/dl	61.1	62.1	62.7	62.2	NS	NS	NS	NS
Serum insulin, ng/ml:								
Mean	.780	.773	.949	.800	NS	NS	NS	NS
Mean pre meal	.816	.717	.835	.850	NS	NS	NS	NS
Mean post meal	.745	.829	1.06	.751	NS	NS	†	NS
Change (post - pre)	-.071	.112	.229	-.099	NS	NS	*	NS

** $P < .01$; * $P < .05$; † $P < .10$

CHAPTER 4

FINAL DISCUSSION AND CONCLUSIONS

Lasalocid treatment, and fine grinding of corn grain improved retention of body condition in early lactation cows, but had no effect on dry matter intake or milk yield. In contrast to other studies, lasalocid increased ruminal lactate concentration in this study, without affecting mean ruminal pH. The increase in lactate concentration did not decrease dry matter intake or subsequent milk yield. Ground corn did not affect lactate concentration or mean pH, but increased ruminal pH range. Again, this did not influence dry matter intake and milk yield. The proposed mechanism of lasalocid and corn grain particle size effect on performance in early lactation cows was that finely grinding corn would increase level of ruminally degraded starch and increase ruminal lactate concentrations. The increase in lactate concentrations was expected to reduce mean ruminal pH or increase pH fluctuations, and decrease dry matter intake and subsequent performance. Lasalocid was expected to decrease lactate concentrations in the rumen, increase mean ruminal pH and improve dry matter intake and performance relative to the control. This hypothesis was not supported by the results of this trial.

The most obvious contradiction of the results with the original hypothesis is the increase in lactate concentration with ionophore treatment. This contradicts *in vitro* data and *in vivo* data from beef cattle with experimentally induced acute acidosis. As noted previously, one possible mechanism for this increase in lactate concentration is an inhibition of protozoa by ionophores, increasing the amount of starch available for hydrolysis and fermentation to

lactate and decreasing lactate utilization. Another possible explanation of the increase in lactate with ionophore treatment is that the expected (although not measured) inhibition of *S. bovis* by lasalocid may have allowed more slowly growing, but ionophore resistant, lactate producers to proliferate. The lactate producers *Bacteroides*, *Selenomonas*, *Succinimonas* and *Succinivibrio* may not be inhibited by ionophores (Dennis et al., 1981). Schelling (1984) suggested that with 'carbohydrate stress', ionophores increase pH and decrease lactate concentrations, but that they increase lactate and do not affect ruminal pH when animals are not stressed with carbohydrate. Although the diets were formulated to 'stress' the animals with rapidly available carbohydrate, the rations fed had 34% starch and 27% NDF, with apparently adequate forage particle size and did not stress these early lactation cows.

Another contradiction with the original hypothesis is the observation that despite the increase in lactate concentration, intake tended to increase, and body condition retention improved with ionophore treatment. One possible explanation is that lactate may not be as important as we originally thought in the control of ruminal pH. The pKa of lactate is one unit lower than that of the other volatile fatty acids, but this fact does not necessarily mean that lactate would have a greater effect on ruminal pH. Acidic or basic compounds in solution have their strongest buffering effects when the pH of the solution is at the pKa of the compound, and the compound is 50% ionized (Voet and Voet, 1990). When the pH is one unit away from the pKa of the compound, it is just 10% ionized, and has little ability to buffer the solution pH. Therefore, as rumen pH declines to around 5, the volatile fatty acids would be expected to strongly buffer the solution, having a much more significant effect on the pH of the 'solution', rumen fluid, than does lactate. The importance of lactate in the etiology of acidosis, therefore, may be when ruminal pH declines to near its pKa. Lactate clearly would make a bad

situation worse, but it may not be of primary importance in bringing about the original decline in pH. This is supported by the observation that a decline in ruminal pH to from ruminal pH greater than 7.0 to below 5 with dosing with a high starch mixture was due more to VFA accumulation than to lactate (Nagaraja et al., 1985).

Another important factor is the relative pool sizes of lactate and the other VFA's in the rumen. In our study, the mean pool size of lactate was .087 moles, as compared to 5.5 moles for acetate, 2.33 moles for propionate, and 1.05 moles for butyrate. Lactate would therefore need to be 12 to 60 times as powerful as the other VFA's in reducing pH to have equal effect at this lower pool size. At pH 6, however, one would expect lactate and the VFA's to have roughly equivalent effects on ruminal pH.

An alternative hypothesis is based on the expectation that the clearance rate of an acid from the rumen increases as ruminal pH declines toward its pKa. Biological membranes are more permeable to uncharged species, as the interior region of the lipid bilayer is hydrophobic, (Chang, 1981), so the fermentation acids from the rumen should be primarily absorbed in their protonated, undissociated form. The proportion of the compound in the protonated form ([HA]) relative to the dissociated form ([A-]) increases as the pH of a solution decreases toward the pKa of the compound. Therefore the absorption of the VFA's would be expected to increase as pH approaches 5, while lactate would not be similarly affected until a lower pH. This may cause shifts in relative pool sizes of the VFA's and lactate, changing their effect on ruminal pH. Thus with cows fed diets higher in ruminally degraded starch, with a lower ruminal pH than in our trial, there may be a stronger correlation of ruminal lactate concentrations and ruminal pH. In steers with induced acute acidosis and very low ruminal pH ($\leq$ 5.5), ruminal pH was more highly correlated with lactate concentration than with

total VFA concentration (Robinson et al., 1991). As we observed neither high concentrations of lactate in the rumen, nor abnormally low ruminal pH in this trial, this discussion about the effect of lactate on ruminal pH is purely conjecture.

The significant parity by treatment interaction with first calf heifers increasing yield of milk, FCM, milk protein and milk lactose in response to lasalocid, and decreasing FCM in response to corn grain is interesting. At first glance, it appears that one possibility is that our original hypothesis of small rumen sizes favoring lactate production was a factor in the heifers, as their mean rumen digesta volume was smaller than those of the older cows (64.8 l vs. 78.2 l). However lactate concentrations, mean pH and pH range were not significantly affected by treatment within the heifer square. No blood metabolite effects were observed in response to lasalocid treatment in this square that would help explain these results.

The decrease in yield of milk, FCM, milk fat and milk lactose in the multiparous cows in square 3 with lasalocid is different from the other square of multiparous cows and difficult to explain. Some of the factors possibly involved include rumen digesta volume, lactate concentration and liquid dilution rate, but examination of these yields few clues. Mean rumen digesta volume was smaller in this square vs. the other square of multiparous cows (74.7 l vs. 81.6 l). Lactate concentrations were lower than in this square vs. the other square of multiparous cows. Lactate concentrations were significantly higher with lasalocid in this square, while the other square of multiparous cows was not affected by treatment. Liquid dilution rates were not different between squares. These parity by treatment interactions are interesting and may point out areas to focus on in future research, but with just four cows per square, the results are preliminary and should not be overemphasized.

One component of our experiment that should be more fully investigated is the competition between rate of digestion and rate of passage of ground corn from the rumen. The quantity of starch digested in the rumen depends upon both of these factors. Although ground corn apparently degrades at a faster rate than does cracked corn, rate of passage of corn grain has also been shown to increase with decreasing particle size, using Ytterbium labeled corn grain (Ewing et al., 1986). However Ytterbium dissociates from the originally labeled feed particles as it passes through the digestive tract, raising serious questions about its suitability as a marker for estimating rates of passage of feed from the rumen (Combs et al., 1992).

We estimated rate of starch passage from the rumen using *in vitro* rates of degradation and total rate of escape from the rumen calculated from rumen pool size of starch and starch intake per hour. We observed no effect of particle size on starch passage from the rumen, but our calculations had weaknesses as well. Representative sampling of rumen contents for starch assay is problematic, as is applying *in vitro* data to *in vivo* situations. When addressing questions of particle size, chewing becomes a potentially important factor that is ignored with *in vitro* methods. The question of effect of particle size of feed on passage from the rumen ought to be explored using a marker that will remain associated with the labeled particles, if and when such a method becomes available.

Another factor worthy of further exploration is the effect of ionophores on ruminal protein degradation, and the importance of this effect to the animal. Ionophores have been shown to decrease ruminal proteolysis, increasing rumen ammonia concentrations. We did not measure these factors in this trial. Reducing ruminal degradation of protein may increase passage of undegraded intake protein form the rumen to the small intestine. With diets high in ruminally degraded protein and deficient in total protein or specific amino acids presented

to the intestine, ionophores may improve performance by increasing the escape of protein from the rumen. With diets high in ruminally undegraded protein, or diets low or deficient in ruminally degraded protein, however, ionophores may not cause a performance response.

The data suggest that the originally proposed mechanism was not appropriate under the conditions of this trial, as lasalocid increased lactate concentrations and ground corn did not affect them, and neither treatment affected mean pH. Examination of this hypothesis under different conditions may be warranted. The fiber content of these diets (27% NDF) may have been high enough to preclude any problem with acid accumulation. The consistency of the forage may also have been a factor. In the field, wide fluctuations in fiber content and dry matter content of the feed often occur undetected. A sudden, unnoticed decline in dry matter of the forage from 40% to 30% could decrease the actual NDF content of the ration from 27.5% to 22% (assuming 50 lb DMI, 50% forage in the ration, with the forage mix averaging 45% NDF, and the concentrate mix averaging 10% NDF). In this situation, a decline in intake due to excessive acid production in the rumen may be more likely. Large, undetected fluctuations in diet NDF concentration may also occur because of changes in forage composition due to cutting, field, and even area within the field.

Also, examination of this hypothesis in very early lactation cows, from 0 to 30 DIM, for example, may be warranted. These cows are the most at risk of metabolic problems, and the most negatively affected by reduced dry matter intakes. Exchanging rumen contents among cows between 21 day periods of a Latin square meant that in effect, we evaluated the effects of continuous treatment on cows from 16 to 100 DIM. That the proposed mechanism does not apply to this relatively long period of treatment is important to know, but our

results do not rule out this mechanism as an explanation for impaired intakes and performance observed in the short term immediately post calving.

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