



Monensin effects on digestion of corn or barley high concentrate diets
by Lisa Marie McKinley Surber

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in
Animal Science

Montana State University

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Abstract:

Two experiments were conducted to determine the effects of monensin addition on digestion of high concentrate diets based on corn or barley. An *in vitro* experiment was designed using a 2 x 4 factorial arrangement to evaluate the main effects of ionophore addition (0 vs 9 ppm monensin; - vs +) and grain source (corn, C; Gunhilde barley, GUN; Harrington barley, HAR; and Medallion barley, MED). The *in vitro* study was replicated three times, with triplicate tubes for each of the 8 above treatments incubated for 0, 3, 6, 9, 12, 18, 24 and 30 h. Rate and extent of *in vitro* DM disappearance (IVDMD) were determined. Four ruminally and abomasally cannulated steers were utilized in a 4 X 4 Latin square design. A 2 X 2 factorial arrangement of treatments was used to test the effects of monensin addition (0 vs 270 mg/d monensin; M- vs M+) and grain source (corn vs Medallion barley; C vs BAR) on *in vivo* diet digestibility. Steers were fed isocaloric (1.87 Mcal/kg NEm, 1.23 Mcal/kg NEg) and isonitrogenous (11.6% CP) high concentrate diets twice daily. Each experimental period consisted of 14 days for diet adaptation followed by 7 days for collection. Beginning on d 1 of the sample collection period abomasal samples were collected at 0, 3, 6, 9, 12, 15, 18, 21, 24, 30, 36, 42, 48, 54, 60, and 72 h after the am feeding, and composited to determine ruminal digestion and flow to the abomasum of DM, OM, N and starch. Boluses containing Cr₂O₃ were used to estimate abomasal DM flow and fecal DM output. On d 4, duplicate nylon bags containing the respective grain source were placed in the rumen and incubated for 0, 1, 2, 3, 4, 5, 6, 9, 12, 15, 18, 21, 24, 30 and 36 h. Rate and extent of *in situ* DM (ISDMD) and starch digestion were measured. An interaction ($P < .10$) was observed between grain source and ionophore addition for IVDMD during 0 through 9 h of incubation. During all times of incubation, C had lower ($P < .10$) IVDMD than the three barley varieties. Ruminal starch digestibility was lower ($P < .01$) for steers fed C than those fed BAR (88.6 vs 92.4%), resulting in more ($P < .10$) starch flowing to the abomasum (321 vs 191 g/d). Microbial N flow was 17% greater ($P < .10$) for steers fed BAR than those fed C (68 vs 58 g/d). Monensin addition reduced ($P < .10$) ruminal digestion of feed N (74.9 vs 81.5%), which resulted in a greater ($P < .10$) feed N flow to the abomasum compared with steers fed M- (34 vs 25 g/d). Steers fed BAR had greater ($P < .10$) ruminal digestion of feed N than steers fed C (81.4 vs 75.0%). No differences ($P > .10$) were seen for time delay, flow rate, retention time and ruminal fill. No interaction ($P > .10$) between ionophore addition and grain source was observed in ISDMD. Between 1 and 21 h, C had a lower ($P < .10$) ISDMD than BAR. During the 4 x 4 Latin square, no differences ($P > .10$) were seen in DM intake, however, C had greater ($P < .01$) starch and N intake than BAR. Total tract DM, OM, starch, and N digestibilities were greater ($P < .10$) for BAR than C. BAR also exhibited a greater *in situ* starch digestibility than C. It appears that grain sources may respond differently to monensin addition. Monensin addition caused a protein sparing effect by reducing ruminal digestion of feed N, and increased feed N flow to the abomasum.

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HIGH CONCENTRATE DIETS**

by

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A thesis submitted in partial fulfillment
of the requirements for the degree

of

Master of Science

in

Animal Science

MONTANA STATE UNIVERSITY
Bozeman, Montana

December, 1995

N378
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ACKNOWLEDGMENTS

This has been the largest undertaking of my life. The completion of my Master's degree would not have been possible without the help of many people whom I owe so much gratitude. To my parents, Warren and Kathie, I cannot begin to thank you enough for giving me the opportunity to receive my secondary education at Montana State. Your patience, understanding and support have been such a blessing. My education gave me one of the luckiest events in life, the chance to meet my husband, Shane. Without you, Shane, I would never have been able to do all the demanding tasks of a Master's thesis project. You spent many hours with me at the Nutrition Center and at home and were sometimes my only help. For this, all I can say is Thank-you and I love you. Your parents, Gene and Vickie, have been so generous and welcomed me into their lives. I would also like to extend my sincerest thanks to Dr. Janice Bowman for taking me on as one of her graduate students. Jan, you have been more than a major professor, teacher and employer, you have been a true friend. I wish you all the success you so justly deserve. Drs. Ray Ansotegui and Bok Sowell, thank you for serving on my committee and being there to listen. I have enjoyed all the classes you have taught. Thank-you to the memorable instructors I have had - Dr. Verl Thomas and Dr. John Paterson. I cannot forget the many special friends I have made while at MSU. Leigh, I cannot imagine life without friends like you - hard times make friends even out of the worst enemies. Thank-you to Ken Bryan and Tim Milner for your help out at the farm and your friendship. Thank-you to Chris, Darrin, Brent, Joanna, Kristie, Scott, Eric, B.J., Tracie, Tanya, Todd, Rob, and Shawn just for being such good friends to me.

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ABSTRACT

Two experiments were conducted to determine the effects of monensin addition on digestion of high concentrate diets based on corn or barley. An *in vitro* experiment was designed using a 2 x 4 factorial arrangement to evaluate the main effects of ionophore addition (0 vs 9 ppm monensin; - vs +) and grain source (corn, C; Gunhilde barley, GUN; Harrington barley, HAR; and Medallion barley, MED). The *in vitro* study was replicated three times, with triplicate tubes for each of the 8 above treatments incubated for 0, 3, 6, 9, 12, 18, 24 and 30 h. Rate and extent of *in vitro* DM disappearance (IVDMD) were determined. Four ruminally and abomasally cannulated steers were utilized in a 4 X 4 Latin square design. A 2 X 2 factorial arrangement of treatments was used to test the effects of monensin addition (0 vs 270 mg/d monensin; M- vs M+) and grain source (corn vs Medallion barley; C vs BAR) on *in vivo* diet digestibility. Steers were fed isocaloric (1.87 Mcal/kg NE_m, 1.23 Mcal/kg NE_g) and isonitrogenous (11.6% CP) high concentrate diets twice daily. Each experimental period consisted of 14 days for diet adaptation followed by 7 days for collection. Beginning on d 1 of the sample collection period abomasal samples were collected at 0, 3, 6, 9, 12, 15, 18, 21, 24, 30, 36, 42, 48, 54, 60, and 72 h after the am feeding, and composited to determine ruminal digestion and flow to the abomasum of DM, OM, N and starch. Boluses containing Cr₂O₃ were used to estimate abomasal DM flow and fecal DM output. On d 4, duplicate nylon bags containing the respective grain source were placed in the rumen and incubated for 0, 1, 2, 3, 4, 5, 6, 9, 12, 15, 18, 21, 24, 30 and 36 h. Rate and extent of *in situ* DM (ISDMD) and starch digestion were measured. An interaction ($P < .10$) was observed between grain source and ionophore addition for IVDMD during 0 through 9 h of incubation. During all times of incubation, C had lower ($P < .10$) IVDMD than the three barley varieties. Ruminal starch digestibility was lower ($P < .01$) for steers fed C than those fed BAR (88.6 vs 92.4%), resulting in more ($P < .10$) starch flowing to the abomasum (321 vs 191 g/d). Microbial N flow was 17% greater ($P < .10$) for steers fed BAR than those fed C (68 vs 58 g/d). Monensin addition reduced ($P < .10$) ruminal digestion of feed N (74.9 vs 81.5%), which resulted in a greater ($P < .10$) feed N flow to the abomasum compared with steers fed M- (34 vs 25 g/d). Steers fed BAR had greater ($P < .10$) ruminal digestion of feed N than steers fed C (81.4 vs 75.0%). No differences ($P > .10$) were seen for time delay, flow rate, retention time and ruminal fill. No interaction ($P > .10$) between ionophore addition and grain source was observed in ISDMD. Between 1 and 21 h, C had a lower ($P < .10$) ISDMD than BAR. During the 4 x 4 Latin square, no differences ($P > .10$) were seen in DM intake, however, C had greater ($P < .01$) starch and N intake than BAR. Total tract DM, OM, starch, and N digestibilities were greater ($P < .10$) for BAR than C. BAR also exhibited a greater *in situ* starch digestibility than C. It appears that grain sources may respond differently to monensin addition. Monensin addition caused a protein sparing effect by reducing ruminal digestion of feed N, and increased feed N flow to the abomasum.

CHAPTER 1

INTRODUCTION

Most cattle in the U.S. are finished on high concentrate diets. These types of diets have increased rate of gain when compared to forage-based diets, and subsequently cattle reach slaughter weight more rapidly. Faster cattle turnover in the feedlot means more profit for the operator and/or owner.

Most high concentrate diets are corn-based due to its wide availability, high energy value and superior animal performance. Corn-based diets can also be the most economical and efficient when used as the primary grain source. However, in the northwestern U.S. and western Canada, barley is the most abundantly grown grain. Here, barley plays an important role in the malting industry, and as a major ingredient in supplements and feedlot diets.

Barley has approximately 90% of the energy value of corn, and a higher protein content (NRC,1984). Barley-based diets have resulted in similar animal performance as corn-based diets (Nichols and Weber, 1988; Dion and Seoane, 1992). However, one possible limitation of using barley as the primary grain source is its extremely rapid rate of starch digestion. This rapid rate of digestion may lead to negative effects, such as decreased ruminal pH and increased incidence of acidosis and bloat. All of these metabolic effects can reduce animal performance and thereby decrease the animal's profitability.

The rapid rate of starch digestion in barley causes starch digestion to take place primarily in the rumen. Ørskov (1986), indicated that up to 40% of the starch in corn

can bypass ruminal fermentation, while 10% or less of the starch in barley escapes the rumen. Reducing ruminal digestion of starch in barley, and shifting a greater proportion of starch digestion to the small intestine would theoretically make more efficient use of the dietary energy by avoiding energy losses that occur during ruminal fermentation.

Monensin is a member of the class of compounds known as monocarboxylic acid ionophores produced by *Streptomyces cinnamonensis* (Haney and Hoehn, 1967). Ionophore addition to high concentrate diets adds greater economic return to feedlot cattle. Ionophores influence animal performance primarily through the modification of ruminal fermentation, by causing a reduction in the activity of gram positive hydrogen and formate producing bacteria. In addition, ionophores destroy primary membrane transport and thereby interfere with cellular solute uptake by ruminal bacteria (Bergen and Bates, 1984).

Monensin is used to alter ruminal fermentation to improve feed efficiency. This compound has been shown to decrease ruminal proteolysis, and to increase the proportion of dietary protein escaping ruminal digestion (Hanson and Klopfenstein, 1979; Poos et al., 1979; Yang and Russell, 1993). Results have not been as conclusive when examining the effects of monensin on ruminal digestion of OM and starch in corn-based diets. Muntifering et al. (1981) found a reduction in ruminal OM and starch digestion with the addition of monensin, while Zinn and Borques (1993) found only a reduction in ruminal OM digestion. Zinn (1987) observed no effect on ruminal OM and starch digestion with monensin addition. Kung et al. (1992) utilized the ionophore lysocellin in continuous culture and found an increase in OM digestion of corn-based diets, but a decrease in OM

digestion of barley-based diets. It appears that grain sources may respond differently to monensin addition.

CHAPTER 2

LITERATURE REVIEW

High Concentrate Diets

Most cattle in the United States and Canada are finished on a high concentrate diet. These types of diets have an increased rate of gain when compared with diets based solely on forages. Elevated rate of gain is essential for high producing ruminants to reach slaughter weight quickly, thereby shortening the time spent in the finishing phase and increasing the animals' profitability. High concentrate diets achieve accelerated performance by providing a tremendous amount of energy (NE_m and NE_g) supplied by the grain source.

The primary cereal grain in cattle high concentrate diets has traditionally been corn. Corn can be the most economical and efficient grain (Anderson and Boyles, 1989), especially in the Midwestern U.S., where corn is widely available and readily produced. When compared with barley (*Hordeum vulgare L.*), corn has higher energy values (2.24 vs. 2.06 Mcal/kg NE_m ; and 1.55 vs. 1.40 Mcal/kg NE_g ; NRC, 1984). Corn-based diets have resulted in superior animal performance (ADG) compared to diets based on other cereal grains (Stock et al., 1990; Zinn, 1993b; Boss et al., 1994).

In the northern U.S. and southern Canada, due to the shorter growing season, and varying climate, corn cannot be readily grown. Because of this, corn must be shipped into local elevators which can make its' inclusion in high concentrate diets costly. However, barley is readily grown in this region, and has higher protein content than corn (13.4 vs. 10.1%, DM basis; NRC, 1984), making it an economical grain source

alternative. Montana produces approximately 85 million bushels of barley annually (Montana Agriculture Statistics Service, 1993), and barley is the primary grain grown in Alberta for finishing cattle diets. It is estimated that 70 to 80% of the barley produced in Alberta is used by the livestock industry, which is equivalent to almost four million acres of the province's production (Barley Country, Summer, 1995).

There is a great deal of variability in barley because of cultivar, region, growing conditions and year (Kemalyan et al., 1990). Different barley varieties can be classified as hulled or hullless, normal or waxy starch type, malting or feed grade, 2-row and 6-row, and by length and type of awn (Middaugh, 1989). Cultivars can also differ in plant height, disease resistance, and whether spring or winter-grown (Middaugh, 1989). Chemical composition including protein, starch and phosphorus content, has also been reported to vary among cultivars (Middaugh, 1989; McDonald et al., 1991). Barley variety has been shown to differ in IVDMD (Kemalyan et al., 1990) and animal ADG (Ovenell and Nelson, 1992).

Barley-based high concentrate diets have resulted in similar animal performance (ADG; quality grade, QG; and yield grade, YG) to corn-based diets (Nichols and Weber, 1988; Dion and Seoane, 1992). A recent study (Boss, 1994) indicated that corn-fed cattle had superior overall ADG, however, a Harrington barley diet did sustain cumulative ADG equal to a corn diet until the final 28 d of the experiment. Steers consuming corn had poorer feed conversion when compared to steers fed Harrington, Medallion, and Gunhilde barleys. Harrington barley-fed steers had superior marbling and QG scores when compared to corn-fed steers. In addition, corn-based high

concentrate diets cost \$.21/kg gain more than the barley diets. The cost advantage of barley in this region is an important factor to consider when formulating a finishing ration.

Protein Digestion In Ruminants

Ruminants have the unique ability to produce a source of protein in addition to dietary protein due to the synthesis of microbial (bacteria, protozoa, and fungi) protein within the rumen (Church, 1988). Microbial protein, together with feed protein that escapes ruminal degradation, supply the small intestine with a source of protein to digest and absorb. The rumen has many sources of nitrogenous inputs: True protein, non-protein nitrogen (NPN), salivary urea, salivary mucoproteins, nucleic acids, and urea that diffuses across the rumen wall. True protein is the primary source of N in high concentrate diets. Dietary or feed N is hydrolyzed into two fractions. Feed N can be completely broken down and deaminated into ammonia (NH_3) and carbon skeletons, or partially broken down into oligopeptides, peptides and amino acids. Degradation involves two steps: (1) hydrolysis of the peptide bond (proteolysis) to produce peptides and amino acids; and (2) deamination of the amino acids (NRC, 1985). Ruminal NH_3 becomes the most important building block of microbial protein. Ammonia can then be transported across the membrane of the existing bacterial cell, and reaminated and transformed into microbial protein. The remaining fraction of feed N (oligopeptides, peptides and amino acids) can either be utilized as a source of N for microbial protein synthesis, or bypass ruminal fermentation and be digested in the small intestine where it can avoid the energy losses that occur in ruminal digestion. Non-protein N such as urea,

can also be a significant source of ruminal NH_3 . Urea is hydrolyzed rapidly to produce two molecules of NH_3 by the enzyme urease.

Ruminal microbes are the most abundant protein N leaving the rumen. Approximately 20 to 60% of their dry matter is crude protein. Microbial flow from the rumen can meet 50% or more of the amino acid requirement of the ruminant in various states of production (Ørskov, 1982).

Ruminant digestion, absorption and metabolism of protein once it arrives in the small intestine is similar to the non-ruminant. The quality of protein is determined by the quantity of essential amino acids present. For non-ruminants, quality is assessed as the amount of essential amino acids ingested. Conversely, ruminants depend on the quality of amino acids coming from the rumen. In a high concentrate diet, ruminal microbes supply the majority of amino acids presented to the small intestine for digestion and absorption.

Once microbial and bypass protein flow to the small intestine proteolysis occurs. As the final product of proteolysis, amino acids enter the blood stream and travel to the liver for metabolism. These amino acids can have three fates: (1) Transamination: resynthesis of non-essential amino acids that can go to animal growth; (2) Deamination: carbon skeletons can be used for energy, and NH_3 excreted via urine; or (3) Liver ammonia: production of urea or production of non-essential amino acids (reamination).

Starch Digestion In Ruminants

Starch is the major constituent of a cereal grain (60-80% of the grain weight) and is concentrated in the endosperm. Starch is also the major source of readily available

energy. Starch is comprised of amylose and amylopectin. Amylose is composed of straight chains of D-glucopyranose units linked by α -(1,4) bonds. Amylopectin contains chains of α -(1,4) D-glucopyranose units branched through α -(1,6) linkages (Newman and Newman, 1992).

Ruminants do not possess salivary amylase, so the first site of starch digestion is the rumen. Starch is rapidly fermented by the ruminal microorganisms to produce energy in the form of volatile fatty acids (VFA). Ruminal starch fermentation rates vary and are influenced by the grain type, the method of processing, diet and ruminant species (Waldo, 1973; Ørskov, 1986; Owens et al., 1986). When diets contain high grain and low forage levels ruminal microflora are dominated by approximately 15 strains of amylolytic organisms. These organisms include *Streptococcus*, *Ruminobacter*, *Bacteroides*, *Butyrivibrio* and *Lactobacillus* (Kotarski et al., 1992). The rate and extent of ruminal starch digestion may influence the composition of the VFA, ruminal pH, the amount of starch available for post-ruminal digestion, and the form in which starch is presented for post-ruminal digestion (Kotarski et al., 1992). If the rate of starch digestion is slow, digestion can be incomplete (Owens et al., 1986; Theurer, 1986). However, if the rate is too rapid, digestive byproducts such as VFA and lactic acid can overwhelm the animal's own buffering and absorption capacity (Kotarski et al., 1992). Ideally, rate of starch digestion should be intermediate.

Starch remaining after ruminal hydrolysis, and microbial polysaccharides begin post-ruminal degradation in the small intestine. In the small intestine starch is attacked enzymatically by amylases. Amylases break starch into glucose which can be absorbed

through the wall of the intestine. Glucose absorbed from the intestine is used with greater metabolic efficiency than glucose derived from ruminal VFA (Owens et al., 1986). Owens and co-workers (1986) stated that starch digested in the small intestine has 42% more value energetically than ruminally digested starch. Also, if starch that escapes the rumen can be digested to an extent exceeding 70% of its digestibility in the rumen, starch escape from the rumen should improve energetic efficiency of production by ruminant animals (Owens et al., 1986). However, there may be a threshold to glucose absorbance. Ørskov (1986) suggested the capacity for absorption of glucose may limit starch digestion in the small intestine.

Type of grain has been shown to affect site and extent of starch digestibility (Waldo, 1973; Ørskov, 1986; Owens et al., 1986). High concentrate diets based on corn commonly had a much slower rate of digestion when compared to barley (McAllister et al., 1990), however, total tract digestibility of starch did not differ (Waldo, 1973; Spicer et al., 1982; Owens et al., 1986). Ruminal digestibility of starch differs greatly between corn and barley. Forty percent of the starch present in corn can escape rumen hydrolysis, while 90% or more of barley starch undergoes ruminal fermentation (Ørskov, 1986).

It is still unclear why there are differences in ruminal starch digestibility between corn and barley. In most barleys, the starch is predominantly in the form of amylopectin (74-78%), the remainder (22-26%) is amylose (Briggs, 1978). Björrek et al. (1990) concluded that the amylose/amylopectin ratio in different barley genotypes (waxy, normal and high amylose starch) had only marginal influence on starch gelatinization. High amylose starch was shown to be less susceptible to α -amylase than normal or waxy

barley starch. Data suggest that high amylose/amylopectin ratios depress starch digestibility (Sandstedt et al., 1962).

McAllister et al. (1993) suggested that regardless of cereal grain type the protein matrix (which contains the starch granules) within the kernel may be the major factor in determining the extent of ruminal starch digestibility rather than the chemistry and physical form of starch. They also stated that there are obvious differences in the protein matrix between corn and barley. Corn protein matrix appeared to be extremely resistant to microbial attachment and penetration, while barley was more readily colonized (McAllister et al, 1994). This may explain the differences in starch digestibility seen between corn and barley.

Monensin Mode Of Action

The polyether ionophores, which include monensin, lasalocid and laidlomycin, are used to alter ruminal fermentation to improve efficiency of feed utilization. These compounds are produced by strains of *Streptomyces* bacteria. Monensin, lasalocid and laidlomycin are approved for use in feedlot diets. Each ionophore has different effects on digestion, intake and efficiency, however, animal performance has been found to be similar. Monensin decreases dry matter intake, while gain remains the same, thus improving feed efficiency (Goodrich et al., 1984). Lasalocid increases ADG, with no change in intake, and improving feed efficiency (Goodrich et al., 1984). The structural formula of monensin sodium is presented in Figure 1. During the manufacturing process, monensin is exposed to sodium ions during a pH adjustment and monensin sodium

results (Rumensin). Monensin has a molecular weight of 692 and is soluble in water and most organic solvents.

Ionophores can have different effects when used with forage and high concentrate diets. Generally, when ionophores are added to high grain diets, animals exhibit depressed intake without a reduction in ADG, thereby improving feed efficiency. When ionophores are added to forage diets animals exhibit increased ADG without depressing intake, and efficiency of feed utilization is improved (Bergen and Bates, 1984).

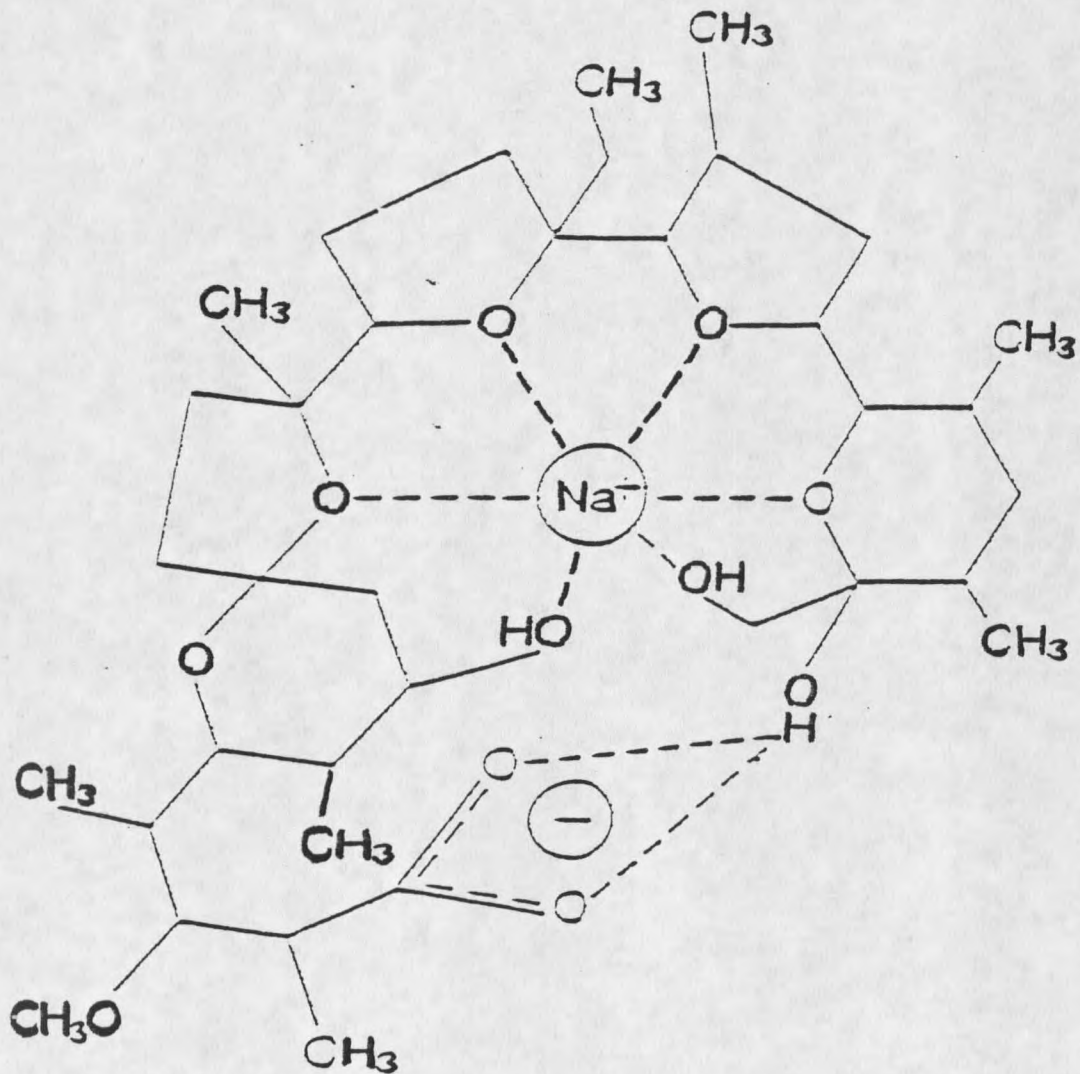


Figure 1. Structural formula of Monensin sodium.

Bergen and Bates (1984) conducted an extensive review of the effects of ionophores on animal production efficiency, ruminal ecology and cellular responses of ruminal anaerobes. These researchers suggested that there are three major areas of animal metabolism that can contribute to or account for the improvement seen when ionophores are added to ruminant diets: (1) Increased efficiency of energy metabolism in the rumen and/or animal; (2) Improved nitrogen metabolism in the rumen and/or animal; (3) Retardation of feedlot disorders, especially lactic acidosis (chronic) and bloat. These authors summarized the effects of monensin on ruminal fermentation, much of which will be discussed later in the chapter (Table 1.)

Table 1. A summary of metabolic effects of ionophores on ruminal fermentation (Bergen and Bates, 1984).

-
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1. Shift in acetate-propionate ratio toward more propionate.
 2. Some increase of lactate to propionate production via the acrylate pathway.
 3. Decreased ruminal protein breakdown and deamination; lower ruminal $\text{NH}_3\text{-N}$.
 4. Primary H^+ or formate producers, gram positive organisms, are inhibited.
 5. Decrease in methane production primarily due to lowered availability of H_2 and formate and depressed interspecies H_2 transfer.
 6. Depression of lactic acid production under acidosis inducing conditions.
 7. Gram negative organisms, of which many produce succinate (source of propionate) or possess capacity for the reductive TCA to use bacterial reducing power, survive.
 8. Some evidence of depressed rumen content turnover.
 9. A mild inhibition of protozoa.
 10. Decrease in rumen fluid viscosity in bloated animals.
 11. Depressed growth yield efficiency of the ruminal microbes.
-
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Bergen and Bates (1984) suggested the underlying mode of action of ionophores is on the transmembrane ion fluxes and the breakdown of cation and protein gradients. This action destroys primary membrane transport of cells, thereby interfering with the

cellular solute uptake coupled to primary transport systems. Bacterial cells that depend on substrate level phosphorylation (Gram positive bacteria) for ATP can not survive, while Gram negative bacteria survive and flourish.

In a study performed by Russell (1987), *Streptococcus bovis*, a gram positive bacterium, was unable to grow in the presence of monensin. Monensin was added in vitro and was shown to immediately decrease growth rates and within three hours no further growth was observed. It appeared that monensin has a very rapid effect on ruminal microorganisms.

Biological Effects Of Ionophores

Ionophores have been shown to affect ruminal microorganisms (Bartley and Nagaraja, 1982; Yang and Russell, 1993). Bartley and Nagaraja (1982) showed the addition of monensin and lasalocid to a forage-based diet reduced protozoal counts. *Diplodinium* and *Ophryoscolex* species of protozoa were most inhibited. Generally, gram positive bacteria developed a sensitivity to monensin and lasalocid, with *Streptococcus bovis* being the exception. However, some gram negative bacteria did develop a sensitivity to both ionophores and this may have been due to the structure of the cell envelope resembling a gram positive cell wall. Ruminal bacteria that produce lactate, butyrate, formate and hydrogen have been found to be susceptible to monensin and lasalocid. Bacterial strains that produce succinate and those that use lactate can develop a resistance to these ionophores.

A recent study evaluated the effects of monensin supplementation on growth of highly active NH_3 -producing bacteria in vivo (Yang and Russell, 1993). Forage-based

diets were supplemented with soybean meal and monensin. The addition of monensin caused a 10-fold decrease in these bacteria. Monensin inhibited these highly active amino acid-fermenting ruminal bacteria and this inhibition, in turn, decreased ruminal amino acid deamination and NH_3 production. Because monensin did not increase protein, peptides, or amino acids in the ruminal fluid, it did not seem that the decrease in NH_3 increased the flow of dietary amino-N to the lower gut. However, monensin increased the concentration of bacterial protein in the ruminal fluid, which could provide additional amino-N for the animals.

Ionophores have been shown to decrease the incidence of feedlot disorders such as lactic acidosis and bloat (Meyer and Bartley, 1972; Bartley et al., 1975; Bartley et al., 1983). Lasalocid appeared to have the greatest effect in reducing bloat symptoms (Bartley et al., 1975). Meyer and Bartley (1972) examined the effects of lasalocid and monensin on feedlot bloat. These researchers initiated bloat-like symptoms and fed one of three diets. The control diet contained no added ionophore and the other two diets contained either 600 mg/kg of body weight of monensin or lasalocid. Both ionophores reduced the degree of bloat, with the greatest reduction occurring at the end of the feeding period. Lasalocid appeared to be the most effective at controlling bloat. This may be due in part to lasalocid's growth inhibiting properties of all *S. bovis* strains of bacteria. *Streptococcus bovis* strains of bacteria have long been associated as the causative bacterias in feedlot bloat problems (Bartley et al., 1975). Because monensin is not as effective as lasalocid at inhibiting strains of *S. bovis*, it did not control bloat as well.

Lactic acidosis can be caused by an over consumption of grain resulting in an increase in lactic acid in the rumen and subsequently in the blood (Dirkson, 1970). When grain is fermented in the rumen by *Streptococcus bovis* bacteria which causes a decrease in ruminal pH allowing *Lactobacillus* to proliferate. These two strains of bacteria are responsible for the initiation of acidotic conditions. It has been shown that salinomycin, monensin and lasalocid are effective in preventing experimentally induced lactic acidosis in cattle (Nagaraja et al., 1981; Nagaraja et al., 1985).

Effect Of Monensin On Protein Digestion

Feeding an ionophore, such as monensin or lasalocid, has been shown to decrease the ruminal fermentation of protein, and to increase the proportion of protein digested in the small intestine (Dinius et al., 1976; Hanson and Klopfenstein, 1979; Poos et al., 1979; Muntifering et al., 1980; Paterson et al., 1983; Newbold et al., 1990; Yang and Russell, 1993).

Research in this area began with a study that examined the influence of feeding monensin on nitrogen digestibility, ruminal NH_3 concentration and types and total numbers of ruminal microorganisms in a forage-based diet (Dinius et al., 1976). In vivo digestion of DM and CP were not affected by feeding monensin, however, ruminal fluid NH_3 concentration appeared to be lower for cattle fed monensin than for the control animals. These treatment differences were not significant because of animal variability within treatments. Nitrogen retention tended to be higher for steers fed monensin. These results are contrary to what has been subsequently reported, however, this study was the

stepping stone that led to further research on the effects of ionophores on protein digestion and metabolism.

Muntifering and Theurer (1978) conducted a study to determine the effect of monensin supplementation on CP digestibility and N retention in steers consuming a high concentrate diet (76% steam-flaked sorghum grain). Monensin addition improved crude protein digestibility in trial 1 but not in trial 2. Nitrogen retention tended to improve when monensin was added. These results suggested that a possible protein sparing action of monensin may account for some of the improvement in feed utilization observed with its use.

The University of Nebraska at Lincoln lead the march into this unexplored area of research. Hanson and Klopfenstein (1979) conducted two steer growth trials using plant protein supplements and monensin. Not only was there an appreciable monensin response in feed efficiency, but there was also decreased ruminal NH_3 levels, which indicated a protein sparing effect. Why was there consistently a decrease in ruminal NH_3 levels? Were the proteolytic organisms being inhibited with addition of monensin? Poos et al. (1979) asked these questions. In two lamb trials using sorghum-based diets, protozoal populations and ruminal NH_3 were reduced with the addition of monensin. A steer trial used abomasally cannulated animals to evaluate monensin effects on nitrogen fractions entering the small intestine. Monensin addition decreased bacterial nitrogen flow. Also, abomasal essential and nonessential amino acid flow was increased, which indicated monensin may spare dietary protein by decreasing ruminal proteolysis. Surber

and Bowman (1995) found a similar protein sparing effect utilizing monensin in corn- and Medallion-barley-based diets.

Most of the research evaluating monensin in feedlot diets has used corn, and few experiments have used barley. Horton et al. (1980) examined the effects of monensin on digestibility and ruminal ammonia levels using different levels of barley fed to lambs and steers. Monensin increased CP digestibility from 61.7% to 69.0%, and protein digestibility increased linearly with barley level in lambs but not in steers. Ruminal NH_3 concentrations were 46% lower in steers fed monensin, but were not affected by barley level. The reduction in ruminal NH_3 in monensin-fed steers was due to lower deaminase activity in the rumen; in monensin-fed lambs, this effect was probably counteracted by the increase in protein digestion. An increase in protein digestion may have been due to a more complete mastication of the diet. Barley variety was not reported. These researchers demonstrated that lambs and steers respond differently to monensin addition.

Monensin, in a corn-based diet, tended to increase CP digestibility (63.4 vs. 61.3%) and decrease ruminal ammonia concentration (Muntifering et al., 1980). In an associated metabolism trial using sorghum-based diets, monensin improved CP digestibility, and tended to improve nitrogen retention. This study agreed with much of the data presented in the literature to this point, and the authors suggested that the slight improvement in nitrogen utilization may account for some of the benefit of feeding monensin with high concentrate diets. In a similar study, lasalocid improved CP digestibility in corn-based diets (Paterson et al., 1983).

The response to the ionophore salinomycin and to increasing forage levels was evaluated on site and extent of protein digestion, microbial synthesis and related efficiency (Zinn, 1986b). Salinomycin is produced by a strain of *Streptomyces albus* and has been shown to act against gram positive bacteria, but has no effect on gram negative bacterial strains. Averaged across forage level, salinomycin addition increased passage of non-NH₃-N to the small intestine. Salinomycin supplementation increased feed-N flow leaving the abomasum. There was no effect on microbial efficiency, however, there was an increase in protein efficiency (non-NH₃-N leaving the abomasum / N intake) when salinomycin was added (Zinn, 1986b).

Tetronasin is more potent than monensin, but has similar spectrum of antimicrobial activity against ruminal microorganisms (Newbold et al., 1988). Newbold et al. (1990) examined the effects of this novel ionophore on the degradation of peptides and amino acids in vitro, and identified how this ionophore inhibits the degradation of protein to ammonia in the rumen. The authors suggested the effect of tetronasin, and probably other ionophores, on amino acid deamination appeared to be twofold. One was the elimination of gram positive deaminating bacteria. The other was the interference in amino acid breakdown in surviving species. It appeared some organisms adapted to grow in the presence of the ionophore. These researchers found similar results in vitro to Hanson and Klopfenstein (1979) and Poos et al. (1979). The increased flow of non-NH₃-N from the rumen when tetronasin was fed observed by Newbold et al. (1988) could be attributed to lower proteolytic, peptidolytic and deaminase activities of the ruminal microorganisms.

Recently, interest in the mechanism behind the protein sparing effect was renewed. Yang and Russell (1993) examined the influence of monensin on the growth of highly active ammonia-producing bacteria and ammonia accumulation in vivo. Mature cows were fed a forage-based diet supplemented with soybean meal. When monensin was added to these diets there was a 30% decrease in ruminal NH_3 , and this decrease could be explained by a 10-fold decrease in bacteria that could utilize peptides and amino acids, but not carbohydrates, as an energy source. Amino acids that were spared from deamination were utilized by other bacteria, and the concentration of bacterial protein increased, which could provide additional amino-N for the animals. These researchers proposed that monensin could provide a means of decreasing the wasteful degradation of dietary amino acids in the rumen.

It is evident that there is no disagreement in the results presented. Ionophores do reduce proteolytic activity in the rumen thereby decreasing ruminal NH_3 . A protein sparing effect can be seen presenting more protein, peptides and amino acids to the small intestine for further digestion and absorption. It appears that ionophores such as monensin prevent ruminal deamination of amino acids but do not inhibit proteolysis as shown by increased total tract digestibility of CP. However, much of this research was done using corn- and sorghum-based diets.

Effect Of Monensin On Organic Matter And Starch Digestion

Results have not been as conclusive when examining the effects of ionophore addition on site of OM and starch digestion.

Horton et al. (1980) evaluated the effect of monensin addition and dietary barley level on digestibility in lambs and steers. Monensin was added at two concentrations (0 vs. 33 ppm), and barley levels were set at 30, 50, and 70% of dietary DM for lambs and 30, 50, 70, and 90% for steers. No mention was made of the specific barley variety. Organic matter (OM) digestibility in lambs and steers increased by .30 and .25%, respectively, for each percentage increase in barley level. Monensin increased total tract digestibility of DM and OM in lambs, but not in steers. Ruminal digestibility was not measured.

Muntifering et al. (1980) saw no differences in OM and starch total tract digestion when monensin was added to a 90% corn-based diet. Monensin was added to a corn based diet (90% shelled corn) to examine its effect on ruminal and post-ruminal utilization in abomasally cannulated steers (Muntifering et al., 1981). Monensin decreased ruminal true digestion of OM and ruminal apparent digestion of starch by 19%. However, monensin appeared to have no effect on apparent total tract digestion of OM or starch. The addition of monensin caused more starch to be presented to the small intestine. This starch could be utilized with possibly greater metabolic efficiency and could explain some of the positive effects seen with the addition of monensin to high concentrate diets.

Miller et al. (1986) were interested mainly in monensin effects on B-vitamins, however, these researchers measured ruminal and total tract OM digestibility as well. Steers were fed an 89% corn grain diet. Apparent ruminal OM digestibility was lower for steers fed monensin. There was no effect on total tract digestion of OM with the addition

of monensin. In contrast, monensin did not alter apparent ruminal digestion of OM in a forage-based diet (Beever et al., 1987).

Zinn (1986a) utilized the ionophore salinomycin in a feedlot trial and in a 4 X 4 Latin square experiment. Salinomycin was fed at 4 levels (0, 5.5, 11, and 16.5 mg/kg). Steers in this experiment were fed a 39% steam-flaked corn, 39% steam-flaked barley high concentrate diet. Ruminal OM digestion was reduced by 6.2% for diets containing higher amounts of ionophore (11 and 16.5 mg/kg). There was no effect on ruminal starch digestion. Total tract digestion of OM and starch was not altered by salinomycin supplementation. Zinn (1986b) saw no effect of salinomycin addition on ruminal digestion of OM and starch. Fecal excretion of starch was reduced when salinomycin was supplemented to corn-based diets, but no differences were seen in total tract digestion of OM and starch. Zinn (1987) evaluated two ionophores (lasalocid and monensin) in corn-based diets. Ionophore supplementation depressed total tract digestion of OM, however, there was no effect on starch digestibility. The addition of lasalocid and monensin did not change the ruminal digestion of OM and starch.

More recently, Zinn and Borques (1993) evaluated the effect of monensin and sodium bicarbonate on utilization of a high energy corn-based diet by finishing steers. Sodium bicarbonate did not affect the ruminal or total tract digestion of OM and starch. Monensin reduced ruminal OM digestion, but had no effect on ruminal starch digestion. However, differences in ruminal digestibility were compensated for by an increase in post-ruminal digestion. These researchers saw no effect of monensin supplementation on total tract digestion of OM and starch.

It is clear that ionophores have no consistent effect on OM and starch digestion. No direct comparisons have been made examining the effects of ionophores on corn and barley diets. It is difficult to compare results from corn-based diets and expect the same results to occur in other grain sources. Comparative effects of ionophores on ruminal metabolism in corn and barley diets is lacking.

One study did evaluate the effect of lysocellin on ruminal fermentation and microbial populations from barley- or corn-based diets in continuous culture (Kung et al., 1992). Lysocellin is a divalent polyether antibiotic from *Streptomyces cacaoi* var. *asoensis*. In this study, corn and barley diets contained 65% grain. Specific barley variety used was not mentioned and when asked did not know. There was a starch source (corn vs barley) X lysocellin interaction for apparent and true OM digestion in vitro. Organic matter digestion was increased in corn-based diets, but was reduced in barley-based diets when supplemented with lysocellin. Surber and Bowman (1994) reported a similar interaction between corn and Harrington barley in vitro.

Since most of the research evaluating ionophores has been done with corn-based diets, it is important to examine equally ionophore effects on barley-based diets. Kung et al. (1992) found that corn and barley responded differently to ionophore addition. Barley is grown and used in large quantities in western areas of the United States and Canada. It is important to remember that there are differences between barley varieties regarding fermentation characteristics (Clark et al., 1987; Kemalyan et al., 1989; Boss and Bowman, 1994). Differences in rate of digestion may result in differences in bypass ability of protein and starch. Each barley variety must be considered separately.

Therefore, this study was designed to examine: (1) The effect of grain source and monensin addition on in vitro dry matter digestion of high concentrate diets, and (2) The effect of grain source and monensin addition on rate, site, and extent of digestion of high concentrate diets.

CHAPTER 3

EXPERIMENTAL PROCEDURE

In Vitro Experiment

An in vitro experiment was conducted using a 2 X 4 factorial design, with two levels of monensin addition (0 vs 9 ppm; M- vs. M+) and four grain sources (corn, C; Gunhilde barley, GUN; Harrington barley, HAR; Medallion barley, MED) as the substrates. Gunhilde barley (8.0 kg/hl) is a European feed barley, while Medallion barley (7.8 kg/hl) is a 6-row cultivar developed as a feed barley and genetically related to Steptoe barley. Harrington barley (7.8 kg/hl) is a 2-row malting barley. The corn (9.0 kg/hl) utilized in the trial was a sample taken from grain purchased at a local elevator in Bozeman, MT. Barleys were grown under irrigated conditions at the Southern Agricultural Research Center, Huntley, MT. Corn had the lowest CP value and had the highest starch content of the four grains utilized in the in vitro experiment (Table 2).

Table 2. Nutrient analysis of grains.

Item	CORN	GUN	HAR	MED
Nutrient composition, %				
DM	92.22	94.24	95.13	95.11
OM	98.40	97.39	97.08	97.19
CP	8.69	11.08	10.71	10.64
Starch	64.96	47.71	49.96	44.29

The eight treatments were evaluated using a modified Tilley and Terry in vitro procedure as described by Harris (1970). There were 3 tubes per treatment per hour and one blank tube containing ruminal fluid and buffer per hour. This experiment was replicated three times. Grain sources were ground to pass through a 1-mm screen. One

half gram samples were incubated at 39 °C in tubes capped with bunsen valves containing 30 ml McDougal's buffer (McDougal, 1948), and 7 ml of ruminal fluid. Ruminal fluid was collected and composited from two ruminally cannulated cows fed grass hay and 3.6 kg barley per day. Composited ruminal fluid was strained through 8 layers of cheesecloth. Microbial populations were not adapted to monensin prior to the experiment. McDougal's buffer was prepared both with and without added monensin.

Microbial fermentation was ceased at 0, 3, 6, 9, 12, 18, 24, and 30 h post-inoculation by cold shocking in ice water for 20 minutes. Tubes were centrifuged at 2000 RPM for 15 minutes, supernatant decanted, dried in a forced air oven at a 60 °C for 48 h, and weighed to measure dry matter (DM) disappearance.

Rate and extent of DM digestibility were calculated according to Bowman and Firkins (1993) using the following equations:

$$R = D_0 e^{-k(t-L)} + U, \text{ when } t > L;$$

$$R = D_0 + U, \text{ when } 0 < t < L$$

where R = percentage of DM remaining at time = t, D_0 = potentially digested DM fraction, k = disappearance rate constant, t = time of incubation, L = discrete lag time, and U = indigestible DM fraction. A nonlinear least squares regression method (NLIN) of SAS (1993) was used to estimate disappearance rate and lag time. To obtain initial estimates of D_0 , K, and L, the natural logarithm (ln) of the percentage of DM remaining was plotted as a function of incubation time. These curves were evaluated visually to determine linearity. The curves had one linear component and a visible end point of disappearance, so disappearance rate was calculated by linear regression of the ln of the

potentially digested DM remaining vs. time for all times after visible lag to give the following equation:

$$R = -kt + R_0$$

where R = ln of percentage of potentially digested DM remaining (Y), k = disappearance rate constant, t = time of incubation (X), and R_0 = ln of percentage of potentially digested DM remaining at $t = 0$ (Y intercept).

Data were analyzed as a 2 X 4 factorial, examining the main effects of monensin addition and grain source and their interactions using the General Linear Models (GLM) procedure of SAS (1993). If significant ($P < .10$) interaction effects were found, means were separated using a least significant difference (LSD) test (SAS, 1993).

In Vivo Experiment

A 4 X 4 Latin square designed experiment was conducted using four ruminally and abomasally cannulated steers. The experiment had a 2 X 2 factorial arrangement of treatments testing the main effects of monensin addition (0 vs 270 mg/d monensin; M- vs M+) and grain source (corn vs Medallion barley; C vs BAR). The grain utilized in this experiment were the same as grains used in the in vitro experiment. Steers were limit fed approximately 6.50 kg/d DM of isonitrogenous (11.6%) and isocaloric (1.87 Mcal/kg NE_m , 1.23 Mcal/kg NE_g) high concentrate diets, with half fed at 0800 and the remainder at 1800 h. Water and trace mineralized salt blocks were available free choice. Steers were penned in individual 15 m² stalls bedded with straw, with bedding changed once during each period.

