EFFECTS OF ZINC AND MANGANESE ON DIGESTION, RUMINAL AND BLOOD PARAMETERS OF CATTLE FED PRAIRIE HAY

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CHAPTER I

INTRODUCTION

On the portal of the second millennium we are facing the most advanced technological achievements in the history of humankind. Among the marvels of this century, human beings have reached the potential to live prolonged lives with better quality than ever before. Undoubtedly, this fact represents a valuable asset appreciated by all past and present civilizations on earth.

In part, this potential exists because of the extraordinary understanding of body functions and responses to different situations through the advances of biological sciences such as genetics, biochemistry, physiology, immunology, and endocrinology. This better understanding has led to unprecedented developments of medical and pharmacological tools and practices. There are even more expectations for the future.

Outstanding developments in other areas of knowledge such as architecture and engineering, education, communications, electronics or even leisure also provided advances to enhance the welfare of people. These aspects also contributed to the potential for increased life expectancy and quality. For all human beings to be closer to this potential is a matter of world politics, cultural adaptations and an authentic interest in cooperation from within and outside the nations. Unfortunately, the wonders of progress and scientific achievement of this century have been obscured by the many confrontations, that generated unparalleled miseries and devastation to the world population. This fact may prove that despite all technological advances, human beings were not able to make much progress in the basic behavior of its nature.

Remaining on the positive side of scientific and technological achievements contributing to increase of life expectancy and quality, those related to nutritional aspects have certainly played key role. A significant example of this contribution is the so-called "Green Revolution" conducted at CIMMYT, Mexico, for which, Dr. Norman Borlaug, earned the1970 Nobel Peace Prize. The development of germplasm for high-yielding wheat varieties enabled a quick response in the mid 1960's to the widespread malnutrition and starvation in the Asian Subcontinent. It was characterized by the introduction of modern wheat varieties with a high yield potential of about 7,000 kg/Ha in 1963. Additionally, due to high input production practices, such as the use of pesticides and fertilizers, the wheat yield of these varieties increased to 8,900 kg/Ha in 1990 (Reeves, 1996).

Also remarkable advances in nutrition, genetics, reproduction as well as mechanized and automated tools, have increased the productivity of farm animals during the 20th century. Animal products represent a source of high quality protein in the form of meat, milk and eggs. Although more expensive and less available that primary plant products, they add nutritional quality to the human diet. Thus, today more people are able to consume a more diverse and healthy diet than just 50 years ago. Undoubtedly, this aspect contributed extensively to the increase in human life span and quality.

However, human society learned in the last few years that some of these achievements in agricultural production, besides providing large amounts of food, can be accompanied also by some undesirable effects. In concert, some developments of world agriculture, together with other human activities are contributing to the deterioration of the environment and natural resources. In addition to environmental issues, other

concerns are negative impacts on human health, especially with overconsumption of red meats and fats, and even, animal rights, in the most developed societies (Owens et al., 1998).

In fact, in a world demanding an increased food supply, these issues add complexity to the political and cultural aspects mentioned above, and present a dilemma that may require a solution in the first years of the second millennium. The world presently faces the greatest population explosion ever (i.e., 200 people per minute) with developing countries having the most rapid population growth. It is expected that in 2020 some 50 % of the population of the developing world will live in urban areas. Food quantity and quality must be available, and a production increase of 2.5 % per year in the coming ten years is needed. This represents a major challenge against a background of decreasing grain supply and stocks, declining soil fertility, decreasing ratio of arable land per person, and poor financial assistance (Reeves, 1996).

Every single resource will have to be utilized to its maximum potential in a sustainable manner. New alternatives to minimize the environmental impact of production must be investigated. However, it should be kept in mind that food production is a priority and it should be impossible to reduce to zero the environmental impact and still make the agricultural production biologically and economically feasible.

With increased demand for food grains, grazing lands will have to play a major role for beef production. Particularly low quality resources, which cannot be used for other purposes, but can be effectively transformed into high quality protein by the ruminant animal.

Low quality roughages are high-fiber, low protein content feeds, poorly consumed and digested by ruminants, unless chemically treated and properly supplemented (Streeter and Horn, 1980, Ternrud, 1987). Additional research should be focusing on maximizing low quality diet utilization by beef cattle development of s effective, economic and manageable supplementation programs.

Urea is a concentrated source of nitrogen (N), and contains 45 % N or 281 % crude protein (CP). Ruminants have the ability to transform non-protein N (NPN) to protein by the action of rumen microorganisms. Therefore, they can theoretically use small amounts of urea in the diet to supply all the protein required for digestion and metabolism. However, a number of biological constraints preclude ruminants from efficiently utilizing all the N supplied by urea, particularly with low quality roughages based diets.

Consequently the objective of this research was to test the hypothesis that urea supplemented to beef cattle consuming low quality hay, can be used more efficiently when fed with high levels of zinc (Zn) and/or manganese (Mn), which can lead to changes in rumen fermentation and total diet utilization.

CHAPTER II

REVIEW OF LITERATURE

Micronutrients in Rumen Metabolism and Digestion

Inorganic elements are required by all living beings to perform their normal biological processes (McDowell, 1992). The ruminant animal obtains micronutrients from the diet in conjunction with its major components (carbohydrates, lipids and proteins) that are combined in the complex structures of feeds. However, single minerals or vitamins can also be obtained from supplementary sources. McDowell (1992) lists 24 mineral elements known to be required by various animals, but the NRC (1996) only recognizes 17 as required by beef cattle. Both publications include Zn and Mn, which are the micronutrients of main concern for this study. Many details were discovered about the metabolic roles of these minerals in the animal body. Conversely, much less is know about the role that may play excesses or deficiencies of different minerals in rumen metabolism.

Even, less information is available on potential interactions between minerals and dietary components or additives. For instance, ionophore antibiotics can alter fermentation patterns and nutrient utilization. Reffett- Stabel et al. (1989) reported that steers receiving corn silage and salinomycin or lasalocid had similar weight gains, but both ionophores decreased feed intake and the molar proportion of rumen acetate. Changes in mineral metabolism and absorption were also observed. Both ionophores increased plasma copper (Cu) concentrations, and calcium (Ca) decreased for steers fed

on salinomycin. Control treatments increased urinary excretion and lowered potassium (K) retention. Consequently, feeding salinomycin may decrease ruminant requirements for K and Cu but may increase requirements for Ca (Reffett- Stabel et al, 1989).

Rumen microorganisms depend on minerals for normal growth, but they are also sensitive to high concentrations. Microorganisms can adapt to different mineral concentrations and sources as well. In the case of oxalate consumption, it can cause poisoning, mineral deficiencies or urolithiasis. When ruminants are fed increasing amounts of oxalates, the oxalate degrading microorganisms also increase and they become more tolerant. Thus, rumen bacteria can utilize up to 50 % of the Ca in tropical grasses containing high levels of Ca oxalate (Barry and Blaney, 1987). Rumen physiological parameters can be altered by micronutrient additions, as well as by their interaction with a diversity of dietary components.

Effects on Osmolality

The osmotic pressure of body fluids allows compounds or elements to freely diffuse through permeable membranes. The most important cations of body fluids are sodium (Na^+) and K^+ and the main anions are chloride (Cl^-) and bicarbonate (HCO^-_3, Berne and Levy, 1993). The osmotic pressure or osmolality in the reticulo-rumen is critical to some physiological functions. The major cation playing a role in ruminal fluid is Na^+. It has received a great deal of attention because of its effect on osmolality in the digestive tract, influencing intake and digestion. Particularly, NaCl significantly correlates with rumen osmolality as well as dry matter (DM) intake (Forbes et al, 1992).

Tonicity of ruminal fluid increases during a meal, and this effect can be enhanced by osmotically active compounds such as NaCl, KCl and VFA's. These increases in

osmolality are involved in the regulation of a meal size. The wall of reticulo-rumen is presumed to be a site sensible to changes in osmolality (Carter and Grovum, 1990a and 1990b).

The physiological response to the addition of high Na levels to the diet may depend on the type of feeds used and their relative proportions. Ruminal infusions of NaCl and Na acetate depressed concentrate intake, but did not affect hay intake. However, the same Na compounds infused into the rumen of dairy cows depressed intake of grass silage supplemented with concentrates (Forbes et al, 1992).

High dietary or injected NaCl stimulates water consumption, which may be attributed to an increased concentration of Na in plasma and cerebrospinal fluid (Rundgren et al., 1990). A consequence of increased water intake and osmolality is an enhanced urine output (Godwin and Williams, 1986 and Hamilton and Webster, 1987). Osmolality regulates water intake and the release of arginine-vasopressin, which alters urinary concentration and flow (Rundgren et al., 1990).

A larger urinary output by increased osmolality implies a loss of other mineral and metabolites. Bone loss and Ca excretion increased in sheep receiving high levels of NaCl. Although blood glucose and K levels did not change, K excretion was reduced, and glucose excretion increased (Godwin and Williams, 1986).

Effects on pH

Calcium, magnesium (Mg) and zinc (Zn) provided in amounts 5 to 10 times the recommended levels were found to be closely correlated with decline in rumen pH after feeding (Gralak et al., 1996). When pH is modified it may affect mineral flow from the rumen. Emanuele and Staples (1994) found that when pH varied from 5.6 to 6.8, it

significantly affected the ruminal dispersion of Ca, Mg and P. Also major fermentation products are influenced by rumen pH variation. Mixed rumen bacteria cultures incubated *in vitro* at decreasing pH values from 6.5 to 5.7 diminished their methane and rates of ammonia (NH_3) production, as well as acetate to propionate ratio (Lana et al., 1998). It was shown that K addition to sheep fed a semipurified diet increased rumen pH (Khorasani and Armstrong, 1990).

There are is not much research relative to how trace minerals may affect rumen pH. More likely because of their characteristics and the normal concentrations in saliva and diet, they would not directly affect pH unless some fermentation products can be changed drastically by supplementation.

Effects on Microorganisms and Synthesis of Products

The main microorganisms that are present in the reticulo-rumen are bacteria and protozoa, although more recently, anaerobic fungal zoospores as well as mycoplasmas and bacteriophages have been observed (Yokoyama and Johnson, 1993). Rumen bacteria are reported to have a wide range of sensitivity to rumen characteristics such as pH, redox potential and osmolality, and these differences may affect how microbes compete against each other by changing substrate availability and concentration (Yokoyama and Johnson, 1993).

These changes in microorganism populations and fermentation products such as volatile fatty acids (VFA) and ammonia (NH_3) can be altered when rumen characteristics or nutrient input changes. Hypothetically micronutient additions to the rumen may have either negative or positive effects on microbial activity and metabolism. Several examples in support of this hypothesis are as follows.

Impact on Rumen Microbial Activity. The rumen microorganisms have nutrient requirements themselves (Suttle, 1987). In the case of P, bacterial requirements are mainly met by salivary flow and only extreme depletion can cause a P deficiency to rumen bacteria (Suttle, 1987). In continuous culture experiments, it was determined that the minimum P concentrations required in rumen fluid to sustain maximum microbial activity was 75 to 100 mg/l (Komisarczuk et al., 1987).

High ruminal Na concentrations have different effects on bacteria and protozoa (Carter and Grovum (1990a). Pure cultures of *Selenomonas ruminantium* and *Ruminococcus albus* adapted to Na concentrations higher than 300 mM (Mackie et al. 1984); but, in buffalos supplemented with 200 g/d of salt, total protozoa counts were reduced by about 39 % (Garg and Nangia, 1993).

This effect of Na on rumen microorganisms, appears to alter rumen fermentation patterns and nutrient utilization. Godwin and Williams (1986) and Garg and Nangia (1993) reported reduced concentrations and changes in molar proportions of VFA's with elevated Na inputs. When fed 200 g/d of NaCl, blood glucose in buffalos increased probably due to a larger dilution rate and lower protozoa counts. This would suggest a shift in starch digestion to the small intestine, with greater efficiency of energy utilization (Garg and Nangia, 1993).

Conversely, high NaCl intake can also be associated to a large energy expenditure. Sodium is pumped by the Na^+- K^+ ATP-ase enzyme from the rumen epithelium into the blood stream (Carter and Grovum 1990a). Because this active transport already is a major consumer of ATP (Bertino, 1987), high Na inputs can increase the demand for ATP.

Increasing Na intake with low or high K in the diet affects rumen fermentation products as well as microbial protein synthesis. It was shown that K addition to sheep fed a semipurified diet increased total VFA concentration but did not affect rumen NH_3 levels or molar proportions of individual VFA. Sodium supplementation resulted in linear increase in the amount of microbial DM and microbial N entering the small intestine (Khorasani and Armstrong , 1990).

Mixtures of Ca, Mg, iron (Fe), Mn, and Zn at two different concentrations were tested *in vitro* with rumen liquid coming from sheep receiving different urea levels and consuming low quality oats straw. At least a 20 % reduction in the NH_3 production was achieved by different mineral combinations and concentrations, which suggested a potential for reducing the rate of urea degradation (Rodriguez et al., 1993a). Also Rodriguez et al. (1993b), in a second *in vitro* study reported that the addition of Zn or the mixture of Zn plus Mn inhibited NH_3 production from urea up to 24 and 18 % respectively compared with the control.

Zinc and Mn were supplemented to wethers at 700 and 525 ppm respectively while receiving wheat straw plus a 35 % CP supplement (40 % total N coming from urea). Daily DM intake and digestibility were not significantly affected, but NH_3 production tended to decrease, and N balance was improved linearly by mineral addition (Rodriguez et al., 1995). Steers fed on alfalfa-corn based diets, and supplied with Zn-sequestered polysaccharide or ZnO tended to increase microbial DM production (Kennedy et al., 1994).

Studies with multiple minerals showed effects on ureolytic bacteria. In washed bacterial suspensions ruminal urease was stimulated by several cations including Mn, Mg,

Ca, Sr and Ba (Jones et al., 1964). However, some other mineral elements such as Cu, Zn, and Cd inhibited at low and high concentrations, whereas Sr, Ca, and Co were inhibitory at high concentrations only (Spears and Hatfield, 1975).

Several metal ions and chelating agents inhibit the dipeptidase activity of *Prevotella ruminicola*, which cleaves peptide chains in the rumen. The cations Cu^{2+}, Cr^{2+} and Hg^{2+} were inhibitory, whereas Co^{2+}, Mn^{2+} and Zn^{2+} stimulated activity by 189, 30 and 26%, respectively. The most effective inhibitory chelators were EDTA, TPEN and the stereoisomer 3,4,7,8-tetramethyl-1, 10-phenanthroline (TMP). However, the inhibitory action on dipeptidases is unlikely to be specific to peptide metabolism (Wallace and McKain, 1996).

Interaction with Other Factors in the Rumen. The effects of micronutrients on rumen microbial population could be synergistic with dietary components, intrinsic animal characteristics, or other environmental factors. These potential interactions have not been studied, probably because of their complexity. Therefore, it is worth to point out a few examples of some of the factors that can interact with micronutrients influencing rumen metabolism.

1. Dietary factors. Type of carbohydrate may affect bacterial counts and microbial activity. In dairy cows partial replacement of carbohydrates from barley by beet molasses lowered the number of cellulolytic bacteria. Also, catabolic enzymes in the rumen were affected differently by the substitution of barley by other carbohydrate sources and certain enzymes were closely correlated with ruminal digestibility of some feed fractions. However, no single parameter of feed composition or rumen status could explain all changes in rumen metabolism (Murphy et al., 1993).

Although not a major component in rumen diets, fat level may induce changes in fermentation. A decrease in rumen acetic: propionic acid ratio, total VFAs, and NH_3 concentrations was noted with increasing tallow levels in the diet in lactating cows. Microbial synthesis in the rumen and flow of amino acids (AA) to the duodenum was highest for medium fat intake at the highest feed intake supplied in this experiment (Weisbjerg et al., 1992). In sheep fed lipids, defaunation increased molar percentage of propionate; whereas, butyrate was decreased as well as rumen digestibility of OM and non-NH_3 N flow to intestine. Microbial growth efficiency was almost doubled. The decrease in protozoal counts or even elimination of protozoa after lipid feeding could not entirely explain the changes in rumen metabolism (Van Nevel et al., 1993).

2. Feeding frequency and forage quality. This aspect seems to modify protozoa numbers. After an 8 h period following once daily feeding of alfalfa hay the number of entodiniomorphs declined by 50%, while the number of holotrichs increased by 60%. Protozoa numbers of steers fed low quality hay once a day or those fed either diet at hourly intervals remained stable (O'Kelly and Spiers, 1992).
3. Genetics. Protozoa may account for differences in ruminal metabolism of Bos indicus and Bos taurus. At the same level of intake of different forages, protozoa numbers in Brahaman cattle was higher than in Hereford contributing to quantitative differences in the end products of digestion. Thus, propionic, butyric, isobutyric, and isovaleric acid concentrations in rumen fluid as well as glucose in plasma were higher in Brahman cattle than in Hereford. Fatty acid concentration in rumen contents from protozoal and bacterial population, and of cholesterol and essential fatty acids in plasma were higher in Brahman than in Hereford (O'Kelly and Spiers, 1992).

Non-Ruminal Anaerobic Digestion

Anaerobic digestion studies have also shown similar effects of minerals on bacteria and end product formation. In experiments using biomass from an anaerobic bioreactor fed continuously with ethanol distillery waste as inoculum; added metals inhibited the biotransformation of acetate, propionate and butyrate. The inhibition occurred over a range of increasing mineral concentrations from 20-200 mg/l. Copper (Cu) and chromium (Cr) were the most inhibitory, and the extent of inhibition varied for the different acids. The activities of methanogens were much less affected by the same minerals (Kong et al., 1994).

In another study (Lin, 1993), the addition of Cr, Cadmium (Cd), lead (Pb), Cu, Zn and nickel (Ni) on the production of VFA in anaerobic bacteria was tested. The relative toxicity of heavy metals to production of acetic acid and n-butyric acid was Cu > Zn > Cr > Cd > Pb > Ni and Cu > Zn > CR > Cd > Ni > Pb, respectively. Copper was the most and Pb was the least toxic heavy metals to VFA-producing organisms. In general, the sensitivity of VFA production to mineral inhibition was n-butyric > acetic. Except for Cu-Ni, mixtures, the minerals caused synergistic inhibition on total VFA production. Some mixes markedly increased the production of acetic acid. Although, these situations do not exactly reproduce rumen conditions, similar effects and interactions may take place by addition of micronutrients.

Effects on Digestibility and Voluntary intake

It has long been recognized that digestibility is a critical determination in assessing the nutritional value of a particular diet. The digestion rate and extent of low quality roughages can be modified by specific treatments and N supplementation (Streeter

and Horn., 1980; Ternrud, 1987). In general the greatest effect by N supplementation is improved intake of digestible DM (Judkins et al., 1987; Caton et al., 1988, McCollum and Horn, 1990). Any positive effect of micronutrients on digestion and intake would represent a low cost, manageable tool to increase animal performance on low quality roughages.

Among the major elements, Ca increased from .25 to .40 or 1.11 % of diet DM, reduced OM and starch digestion in the rumen but increased postruminal digestion of these components (Goestch and Owens, 1985). There is extensive recycling of P through saliva. Only complete depletion for 24 hr depressed cellulose digestion (Hubbert et al., 1958). Roughage diets low in P had decreased DM digestibility, but voluntary intake was more related to plasma levels of P (Ternouth and Sevilla, 1990).

The addition of $NaCO_3H$ to result in concentrations of 4,000 μg/ ml of fermentation medium had no effect on cellulose digestion (Hubbert et al., 1958). Also Hubbert et al. (1958) reported that K addition was essential for cellulose digestion. More recently, rumen OM digestion was reported to decrease by K supplementation, and again Na supplementation did not affect rumen OM digestion (Khorasani and Armstrong , 1990).

Some controversy exists on the impact that NaCl may have on diet intake and digestibility. More than 750 mmol/d of NaCl in sheep increased rumen turnover rate, but lowered DM and N digestibility, as well as rumen NH_3 and plasma urea levels. The enhanced rumen turnover rate and poor N efficiency contributed to the decline in digestibility (Godwin and Williams, 1986). According to this data high Na levels may not be compatible with low protein diets. However, in steers consuming bermuda hay *ad*

libitum, plus a corn-salt limited supplement, the intake of NaCl did not affect particulate passage rate or fiber digestion (Galloway et al., 1991). Also, larger water intakes with high NaCl inputs seems to enhance dilution rate and total digesta outflow rate from the rumen (Godwin and Williams , 1986; Garg and Nangia, 1993); which may be detrimental to digestibility.

Sulfur (S) requirements of rumen microorganisms increase with poor quality diets, especially when supplemented by urea (Elliott and Armstrong, 1982). The addition of S from either Na_2SO_4 or methionine increased cellulose digestion *in vitro* (Guardiola et al., 1980), as well as *in vivo* (Guardiola et al., 1983).

Little is known about the effects of trace elements on rumen digestion and voluntary intake. Martinez and Church (1970) reported improved microbial activity after addition of Co, Mn, F,e and Zn. They also evaluated the effect of several microelements added to washed suspensions of rumen microorganisms on cellulose digestion and toxic levels (Table 1).

Other experiments have shown that different trace elements can affect digestion and intake differently under a variety of conditions. Copper and Mo fed to sheep increased *in vitro* cellulose degradation (Ellis et al., 1958). Early studies showed that Zn or Mn additions decreased cellulose digestion *in vitro* (Chamberlain and Borroughs, 1962). In agreement with these findings, wethers supplemented with 0, 3, 6 or 9 g/kg of Mn as MnO decreased feed intake with increasing Mn levels (Black and Ammerman, 1985). These results may appear contradictory with more recently reported data. Steers supplemented with Zn methionine or oxide had increased DM intakes by 5.2 and 4.4% respectively, compared with control treatments (Spears et al., 1991).

Table 1. Effects of microminerals on cellulose digestion by washed suspensions of rumen microorganisms (adapted from Martinez and Church, 1970).

Mineral element	Optimal concentration[1]	Toxic concentration[2]
	ppm	ppm
Boron	0-200	300
Cadmium	1-7	10
Cobalt	1-3	7
Cromium	1-3	10
Copper	0-.1	1
Fluor	0-.05	.5
Iron	2-5	100
Iodine	20	>1,000
Manganese	5-30	100
Molibdenum	10-200	>500
Nickel	0-.1	.5
Selenium	0-.1	7
Zinc	3-10	20

[1] Concentration that supported maximum cellulose digestion.

A probably overlooked aspect by which microelements can affect rumen digestion is by facilitating bacterial attachment to plant cell walls. Copper and Co may act as bridges between bacteria and plant cell walls, allowing a negative charged microorganism to attach to a fiber particle of the same charge by using a divalent cation bridge. Copper and Co sulfates added as 12 and .25 ppm of Cu and Co respectively, to corn cobs-alfalfa or corn crop residue-alfalfa silage diets, tended to increase the rate of DM degradation by rumen microorganisms (Lopez-Guisa and Satter, 1992).

Progressive solubilization of cellulose and hemicellulose in low quality roughages may leave increasing proportions of negatively charged lignin (Agosin et al., 1986). Carboxyl and phenyl groups attached to the structural carbohydrates of digested cell walls would increase the ionic interchange capacity (Villalba et al., 1992). These last two

examples may insert some mineral elements within a different prospective with respect to requirements.

Requirements and Tolerance Levels for Zinc and Manganese

Zinc and Mn requirements can be established at the rumen and at the animal level. Martinez and Church (1970) reported that the optimum concentrations for cellulose degradation by rumen bacteria was 3-10 and 5- 30 ppm, and toxic levels were 20 and 100 ppm for Zn and Mn, respectively (Table 1). The requirements and maximum tolerable concentrations in the diet for the animal as a whole are 30 and 500 ppm for Zn, and 20-40 and 1,000 ppm for Mn (NRC, 1996).

Zinc and Mn are associated with a number of important body metabolic functions. Zinc has a catalytic role for enzymes such as alkaline phosphatase, carbonic anhydrase and nucleotide transferases, a structural function in the cytosolic Cu/Zn superoxide dismutase and more recently found roles of Zn in regulation of gene expression have been reported (Cousins, 1996). Biochemical functions of Mn include: enzyme activator for a number of hydrolases, kinases, decarboxylases and transferases; structural function in metaloenzymes that includes arginase, pyruvate carboxylase and Mn superoxide dismutase (Keen and Zindenberg-Cherr, 1996).

A broader discussion of the general metabolic functions of these elements goes beyond the scope of this review. Nevertheless, some recent findings may provide some additional criteria for evaluating Zn and Mn requirements. Requirements and tolerance levels may not be clearly defined any more, rather they may change dynamically with the interaction of multiple factors.

Immune Response

Zinc and Mn play a role in the immune response of young ruminants. Steers before weaning and their dams were given free-choice mineral supplement Zn methionine + Mn methionine which sustained body weight (BW) change and plasma Zn concentrations of IBRV-stressed steers (Chirase et al., 1994). Antibody titers against bovine herpesvirus-1 tended to be higher in steers supplemented with Zn methionine (Spears et al., 1991).

Calves 2 to 8 weeks old were given 500 mg of Zn, and/or 2,500 mg of Mn daily. The combined administration of Zn and Mn significantly increased percentage of phagocytes and phagocytic index in blood samples examined at for 6 weeks thereafter. Calves that received only Mn showed the highest concentration of alkaline phosphatase (45.18 IU/L; Bednarek and Kondracki, 1994).

Production parameters

For finishing steers receiving high-concentrates low-fiber corn silage-corn based diets, no effect of dietary Zn level and no interactions between protein levels and Zn were found. The authors suggest that a basal level of 24 ppm Zn in the diet is adequate for maximum daily gain and gain:feed ratio (Pond and Oltjen, 1988). In practice feedlot steers typically receive much larger dietary Zn concentrations than those recommended by the NRC (Gaylean, 1996).

No particular requirements of Zn and Mn were found in the literature for diets containing NPN, except for the fact that higher levels of both Zn and Mn were reported to depress ureolytic activity in sheep (Rodriguez et al., 1993a and 1993b).

Zinc and Manganese Sources

Several Zn and Mn inorganic and organic sources can be used as supplements for ruminants (NRC, 1996). The extent to which different sources of these minerals may impact rumen metabolism is not known. As reported in a recent review, some studies found that organic trace minerals increased growth, milk production and reproductive performance probably because of better absorption. However, it was not possible to determine if the response was due to the source or simply due to increased mineral intake (Spears, 1996). Conversely, Zn proteinate was not advantageous over inorganic sources for health of dairy cows in a study reported by Whitaker et al. (1997).

Toxic Effects

Levels of toxicity for several microelements including Zn and Mn were investigated early by Martinez and Church (1970) and discussed above. Other studies include calves given milk replacers containing different Mn or Zn levels varying from 40 to 5,000 ppm, and 40-1,000 ppm for Mn, and Zn respectively (Jenkins and Hidiroglou, 1991). A decrease in weight gain and feed efficiency was observed by supplying 1,000 ppm Mn, while those given 5,000 ppm did not survive. The largest amount of Mn absorbed appeared in liver and bile.

At 700 and 1,000 ppm of Zn, weight gains, DM intake, and feed efficiency were decresed. Largest Zn increases were found in liver, kidney and plasma. Calf performance was not affected by 500 ppm of either Mn or Zn in milk replacer. However, these concentrations are substantially larger than those recommended by NRC (1996). The authors suggest that at these levels toxicity might have arisen if the trial had been continued, since Mn and Zn concentrations increased in some tissues.

No beneficial effects are apparent from feeding these minerals over the NRC recommended levels. However, in adult ruminants, with a developed rumen and different combinations of basal diets and supplements, a different impact of various Zn and Mn levels on digestion parameters and overall performance might be expected.

Protein Supplementation of Low Quality Roughages

Low quality roughages are abundant and can be used for maintenance or support intermediate levels of production when fed to ruminants. About 3,000 million tones of fibrous materials are available yearly worldwide as reviewed by Ternrud (1987). Part of it is used by different industries, but a major amount is discarded. Assuming the discarded material represents only one fifth of the amount produced, then about 200,000,000 cows could theoretically be fed yearly. Low quality roughages have a large proportion of highly lignified cell wall components. Phenylpropanoids associated with lignin, tannins, cutin and silica prevent microbial colonization (Hoover, 1986). The difficult access of microorganisms to the cell wall structure limits its digestion (Wilson and Kennedy, 1996).

Several methods for improving the digestibility of low quality roughages have been described and practiced with different degrees of success (Streeter and Horn, 1980, Ternrud, 1987). Chemical treatments of fibrous forages are the most widely accepted and practically used. Treatment with sodium hydroxide or ammonia promotes changes in the structural components of the cell wall, mainly dissociation of lignin (Ternrud, 1987).

Nitrogen is also limiting in these diets and has to be provided by supplementation. The rate of digestion of fiber components in cell walls depend on

sufficient supply of N and energy for rumen growth of microorganism in gaining accessibility to cell walls and to decrease particle size (Mawuenyegah, 1997). Additive effects of chemical treatment and N supplementation may not always exist. Beef cattle performed similarly when low quality *Eragrostis curvula* hay was fed untreated or treated with NaOH, both with N supplementation (Arelovich et al., 1987). This aspect can make N supplementation more critical than forage treatment when feeding this type of diets.

The role of N supplement for low quality roughage diets has been well defined by Kempton (1982). A feed supplement should maximize utilization of the low cost basal diet, by being palatable, maximizing microbial protein outflow from the rumen, increasing metabolizable energy intake, and efficiency of intestinal absorption. Among other aspects, cattle breeds may exhibit different abilities to digest fibrous components. Dry matter disappearing from nylon bags in the rumen of steers fed low quality roughage was higher in Brahman than in Hereford (O'Kelly and Spiers, 1992).

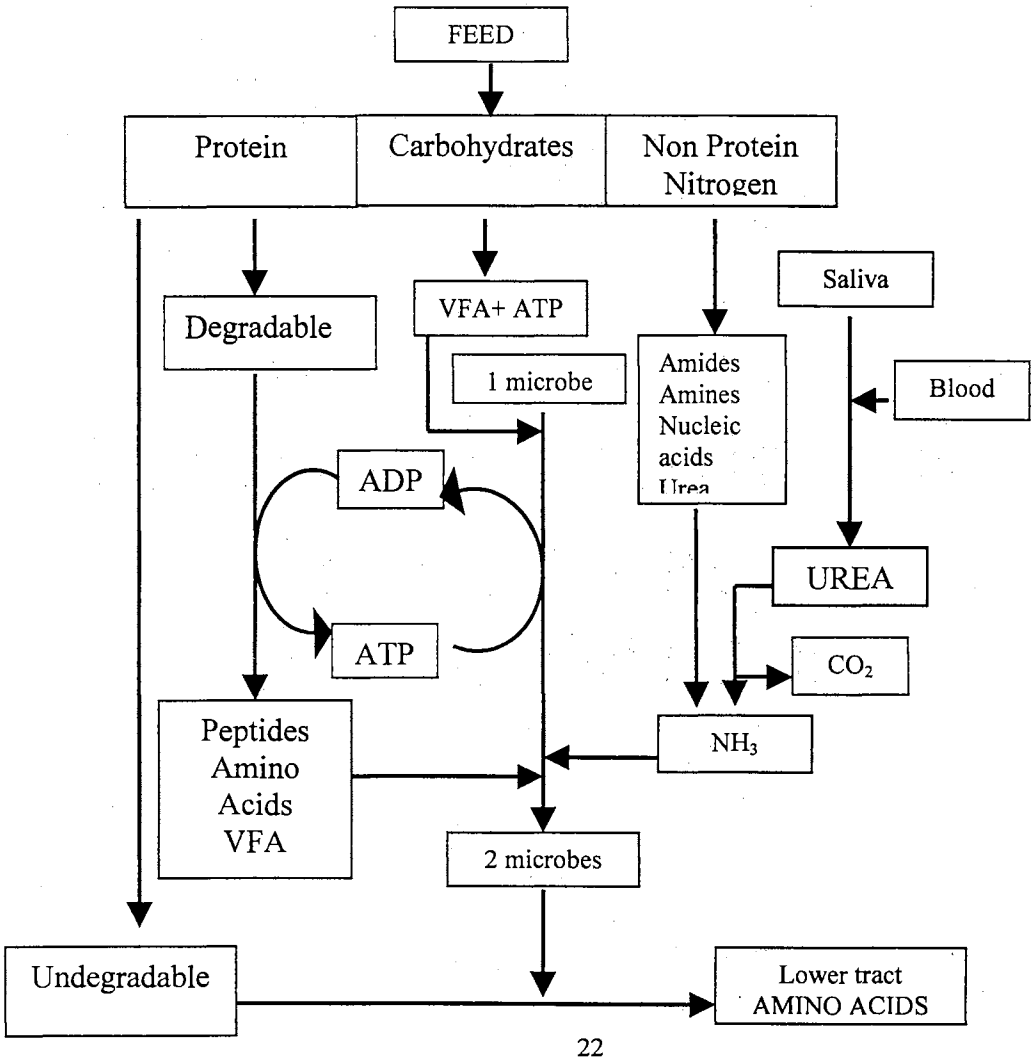
Rumen Metabolism of Dietary Nitrogen Compounds

The major source of protein available to ruminant animals are the rumen microorganisms for the vast majority of dietary conditions (Cotta and Russell, 1982). In fact, microbial protein accounts for more than 50 % of the total duodenal flow (Dijkstra et al., 1998). In turn, rumen microorganisms, satisfy their own N needs from dietary compounds such as soluble and insoluble proteins, nucleic acids and urea (Baldwin, 1995). Cross-feeding of microbial protein in the rumen affects efficiency of utilization of feed substrates and requiring extra energy for resynthesis. Several factors such as bacteriophage infection, substrate excess or deprivation and predation by protozoa,

contribute to microbial recycling which can interact with dietary composition and DM intake in affecting the efficiency of microbial protein synthesis (Dijkstra et al., 1998).

Nitrogen N is also continuously recycled into the rumen from urea entering with saliva and (or) diffusing freely from the blood though the rumen wall (Owens and Zinn, 1993). The latter represent a remarkable survival mechanism particularly with low quality roughages, that have very low N content. Additional changes in N partitioning within the rumen also occur with low quality roughages. Sodium hydroxide-treated wheat straw supported a smaller protozoal population and was rich in rumen-degradable N (Ushida et al., 1986). A general scheme for N metabolism in the rumen is shown in Figure 1.

Figure 1. Degradation, utilization and recycling of nitrogen compounds in the reticulo-rumen (modified from Baldwin, 1995).



This scheme is in agreement with the new suggested expression for protein requirements of cattle (NRC, 1996). The NRC model utilizes metabolizable protein (MP) which represents absorbed protein, separating the requirements into the needs for rumen microorganisms and the needs for the host animal. The system accounts for bacterial crude protein (BCP) synthesis, undegraded intake protein (UIP) and degraded intake protein (DIP).

However, the level of DIP required by rumen microorganisms or UIP required by the host animal varies for different productivity levels or physiological stages and combination of dietary components. The addition of urea to corn based finishing rations made steers gain 6.6 % faster and were 5.4 % more efficient than those fed diets without a readily source of DIP (Shain, et al., 1998).

The BCP synthesis is estimated as 13 g BCP/100 g of total digestible nutrients (TDN) consumed for a variety of situations, but it is not a constant value (NRC, 1996). Conversely, BCP synthesis can be highly variable, especially when forages are the main components of the diet. Minson (1990) concluded from a variety of literary sources, that production of microbial protein ranges from 34-162 g/kg of DM consumed; or microbial CP efficiency ranges from 98-308 g/ kg of dietary OM apparently digested. The lowest values are associated with the less digestible low protein roughages, which led to a poor amino acid supply to the intestine. Dijkstra et al. (1998) used a mechanistic model of rumen fermentation, and reported that the OM required will be affected by the recycling

of microorganisms; thus to synthesize 1 g of microbial protein at 35 % or 75 % recycling requires 4.7 or 12.1 g of OM respectively.

Rumen NH_3 and amino acids are the primary sources of N for growth of microorganisms. Because NH_3 can be provided from inexpensive sources of NPN, there should be an optimum dietary mix of both NPN and true protein to maximize efficiency of synthesis in the rumen (Cotta and Russell, 1982). Other factors may be involved in the efficiency of NPN use as will be discussed later.

Amino acid supplementation may not improve rumen metabolism and animal performance when diets contain more than 10 % CP. Ewes receiving either 10.2% or a 16.2% CP diets fed encapsulated methionine and lysine did not show changes in DM intake, BW gain, or milk yield. However, BW gains and N balance were increased in their nursing lambs (Lynch et al., 1991)

The amino acids required by the ruminant animal can be obtained the from the UIP, DIP fractions or BCP synthesis as shown in Figure 1. The more microbial protein produced in the rumen the less the need for expensive UIP, which means significant savings in feed costs. Besides energy, the amount of BCP produced is also a function of rumen N availability and efficiency of use for microbial growth. Energy and N availability in the rumen are often unbalanced resulting in low effective substrate availability which decrease microbial growth rate. Balancing N and energy inputs into the rumen can increase effective substrate availability. This can be obtained by combining the correct N and energy sources in the diet (Henning, 1990).

Effects of Supplementation on Voluntary Intake and Digestion

The intake of forages containing less than 7 % of CP is limited by N supply (Doyle, 1987). Nitrogen supplementation increases the voluntary intake of these roughages in most cases (Arelovich, 1983; Caton et al., 1988, Hunt et al., 1989, Koster et al., 1996, Stafford et al., 1996). An increased voluntary consumption and consequently an enhanced total intake of digestible energy are likely the major effect that can be expected from N supplementation of low quality roughage diets.

Highly lignified cell wall contents and bulkiness characterize low quality forages. When consumed by ruminants a critical level of rumen fill is reached sooner than with a more digestible diet. Grovum (1993) reported that once the critical fill level is reached in the reticulo-rumen it signals satiety, in combination with other short term and long term physiological mechanisms that regulates voluntary consumption. Then, for low quality roughages rapid rumen distention, reached at low intake levels, is the primary limitation to the efficient utilization of these feed sources.

Any means to increase the rate of breakdown in the reticulo rumen such as improved microbial activity, or rumination time, or chemical treatment of the forages, should enhance the rate of production of small particles and consequently rumen turnover. The intake at which the same distention level is attained is then increased (Grovum, 1993). Therefore, protein supplementation may improve utilization of fibrous materials by increased digestion and intake and increased microbial protein supply to the lower tract (Doyle, 1987). Under these circumstances an overall improved animal performance can be expected.

Feeding level and type of supplement can influence maximum utilization of low quality roughages. Steers consuming to low-quality, tallgrass-prairie forage *ad libitum* were supplemented with moderate to high CP mixtures of sorghum grain-soybean meal, or long-stem alfalfa hay or alfalfa pellets at different intake levels. Forage and digestible DM intake as well as passage rate were increased for all supplemented steers compared with unsupplemented controls. Also, most fermentation variables displayed positive responses to supplementation per se and to increasing amounts of supplements. However, type of supplement and high supplementation levels affected substitution rate (Stafford et al., 1996).

The impact of protein supplementation on digestion patterns, voluntary consumption, and animal performance has been extensively studied worldwide with a large diversity of low quality diets, conditions and types of animals. Therefore a variety of biological responses have been observed as a consequence of changes in feed intake and digestion.

Sheep fed rapeseed meal, sunflower meal, or urea supplements increased the DM intake of a low quality basal diet, and improved weight gain and wool growth. Digestibilities of organic matter and cell wall constituents did not differ much among supplemental treatments. Also six hours after feeding, rumen pH and VFA levels showed few differences among diets (Coombe, 1985).

Smith and Warren (1986) reported that intake of low quality roughage by sheep was increased 67, 49, 46 and 35% after feeding cottonseed meal, soybean meal, or pelleted or rolled lupines, respectively. Wethers fed isoenergetic diets based on grass silage, barley straw and molasses were unsupplemented or supplemented with urea,

soybean meal or soybean meal plus urea. Molar proportions of propionate and butyrate were higher and acetate was lower for the control than the other 3 diets. Apparent digestibilities of DM did not differ significantly across treatments (Yan et al., 1996).

Growth of steers grazing dry pastures supplemented with increasing amounts of pelleted cottonseed (200 to 1000 g/d) was increased, and partially attributed to a greater forage intake (Smith and Warren, 1986). Steers grazing dormant pastures without or with a 45.5% CP supplement exhibited increased forage consumption and digestibility. Also supplemented animals had greater rumen NH_3 concentrations at -3, 0, 1 and 4 h after supplementation and total VFA concentrations 1 h after supplementation. Rumen fluid and particulate passage rates were greater and retention time lower in supplemented than in control steers (Caton et al., 1988). Hunt et al. (1989) found greater DM and NDF intake, NDF and ADF *in situ* disappearance, rumen VFA concentrations and faster passage rate for beef cattle when meadow fescue grass hay (6.6% CP) was supplemented with cottonseed meal. These variables where not affected by frequency of supplementing cottonseed meal every 12, 24 or 48 h.

This selected data indicates that protein supplementation mostly increased intake of the basal diet, probably through increased digestion rate. Extent of digestion is not always improved, but in general VFA and microbial protein supply is enhanced. Other aspects like grazing behavior can also be affected by protein supplementation.

Diet selectivity. It is widely accepted that diet selectivity by ruminants might be partially governed by the composition of the diet. On native range animals may be selecting a diet with the highest N content possible, and protein supplementation may be expected to affect this aspect. Nevertheless, supplementation with cottonseed cake or

ground, pelleted alfalfa hay fed at isonitrogenous levels every other day did not affect selectivity patterns of grasses and forbs by steers when grazing on rangeland (Judkins et al., 1985). However, Coombe and Mulholland (1983) found that urea-supplemented sheep selected diets lower in grain and N content than those on unsuplemented diets. Also in sheep, long term diet selection may be affected by degree of synchrony of energy and protein in the rumen (Kyriazakis and Oldham, 1997).

Interaction with diverse factors. Intake and digestion of the basal diet as well as animal performance responses to N supplementation may be influenced by interactions with basal diet, genetics, type of supplement, CP concentration, and physiological state of the animals being supplemented.

The response to N supplementation seems to be associated with the type of forage and breed. On speargrass, N (6.2 g N/kg OM) plus S supplementation increased intake in Hereford by 24%, but not in Brahman. However, with 7.9 g N/kg of pangola grass OM there was a significant increase in intake by both breeds with the magnitude of the response in Hereford being greater than that in Brahman (42 vs. 15%). Plasma urea concentrations were higher in Brahmans than in Herefords on all diets (Hunter and Siebert, 1985). Studying digestion aspects of a low quality Pangola grass diet, Hunter and Siebert (1986) showed that there was no significant difference between breeds in digestion of OM and cell wall constituents. Also, the flow of non-ammonia N through the abomasum was greater in Hereford than in Brahman steers.

The biological response of some parameters is related to protein concentration of the supplement. Steers fed ammoniated wheat straw plus protein supplements varying from 12 to 31.7 % CP, responded to increased CP concentration by improving DM and

NDF digestibilities, ruminal NH_3-N, and other fermentation characteristics. The same supplements fed to beef cows, improved forage consumption, cow weight, and body condition score with increasing CP concentrations. However, the response was not sufficient to affect subsequent reproduction performance or calf gain (Fike et al., 1995). Sometimes, with low quality forages the benefits achieved with supplementation may not be reflected in some economic traits, particularly reproductive performance.

Furthermore, production objectives and physiological state of the animal influence the design of N supplementation programs. Supplementation with urea and soybean meal increased intake as well as milk production in lactating dairy cows by using high levels of molasses (up to 250 g/kg DM) on grass silage-based diets (Yan et al., 1997). However, when cottonseed meal was used to supplement meadow fescue grass hay for growth of steers no additional energy source was necessary to increase digestible DM consumption and BW gain (Hunt et al., 1989).

The source of protein also impacts basal diet utilization and nutrient supply. The protein of some supplementary sources has lower rumen solubility, bypassing rumen degradation, to deliver amino acids more efficiently directly to the absorption site. For low quality diets, it is critical to sustain rumen supply of NH_3, to maximize microbial synthesis. All protein fed above the level required to satisfy rumen requirements for degradable protein, would be more efficiently utilized by the animal if it is undegraded in the rumen (NRC, 1996).

The requirement for rumen degradable protein is 130 g/kg of OM digested (NRC, 1996). Low quality forages decrease rate of passage, microbial growth rate and feed efficiency, which probably reduces requirements for DIP, and increase the necessity of

using escape protein to meet the requirements for production (Klopfenstein and Blair, 1996).

The solubility and degradability of some proteins are naturally low, such as those of animal origin (NRC, 1996). Degradability of soluble sources of protein can also be decreased by chemical and physical treatments. In sheep fed about 80% oat straw, supplemented with untreated or formaldehyde treated sunflower or rapeseed meals, ruminal degradability of dietary N varied from 74 to 42 %, and rumen NH_3 concentrations were decreased by formaldehyde treatment (Coombe, 1985). Estimation of NH_3 and amino acid production *in vitro* also showed that pelleting of cottonseed meal and soybean meals makes them more resistant to degradation (Smith and Warren, 1986).

Metabolic Effects of Protein Supplementation. McCollum and Horn (1990), suggest that protein supplementation may increase amino acid availability at the intestine and at tissue levels, promoting tissue deposition and increasing availability of glucogenic amino acids. In turn, these glucogenic amino acids would enhance overall energy use and stimulate forage intake.

Intake of low quality forage increased as well as the flow of microbial non-NH_3-N from the abomasum by urea supplementation of sheep (Egan and Doyle , 1985). The authors suggested that the stimulation of intake by urea was more associated with the provision of additional microbial protein for digestion in the intestines, rather than changes in the rate or extent of DM fermentation in the reticulo-rumen. Ruminally protected methionine and lysine fed to dairy cows with a 14 % or an 18 % CP diet increased plasma amino acids in both. However, DM intake and productivity was only increased with the higher CP diet (Piepenbrink et al., 1996).

One of the symptoms of protein deficiency is reduced voluntary consumption (NRC, 1996). A decreased concentration of plasma free amino acids may then signal an intake depression or satiety. However, de Jong (1988) stressed that it is very unlikely that changes in plasma amino acid concentration govern initiation or termination of a meal. The role of plasma amino acids on voluntary consumption is less understood than the effect of protein supplementation on rumen digestion rate in explaining increased voluntary consumption.

Steers were given complete diets containing 10.7, 20.2, 32.5 or 40.0% CP from soybean meal or isolated soybean protein. Increased CP in the diet depressed intake the first days of the experiment but the steers recovered intake level afterwards. The high-protein diets also promoted higher rumen NH_3 and plasma urea values. However significant changes in either total essential and nonessential or most individual amino acids were not found. The depression in feed intake in the early stages on high-protein diets was attributed to high rumen NH_3 content. (Fenderson and Bergen, 1976). When a deficient protein diet is supplemented with N, plasma amino acids seems to increase, as shown by the addition of urea to a semipurified diet. Increased plasma values of aspartic acid, citrulline, glutamic acid, glycine and proline were noted with 76 % of added dietary N was urea (Salem et al., 1973).

Some studies may suggest an association between plasma free AA concentrations and food intake, but such association may also be affected by characteristics such as age or weight. This is the case for lambs of different weights given diets in which the proportion of essential amino acids varied from 120 to 876 g/kg CP. The heavier animals had decreased concentrations of all plasma free essential amino acids. Free amino acid

values of lighter lambs changed in response to the proportion of essential amino acids in the diet. The response in plasma free amino acids to diets with a low proportion of essential amino acids in this study is similar to the plasma free amino acid pattern characteristic of kwashiorkor development in experimental animals (Dove, 1978). Kwashiorkor is induced by a chronic deficiency of protein in the diet, with lack of appetite being an associated symptom.

Non Protein Nitrogen Sources

A distinctive characteristic of the ruminant is that rumen microorganisms can synthesize their own protein from NPN sources. Also, supplementation of low quality roughages with NPN is less expensive than with traditional protein concentrates. The NPN compounds are concentrated sources of N. Nevertheless, the use of NPN usually results in lower animal response than true protein supplements (Arelovich, 1992), which may be attributed absence of amino acids and energy in NPN compounds.

Research has been conducted with N containing organic and inorganic compounds as potential sources of NPN. Among them are included some very unusual organic structures such as the glucopyranosylamines. At least one form of them was degraded less rapidly than urea and soybean meal (Martin et al., 1982). Inorganic sources such ammonium salts have been studied, but the most common forms of NPN for ruminants are organic compounds such as uric acid, biuret and urea.

Uric Acid. Uric acid is a waste product of N metabolism of birds, and is normally excreted into urine. Broiler litter or laying hen wastes are rich in N from uric acid. Although some problems exist related to their processing, handling and feeding, they have been extensively researched and used in diverse feeding programs. One of the major

concerns with uric acid containing products is contamination with pathogens. Leterme et al. (1992) found that sugarbeet pulp ensiled with laying hen excreta, all pathogens except Clostridium were eliminated after ensiling. Another constraint is bulkiness, so they can only be economically used within a geographical region.

Uric acid seems to be less soluble in the rumen than other NPN compounds (Slyter et al., 1968). Jacobs and Leibholz (1977) reported that replacing urea by uric acid in semipurified diets fed to cattle increased the OM flow to the duodenum, from 35.8 to 40. 6 % of total intake. This may not affect animal performance or apply to all feeding conditions. Wethers exhibited a lower OM and crude fiber digestibility when supplemented with laying hen excreta compared witho urea-molasses or soybean meal (Leterme et al., 1992). In the same study, rumen NH_3-N concentration and pH was higher for laying hen excreta; whereas, VFA profiles were similar for all diets. Bacterial-N synthesis was higher for urea, the proportion of duodenal bacterial N was higher for laying hay excreta, and duodenal flow of amino acids was lowest for the uric acid supplement.

Biuret. Biuret is a condensation product of two urea molecules. It has been studied as an alternative to urea (Fonnesbeck et al., 1975). Some other advantages may be that biuret is more palatable and less toxic than urea, but it cost more and is less available in the market than urea (Bartley and Deyoe, 1977).

The slower ruminal degradation of biuret to NH_3 compared with urea, may be an advantage to supplement slow degrading low quality roughages. However, it does not appear to be superior to urea when fed with high concentrate diets (Bartley and Deyoe, 1977).

Other studies indicate that biuret is generally less efficiently utilized than urea (Fonnesbeck et al., 1975). One of the reasons may be that animals may require 3 to 8 weeks to adapt to biuret, and then it has to be consumed regularly to maintain the ruminal activity of biuretase (Bartley and Deyoe, 1977).

Urea. Urea has been the most researched NPN source, and it is still the most commonly used NPN source. The lower price per N unit, market availability, and scientific knowledge of benefits and limitations are the most likely reasons for its widespread use. Because urea utilization is a central topic of this dissertation, its use is discussed more extensively in the following section.

Urea Utilization and Limitations

Urea is very easy to obtain and cost less per unit N when compared with other NPN compounds. Some problems associated with urea supplementation of ruminants are very well known: rapid rumen degradation rate, waste and or toxicity, low palatability, and limited animal productivity.

Urea is rapidly hydrolyzed by the rumen microbial population to NH_3 (Doyle, 1987). Under certain circumstances, the rate of NH_3 release was determined to be 4 times the rate at which it could be assimilated (Bloomfield et al., 1960). Ammonia can also be produced from dietary protein by a variety of bacterial and protozoa proteases (Wallace, 1988). However, NH_3 from urea seems to be released only by urease, a specific Ni dependent enzyme produced by bacteria that lives nearby or attached to the rumen epithelium (Spears and Hatfield, 1978).

Maximum NH_3 production from urea is achieved 1 to 2 hr after urea is ingested. In contrast this peak for natural protein is reached between 3 and 5 h (Owens and Zinn, 1993). Theoretically, this aspect gives an advantage to natural protein over urea, because maximum NH_3 utilization for microbial protein synthesis occurs when synchronized with the rate of release of carbon skeletons from dietary carbohydrates (Doyle, 1987). When low quality roughages are fed, the slow degradation of structural carbohydrates from plant cell walls presents a limitation to maximize NH_3 uptake into microbial protein.

Ammonia is fixed to carbon by the action of two bacterial enzymes, glutamine synthetase (GS) and glutamate dehydrogenase (GDH; Owens and Zinn, 1993). All strains of the ruminal bacteria Prevotella ruminicola possess GDH activity, which is greatest following NH_3-limited growth. In P. bryantii, GDH activity can be attributed to a single protein; whereas P. brevis produces an additional protein or proteins in response to growth with peptides (Wen et al., 1997).

Edwards et al. (1991) studied the regulation of these enzymes in sheep. The addition of 0.005 mole NH_4Cl added to a low N-energy diet resulted in a 6-fold increase in rumen NH_3 concentration and a 17% decrease in GS activity 30 minutes after infusion. The GS activity decreased in a dose dependent manner, suggesting rapid regulation of activity in response to NH_3. No effects were observed after addition of NH_4Cl on GS activity with a high N- energy diet. The GS activity in bacteria from this diet were 5-fold lower than in the low N /low energy diet, whereas NH_3 concentration was 14-fold higher. The activity of GDH was not affected by these treatments.

When rumen NH_3 concentration is low and GS is active, it spends 1 ATP/mole per NH_4^+ fixed, conversely GDH acts at higher NH_3 concentrations without energy

expenses (Owens and Zinn, 1993). Sustaining rumen NH_3 concentrations compatible with the energetically inexpensive fixation pathway by using urea as the main N source is highly unlikely, unless urea degradation rate can be prolonged in time.

Recycling and toxicity

The NH_3 not incorporated into microbial protein diffuses through the rumen wall, and this transfer may vary from 1-13 g/day under diverse dietary circumstances (Cotta and Hespell, 1986). The NH_3 diffusion through rumen epithelium is pH dependent At neutral or basic pH a much larger proportion is in the ionized form which is absorbed less readily (Owens and Zinn, 1993). It is very unlikely that ruminants eating low quality diets supplemented with urea will have ruminal pHs below 6.5, which may moderate rate of ammonia absorption. Nevertheless, the actual NH_3 concentration and the rate at which bacteria capture NH_3 may also affect diffusion.

The NH_3 absorbed through the rumen wall is transported to the liver where it serves as a substrate for urea synthesis. The ruminant has a quiet efficient system of urea-N recycling from body fluids such as saliva and blood. Rumen NH_3 concentrations increase with the N concentration of the supplement. Highest values are observed with larger NPN intakes and this is followed by a similar pattern in blood urea N (Egan and Kellaway, 1971; Schmidt et al., 1973; Arelovich et al., 1992). About 23 to 92 % of plasma urea is recycled to the rumen (Owens and Zinn, 1993). Blood urea concentrations can be effectively increased by reduced kidney clearance particularly in the ruminant (Egan et al., 1986).

Although urea synthesized by the liver is partially excreted into urine, this recycling system represents a critical survival mechanism for ruminants consuming N

deficient diets. The effectiveness of use of recycled N in the rumen depends on urea concentration in blood and saliva, permeability of the rumen wall, and efficiency of capture by rumen microorganisms (Egan et al., 1986). Rumen NH_3 concentration is a major factor in controlling urea transfer through the rumen wall (Egan et al., 1986; Owens and Zinn, 1993). Other control mechanisms include pH at both sides of the wall, a potential boundary layer of CO_2 and NH_3 derived from urea hydrolysis at the epithelium surface, and changes in functional permeability of the wall to urea were also discussed by Egan et al.(1986).

A major limitation to urea utilization is the risk of toxicity that may occur with high urea consumption by non-adapted animals. Rumen concentrations exceeding 100 mg/dL often induce toxicity (Owens and Zinn, 1993). Under these conditions large amounts of NH_3 are absorbed into the blood, the urea cycle in the liver becomes saturated, and NH_3 buildups in blood resulting in toxicity. Symptoms of urea toxicity are described elsewhere.

The implications of NH_3 detoxification to energy and N metabolism of the ruminant are discussed by Lobley et al. (1995). In lambs injected with 25 or 235 μmol/min $^{15}NH_4Cl$ by mesenteric vein for 5 days, portal drained viscera and liver blood flow were not affected by the additional NH_3 loading. However, at the higher rate there was a trend for increased liver O_2 consumption and NH_3-N extraction by the liver accounted for 64-70% of urea-N synthesis, and additional sources of N were apparently required for urea synthesis. Substantial synthesis of [^{15}N] glutamine occurred across the liver, particularly with the greater NH_3 supply and enrichments exceeded considerably those of glutamate. Therefore, ruminants consuming diets with soluble N content, or non-

adapted to urea supplements may increase energy and amino acid requirements to sustain NH_3 detoxification.

Animal performance

Of all biologic variables that may be measured, animal performance variables such as product output and feed efficiency are likely the most crucial responses in determining the usefulness of a nutritional practice. Cows fed a urea-molasses liquid supplement or urea-mineral blocks in early pregnancy, while grazing low quality oat stubble, increased intake and rumen NH_3. However, little benefit on animal performance was found (Coombe and Mulholland, 1989). Unfortunately not all available data on evaluation of urea as a N supplement for ruminants is accompanied by performance data, which sometimes do not correlate very well with other biological data.

The addition of 1% urea to low-quality fescue hay increased *in vitro* cellulose digestion (Guardiola et al., 1980). Certain levels of supplemental DIP seem to improve forage utilization as shown by increasing amounts of sodium caseinate infused into the rumen of cows receiving low-quality tallgrass. This resulted in increased microbial flow to the small intestine, and increased rumen VFA and NH_3 concentrations. Rumen digestion of OM and NDF increased with the addition up to180 g/d supplemental DIP, but the response was variable with greater amounts. Intake of total OM and duodenal flow of N increased quadratically and peaked at 540 g/d supplemental DIP or 11 % supplemental DIP in the diet (Koster et al., 1996).

Egan and Doyle (1985) also reported increased DM intake of low quality roughage and microbial non ammonia- N flow from the abomasum, in sheep supplemented with urea. In another study, supplementation with a urea-mineral block, a

molasses lick or a urea-molasses lick had only small effects on the intake of digestible OM by sheep grazing oat stubble (Coombe and Mulholland, 1983).

Lower concentrations of essential amino acids were found in plasma or liver when urea replaced true protein (Salem et al., 1973). Ammonia, amino acids and peptides are important nutrients for many species of rumen microorganisms (Wallace, 1987). Nevertheless, microbial response to preformed amino acids can only be expected when the energy source is rapidly fermented (Chikunya, 1996).

Amino acids such as leucine, isoleucine and valine are converted to isovalerate, 2-metylbutyrate, and isobutyrate in the rumen, and stimulate growth of fiber digesting organisms (Cotta and Hespell, 1986). Urea supplementation can change the total amount of microbial protein and amino acids reaching the small intestine. If microbial species differ in amino acid composition, and some microorganisms are more affected than others by rumen N availability, then urea supplementation may induce changes in amino acid composition reaching small intestine.

These critical factors may partially explain diminished animal performance when true protein is substituted with urea in protein supplements for fibrous forages. Sources of UIP may also change digestion patterns and basal diet utilization, when replacing urea. The extent of digestion of β-linked glucose (cellulose) and arabinose (part of the hemicellulose fraction) from straw was improved when fish meal replaced urea as the main N source. However, it did not affect efficiency of microbial protein synthesis (Merry et al., 1990). Many examples of improved performance of ruminants receiving true protein supplements than urea are reported in the literature.

Arelovich et al (1992) fed heifers low quality weeping lovegrass hay plus supplements based on oats grain, sunflower meal and urea with increasing urea concentrations. Animal growth was decreased by increased urea feeding, but animals supplemented with urea-oats performed better than control animals receiving only oats.

In a comparison of sheep fed pelleted oat straw supplemented with either rapeseed, sunflower meal or urea, those receiving the true protein supplements consumed 43 % more straw, gained 5 times more weight, and grew 2 times more clean wool than those given urea. Additionally, the N balance for the natural protein diet was a 2.68 g/d versus -3.77 g/d for the urea supplement. Apparent disappeared NDF in the rumen was lower for unsuplemented control and urea than for sunflower or sunflower plus urea. Molar proportions of isobutyrate and isovalerate were also greater for true protein supplements than to urea (Yan et al., 1996). Daily gains of steers offered freely low-quality grass pasture hay supplemented with protein meal were 210 g more than those offered urea, indicating an effect of protein on the efficiency of utilization of absorbed nutrients (Lee et al., 1987).

Despite differences observed when natural protein is compared with urea supplements, urea seems to perform better than other sources of NPN. Bacterial protein synthesis was improved by supplementation with urea and molasses compared with laying hen excreta (Leterme et al., 1992). Additionally, a large proportion of the NH_3 released was not assimilated into protein because of a lack of synchrony with the energy available to promote microbial growth (Wallace, 1988). Provision of readily available carbohydrates should improve urea utilization. No differences in microbial CP synthesis

were found when a diet containing molasses was supplemented with urea or true protein (Yan et al; 1996).

Synthesis of S-containing amino acids is an additional constraint with urea supplements. It was found that with S-urea or cottonseed meal supplementation, steers increased digestible OM intake of spear grass. However, non ammonia-N reached the intestine only when the supplement was cottonseed meal (Hunter and Siebert, 1980).

Palatability and adaptation

The exact reasons for reduced consumption of urea supplements are not very clear. Chalupa et al. (1978) suggested that decreased consumption might be the result of an aversion determined by the association with malaise developed after ingestion of urea. Much more widespread is concept of low "palatability" of urea as sensed by taste and smell. Urea has to be mixed with a palatable carrier to be fed to ruminants.

Supplements containing 2-3 % urea or biuret took about 7 and 21 days for adaptation, respectively. Abrupt switches in N source affected feed intake (Bond et al., 1978). Acceptability of feeds with 5 to 10 % urea seems to be very variable, particularly with urea-molasses blocks or liquid supplements. A large variability in supplement intake among different sheep flocks was reported when the same urea supplements where fed *ad libitum* (Doyle, 1987).

Also, a high variability in individual supplement intake was reported for Friesian cows in early pregnancy while grazing oat stubble supplemented with liquid urea-molasses supplements or urea-mineral blocks. However mean intake of digestible OM was generally increased by supplementation (Coombe and Mulholland, 1989). This variability in intake of urea containing supplements can probably be overcome by feeding

low urea supplements in frequent, small meals. Nevertheless, this is a highly unpractical alternative under production conditions.

Interaction of Urea with Type of Diet

For ruminants receiving moderate or adequate protein diets, additional urea supply does not improve basal diet utilization. Rams fed Italian ryegrass and ladino clover mixed hay (8.2 % CP), were supplemented with oral drenches of urea at 0, 15 and 30% of the dietary N intake. Fiber and OM digestibilities were unaffected by urea supplementation. Rumen NH_3-N and blood urea as well as urinary N excretion was increased with increased urea supplementation in this study, indicating wastage of the N supplemented. Rumen VFA concentrations tended to decrease with urea supplementation (Fujihara, 1993). The imposed restricted hay intakes of the latter experiment probably resulted in a lower energy availability. Supplements of isobutyraldehyde monourea, urea, soybean meal, or rapeseed meal had no effect on N balance, DM digestibility, or energy digestibility in sheep fed 10.4 % CP bromegrass hay (Mathison, 1994).

Recent reports from Kansas State University have shown a beneficial effect of increasing the proportion of supplemental degradable intake protein (DIP) derived from urea on forage intake and digestion. Results indicated that urea can replace up to 40 % of the supplemental DIP without affecting forage intake and digestion in beef cattle consuming low quality forages (Köster et al., 1996).

Potential for Reducing Rumen Ureolysis

Present uses and limitations of urea use were discussed above. Nevertheless, at least theoretically, urea could be used more efficiently and in greater amounts by decreasing its rate of degradation in the rumen and increasing its capture for microbial

protein. Following this hypothesis a variety of alternatives have been evaluated, including alteration of physical or chemical properties of urea, complexing urea with organic or inorganic compounds, synchronization of N and energy availability in the rumen, or decreasing rumen ureolytic activity.

The rate of NH_3 release in the rumen can be controlled by decreasing the activity of rumen urease by the use of specific urease inhibitors or by modification of urea into products that release NH_3 slowly (Makkar and Negi, 1988). The study of ways to improve animal performance by using urea more efficiently has continued, and it is important for countries that do not have other affordable source of protein supplements, as well as developed countries to decrease protein supplementation costs for ruminants.

Treatments for urea compounds. Several urea treatments have been shown to decrease rumen degradation. Lactosyl urea, a low release variant of glucosil ureides, is prepared from a mixture of urea, precipitated whey, and sulfuric acid (McAllan et al., 1975). Slower N-release than urea has also been shown from compounds such as fenilurea or hydroxurea (Mahadevan et al, 1976).

Feeding cows on native range a slow release urea compound, made by coating individual urea prills with linseed meal and talc, resulted in improved palatability and slowed NH_3 release (Forero et al., 1980). Other studies were performed by altering the physical or chemical properties of urea (Huston et al., 1974, Owens et al., 1980, Makkar et al., 1981) . More recently studies with a urea and glucose mixture were performed in dairy cows (Robinson et al., 1991).

Addition of .75 N sulfuric acid to a mix of wheat straw and urea with 70 % moisture was resistant to urease activity. This mix increased DM digestibility by 20 to 30

units above that of untreated wheat straw. The authors suggested that monosaccharides released by acidic autoclaving improved the binding of urea considerably, and the bound N was about 50% degradable in the rumen (Jinderpal and Kaushal, 1991). A boiled urea-molasses mix absorbed onto ground wheat straw (Uromol) and an autoclaved starch and urea mix (Starea) resulted in about 50 % of the urea being resistant to urease activity (Jinderpal and Kaushal, 1993).

Isobutyraldehyde monourea (IBMU; propanal, 2-methyl-monourea) may be a useful slow releasing NPN source. Sheep fed bromegrass hay and supplemented with IBMU had higher N- digestibility (70.2%) as compared with control, urea, soybean meal, or rapeseed meal supplements. Ruminal NH_3 levels were higher before and 8 h after feeding IBMU compared with the other supplements. The sheep that received IBMU also had the highest rumen concentration of isobutyric acid, but lower concentrations of acetic, propionic, and butyric acid than those fed the natural protein supplements (Mathison et al, 1994).

Some of these studies showed inconclusive results or no marked differences with feeding untreated urea. Doyle (1987) stresseed that untreated urea is the most common NPN source until other NPN products are conclusively shown to be utilized more efficiently for microbial protein synthesis to justify major costs.

Synchronizing ammonia and energy release. Rapidly released N from urea supplements requires additional C-chains for efficient microbial protein synthesis. The ratio between energy of rumen fermentable carbohydrates and NPN affects efficiency of urea supplementation with low quality forages. An efficient utilization of these forages depends on an active population of cellulolytic microorganisms within the rumen. The

inhibition of cellulose digestion by dietary starch was attributed to competition between cellulolytic and amylolitic bacteria for nutrients, mainly N, and N supplementation alleviated this inhibition (el-Shazly et al., 1961).

Moreover, the source of starch was noted to affect intake and ruminal parameters (Fulton et al., 1979). Therefore, the rate at which supplementary carbohydrate sources provide C-chains for bacterial protein synthesis may also improve the efficiency of rumen NH_3-N utilization. However, recent data has shown that little improvement in total energy supply should be expected with starch addition within DIP level, and that ruminally available protein was limiting to the utilization of low quality roughages (Jones et al., 1996).

Inhibition of ureolysis by organic compounds. An interesting approach to improving urea utilization has been the inhibition of rumen urease activity. Some compounds inhibiting urease activity such as acetohydroxamic acid (Streeter et al, 1969; Makkar et al., 1980) have been studied. However, rumen bacteria seem to adapt after a period of time. Other complex organic compounds were also reported to inhibit urease activity (Sharma and Skula,1974, Mahdevan et al, 1976, Viogt et al., 1982), with no practical implications in animal performance. Production of specific antibodies by jack-bean urease injection has also been studied as means of decreasing ureolytic activity (Sidhu et al., 1968).

Recently a 25 % NBPT solution was added to supply 0, 6.5, 13, 26, or 52 μg NBPT per *in vitro* tube. Measurements were done on NH_3 and urea concentrations at 0, 10, 30, 60, 120, 240 and 360 minutes. VFA concentrations were determined at 6 h, and digestibility estimated after 48 h incubation based on NDF analysis. NBPT addition

resulted in a decrease in rate of urea hydrolysis and subsequent ammonia release. The acetate to propionate ratio as well as estimated true digestibility was decreased by NBPT addition (Ludden et al., 1998).

Inhibition of ureolysis by minerals. As previously discussed minerals such as Cu, Zn, Cd, Sr, Ca, Co, Mn, Ba and Mg induced inhibition of rumen urease to different extents (Spears and Hatfield, 1978). Copper sulfate decreased intestinal urease activity in swine in a manner similar to Aureomycin (Varel et. al., 1989). Minerals may probably decrease NH_3 release by affecting urease activity, depressing rate of growth of microorganisms (toxicity), or promoting conditioned deficiencies by competing with other essential minerals for incorporation into microbial cells.

Rodriguez et al. (1993a and 1993b), have studied the *in vitro* inhibition of urease activity by several minerals and their combinations at different concentrations. Zinc or Mn plus Zn diminished NH_3-N production by 20 % in rumen liquor from sheep, fed high-urea supplements and low quality roughages. Other *in vivo* experiments showed improved N balance and a trend to increase weight gain, when high urea supplements with added Zn or Mn plus Zn were fed to sheep (Rodriguez et al., 1995). Market accessibility of mineral compounds and low cost due to low dosage, make them an interesting alternative to explore their potential for modification of NH_3 release and other fermentation patterns.

Summary of Literature Review

Some minerals alter rumen fluid osmolality, pH, microbial growth, and fermentation products (such as VFA and NH_3). The effects of micronutrients upon rumen population and environment could be synergistic with dietary components, genetics, or

other factors, which by themselves may influence rumen microbial population and end products.

Minerals may also affect digestibility and voluntary intake. These parameters are critical when low quality roughages are fed to ruminants. Their digestion rate and extent can be modified by N supplementation. The usual effect is improvement of digestible DM intake.

To a certain extent and in some circumstances addition of minerals may help to manipulate rumen fermentation. Any positive effect of micronutrients on the digestion of a diet could represent a low cost, manageable tool to increase animal performance. Particular attention has been given to Zn and Mn, which at concentrations many times higher than those reported as required, may influence on rumen digestion and fermentation products. Additionally, particular aspects of their metabolic roles and beneficial effects on immune response, as well as toxic effects were discussed. Some Zn and Mn effects are expected to influence rumen N metabolism

When ruminants consume low-quality roughages N content is limiting and should be additionally provided by supplementation. The rate of cell wall digestion depends on a sufficient supply of N and energy for growth of rumen microorganisms, accessibility to cell wall, and particle size reduction.

The major source of protein available to the ruminant animal is the rumen microorganisms. In turn, they satisfy their own N needs from dietary compounds such as soluble and insoluble proteins, nucleic acids and urea. Dietary N is degraded to NH_3, amino acids, and peptides. While all of these N compounds are necessary for microbial

protein synthesis, NH_3 constitutes the primary source of N for growth of rumen microorganisms.

Increased voluntary consumption and consequently an enhanced total intake of digestible energy are likely the major effect that can be expected from N supplementation of low quality forages. The mechanism of action is an increase in the rate of breakdown in the reticulo rumen, by improved microbial activity, rumination time, and rate of production of small particles, and consequently increased rumen turnover. These results in enhanced voluntary intake of the basal diet, which is limited primarily by rumen distension.

A distinct characteristic of the ruminant is that rumen microorganisms can synthesize their own protein from NPN. Also, supplementation of low quality roughages with NPN is less expensive than the use of traditional protein concentrates. The NPN sources primary experimented and practically used are uric acid, biuret and urea. Urea is very easy to obtain and cheaper per unit N as compared with other NPN compounds.

The rapid degradation of urea in the rumen generates spikes of NH_3 that can be lost by absorption through the rumen epithelium, transformed back into urea by the liver and excreted into urine. However, the ruminant has an efficient system of urea-N recycling from saliva and blood. This system is particularly important for survival of ruminants under conditions of limited N intake. Rumen NH_3 concentration is a major factor in controlling urea transfer through the rumen wall. Toxicity may occur if ammonia concentration surpasses 100 mg/dl of rumen fluid. Synthesis of urea by the liver increases energetic and amino acid demands by the liver.

Use of urea has usually resulted in decreased animal performance compared with natural or true protein supplements. Effects of urea on animal performance have been variable depending on type of supplement, carrier, urea concentration and type of basal diet. Large variability in supplementary urea consumption by individual animals is attributed to poor acceptability. Also, lower concentrations of essential amino acids appear in plasma or liver when urea in the diet replaces true protein. Amino acids and peptides are important nutrients for many species of rumen microorganisms.

Theoretically, urea could be used more efficiently and in greater amounts by decreasing rumen NH_3 release and improving its incorporation into microbial protein. The rate of NH_3 release in the rumen can be influenced by decreasing rumen urease activity or by conversion of urea into products that release NH_3 slowly. Several minerals have been shown to inhibit rumen ureolysis. Recent studies with sheep proved that Zn or Mn plus Zn decreased the rate of NH_3-N production *in vitro*, improved N balance, and tended to increase weight gain *in vivo*. Market accessibility of minerals, and their low cost related to low dosage, make it attractive to further explore their potential for modification of rumen NH_3 release and other fermentation characteristics in ruminants receiving diets of low quality roughage and urea.

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CHAPTER III

IMPACT OF ADDED ZINC AND MANGANESE ON IN VITRO UREA DEGRADATION AND PRAIRIE HAY DISAPPEARANCE

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ABSTRACT

Rates of disappearance of urea and prairie hay *in vitro* with various zinc (Zn) and manganese (Mn) concentrations were appraised. Five concentrations of Zn (0, 5, 10, 15 and 20 ppm) and two of Mn (0 and 100 ppm) were tested in a triplicated 2 x 5 factorial experiment. Each fermentation tube contained .5 g of prairie hay, 1 ml of Zn and 1 ml of Mn solution (both added as chloride), 1 ml of an urea solution (35g/L), 20 ml of ruminal fluid, and 20 ml of McDougall's artificial saliva. These mineral concentrations equate to daily steer intakes of 0 to 2.62 g Zn, 0 to 13.10 g Mn, and 100 g of urea. Ruminal fluid was obtained from three 540-kg cannulated steers 24 h after the last meal of 7.5 kg of prairie hay plus 500 g of a supplement (89.5% ground corn, 10 % urea, .5 % NaCl). Tubes were incubated at 39°C to measure dry matter (DM) disappearance using the single stage *in vitro* system during a 48-h period. One-ml samples were removed from each tube at 0, 60, 120 and 180 min after incubation began and analyzed colorimetrically for residual urea. Incubations were repeated on three different dates. In vitro DM disappearance of prairie hay was increased by added Mn but decreased linearly with added Zn. Urea concentration decreased over time (38.0, 27.6, 16.8 and 15.2 mg/dl at 0, 60, 120 and 180 min, respectively). Analyzed within specified time intervals, added Zn linearly decreased ureolysis at 60, 120, and 180 min. An interaction with Mn was

apparent at 120 and 180 min. Ureolysis seemed less inhibited by Zn when Mn was added. Results support previous suggestions that an equivalent Zn intake above 600 ppm retard conversion of urea to ammonia *in vitro*.

(Key Words: Zinc, Manganese, Dry matter disappearance, Ureolysis.)

Introduction

In vitro digestibility techniques provide a fast, economical and precise prediction of *in vivo* digestibility in ruminants. Tilley and Terry (1963) developed the most commonly used *in vitro* procedure. Modifications of this technique have been used to improve these predictions, and to study rumen fermentation variables as well.

Examples of such applications can be found in the scientific literature for a wide range of experimental objectives. Some studies included the determination of pH effects on methanogenesis and ammonia (NH_3) production (Lana et al. 1998). Others have examined the influence of various minerals on cellulose digestion by bacteria (Martinez and Church, 1970). The addition of different minerals and concentrations (Spears and Hatfield, 1978, Rodriguez et al., 1993 a and b) and organic compounds (Ludden et al., 1998) on rate and extent of ureolysis were evaluated as well.

Through retarding ureolysis, mineral elements may improve utilization of diets containing urea. A presumed constraint to urea use in ruminant diets is rapid hydrolysis to NH_3 within the rumen; this can decrease N retention and result in toxicity and reduced productivity (Doyle, 1987). Urea could be used more safely and might prove useful in larger amounts if NH_3 release rate in the rumen were prolonged. Many attempts have

been made to reduce rate of ruminal urea degradation rate by complexing urea with a variety of compounds such as oil, carbohydrates, and treatment with formaldehyde or acids (Forero et al., 1980, Makkar and Negi, 1988, Jinderpal and Kaushal, 1993). However, fewer studies report has examined the impact of elevated mineral concentrations on rumen urea utilization.

The objectives of this experiment were: 1) to measure the rate of disappearance of an urea solution incubated with ruminal fluid containing different concentrations of Zn and Mn; and 2) to determine the one stage-*in vitro* dry matter disappearance (IVDMD) of prairie hay incubated with an urea solution at various Zn and Mn concentrations.

Materials and Methods

Rates of disappearance of urea and DM *in vitro* at various Zn and Mn concentrations were appraised by incubating prairie hay with ruminal fluid obtained from steers fitted with rumen cannulas. The experiment started on September 10, 1996 and continued for a 4-month period during which the animals were sampled to obtain rumen fluid.

Ruminal fluid

Ruminal fluid was obtained from three 540-kg cannulated steers that were housed in individual pens. The steers received daily a diet of 7.5 kg of prairie hay plus 500 g of a supplement containing 89.5% ground corn, 10 % urea and .5 % NaCl. The steers were adapted to this diet for 15 days before rumen liquor was collected for the first time.

Between 0800 and 0900, about 750-ml of ruminal fluid was obtained from each steer 24 h after the last meal, for each replication of the experiment. The ruminal fluid of

each steer was filtered through four layers of cheesecloth into a insulated bottle, and transported to the lab. This mixture of fluid from the three steers was used as a source of inoculum.

Assumptions to establish mineral treatments

Maximum concentrations of Zn and Mn to use were 20 and 100 ppm (Martinez and Church, 1970). Equivalent daily intakes were estimated from predictions of rumen capacity and turnover rate, mineral intakes and availability from the hay and the mineral supplements (Table 1).

The potential contributions of Zn and Mn to the rumen contents were estimated from their concentrations in prairie hay (20 and 85 ppm for Zn and Mn respectively (Lusby and Selk, 1994) and their estimated ruminal release. Because the extent of ruminal release for Zn and Mn in prairie hay is unknown, average estimates were used from determinations made in similar species and plant maturity stages (Kabaija and Smith, 1988, Emmanuel and Stapples, 1990). Although absorption through the rumen wall may approach zero, absorption was considered to equal ruminal input via salivary recycling (Owens, 1996). According to values reported by Martinez and Church (1970) the concentrations at which these minerals become toxic to rumen bacteria are about 100 and 300 times higher for Zn and Mn respectively than those supplied by the forage alone. Therefore, for the objectives of this study, the amounts provided by prairie hay were considered negligible and not subtracted from the mineral solutions to be assayed.

Based on these calculations, the maximum *in vitro* mineral concentrations were 20 ppm Zn and 100 ppm Mn, which would be equivalent to supplemental daily dietary intakes of 0 to 2.62 g Zn and 0 to 13.10 g Mn (Table 2). These maximum mineral

concentrations were thought to provide concentrations reported previously to depress activity of ruminal bacteria (Martinez and Church, 1970). Similarly, a urea solution of 35 mg/l, potentially equal to a supplementary intake of 100 g/d, was added to all mineral treatments. The minerals were added as Zn chloride and tetrathydrated Mn chloride, and were equally spaced at five levels between no added minerals and the maximum concentrations.

In a preliminary *in vitro* trial, all possible combinations of five different Zn and five Mn concentrations, ranging from 0 to 20 ppm and 0 to 100 ppm were assayed. These determinations indicated that urea degradation response was greater to Zn than to Mn. Therefore, only two Mn concentrations, 0 and 100 ppm were tested in this experiment. Then, the concentrations of supplemental solutions for Zn0, Zn1, Zn2, Zn3, Zn4 and Zn5 contained 0, 5, 10, 15 and 20 ppm of Zn, and for Mn0 and Mn1 0 and 100 ppm of Mn respectively in a 2 x 5 factorial experiment. These solutions resulted in estimated *in vitro* concentrations of 0, 5, 10, 15 and 20 ppm, and 0 and 100 ppm for Zn and Mn, respectively (Table 2).

In vitro procedure

The method was adapted from Tilley and Terry (1963) procedures. The ruminal fluid was filtered through 4 layers of cheesecloth again and mixed 1:1 with the buffer solution (McDougall's artificial saliva) bubbled in with carbon dioxide and continuously stirred on a hot plate. A separate set of 100-ml polypropylene tubes and 100-ml glass vials were used to measure urea degradation and DM disappearance, respectively.

Each *in vitro* tube containing .5 g of prairie hay, 1 ml of Zn solution, 1 ml of Mn solution, and 1 ml of the urea solution was added with 40 ml of ruminal fluid-buffer mix.

For zero supplemental Zn or Mn, 1-ml of distilled water replaced the mineral solutions. Then, carbon dioxide was bubbled into the containers to maintain an anaerobic environment. Rubber stoppers with Bunsen valves were used to seal the tubes, and a crimper was used to tighten aluminum-rubber caps to the vials. Tubes and vials were placed at 39°C into different water baths. Duplicate tubes were incubated for a single-stage 48-h *in vitro* DM disappearance (IVDMD - Tilley and Terry, 1963). Determination of IVDMD was repeated in three different dates.

For urea analysis, 1-ml aliquots, removed from each vial at 0, 60, 120 and 180 min after incubation began, were transferred into centrifuge tubes and immediately immersed on ice to stop fermentation. These samples were centrifuged at 13,600-x g for about one minute and kept refrigerated for urea analysis. Twenty µl subsamples were analyzed colorimetrically for residual urea using a blood urea-N kit from Sigma Chemical Co. (Crocker, 1967), and a Gilford Response UV-VIS spectrophotometer at a wavelength of 540 nm. The incubations were repeated on three different dates.

Statistical Analysis

The experiment was analyzed statistically as a completely randomized factorial arrangement of treatments. The IVDMD and urea degradation values were analyzed using the GLM procedure of SAS (1990). For IVDMD, the effect of treatment levels for Zn and Mn, and Zn x Mn interaction was included in the model. The term sampling date nested within the interaction Zn x Mn was used as error term to test main effects.

For the urea-N concentration measurements, the design included a split-plot for the different incubation periods in time. The error term to test the time effect and the interactions time x treatment (Zn or Mn) was sampling date nested within the triple

interaction of time x Zn x Mn. Pre-planned orthogonal linear, quadratic and cubic contrasts were used to interpret mineral effects on IVDMD of prairie hay and urea degradation at specific times.

Results and Discussion

Prairie hay dry matter disappearance

The Zn x Mn interaction for IVDMD was not significant (P=. 93). The IVDMD mean values for Zn across Mn levels after 48 h of incubation with ruminal fluid were 44.8, 44.3, 43,8, 43.2 and 40.9 (SE= 1.12) for Zn0, Zn1, Zn2, Zn3 and Zn4 respectively, for a linear decrease ($P < .05$) with added Zn. The IVDMD mean values for Mn averaged across Zn levels were 42.0 and 44.7 (SE= .70) for Mn0 and Mn1, respectively, for an increased ($P < .05$) IVDMD with added Mn. Despite not detecting an interaction, the impact of Mn concentration seemed 11.5 % greater with the highest Zn level. A graphic representation of IVDMD values is shown in Figure 1.

Mineral balances in ruminal fluid may affect substrate digestion by microorganisms *in vitro* as well as *in vivo*. Restricting intake of P (Hubbert et al., 1958, Ternouth and Sevilla, 1990) or K (Hubbert et al., 1958) depressed digestion of organic matter and cellulose respectively. Addition of Ca (Goestch and Owens, 1985), K (Khorasani and Armstrong, 1990) or Na (Godwin and Williams, 1986) decreased organic matter digestion, whereas cellulose degradation was promoted by additions of Cu and Co (Ellis et al., 1958) or S (Guardiola et al., 1980).

In agreement with the results of this study Chamberlain and Borroughs (1962) found that higher amounts of added Zn or Mn decreased cellulose digestion *in vitro*.

Using washed suspensions of rumen microorganisms, they found that maximum cellulose digestion occurred at concentrations of 5 and 15 ppm for Zn and Mn, respectively. Additions of 20 ppm of Zn or 100 ppm of Mn decreased cellulose digestion by 31 and 24 % respectively (Martinez and Church, 1970).

Stimulation of IVDMD with added Mn does not agree with previous results; however, results support the idea that Zn can depress fiber digestion. However Mn addition may partially relieve the depression in IVDMD that occurs at high Zn levels. Different strains of bacteria may be stimulated or inhibited differently by high levels of Zn or Mn addition, which may have altered enzymatic activity.

Urea degradation

Mean urea-N concentration decreased linearly ($P < .01$) over time, being 38.0, 27.6, 16.8 and 15.2 mg/dl (SE = .45) at 0, 60, 120 and 180 min, respectively ($P < .01$). The rate of urea degradation was slowest from 120 to 180 min. Residual urea-N values are shown in Table 2. When analyzed within specified time intervals, added Zn linearly increased residual urea at 60, 120, and 180 min ($P = .08$; .10 and .02, respectively) although interactions with Mn were detected ($P = .03$) at 120 and 180 min. To illustrate the general trends across all treatments, data on urea-N concentrations were transformed into percentages of the initial urea concentration. Percentages of *in vitro* urea lost after 120 minutes incubation period are shown in Figure 2.

Urea disappeared rapidly for the first 120 minutes but less rapidly thereafter. Ureolysis appeared to be inhibited less by Zn when Mn was supplemented; thus, higher urea-N concentrations were found in those samples containing the highest Zn levels, except when combined Mn also was added. Nevertheless, absolute values varied with

sampling dates. Although procedures were standardized with respect to differences in rumen fluid and temperature, small differences in sampling time or agitation of tubes may have contributed to this variability.

The rate of NH_3 release in the rumen can be controlled by decreasing the activity of rumen urease by the use of specific urease inhibitors or by modification of urea into products that release NH_3 slowly (Makkar and Negi, 1988). Minerals such as Cu, Zn, Cd, Sr, Ca, Co, Mn, Ba and Mg seem to inhibit ruminal urease activity (Spears and Hatfield, 1978).

If supplied with urea, these minerals might reduce ruminal urea degradation rate and improve its utilization. A 20 % reduction in the *in vitro* NH_3 production at 3 h was achieved by different mineral combinations and concentrations of Ca, Mg, Fe, Mn and Zn (Rodriguez et al., 1993a). In particular Zn or Zn plus Mn inhibited NH_3 production from urea incubated *in vitro* by 24 and 18 % respectively (Rodriguez et al., 1993b). Similarly, several metal ions (Cu, Cr and Hg) inhibit the dipeptidase activity of *Prevotella ruminicola* (Wallace and McKain, 1996). These results further suggest that enzymatic action can be modified by high mineral concentrations in rumen fluid. Certain types of bacteria may be more susceptible than other types to a specific mineral effect. The ureolytic activity of rumen microorganisms also can be depressed also by certain organic compounds. In an *in vitro* study N-(nbutyl) thiophosphoric triamide decreased linearly the rate of urea hydrolysis and subsequent NH_3 release (Ludden et al., 1998).

Relationship between prairie hay disappearance and ureolysis

Although rate of IVDMD was not related to rate of ureolysis, dry matter disappearance decreased linearly as Zn concentration was increased and tended to increase with Mn. One might expect a depression in intake from this reduction in digestibility. In agreement with these findings, wethers supplemented with Mn at 0, 3, 6 or 9 g/kg as Mn oxide had decreased feed intake when Mn levels were increased (Black and Ammerman, 1985).

The decrease in the rate of urea degradation with added Zn supports previous reports on the effect of Zn concentration on decreased *in vitro* NH_3 accumulation. Added minerals may probably reduce NH_3 release in several ways; these include an effect on enzymatic activity or, by having toxic effects on ruminal microbes, by depressing rate of growth of microorganisms. The latter if acting on fiber digesting microbes, may decrease IVDMD.

Ready accessibility of Zn and Mn salts and its low cost at the dose needed, make them useful to inhibit NH_3 release and alter fermentation patterns of ruminants consuming urea.

Implications

Moderate Zn concentrations (10 to 15 ppm) inhibited urea hydrolysis in ruminal fluid. Thereby, added Zn potentially could increase usefulness of urea by ruminants. However, added Zn may also interact *in vivo* with factors such as ruminal turnover, recycling of urea and microbial adaptation.

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Table 1. Summary of assumptions and calculations used to estimate daily ruminal levels of Zn and Mn in a 500-kg steer consuming prairie hay.

Item	Data	Computed values
Estimated DMI	DMI= 1.5 % of BW; BW= 500 kg	7.5 kg/DM daily
Rumen capacity	[a]RC (%BW)= 18.4-6.21 FI +2.45 $(FI)^2$	73 l
Turnover rate	[a]FPR (%/hr) = 4.12 + .77 CI + 2.32 RI	1.8 times/day
	TFTR (times/day) = FPR*24hr	
Total rumen volume	(73L * 1.8 times/day)	131 l/d
Rumen availability from PH		
Zn	[b]PH= 20 ppm; [c]RA= 8%; (20 ppm* .08)	1.6 ppm
Mn	[b]PH= 85 ppm; [c]RA= 15 %; (85 ppm*.85)	12.8 ppm
Maximum tolerable levels		
Zn	[d]TL= 20 ppm; (20 ppm * 131 l)/ 7.5 kg DM/d	350 ppm
Mn	[d]TL= 100 ppm;(100 ppm * 131 l)/ 7.5 kg DM/d	1,750 ppm

DMI= dry matter intake; BW= body weight; RC= rumen capacity
FPR= fractional passage rate of liquid phase; TFTR= total fractional turnover rate of liquid phase; PH= prairie hay; RA= rumen availability; TL= toxic levels
[a](Owens and Goetsch, 1986)
[b](Lusby and Selk, 1994).
[c](Kabaija and Smith, 1988).
[d](Martinez and Church,1970)

Table 2. *In vitro* concentrations for Zn and Mn and estimated equivalent daily intake in a 500-kg steer.

Added levels[a]		$ZnCl_2$[b]	$MnCl_2$[c]	Final *in vitro* concentration, ppm[d]		Daily intake equivalent g/d[e]	
Zn, ppm	Mn, ppm	g/l	g/l	Zn	Mn	Zn	Mn
0	4300	.00	15.48	0	100	.00	13.10
215	0	.45	.00	5	0	.66	.00
430	0	.90	.00	10	0	1.31	.00
645	0	1.35	.00	15	0	1.96	.00
860	0	1.80	.00	20	0	2.62	.00

[a] Zn and Mn solution concentrations

[b] Anhydrous zinc chloride.

[c] Manganese chloride crystallized with 4 molecules of water.

[d] Excluding endogenous Zn or Mn in the buffered ruminal fluid

[e] Equivalent to total daily intake from the mineral supplement.

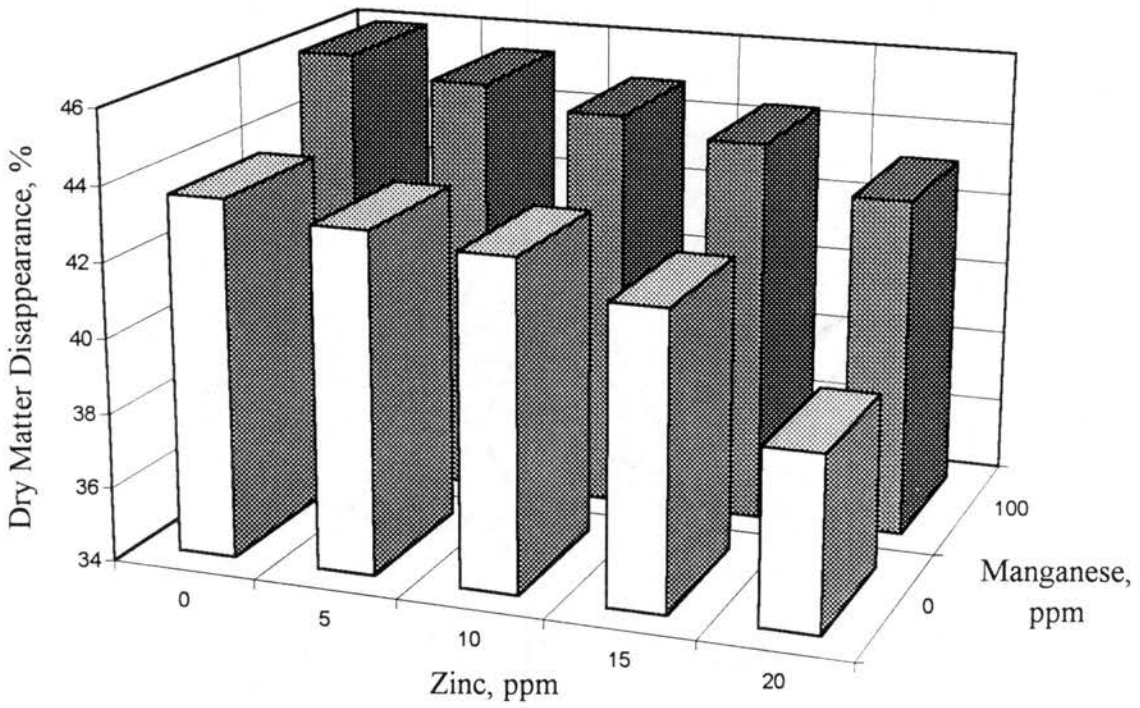

Figure 1. *In vitro* dry matter disappearance of prairie hay after 48-h incubation with different Zn and Mn concentrations. Overall effect of Mn and linear of Zn ($P < .05$, SE= 1.00).

Table 3. *In vitro* urea-N concentration after incubation at 0, 60, 120 and 180 minutes with different Zn and Mn concentrations.

Time (min)[a]		0	60	120	180
Concentration, ppm		Urea-N mg/dl			
Zn	Mn				
0	0	39.09	27.22	15.58	14.77
5	0	40.54	29.53	16.97	15.90
10	0	38.50	28.00	21.17	16.92
15	0	37.41	27.90	18.39	16.42
20	0	36.58	27.84	17.75	15.65
0	100	38.45	27.20	16.08	13.82
5	100	37.47	28.09	15.78	16.01
10	100	38.55	27.41	16.67	14.46
15	100	35.55	26.66	15.73	14.21
20	100	37.45	25.84	14.21	13.41

[a] Sampling time after incubation ($P < .01$)

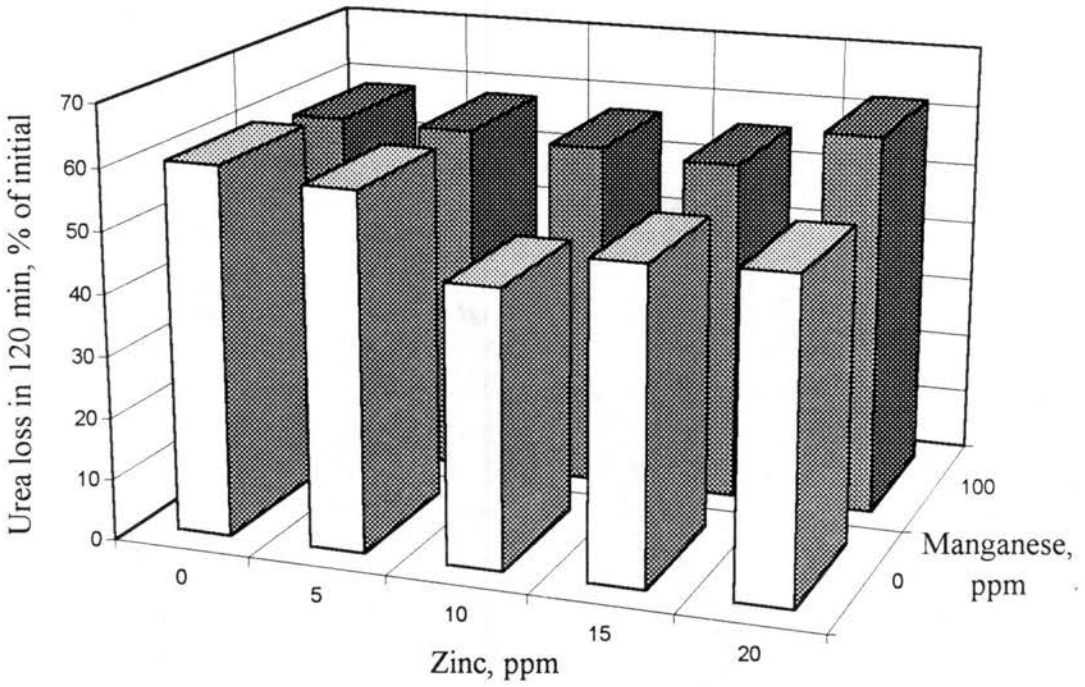

Figure 2. Percentage *in vitro* urea loss at two hours with various concentrations of Zn and Mn.

CHAPTER IV

EFFECT OF ZINC SUPPLEMENTATION ON DIETARY DRY MATTER UTILIZATION AND RUMEN METABOLISM IN BEEF CATTLE FED LOW QUALITY PRAIRIE HAY

H.M. Arelovich, F.N. Owens, G. W. Horn, and J.A. Vizcarra

ABSTRACT

Six 363-kg ruminally cannulated heifers were fed prairie hay (PHAY) and one of two levels of urea in two simultaneous 3x3 Latin squares with one square at each urea level (45 and 90 g urea/animal daily); Zn levels were assigned within each square. The basal diet, PHAY (5.1 % crude protein), was fed at fixed level of 4.8 kg of DM split in two daily meals. Zinc chloride provided the dietary equivalent of 30 ppm (Zn1- dietary requirement), 250 ppm (Zn2) or 470 ppm (Zn3) of Zn. Also, Mn chloride was added to provide a dietary concentration of 40 ppm of Mn. The urea-mineral supplements were dosed via cannula once daily. After 7 days of adaptation, the rumen was sampled 2, 4, 6, 12, 18, 21 and 24 h after feeding the supplement on day 8; ad lib intake of PHAY was measured on days 9-16. PHAY intake averaged 4.9 kg/animal daily. Either urea or Zn levels did not affect PHAY intake and digestibility. There was a trend (P = .18) to decrease digestible DM intake at the highest Zn level. The rumen mineral concentration of several elements was affected by sampling period, but not by supplemental treatments. High concentrations of Zn and Mn reflected their dietary addition. Ruminal pH and NH_3 response to urea and Zn levels differed with time, with Zn x time interactions for both pH (P = .049) and NH_3 (P = .066). In general, pH was lower from 12-24 h than during the 2-6

h (6.68 vs 6.98; P < .01). Concentrations of NH_3, highest for all treatments at 2 h (56, 43 and 35 mg/dl for ZN1, ZN2 and ZN3), decreased (P < .01) with added Zn. This may reduce toxicity. Rumen NH_3 concentrations decreased over time, being higher from 2-6 h than 12- 24 h (P < .01). The Zn x time interaction was best described by a cubic regression on time (P = .10, R^2 = .98). The regression study showed that NH_3 concentrations over time differed between ZN1 vs. ZN3 (P = .039). Ammonia concentrations fell below 5 mg/dl sooner with ZN1 than ZN2 (8.9 vs. 13.4-h postfeeding). Neither urea nor Zn significantly affected intake or digestibility, but digestible DM intake tended to be reduced at the highest Zn level. In response to zinc additions, molar proportions of propionate of 12.96, 15.50 and 16.87, increased (P < .01); and acetate: propionate ratios of 4.71, 3.68 and 3.83 decreased (P = .0001) in a quadratic manner for Zn1, Zn2 and Zn3 respectively. Prolonging the time period with adequate NH_3 concentrations and increased propionate values should favor Zn supplementation.
Key Words: Urea, Zinc, Cattle.

Introduction

Urea is an economical source of non-protein N for ruminants, and is widely used because of its convenience, availability, and low cost. Although urea is frequently used in high concentrate feedlot rations, its potential for increased use should be greater for poor quality, low protein diets. Supplementing increasing levels of urea from 0 to 65 g to beef heifers consuming a low quality hay increased basal diet intake and rumen ammonia (NH_3) which improved microbial protein synthesis (Bowen et al., 1998). Overall,

increasing microbial protein yield in the rumen should increase digestibility and feed intake, but the efficiency of urea utilization for this purpose needs to be improved.

When urea is converted to NH_3 in the rumen too rapidly, its incorporation into microbial protein is inefficient; and excess NH_3 is absorbed and partially wasted through urea excretion. An excess of NH_3 in the blood can also cause toxicity to the animal. Additionally, a shortage of carbon skeletons to capture the NH_3 released from urea limits microbial protein synthesis (Johnson, 1976; Mizwicki et al., 1980), as may occur with low quality forages.

Decreasing the rate of ammonia release and avoiding spikes of NH_3 in the rumen may improve the usefulness of urea. Several minerals can influence NH_3 production rate. One study showed that Cu, Zn, Cd, Sr, Ca, Co, Mn, Ba and Mg inhibit ruminal urease activity *in vitro* (Spears and Hatfield, 1978). Additionally, supplementing with 860 ppm Zn significantly decreased rate of NH_3 production (Arelovich et al., 1997). High levels of Zn or Zn plus Mn have improved urea utilization, N balance, and weight gain of sheep (Rodriguez et al., 1995). The objective of this study was to assess the effect of supplemental Zn and urea on ruminal metabolism and dietary utilization of prairie hay.

Materials and Methods

Animals and treatments

Six 363-kg ruminally cannulated heifers were randomly allocated to individual pens. The animals were fed 2% dry matter (DM) of their average body weight (BW) from medium quality (5.1 % crude protein) chopped prairie hay (PHAY). Once the maximum hay consumption was reached for each heifer, they received only a fixed level of 4.8-kg

average of DM, which was 10 to 20 % below their previous average consumption. The PHAY was split in two daily meals given at about 0900 and 0500 h. The animals were randomly allotted to two supplemental levels of urea 45 (U1) or 90 g (U2) /day, plus Zn chloride to result in dietary concentrations of Zn of 30 (Zn1), 250 (Zn2) or 470 (Zn3) ppm in a 2 x 3 factorial arrangement of treatments. Additionally, Mn chloride was added to result in a dietary concentration of 40 ppm of Mn. The Zn levels were designed to meet the Zn requirements (Zn1) suggested by NRC (1996), provide a level estimated to be the maximum tolerated (ZN3) estimated from a previous *in vitro* experiment (Arelovich et al., 1997), and one intermediate equally spaced level (ZN2). The supplements were prepared as single doses of urea, zinc chloride and manganese chloride and inserted through the rumen cannula with the morning feeding. The experimental design was two simultaneous 3 x 3 Latin squares, with one urea level per square and the zinc levels distributed within each square.

Sampling procedures and determinations

Each period of the Latin square was divided into adaptation (day 1 through 7), rumen sampling at 2, 4, 6, 12, 18, 21 and 24 h (day 8), and PHAY consumption and digestibility (days 9 through 16). Immediately after rumen sampling and for the remaining portion of each period all animals received the PHAY *ad libitum*.

Rumen fluid was obtained through the rumen cannula of each heifer and filtered through four layers of cheesecloth at the specified time intervals. The pH of ruminal fluid was measured immediately after sampling. A 100-ml subsample was acidified with 2 ml of a 20 % v/v sulfuric acid solution and frozen. When thawed later, it was centrifuged for

15 min at 16,000-x g and analyzed for mineral content, ammonia-N (NH_3), and volatile fatty acid (VFA) concentration.

Prairie hay, orts, and fecal grab samples were collected, composited within animal and period and dried at 55°C. After drying, all samples were ground through a 2-mm screen in a Wiley mill (Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA) and stored for DM determination. Dry matter intake (DMI) was computed by subtracting dry orts weight from offered PHAY. Dry matter digestibility (DMD) was calculated by using lignin as the marker, and digestible DMI (DDMI) was calculated for each animal from DMI and DMD values.

Laboratory analyses.

Rumen fluid samples were analyzed for Ca, P, Mg, Na, K, S, Zn, Mn, Cu, Fe, Cr, Co, Se and Mo using an Inductively Coupled Plasma Spectrometer (Spectroflame FTM-08, Spectro Analytical Instruments, and Fitchburg, MA). Stock solution standards (High Purity Standards Co., Charleston, SC) were independently diluted with 5 % v/v nitric acid (Fisher Scientific-trace mineral grade) to suitable ranges for each element of concern. A minimum four-point regression comprised the instrument calibration.

For rumen NH_3-N concentration, 50 µl aliquots were analyzed colorimetrically at 630 nm using a Beckman spectrophotometer (Beckman, DU 64, Beckman Instruments, Inc, Fullerton, CA), following the procedure of Broderick and Kang (1980). Rumen fluid VFA content was determined by using a Perkin-Elmer Autosystem gas chromatograph (Perkin-Elmer 9000 Model Series, Norwalk, CN), equipped with a Megabore DB-FFAP liquid phase column that used helium as a carrier, and 2-ethylbutyric acid as internal

standard. Samples of PHAY, orts and feces were analyzed for acid-detergent lignin (Goering and Van Soest, 1970) and gravimetrically for DM content.

Statistical analysis

Analysis of variance was performed using the GLM procedure of SAS (1990). Treatment effects were tested on DMI, DMD and DDMI using the residual error. Rumen pH, minerals, NH_3-N and VFAs data were treated as repeated measures and tested for the effects of urea level using animal nested in urea level as the error term. Effects of Zn levels were evaluated by using the interaction animal x period x Zn treatment as the error term. Means were separated by least significant differences when a significant treatment effect was detected. Also pre-planned orthogonal contrasts were used to compare treatment means for the early (2-6 h) vs. the late (12-24 h) periods after urea dosing. A regression study with dummy variables was also performed to evaluate the effects of Zn level on NH_3 concentrations over time. Linear and quadratic contrasts were used to interpret Zn effects on VFA concentrations.

Results and Discussion

Prairie hay utilization

Average DMI by animals given free choice access to feed was 4.94-kg daily or 1.36 % of the mean BW. This level of consumption was lower than expected and only slightly over the 4.75 kg of DM fed in the restricted intake phase of each period. Neither urea nor Zn levels significantly affected DMI, DMD or DDMI (Table 1). However, DDMI expressed as g/ kg of metabolic weight ($BW^{.75}$) at the highest Zn level tended to be reduced ($P = .18$) as a result of slightly depressed digestibility values.

The activity of rumen microbes may be reduced at high Zn concentrations. Twenty to 30 ppm of added Zn resulted in significant decrease of *in vitro* cellulose digestion (Martinez and Church, 1970). In a previous *in vitro* study DM disappearance of prairie hay decreased linearly with added Zn (Arelovich et al., 1997), but increased with added Mn. The omission of Mn from a rumen fluid fermentation medium was found to depress cellulose digestion (Chamberlain and Borroughs, 1962). In this study, Mn chloride was added to all treatments at the level suggested by NRC (1996). Although the effect of Mn was not measurable and the level supplied was much lower than that used in the previous *in vitro* study (Arelovich et al., 1997), it may partially alleviate the impact of high Zn levels on rumen digestion of PHAY. This decrease in fiber digestion reduces feed consumption. Reduced feed consumption for sheep should be expected with high dietary levels of Zn (Puls, 1990).

Ruminal fluid parameters

Mineral concentrations of ruminal fluid were not affected by urea or Zn addition ($P > .10$). However the Zn x time interaction was significant ($P = .06$). Figure 1 illustrates that Zn concentrations were initially higher for Zn2 and Zn3 and tended to remain higher over time as compared with Zn1. Concentrations of Ca, P, Mg, Na, K, S, Mn, Cu, Fe and Cr varied with time ($P < .05$). Concentrations of Se, Co and Mo were very low and remained beyond 2.1, 1.7 and 35 ppb, which are the detection limits of the instrument. When Ca, Mg, Na, K and Cu were analyzed by time intervals there were no differences in the least square means. All tended to have the lowest concentrations at 2 h except for Cu, which exhibited the largest variability over time (Table 2). Phosphorus, S, Fe and Cr were present in lowest concentrations at 2 h but increased later ($P < .05$). The lower initial

mineral concentrations may be explained by a larger proportion of undigested PHAY early in the morning accompanied by less nutrient release. Manganese concentrations were about 2.5 times higher at 2 h ($P < .05$) compared with the other time intervals. This supports the inclusion of Mn chloride in the Zn-urea supplement.

Except for the added Zn and Mn, rumen fluid concentrations of other minerals may be influenced by other factors than Zn or Mn addition. Dietary bioavailability, microbial utilization, absorption through the rumen wall (Mg), rate of passage to the lower tract and recycling via saliva would all contribute to changes in mineral concentration. The solubility of minerals from forages into ruminal contents depends on plant maturity (Kabaija and Smith, 1988), the mineral itself, the fraction of the plant to which is associated, rumen pH, and type of forage (Emanuele and Staples, 1990 and 1994). All these factors will influence mineral concentration in rumen fluid.

Interactions of sampling period with both urea and Zn level were found for pH ($P < .01$ and $P = .08$) and NH_3 concentration ($P < .01$ and $P = .07$). The average pH and NH_3 concentration for different time intervals by urea and Zn levels are reported in Tables 3 and 4, respectively. When analyzed within time intervals, pH decreased by Zn addition at 2 h ($P < .05$). Also, pH was higher during the early (2-6 h) than the later (12-24 h) period (6.98 vs. 6.68; $P < .01$) after dosing urea.

This pH drop probably did not affect the rumen environment enough to decrease in cellulose and fiber digestion. The activity of cellulolytic bacteria is inhibited when rumen pH falls below 6.0 (Owens and Goestch, 1993). The high pH values probably reflect the basic nature of NH_3 liberated from urea.

Concentrations of NH_3 (Table 4), were decreased ($P < .01$) by added Zn 2 h after feeding (56, 43 and 35 mg/dl for Zn1, Zn2 and Zn3, respectively). Rumen NH_3 concentrations decreased similarly for all Zn treatments over time being higher from 2-6 h than 12- 24 h ($P < .01$). The Zn x time interaction was best described by a cubic regression as shown in Figure 2. Regression analysis revealed a significant difference in the NH_3 concentration pattern between Zn1 and Zn3 ($P = .04$). Rumen NH_3 is primary source of N for rumen microorganisms (Cotta and Russell, 1982). A minimum of 5 mg/dl is suggested to be needed for optimal activity of ruminal bacteria (Satter and Slyter, 1974), concentrations fell below this level sooner with Zn1 than Zn3 (8.9 vs. 13.7 h).

Zinc concentrations of 130 to 1,300 ppm were shown to decrease NH_3-N release *in vitro* by inhibiting urease activity in sheep ruminal fluid (Spears and Hatfield, 1978). Other studies have also shown that Zn addition decreased rumen NH_3 production when low-quality roughages were supplemented with urea (Rodriguez et al., 1993; Arelovich et al., 1996). These results favored Zn supplements, and should improve efficiency of urea utilization and decrease the risk of toxicity, particularly when large amounts of urea are fed. Additionally decreasing the NH_3 releasing rate would better synchronize with carbohydrate fermentation to improve microbial protein synthesis (Johnson, 1976; Mizwicki et al., 1980)

The effect of urea level on VFA molar proportions, acetate: propionate ratio, and total VFA concentrations was not significant. Molar proportions of VFAs and total VFA concentrations are reported for the different time intervals in Table 5. The mean molar proportions of propionate were 12.96, 15.50 and 16.87, and the acetate: propionate ratios were 4.71, 3.68 and 3.83 for Zn1, Zn2 and Zn3, respectively. Zinc levels affected the

molar proportions of propionate (P = .03) as well as the acetate: propionate ratio (P = .03). Sampling time also affected molar proportions of acetate, propionate, isobutyrate and acetate: propionate ratio (P = .04). For the molar proportion of valerate there was an interaction time x Zn (P = .02). Higher concentrations of NH_3, early after the supplement was fed, were followed by the greater total VFA production at 2 h, which may have influenced the significant pH drop at 2 h.

The effect of Zn levels on molar proportion of propionate and acetate: propionate ratio are further described in Figures 3 and 4, respectively. Zinc addition resulted in linear (P = .01) and quadratic (P = .02) increases in propionate concentration. Least significant differences showed that propionate in Zn1 were lower than in the other two Zn levels (P < .05). Responses of acetate: propionate ratio to Zn addition were also linear (P = .02) and quadratic (P < .01), being larger for Zn1 vs. Zn2 or Zn3 (P < .05).

Other compounds such as the ionophore antibiotics monensin, lasalocid and salynomicin also change VFA molar proportions. These compounds inhibit gram-negative bacteria such as the hydrogen producer's Ruminococcus and Butyribivrio genera (Van Soest, 1982), and stimulate propionate production, reducing acetate and butyrate, as well as methanogens (Van Nevel and Demeyer, 1988). An inhibitor of urease activity, N-(n-butyl) thiophosphoric triamide, was also found to decrease the acetate: propionate ratio after 6 h of in vitro incubation with ruminal fluid (Ludden et al., 1998). However, we are not aware of any previous reports on high levels of Zn modifying rumen fermentation patterns.

It appears that high concentrations of added Zn in the rumen do not act selectively on the growth or the metabolic activity of a single group of microbes. It may depress the

activity of fibrolytic and ureolytic bacteria, but may stimulate propionic producing bacteria. Thus, the trend to decrease digestibility and intake may be balanced by a more efficient rumen utilization of the PHAY, by increasing propionate production. These effects would depend on Zn concentration and may interact with the type of diet. If a prolonged rate of NH_3 release from urea is not coupled with availability of carbon skeletons, then microbial protein synthesis may not increase and the advantage of slower NH_3 release can be partially offset.

Implications

By providing ruminants with Zn above concentrations of 40 ppm of the dry matter, the rate of release of ammonia from urea was decreased; thus, improving potential of urea utilization. Above 40 ppm of added Zn, ruminal propionate and acetate: propionate ratio decreases, which will improve overall feed efficiency. Further the decrease in acetate: propionate ratio may decrease methane produced, and thus, have a positive environmental impact.

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Table 1. Daily prairie hay dry matter (DM) intake and digestibility in heifers receiving different levels of urea and Zn.

Item	Urea level[a]			Zinc level[b]			
	U1	U2	SE	Zn 1	Zn 2	Zn 3	SE
DM intake, kg	4.89	4.99	.22	5.26	4.63	4.92	.27
DM intake, g/BW$^{.75}$	57.32	58.83	2.59	61.45	54.64	58.14	3.18
DM digestibility, %	47.11	46.49	2.06	48.48	49.11	42.82	2.53
Digestible DM intake, kg/d	2.30	2.26	.14	2.56	2.26	2.02	.17
Digestible DM intake, g/BW$^{.75}$	26.7	27.0	1.52	29.89	26.66	24.28	1.86

[a] Urea levels 45 g (U1) or 90 g (U2) /animal daily)

[b] Zn levels 30 ppm (Zn1), 250 ppm (Zn2) or 470 ppm (Zn3)

No significant effect of urea or zinc level (P> .10).

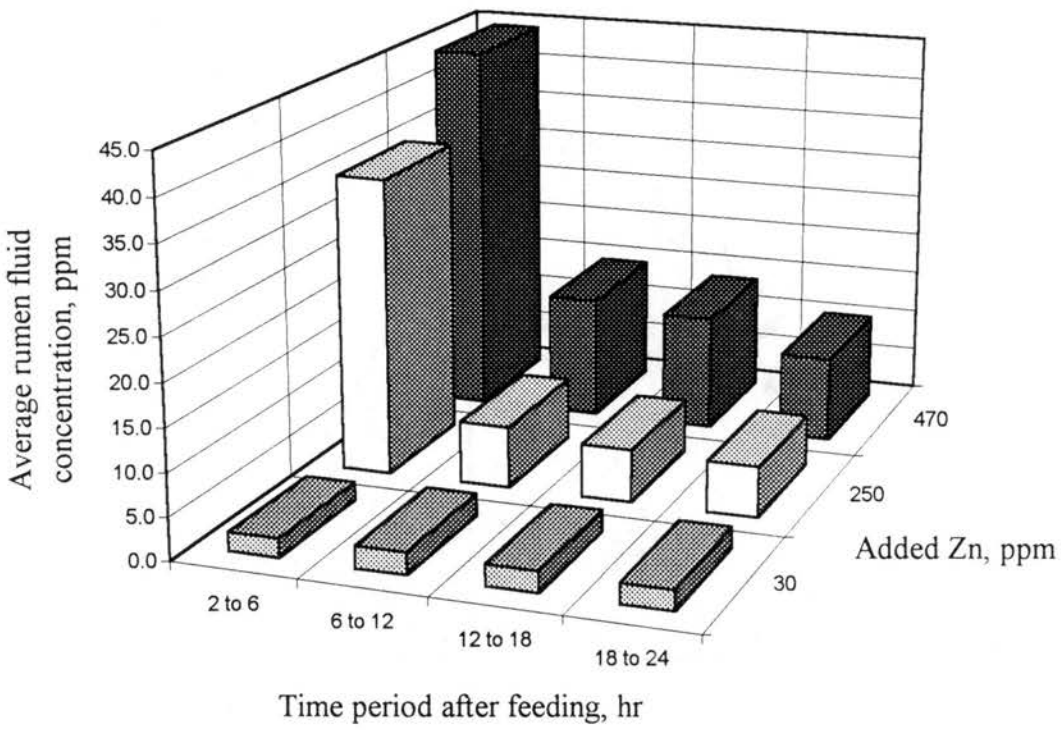

Figure 1. Average ruminal fluid concentration of zinc at different time intervals after addition of zinc chloride through the rumen cannula.
Time x zinc (P = .0602)

Table 2. Least square means across urea and zinc levels for mineral concentrations in ruminal fluid at different time periods in beef heifers consuming prairie hay.

Item	Time, h[1]							SEM
	2	4	6	12	18	21	24	
Calcium, ppm	167.3	155.2	192.1	201.6	174.5	191.2	198.4	7.7
Phosphorus, ppm	189.5^{a}	209.5^{ab}	208.3^{ab}	227.2^{ab}	255.9^{bc}	263.3^{c}	251.1^{bc}	7.7
Magnesium, ppm	45.6	46.1	60.1	58.1	45.4	53.9	58.2	2.4
Sodium, ppm	1317.1	1299.4	1218.6	1273.4	1390.3	1414.8	1345.4	21.5
Potassium, ppm	2354.5	2331.8	2307.3	2318.2	2414.1	2487.8	2466.5	46.3
Sulfur, ppm	1549.3^{a}	1620.5^{ac}	1747.6^{bc}	1860.9^{b}	1895.3^{b}	1814.1^{b}	1902.1^{b}	72.8
Manganese, ppm	13.1^{a}	4.5^{b}	5.5^{b}	5.2^{b}	4.3^{b}	4.6^{b}	4.8^{b}	1.6
Copper, ppb	74.8	15.4	26.9	21.7	22.6	16.3	22.7	16.6
Iron, ppb	266.8^{a}	317.0^{ad}	932.6^{b}	732.6^{bc}	607.4^{ce}	498.9^{de}	670.5^{e}	105.8
Chromium, ppb	6.8^{a}	5.3^{a}	7.1^{abc}	7.1^{ac}	7.6^{ac}	7.3^{ac}	8.7^{ac}	.6

[1]Overall time effect ($P < .05$), except for copper.

^{a,b,c,d,e}Means in the same row with different superscripts differ ($P < .05$)

Table 3. Rumen pH in fistulated heifers consuming prairie hay supplemented with urea and different levels of added zinc.

Item	Time, h[c]							SEM
	2	4	6	12	18	21	24	
Urea level[a]								
U1	6.94	7.03	6.90	6.68	6.81	6.86	6.78	.06
U2	7.05	7.14	6.82	6.48	6.59	6.68	6.55	.06
Zinc level[b]								
Zn1	7.18[d]	7.03	6.77	6.52	6.71	6.82	6.60	.07
Zn2	6.95[de]	7.19	6.87	6.58	6.70	6.72	6.63	.07
Zn3	6.87[e]	7.03	6.95	6.66	6.69	6.77	6.71	.07

[a] Urea levels 45 g (U1) or 90 g (U2) /animal daily)

[b] Zn levels 30 ppm (Zn1), 250 ppm (Zn2) or 470 ppm (Zn3)

[c] Interactions time by urea level ($P < .01$) and time by zinc level ($P = .08$)

[d,e] For zinc level, values within a time period with different superscripts differ ($P < .05$)

Table 4. Rumen ammonia concentration in fistulated heifers consuming prairie hay supplemented with urea and different levels of added zinc.

Item	Time, h[c]							SEM
	2	4	6	12	18	21	24	
Urea level[a]								
U1	32.60	22.20	11.69	1.30	.81	1.49	.82	2.94
U2	56.84	51.58	27.42	5.80	1.72	2.34	1.35	2.94
Zn level[b]								
Zn1	55.96[d]	34.44	15.46	2.36	1.38	2.23	1.21	3.60
Zn2	43.35[de]	42.29	22.31	2.87	1.30	1.77	1.23	3.60
Zn3	34.85[e]	33.94	20.89	5.42	1.13	1.73	.83	3.60

[a] Urea levels 45 g (U1) or 90 g (U2) /animal daily)

[b] Zn levels 30 ppm (Zn1), 250 ppm (Zn2) or 470 ppm (Zn3)

[c] Interactions time by urea level ($P < .01$) and time by zinc level ($P = .07$)

[d,e] For zinc level, values within a time period with different superscripts differ ($P < .05$)

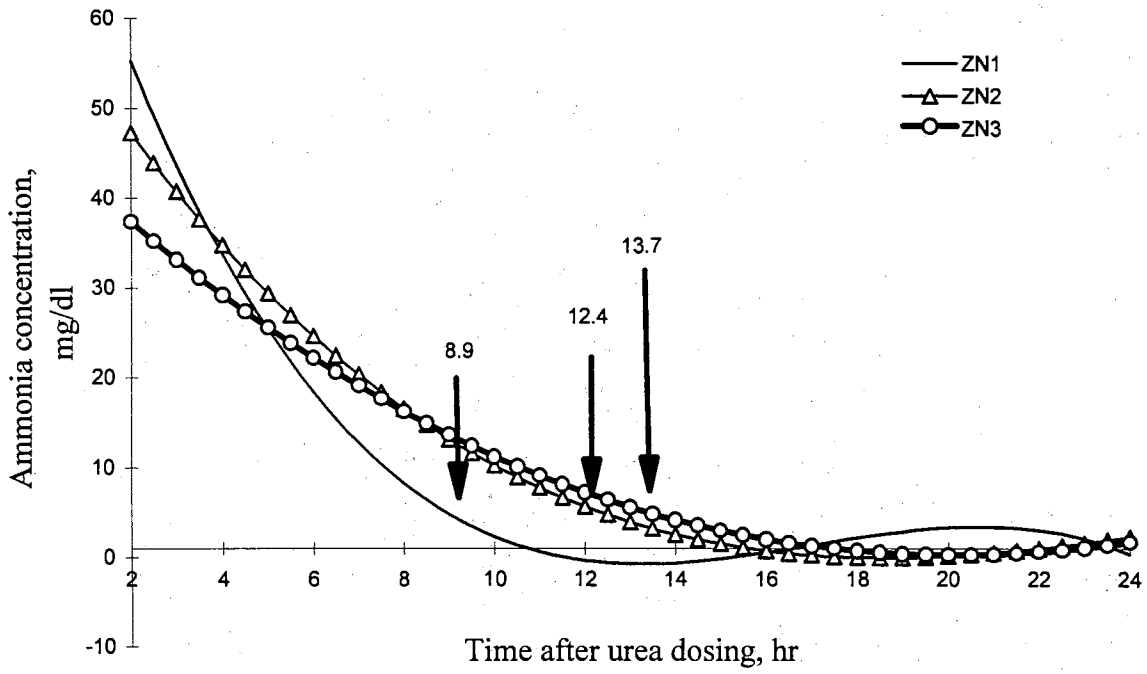

Figure 2. Relationship between rumen ammonia-N concentration and time elapsed after supplements were fed.

Zn levels: 30 ppm (Zn1), 250 ppm (Zn2) or 470 ppm (Zn3)
Zn1 : $NH_3 = -.01197\ h^3 + 1.0040\ h^2 - 16.23196\ h + 83.88960$; $R^2 = .99$
Zn2 : $NH_3 = -.00416\ h^3 + .33474\ h^2 - 8.13704\ h + 62.26731$; $R^2 = .96$
Zn3 : $NH_3 = -.00080\ h^3 + .14673\ h^2 - 4.93607\ h + 46.68631$; $R^2 = .97$
Zn1 vs Zn3 (P = .04)

Table 5. Rumen volatile fatty acids averaged by sampling periods and probability levels for time and zinc effects in fistulated heifers consuming prairie hay supplemented with urea and three zinc levels.

Item	Time, h							SE	Probability[1]		
	2	4	6	12	18	21	24		Time	Zinc	Time x Zinc
Acetate, mol/ 100 mol	72.95	72.76	72.78	71.82	71.14	71.29	71.53	1.16	.0005	.0890	.2727
Propionate, mol/100 mol	17.01	17.54	17.42	18.51	18.49	19.24	18.33	1.07	.0001	.0265	.9992
Butyrate mol/ 100 mol	7.53	7.31	7.76	7.60	7.56	7.14	7.83	.46	.3073	.0711	.7121
Isobutyrate mol/100 mol	.50	.32	.31	.24	.54	.61	.60	.09	.0001	.4307	.4561
Isovalerate mol/ 100 mol	.59	.67	.51	.48	1.01	.71	.54	.16	.0100	.2849	.8559
Valerate mol/ 100 mol	1.42	1.40	1.22	1.36	1.25	1.01	1.17	.19	.6397	.9094	.0219
Total VFA, mM	87.09	82.20	86.48	84.01	80.69	80.83	82.86	5.76	.3920	.7358	.4598
Acetate:propionate ratio	4.37	4.22	4.25	3.96	3.95	3.80	3.99	.31	.0001	.0297	1.0000

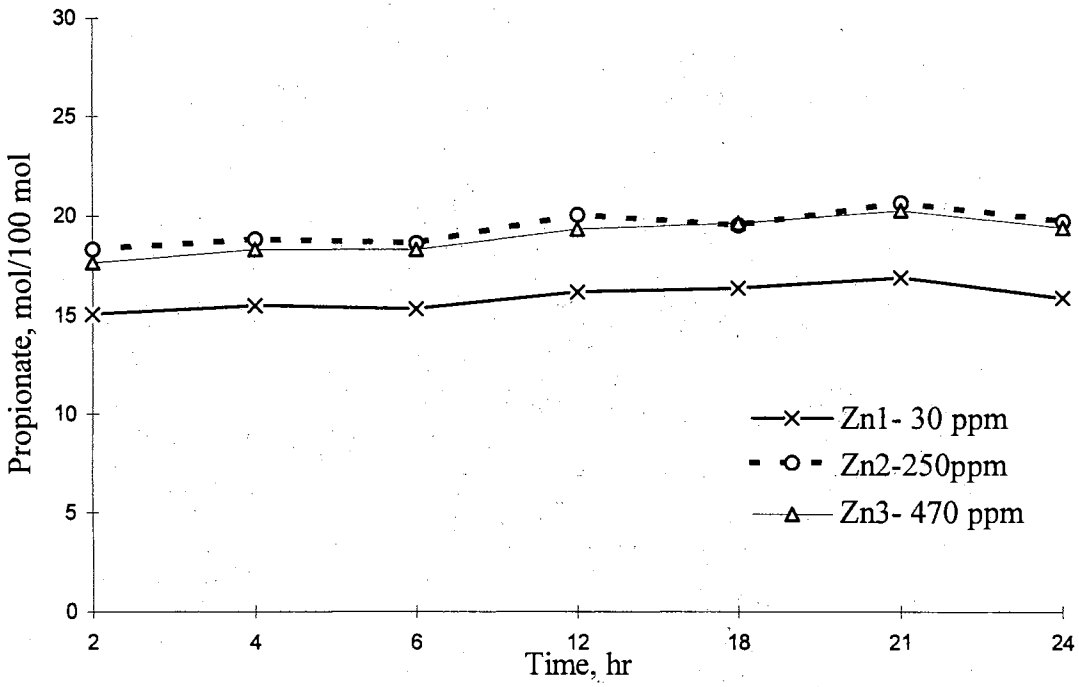

Figure 3. Molar proportions of propionate in fistulated heifers consuming prairie hay supplemented with two urea and three zinc levels averaged by sampling periods.
Zn linear (P =.01); Zn quadratic (P < .01)
LSD: Zn1 vs Zn2 (P < .01); Zn1 vs Zn3 (P =.02)

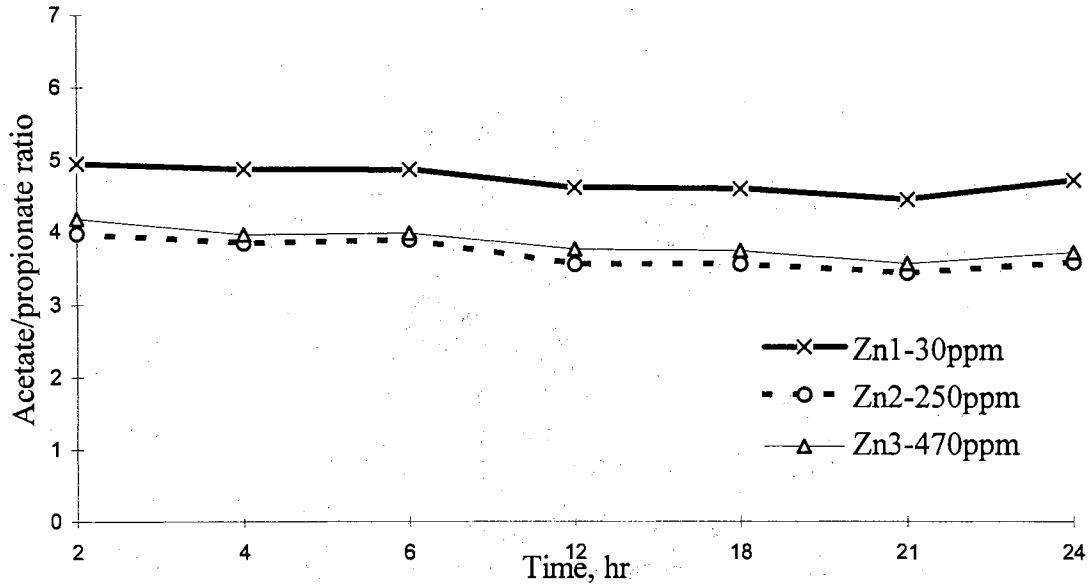

Figure 4. Acetate to propionate ratio in fistulated heifers consuming prairie hay supplemented with two urea and three zinc levels averaged by sampling periods.
Zn linear (P=.02); Zn quadratic (P < .01)
LSD: Zn1 vs Zn2 (P = .01); Zn1 vs Zn3 (P =.02)

CHAPTER V

PRAIRIE HAY UTILIZATION, RUMEN AND BLOOD PARAMETERS IN BEEF CATTLE SUPPLEMENTED WITH UREA AND ZINC AND MANGANESE OXIDES

H.M. Arelovich, G. W. Horn, and F.N. Owens

ABSTRACT

Twenty Charolais steers plus four crossbred ruminally cannulated heifers were used in six 4 x 4 Latin squares to determine how supplemental urea plus Zn and Mn oxides influenced prairie hay (PHAY) intake and digestion, and rumen and blood parameters. PHAY (4.9 % crude protein) was fed *ad libitum* plus the supplement of either 1) control, including wheat middlings, cottonseed hulls, dehydrated alfalfa and molasses (CON), 2) CON + urea (U), 3) treatment 2 + Zn (UZ), and 4) treatment 3 + Mn (UZM). Urea, Zn and Mn were 5.6 %, 2,300 mg/kg and 4,700 mg/kg of supplement dry matter (DM), respectively. Supplements were fed at a rate of 900 g DM/animal once daily. Each period lasted 18 d, with 10 d for adaptation. Cannulated heifers were used once each period to obtain rumen samples at 2, 4, 8, 12, 18 and 24 h. Mean DM intake of PHAY was lower for CON (5.08 kg/animal) vs urea supplements (6.81 kg/animal, $P < .05$). Total diet digestibility averaged 53.9 %, slightly reduced in U ($P = .06$). Digestible DMI was 3.18 (CON) vs. 4.15 kg /animal daily with urea supplements ($P < .05$). Treatment affected ruminal concentrations of Zn and P ($P < .01$), but not S, Na, K, Cu and Fe ($P > .10$). Time x treatment interaction was apparent for Ca, Mg and Mn concentrations ($P < .05$). A time x treatment interaction affected rumen pH and NH_3 ($P < .05$). Overall mean pH was 6.5. CON has higher ruminal pH than urea supplements at 12 and 18 h ($P < .05$); pH did not

differ among urea supplements at 12 h, but in UZ was lower than in U at 18 h ($P < .05$). Rumen NH_3 concentration was lower at 2 and 4 h after feeding for CON vs. urea supplements ($P < .01$). Added Mn increased ruminal NH_3 further at 2 h ($P < .05$). Regression analysis revealed that rumen NH_3 was lower for CON than for urea supplemented cattle during the 24 h period ($P < .01$). Interaction time x animal was found for acetate, propionate and acetate: propionate ratios ($P < .05$). Total VFA averaged 88.0 and 104.7 mM/l for CON and urea supplements respectively ($P < .01$). Mean serum glucose, at 78.5 mg/dl, was not affected by changes in digestible DM intake or VFA concentration. Hematocrit (40.2 %), creatinine (1.90 mg/dl) and total protein (6.38 g/dl) values were greater with the unsupplemented diet ($P < .05$). Alkaline phosphatase was lower the unsupplemented diet (182.3 mg/dl) than the average of diets with supplemental urea (213.1 mg/dl, $P < .05$). Alkaline phosphatase values may reflect increased liver activity to convert blood NH_3 into urea. Correlation of serum creatinine with total protein ($P < .01$) and glucose ($P < .05$) for CON may reflect enhanced muscle turnover of cattle not receiving the urea supplement. Blood urea N was greater in steers receiving urea supplements (6.01 vs. 1.37 mg/dl, $P < .01$). UZ and UZM had larger values of serum urea N than U ($P < .01$) which may indicate prolonged ruminal availability of urea. Urea supplementation improved basal diet utilization, modified ruminal and blood metabolites. However, Zn and Mn oxide addition did not substantially affect rumen urea degradation and VFA molar proportions as found previously. Mineral source and components of the supplement other than urea may have diminished the impact of added Zn and Mn in this study.

Key words: Urea, Zinc, Manganese, Cattle, Prairie hay utilization

Introduction

Beef cattle consuming low quality forage have a slow rate of passage of undigested feed as well as microbial growth that is slow and inefficient (Klopfenstein, 1996). Consequently, low feed intake and animal performance can be expected from these diets. Overall, N supplementation increases feed intake and improves animal performance when low quality diets are fed (Caton et al., 1988; Hunt et al., 1989, Arelovich et al, 1992). Urea alone can enhance intake of mature forages by grazing cattle (Dixon et al., 1998 and Bowen et al., 1998). Urea is less expensive per unit of N than other non-protein N sources or natural protein supplements. However, urea is rapidly degraded to ammonia in the rumen. The ammonia is absorbed or flushed through the rumen, which diminishes the ability of rumen microorganisms to integrate the N into their own protein (Bloomfield et al., 1960). This can result in N waste or under-utilization.

Ammonia release from urea in the rumen depends on the activity of bacterial urease. This enzyme can be stimulated or depressed by a number of inorganic ions (Spears and Hatfield, 1978), and depressed by organic compounds (Ludden et al., 1998). Zinc and Mn inhibited ammonia liberation from urea *in vitro* (Rodriguez et al., 1993). These minerals also improved N retention in sheep fed low quality hay supplemented with urea-grain mixtures (Rodriguez et al., 1995). Adding 860 ppm of Zn from Zn chloride solution to an *in vitro* incubation flask, decreased urea degradation rate, and also tended to depress dry matter degradation of prairie hay. This depression was partially relieved by Mn addition (Arelovich et al., 1997). Directly supplying 470 ppm of Zn as Zn-chloride with urea into the rumen, decreased rumen ammonia in cattle fed prairie hay

(Arelovich et al., 1998). Moreover, zinc addition stimulated propionate production (Arelovich et al., 1998, see Chapter IV). Mineral sources may differ in rumen degradability, making it more or less available at different sites of the digestive tract (Kennedy et al., 1994; Spears, 1996). Thus, minerals may be one alternative tool to manipulate rumen fermentation, particularly for low quality diets when urea is used as the source of supplementary N. The objective of this study was to determine how supplemental urea plus Zn and Mn in the oxide form influenced prairie hay intake and digestion, and rumen and blood parameters in beef cattle.

Materials and Methods

Animals and treatments

Twenty beef steers (Charolais crosses) and four ruminally cannulated heifers (British crosses) weighing 302 and 363 kg, respectively, were assigned to four treatments in a 4 x 4 Latin square replicated five times. The animals were randomly allotted to individual 3 x 5 m indoor pens. They received low quality prairie hay (PHAY) *ad libitum* as a basal diet. The supplemental treatments included 1) control, based on wheat middlings, cottonseed hulls, dehydrated alfalfa and molasses (CON), 2) CON + urea (U), 3) treatment 2 + zinc oxide (UZ) and 4) treatment 3 + manganese oxide (UZM). The minerals were calculated to provide 2300 mg Zn and 4700 mg Mn per kg of supplement DM. Based on an estimated daily DMI of about 5.75 kg/animal, these levels would be dietary equivalents of 400 and 800 ppm for Zn and Mn, respectively. Both figures were 20 % below maximum tolerable levels (NRC, 1996). All supplements were fed daily at

0830 at a rate of 900 g DM/animal. The PHAY and the supplements were provided in separate feeders. The ingredient and chemical composition of the PHAY and the supplements is shown in Table 1.

Sampling procedures and determinations

Each period lasted 18 d with 10 d for adaptation. Although all animals were placed simultaneously on the treatments, only the four cannulated heifers (no replicates) were used for rumen sampling at 2, 4, 8, 12, 18 and 24 h on day 11 of each period. The ruminal fluid obtained through the rumen cannula of each individual was filtered through 4 layers of cheesecloth at the specified time intervals. The pH of ruminal fluid was measured immediately after sampling. A 100-ml subsample was acidified with 2 ml of a 20 % v/v sulfuric acid solution and frozen. When thawed later, it was centrifuged for 15 min at 16,000 x g and analyzed for mineral content, ammonia-N (NH_3), and volatile fatty acid (VFA) concentration.

The steers (n = 20) were used to measure PHAY intake and DM digestibility during the last 7 d of each period. Prairie hay, orts, and fecal grab samples were collected from each animal once a day. After finishing each period, the samples were composited within animal and period and dried at 55°C. After drying, all samples were ground through a 2-mm screen in a Wiley mill (Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA) and stored for DM determination. Dry matter intake (DMI) was computed by subtracting weight of dry orts from offered PHAY. Dry matter digestibility (DMD) was calculated by using lignin as a marker, and digestible DMI (DDMI) was calculated for each animal from DMI and DMD values. Also, in these animals, blood samples were obtained by coccygeal venipuncture on the last day of each period 5 h after

the supplement was fed. Ten-ml blood samples were obtained by using vacuum containers with no additives for serum collection. All animals were weighed (unshrunk) at the beginning and end of each period.

Laboratory analyses.

Rumen fluid samples were analyzed for Ca, P, Mg, Na, K, S, Zn, Mn, Cu, Fe, Cr, Co, Se and Mo using an Inductively Coupled Plasma Spectrometer (Spectroflame FTM-08, Spectro Analytical Instruments, and Fitchburg, MA). Stock solution standards (High Purity Standards Co., Charleston, SC) were independently diluted with 5 % v/v nitric acid (Fisher Scientific-trace mineral grade) to suitable ranges for each element of concern. A minimum four-point regression comprised the instrument calibration.

For rumen NH_3-N concentration, 50 µl aliquots were analyzed colorimetrically at 630 nm using a Beckman spectrophotometer (Beckman, DU 64, Beckman Instruments, Inc, Fullerton, CA), following the procedure of Broderick and Kang (1980). Rumen fluid VFA content was determined by using a Perkin-Elmer Autosystem gas chromatograph (Perkin-Elmer 9000 Model Series, Norwalk, CN), equipped with a Megabore DB-FFAP liquid phase column that used helium as a carrier, and 2-ethylbutyric acid as an internal standard. Samples of PHAY, orts and feces were analyzed for acid lignin (Goering and Van Soest, 1970) and gravimetrically for DM content (AOAC, 1980). Prairie hay and supplements were further analyzed for NDF, ADF (Goering and Van Soest, 1970) total N and ash (AOAC, 1980).

Hematocrit was determined immediately after blood samples were obtained, then, the samples were centrifuged and frozen. Later when thawed, serum samples were analyzed for alkaline phosphatase, creatinine, glucose, urea-N and total protein using

blood chemistry automated analyzer (Roche-Cobas Mira, Roche Diagnostics Systems, Inc, Montclair, NJ).

Statistical analysis

Statistical analyses were performed using the GLM procedure of SAS (1990). Period, animal and treatment were the sources of variation. Orthogonal contrasts were used to compare the CON vs. urea containing supplements, U vs. UZ + UZM, and UZ vs. UZM. For DMI, DMD, digestible DMI, and blood chemistry; treatment effects were tested using treatment x period x animal as the error term. Correlations among blood parameters were calculated by using the PROC CORR of SAS (1990). Ruminal pH and mineral, NH_3-N and VFA concentrations were considered as split plot in time and treated as repeated measures. Treatment x animal x period was used as error term to test treatment effects in the main plot, while the residual was used for treatment and time effects and 2 way- interactions in the subplot.

Results and Discussion

Intake and digestion

The mean DMI of PHAY was 5. 08 and 6.81 kg/animal daily for unsupplemented and urea supplemented diets, respectively. Voluntary consumption of PHAY was larger for urea supplements than CON supplement ($P < .05$) as shown in Table 2. When compared among urea supplements, PHAY intake was slightly decreased by added Zn ($P = .05$), and Mn ($P = .07$). For all treatments, total tract digestibility of DM diet averaged 53.9%, being slightly lower without added minerals ($P = .06$). Digestible DMI

was increased by a mean of 31% by including urea in the supplement, averaging 3.18 without vs. 4.15 kg/animal daily with added urea.

In agreement with previous reports, feeding high N supplements increased intake of low quality roughage (Caton et al., 1988, Hunt et al., 1989, Arelovich et al, 1992). Supplemental urea as the only N source can stimulate intake of mature hay in cattle as well (Dixon et al. and 1998, Bowen et al., 1998). Urea is 100 % degraded intake protein (DIP, NRC, 1996). Increasing DIP by sodium caseinate addition has not improved low quality hay intake but it has increased DM digestibility and digestible DMI (Köster et al., 1997). In our study, digestible DMI was also increased but digestibility was not increased. Therefore, the increase in digestible DMI can be primarily attributed to a larger PHAY intake. Total diet digestibility for CON (53.4 %) was similar to the average of N supplements (54.1 %). These values were 15 % higher than the average digestibility of 46.8 % observed for PHAY plus urea-mineral supplements in a previous experiment (Arelovich et al., 1998). This improvement in digestibility might be explained by larger supply to rumen microorganisms of energy, amino acids and peptides from other components of the supplement, not present in the previous study.

Besides NH_3, true protein is also required by rumen microorganisms (Cotta and Russell, 1982) and can potentate urea utilization. Digestible organic matter intake was maximum with low quality hay when the supplement contained about 11 % DIP as a percentage of total organic matter (Köster et al., 1996). For the high N supplements in this experiment, DIP was estimated to be over 15 % of DM. If extent of digestion of the total diet was not improved by urea addition to the CON supplement, then the effect on intake may be due to an increased digestion rate. Also, NH_3 released from non protein N

would be more synchronous with carbohydrate fermentation to improve microbial protein synthesis (Johnson, 1976 and Mizwicki et al., 1980)

The treatments including Zn and Mn decreased hay DMI by an average of 3.1 % (P = .06), and increased total diet digestibility by an average of 4.6 % as compared with supplementing urea without minerals. Although statistically significant, these differences may be of minor biological importance.

High levels of Zn and Mn have been reported to depress *in vitro* cellulose digestion (Chamberlain and Borroughs, 1962). Martinez and Church (1970) established that the addition of 20 ppm of Zn and 100 ppm of Mn to washed suspensions of rumen microorganisms depressed cellulolytic activity.

Arelovich et al. (1997) also found a trend for decreased DM disappearance of PHAY *in vitro* with Zn addition. In the same experiment, Mn addition diminished Zn negative effect on digestibility. Both Zn and Mn were added as chlorides, whereas in the present study the oxide forms were used.

Minerals from different sources differ in rumen solubility. Organic forms such as Zn or Mn methionine reportedly yield higher rumen concentrations than sulfate and oxide forms (Ward et al., 1992). Also chlorides are more soluble than oxide and sulfate forms, but they are highly hygroscopic and consequently difficult to manage under practical conditions. Oxides of Zn and Mn were used in this experiment. Differences in solubility of mineral sources affect mineral rumen availability, which in turn may have a different impact on metabolism. In fact, about 400 ppm of Zn added to the diet per day yielded an average rumen fluid concentration of Zn of 25.4 ppm from Zn chloride (Arelovich et al., 1998, see Chapter IV), vs. 3.4 ppm from Zn oxide in the present experiment. This lower

Zn level could also be influenced by an increased turnover rate of rumen contents with U, UZ and UZM compared with CON.

Ruminal fluid characteristics

Treatment means, main effects and interactions of treatment and time affected the concentration of several mineral elements in rumen fluid (Table 3). Ruminal concentrations of S, Na, K, Fe and Cr were not affected by treatment ($P > .10$), but rumen levels of Zn and P were affected ($P < .01$). A non-significant treatment trend was observed for Cu ($P < .10$). Sampling time by treatment interactions were apparent for Ca, Mg and Mn concentrations ($P < .05$) The mineral concentrations of Se, Co and Mo were very low, in most cases remaining below the detection limits for the equipment of 2.1, 1.7 and 35 ppb respectively.

Phosphorus concentrations were larger for CON compared with urea supplements ($P < .01$). Ruminal absorption of P has not been demonstrated (Yano et al., 1991). Therefore, increased disappearance of rumen P with urea supplements may be due to increased incorporation into nucleic acids, or greater dilution with saliva, or, although less likely, a larger ATP demand for enhanced rumen fermentation.

Manganese additions by supplement UZM reduced ruminal P concentrations further ($P < .01$). Because of the high dietary Mn concentration, the formation of insoluble complexes with phytic acid from the wheat middlings in the supplement is possible (McDowell, 1992). Copper in rumen fluid was very low compared with the ruminal concentration of other trace elements required at about the same levels in the diet (NRC, 1996). It was also highly variable and decreased with the addition of urea in the

supplements compared with CON ($P < .01$). This reduction may also be due to an increased turnover rate of ruminal fluid or a larger demand by rumen microorganisms.

Mean Zn concentrations were increased by Zn oxide addition in the UZ and UZM supplements ($P < .01$). The 24-h average ruminal concentration of CON and U was 1.2 ppm, for UZ and UZM was 3.4 ppm for a daily supply of 2,300 mg/kg of supplement. This difference reflects an increase of 2.85 times from added Zn. Similarly, the provision of 806 mg/d of Zn from Zn oxide resulted in cell free rumen fluid mean concentration of 2.35 ppm across 8 h sampling period for animals receiving an alfalfa-corn based diet (Kennedy et al., 1994). An *in vitro* concentration of 20 ppm has been reported to be toxic for cellulolytic bacteria (Martinez and Church, 1970).

Rumen pH values and NH_3 concentrations are reported in Table 4. Both variables were affected by the treatment x time interaction ($P< .05$). The mean pH value across treatments and different time intervals was 6.51. The pH fluctuations observed throughout the day varied between a minimum of 6.06 and a maximum of 6.79. Only pH values below 6.0 inhibit cellulolytic bacterial activity in the rumen (Owens and Goestch, 1993). Treatment effects analyzed within each time period showed that CON exhibited a higher ruminal pH than urea supplements at 12 and 18 h ($P < .05$). No differences in pH were found among urea supplements at 12 h, but UZ had lower pH than U, although not different from UZM at 18 h ($P < .05$).

Treatment effects within each time period were also analyzed for rumen NH_3 concentration. It was initially lower at 2 and 4 h after feeding the CON supplement compared with the urea supplements ($P < .01$). Added Mn seems to increase ruminal NH_3 further at 2 h. *In vitro* DM disappearance of prairie hay was increased by Mn addition

(Arelovich et al., 1997). Therefore, higher ruminal availability of Mn of UZM may be increasing N release from PHAY protein fraction.

For rumen NH_3 the treatment x sampling time interaction was best described by a cubic regression as shown in Figure 1. Regression analysis using Dummy variables revealed a significant difference in the NH_3 concentration when compared CON individually vs each urea supplement ($P < .01$). No differences were found in rumen NH_3 concentrations among the supplemental treatments U, UZ or UZM ($P > .10$). However, a non significant trend for decreased NH_3 values from 2 to 8 h and increased from 12 to 24 h in the UZ compared with U or UZM can be noted numerically (Table 4) as well as graphically (Figure 2). Zinc concentrations of 130 or 1,300 ppm have been shown to decrease NH_3-N release *in vitro* by inhibiting urease activity in sheep ruminal fluid (Spears and Hatfield, 1978). Other studies have also shown that Zn addition will depress rumen NH_3 production when low quality roughages are supplemented with urea (Rodriguez et al., 1993; Arelovich et al., 1997; Arelovich et al. 1998).

Overall NH_3 values ranged from 2.3 to less than 1 mg/dl (CON) and 21.0 to 1.6 mg/dl (urea supplements) during the first 8 h period after feeding. The NH_3 (U, UZ and UZM) and Zn levels (UZ and UZM) were substantially lower in this experiment than in the previous one (Arelovich et al., 1998 see Chapter IV). Perhaps in the current experiment, Zn impact on NH_3 liberation from urea was masked by a faster NH_3 utilization because of additional carbohydrate availability. However, higher rumen levels of Zn may be necessary to inhibit rapid NH_3 release from urea as well. Faster turnover rates of rumen fluid may have also influenced NH_3 utilization by rumen microbes.

Molar proportions and total VFA concentrations are reported for the different time intervals in Table 5. We found time x treatment interactions for molar proportions of acetate, propionate and acetate to propionate ratio ($P < .05$). Total VFA concentration was affected by treatment ($P = .02$) and time period. Means across sampling periods were 88.0 vs 104. 7 mmol/l for CON vs the average of U, UZ and UZM, respectively. The urea supplements increased total VFA concentration ($P < .01$), compared with CON treatment, but Zn or Mn addition did not significantly affect concentration.

WhenVFA concentrations (mM/l) were analyzed no interactions were found for acetate or propionate. The mean acetate and propionate concentrations were 63. 3 and 13.9 mmol/l (CON) vs 75.2 and 17.6 mM/l (urea supplements). Molar concentration of propionate was lower for CON ($P < .01$) than urea supplements. Molar proportions are normally reported for VFA.

However, because of critical role of propionate as glucose precursor in ruminants, and the potential to be affected by the treatments tested, treatment effects on propionate concentration (mM/l) are shown in Figure 2. Ionophore antibiotics are classic examples of additives favoring propionate production (Van Soest, 1982,Van Nevel and Demeyer, 1988). An inhibitor of urease activity, N- (n-butyl) thiophosphoric triamide, was also found to decrease the acetate: propionate ratio after 6 h of *in vitro* incubation with ruminal fluid (Ludden et al., 1998). We discovered that the addition of 400 ppm of Zn from Zn chloride increased the molar proportion of propionate in a previous experiment (Arelovich et al., 1998, see Chapter IV). In contrast, a similar level of Zn added from Zn oxide did not have the same effect on propionate. Nevertheless, a non-significant trend for increased propionate concentrations with Zn additions seems apparent in Figure 2.

Ruminal concentrations of Zn may not have been sufficiently high to markedly influence propionate production. However, any Zn effect might also have been masked by the additional energy supply of supplement components other than urea. Owens and Goestch (1993) suggested that the rates of VFA production seem to be controlled primarily by substrate availability for amylolytic and saccharolytic bacteria.

Overall, ruminal NH_3 and total VFA concentrations increased in response to urea supplementation ($P < .01$). A similar effect was found by increased DIP addition with sodium caseinate to supplementation of a low quality prairie forage (Köster et al., 1996).

Blood parameters

Means for blood constituents are reported in Table 6. The concentration of GLU was constant across treatments averaging 78.5 mg/dl. However, statistical analysis revealed effects of the supplemental treatments on the other blood measurements ($P < .05$).

Hematocrit values were lower with urea supplementation compared with CON ($P < .01$); this may be attributed to increased water intake that may occur with urea consumption, resulting in dilution of packed cell volume. Alkaline phosphatase values were larger for all treatments than normal reference values reported by The Merck Veterinary Manual (1991). Serum alkaline phosphatase may vary over a wide range of values in large animals, however, increased levels reflect bone and liver disorders (Kramer, 1989). Alkaline phosphatase was lower for CON than the average of urea supplements ($P < .05$). Elevated serum alkaline phosphatase in treatments with supplemental urea may relate to increased hepatic activity arising from increased

transformation of absorbed NH_3 into urea. In fact, blood urea-N values were five times lower in CON.

Enhanced muscle tissue turnover seems to occur in animals receiving CON supplement as indicated by larger serum creatinine levels (1.90 mg/dl, $P < .01$) as compared with the average for urea supplements (1.72 mg/dl). Since DDMI was lower in CON, more amino acids may have been used for gluconeogenesis than in the other treatments. This fact is supported by the higher level of total protein in serum for CON ($P < .01$). Moreover, creatinine levels were positively correlated with total serum protein ($P < .01$; correlation coefficient = .3886) and negatively with serum glucose ($P < .05$; correlation coefficient = -. 2281).

Serum urea N was increased by urea addition to the supplements ($P < .01$). In agreement with previous research, an increased blood urea N would be expected from the addition of urea to the supplement (Egan and Kellaway, 1971; Schmidt et al., 1973; Arelovich et al, 1992). Five hours after feeding the supplement serum urea N was very low in the CON treatment (1.37 mg/dl) compared with the average of U, UZ and UZM supplement (6.01 mg/dl; $P < .01$). Additionally, UZ and UZM produced serum N values larger than U ($P < .01$) which may be due to a slower ruminal urea degradation. A significant effect of Zn on urea degradation rate was observed *in vitro* (Arelovich et al., 1997). In this experiment rumen NH_3 levels were not significantly changed with Zn addition. However, in a previous experiment they were modified by equal levels of added Zn chloride when urea was directly inserted through the rumen cannula. This difference may be attributed to a lower Zn availability from Zn oxide in the rumen, a masking effect of the natural protein from other supplement components, or both.

Implications

Urea supplements improved basal diet utilization and modified ruminal and blood chemistry in beef cattle fed low quality prairie hay. In general, the addition of 400 ppm of Zn and 800 ppm of Mn to the diet together with urea did not substantially change total diet utilization, rumen or blood constituents. However, mineral source and components of the supplement other than urea may have diminished or masked the impact of these minerals on rumen metabolism and diet utilization with urea supplement.

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Table 1. Ingredient and nutrient composition of supplements and prairie hay (DM basis).

Item	Supplements[a]				Prairie hay
	CON	U	UZ	UZM	
Ingredient, %					
Dehydrated alfalfa, pelleted	7.1	7.1	7.1	7.1	-
Molasses	6.7	6.7	6.7	6.7	-
Wheat middlings	54.1	53.8	53.5	53.0	-
Cottonseed hulls	30.7	25.4	25.4	25.4	-
Urea	-	5.6	5.6	5.6	-
Zinc Oxide	-	-	0.3	0.3	-
Manganese Oxide	-	-	-	0.6	-
Salt	1.4	1.4	1.4	1.4	-
Nutrient composition, %					
DM	90.3	89.5	89.6	89.6	90.0
CP	13.0	20.3	20.0	21.0	4.9
NDF	51.5	44.7	43.6	44.9	69.2
ADF	27.0	25.7	26.4	25.7	48.0
ADL	9.1	9.3	9.4	9.5	6.8
Ash	6.1	5.7	5.7	6.2	6.9

[a] Supplements CON= control, wheat middlings, cottonseed hulls, dehydrated alfalfa and molasses; U= CON + urea; UZ= U + zinc oxide; UZM= UZ + manganese oxide.

Table 2. Daily prairie hay and supplement consumption, total tract dry matter digestibility and digestible dry matter intake in steers fed supplements with or without urea, zinc and manganese.

	Treatments[a]					Contrast[b] (P <.05)		
Item	CON	U	UZ	UZM	SE[c]	1	2	3
Feeds								
Prairie hay DMI, kg	5.08	6.95	6.85	6.62	.08	.0001	.05	.07
Supplement intake, g	903	895	896	896	-	-	-	-
Total tract digestibility, %	53.35	52.47	54.29	55.49	.01	.51	.06	.38
Digestible DMI, kg	3.18	4.10	4.20	4.16	.07	.0001	.40	.71
Digestible DMI, g/ kg $(BW)^{.75}$	42	54	55	54	.98	.0001	.46	.77

[a] Supplements CON= control, wheat middlings, cottonseed hulls, dehydrated alfalfa and molasses; U= CON + urea; UZ= U + zinc oxide; UZM= UZ + manganese oxide

[b] Observed significant level for contrast 1 = CON vs (U + UZ + UZM)/3; 2 = U vs (UZ + UZM)/2; 3 = UZ vs UZM

[c] Standard error of treatment means (n=20)

Table 3. Treatment means for rumen mineral concentrations of heifers fed prairie hay supplemented with or without urea, zinc and manganese.

	Treatments[a]					Effects[b]			Contrast[c] (P <.05)		
Item	CON	U	UZ	UZM	SE[c]	TR	TI	TR x TI	1	2	3
Calcium, ppm	182	235	243	241	4.7	-	-	*	-	-	-
Phosphorus, ppm	292	256	256	232	3.6	***	NS	NS	<.01	.30	<.01
Magnesium, ppm	86	98	103	99	2.0	-	-	**	-	-	-
Sulfur, ppm	2001	2082	2044	1969	52.9	NS	***	NS	-	-	-
Sodium, ppm	1610	1496	1513	1413	13.6	NS	NS	NS	-	-	-
Potassium	2277	2512	2444	2578	23.6	NS	NS	NS	-	-	-
Zinc, ppm	1.2	1.2	3.4	3.4	.2	***	NS	NS	.01	<.01	.56
Manganese, ppm	2.6	3.4	3.5	12.4	.3	-	-	***	-	-	-
Iron, ppb	452	373	376	375	4.1	NS	NS	NS	-	-	-
Chromium, ppb	8.5	7.4	6.8	7.9	.3	NS	NS	NS	-	-	-
Copper, ppb	25.4	13.1	14.9	10.3	2.0	*	***	NS	.02	.91	.39

[a] Supplements CON= control, wheat middlings, cottonseed hulls, dehydrated alfalfa and molasses; U= CON + urea; UZ= U + zinc oxide; UZM= UZ + manganese oxide

[b] Effects TR= treatment, TI = time, TR x TI =interaction treatment and time
NS = P ≥ .10, * = P < .10, ** = P <.05 and *** = P < .01.

[c] Observed significant level for contrast 1 = CON vs (U + UZ + UZM)/3; 2 = U vs (UZ + UZM)/2; 3 = UZ vs UZM

[c] Standard error of treatment means (n = 20)

Table 4. Rumen pH and ammonia-N concentrations at different time intervals in heifers fed prairie hay supplemented with or without with urea, zinc and manganese.

	Time, h					
Item[a]	2	4	8	12	18	24
pH[b]						
CON	6.65	6.62	6.63	6.58[d]	6.76[d]	6.78
U	6.79	6.45	6.28	6.18[e]	6.44[e]	6.72
UZ	6.76	6.45	6.32	6.21[f]	6.18[f]	6.63
UZM	6.77	6.48	6.36	6.06[e]	6.41[ef]	6.67
Ammonia-N, mg/dl[c]						
CON	2.29[d]	1.47[d]	.63	.41	.45	.52
U	19.63[e]	11.36[e]	1.71	.93	.69	.60
UZ	20.50[e]	9.84[e]	1.48	.98	1.69	.94
UZM	22.80[ef]	11.56[e]	1.68	1.26	.82	.63

[a] Supplements CON= control, wheat middlings, cottonseed hulls, dehydrated alfalfa and molasses; U= CON + urea; UZ= U + zinc oxide; UZM= UZ + manganese oxide

[b] Interaction treatment x time (P=.0097, standard error of mean = .07)

[c] Interaction treatment x time (P=.0001, standard error of mean = .79)

[d,e,f] Means in the same column with different superscripts differ (P < .05)

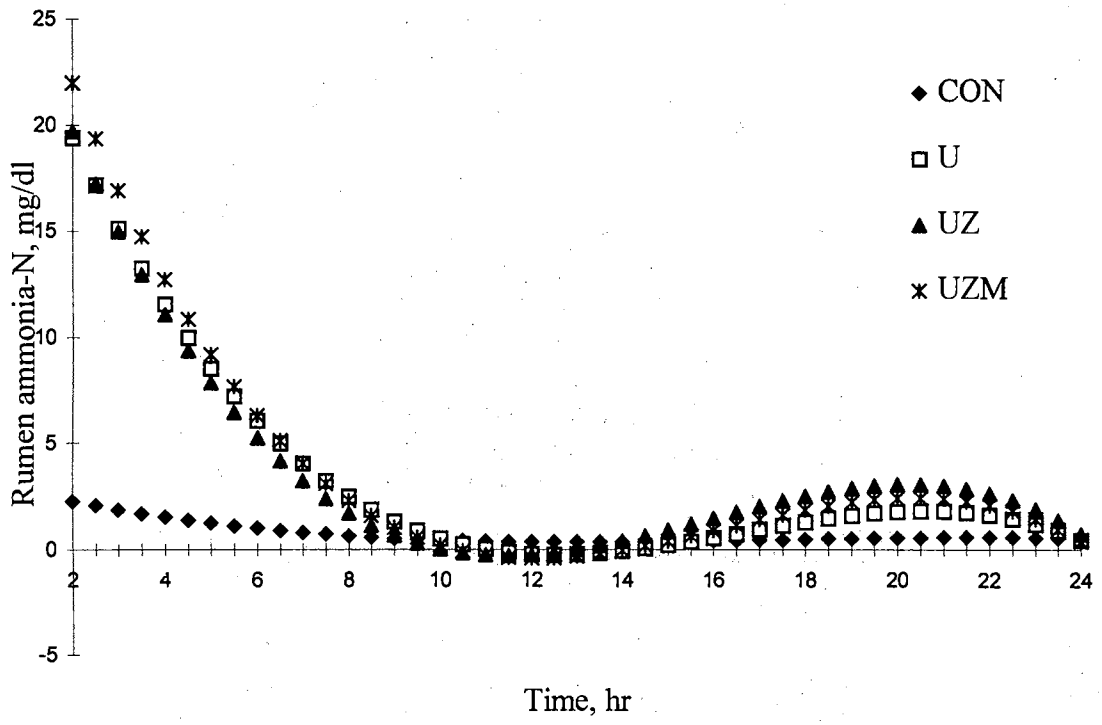

Figure1. Relationship between rumen N-NH_3 concentration and time elapsed after supplements were fed in heifers fed prairie hay supplemented with or without urea, zinc and manganese.

Supplements CON= control, wheat middlings, cottonseed hulls, dehydrated alfalfa and molasses; U= CON + urea; UZ= U + zinc oxide; UZM= UZ + manganese oxide

CON: $NH_3 = -.00066h^3 + .03441\ h^2 - .55772\ h + 3.24058$; $R^2 = .99$
U: $NH_3 = -.00773\ h^3 + .38544\ h^2 - 6.02526\ h + 29.95674$; $R^2 = .99$
UZ: $NH_3 = -.00982\ h^3 + .46751\ h^2 - 6.84973\ h + 31.59848$; $R^2 = .98$
UZN: $NH_3 = -.00978\ h^3 + .47624\ h^2 - 7.22420\ h + 34.59385$; $R^2 = ..98$
CON vs all urea supplements ($P < .01$)

Table 5. Volatile fatty acid molar proportions and total concentration at different time intervals in heifers fed prairie hay supplemented with or without urea, zinc and manganese.

	Time, h					
Item[a,b]	2	4	8	12	18	24
Acetate[c]						
CON	70.8	70.8	72.3	72.3	73.3	73.0
U	70.9	71.9	72.1	72.6	72.9	73.1
UZ	70.7	71.1	70.9	71.2	71.3	71.7
ZM	71.2	71.7	72.5	71.9	72.8	72.9
Propionate[c]						
CON	16.4	16.6	15.3	15.2	14.6	15.4
U	17.4	17.1	16.6	16.4	15.9	15.9
UZ	17.5	17.4	17.4	17.1	16.7	16.5
ZM	17.1	17.0	16.4	16.5	16.0	16.1
Acetate:Propionate[c]						
CON	4.4	4.3	4.7	4.8	5.1	4.8
Urea	4.1	4.2	4.4	4.5	4.6	4.6
UZ	4.2	4.1	4.1	4.2	4.3	4.4
ZM	4.2	4.2	4.4	4.4	4.6	4.6
Total VFA, mmol/l[d]						
CON	103.6	86.4[e]	84.5[e]	90.4[e]	81.8[e]	81.8
U	106.7	104.9[f]	102.4[f]	112.9[f]	102.1[f]	91.5
UZ	98.5	103.3[f]	104.6[f]	110.6[f]	112.9[f]	98.2
ZM	99.6	108.5[f]	102.8[f]	117.9[f]	108.3[f]	99.4

[a] Supplements CON= control, wheat middlings, cottonseed hulls, dehydrated alfalfa and molasses; U= CON + urea; UZ= U + zinc oxide; UZM= UZ + manganese oxide

[b] Standard error of means for Acetate = .37, Propionate = .27, Acetate: Propionate =.08 and Total VFA = 5. 0.

[c] Time x animal interaction ($P < .01$)

[d] Treatment effect ($P = .02$). Contrasts CON vs. (U + UZ + UZM)/3 ($P < .01$)

[e,f] Time effect ($P < .01$), means different subscript differ

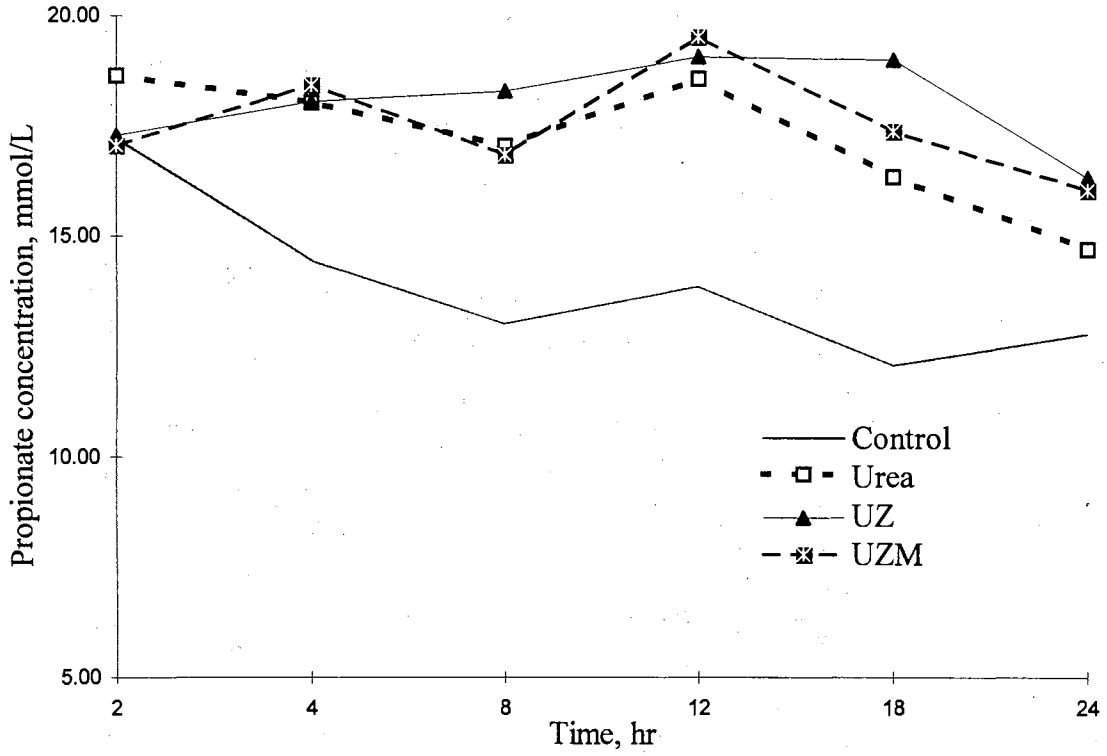

Figure 2. Rumen propionate concentration after supplements were fed in heifers fed prairie hay supplemented with or without urea, zinc and manganese.

Supplements CON= control, wheat middlings, cottonseed hulls, dehydrated alfalfa and molasses; U= CON + urea; UZ = U + zinc oxide;
UZM = UZ + manganese oxide
CON vs. urea supplements ($P < .01$)

Table 6. Blood serum parameters in steers fed prairie hay as basal diet and supplements with or without urea, zinc and manganese.

	Treatments[a]					Contrast[b] (P <.05)			Reference
Item	CON	U	UZ	UZM	SE[c]	1	2	3	ranges[d]
Hematocrit, %	40.28	38.28	38.35	38.60	.30	.0001	.60	.59	24-26
Alkaline phosphatase, μ/l	182.33	209.45	198.70	231.15	10.72	.02	.68	.041	7.5-152.7
Creatinine mg/dl	1.90	1.73	1.71	1.72	.02	.0001	.66	.58	.6-1.8
Glucose, mg/dL	78.58	77.75	78.75	78.80	.80	.88	.30	.96	42.1-74.5
Blood Urea Nitrogen, mg/dl	1.37	5.61	6.10	6.31	.16	.0001	.0054	.38	7.8-24.6
Total Protein, g/dl	6.38	6.20	6.20	6.29	.04	.0039	.4821	.1452	6.16-8.22

[a] Supplements CON= control, wheat middlings, cottonseed hulls, dehydrated alfalfa and molasses; U= CON + urea; UZ= U + zinc oxide; UZM= UZ + manganese oxide

[b] Observed significant level for contrast 1 = CON vs (U + UZ + UZM)/3; 2 = U vs (UZ + UZM)/2; 3 = UZ vs UZM

[c] Standard error of treatment means (n=20)

[d] The Merck Veterinary Manual (1991). Serum reference range for biochemical constituents.

APPENDIXES

APPENDIX A

Preliminary *in vitro* determination of urea-N disappearance at various Zn and Mn levels, and zinc level at which maximum urea degradation rate occurred *in vitro* (Chapter III).

Table 1. *In vitro* urea-N concentration of rumen fluid after incubation with urea and different Zn concentrations averaged across levels of added Mn in a preliminary assay[a].

	Time, min[b]		
Item	60	120	180
Zn, ppm[c]			
0	57.65	5.37	.31
5	60.96	5.69	1.33
10	67.17	9.67	5.09
15	70.66	12.39	10.89
20	67.74	12.20	10.03

[a] Preliminary assay. It tested *in vitro* technique and defined Zn and Mn levels for the experiment in Chapter III. No significant effect of 0, 25, 50, 75 and 100 ppm of Mn, or Zn x Mn interaction ($P < .10$), Zn concentration ($P < .01$).

[b] Initial urea-N concentration at time 0 was 75.0mg/dl, unaffected by Zn or Mn levels

[c] Time period x Zn level interaction ($P<.01$), SEM= 1.77

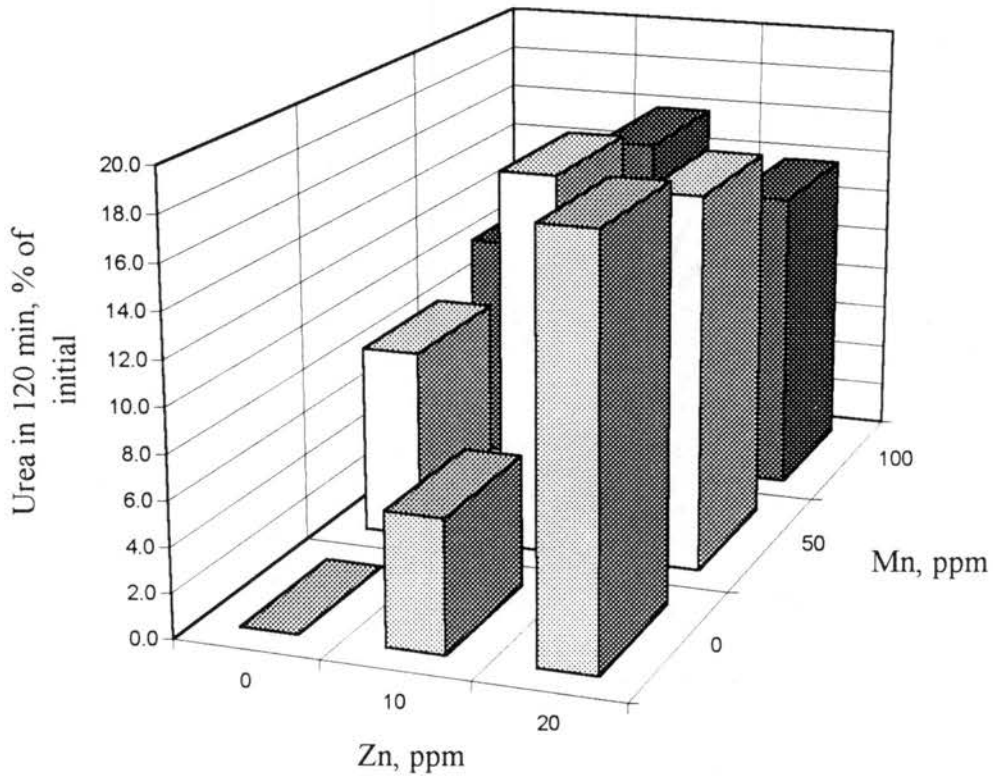

Figure 1. Percentage *in vitro* urea loss at various concentrations of Zn and Mn in a preliminary study.

Table 2. Zinc concentration at which maximum urea concentration occurred at different time periods after addition of urea solution incubated *in vitro* with different levels of Zn and manganese.

Incubation time, min[a]	Urea degradation mg/dl[b]	Maximum degradation[c]	Zn level for maximum degradation[d]	Contrasts for degradation		
				L	Q	C
60	.1915 - .0179 Zn + .0029 Zn^2	-.0179 + (2) .0029 Zn	Zn ≅ 3	.08	.33	.78
120	.1957 - .0218 Zn + .0040 Zn^2	-.0218 + (2) .0040 Zn	Zn ≅ 3	.02	.05	.12
180	.1364 - .0096 Zn + .0029 Zn^2	-.0096 + (2) .0016 Zn	Zn ≅ 3	.01	.13	.43

[a] Samples were obtained from *in vitro* tubes at equally separated intervals

[b] Regression equation where Zn was equivalent to the digits 0,1,2,3 and 4 for the levels Zn0 (0 ppm), Zn1 (5ppm), Zn2 (10 ppm), Zn3 (15 ppm) and Zn4 (20 ppm) respectively

[c] First derivative of degradation equation

[d] Value obtained by solving for Zn using the derived equation. Zn level was near 3 at all time intervals (Zn3 = 15 ppm Zn)

[e] Linear, quadratic and cubic effects for urea concentration at different sampling times minus that at time 0

APPENDIX B

Prairie hay composition and ruminal fluid volatile fatty acid concentrations for heifers receiving two urea and three zinc levels (Chapter IV).

Table 1. Dry matter composition of prairie hay fed to beef heifers receiving two levels of urea plus three of Zn chloride through the rumen cannula.

Item	Percentage
Dry matter	92.9
Crude protein	5.1
Neutral detergent fiber	74.8
Acid detergent fiber	48.6
Acid detergent lignin	7..9
Ash	6..9

Table 2. Rumen volatile fatty concentration in beef heifers consuming prairie hay supplemented with two levels of urea and three levels of zinc, averaged across urea levels.

Zinc level, ppm[a]	30							250							470							
Time, h[b]	2	4	6	12	18	21	24	2	4	6	12	18	21	24	2	4	6	12	18	21	24	SE
VFA, mmo/l																						
Acetate	59.4	58.5	64.7	59.2	60.6	56.6	58.8	68.8	63.7	60.3	62.3	55.2	61.1	60.0	62.6	56.9	63.6	59.4	57.5	55.3	59.0	3.0
Propionate	12.0	12.4	13.4	13.2	13.5	13.3	12.9	17.4	16.8	15.8	17.6	16.0	17.8	12.9	15.0	14.4	16.1	16.0	15.5	15.6	16.0	.9
Butyrate	6.5	6.0	7.3	6.5	6.3	5.9	6.6	7.4	6.8	6.6	6.7	6.2	6.1	6.7	5.9	5.2	6.2	5.9	5.3	5.2	6.1	.4
Isobutyrate	.45	.18	.34	.19	.57	.48	.53	.41	.33	.24	.18	.29	.60	.55	.44	.34	.30	.27	.50	.38	.54	.09
Isovalerate	.67	.59	.55	.50	.69	.70	.59	.39	.50	.34	.29	.69	.48	.36	.47	.54	.47	.37	.74	.54	.43	.10
Valerate	1.45	1.07	.98	1.12	.52	.89	.92	1.04	1.00	1.08	1.13	1.42	.71	1.00	1.07	1.37	1.15	1.07	.65	.78	.91	.18

[a] Zn level no significant

[b] Time effect for butyrate and isovalerate ($P < .01$)

APPENDIX C

Nutrient intake, actual and predicted body weight changes, and ruminal fluid volatile fatty acid concentrations (Chapter V)

Table 1. Actual and predicted values for nutrient intake and body weight change in beef heifers consuming low quality prairie hay and supplements with or without urea plus zinc and manganese.

	Treatment[a]			
Item[b]	CON	U	UZ	UZM
DMI predicted kg/d[c]	7.42	7.34	7.34	7.35
DMI actual, kg/d	5.97	7.85	7.75	7.51
DIP balance g/d[c]	-83.3	26.0	28.0	32.0
ADG allowed by ME, g/d[c]	20	240	230	200
ADG allowed by MP, g/d[c]	130	420	400	360
ADG actual g/d[d]	157	760	681	496
SEM for actual ADG	81	78	77	75

[a] Supplements CON= control, wheat middlings, cottonseed hulls, dehydrated alfalfa and molasses; U= CON + urea; UZ= U + zinc oxide; UZM= UZ + manganese oxide

[b] DMI = dry matter intake, DIP = degradable intake protein, ADG= average daily gain, ME= metabolizable energy, MP= metabolizable protein, SEM = standard error of the mean.

[c] Calculated by using NRC Model Level 1 (1996)

[d] Treatment effect ($P < .01$). Contrasts: CON vs. Urea supplements ($P < .01$), U vs. (UZ + UZM)/2 ($P = .09$)

Table 2. Ruminal fluid volatile fatty concentration in beef heifers consuming prairie hay and supplements with or without urea plus zinc and manganese.

	Time, h					
VFA, mmo/l	2	4	8	12	18	24
Acetate						
CON	72.9	61.1	61.1	65.3	59.8	59.5
U	75.6	75.4	73.9	82.0	74.3	66.8
UZ	69.7	73.5	74.2	78.7	80.3	70.2
UZM	70.9	77.9	74.7	84.7	78.9	72.4
Propionate						
CON	17.2	14.4	13.0	13.9	12.1	12.9
U	18.6	18.0	17.0	18.6	16.3	14.7
UZ	17.3	18.0	18.3	19.1	19.0	16.3
UZM	17.0	18.4	16.8	19.5	17.4	16.0
Butyrate						
CON	11.0	9.3	8.7	9.3	7.9	7.6
U	10.5	10.1	9.9	10.9	9.5	8.0
UZ	9.9	10.4	10.6	11.2	11.5	9.4
UZM	9.9	10.6	9.8	11.7	10.1	9.0
Isobutyrate						
CON	.60	.32	.36	.43	.40	.45
U	.40	.05	.00	.06	.44	.48
UZ	.45	.10	.20	.15	.48	.54
UZM	.43	.05	.00	.24	.35	.47
Isovalerate						
CON	1.03	.45	.59	.74	.76	.71
U	.68	.42	.70	.58	.61	.63
UZ	.50	.34	.51	.49	.70	.80
UZM	.65	.64	.58	.67	.83	.78
Valerate						
CON	.96	.82	.73	.86	.86	.76
U	.88	.89	.80	.80	.90	.82
UZ	.70	.86	.81	1.03	.95	.89
UZM	.70	.89	.91	1.00	.83	.80

[a] Supplements CON= control, wheat middlings, cottonseed hulls, dehydrated alfalfa and molasses; U= CON + urea; UZ= U + zinc oxide; UZM= UZ + manganese oxide

Table 3. Statistics for volatile fatty acid concentration presented in Table 2.

		Main Effects		Contrasts (P < .05)[a]		
VFA	SE[b]	Treatment[c]	Time period[d]	1	2	3
Acetate	3.4	.0001	.0065	.0001	.6307	.2835
Propionate	1.0	.0001	.0032	.0001	.2780	.4254
Butyrate	.60	.0014	.0008	.0004	.1061	.3456
Isobutyrate	.09	.0050	.0010	.0011	.2888	.2284
Isovalerate	.11	.0777	.0149	.0833	.7264	.0494
Valerate	.08	.8258	.2899	-	-	-

[a] Observed significant level for contrast 1 = CON vs. (U + UZ + UZM)/3; 2 = U vs. (UZ+ UZM)/2; 3 = UZ vs. UZM

[b] Standard error of treatment means (n=20)

[c] Prairie hay *ad libitum* plus supplements CON= control, wheat middlings, cottonseed hulls, dehydrated alfalfa and molasses; U= CON + urea; UZ= U + zinc oxide; UZM= UZ + manganese oxide

[d] Rumen sampling periods: 2, 4, 8, 12, 18 and 24 h after feeding the supplement

VITA

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