DISSERTATION

# THE EFFECTS OF LONG-TERM MOLYBDENUM EXPOSURE IN DRINKING WATER ON MOLYBDENUM METABOLISM AND PRODUCTION PERFORMANCE OF BEEF CATTLE CONSUMING A HIGH FORAGE DIET

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ABSTRACT

THE EFFECTS OF LONG-TERM MOLYBDENUM EXPOSURE IN DRINKING WATER ON MOLYBDENUM METABOLISM AND PRODUCTION PERFORMANCE OF BEEF CATTLE CONSUMING A HIGH FORAGE DIET

In the current series of experiments the influence of long term molybdenum (Mo) exposure in the feed or water on copper (Cu) status, metabolic, reproductive, and carcass characteristics was investigated.

The objective of experiment 1 was to conduct a life-cycle production and health assessment of lactating and gestating beef cattle, and their calves, exposed to varying doses of Mo. In this experiment Commercial, multiparous beef cows (n=54 in year 1; n= 51 in year 2) with calves (approximately 2 months of age) were used to evaluate the effects of Mo source (feed or water) on reproduction, mineral status, and performance in cows and calves receiving a grass hay diet [dry matter (DM) basis: 6.6% crude protein; 0.15% S, 6.7 mg Cu/kg, 2.4 mg Mo/kg] for 553 d. Cows were stratified by age, body weight (BW), and liver Cu and Mo status, and were then randomly assigned to one of six treatment groups. Treatments were: 1) Negative control (NC; basal diet with no supplemental Mo or Cu); 2) Positive control (PC: NC + Cu; 3 mg of supplemental Cu/kg diet DM from $CuSO_4 \cdot 5H_2O$); 3) NC + 500 µg Mo/L from $Na_2MoO_4 \cdot 2H_2O$ supplied in drinking water (Mo 500-water); 4) NC + 1000 µg Mo/L of $Na_2MoO_4 \cdot 2H_2O$ supplied in drinking water (Mo 1000-water); 5) NC + Mo 1000-water + 3 mg of supplemental Cu/kg diet DM from $CuSO_4 \cdot 5H_2O$ (Mo 1000-water+Cu); and 6) NC + 3.0 mg of supplemental Mo/kg diet DM from $Na_2MoO_4 \cdot 2H_2O$ (3.0 Mo-diet). During the winter months,

animals were housed in three replicate pens per treatment and during the summer months animals were housed in separate pastures by treatment. Animals were allowed ad libitum access to both feed and water throughout the experiment. Cows were bred via artificial insemination during the summer months of both years of the experiment and calves were weaned at approximately 6 months of age in the fall of both years. All cows and calves were weighed, bled, and feed and water intake were determined every 28d. Cattle receiving diets containing less than 10 mg Cu/kg DM total diet became Cu deficient over the course of the experiment as determined by liver and plasma Cu concentrations. However, no Mo toxicity or Cu deficiency signs (e.g., reduction in growth rates, reproductive performance, or immune function) were observed throughout the course of the experiment for any treatment. Results suggest that Mo supplemented in water or feed at concentrations used in this experiment had minimal impact on Cu status and overall animal performance. However, dietary Cu concentration below 10.0 mg Cu/kg DM total diet reduced liver and plasma Cu concentrations to values indicative of a marginal Cu deficiency in beef cows.

The objective of the chapter 3 review was to examine the impact of Mo in drinking water on cattle performance and Mo and Cu metabolism. The majority of Mo research has focused on the antagonist effect of Mo, alone or in combination with elevated dietary S, on Cu absorption and metabolism in ruminants. Diets containing both >5.0 mg of Mo/kg DM and >0.33% S have been reported to reduce the Cu status in cattle and sheep. Therefore, due to the potential for inducing Cu deficiency, Mo and S concentrations in the diet should be monitored and kept within appropriate values. Elevated sulfate concentrations in drinking water can also be detrimental to livestock production, especially in ruminants. High concentrations of sulfate in water have been extensively studied in cattle because high-sulfate water induces polioencephalomalacia in ruminants. However, little research has been conducted investigating the impact of Mo in water on Cu metabolism in ruminants. Based on the limited number of published experiments, it appears that Mo in drinking water may have a lower antagonistic impact on the cu status in cattle when compared to Mo consumed in the diet. This response may be due to a certain percentage of water bypassing the rumen when consumed by ruminants.

The chapter 4 experiment objective was to of this experiment was to investigate the influence of prolonged exposure to elevated Mo water concentrations on apparent absorption and retention of both Cu and Mo in pregnant multiparous beef cows. In this experiment twelve multiparous beef cows of similar BW, age, and gestational length, from a larger cow-calf study, were utilized to evaluate the effects of molybdenum (Mo) consumption method (feed or water) on apparent absorption and retention of copper (Cu) and Mo. Cows (n=54) with calves had been assigned to one of six dietary and/or water treatments (n=9 cow-calf pairs per treatment) 301 d prior to selecting a sub-group of 12 cows. Treatments consisted of: 1) negative control (control; basal diet with no supplemental Mo or Cu), 2) positive control (control + 3 mg of supplemental Cu/kg DM), 3) control + 500 µg Mo/L from $Na_2MoO_4 \cdot 2H_2O$ supplied in drinking water, (4) control + 1000 µg Mo/L of $Na_2MoO_4 \cdot 2H_2O$ supplied in drinking water, (5) positive control + 1000 µg Mo/L of $Na_2MoO_4 \cdot 2H_2O$ supplied in drinking water, and (6) control + 3.0 mg of supplemental Mo/kg diet DM from $Na_2MoO_4 \cdot 2H_2O$. The sub-group of cows were individually fed a low quality grass hay diet with their respective treatments, n=2 cows/treatment) for 14 d. On day 15, dry matter intake (DMI) was held at 90% of the group's average intake. Total fecal and urine output were then collected for 3 d. Dry matter digestibility and water intake were similar across treatments. Copper intake and apparent absorption and retention of Cu were greater ($p < 0.05$) in cows receiving supplemental Cu when compared to non-Cu supplemented cows. Apparent absorption of Mo was similar across all treatments. Apparent retention of Mo was greater while apparent absorption of Cu was lesser ($p < 0.05$) in cows receiving 3 mg of Mo/kg dm and cows receiving 1000 µg Mo/L when compared to all other treatments. These data indicate that Mo source (feed vs water) may impact apparent absorption of cu in cows receiving a low quality forage diet.

The chapter 5 survey of central Rocky Mountian livestock producers objective was to understand the mineral consumption within the forage and water as well as Cu and Mo status of the grazing cattle. Commercial, multiparous, crossbred beef cows from 3 independent cow-calf production operations were selected to assess the molybdenum (Mo) and copper (Cu) status of cattle raised in the Rocky Mountains. Fifteen cows from each operation were selected at random, during early summer and late fall processing. At the time of cattle processing, both jugular venipuncture blood samples and liver biopsies were obtained from each cow. Furthermore, all diet components, forage, water, and supplement samples were obtained from each location. Plasma, liver, water, and feed samples were analyzed for Mo and Cu concentrations via inductively coupled plasma mass spectrometry. Feed samples were also analyzed for moisture, crude protein (CP), ash, ADF and NDF. Water samples were sent to an established laboratory for general water quality analysis. On average, in the current survey forages samples contained 55.61% DM, 8.37% CP, 34.91% ADF, and 54.98 NDF with a Cu:Mo ratio of 2.8:1. Additionally, the water quality of the samples obtained were well within the "safe and should pose no health problems" category for beef cattle. Plasma Mo concentrations of 0.22 (±0.10) mg/kg DM were considered to be elevated in 64% of all samples obtained, likely a result of the elevated Mo forage concentrations in the grazed plant material. Plasma Cu of 0.83 (±0.11) concentrations were within normal ranges for all samples obtained. Molybdenum and Cu liver concentrations of 3.74 (±1.29) and 82.54 (±22.76) respectively, were within ranges considered to be normal for beef cattle for all samples collected. Based on the results of this survey, Mo and Cu plasma and liver concentrations in Colorado cow-calf operations in the central Rocky Mountians were similar to Mo and Cu plasma and liver concentrations in Cu supplemented cows in the previously described 2-year Mo supplementation cow-calf experiment. Furthermore, these data suggest that Cu supplementation at NASEM (2016) recommended concentrations of 10 mg Cu/kg DM total diet (or greater) meets the animals dietary Cu requirement for cattle consuming forages sampled.

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# CHAPTER 1 – REVIEW OF LITERATURE

## INTRODUCTION

There are six essential nutrients required for life by all organisms: water, carbohydrates, protein, fat, vitamins, and minerals. Essential minerals can further be classified based on intake requirements into macro- or trace-minerals. Currently NASEM (2016), has classified seven macro minerals, calcium (Ca), magnesium (Mg), phosphorus (P), potassium (K), sodium (Na), chlorine (Cl), and sulfur (S) and as the name suggests they are required in amounts larger than the trace minerals. Macro minerals are represented as a percentage of the diet and required in gram amounts whereas trace minerals are required in concentrations of milligrams or even micrograms (Paterson and Engle, 2005; NASEM, 2016). The roles of macro minerals in the body include but are not limited to fluid balance, osmotic pressure, membrane potential, and structural components of bone and other tissues (NASEM, 2016). Trace minerals are involved in various biological reactions as integral parts of metalloenzymes, enzyme cofactors, or hormone components (NASEM, 2016). Trace minerals for beef cattle have also been defined by NASEM (2016) as chromium (Cr), cobalt (Co), copper (Cu), iodine (I), iron (Fe), manganese (Mn), molybdenum (Mo), nickel (Ni), selenium (Se), and zinc (Zn). However, based on the findings of current research, Cr, Mo, and Ni requirements have not been defined for beef cattle (NASEM,2016).

Mineral consumption within narrow ranges is critical for health and optimal performance for cattle. In cow-calf systems minerals play roles in growth, health, and reproduction through their integral parts in the metabolism of other nutrients and key contributions in enzyme functionality. Both under and over consumption of trace minerals can result in the body compensating for the imbalances by depressing performance and health, and occasionally by causing noticeable metabolic diseases (Paterson and Engle, 2005; Spears et al., 2022). Primary trace mineral deficiencies are a consequence of inadequate consumption of the appropriate amounts needed to meet their requirements given their physiological status (Olson, 2007). Secondary deficiencies are a result of abnormal absorption, transport, retention, or excretion of a given element often stemming from diseases, parasites, or antagonistic effects of other components in the diet (Olson, 2007). As previously mentioned, not all trace elements have a clearly established requirement concentrations similarly, in some cases, no upper tolerable limits have been determined. Toxicities occur when animals consume excess quantities which similar to deficiencies can result in depressions in health and performance (NASEM, 2016). Additionally, over supplementation of trace minerals results in increased excretion of these elements into the environment which could potentially cause issues with runoff or the spreading of manure on farmlands (NASEM, 2016).

Cow-calf producers manage several variables at any given time. These variables include but are not limited to, basal diet characteristics, animal physiological state, environmental conditions, economics, and facility limitations all of which make these production systems challenging to manage. While certain variables may be dependent on location/region, they are particular to the individual operation and production goals of the system. However, because these animals rely heavily on a forage based diet (grazed and/or stockpiled), trace minerals (Se, Cu, Zn, Mn, Co, and I) naturally occurring within forages can be below or above the animal's requirements during certain times of the year. Furthermore, dietary antagonists such as excess S, Mo, and Fe can reduce the availability of certain trace minerals (e.g., Cu, Se, etc.) and cause deficiencies to occur (Arthington and Ranches, 2021). Therefore, if basal dietary ingredients do not meet the animal's requirement, supplementation strategies should be implemented. Thorough management strategies are required when supplementing trace minerals, one must pay careful attention to concentrations in order to meet the animal's requirement and at the same time not to over supplement required trace minerals.

Supplementation methods are a balancing act of animal needs, cost, and environmental implications. There are indirect supplementation techniques that can be used to supplement minerals to grazing cattle. These methods involve the manipulation of plant growing conditions and mineral availability in the soil. However, the more commonly applied direct mineral supplementation methods are with free-choice mineral feeders, protein/energy supplement mineral fortification, injection, and long-term slow release boluses. Therefore, this review will focus on the role of trace minerals in beef cow-calf production systems as well as common methods of trace mineral supplementation strategies implemented by cow-calf producers.

## TRACE MINERALS

### *Chromium*

Chromium functions as a component of glucose tolerance factor intensifying insulin and insulin like growth factor I activity (McCarty, 1980; Mertz, 1992; Spears et al., 2012). Thus, it is involved in the metabolism of carbohydrates, lipids, and proteins (Stahlhut et al., 2006; Bernhard et al., 2012; Baggerman et al., 2020). Stahlhut et al. (2006) conducted a long-term experiment where 152 gestating Angus and Simmental cows of varying ages were randomly assigned to a control of no supplemental Cr or a treatment of 40 mg Cr/kg DM of mineral (from Cr picolinate) resulting in an average intake of 3.5 mg Cr/cow/d. In this experiment cows grazed tall fescue pastures during the grazing season, and in the winter months animals consumed primarily a grass hay diet. Animals were offered a free choice mineral supplement year-round, which depending on treatment group included or was absent of supplemental Cr (Stahlhut et al, 2006). On day 65 Stahlhut et al. (2006) conducted a prepartum glucose tolerance test and reported that Cr supplementation resulted in lower plasma glucose concentrations but glucose clearance rates were not affected by Cr supplementation. However, decreasing concentrations of plasma glucose (from Table 2 in Stahlhut et al., 2006) over time with improved glucose clearance and insulin sensitivity following a glucose tolerance test on day 126 postpartum in Cr supplemented cows, suggests that Cr supplementation improved glucose sensitivity to less insulin in plasma (Stahlhut et al., 2006). Non-esterified fatty acids (NEFA) concentrations in plasma after parturition of cows receiving supplemental Cr was lower suggesting Cr impacts lipid mobilization. Furthermore, similar decreases in NEFA concentrations were observed in growing feedlot steers (Bernhard et al., 2012). This experiment included 20 steers and was conducted during the receiving feedlot period. Animals were randomly assigned to either a control group, with no supplemental Cr, or a treatment group, receiving 0.2 mg Cr/kg DM as Cr Propionate. (Bernhard et al., 2012). Steers were stepped up to a high concentrate steam-flaked corn diet over a 28- day period, and treatment duration continued till day 53 with animals in the treatment group receiving Cr additions to the typical vitamin and mineral premix. On d 52 blood samples were collected from each steer before, during, and after a glucose tolerance test, samples were processed, and serum was analyzed for NEFA concentrations via a NEFA-HR assay (Bernhard et al., 2012). Similar to Stahlhut et al. (2006), Bernhard et al (2012) reported lesser NEFA concentrations in steers receiving Cr supplementation both pre- and post-glucose infusion when compared to control steers, $0.231 \pm 0.018$ and $0.172 \pm 0.009$ mmol/L, and $0.296 \pm 0.018$ and $0.216 \pm 0.009$ mmol/L, respectively ($P \leq 0.01$). Baggerman et al. (2020) reported increased translocation of GLUT4 to the interior of the skeletal muscle fiber from the sarcolemma in feedlot steers supplemented with 0.45 mg/kg DM Cr propionate. These changes in metabolism have the potential to positively impact animal performance as described with an improvement of milk production in dairy cows (Vargas-Rodriguez et al., 2014), and growth in feedlot steers (Baggerman et al., 2020).

Chromium in fresh forage or feed is usually found in amounts assumed to meet beef cattle requirements (Puls, 1994). However, given performance improvements upon supplementation of Cr it is important to evaluate feed Cr concentrations. While variable across feed types, Cr concentrations are typically low, with grass hay averaging 0.155mg/kg DM, with ranges between 0.098 to 0.320 mg/kg DM, and alfalfa hay samples averaging 0.522 mg/kg DM, with ranges between 0.199 to 0.889 mg/kg DM (Spears et al., 2017). Specifics relating to livestock species absorption and transport mechanisms of Cr are unknown and studies on such Cr metabolic involvement are assumptions based on findings in humans and laboratory animals (Hayirli, 2005).

Absorption of Cr occurs via passive diffusion through the small intestines (Dowling et al., 1989), where it is then transported through the blood bound to albumin and β-globulin (Hayirli, 2005), and transferred to varying tissues by transferrin (Linder, 1991). The assessment of Cr status has been a challenge for researchers because typical feed stuffs contain Cr concentrations in the ppb range. Such low concentrations can result in result variability dependent on sample preparation methods (Hayirli, 2005). Currently, there are no outlined Cr requirements for beef cattle and further research is needed before dietary standards are set. As of 2009 chromium propionate (CrP) is and remains the only form of Cr approved by the Food and Drug Administration (FDA) for inclusion in beef cattle diets in the United States at 0.50 mg Cr/kg (Spears et al., 2017; Baggerman et al., 2020).

***Cobalt***

Ruminants lack a vitamin $B_{12}$ dietary requirement as rumen microbial production of $B_{12}$ will meet biological needs for the host animal (NRC, 1996). Cobalt functions as a component in microbial cobalamin (vitamin $B_{12}$) synthesis, making Co inclusion in ruminant diets essential. Anerobic production of cobalamin by various microbial populations in the rumen is essential for the production of energy metabolism and propionate production (González-Montaña et al., 2020). The conversion rate is low, as it has been estimated that 3 to 13% of dietary Co will be incorporated into ruminal cobalamin and even less will be absorbed by the ruminant (Smith, 1987). Vitamin $B_{12}$ is critical for several biologic reactions and is involved in lipid, carbohydrate, and amino acid metabolism (González-Montaña et al., 2020). The vitamin $B_{12}$ dependent enzymes are methylmalonyl-CoA mutase and methionine synthase which are involved in blood glucose synthesis from propionate production in the rumen and methionine synthesis respectively (McDowell, 2017). Currently, there is no known specific function of Co in the body except for its inclusion in the corrin ring complex of vitamin $B_{12}$. therefore, Co status is really a function of $B_{12}$ status (Tiffany et al., 2003; González-Montaña et al., 2020). A study conducted at North Carolina State University randomly assigned 60 growing angus steers to one of six treatments containing 0.0, 0.05, 0.10, or 1.00 mg of supplemental Co/kg DM from Co carbonate ($CoCO_3$) or Co propionate (CoPr; Tiffany et al., 2003). In this experiment cattle supplemented with Co had higher vitamin $B_{12}$ concentrations in the plasma and liver than control cattle receiving no supplemental Co, indicating dietary consumption of Co increases ruminal cobalamin synthesis. In some cases, Co supplementation has translated to performance improvements. For example, milk yield increases in dairy cattle (Kincaid et al., 2003), or improved marbling scores of feedlot steers (Tiffany et al., 2003) have been reported in Co supplemented animals. Other researchers have reported no impact of Co supplementation on animal performance.

Cobalt consumption among grazing ruminants is dependent on soil characteristics, and abundance and availability within the feed source. Typically, the concentration of Co is greater in legumes than grasses, and concentrations of Co will decrease with plant maturity and increase in plants as soil pH increases (Spears, 1994; Underwood and Suttle, 1999). Cobalt will need to be consumed in quantities that result in ruminal fluid concentrations of at least 0.5 mg/L for optimum vitamin $B_{12}$ production (González-Montaña et al., 2020). These concentrations will allow for adequate synthesis of vitamin $B_{12}$ by the rumen vitamin $B_{12}$ synthesizing microbes as there is no usable vitamin $B_{12}$ in the diet (González-Montaña et al., 2020). While the exact mechanisms of absorption and transport of vitamin $B_{12}$ from the gastrointestinal tract of ruminants is unknown, it is theorized that vitamin $B_{12}$ is absorbed through endocytosis in the distal small intestine with help from intrinsic factor binding protein. Vitamin $B_{12}$ is then transferred through the blood bound to transcobalamin and with a majority (60% of body reserves) being stored in the liver with some distribution in other body tissues or it is secreted in milk (González-Montaña et al., 2020). Cobalt status of ruminants is normally assessed through analysis of Co or vitamin $B_{12}$ concentrations in the liver or serum, with other methods including measurements of metabolites methylmalonic acid, homocysteine, or holotranscobalamin in the blood or methylmalonic acid in the urine (Spears, 1994; González-Montaña et al., 2020).

While imbalances in Co consumption among grazing ruminants is not prevalent in the United States, it does occur, and most supplements will target Co intakes at or above 0.15 mg/kg dry matter intake (DMI; Arthington and Ranches, 2021). The current approved sources of Co supplements for beef cattle in the United States include the organic acetate or inorganic carbonate and sulfate sources (Raths et al., 2023). Currently, the sulfate form of Co is not economically feasible for most production systems and the lack of abundance of Co acetate in the marketplace makes Co carbonate the most widely used source in cattle supplements (Raths et al., 2023). The target intake among mineral supplements is consistent with the 0.15 mg Co/kg DM requirement set by NASEM (2016) which is an increase from the previous 0.10 mg Co/kg DM requirement (NRC, 1996). Animals grazing Co-deficient pastures for an extended period of time may exhibit deficiency symptoms from decreased appetite and moderate weight loss to liver steatosis and anemia (Tiffany et al., 2003; NASEM, 2016; González-Montaña et al., 2020; Arthington and Ranches, 2021). Given the relation of Co to vitamin $B_{12}$ in ruminants, and the implications of methylmalonyl-CoA mutase in the conversion of succinate to propionate, consumption outside of the requirement may disrupt lipid metabolism and gluconeogenesis (Tiffany et al., 2003; NASEM, 2016; González-Montaña et al., 2020). Cattle can handle approximately 100 times the dietary requirement making Co toxicity rare in ruminants and likely a supplement formulation error if it occurs (NASEM, 2016; Arthington and Ranches 2021).

***Copper***

Copper is incorporated into several metalloenzymes including lysyl oxidase, cytochrome oxidase, superoxide dismutase (SOD), ceruloplasmin (Cp), and others which are critical to immune function, erythrocyte formation, energy production, hormone construction, collagen synthesis, pigmentation, protection from free radicals and is essential for life (McDowell, 2003; Spears, 1994; López-Alanso and Miranda, 2020; Arthington and Ranches 2021). Concentrations of Cp, a major acute-phase protein carrying up to 90% of the circulating Cu, will increase in instances of inflammatory incident in Cu adequate cattle (Arthington et al., 1996; Arthington and

Ranches 2021). However, this response is not observed when Cu status is deficient (Arthington et al., 1996). Superoxide dismutase is an antioxidant that functions to control oxidative stress by converting harmful superoxide free radicals into hydrogen peroxide (Fridovich, 1975). A study conducted by Arthington et al. (1996), 12 heifers were assigned to one of two treatment groups including a control group receiving supplemented Cu and a treatment group with supplemented Mo (Mo:Cu ratio of 2.5:1), after 129 days a secondary Cu deficiency was achieved and verified through measurement of liver copper concentrations. Following established Cu status (adequate or deficient depending on treatment) an immune challenge was conducted resulting in increased Cp by 48-h in Cu adequate control group but not in the Cu deficient heifers. The researchers also reported that Cu treatment and immune challenge had no effect on SOD activity, however Mo supplemented treatment steers did have less erythrocyte activity prior to an immune challenge. These data suggest that copper status may alter acute-phase protein response and immune function.

Copper and its antagonist Fe, Mo, and S vary in concentration through pastures and forages based on plant species and strain, plant maturity and part (leaf vs stem), soil conditions and fertilizer usage, and environmental conditions (Gilbert, 1952; MacPherson, 2000). The consumption of elevated concentrations of Cu antagonist can result in secondary Cu deficiency. As increased abundance of antagonists can cause increased binding competition or the formation of insoluble Cu complexes in the rumen or systemically, impairing absorption or activity in the body (Gould, and Kendall, 2011). Furthermore, because so many variables play a role in forage Cu concentrations it is difficult to predict Cu concentrations in forages. Legume Cu concentrations are typically higher than Cu concentration in grasses (NASEM, 2016), however the opposite is true for tropical legumes and grasses (3.9 vs 7.8 mg/kg DM; Underwood and

Suttle, 1999; MacPherson, 2000). Leaves and flower heads typically contain greater concentrations than stems. However, total plant Cu content decreases with maturity (MacPherson, 2000). Upon consumption and solubilization in the rumen, $Cu^{2+}$ will be reduced to $Cu^{+}$ where it will be absorbed into the small intestine primarily by a specific Cu transporter: Ctr1, and the remaining by a non-specific Cu transporter, divalent metal transporter 1 (DMT1) (López-Alanso and Miranda, 2020). It is important to mention that most Cu will travel through the gastrointestinal track and be excreted in the feces (Gooneratne et al., 1989). Meanwhile the Cu that is absorbed is transported through systemic circulation from the gut to the liver bound to a specific copper carrier, transcuprein, or albumin (López-Alanso and Miranda, 2020). Once in the liver Cu can be incorporated into various Cu containing/dependent enzymes, be stored in lysosomes, or due restricted regulatory mechanisms – low concentrations of Cu can be excreted in the bile in times of excess consumption (López-Alanso and Miranda, 2020).

As can be speculated given the various factors that impact Cu status of ruminants, management of this trace mineral proves challenging for producers and nutritionists. The current Cu requirement for beef cattle is set at 10 mg Cu/kg DM (NASEM, 2016), however recommendations can vary depending on the concentration of present antagonist in the feedstuffs. In general, 10 mg Cu/kg DM should be sufficient if S concentrations don't exceed 0.25% diet DM and Mo concentrations remain under 2 mg/kg DM (Spears 2003; NASEM, 2016). Additionally, maximum tolerable limits of Cu have been set at 40 mg Cu/kg DM (NRC, 2005). Currently there is no evidence that grazing beef cattle need more than 10 mg Cu/kg DM when critical concentrations of antagonists are not present. Regardless, Mortimer et al. (1999) reported that in 23 states (n = 709) that 66.7% of forage samples were below the 10 mg Cu/kg DM requirement, and over 40% of samples had Cu:Mo ratios that would suggest marginal deficiency in beef animals grazing only those forages (Spears et al., 2022). The best method for determining the Cu status of ruminants is analysis of liver Cu concentrations (Spears et al., 2022). Other methods to determine Cu status are through analysis of Cu concentrations in the plasma, serum, and hair, as well as SOD activity in erythrocytes (Spears et al., 2022). Liver Cu concentrations are more sensitive to changes in dietary consumption of Cu and therefore are a better method for establishing animal Cu status than blood Cu concentrations (Spears et al., 2022). Normal plasma Cu concentrations range from 0.6 to 1.5 mg/L (Underwood and Suttle, 1999), and Puls (1994) defined adequate liver Cu concentrations 25 – 100 mg Cu/kg wet weight, with plasma Cu concentrations not decreasing below normal ranges until liver Cu concentrations were below 40 mg Cu/kg DM (Claypool et al., 1975).

With such narrow ranges between deficient, adequate, and toxic dietary concentrations of Cu, the potential for both deficiencies and toxicities are present. Copper deficiency is characterized by symptoms such as anemia, depigmentation and changes in hair growth, bone fragility, cardiac issues, diarrhea, impaired reproduction, and reduced growth (Spears, 1994; Underwood and Suttle, 1999; NASEM, 2016). The diagnosis of Cu deficiency is evident through liver Cu concentrations 0.5 – 10.0 mg/kg wet weight with marginal status defined with concentrations of 5. 0 – 25.0 mg/kg wet weight (Puls, 1994). Plasma Cu concentrations less than 0.4 mg/L are indicative of Cu deficiency and 0.5 mg/L is suggestive of a marginal Cu status (Spears et al., 2022). While most forages are not high enough to cause Cu toxicity in grazing cattle, occasionally, it may be a consequence of over supplementation or a supplement formulation error. Unfortunately, a ruminant’s lack of regulatory methods to excrete excess Cu in the body can result in substantial accumulation in the liver when consuming elevated amounts. Copper toxicosis may be characterized by elevated blood liver enzymes but can occur absent of clinical signs indicative of a nutritional issue (Spears et al., 2022). However, toxicity may present itself through hemolytic crisis, hemolysis, methemoglobinemia, hemoglobinuria, jaundice, icterus, necrosis, and death (NRC, 2005). Health concerns and decreases in performance associated with Cu toxicosis and deficiency, paired with the optimization of performance during adequate Cu status makes appropriate supplementation critical and advantageous. Copper is often supplemented in the diet or through free-choice mineral mixes and can be from inorganic, hydroxy, or organic sources. Each production system is unique and in order to determine the right Cu source the producer must weigh bioavailability, cost, and purchasing abundance.

***Iodine***

Iodine is a necessary component of thyroid hormones, thyroxine (T4) and triiodothyronine (T3), which are involved in energy metabolism, oxidation, thermoregulatory processes, and reproduction (Underwood and Suttle, 1999; Arthington and Ranches, 2021; Żarczyńska and Świerczyński, 2023). Iodine is relatively abundant in the environment. Plants contain variable concentrations of I which are dependent on plant species, environment conditions, and soil characteristics. Plants growing in rich soils have the potential to have greater concentrations of I. However, the plants' ability to utilize I in the soil is quite variable among species and there can be a ten-fold difference among species within a pasture and those variations can be impacted by seasonal effects (Underwood and Suttle, 1999). In addition to the basal diet, I can be supplemented in cattle diets as an inorganic salt (sodium iodine, potassium iodate, sodium iodate, calcium iodide, etc.), elemental I, and organic compounds (methyl iodide and ethylenediamine dihydroiodide, EEDI). Lejeune et al. (2010) reported significantly higher blood I concentrations in cattle raised in more intensive indoor operations consuming a high concentrate total mixed ration with mineral/vitamin premix compared to a group of cattle raised in a pasture environment outdoors. Once consumed I is readily absorbed mainly as iodine in the rumen (70-80%), omasum, and abomasum where it then travels to its target organ via plasma proteins (Miller et al., 1975). A majority of the I will be transported to the thyroid gland where it will be incorporated into T4 and T3 hormones. Other target organs include the uterus, mammary gland, kidney, and liver (Miller et al., 1975; Żarczyńska and Świerczyński, 2023). The primary route of excretion is through the kidney and urine elimination, smaller amounts can be excreted in the feces, milk, and bile (Miller et al., 1975; Żarczyńska and Świerczyński, 2023).

Urine excreted I, protein bound-I, thyroid hormones, and concentrations of I in the milk, liver and thyroid gland have been used as indicators of body supply and animal status (Puls, 1994; Underwood and Suttle, 1999; Żarczyńska and Świerczyński, 2023). The current I requirement is not well verified but has been set at 0.5 mg I/kg DM which has been sufficient in most grazing production settings given low abundance of goitrogenic substances in the feed which can increase the I requirement (NASEM, 2016). While historically I deficiencies were widespread issues in livestock, I fortification of salt has rectified many of issues. In recent cases of I deficiency, the presence of goitrogenic substances in the ration has been the primary cause of I deficiency. However, in most cow-calf grazing systems I-fortified salt along with basal diet I consumption should prevent I deficiency (Arthington and Ranches 2021). Enlarged thyroid gland/goiter, weak unthrifty calves, and reproduction impairments such as poor conception rates and retained placentas have been reported as classical symptoms of I deficiency (Miller et al., 1988; McDowell, 2003). Puls (1994) has defined deficient I concentrations as: protein bound-I between 3.0 – 5.3 μg/dL, milk concentrations between 3 – 25 μg/L, liver concentrations less than 0.094 mg/kg wet weight, and serum T4 concentrations $< 7 – 30$ ng/ml. The maximum tolerable limit for I has been set at 50 mg/kg diet (NRC, 2005). Instances of I toxicity in grazing cattle are rare and concentrations associated with toxicity may be related to source of I intake. For example, Newton et al, (1974), fed 32 Holstein bull calves varying levels of I (0, 10, 25, or 50 mg/kg DM) from calcium iodate and reported some calves in the 50 mg/kg treatment groups had severe coughs, persistent nasal drainage and decreased weight gain when compared to other treatment groups but this is not the case with calves and lactating cows consuming 50 mg/kg EDDI (NRC, 2005).

***Iron***

The trace mineral with the greatest abundance in the body is Fe which is incorporated into many proteins associated with the utilization or transport of oxygen (Underwood and Suttle, 1999; NASEM 2016). Iron has many modes of action through enzyme activators or being integrated into cytochromes and F-S proteins involved in the electron transport chain and most notably as a component of hemoglobin and myoglobin (Spears, 1994; Underwood and Suttle, 1999; McDowell, 2003; NASEM, 2016). The requirement of Fe for beef cattle has been set at 50 mg Fe/kg DM (NASEM, 2016). Bernier et al. (1984) reported mild anemia at 6-wk and severe anemia and feed refusal at 14-wk in veal calves consuming milk replacer devoid of Fe. The requirement in adult cattle is less defined and previous literature theorizes it would be less than young animals because blood volume isn't increasing in most cases and Fe is recycling during erythrocyte turn over (NASEM, 2016).

Most forages contain between 70 to 500 mg Fe/kg (NASEM, 2016). Campell et al. (1974) reported results of pastures in New Zealand with Fe concentrations ranging from 111 to 3850 mg/kg. These extremely high concentrations in forages are likely a result of soil contamination (NASEM, 2016). Other factors contributing to Fe variability are plant species, environmental climate, and soil type (Underwood and Suttle, 1999). Water and soil Fe consumption also contribute to the total Fe intake in ruminants (NASEM, 2016). Iron will be absorbed according to need from the small intestine, transferred to the plasma, and bound to transferrin, a non-heme glycoprotein, for transport to cells throughout the body (Underwood and Suttle, 1999; Harvey, 2008). Absorbed Fe is efficiently recycled in the body (Harvey, 2008). A majority of the Fe in plasma will be incorporated into hemoglobin and erythrocytes, the remaining will be taken up by nonerythroid tissues such as the liver where it can be stored as ferritin and hemosiderin (Underwood and Suttle, 1999; Harvey, 2008).

With the consumption of practical feedstuff and Fe recycling, deficiency does not occur often in grazing cattle in the United States (Greene, 2000). Occasionally, Fe deficiency can result in young calves only consuming milk in highly controlled environments and cattle with parasite infections or disease in the animal that's causing blood loss (McDowell, 2003; NASEM, 2016). If deficiency occurs the resulting symptoms are anemia, lethargy, depressed feed intake and weight gain, and pale mucus membranes (NASEM, 2016). On the other hand, Fe toxicity exhibits symptoms such as diarrhea, metabolic acidosis, hypothermia, and depressed feed intake and weight gain (NASEM, 2016). The upper tolerable limits have been set at 500 mg Fe/kg DM (NRC, 2005). Additionally, excess Fe consumption can have antagonistic effects on Cu availability to the animal resulting in a secondary Cu deficiency (Greene, 2000; NASEM, 2016). A study by Bremner et al. (1987) 27 Friesian steers calves barley straw with 0, 729.9, 1459.8, or 2189.7 mg Fe/kg for 24-wk which resulted in reduced Cu concentrations in the liver and plasma indicating this antagonistic effect of Fe on Cu status. Therefore, it is important to know Fe concentrations in the water, soil, and forages grazing animals are consuming as excess Fe ingestion may be unavoidable and, in such situations, Cu supplementation over requirements may be warranted (Underwood and Suttle, 1999; NASEM, 2016).

***Manganese***

Manganese is involved in the activation of several metalloenzymes, such as SOD, pyruvate carboxylase, arginase, several hydrolases, kinases, decarboxylases, and transferases, and is essential for the activation of glycotransferases (Underwood and Suttle, 1999; NASEM, 2016. In an experiment from Hansen et al. (2006a) 80 heifers consuming a basal diet of 15.8 mg Mn/kg DM were supplemented 0, 10, 30, or 50 mg Mn/kg DM from $MnSO_4$ for 196-d. The authors reported an increased liver Mn concentrations in Mn supplemented heifers when compared to non-supplemented heifers. However, Mn supplementation in this study did not affect measures of growth (ADG, DMI, gain to feed ratio) or reproductive performance (pregnancy rate, conception rate, and services to conception) for the extent of the experiment (Hansen et al., 2006a). In another experiment, 10 heifers from the control (0 mg Mn/kg DM) and 50 mg Mn/kg DM supplemental groups (Hansen et al., 2006a) were retained for a long-term 267-d Mn supplementation study (Hansen et al, 2006b). This experiment reported no differences in whole blood Mn concentrations of calves born to supplemented heifers however, calves born to control group heifers weighed less and exhibited some Mn deficiency symptoms such as superior brachygnathism, disproportionate dwarfism, and swollen joints (Hansen et al., 2006b). These results indicate the 15.8 mg Mn/kg DM may meet requirements for growth, onset of estrus, and conception in heifers, but it is not sufficient to meet fetal development requirements. While 50 mg Mn/kg DM is enough to meet fetal development requirements it is impossible to estimate a closer requirement based on this experiment.

Similar to most trace minerals, Mn found in plant material is variable and dependent on plant species and soil characteristics such as soil drainage and pH. Plant materials contain 30 to 50 mg Mn/kg on average, with inorganic (manganese sulfate and manganese oxide) and organic (manganese methionine, manganese amino acid chelate, etc., NASEM, 2016) forms of Mn being used for Mn supplementation. There is little information regarding the mechanisms in which Mn utilizes for absorption, transport, and excretion in the body of cattle and other ruminants. If Mn absorption resembles that of other species after consumption it will be absorbed through the small intestine via divalent metal transporters indicating a competition on binding sites for transition metals (Goff, 2018). In a 13-d study looking at the oral dose retention of $^{54}$Mn, Vagg (1976) reported <1% of Mn retained in the body, and Weiss and Socha (2005) reported Mn absorption at 6 – 7% when Mn consumption of 700 mg Mn/d. Even though these exasperated ingestion concentrations exhibit a greater absorption efficiency it still indicates most Mn will be excreted. A small amount of free Mn exists in plasma but most absorbed Mn will be bound to α2-macroglobulin and albumen then transported to cells or target organ (Goff, 2018). While Mn stores in the body are not large most of what is found is located in the skeleton, liver and hair. Homeostatic control on Mn in the body seems to be coordinated by biliary mechanisms in which most of the Mn absorbed will make its way to the bile where it will be excreted in the feces (Goff, 2018).

The Mn requirement for reproducing beef cattle has been set at 40 mg/kg DM and 20 mg/kg DM for growing and finishing cattle (NASEM, 2016). Regardless of variability in Mn concentrations in feedstuffs, in most practical situations Mn content in forages is enough to meet cattle requirements. However, due to low cost and being relative non-toxic to cattle, Mn is often supplemented in trace mineral mixes to prevent any deficiencies from arising (Greene, 2000). For Mn, analysis of feedstuffs and understanding mineral antagonism are the best way to assess if animals are consuming appropriate amounts for the required level of production expected (Greene, 2000; Spears et al., 2022). This is because assessing Mn status of the animal has proven difficult as there are currently no standards to accurately estimate Mn status and without observed clinical symptoms (Spears et al., 2022). Manganese concentrations are measured on µg/L basis in the serum or plasma, whereas concentrations of Zn or Cu are measured on a mg/L basis ultimatly indicating greater chance for analysis error and the requirement for more sensitive analytical instrumentation (Spears et al., 2022). Great variability in reported values of Mn concentrations in liver, plasma, serum, whole blood, and hair as well as Mn-dependent SOD activity has been seen across studies. Even animals exhibiting deficiency symptoms in growth, reproduction, structural integrity do not always display differing metrics from controls. Hansen et al. (2006b) reported no differences in whole blood Mn concentrations of control and treatment calves when non-supplemented control calves exhibited signs of Mn deficiency. Manganese deficiency in grazing cattle is rare and symptoms include impaired reproductive soundness in both males and females, pregnancy terminations, and structural birth abnormalities (Arthington and Ranches, 2021; Spears et al., 2022). Manganese toxicities are rare in grazing ruminants due to low absorption and homeostatic control mechanisms (e.g., increased Mn excretion in the bile (Arthington and Ranches, 2021), however NRC (2005) set upper tolerance limits at 1,000 mg Mn/kg DM.

***Molybdenum***

The enzymes, xanthine oxidase, sulfide oxidase, and aldehyde oxidase all require Mo for proper function (Mills and Davis, 1987). Because of Mo involvement as a component in these enzymes Mo is involved in growth, reproduction, cellular oxidation, purine metabolism, and possibly Fe metabolism (Underwood and Suttle, 1999; Arthington and Ranches, 2021). Interactions among Mo-S-Cu have the potential to negatively impact ruminants via a secondary Cu deficiency. Microbial populations in the rumen will reduce sulfate to sulfide through fermentation, sulfide in turn can react with molybdate to form thiomolybdates in the rumen (Miller et al, 1988). Thiomolybdates produced in the rumen can: 1) interact with Cu in the rumen forming a cupric thiomolybdate which are insoluble complexes or 2) thiomolybdates can be absorbed where they have the ability to bind to Cu in the circulatory system, making Cu biologically unavailable (Miller et al., 1988; Suttle, 2010; Gould and Kendall, 2011).

The concentrations of Mo in plants varies based on soil pH and type, as well as plant species. Molybdenum in neutral or alkaline soils is more available to plants than Mo found in acidic soils (McDowell, 2003). Miltimore and Mason (1971) reported intermediate Mo concentrations of grasses at 2.0 mg/kg DM and legumes at 1.8 mg/kg DM. It is important to also calculate the Mo:Cu ratios as well as S concentrations in the forage to determine the Cu concentration needed in supplements for grazing cattle. It has been recommended to keep S in diets less than 0.29% and Cu:Mo ratios no less than 2:1 with 4:1 being more adequate to prevent Cu deficiency (Miltimore and Mason, 1971; NASEM, 2016).

Once consumed molybdenum will be solubilized in the rumen as molybdate where it can: 1) form thiomolybdates in the rumen that interact with Cu or 2) be absorbed as molybdate in the small intestines and transferred to the circulatory system (Miller et al., 1988; Underwood and Suttle, 1999; Gould and Kendall, 2011). Molybdate that is absorbed will be transported in plasma where it will be stored in tissues bound to xanthine dehydrogenase or aldehyde oxidase, or in the mitochondrial membranes bound to sulfite oxidase (Underwood and Suttle, 1999). Molybdenum that has been absorbed as molybdate will be excreted in the urine whereas, Mo that is incorporated in thiomolybdates will mainly be excreted in the feces (Miller et al., 1988; Underwood and Suttle, 1999).

There have been no requirements set for Mo consumption as there has never been a recorded event of Mo deficiency in beef cattle (NASEM, 2016). Therefore, the intake recommendations are associated with the Cu:Mo ratios to prevent a secondary Cu deficiency. There is the potential for Mo toxicity in ruminants and the Mineral Tolerance of Domestic Animals set upper limits between 5 and 10 mg Mo/kg DM (NRC, 2005). Molybdenum toxicity is described with symptoms including diarrhea, anorexia, loss of weight, stiffness, and changes in hair color (NASEM, 2016). An experiment by Phillippo et al. (1987) fed heifers diets supplemented with 0 mg Mo/kg DM or 5 mg Mo/kg DM and reported decreased live weights and conception rates among heifers consuming the increased Mo diets. Molybdenum status is typically assessed through liver and plasma Mo concentrations, Puls (1994) reported normal liver Mo concentrations at 0.14 – 1.40 mg/kg wet weight and plasma concentrations of 0.1 – 0.10 mg/L wet weight. Additionally, Mo concentrations in blood and milk are sensitive to Mo intake and therefore make them meaningful measures to estimate animal status (Miller et al., 1988).

***Nickle***

There are many uncertainties regarding the biologic functions, metabolism, and requirements of Ni in the ruminants and research findings regarding Ni have been variable. Nickle is required for the appropriate function of the enzyme urease. Urease is produced by ureolytic bacteria which are found in the rumen. Therefore, Ni is required by ureolytic bacteria for appropriate function (Spears, 1984; Underwood and Suttle, 1988). Ureases is used by bacteria to hydrolyze non-protein sources of N to ammonia for use in microbial protein synthesis (Spears and Harfield, 1978; Hailemariam et al., 2021). Nickel has been shown to activate enzymes in mammalian systems but has not been shown to be essential for mammalian enzymes (Miller et al., 1988).

Nickel consumption in basal ruminant diets is low and Ni abundance in plants, soil, and water is correspondingly low. Generally, the Ni content of grasses is lower than legumes and in pasture, plants with increasing maturity or soils that have been fertilized with Ca carbonate (to increase soil pH), will have reduced Ni concentrations (Miller et al., 1988). Welch and Cary (1975) reported Ni concentrations of 80 to 350 μg Ni/kg DM in pastures across the United States with average Ni content at 0.18 mg/kg DM. While there are many unknowns regarding the metabolism of Ni in ruminants it is speculated that absorption is low, around 1 – 5% of dietary consumption. Nickel excretion in calves receiving a basal diet with 62.5, 250, or 1000 mg Ni/kg DM supplemented to the diet excreted 97.3, 98.1, and 95.8% of the dietary daily supplementation in feces respectively (O'Dell et al., 1971). Intravenous supplementation of Ni increases Ni urine concentrations further suggesting dietary Ni excreted in the feces is not absorbed (Miller et al., 1999). It is hypothesized that Ni is transported via albumin in the circulatory system (Underwood and Suttle, 1999). Suspected, adequate concentrations of Ni in liver and serum range from 0.1 – 0.6 mg/kg wet weight and 1.2 – 5.6 μg/L respectively (Puls, 1994).

There is currently no established requirement for Ni in beef cows, and there are no naturally occurring Ni deficiencies in ruminants reported, suggesting the requirement is met by forages and there is unlikely a need for Ni supplementation under practical conditions (Miller et al., 1988; Underwood and Suttle, 1999). The maximum tolerable level has been set to 50 mg Ni/kg DM (NRC, 2005). O'Dell et al. (1970) supplemented 0, 62.5, 250, and 1000 mg Ni/kg DM from nickel carbonate to young dairy calves for 8-wks. Researchers reported calves consuming 62.5 mg Ni/kg DM has similar intake and weight gain to control animals whereas animals in the 250 and 1000 mg Ni/kg DM treatments had significant reductions in feed consumption and weight gain when compared to the controls (O'Dell et al., 1970). Furthermore, there were no gross anatomical differences relating to toxicity upon necropsy other than nephritis in bull calves consuming 250 mg Ni/kg or greater (O'Dell et al., 1970). This suggests that toxicity of Ni is a function of rumen microbial health (Underwood and Suttle., 1999)

***Selenium***

Selenium is involved in several biological functions within the body to help optimize reproductive efficiency, performance, and health. Within the immune system, selenium is critical for the formation and activation of helper T, cytotoxic T, and Natural killer cells, and its antioxidant role is accounted for because Se is an integral component of several selenoproteins such as, glutathione peroxidase, iodothyronine deiodinases, and thioredoxin reductases (Spears and Weiss, 2008; Mehdi and Dufrasne, 2016).

The presence of Se in forages is highly dependent on the soil Se concentration as well as the plant species and environmental conditions (Underwood and Suttle, 1999). A majority of Se concentrations in forages are based on geographical trends of the soil, though generally grasses tend to be higher in Se than legumes and fertilizing with phosphate will reduce Se concentration in plants (Underwood and Suttle, 1999). In the United States there are both regions of high and low Se. For example, the northern plains soil Se concentrations are relatively high, translating to Se uptake from plants that can eventually be grazed by ruminants that will in turn have sufficient Se status. This is evident through accumulation of Se in meat of harvested animals in these areas indicating animals may not need additional Se supplementation outside of the basal diet (Hintze et al., 2002; Arthington and Ranches, 2021). It is important to note, there are several geographical areas within the United States where this is not the case; when soil concentrations are low this will result in lesser concentrations in plants (0.02 to 0.05 mg Se/kg) and deficiency symptoms may be expressed in cattle, in these regions Se supplementation would be beneficial (NASEM, 2016; Spears et al., 2022). Ruminants consume both organic and inorganic sources of selenium. Selenomethionine (SeMet) is a naturally occurring form of organic Se in feedstuffs and is a common component of selenized yeast that is supplemented to cattle (Spears et al, 2022). Inorganic forms include selenate found in plants as well as sodium selenite, a common ruminant Se supplement (Spears et al., 2022). The source of Se consumed helps to predict its route of metabolism. Once consumed Se can be taken up by rumen microbes and incorporated into selenoamino acids or into insoluble Se forms such as elemental Se or selenide (Miller et al., 1988; Spears et al., 2022). Low absorption of Se in ruminants is suspected because of the formation of these insoluble Se compounds as most excreted Se found in feces is in the inorganic or insoluble forms (Spears et al., 2022). The majority of Se absorption occurs in the small intestine (Miller et al., 1988, Underwood and Suttle, 1999). Once in the body selenium trioxide will be reduced to selenide which will go through a set of complex reactions to form selenocysteine (SeCys) which can then be incorporated into Se metalloenzymes such as glutathione peroxidase (Spears et al., 2022). Absorbed SeMet can either enter the methionine pool and be incorporated into proteins in place of methionine or it can also be metabolized into SeCys (Spears et al., 2022). Selenium absorbed but not used will be excreted in the urine (Spears et al., 2022).

The current Se requirement for beef cattle has been set at 0.1 mg/kg DM by NASEM (2016), as consumption of concentrations 0.02 to 0.05 mg Se/kg DM resulted in reported clinical and subclinical signs of deficiency (Spears et al., 1986). Symptoms of Se deficiency include degeneration of the skeletal and cardiac muscle, lethargy, weight loss and diarrhea, anemia, and muted immune response which are further explained by measured downregulated iodothyronine

5’-deiodinase and glutathione peroxidase activity (Spears et al., 1986, Spears and Weiss, 2008; NASEM, 2016; Mehdi and Dufrasne, 2016). A study conducted at North Carolina State University assigned 72 crossbred cows consuming marginally deficient Se basal diet (0.03 to 0.05 mg/kg) to a control group with no additional supplements or to a treatment group receiving 30 mg Se from sodium selenite and 408 IU of vitamin E subcutaneously at 60-d intervals for 2-years beginning at an estimated 3.5 months prepartum (Spears et al., 1986). Cows receiving injections had higher plasma Se concentrations and glutathione peroxidase activity in whole blood and calves receiving Se injections had a lower death loss and higher weaning weights (Spears et al., 1986). These results suggest that clinical deficiencies will begin to present with marginal 0.03 to 0.05 mg Se/kg DM consumption. Given the small window of safety and potential for toxicity the FDA has established regulations restricting supplementation to no more than 0.3 mg of supplemental Se/kg DM (Greene, 2000; NASEM, 2016; Arthington and Ranches 2021). There has been no evidence that feeding concentrations in excess of 0.3 mg Se/kg provide any beneficial performance or immunity to the ruminant (Arthington in Ranches, 2021). The maximum tolerable Se limits have been set 5 mg Se/kg DM with signs of selenosis presenting as lameness, feed refusal, weight loss, cracked and sore hoofs, hair loss, liver cirrhosis, blind staggers, alkali disease, and nephritis (NASEM, 2016). Because Se abundance in the environment is regional, management of Se toxicity is difficult and should include removal of animals from toxic areas, soil treatment to prevent absorption from plants, diet modification and incorporation of low Se containing feedstuff into ration (McDowell, 2003; NASEM, 2016). Once environmental Se conditions are known, several methods can be used to estimate animal Se status. Selenium concentrations in the plasma, serum, whole blood, liver, kidney, muscle as well as glutathione peroxidase activity in plasma, whole blood, and erythrocytes has been used to evaluate status (Puls, 1994; NASEM, 2016). While the information is useful, it is important to recognize values reported for glutathione peroxidase activity vary from laboratory to laboratory based on the type of assay used. Furthermore, source of consumed Se may impact the responsiveness of Se concentrations in blood parameters (Spears et al., 2022). It has been recognized that Se concentrations in whole blood and liver are best indicators of Se status in cattle (Arthington and Ranches, 2021; Spears et al., 2022).

***Zinc***

Zinc has several different roles and functions within the body making it essential. One of the primary roles of Zn, similar to other trace minerals, is an essential component to several metalloenzymes, most notably, glutamic dehydrogenase, Cu-Zn superoxide dismutase, carbonic anhydrase, lactic dehydrogenase, alcohol dehydrogenase, carboxypeptidase, alkaline phosphatase, and RNA polymerase (Hambidge et al., 1986; Miller et al., 1988). The integration of Zn into a vast number of metalloenzymes as well as its role in enzyme activation make it critical to the proper metabolism of carbohydrates, protein, lipids, and nucleic acids, reproduction, and proper immune function (Hambidge et al., 1986; Miller et al., 1988; Graham, 1991; NASEM, 2016). Additionally, Zn is involved in DNA, RNA, and protein synthesis through 'zinc-fingers', this role makes Zn critical for transcription, cellular division, and genetic potential, explaining its function in cells with rapid growth and turn over (Miller et al., 1988; Underwood and Suttle, 1999; Arthington and Ranches, 2021).

Plant species, plant maturity, fertilizer application, and soil Zn concentrations are all factors that contribute to Zn abundance in forages with grasses commonly being lower in concentration than legumes (NASEM, 2016). Even with the variability Zn concentrations Mortimer et al. (1999) reported that analysis of 164 pasture grasses samples abundance ranged from 19.5 to 42.9 mg Zn/kg DM, indicating marginal to adequate Zn concentrations. It is also important to point out that forage Zn can be found in one of three forms (Miller et al., 1988). Most available of the three is the soluble fraction, then Zn bound to NDF that is available after digestion, and finally the least available form is bound to ADF (Miller et al., 1988). While Zn deficiency is uncommon in ruminants, supplementation sources include Zn oxide, Zn sulfate, Zn methionine, and Zn proteinate (NASEM, 2016; Arthington and Ranches 2021). In the ruminant Zn consumed will be absorbed and excreted from the small intestine based on need, in a carrier mediated process (Suttle et al., 1982; Miller et al, 1988; Underwood and Suttle, 1999). After absorption Zn will be bound to albumin and enter portal circulation (Underwood and Suttle, 1999). Once in the liver Zn metallothionein synthesis will be upregulated, outside of the liver mechanism for Zn uptake and incorporation other target organ mechanisms are not clear (Underwood and Suttle, 1999). The primary mechanism for excretion is through the feces with smaller concentrations found in urine (Miller et al., 1988; Underwood and Suttle, 1999).

The Zn requirement set for beef cattle is 30 mg of Zn/kg DM, however grazing beef cow requirements are not well defined (NASEM, 2016; Arthington and Ranches 2021). A multi-year experiment by Mayland et al. (1980) using cow calf pairs in a grazing system consuming crested wheatgrass pasture were stratified into a control group, consuming a basal diet with < 20 mg Zn/kg DM in the basal diet with no supplemental Zn, or into a treatment group, with 86 or 90 mg Zn/kg DM supplemented as Zn oxide and provided free choice. While no clinical signs of Zn deficiency were apparent, calves receiving supplemental Zn had significantly higher weight gain when compared to the controls, indicating a potential marginal status in calves consuming less than 20 mg Zn/kg DM (Mayland et al., 1980). Documented clinical symptoms of Zn deficiency include decreased growth, feed intake and feed efficiency, lack of appetite, listlessness, skin lesions and hair loss, excess salivation, decreased testicle growth (Miller et al., 1988; Paterson and Engle, 2005). While severe deficiencies may be rare in practical production systems, marginal deficiencies may occur. However, there is no reliable analysis regarding these types of Zn deficiencies (Spears et al., 2022). In instances where plasma or serum Zn concentrations are below the normal range of 0.6 to 1.2 mg/L, the animal is said to be Zn deficient. However, analysis procedures are not sensitive enough to detect marginal deficiency (Spears et al., 2022). The only mechanism to estimate a marginal deficiency is through analysis of feedstuff and positive performance response to supplemented Zn (Spears et al., 2022). Ruminants have a high tolerance to elevated Zn concentrations, the maximum tolerable limit has been set to 500 mg/kg DM in beef cattle (NRC, 2005). In a study where calves were fed 40, 200, 500, 700, or 1000 mg Zn/kg DM for 35-d to determine the dietary Zn concentrations indicative of Zn toxicity, researchers reported reductions in weight gain, intake, and efficiency of calves receiving 700 or 1000 mg Zn/kg DM s with no adverse effects in calves consuming 500 mg Zn/kg DM or less (Jenkins and Hidiroglou, 1991).

## COMMON METHODS OF SUPPLEMENTATION

Cattle are efficient at turning land unsuitable for other crops into high quality protein for human consumption. Cow-calf producers rely heavily on grazing pastures for their animals' nutritional needs. As mentioned above, trace mineral concentrations in forages and pastures can vary greatly depending on a wide variety of environmental and plant conditions (Greene, 2000). Basal diet composition greatly influences animal mineral status, and while pastures are often not a complete feed, animal requirements are more complicated than total concentrations consumed. With that in mind, in the United States, trace minerals contained in forages that are often deficient in meeting beef cattle requirements under practical conditions are Cu, Se, and Zn

(Olson, 2007). Basal diet mineral concentrations, mineral-to-mineral interactions, mineral bioavailability, physiological status, performance expectations, breed, age, and occurrence of biological stress must be considered when determining animal supplemental trace mineral needs outside grazing intake to optimize performance and health. All the above factors make trace mineral supplementation a challenging obstacle for cattle operations and is a balance of animal health and performance, cost, and environmental impacts. The more information the producer can obtain in terms of mineral concentrations consumed in the feed and water, in combination with mineral status of the animal, can be critical in formulating specialized trace mineral supplements for their animals. There are serval methods producers and nutritionists can go about supplementing trace minerals to grazing ruminants to improve mineral status. This section will discuss both indirect and direct supplementation methods available.

***Indirect Methods***

Mineral supplementation by indirect means involves the improvement of minerals in forages consumed by cattle with efforts to meet animal requirements (Greene, 2000). Biofortification methods such as using mineral containing fertilizers, amendments to the soil, and establishment of certain plants known to be rich in minerals of concern are all examples of indirect mineral supplementation (Greene, 2000; Olson, 2007; Arthington and Ranches, 2021). While mineral composition of forages can be altered through fertilization methods, this technique is typically economically unfeasible for most producers. Greene (2000) reported that based on the cost analysis, fertilizers should be targeting plant growth needs rather than animal needs, and additional direct supplementation of deficient minerals for animals is more economically effective. Furthermore, if fertilizer amendments made are not readily absorbed by plants given the soil conditions or if the bioavailability of added minerals, these efforts may be without cause (McDowell, 1996). Altering soil pH can also affect mineral status of plants, more alkaline soil may increase the uptake of Se but at the same time Mo uptake will increase and Cu and Co plant absorption will decrease (McDowell, 1996; Greene, 2000). This method has the potential to manage one nutritional issue while creating another.

Diversification of the plant species to a variety of grasses and legumes is a management technique that allows producers to control mineral trends of plants consumed in a pasture setting. Often legumes are higher in Ca, K, Mg, Cu, Zn, Fe, and Co than grasses, and grasses contain higher concentrations of Mn than legumes grown in the same soil (McDowell, 1996; Olson, 2007). However, these are related to the environmental conditions in which they grow. Biofortification as a method of trace mineral supplementation in cattle has potential but future exploration is required (Arthington and Ranches, 2021). Most common methods of trace mineral supplementation in cattle are through direct supplementation techniques which are more efficient and cost effective at supplying minerals to meet the animal's needs.

***Direct Methods***

Direct supplementation of trace minerals to meet ruminant requirements is common amongst cattle producers. General methods include free-choice supplementation, fortification of energy or protein supplements, injections, and bolus or drenches. Several variables such as, cost, management practices, basal diet mineral concentrations, mineral source bioavailability, animal requirement, and production targets must be considered when determining the best mineral supplement for the specific operation especially in a competitive market (Greene, 2000). Noting that supplementation is more cost effective than deficiencies and production losses associated with not meeting requirements for their anticipated physiological status (Cockwill et al, 2000). However, this idea relies on adequate supplementation, under supplementation will not meet animal requirements and over supplementation can have negative performance results and increased nonutilized mineral translating to wasted money and increased mineral concentrations in animal waste (Bowman and Sowell, 1997). Supplementation can be as extensive as compressed trace mineral salt blocks or as intensive as routine injections or oral supplementation and will be based on investment return and animal health and performance responses.

*Free-Choice Supplements*

Free-choice mineral supplementation, or the voluntary consumption of blended trace mineral and salt, to grazing beef cattle is the most common mineral supplementation method (Arthington and Ranches, 2021). While free-choice trace mineral supplementation is an effective technique of supplementation, it is not without complications as management of intake is crucial and can be difficult to estimate or predict for individual animals.

This method of supplementation relies on all cattle exposed to the free-choice mineral to voluntarily consume salt in sufficient but not excess amounts to meet nutritional requirements (Cockwill et al., 2000; Arthington and Ranches 2021). Cockwill et al. (2000) used multiplex RF mineral feeder systems (GrowSafe Systems Ltd., Airdrie, AB) to estimate individual mineral consumption of cows and calves on pasture. The authors reported that daily intake was highly variable; average daily consumption for cows was 241.6 g/hd with ranges from 0 g/d to 974 g/d with a 100 g/hd/d target, for calves the average daily consumption of the salt-based mineral was 39.3 g/hd with ranges of 0 g/d to 181 g/d (Cockwill et al., 2000). Additionally, as the percentage of salt was increased in the trace mineral supplement, intake decreased but was still highly variable among individuals (Cockwell et al., 2000). Furthermore, supplement consumption was increased in cows and calves consuming free-choice mineral mixture with lower salt inclusion (9.8% salt vs 22.5% salt; Cockwell et al., 2000). These variations in intake results suggest that while uniform consumption among grazing herds is unlikely and individual animal estimates of intake are nearly impossible without sophisticated technology, it is still a valuable management technique to supply trace minerals. Furthermore, consumption of the trace mineral can be decreased or increased with the addition or removal of salt from the trace mineral mixture.

When determining herd mineral consumption and proportion of salt inclusion in the trace mineral a 1 to 2-wk herd consumption average will provide great insight into target intakes. However, the producer must routinely check for salt craving changes to ensure target intakes stay within an appropriate range (Arthington and Ranches, 2021). As mentioned previously, greater salt inclusion will deter mineral consumption while lower concentrations of salt in the free-choice trace mineral supplement will encourage intake (Bowman and Sowell, 1997; Arthington and Ranches 2021). Loose or blocked forms of free-choice trace mineral supplements will also influence intake. It's important to recognized that intake of minerals provided in a salt-based block form can be 10% lower than that of lose form minerals (McDowell, 1996; Arthington and Ranches 2021), but compressed trace mineral salt blocks can be implemented in very extensive grazing operations. Furthermore, free-choice trace mineral intake can be enhanced when molasses is the carrier rather than salt (Bowmen and Sowell, 1997; Bailey and Welling, 2007). Bailey and Wellington (2007) conducted an experiment to compare the impact of blocked molasses-based trace mineral supplementation to the traditional loose salt-based trace mineral supplementation on supplement intake and performance in mature grazing cows. Researchers reported greater intake of molasses-based trace mineral blocks compared to loose salt-based trace minerals 213 ± 38 g/d vs 128 ± 15 g/day, respectively (Bailey and Welling, 2007). The greater consumption of molasses-based blocked free-choice trace minerals over the lose form is likely related to increased palatability among animals.

*Energy/Protein Supplement Fortification*

During certain times of the year, forage quality or abundance can be insufficient to realistically meet an animal's protein, energy, vitamin, and mineral requirements. Therefore, supplementation is required with mineral fortified protein and energy (e.g., range cubes, protein blocks, etc.) supplements (Arthington and Ranches, 2021). Similar to salt-based free-choice supplementation, producers rely on voluntary animal consumption for adequate supplementation to be achieved. However, while intake variation is expected with this type of supplementation, it is assumed that protein or energy supplement intake is less variable than salt-based trace mineral free-choice supplements (Bowman and Sowell, 1997; Arthington and Ranches, 2021). Mineral consumption with these types of supplements is guaranteed because even unfortified energy/protein supplements contain trace minerals in their ingredients. Moreover, mineral fortification of many commercially advertised energy or protein supplements is available (Greene, 2000; Arthington and Ranches, 2021). Adequate fortification of trace minerals into energy/protein supplements can potentially be the sole form of mineral supplementation during times when energy/protein additions are being made (Arthington and Ranches, 2021). However, in most cases year-round energy or protein supplementation in grazing systems is economically inefficient and unnecessary when forage quality and abundance is adequate for cattle. This indicates careful formulation, accurate intake targets, economic and performance tradeoffs, as well as increased management requirements are critical to the success of this trace mineral supplementation program (Bowman and Sowell, 1997; Arthington and Ranches, 2021). Producers must be aware this supplementation method is more intensive than free-choice supplementation methods and must be aware of feeding methods, dominant animals and social hierarchy, and palatability for instance (Bowman and Sowell, 1997).

*Injections*

Trace minerals can also be supplemented through injection; however, this is labor intensive and requires facilities to implement such strategies. The most commonly applied injectable trace mineral supplement, MultiMin 90 (MultiMin North America, Fort Collins, CO, USA) contains 60 mg Zn, 10 mg Mn, 5 mg Se, and 15 mg Cu. In a study comparing several trace mineral supplementation methods researchers reported utility in MultiMin 90 trace mineral injections for the rapid improvement in animal mineral status for approximately 30-d post injection as liver concentrations were blow other bolus methods at 91-d (Jackson et al., 2019).

Mundell et al. (2012) conducted an experiment stratifying 460 cows and heifers into either a control group with sterile physiological saline injections or the treatment group receiving MultiMin 90 trace mineral injections 105-d prior to projected calving and again 30-d before fixed-time AI. Additionally, researchers reported variable mineral status of cattle prior to study initiation, and all animals, regardless of treatment, had free-choice trace mineral supplement available during the duration of the study. Mundell et al. (2012) reported greater conception rates ($P = 0.05$) in cows receiving trace mineral injections (60.2%) than controls (51.2%) and while overall pregnancy rate between the two treatment groups cows receiving trace mineral injections calved earlier in the calving cycle than controls ($P = 0.01$). However, in another experiment researchers stratified 290 heifers into either a control group, receiving saline injections, or a treatment group, receiving MultiMin 90 injections several times over a 1.5-year period (Stokes et al., (2018). Similar to the Mundell et al. (2012) experiment, Stokes et al (2018) supplied a free-choice trace mineral supplement to both treatment groups grazing native range, but within this experiment animals were all mineral adequate prior to study initiation. Within this experiment no treatment differences related to artificial insemination or overall pregnancy rates was observed in heifers, however trace mineral injected heifers did have greater ($P \leq 0.01$) Cu and Se concentrations in the plasma and liver when compared to control animals. These data suggest that animal trace mineral status can impact injection trace mineral response to reproduction. Furthermore, injectable trace minerals may be a useful tool during particularly critical or stressful times such as weaning, shipping, or breeding and should be used in combination with free-choice mineral supplementation.

*Boluses/Drenches*

Another management technique for supplementing trace mineral is through oral provisions of boluses or drenches. While researching is limited and implementation of this supplementation method is not common amongst producers, there are products with this intended route of supplementation. Historically, there has been interest in bolusing Cu oxide needles as a slow-release method to improve copper status. However, previous studies found minimal bioavailability (Kegley and Spears, 1994), reduced calf weight gain (Arthington et al., 1995), and reduced digestibility of forages (Arthington, 2005), resulting in decreased implementation of Cu oxide boluses. More recently, long-term slow-release boluses containing Cu, Se, and Co have become commercially available (Arthington and Ranches, 2021). Sprinkle et al. (2021) conducted a four-year long experiment on cows grazing central Arizona range investigating strategic trace mineral supplementation of a slow release 6 month Cu, Se, and Co bolus (Cosecure, Bimeda UK, Anglesey, Wales) on calving interval, body condition, milk production, and calf weaning weights. While researchers reported strategic supplementation of Cosecure long-acting boluses did not affect milk production across treatments but there was a decrease in calving interval and improved adjusted calf weaning weights (Sprinkle et al., 2021). Furthermore, a study out of Iowa State University comparing various single-use or pulse-dose trace mineral supplementation methods reported gradual improvement of mineral liver status in cattle with long-term boluses (Jackson et al., 2020). Additionally, Jackson et al (2020) reported drench and paste trace mineral supplements did not alter mineral concentrations in the liver and plasma of mineral-adequate Angus-cross steers. These data suggest that long term Bolus supplementation methods may be beneficial to producers in extensive range operations given the trace mineral source is biologically available to the animal.

As previously discussed, the interactions between animal production and trace minerals and supplementation to provide these minerals are complex. The subsequent chapter will discuss primary research designed to determine the impact of Mo exposure in water on Cu status and cow-calf production over a 2-y period.

There is the potential for elevated Mo and low Cu concentrations in some areas of the Rocky Mountains given these conditions an antagonistic interaction between Cu and Mo may appear in the rumen. This possible occurrence in combination with the opportunity for potential water contamination from mining or natural of rock formations enticed the experiments in the following chapters of this dissertation. Molybdenum water quality standards for domesticated bovines has been set to 160 µg of Mo/L by the Environmental Protection Agency. While the interaction between Mo and Cu consumption in feedstuffs is well explored and it's known that elevated Mo can reduce Cu absorption in ruminants. Previous investigation of elevated Mo exposure through consumption of drinking water in beef cattle was limited to two short term studies by Kincaid (1980) in dairy calves and Kistner et al. (2017) in growing feedlot steers. The subsequent chapters aim to investigate the effects of long term Mo exposure in the drinking water on Cu status in beef cattle of various life stages.

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# CHAPTER 2 – MOLYBDENUM EXPOSURE IN DRINKING WATER VS FEED IMPACTS APPARENT ABSORPTION OF COPPER DIFFERENTLY IN BEEF CATTLE CONSUMING A HIGH FORACE DIET

## SUMMARY

Commercial, multiparous beef cows (n=54 in year 1; n= 51 in year 2) with calves (approximately 2 months of age) were used to evaluate the effects of Mo source (feed or water) on reproduction, mineral status, and performance in cows and calves receiving a grass hay diet [dry matter (DM) basis: 6.6% crude protein; 0.15% S, 6.7 mg Cu/kg, 2.4 mg Mo/kg] for 553 d. Cows were stratified by age, body weight (BW), and liver Cu and Mo status, and were then randomly assigned to one of six treatment groups. Treatments were: 1) Negative control (NC; basal diet with no supplemental Mo or Cu); 2) Positive control (PC: NC + Cu; 3 mg of supplemental Cu/kg diet DM from $CuSO_4 \cdot 5H_2O$); 3) NC + 500 µg Mo/L from $Na_2MoO_4 \cdot 2H_2O$ supplied in drinking water (Mo 500-water); 4) NC + 1000 µg Mo/L of $Na_2MoO_4 \cdot 2H_2O$ supplied in drinking water (Mo 1000-water); 5) NC + Mo 1000-water + 3 mg of supplemental Cu/kg diet DM from $CuSO_4 \cdot 5H_2O$ (Mo 1000-water+Cu); and 6) NC + 3.0 mg of supplemental Mo/kg diet DM from $Na_2MoO_4 \cdot 2H_2O$ (3.0 Mo-diet). During the winter months, animals were housed in three replicate pens per treatment and during the summer months animals were housed in separate pastures by treatment. Animals were allowed ad libitum access to both feed and water throughout the experiment. Cows were bred via artificial insemination during the summer months of both years of the experiment and calves were weaned at approximately 6 months of

age in the fall of both years. All cows and calves were weighed, bled, and feed and water intake were determined every 28d. Cattle receiving diets containing less than 10 mg Cu/kg DM total diet became Cu deficient over the course of the experiment as determined by liver and plasma Cu concentrations. However, no Mo toxicity or Cu deficiency signs (e.g., reduction in growth rates, reproductive performance, or immune function) were observed throughout the course of the experiment for any treatment. Results suggest that Mo supplemented in water or feed at concentrations used in this experiment had minimal impact on Cu status and overall animal performance. However, dietary Cu concentration below 10.0 mg Cu/kg DM total diet reduced liver and plasma Cu concentrations to values indicative of a marginal Cu deficiency in beef cows.

## INTRODUCTION

Numerous experiments have investigated the impact of water quality on beef cattle production. Nitrates, sulfates, microbial contamination, and excessive cations are water contaminants that can negatively impact beef cattle production (Wright, 2007; Wagner and Engle, 2021). As described by Thorndyke et al., (2021) certain locations within the Rocky Mountain region in the US have natural rock formations or human activity that can contribute to elevated concentrations of molybdenum (Mo) in surface and/or ground water that may impact livestock performance. Elevated dietary Mo concentrations have been reported to reduce copper (Cu) status in ruminants (Dick and Bull, 1945; Vandereen and Keener, 1964; Suttle, 1991). The negative impact of Mo on Cu status in ruminants is substantially increased when elevated concentrations of sulfur (S) are added to the diet (Suttle, 1991; Dick, 1953; Suttle, 1974a, b; Mills et al., 1977).

Limited controlled research has been conducted investigating the influence of Mo water concentrations on cattle production and Cu status. Kincaid investigated the short-term exposure (21 d) of weaned calves (5 wk. old) to varying concentrations of Mo in water (0.0, 1,000, 10,000, or 50,000 µg of Mo/L) (1980). The author reported no difference in body weight gain across all treatments and a safe ratio of total dietary Cu to Mo of 0.5:1.0. Kistner et al. conducted a longer duration experiment (approximately 131d) utilizing beef steers with lower doses (0 – 960 µg Mo/L) of Mo added to the water (2017). Diets used in this study contained approximately 10.4 mg Cu/kg DM, and Mo dose had no impact on growth, animal health, Cu status, or carcass characteristics. More recently, Thorndyke et al. conducted an experiment investigating the impact of Mo in water or feed on Cu absorption in beef cattle (2020). They reported that short term Mo exposure in water may impact Cu absorption and retention to a lesser degree that when Mo is supplemented in the diet.

The impact of prolonged exposure to elevated Mo water concentrations on beef cattle consuming a high forage-based diet is unknown, yet these systems may be realized in areas where Mo in cattle drinking water sources are due to natural or anthropogenic inputs. Therefore, the objective of the current experiment was to conduct a life-cycle production and health assessment of lactating and gestating beef cattle, and their calves, exposed to varying doses of Mo. We hypothesized that as duration of Mo exposure increased, animal performance and Cu status would be impaired.

## MATERIALS AND METHODS

### *Experimental Design*

Prior to the initiation of the experiment, all animal use, handling, and sampling techniques described herein were approved by the Colorado State University Animal Care and

Use Committee (IACUC approval #18-7819A). The overall project lasted 553 d with the animals housed in pastures and feedlot pens, depending on time of year (described below).

Thirty-five days prior to the initiation of the experiment, 54 multiparous commercial (Angus & Angus x Hereford) beef cows and 54 nursing calves purchased from a local cow-calf producer in Grand County, CO. Cows were stratified based on age, BW, and initial liver Mo and Cu status, and then randomly assigned to one of six treatments (n = 9 cow-calf pairs per treatment). The study was initiated in June. Cows calved each year in February and March, and calves were weaned in October. Treatments consisted of: 1) negative control (NC; basal diet with no supplemental Mo or Cu); 2) positive control (PC: NC + Cu; 3 mg of supplemental Cu/kg diet DM from $CuSO_4 \cdot 5H_2O$); 3) NC + 500 µg Mo/L from $Na_2MoO_4 \cdot 2H_2O$ supplied in drinking water (Mo 500-water); 4) NC + 1000 µg Mo/L of $Na_2MoO_4 \cdot 2H_2O$ supplied in drinking water (Mo 1000-water); 5) NC + Mo 1000-water + 3 mg of supplemental Cu/kg diet DM from $CuSO_4 \cdot 5H_2O$ (Mo 1000-water+Cu); and 6) NC + 3.0 mg of supplemental Mo/kg diet DM from $Na_2MoO_4 \cdot 2H_2O$ (3.0 Mo-diet). Total calculated intake of Cu and Mo (water and feed) expressed on a mg/kg diet DM (Cu/Mo) basis for each treatment were as follows: NC 6.8/2.6; PC 9.8/2.6; Mo 500-water 6.8/4.3; Mo 1000-water 6.8/6.0; Mo 1000-water+Cu 9.8/6.1 and; 3.0 Mo-diet 6.8/5.6

All procedures described below were repeated following the weaning of the first calf crop, except where noted. Cows remained on the same treatment for the duration of the experiment, while calves were removed from treatments at the time of weaning. All cows and calves received standard vaccinations (Bovi-Shield Gold; Zoetis, Kalamazoo, MI and Covexin 8; Merck, Omaha, NE) and were dewormed (Eprinex; Boehringer Ingelheim, St. Joseph, MO) yearly per recommendations of the USDA APHIS – Veterinary Services Foreign Animal Disease

Preparedness and Response Plan (2016) in consultation with the local veterinarian in Grand County, CO. Additionally, all cows and calves were tested for bovine viral diarrhea virus by obtaining an ear notch from each animal prior to the initiation of the experiment. Bovine viral diarrhea virus was not detected in any animal used in this experiment.

***Animal Housing***

Each year, animals were housed in two different locations. In the summer and early fall months (northern hemisphere), animals were housed in one of 6 pastures (≈ 1.2 hectares per pasture) by treatment. Pastures were located in Grand County, CO and had no standing water and minimal naturally occurring forage cover. Each pasture contained four-1650 L water tanks. Each water tank was fenced to allow only cows access to water tanks. Water utilized in this experiment was transported from the Williams Fork River to the water tanks using a water truck. Each pasture was also equipped with a creep feeder that contained two-265 L water tanks only accessible to calves within that pen. This allowed determination of water intake for cows and calves separately within a pasture. Each pasture also contained one round bale feeder that was placed in an empty water tank to limit calves from consuming hay from the round bale feeder. Feed troughs were placed in each creep feeder to allow calves access to hay. This allowed for feed intake determination for cows and calves independently while housed together within a pasture. In year one, cow/calf treatment groups were rotated to a new pasture location once. In year two, pasture rotation occurred approximately every 28 d so that all cows were exposed to each pasture location. For each rotation, all waterers, feeders and loose salt feeders were moved to the appropriate pasture in order to maintain treatment integrity.

During the winter and early spring months, all animals were transported to the Agriculture, Research, Development, and Education Center (ARDEC) feedlot in Fort Collins, CO and housed in feedlot pens (7 m x 40 m) containing 3 cow-calf pairs from the same treatment per pen (3 replicates per treatment). Each pen was equipped with a concrete feed bunk, a 3 m x 7 m concrete bunk pad, and a 1050 L water tank. Feed was delivered daily in amounts that allowed ad libitum access to feed for cows and calves.

***Feed, Supplement, and Water Delivery***

For both years, grass hay was purchased from local hay producers in Grand County, CO. All hay bales were sampled and analyzed for nutrient composition (Table 2.1). While cattle were on pasture in Grand County, CO, all hay was weighed prior to being placed in the round bale feeders or feed troughs within the creep feeders. Loose white salt was also provided to all cattle while on pasture. For a given pen, loose white salt was placed in rubber troughs and hung on the outside of the creep feeder at a height to only allow cow access. Loose white salt was also placed in a rubber tub within the creep feeder to allow calves access to white salt. Supplemental Cu (as $CuSO_4 \cdot 5H_2O$) or molybdenum ($Na_2MoO_4 \cdot 2H_2O$) was added to the loose salt supplement for those cows and calves receiving dietary Cu or Mo treatments (Table 2.2). Weekly intake of white salt was determined for cows and calves. Molybdenum and Cu concentrations of the loose salt supplement, as well as the Cu:Mo ratio for each treatment was calculated using the actual intake of water, feed, and supplement consumed by cows and calves.

Due to the low nutrient quality of the hay being fed, a custom molasses-based protein hard lick-tub supplement (30% CP on a DM basis; 16% equivalent CP units from urea and 14% CP units from soybean meal) was formulated to supply the appropriate amount of protein and vitamins A, D, and E for gestating and lactation beef cattle with a targeted intake of 0.34-0.57 $kg \cdot animal^{-1} \cdot day^{-1}$). Supplemental protein contained no added Cu and was provided to all cattle in tubs throughout the duration of the experiment. Protein intake was quantified weekly by weighing the protein tub. All cows and calves within a pen had ad libitum access to the same protein tub.

After transporting cattle to the ARDEC facility in Fort Collins, CO, dried distillers grains (DDG) were used as the carrier for all Mo and Cu dietary treatments. Dried distillers grains were added to each pen daily (0.25 kg animal/d) at the time of hay delivery. Cattle on treatments not receiving supplemental Mo or Cu were fed the same amount of DDG without additional Mo or Cu (Table 2.2). The same hay fed to cattle in pastures was fed at the ARDEC facility. Water intake was monitored twice a week by measuring the disappearance of water over a given time period as described by Kistner et al. (2017). Sodium molybdate dehydrate ($Na_2MoO_4 \cdot 2H_2O$) was added to each water tank at the appropriate concentration, via a dilution of a 65,000 µg of Mo/L stock solution. Water Mo and Cu concentrations for each treatment over the entire experiment are shown in Table 2.2.

***Animal Sampling***

For all cows and calves, BW and jugular blood samples were obtained approximately every 28 d. Blood samples were collected into three 7 ml vacutainer tubes [1) heparinized trace-mineral-free vacutainer tubes; 2) vacutainer tubes containing EDTA; and 3) vacutainer tube containing no additive; Becton Dickinson Co., Franklin Lakes, NJ]. Once collected, blood samples were placed on ice and transported back to the laboratory (approximate time from collection until processing was 6 h). Heparinized trace-mineral-free vacutainer tubes and vacutainer tubes with no additive were centrifuged at 2,000 × g for 15 min at room temperature and plasma or serum was then transferred to acid-washed storage vials and stored at –20°C. One ml of red blood cells (RBC) from each tube was lysed in 4 mL of cold deionized H2O and stored at –80ºC until superoxide dismutase activity analysis could be performed. Vacutainer tubes containing EDTA were submitted to the Colorado State University Diagnostic Laboratory for complete blood count (CBC) determination. Whole blood was also analyzed for pH, pCO2, and pO2 using an I-STAT blood chemistry device.

Milk samples (approximately 50 mL) were obtained from all cows at approximately 3 month post calving, for both years, by placing each cow in a squeeze chute and manually hand milking each cow. Liver biopsies (approximately 150 mg wet weight) were obtained from all cows at the beginning of the experiment, prior to transport to the ARDEC facilities, prior to transport back to pastures in Grand County, CO, and prior to transport back to ARDEC in the second year of the experiment (Pearson and Craig, 1980; Engle and Spears, 2000). Liver samples (approximately 100g wet weight) were also obtained from the right lobe of the liver from all cows at the time of slaughter. Calf liver biopsies were obtained from all calves at approximately 3 months of age for year 1 calves and 1.5 months of age for year two calves.

Once weaned, all calves were removed from their respective treatments, commingled by sex, and fed a standard feedlot finishing diet for approximately 220 d. On the day of slaughter, cattle were transported to a commercial abattoir, and individual carcass data and liver samples were collected. Hot carcass weight was determined at the time of slaughter. Carcasses were allowed to chill for approximately 36 h. Standard carcass data measurements were collected by trained personnel. After weaning the second calf crop, all cows were slaughtered as described above. Kidney, muscle (longissimus dorsi), pancreas, spleen, and subcutaneous fat were obtained post slaughter and analyzed for Mo and Cu concentrations.

***Cow Reproductive Performance***

To determine the effects of treatments on cow reproductive performance, every cow was inseminated once following a modified Select-Synch method (Geary et al., 2000) with the addition of a controlled internal drug-release insert (CIDR) estrus synchronization protocol as described by Ahola et al. (2005). Cows and calves were then returned to their appropriate pastures. Fourteen days after mass insemination, six Angus bulls, that had passed breeding soundness exams, were housed (1 per pasture) with the cows and calves for 60 d (1 bull per pasture). Pregnancy was determined at 40 d after mass insemination via rectal ultrasonography to classify fetuses as either artificial insemination pregnancies or natural service pregnancies.

Cow and calf performance: Cow body weights and body condition scores (BCS; 1 = emaciated, 9 = obese; (Richards et al., 1986) were collected at approximately 28-d intervals throughout the experiment. Over both years, body weight gain and 205 d adjusted weaning weights were collected to evaluate calf performance.

***Health Status, Immune Parameters, and Blood Chemistry***

Throughout the entire experiment, all animals were visually monitored daily to detect signs of morbidity by trained personnel as described by Caldera et al. (2017). Necropsies were performed on animals that died during the experiment (n = 3 cows, and n = 6 calves). Briefly, 3 cows died during the experiment. One cow from the 3.0 Mo-diet died on day one of the experiment due to bloat and one cow from the same treatment (3.0 Mo-diet) was euthanized on day 401 of the experiment due to bovine traumatic reticulopericarditis (hardware disease). A cow receiving the Mo 500-water treatment was euthanized on day 168 of the experiment because of loss of teeth. Six calves died during the course of the experiment. A calf on the PC treatment died of respiratory disease; a calf from the NC died but the cause of death was not able to be determined. Three calves in the Mo 500-water treatment died: one calf was inadvertently separated from its mother during a winter storm and died of failure to thrive; one calf was stillborn, and one calf was stepped on by its dam during calving and died of a crushed thorax;

and one calf receiving Mo 1000-water died of respiratory disease. In order to assess immune function, interferon gamma concentrations, total IgG and IgM concentrations, RBC superoxide dismutase (SOD) enzyme activity, complete blood counts (CBC), pH, pO2, and pCO2, were determined on blood samples collected on d 0 and approximately every 28 d throughout the experiment on all cows and calves.

***Analytical Procedures***

Feed samples were analyzed for: moisture using the AOAC (2006) Official Method 950.46 moisture removal process; CP using the AOAC (2006) Official Method 992.15 (TruSpec CN, 2004); ash using the ash oven method described in the AOAC (2006) Official Method 920.153; and acid and neutral detergent fiber (ANKOM Technology 2015). Feed, water, plasma, milk, and liver samples were wet ashed and analyzed for Mo and Cu concentrations using inductively coupled plasma mass spectrometry (EPA 200.8, rev. 5.4, 1994; PerkinElmer; NexION 2000 B). Water quality was analyzed using standard analytical techniques (EPA, 1983 and EPA, 1986).

Complete blood counts from whole blood were determined using a Siemens Advida 120 Hematology Analyzer (Siemens Healthineers, Erlangen, Germany). Serum ceruloplasmin activity was determined using a spectrophotometric procedure described by Houchin (1958). Total serum IgG and IgM concentrations were determined using single radioimmunodiffusion assay kits (VMRD 240-30 and 246-30; VMRD, Inc., Pullman, WA) as described by Stabel et al. (1993). Plasma samples were analyzed for INF-ɣ concentrations using an ELISA assay (Biosource KBC1231, Biosource International, Inc., Camarillo, CA). The assay was designed as a qualitative assay but for this study was modified into a quantitative assay. Briefly, a positive control that was supplied with the kit was diluted with a negative control to make standards of known concentrations. A standard curve was created using linear regression to quantitate the INF-ɣ concentrations in the unknown samples. The regression coefficient of the standard curve was 0.991 and samples were reported as log10. Lysed RBC were analyzed for SOD activity using a SOD 525™ Assay Kit (Biotech® 21010; Oxis Health Products, Inc., Portland, OR). Superoxide dismutase activity was expressed as SOD activity per milligram hemoglobin. Hemoglobin concentration was determined using a Total Hemoglobin Assay Kit (Sigma 525-A; Sigma-Aldrich, St. Louis, MO). Blood chemistry (pH, pO2 and pCO2) were analyzed using an i-STAT analyzer (Abbott, North America, Orlando FL).

***Statistical Analysis***

Cow and calf performance data, mineral status, nutrient analysis, and immune measurements were assessed using a restricted maximum likelihood-based, mixed-effects model, repeated-measures analysis (PROC MIXED; SAS Inst., Inc., Cary, NC) where appropriate. Initial cow performance and mineral status models contained fixed effects of treatment, time, and treatment × time interaction. Initial calf performance models included fixed effects of treatment, year, age of dam, age of calf, sex of calf, and all relevant two- and three-way interactions. A spatial power covariance structure was used in the analysis and the containment approximation was used to calculate denominator degrees of freedom. Pen was considered the experimental unit for all response variables measured. Reproductive response data were analyzed using logistic regression (PROC GENMOD of SAS). Initial models for reproductive response contained fixed effects of treatment, BCS, BW, and year, in addition to all relevant two- and three-way interactions. When an interaction was not significant, it was removed from the model. If the interaction of year × treatment was not significant, data were pooled across years; otherwise, data were reported for each year separately. Significance was determined at $P \leq 0.05$, and tendencies were determined if $P > 0.05$ and $\leq 0.10$.

## RESULTS AND DISCUSSION

### *Feed and Water*

Table 2.1 describes the chemical composition of the grass hay used throughout the experiment. There were no year by grass hay interactions for any of the components analyzed. Therefore, all data are presented as overall means ± standard deviations. The basal grass hay used in this experiment was a low quality grass hay typically fed to gestating and lactating beef cows and calves and contained 6.84±2.91 and 2.58±0.26 mg of Cu and Mo/kg diet DM, respectively.

Molybdenum and Cu concentrations in the water, white salt, and dried distiller's grains (DDG) supplements are shown in Table 2.2. Targeted concentrations for Mo in the Mo water treatments were 500, 1000, and 1000 mg/L for treatments Mo 500-water, Mo 1000-water, and Mo 1000-water+Cu, respectively. Analyzed Mo water concentrations were slightly greater than targeted values for all Mo water treatments. Molybdenum and Cu concentrations were below detection limits for the basal white salt supplement and within normal concentrations for dried distiller's grains (DDG) (NASEM, 2016). Molybdenum and Cu concentrations of the white salt and DDG supplements for PC, Mo 1000-water+Cu, and the 3.0 Mo-diet treatments were formulated to supply an additional 3.0 mg of Mo and/or Cu/kg total diet DM (pasture targeted salt intake was 0.7% of DM intake; feedlot targeted DDG intake was 0.25 $kg \cdot animal^{-1} \cdot d^{-1}$ or 1.9% of diet DM). Overall, total dietary Cu concentrations were slightly below (9.8 mg Cu/kg DM) targeted NASEM (2016) Cu intake concentrations of 10.0 mg Cu/kg DM for the PC and Mo 1000-water+Cu treatments. Whereas, Mo concentrations in the 3.0 Mo-diet treatment was slightly above (3.1 mg Mo/kg DM) the targeted supplemental intake of 3.0 mg Mo/kg diet DM. The Cu:Mo ratio was calculated based on the actual intake of water, feed, and supplements consumed by the cows throughout the experiment. The Cu:Mo ratios of the NC, PC, Mo 500-water, Mo 1000-water, Mo 1000-water+Cu, 3.0 Mo-diet based on forage and supplement intake were 2.63:1, 3.79:1, 1.56:1, 1.14:1, 1.59:1, and 1.21:1, respectively (Table 2.2).

Water hardness, nitrate concentrations and total dissolved solids were within the "safe and should pose no health problems" category for beef cattle for all water sources used (Table 2.3) (NASEM, 2016). Molybdenum and Cu water concentrations were below detection limits in both water sources at all sampling timepoints.

Due to the low protein content of the hay, a free choice molasses-based protein hard lick-tub supplement was formulated to meet the NASEM (2016) protein requirements for mature lactating and gestating cows with a targeted intake of 0.34-0.57 $kg{\cdot}animal^{-1}{\cdot}day^{-1}$. No supplemental Cu was added to the protein supplement. The Mo and Cu content of the protein supplement was 0.67 and 2.34 mg/kg DM, respectively. Overall protein intake was 0.41, 0.39, 0.44, 0.43, 0.38, and 0.42 (SEM = 0.06) $kg{\cdot}animal^{-1}{\cdot}day^{-1}$ for NC, PC, Mo 500-water, Mo 1000-water, 1000-water+Cu, and 3.0 Mo-diet treatments, respectively (data not shown).

***Cow Performance and Mo and Cu Status***

Morbidity and mortality rates were low throughout the experiment and no signs of Mo toxicity were observed. The influence of chronic molybdenum exposure in drinking water or feed on cow performance and water intake over the duration of the experiment is shown in Table 2.4. There were no treatment x time interactions for any cow performance response variables. Therefore, data were pooled across year. Body weights, DM intake, water intake, and overall pregnancy rates were similar across treatments and within normal ranges for moderately framed beef cows (NASEM, 2016).

The influence of chronic Mo exposure in drinking water or feed on cow liver, plasma, and milk Mo and Cu concentrations and plasma ceruloplasmin activity over the duration of the experiment is shown in Table 2.5. Initial and final liver Mo concentrations and initial liver Cu concentrations were similar across treatments. Final liver Cu concentrations were greater ($P < 0.05$) in cows receiving supplemental Cu (PC and 1000 Mo-water+Cu treatments) when compared to cows not receiving supplemental Cu. At the beginning of the experiment, initial liver Cu concentrations were slightly above concentrations indicative of a marginal Cu deficiency (deficiency defined as less than 20 – 30 mg Cu/kg) (Mills, 1987). Final liver Cu concentrations were adequate for cattle receiving supplemental Cu and deficient in all other treatments.

Similarly, initial and final plasma Mo concentrations and initial plasma Cu concentrations were similar across treatments. Plasma Cu concentrations were greater ($P < 0.05$) in cows receiving supplemental Cu. Initial plasma Cu concentrations were adequate (0.6 mg Cu/L) for all cattle whereas final plasma Cu concentrations were considered deficient for cows receiving no supplemental Cu (deficiency defined as plasma Cu concentrations < 0.6 mg Cu/L) (Puls, 1994). Serum ceruloplasmin activity followed a similar pattern as plasma Cu concentrations. Initial ceruloplasmin activity was similar across treatments whereas final ceruloplasmin activity was greater ($P < 0.01$) in cows receiving supplemental Cu when compared to all other treatments.

Initial and final Mo liver concentrations were within ranges considered to be normal (0.6-6.0 mg Mo/kg DM; CSU Veterinary Diagnostic Laboratory). Initial and final plasma Mo concentrations were considered to be elevated in all treatments (0.08-10.0 mg Mo/L) (Puls, 1994). Furthermore, the range of values proposed by Puls (1994) indicative of "elevated" plasma Mo concentrations (0.08-10.0 mg Mo/L) is broad.

There were no treatment x time interactions for milk Mo or Cu concentrations (Table 2.5). Initial and final Mo and Cu concentrations in milk obtained from all cows within the experiment were similar across treatments. For all treatments, milk Mo concentrations were slightly elevated above normal (normal milk Mo concentrations = 0.018-0.120 mg Mo/L; elevated 0.200 – 0.400 mg Mo/L) and milk Cu concentrations were within normal ranges (adequate milk Cu concentrations = 0.05 – 0.60 mg/L) (Puls, 1994).

Shorter term experiments have been conducted examining the impact of Mo in water and feed on cattle performance. Earlier research by Kincaid indicated that short-term exposure (21 d) of 5 wk. old, weaned calves to different doses of Mo in water (0.0, 1,000, 10,000, or 50,000 µg of Mo/L) had no impact on animal performance (1980). Based on these data, the author suggested a safe ratio of total dietary Cu to Mo to be 0.5:1.0. Kistner et al. conducted a longer duration experiment (approximately 131d) in feedlot steers with lower doses of Mo in drinking water, ranging from 0 – 960 µg Mo/L (2017). The high concentrate feedlot diet contained 10.4 mg Cu/kg DM. The authors reported no impact on growth, animal health, Cu status, or carcass characteristics at any of the Mo doses examined in this experiment. More recently, Thorndyke et al. (2020) conducted an experiment investigating the impact of Mo in water or feed on Cu absorption in beef cattle. They reported that short term Mo exposure in water may impact Cu absorption and retention to a lesser degree than when Mo is supplemented in the diet.

Although there is limited research investigating the impact of long term Mo supplementation on cow-calf production parameters, cow grazing studies have been conducted in areas of reclaimed mines with elevated forage Mo concentrations. Gardner et al. (2003)

investigated the impact of reclaimed mining area forages containing between 21- 44 mg Mo/kg DM on cow and calf performance. Cows were allowed to graze for 12 weeks each year for three consecutive years. The authors reported no signs of Mo toxicity, Cu deficiency, or impacts on health in Cu supplemented or non-Cu supplemented cows. The reason for the lack of observed molybdenosis in these cattle (Gardner et al., 2003) may be due to the short duration of Mo exposure. In the current experiment, animals receiving 3.0 Mo-diet showed no adverse effects from consuming a basal forage diet containing 2.58 mg/kg Mo with 3.0 mg Mo/kg DM added to the diet with a Cu:Mo ratio of 1.21:1 for greater than 500 d. Animals consuming Mo in the drinking water treatments (Mo 500-water; Mo 1000-water) with no added Cu also exhibited no adverse effects based on a Cu:Mo ratio of (1.56 and 1.14: 1, respectively) over the duration of the experiment. This suggests that a lower Cu:Mo ratio may be safe for cattle.

***Immune and Blood Chemistry Parameters in Cows***

Long-term Mo exposure in drinking water or diet did not affect CBC, blood pH, $pO_2$, $PCO_2$, RBC SOD activity, or concentrations of interferon gamma, total IgG and IgM (data not shown). The impact of Cu deficiency on beef cattle immunity has been variable. In vitro neutrophil killing ability of C. *albicans* (Jones and Suttle, 1981; Boyne and Arthur, 1981) and *S. aureus* (Gengelbach et al., 1997; Torre et al, 1996) were reduced in Cu deficient or marginally Cu deficient cattle, respectively. Supplementing Cu improved the neutrophil killing ability of *C. albicans* of previously Cu deficient calves, indicating that Cu may play a role in neutrophil function (Jones and Suttle, 1981). However, Jones and Suttle (1981) and Boyne and Arthur (1981) reported that Cu deficiency had no impact on the phagocytosis of yeast by neutrophils. Lymphocyte function, cytokine production, and antibody production in Cu deficient cattle have been reported to be reduced (Gengelbach et al., 1997; Torre et al., 1995; Gengelbach and Spears

1998; Ward and Spears, 1999) or not impacted (Stabel et al., 1993; Torre et al., 1995; Gengelbach and Spears 1998; Ward et al., 1993; Ward et al., 1997; Arthington et al., 1995; Arthington et al., 1996) when compared to Cu adequate cattle in each respective experiment. In the current experiment, it appears that Cu status was not low enough in non-Cu-supplemented cattle to impact any of the immune parameters measured.

***Calf Performance and Mo and Cu Status***

Table 2.6 shows the effects of long-term Mo exposure in drinking water or diet on performance of both calf crops throughout the experiment. Year 1 and 2 initial and final BW, DMI, average daily gain (ADG) and water intake were similar across treatments and are representative of typical calf growth rates and water intake (NASEM, 2016).

The influence of chronic Mo exposure in drinking water or feed on calf liver and plasma Mo and Cu concentrations and serum ceruloplasmin activity of both calf crops is shown in Table 2.7. Initial and final liver and plasma Mo and Cu concentrations and ceruloplasmin activity were similar across treatments.

***Immune and Blood Chemistry Parameters in Calves***

The effects of long term Mo exposure in drinking water or diet on CBC, blood pH, $pO_2$, $pCO_2$ and immune parameters were determined for all calves throughout the experiment (data not shown). No treatment x year or treatment x time interactions were detected for any blood chemistries or immune measurements in calves. All blood chemistry parameters (CBC, blood pH, pO2, and $pCO_2$) were similar across treatments and were within normal values for beef calves. Initial and final IgG, IgM, INFɤ, were similar across treatments for years 1 and 2. Initial and final RBC SOD activity was similar across treatments in year 1. There was a tendency ($P <$

0.07) for year 2 calves receiving PC and Mo 1000-water+Cu treatments (Cu supplemented treatments) to have lower SOD activity when compared to all other treatments (data not shown).

***Cow and Calf Slaughter Data***

At the end of the experiment cows were transported to a commercial slaughter facility. Kidney, muscle (longissimus dorsi), pancreas, spleen, and subcutaneous adipose tissue were obtained post slaughter and analyzed for Mo and Cu concentrations for all cows (data not shown). Kidney Mo concentrations were greater ($P < 0.05$) in animals receiving supplemental Mo when compared to non-Mo-supplemented cattle (NC = 0.62; PC = 0.58; 500 Mo-water = 0.73; 1000 Mo-water = 0.71; 1000 Mo-water+Cu = 0.81; Mo-diet = 1.08 mg Mo/kg DM). Furthermore, kidney Mo concentrations were greater ($P < 0.05$) in animals receiving Mo in their diet than when compared to animals receiving Mo in drinking water. Kidney Cu concentrations were greater in cows receiving supplemental Cu when compared to non-Cu supplemented cows (NC = 4.08; PC = 5.12; 500 Mo-water = 4.32; 1000 Mo-water = 4.21; 1000 Mo-water+Cu = 5.01; Mo-diet = 4.08 mg Cu/kg DM). Treatment had no impact on the Mo or Cu concentrations in all other tissues collected.

All offspring from both years were removed from their respective treatments at weaning and fed a standard commercial finishing diet until they reached an appropriate slaughter weight. Animals were then transported to a commercial abattoir and slaughtered using standard U.S. beef industry practices and USDA/Food safety inspection service criteria and individual carcass data were collected. Hot carcass weight, dressing percentage, yield grade, and marbling score were similar across treatment for all calves across both years (data not shown).

The lack of a Mo impact on the response variables measured in the current experiment is similar to data reported by Gardner et al. (2003) and Raisbeck et al. (2006) when variable doses of Mo were consumed in feed. However, to our knowledge, the current experiment is the first experiment to examine the impact of long term Mo supplementation in drinking water or feed on life-cycle production parameters, health, and Mo and Cu status of gestating and lactating beef cows and their calves consuming a low quality forage diet. Under the conditions of the current experiment, no signs of molybdenosis were observed. Cattle that received diets containing less than the NASEM (2016) (10 mg Cu/kg DM) Cu requirement for beef cattle became Cu deficient over the course of the experiment, as determined by liver and plasma Cu concentrations, regardless of Mo treatment. However, no Cu deficiency signs (e.g., reduced growth rate, reproductive performance, or immune function) were observed throughout the course of the experiment. Furthermore, offspring Cu status was not different across treatments. This indicates that adequate maternal transfer of Cu occurs when dams are marginally deficient in Cu in current experiment.

Although the current experiment was designed to examine the impact of Mo in feed or water on Cu status in beef cattle, it is important to note that dietary S (feed and water) can influence the impact of Mo on Cu metabolism in ruminants. Dick was the first to determine that elevated dietary sulfur can greatly influence the antagonistic impact of Mo on Cu metabolism in ruminants (Dick, 1953). Subsequent research determined that S and Mo form thiomolybdates under the reducing conditions of the rumen and can bind to Cu in the gastrointestinal tract and either prevent Cu absorption or reduce the availability of Cu once absorbed into the blood stream (Suttle, 1974a; Suttle, 1974b; Suttle and Field, 1983; Price et al., 1987; Allen and Gawthorne, 1987; Allen and Gawthorne, 1988). In the current experiment, the S content of the diet was slightly above the NASEM (2016) dietary requirement of 0.15% for ruminants and well below dietary (0.30-0.50% S) and water (333 mg S/L of drinking water) concentrations know to impact Cu metabolism in ruminants.

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Table 2.1. Nutrient composition of grass hay (dry matter basis).

| Nutrient | Mean | Standard deviation |
|---|---|---|
| Dry matter, % | 86.45 | 5.69 |
| Crude Protein, % | 6.27 | 0.72 |
| Acid detergent fiber, % | 35.01 | 1.78 |
| Neutral detergent fiber | 57.91 | 3.29 |
| Net energy for lactation, Mcal/kg | 0.53 | 0.07 |
| Net Energy for gain, Mcal/kg[b] | 1.19 | 0.13 |
| Net energy for maintenance, Mcal/kg[c] | 1.06 | 0.08 |
| Total digestible nutrients, % | 53.47 | 2.69 |
| Digestible energy, Mcal/kg | 2.39 | 0.12 |
| Metabolizable energy, Mcal/kg | 2.09 | 0.14 |
| Calcium, % | 0.42 | 0.08 |
| Phosphorus, % | 0.14 | 0.04 |
| Potassium, % | 1.81 | 0.14 |
| Magnesium, % | 0.15 | 0.03 |
| Sodium, % | 0.03 | 0.01 |
| Sulfur, % | 0.15 | 0.06 |
| Cobalt, mg/kg | 0.24 | 0.06 |
| Copper, mg/kg[a] | 6.84 | 2.91 |
| Iron, mg/kg | 99.23 | 14.42 |
| Manganese, mg/kg | 180.11 | 20.12 |
| Molybdenum, mg/kg | 2.58 | 0.26 |
| Selenium, mg/kg | < 1.50 | --- |
| Zinc, mg/kg | 20.54 | 1.02 |

[a]Copper inclusion to copper containing treatments was adjusted based on the copper concentration of each hay source. Samples (n=525) were included in this analysis.

[b]NEg = $\{[1.42 \times (TDN \times 0.0361)] - [0.174 \times (TDN \times 0.0361) \times (TDN \times 0.0361)] + [0.0122 \times (TDN \times 0.0361) \times (TDN \times 0.0361) \times (TDN \times 0.0361) - 1.65]\} \div 2.205$.

[c] NEm = $\{[1.37 \times (TDN \times 0.0361)] - [0.0138 \times (TDN \times 0.0361) \times (TDN \times 0.0361)] + [0.0105 \times (TDN \times 0.0361) \times (TDN \times 0.0361) - 1.12]\} \div 2.205$.

Table 2.2. Molybdenum (Mo) and copper (Cu) concentrations of water, white salt, and dried distiller's grains (DDG) utilized to deliver experimenal treatmtens (mean ±SD).

| Item | Treatment | | | | | |
|---|---|---|---|---|---|---|
| | Negative Control[a] | Positive Control[b] | 500 µg Mo/L $H_2O$[c] | 1000 µg Mo/L $H_2O$[d] | 1000 µg Mo/L $H_2O$ + dietary Cu[e] | Mo diet[f] |
| Water | | | | | | |
| Mo, µg/L | < 10.0 | < 10.0 | 531.4 ±22.47 | 1037.0±117.62 | 1087.3±121.12 | < 10.0 |
| Cu, µg /L | < 10.0 | < 10.0 | < 10.0 | < 10.0 | < 10.0 | < 10.0 |
| White Salt[g] | | | | | | |
| Mo, mg/kg DM | < 0.01 | < 0.01 | < 0.01 | < 0.01 | < 0.01 | 458.21±20.34 |
| Cu, mg/kg DM | < 0.01 | 441.43±30.31 | < 0.01 | < 0.01 | 445.71±29.27 | < 0.01 |
| DDG[h] | | | | | | |
| Mo, mg/kg DM | 0.91±0.07 | 0.89±0.06 | 0.94±0.10 | 0.87±0.14 | 0.92±0.13 | 161.11±10.18 |
| Cu, mg/kg DM | 6.21±0.14 | 158.12±12.20 | 6.29±0.18 | 6.18±0.12 | 160.18±10.25 | 6.07±0.19 |
| Cu:Mo ratio[i] | 2.63:1 | 3.79:1 | 1.56:1 | 1.14:1 | 1.59:1 | 1.21:1 |

[a]Negative Control: no supplemental Mo or Cu added to the diet or water.
[b]Positive Control: 3.0 mg Cu/kg DM from $CuSO_4 \cdot 5H_2O$ added to the basal diet.
[c]500 µg Mo/L $H_2O$: Negative Control diet + 500 µg Mo/L from $Na_2MoO_4 \cdot 2H_2O$ supplied in the drinking water.
[d]1000 µg Mo/L $H_2O$: Negative Control diet + 1000 µg Mo/L of $Na_2MoO_4 \cdot 2H_2O$ supplied in the drinking water.
[e]Mo 1000-water plus 3 mg Cu/kg DM from $CuSO_4 \cdot 5H_2O$ added to the basal diet.
[f]Mo Diet: Negative Control diet plus 3.0 mg Mo/kg DM from $Na_2MoO_4 \cdot 2H_2O$ added to the basal diet.
[g]Target intake was 0.7% of DM intake.
[h]Dried distillers grains; supplemented at 0.25 kg·animal$^{-1}$·d$^{-1}$ or 1.9% of diet DM and contained 0.48% sulfur.
[i]Calculated based on the actual intake of water, feed, and supplement consumed by cows in this experiment.

Table 2.3. Chemical composition of water supplied to cattle while housed in Grand County, CO (Williams Fork River) and at the Agricultural, Research, Development, and Education Center (ARDEC-well water).

| Item | Williams Fork River water Result (mean ±standard deviation) | ARDEC-well water Result (mean±standard deviation) |
|---|---|---|
| n= | 115 | 126 |
| pH, s.u. | 7.24 (±0.17) | 7.42 (±0.16) |
| Chloride, mg/L | 5.2 (±0.10) | 28.6 (±3.24) |
| Total Hardness, mg/L | 26.4 (±1.21) | 75.4 (±58.24) |
| Nitrate-Nitrogen, mg/L | <1.0 (±N/A) | <1.0 (±N/A) |
| Calcium, mg/L | 7.93 (±0.21) | 174.2 (±12.54) |
| Magnesium, mg/L | 1.53 (±0.06) | 54.23 (±0.071) |
| Phosphorous, mg/L | <0.1 (±N/A) | <0.1 (±N/A) |
| Potassium, mg/L | <5.0 (±N/A) | <5.0 (±N/A) |
| Sodium, mg/L | <5.0 (±N/A) | 56.3 (±9.29) |
| Sulfate, mg/L | 2.72 (±0.08) | 432.7 (±29.70) |
| Aluminum, mg/L | 0.21 (±0.01) | 0.06 (±0.004) |
| Cobalt, mg/L | <0.01 (±N/A) | <0.01 (±N/A) |
| Copper, µg/L | <10.0 (±N/A) | <10.0 (±N/A) |
| Iron, mg/L | 0.27 (±0.01) | 0.12 (±0.02) |
| Manganese (mg/L) | <0.01 (±N/A) | 0.08 (±0.05) |
| Molybdenum (µg/L) | <10.0 (±N/A) | <10.0 (±N/A) |
| Selenium (µg/L) | <30.0 (±N/A) | <30.0 (±N/A) |
| Total Dissolved Solids (mg/L) | 42.5 (±3.97) | 985.7 (±85.24) |

Table 2.4. The influence of chronic molybdenum exposure in drinking water or feed on cow performance.

| Item | Treatment | | | | | | | *P* < | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Negative Control[a] | Positive Control[b] | 500 µg Mo/L $H_2O$[c] | 1000 µg Mo/L $H_2O$[d] | 1000 µg Mo/L $H_2O$ + dietary Cu[e] | Mo diet[f] | SEM | TRT | Time | Trt x time |
| Initial body weight, kg | 522.5 | 571.3 | 529.0 | 548.6 | 540.9 | 579.5 | 12.3 | 0.72 | --- | --- |
| Final body weight, kg | 553.1 | 557.4 | 561.2 | 554.9 | 562.3 | 561.7 | 8.9 | 0.64 | 0.001 | 0.59 |
| Body condition score[g] | 4.9 | 5.1 | 5.3 | 5.0 | 5.1 | 5.2 | 0.05 | 0.81 | 0.0001 | 0.83 |
| Dry matter intake, kg/d | 13.6 | 14.8 | 14.2 | 14.4 | 13.8 | 14.0 | 1.3 | 0.73 | 0.05 | 0.68 |
| Water intake, $L\cdot animal^{-1}\cdot d^{-1}$ | 44.2 | 45.3 | 46.9 | 46.9 | 45.1 | 46.9 | 7.2 | 0.54 | 0.001 | 0.81 |
| Pregnancy rate to artificial insemination, % | | | | | | | | | | |
| Year 1, % | 56 | 44 | 56 | 44 | 56 | 57 | --- | 0.89 | --- | --- |
| Year 2, % | 44 | 56 | 56 | 44 | 56 | 71 | --- | 0.56 | --- | --- |
| Overall pregnancy rate after a 60-d breeding season, % | | | | | | | | | | |
| Year 1, % | 100 | 100 | 89 | 100 | 100 | 88 | --- | 0.82 | --- | --- |
| n= | 9 | 9 | 8 | 9 | 9 | 7 | --- | --- | --- | --- |
| Year 2, % | 100 | 89 | 100 | 89 | 89 | 100 | --- | 0.79 | --- | --- |
| n= | 9 | 9 | 8 | 9 | 9 | 7 | --- | --- | --- | --- |

[a]Negative Control: no supplemental Mo or Cu added to the diet or water.
[b]Positive Control: 3.0 mg Cu/kg DM from $CuSO_4\cdot 5H_2O$ added to the basal diet.
[c]500 µg Mo/L $H_2O$: Negative Control diet + 500 µg Mo/L from $Na_2MoO_4\cdot 2H_2O$ supplied in the drinking water.
[d]1000 µg Mo/L $H_2O$: Negative Control diet + 1000 µg Mo/L of $Na_2MoO_4\cdot 2H_2O$ supplied in the drinking water.
[e]Mo 1000-water plus 3 mg Cu/kg DM from $CuSO_4\cdot 5H_2O$ added to the basal diet.
[f]Mo Diet: Negative Control diet plus 3.0 mg Mo/kg DM from $Na_2MoO_4\cdot 2H_2O$ added to the basal diet.
[g]BCS: 1 = emaciated; 9 = obese [19].

Table 2.5. The influence of chronic molybdenum exposure in drinking water or feed on cow liver, plasma, and milk molybdenum (Mo) and copper (Cu) concentrations and serum ceruloplasmin activity.

| Item | Negative Control[a] | Positive Control[b] | 500 µg Mo/L $H_2O$[c] | 1000 µg Mo/L $H_2O$[d] | 1000 µg Mo/L $H_2O$ + dietary Cu[e] | Mo diet[f] | SEM | *P*< TRT | *P*< Time | *P*< Trt x time |
|---|---|---|---|---|---|---|---|---|---|---|
| Liver | | | | | | | | | | |
| Mo, mg/kg DM | | | | | | | | | | |
| Initial | 3.62 | 4.44 | 4.07 | 4.14 | 2.86 | 2.93 | 0.63 | 0.94 | --- | --- |
| Final | 3.94 | 4.19 | 3.79 | 3.98 | 3.61 | 4.09 | 0.49 | 0.68 | 0.05 | 0.37 |
| Cu, mg/kg DM | | | | | | | | | | |
| Initial | 31.2 | 30.9 | 31.7 | 32.1 | 32.7 | 32.2 | 1.08 | 0.83 | --- | --- |
| Final[g] | 24.3[x] | 70.2[y] | 26.3[x] | 27.1[x] | 61.9[y] | 26.3[x] | 1.23 | 0.01 | 0.001 | 0.01 |
| Plasma | | | | | | | | | | |
| Mo, mg/L | | | | | | | | | | |
| Initial | 0.17 | 0.16 | 0.20 | 0.21 | 0.22 | 0.18 | 0.04 | 0.92 | --- | --- |
| Final | 0.19 | 0.22 | 0.18 | 0.24 | 0.19 | 0.23 | 0.05 | 0.87 | 0.95 | 0.98 |
| Cu, mg/L | | | | | | | | | | |
| Initial | 0.96 | 1.02 | 0.93 | 1.20 | 0.95 | 1.11 | 0.15 | 0.91 | --- | --- |
| Final[g] | 0.59[x] | 1.17[y] | 0.62[x] | 0.58[x] | 0.81[y] | 0.53[x] | 0.21 | 0.02 | 0.01 | 0.04 |
| Serum Ceruloplasmin, IU/L | | | | | | | | | | |
| Initial | 32.12 | 35.68 | 34.94 | 34.17 | 36.90 | 35.43 | 0.89 | 0.78 | --- | --- |
| Final[g] | 10.32[x] | 40.21[y] | 12.71[x] | 11.39[x] | 41.47[y] | 10.97[x] | 1.02 | 0.01 | 0.001 | 0.65 |
| Milk | | | | | | | | | | |
| Mo, mg/L | | | | | | | | | | |
| Initial | 0.15 | 0.16 | 0.13 | 0.16 | 0.17 | 0.11 | 0.01 | 0.92 | --- | --- |
| Final | 0.18 | 0.17 | 0.22 | 0.21 | 0.18 | 0.20 | 0.01 | 0.18 | 0.05 | 0.39 |
| Cu, mg/L | | | | | | | | | | |
| Initial | 0.081 | 0.084 | 0.092 | 0.063 | 0.059 | 0.064 | 0.001 | 0.76 | --- | --- |
| Final | 0.086 | 0.091 | 0.083 | 0.079 | 0.077 | 0.080 | 0.001 | 0.57 | 0.19 | 0.61 |

[a]Negative Control: no supplemental Mo or Cu added to the diet or water.
[b]Positive Control: 3.0 mg Cu/kg DM from $CuSO_4 \cdot 5H_2O$ added to the basal diet.
[c]500 µg Mo/L $H_2O$: Negative Control diet + 500 µg Mo/L from $Na_2MoO_4 \cdot 2H_2O$ supplied in the drinking water.
[d]1000 µg Mo/L $H_2O$: Negative Control diet + 1000 µg Mo/L of $Na_2MoO_4 \cdot 2H_2O$ supplied in the drinking water.
[e]Mo 1000-water plus 3 mg Cu/kg DM from $CuSO_4 \cdot 5H_2O$ added to the basal diet.
[f]Mo Diet: Negative Control diet plus 3.0 mg Mo/kg DM from $Na_2MoO_4 \cdot 2H_2O$ added to the basal diet.
[g]Means within a row lacking common superscripts differ $P < 0.05$.

Table 2.6. The effects of long term molybdenum exposure in drinking water or diet on performance of two calf crops from dams fed a high forage diet[1].

| | Treatment[1] | | | | | | | P < |
|---|---|---|---|---|---|---|---|---|
| Item | Negative Control[a] | Positive Control[b] | 500 µg Mo/L $H_2O$[c] | 1000 µg Mo/L $H_2O$[d] | 1000 µg Mo/L $H_2O$ + dietary Cu[e] | Mo diet[f] | SEM | Trt |
| Initial body weight, kg | | | | | | | | |
| Year 1 | 138.9 | 153.3 | 146.0 | 136.6 | 153.7 | 143.5 | 2.9 | --- |
| Year 2[g] | 33.1 | 34.2 | 32.7 | 31.8 | 32.9 | 33.1 | 0.92 | 0.67 |
| Final body weight, kg[h] | | | | | | | | |
| Year 1 | 170.5 | 166.8 | 165.6 | 160.4 | 172.7 | 161.4 | 3.0 | 0.74 |
| Year 2 | 180.2 | 172.3 | 171.9 | 174.6 | 173.8 | 170.9 | 3.4 | 0.67 |
| Dry matter intake, $kg \cdot animal^{-1} \cdot d^{-1}$ | | | | | | | | |
| Year 1 | 4.4 | 3.5 | 3.9 | 3.9 | 3.8 | 4.1 | 0.29 | 0.57 |
| Year 2 | 4.6 | 4.2 | 4.3 | 4.4 | 4.6 | 4.2 | 0.31 | 0.49 |
| Average daily gain, $kg \cdot animal^{-1} \cdot d^{-1}$ | | | | | | | | |
| Year 1 | 0.96 | 0.79 | 1.06 | 1.12 | 0.87 | 0.82 | 0.18 | 0.62 |
| Year 2 | 1.02 | 0.97 | 0.96 | 1.1 | 1.0 | 0.91 | 0.14 | 0.47 |
| Water intake, $L \cdot animal^{-1} \cdot d^{-1}$,[I] | | | | | | | | |
| Year 1 | 5.2 | 5.7 | 4.6 | 6.0 | 5.5 | 5.9 | 0.39 | 0.58 |
| Year 2 | 6.1 | 5.9 | 6.2 | 5.7 | 5.9 | 5.4 | 0.29 | 0.49 |

[1]Data presented are from birth or experiment initiation up to weaning.
[a]Negative Control: no supplemental Mo or Cu added to the diet or water.
[b]Positive Control: 3.0 mg Cu/kg DM from $CuSO_4 \cdot 5H2O$ added to the basal diet.
[c]500 µg Mo/L $H_2O$: Negative Control diet + 500 µg Mo/L from Na2MoO4·2H2O supplied in the drinking water.
[d]1000 µg Mo/L $H_2O$: Negative Control diet + 1000 µg Mo/L of Na2MoO4·2H2O supplied in the drinking water.
[e]Mo 1000-water plus 3 mg Cu/kg DM from $CuSO_4 \cdot 5H2O$ added to the basal diet.
[f]Mo Diet: Negative Control diet plus 3.0 mg Mo/kg DM from Na2MoO4·2H2O added to the basal diet.
[g]Birth weight for Year 2 calves. Year 1 calves were approximately 5 months of age at the initiation of the experiment.
[h]Adjusted 205 day weaning weight.
[i]Measured during the pasture phase only.

Table 2.7. The influence of chronic molybdenum exposure in drinking water or feed on calf liver and plasma molybdenum and copper concentrations and serum ceruloplasmin activity of two calf crops from dams fed a high forage diet.

| Item | Negative Control[a] | Positive Control[b] | 500 µg Mo/L $H_2O$[c] | 1000 µg Mo/L $H_2O$[d] | 1000 µg Mo/L $H_2O$ + dietary Cu[e] | Mo diet[f] | SEM | P< TRT[g] | P< Time | P< Trt x time |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Liver Mo, mg/kg DM | | | | | | |
| Year 1-Initial | 3.05 | 3.40 | 3.25 | 5.19 | 5.64 | 4.24 | 0.98 | 0.39 | --- | --- |
| Year 1-Final | 4.91 | 4.76 | 5.01 | 5.24 | 5.19 | 4.69 | 0.81 | 0.76 | 0.61 | 0.62 |
| Year 2-Initial | 5.31 | 5.28 | 4.97 | 5.47 | 5.21 | 5.63 | 0.94 | 0.58 | --- | --- |
| Year 2-Final | 5.01 | 5.21 | 5.07 | 5.41 | 4.98 | 5.14 | 0.86 | 0.68 | 0.39 | 0.84 |
| | | | | Liver Cu, mg/kg DM | | | | | | |
| Year 1-Initial | 52.14 | 49.73 | 53.61 | 52.76 | 54.47 | 50.29 | 1.78 | 0.74 | --- | --- |
| Year 1-Final | 44.25 | 54.17 | 46.98 | 47.96 | 45.39 | 49.18 | 2.36 | 0.43 | 0.01 | 0.67 |
| Year 2-Initial | 103.03 | 120.41 | 106.21 | 111.32 | 117.12 | 108.65 | 4.12 | 0.49 | --- | --- |
| Year 2-Final | 52.97 | 63.02 | 54.01 | 53.64 | 60.98 | 57.36 | 2.31 | 0.64 | 0.41 | 0.54 |
| | | | | Plasma Mo, mg/kg DM | | | | | | |
| Year 1-Initial | 0.17 | 0.19 | 0.18 | 0.19 | 0.16 | 0.13 | 0.07 | 0.90 | --- | --- |
| Year 1-Final | 0.19 | 0.17 | 0.20 | 0.16 | 0.17 | 0.21 | 0.08 | 0.67 | 0.81 | 0.93 |
| Year 2-Initial | 0.19 | 0.16 | 0.21 | 0.18 | 0.19 | 0.22 | 0.07 | 0.74 | --- | --- |
| Year 2-Final | 0.21 | 0.22 | 0.18 | 0.20 | 0.23 | 0.24 | 0.08 | 0.34 | 0.59 | 0.61 |
| | | | | Plasma Cu, mg/kg DM | | | | | | |
| Year 1-Initial | 0.70 | 0.76 | 0.67 | 0.65 | 0.71 | 0.57 | 0.19 | 0.85 | --- | --- |
| Year 1-Final | 0.79 | 0.82 | 0.87 | 0.79 | 0.82 | 0.72 | 0.23 | 0.91 | 0.05 | 0.69 |
| Year 2-Initial | 0.72 | 0.81 | 0.69 | 0.75 | 0.79 | 0.70 | 0.19 | 0.64 | --- | --- |
| Year 2-Final | 0.70 | 0.83 | 0.65 | 0.71 | 0.77 | 0.65 | 0.14 | 0.41 | 0.19 | 0.39 |
| | | | | Serum Ceruloplasmin, IU/L | | | | | | |
| Year 1-Initial | 29.93 | 28.70 | 31.94 | 30.47 | 29.78 | 31.87 | 1.74 | 0.89 | --- | --- |
| Year 1-Final | 31.24 | 32.98 | 34.39 | 35.74 | 32.68 | 31.93 | 1.31 | 0.87 | 0.01 | 0.76 |
| Year 2-Initial | 30.21 | 31.63 | 29.98 | 30.87 | 31.07 | 30.22 | 1.21 | 0.64 | --- | --- |
| Year 2-Final | 31.03 | 32.61 | 29.07 | 29.96 | 31.24 | 30.97 | 1.07 | 0.74 | 0.65 | 0.81 |

[a]Negative Control: no supplemental Mo or Cu added to the diet or water.
[b]Positive Control: 3.0 mg Cu/kg DM from $CuSO_4 \cdot 5H2O$ added to the basal diet.
[c]500 µg Mo/L $H_2O$: Negative Control diet + 500 µg Mo/L from Na2MoO4·2H2O supplied in the drinking water.
[d]1000 µg Mo/L $H_2O$: Negative Control diet + 1000 µg Mo/L of Na2MoO4·2H2O supplied in the drinking water.
[e]Mo 1000-water plus 3 mg Cu/kg DM from $CuSO_4 \cdot 5H2O$ added to the basal diet.
[f]Mo Diet: Negative Control diet plus 3.0 mg Mo/kg DM from Na2MoO4·2H2O added to the basal diet.
[g].There are no significant differences between means within a row at $P < 0.05$.

# CHAPTER 3 – INFLUENCE OF MOLYBDENUM IN DRINKING WATER OR FEED ON COPPER METABOLISM IN CATTLE – A REVIEW


## SUMMARY

The majority of Mo research has focused on the antagonist effect of Mo, alone or in combination with elevated dietary S, on Cu absorption and metabolism in ruminants. Diets containing both >5.0 mg of Mo/kg DM and >0.33% S have been reported to reduce the Cu status in cattle and sheep. Therefore, due to the potential for inducing Cu deficiency, Mo and S concentrations in the diet should be monitored and kept within appropriate values. Elevated sulfate concentrations in drinking water can also be detrimental to livestock production, especially in ruminants. High concentrations of sulfate in water have been extensively studied in cattle because high-sulfate water induces polioencephalomalacia in ruminants. However, little research has been conducted investigating the impact of Mo in water on Cu metabolism in ruminants. Based on the limited number of published experiments, it appears that Mo in drinking water may have a lower antagonistic impact on the cu status in cattle when compared to Mo consumed in the diet. This response may be due to a certain percentage of water bypassing the rumen when consumed by ruminants. Therefore, the objective of this review was to examine the impact of Mo in drinking water on cattle performance and Mo and Cu metabolism.


## INTRODUCTION

The impact of water quality on beef cattle production has been extensively studied. Common water quality contaminants that negatively impact livestock production have been reported in the literature and have been summarized (Wright, 2007; NASEM, 2016). These include microbial contamination, elevated soluble salts, nitrates, sulfates, and soluble cations such as calcium (Ca) and magnesium (Mg). Other elements such as copper (Cu), manganese (Mn), and zinc (Zn) can also be detrimental to water quality. However, these elements are not typically found in concentrations great enough to impact water consumption or animal performance.

Certain areas within the Rocky Mountain region contain natural deposits of rock and/or sediment that present high concentrations of molybdenum (Mo). Due to natural geological events or human activity, Mo deposits can become soluble and enter ground or surface water systems (Kistner et al, 2016). In 2012, the Environmental Protection Agency set a Colorado Mo agricultural water standard of 160 μg/L. Although the impact of elevated water concentrations of Mo on livestock production has not been extensively studied, the in-fluence of dietary Mo concentrations on livestock production in conjunction with dietary sulfur (S) has been extensively investigated. In ruminants, dietary concentration of Mo (ranging from 4 to 50 mg Mo/kg DM) have been reported to reduce the Cu status in cattle and sheep (Dick and Bull, 1945; Vandereen and Keener, 1964; Dowdy and Matrone, 1968). However, elevated dietary Mo concentrations in combination with elevated dietary S (>0.33%) concentrations have been reported to substantially reduce the Cu status in cattle and sheep (Mills et al., 1977). This is most likely through the formation of thiomolybdates in the gastrointestinal tract of the animals (Dick, 1953; Mylrea, 1958; Suttle, 1974a; Suttle, 1974b; Suttle, 1991). Because Cu is an essential dietary element involved in numerous biochemical reactions in ruminants (Davis and Cooper, 1987; Underwood and Suttle, 1999), understanding the impact of elevated Mo and/or S concentrations on Cu absorption and tissue deposition is important.

Due to the negative impact of high dietary concentrations of Mo (>5.0 mg Mo/kg DM), alone and especially, in combination with elevated dietary S concentrations, on the Cu status in ruminants, the Mo and S concentrations of water should be considered when formulating diets for ruminants to prevent Cu deficiency and optimize animal performance. The impact of high S concentrations in water on animal performance is beyond the scope of this review. For in-depth reviews of the impact of S in water and diets on livestock production, see Wright (2007) and Drewnoski et al., (2014), respectively. This review will focus on the impact of Mo in drinking water on cattle performance and Mo and Cu metabolism.

## MOLYBDENUM

### *Soil*

Molybdenum is typically found in soils as sesquioxide, water-soluble Mo or organically bound Mo (Albasel and Pratt, 1989). The availability of Mo in soil to the plant depends on soil pH and on the concentration of other elements such as phosphate (Albasel and Pratt, 1989; Barshad, 1948). In acidic soils, Mo is mainly in sesquioxide forms (e.g., iron oxides) and thus unavailable for plant uptake (Albasel and Pratt, 1989) or entry into water systems as soluble Mo. Soils with an alkaline pH (e.g., soils that are poorly drained and aerated) have typically greater concentrations of water-soluble Mo. which is available for uptake by plants and possible movement into water systems (Kincaid, 1980). Therefore, the total Mo soil concentration is not a good indicator of Mo concentrations in the vegetation.

### *Plants*

In general, Mo concentrations in the vegetation used for livestock production typically range from 0.6 to 3.5 mg Mo/kg DM but have been reported to be in excess of 50 mg Mo/kg DM in areas with alkaline soil and elevated soil Mo concentrations (NASEM, 2016; Albasel and Pratt, 1989; Barshad, 1948; Legendre and Runnells, 1975). Molybdenum absorption in plants is mostly from soluble forms of Mo in the soil, pre-dominantly molybdate ($MoO_4^{-2}$; Gupta and

Lipsett, 1981). Molybdenum plays a functional role in nitrogenase in plants. Nitrogenase functions to convert nitrogen to ammonia, which is then used by plants (Chatt and Leigh, 1972). Molybdenum also works in nitrate reduction as an electron carrier in the enzyme nitrate reductase. Nitrate reductase reduces nitrate to nitrite in plants and in some microbial populations associated with plant roots and within the gastrointestinal tract of animals (Nicholas and Nason, 1955). Reddy (1964) reported that the application of Mo to the soil can in-crease the crude protein content of plants.

Due to the role of Mo in nitrogen fixation in plants, Mo deficiency can cause a reduction in plant nitrogen metabolism and overall nitrogen fixation (Gupta and Lipsett, 1981). Legume plants such as ladino clover and birdsfoot trefoil have a higher Mo requirement than non-legume plants. Legumes grown in alkaline soils (pH 7.0–7.5) are the most efficient in absorbing Mo (Cameron and Goss. 1948), most likely because Mo becomes soluble at alkaline pH. Although little data are available as to what is deemed normal for Mo concentrations in soil, normal soil Mo concentrations are suggested to range from 0.5 to 5 mg Mo/kg DM (Gupta and Lipsett, 1981). Legumes grown in areas with naturally high concentrations of Mo in the soil have been found to contain between 20 and 40 mg of Mo/kg DM (Kubota et al., 1961).

In a three-year plant study, Jensen and Lesperance (1971) compared Mo accumulation by several forage plant species with variables indicative of plant growth. They reported that Mo accumulation by plants typically used as feed for grazing livestock was affected by soil pH, plant species, location within the plant, depth to the water table, and agronomic practices. Other researchers have confirmed the findings of Jensen and Lesperance (Legendre and Runnells, 1975; Jensen and Lesperance, 1971; Wittenberg and Boila, 1988). Overall, Mo accumulation in plant tissues, in the aforementioned experiments, varied significantly. Legumes generally accumulated more Mo than grasses, likely due to the role of Mo in nitrogen fixation in legumes (Legendre and Runnells, 1975; Jensen and Lesperance, 1971; Lesperance and Bohman, 1963), although there was a large variation in Mo concentrations between individual plant species (Legendre and Runnells, 1975; Kubota et al., 1961). The Mo content of legumes and grasses appeared to increase with the age of the plant (Barshad, 1948; Kubota et al., 1961). Growing plants are able to absorb soluble Mo from an alkaline soil, and Mo concentration appears to be the greatest in the blades and leaves of plants (in concentrations of 1.5 to 5.0 mg Mo/kg DM, which may impact Cu metabolism in grazing ruminants) (Barshad, 1948; Kubota et al., 1961). Forage grown where the water table was near the soil surface contained more Mo than forage grown on soil with a greater water depth (Jensen and Lesperance, 1971). Concentrations of Mo in plants ranged from values indicative of plant Mo deficiency (<0.10 mg Mo/kg DM) to values as high as 300 to 400 mg Mo/kg DM (Legendre and Runnells, 1975).

***Water***

Water is frequently suggested as a source of excess Mo, although no cases of Mo toxicity due to consumption of Mo in water have been confirmed under practical production conditions. Since cattle consume much larger quantities of water than dry matter, the expectation of Mo toxicity (diarrhea, anorexia, weight loss, stiffness, changes in hair color) under lower water Mo concentrations would be logical (Ward, 1978). For approximate water intakes for beef cattle, see Table 3.1, adapted from the NASEM (2016). Estimates of water consumption are related to cattle production classification, body weight, and ambient temperature. Numerous factors, such as dry matter intake, animal species, breed, stage of production, water quality, and the environment can influence water in-take. Table 3.2 shows the calculated influence of dietary and water Mo concentrations on total Mo consumed (mg) per day for different dietary and water Mo concentrations. Based on these calculations, assuming that a mature beef animal would consume ap-proximately 50 L of water and 10 kg of dry matter per day, if water and feed Mo concentration were equal, water would contribute 5 times the amount of total Mo intake with respect to feed.

***Functions***

Molybdenum functions as a component of several oxidase enzymes in animals (xanthine oxidase, sulfite oxidase, and aldehyde oxidase) (Johnson et al., 1980; Mills and Davis, 1987). Numerous reviews have been published on the impact of dietary Mo on ruminants' Cu status and overall production (NASEM, 2016; Davis and Copper, 1987; Underwood and Suttle, 1999; Ward, 1978; Mills and Davis, 1987; Dick, 1956; Underwood, 1977; Friberg and Lener, 1986; Hall, 2007; Gould and Kendall, 2011). However, as mentioned previously, the dietary requirements for Mo are not well defined for beef cattle because, under practical feeding conditions, Mo deficiency has not been reported (NASEM, 2016; Mills and Davis, 1987). There is limited evidence that the addition of Mo to sheep and beef cattle diets may improve diet digestibility depending on diet type (roughage compared to concentrate). Adding 10 mg of Mo/kg diet DM to a high-fiber diet (basal diet contained 1.7 mg of Mo/kg DM) improved in situ DM digestibility in steers compared to control animals but had no impact when animals were fed a ground barley-based diet supplemented with Mo (Shariff et al., 1990). Earlier research by Ellis et al. (1958) reported an improvement in growth rate and cellulose digestion in lambs supplemented with 2 mg of Mo/kg DM (basal diet contained 0.36 mg Mo/kg DM). However, Ellis and Pfander (1960) were unable to reproduce this improvement in growth and cellulose digestibility in subsequent experiments.

Arguably, the most extensively studied aspect of Mo in ruminant diets is in relationship to dietary S and Cu concentrations. Ferguson et al. (1938) originally reported that excess Mo in the form of 'teart' herbage (herbage with elevated Mo concentrations) caused: 1) a decrease in milk production, 2) scours, and 3) a reduction of body condition in grazing cattle. Ferguson et al. (1938) were unsure if Mo was directly responsible for the symptoms reported or if it was metabolized to a toxic product in the rumen. Dick and Bull (1945) were the first to report that long-term Mo supplementation reduced the Cu status in cattle. Later, Dick (1952) reported similar impacts of elevated dietary Mo on reducing the Cu status in sheep. Upon further investigation, Dick (1953) determined that the dietary S content substantially increases the antagonistic impact of Mo on Mo and Cu metabolism in sheep. Since this discovery, several researchers have examined the impact of dietary Mo and S concentrations on Cu metabolism in cattle and sheep (Dowdy and Matrone, 1968; Suttle, 1974a; Suttle, 1974b; Ward and Spears, 1997). Collectively, these data indicate that elevated dietary concentrations of Mo (≥5.0 mg Mo/kg DM) in the presence of adequate dietary S concentrations (≈0.2% S) or elevated dietary S (≥0.33 %S) concentrations in the presence of moderate Mo (<2.0 mg Mo/kg DM) concentrations can modestly reduce Cu absorption in ruminants by 0.5 to 5%. This antagonism is most likely through the formation of insoluble Cu–Mo or Cu–S complexes. The location of the formation of Cu–Mo and Cu–S complexes in the gastro-intestinal track is not completely understood (Suttle, 1974b). Additionally, elevated dietary S (≥0.33% S) in the presence of elevated dietary Mo (≥5.0 mg Mo/kg DM) concentrations can drastically reduce Cu absorption in cattle and sheep by approximately 60%. The reduction in Cu status with elevated dietary Mo and S is most likely due to the formation of insoluble Cu–Mo–S complexes in the rumen. For extensive reviews of the formation and impact of thiomolybdates on Cu metabolism in ruminants, see (Suttle, 1991; Underwood and Suttle, 1999; Gould and Kendall, 2011).

***Dietary Molybdenum and Copper***

Molybdenum toxicity can be of concern due to possible depletion of Cu stores through the formation of thiomolybdates. For a thorough review of the formation of thiomolybdates in ruminants, see Suttle (1991). A physiological Cu deficiency in animals from excess Mo consumption can lead to a multitude of issues affecting growth and overall animal health. Molybdenum intake in feed has been closely studied. In 1975, Kubota examined the geographic distribution of Mo in western states of the USA in relation to historical reports of molybdenosis and Cu deficiency in grazing beef cattle. Kubota (1975) concluded that cattle grazing forages with 10 to 20 mg of Mo/kg DM were likely to exhibit Mo toxicity symptoms. In a review by Underwood and Suttle (Underwood and Suttle, 1999; Ferguson et al., 1938), cattle grazing teart pastures that contained between 20 and 100 mg of Mo/kg plant DM experienced mild to extreme forms of scouring (Ferguson et al., 1938).

The Cu:Mo ratio is important, as well as the total concentration of Mo in the diet. Ward (1978) reviewed previously published data investigating the influence of Mo dietary intake on cattle performance and Cu status. Ward (1978) concluded that cattle fed more than approximately 100 mg of Mo/kg DM or fed forage with a Cu:Mo ratio of 2:1 or less can experience Cu deficiencies. Gardner et al. (2003) investigated the impact of Mo on gestating cows nursing calves and grazing standing forages containing between 21 and 44 mg of Mo/kg DM in reclaimed mining areas. Cow–calf pairs were allowed to graze for 12 weeks each year for three consecutive years on the reclaimed mining area pastures. No signs of molybdenosis, Cu deficiency, or any adverse health effects, regardless of whether cattle received a Cu bolus or not, were reported. The authors concluded that the risk assessment value of 10 mg of Mo/kg DM reported by O'Conor et al. (2001) to establish Mo standards for land application of biosolids is conservative for grazing cattle. However, the reason for the lack of observed molybdenosis in these cattle may be the short duration of Mo exposure in the Gardner et al. (2003) experiment. In 2006, Raisbeck et al. (2006) conducted a long-term grazing experiment where pregnant cows (n = 306) were grazed on one of three pastures containing different Mo concentrations in the standing forage (2.0, 13, and 230 mg of Mo/kg DM for pastures 1, 2, and 3, respectively). Pasture 1 contained 59 cows, pasture 2 contained 241 cows, and pasture 3 contained 6 cows. For pastures 2 and 3, all cattle were given a protein supplement formulated to provide 17 mg of Cu/kg DM total diet. Furthermore, half for the cows in pastures 2 and 3 received a 25 g CuO bolus (21.8 g elemental Cu equivalent) every 60 d throughout the 12-month experiment. Cattle in pasture 1 received protein supplementation but minimal Cu supplementation (2 mg Cu/kg DM total diet), and no CuO boluses were administered to these cows. Although not described fully in the publication, Raisbeck et al. (2006) indicated that standing forage Cu concentrations varied across season and that cattle were fed supplemental alfalfa hay during the winter months. Therefore, the approximate Cu:Mo ratios for cattle in pasture 1 and cattle not receiving CuO boluses in pastures 2 and 3 were 3.7:1, 1.8:1, and 0.10:1, respectively. Raisbeck et al. (2006) reported no adverse effects of Mo on cattle housed in pastures 1 and 2 and noted that two cattle in pasture 3 (230 mg Mo/kg DM in standing forages) exhibited diarrhea and lameness (signs indicative of molybdenosis) in the last two weeks of the experiment. However, the bull was diagnosed with a spinal injury and treated with flunixin meglumine, and the cow was diagnosed with a bacterial infection and treated with an antibiotic. Both animals recovered within a week of being treated and were physiologically normal. Raisbeck et al. (2006) also reported that the Mo and Cu statuses were not impacted in cattle grazing pastures containing 13 mg of Mo/kg DM. However, cattle grazing pastures containing 230 mg of Mo/kg DM exhibited an increase in liver Mo concentrations as well as an increase in soluble serum Cu and liver Cu concentrations, regardless of CuO bolus administration. The authors concluded that cattle receiving the greatest Mo concentrations (230 mg Mo/kg DM) had an increased Mo status indicative of molybdenosis but never showed signs of molybdenosis and that no negative impacts on animal growth, calf weaning weighs, or reproductive performance in any of the animals in the experiment were observed. The Mo and Cu concentrations consumed by cattle in pasture 2 (13 mg Mo/kg DM) of the Raisbeck et al. (2006) experiment were approximately 156 mg of Mo · head$^{-1}$ · d$^{-1}$ and 240 mg of Cu · head$^{-1}$ · d$^{-1}$ (using an estimated DMI of 12 kg · head$^{-1}$ · d$^{-1}$, a Mo forage concentration of 13 mg Mo/kg DM, and an estimated total diet Cu concentration of 20 mg Cu/kg DM), the dietary Cu/Mo ratio was 1.8:1 and did not impact Cu status or animal performance.

***Molybdenum and Water***

As previously described, the majority of research investigating the impact of Mo on Cu metabolism in ruminants has been conducted by supplementing varying concentrations of dietary Mo and S and monitoring the Cu status of the animal. Very few studies have investigated the impact of Mo supplied in drinking water on Cu metabolism in ruminants.

In 1980, Kincaid conducted an experiment utilizing 12 male, 5-week-old Holstein calves. Calves were allowed ad libitum access to drinking water containing targeted concentrations of 0.0, 1.0, 10.0, and 50.0 mg of Mo/L (analyzed Mo concentrations were <1.0, 1.0, 8.0, and 53.0 mg Mo/L, respectively) for 21 days. The basal diet for these calves contained 13 mg of Cu/kg DM (<1 mg of Mo/kg diet DM and 0.29% S). There was no difference in body weight gain across all treatments. At the greatest Mo water concentration (50.0 mg of Mo/L), Kincaid (1980) reported an increase in plasma Cu concentrations and a numeric decrease in liver Cu concentrations. Calves receiving 0.0, 1.0, and 10.0 mg of Mo/L in drinking water had similar plasma and liver Cu concentrations and ceruloplasmin levels. Kincaid (1980) indicated that the safe Cu-to-Mo ratio in this experiment was 0.5:1.0. Kincaid (1980) also suggested that Mo in water could be less toxic than Mo in forage and that the minimum toxic concentration of Mo in water for calves in this experiment was between 10 and 50 mg of Mo/L.

In 2017, Kistner et al. performed an experiment with 30 Angus, Hereford, and Angus × Herford steers exposed to varying doses of Mo in drinking water. Water treatments consisted of: (1) 0.0 mg/L, (2) 0.16 mg/L, (3) 0.32 mg/L, (4) 0.48 mg/L, and (5) 0.96 mg/L of supplemental Mo added as $Na_2MoO_4$ to drinking water. Steers were housed in individual pens and fed a growing diet for 28 days and then transitioned to a finishing diet. Mo exposure was maintained for a period of 112 to 151 days. No adverse effects were observed in any animals at any Mo dose. Total dietary Cu concentrations ranged from 9.7 to 11.1 mg of Cu/kg DM in order to meet the NASEM (2016) guidelines of 10 mg of total Cu/kg diet DM. A Cu-to-Mo no-effects ratio (no impact on Cu status or growth performance) of 2.8:1 was measured in this experiment. Kistner et al. (2017) hypothesized that Mo in drinking water may have a lower impact on the Cu status in cattle, possibly due to water bypassing the rumen when consumed.

Ruminal bypass of drinking water via the esophageal groove has been estimated to be between 18 and 80% of the water consumed by mature cattle (Warner and Stacy, 1968; Woodford et al., 1984; Garza and Owens, 1989; Zorrilla-Rios et al., 1990; Garza et al., 1990). The wide range of the proportion of drinking water that can bypass the rumen may be due to diet type. Garza et al. (1990) estimated drinking water ruminal bypass (using two different markers, i.e., polyethylene glycol and chromium-EDTA) in cattle consuming a high-concentrate diet compared to cattle consuming a high-forage diet. The authors estimated that approximately 49% of the drinking water consumed bypassed the rumen when cattle were consuming a high-forage diet compared to 79% water bypass in cattle consuming a high-concentrate diet. Other factors such as the height of the water trough, drinking frequency and duration, and other dietary ingredients may also impact the ruminal bypass of drinking water.

Based on the findings of Kincaid (1980) and Kistner, et al. (2017), it appears that Mo in water may have a low impact on Cu metabolism in ruminants, possibly due to a portion of the water consumed bypassing the rumen. If this theory is correct, a portion of Mo consumed through water would theoretically not be exposed to the reducing environment of the rumen and therefore be less available to form thiomolybdates. To test this hypothesis, Thorndyke et al. (2020) utilized 12 Angus steers and investigated the influence of Mo in drinking water or feed on the apparent absorption and retention of Cu and Mo. Steers were fed a low-quality grass hay diet (basal diet: 6.4% CP; 0.12% S, 6.8 mg Cu/kg DM; 2.5 mg Mo/kg DM) for a period of 14 days. The steers were then housed in metabolism stalls, and total fecal and urine output were collected for 5 days. During the collection period, treatments consisted of: (1) control, no supplemental Mo, (2) 5.0 mg Mo/kg DM from sodium molybdate dihydrate ($MoNa_2O_4{\cdot}2H_2O$; Mo-diet), and (3) 1.5 mg Mo/L from $MoNa_2O_4{\cdot}2H_2O$ delivered in drinking water (Mo-water). The control steers consumed 25 mg Mo/d while Mo-supplemented animals consumed 79.7 and 72.3 mg Mo/d (Mo-diet and Mo-water steers, respectively). Dry matter intake, DM digestibility, water intake, and Cu intake were similar across treatments. Mo intake was lower in controls compared to Mo-supplemented steers. Molybdenum-supplemented steers had lower apparent absorption and retention of Mo (when expressed as a % of Mo intake). However, the apparent absorption of Mo was similar between Mo-supplemented steers (Mo-diet and Mo-water). When expressed as mg Mo absorbed/d, Mo apparent absorption was greatest for steers receiving Mo-diet, followed by steers on Mo-water and control steers. The apparent retention of Mo (mg/d) was greater for Mo-diet steers when compared to Mo-water steers which had greater Mo apparent retention than control steers.

Urinary Cu excretion (mg/day) was lower in Mo-diet steers when compared to control and Mo-water steers. The apparent absorption of Cu (expressed as a % of Mo intake) was greater in controls compared to Mo-diet steers but similar to Mo-water steers. The percent apparent absorption of Cu tended to be lower in Mo-diet compared to Mo-water steers. When expressed as mg Cu/d, the apparent absorption of Cu was greater in control compared to Mo-water steers and in Mo-water than in Mo-diet steers. The apparent retention of Cu expressed as a percent of intake or mg/d was similar between control and Mo-water steers and different compared to Mo-diet steers. However, the apparent retention of Cu (expressed as either a % of Cu intake or mg Cu/day) was similar between Mo-water and Mo-diet steers. The results from Thorndyke et al. (2020) indicate that Mo in water may have a lower impact on apparent absorption and retention of Cu than Mo in feed.

## CONCLUSION

Based on the limited number of experiments available for this review, it appears that Mo in drinking water may have a lower antagonistic impact on Cu status in cattle when compared to Mo consumed in the diet. However, the experiment conducted by Kincaid (1980) were performed on a small sample, had a short exposure period, and used young calves. Experiment with a longer in duration (≈ 5 months) were performed by Kistner et al. (2017), who supplied Mo in drinking water to feedlot steers consuming a high-concentrate diet that contained 0.15% dietary S, whereas Thorndyke et al. (2020) examined an acute exposure of Mo in the water or diet over a 5-day period. In all experiments, dietary S concentrations were low to adequate. Future research should examine how diet type, duration of Mo exposure, and dietary and water S concentrations influence the impact of Mo supplied in the water on the Cu status of ruminants.

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Table 3.1. Influence of temperature, cattle type, and body weight on estimated daily water intake (L) for beef cattle[a,b].

| Body Weight, kg | Temperature, °C [b] | | | | | |
|---|---|---|---|---|---|---|
| | 4.4 | 10.0 | 14.4 | 21.1 | 26.6 | 32.2 |
| | Growing Heifers, Steers, and Bulls | | | | | |
| 182 | 15.1 | 16.3 | 18.9 | 22.0 | 25.4 | 36.0 |
| 273 | 20.1 | 22.0 | 25.0 | 29.5 | 33.7 | 48.1 |
| 364 | 213.0 | 25.7 | 29.9 | 34.8 | 40.1 | 56.8 |
| | Finishing Cattle | | | | | |
| 273 | 22.7 | 24.6 | 28.0 | 32.9 | 37.9 | 54.1 |
| 364 | 27.6 | 29.9 | 34.4 | 40.5 | 46.6 | 65.9 |
| 454 | 32.9 | 35.6 | 40.9 | 47.7 | 54.9 | 78.0 |
| | Cows Pregnant/Lactating [c, d] | | | | | |
| 409 | 24.4/43.1 | 27.3/47.7 | 31.4/54.9 | 36.7/64.0 | NA[e]/67.8 | NA[e]/61.3 |
| | Mature Bulls | | | | | |
| 636 | 30.3 | 32.6 | 37.5 | 44.3 | 50.7 | 71.9 |
| 727 | 32.9 | 35.6 | 40.9 | 47.7 | 54.9 | 78.0 |

[a] Adapted from NASEM (2016) and Winchester and Morris (1956). [b] Water intake of a given class of cattle in a specific management regime is a function of dry matter intake and ambient temperature. Water intake is quite constant up to 4.4 °C. [c] Dry matter intake has a major influence on water intake. Heavier cows are assumed to be higher in body condition and to require less dry matter and, thus, lower water intake. [d] Cows larger than 409 kg are included in this recommendation. [e] Data not available.

Table 3.2. Calculated influence of water molybdenum (Mo) concentrations and dietary molybdenum concentrations on total Mo intake, total mg of Mo consumed per day for beef cattle [a].

| Diet Mo Concentration, mg Mo/kg DM | Water Molybdenum Concentration, mg/L [b] | | | | |
|---|---|---|---|---|---|
| | 0.0 | 0.1 | 0.5 | 1.0 | 2.0 |
| 0.0 | 0.0 | 5.0 | 25.0 | 50.0 | 100.0 |
| 1.0 | 10.0 | 15.0 | 35.0 | 60.0 | 110.0 |
| 2.0 | 20.0 | 25.0 | 45.0 | 70.0 | 120.0 |
| 3.0 | 30.0 | 35.0 | 55.0 | 80.0 | 130.0 |
| 4.0 | 40.0 | 45.0 | 65.0 | 90.0 | 140.0 |
| 5.0 | 50.0 | 55.0 | 75.0 | 100.0 | 150.0 |
| 10.0 | 100.0 | 105.0 | 125.0 | 150.0 | 200.0 |
| 50.0 | 500.0 | 505.0 | 525.0 | 550.0 | 600.0 |

[a] Adapted form Neuhold (2012). [b] Based on 50 L/animal daily water intake and 10.0 kg/animal dry matter intake.

# CHAPTER 4 – THE IMPACT OF LONG-TERM MODERATE MOLYBDENUM EXPOSURE IN DRINKING WATER ON APPARENT ABSORPTION AND RETENTION OF MOLYBDENUM AND COPPER OF PREGNANT FORAGE FED BEEF COWS


## SUMMARY

Twelve multiparous beef cows of similar BW, age, and gestational length, from a larger cow-calf study, were utilized to evaluate the effects of molybdenum (Mo) consumption method (feed or water) on apparent absorption and retention of copper (Cu) and Mo. Cows (n=54) with calves had been assigned to one of six dietary and/or water treatments (n=9 cow-calf pairs per treatment) 301 d prior to selecting a sub-group of 12 cows. Treatments consisted of: 1) negative control (control; basal diet with no supplemental Mo or Cu), 2) positive control (control + 3 mg of supplemental Cu/kg DM), 3) control + 500 µg Mo/L from $Na_2MoO_4 \cdot 2H_2O$ supplied in drinking water, (4) control + 1000 µg Mo/L of $Na_2MoO_4 \cdot 2H_2O$ supplied in drinking water, (5) positive control + 1000 µg Mo/L of $Na_2MoO_4 \cdot 2H_2O$ supplied in drinking water, and (6) control + 3.0 mg of supplemental Mo/kg diet DM from $Na_2MoO_4 \cdot 2H_2O$. The sub-group of cows were individually fed a low quality grass hay diet with their respective treatments, n=2 cows/treatment) for 14 d. On day 15, dry matter intake (DMI) was held at 90% of the group's average intake. Total fecal and urine output were then collected for 3 d. Dry matter digestibility and water intake were similar across treatments. Copper intake and apparent absorption and retention of cu were greater ($p < 0.05$) in cows receiving supplemental cu when compared to non-cu supplemented cows. Apparent absorption of Mo was similar across all treatments. Apparent retention of Mo was greater while apparent absorption of Cu was lesser ($p < 0.05$) in cows receiving 3 mg of Mo/kg dm and cows receiving 1000 µg Mo/L when compared to all

other treatments. These data indicate that Mo source (feed vs water) may impact apparent absorption of cu in cows receiving a low quality forage diet.

## INTRODUCTION

The impact of water quality on beef cattle production has been extensively studied. While some water contaminants have been shown to affect animal performance and health (NASEM, 2016; Wagner and Engle, 2021), the presence of soluble minerals in drinking water rarely occurs in concentrations great enough to influence nutritional status of an animal (Thorndyke et al., 2021). However, there are instances that occur in nature where elevated concentrations of minerals dissolved in water that may be consumed by livestock that has the potential to impact the availability of other minerals consumed in the diet (Thorndyke et al., 2021).

Given the high concentration of Mo deposits in the Rocky Mountains paired with natural environmental events or human activity, streams or groundwater may exceed the Colorado Mo agriculture water standard of 160 μg/L (Environmental Protection Agency, 2012; Kistner et al. 2017). Additionally, dietary Mo concentrations of 2-5 mg Mo/kg DM have been reported to moderately reduce the availability of dietary Cu in sheep and cattle (Underwood and Suttle, 1999). However, the impact of elevated dietary Mo concentration on Cu availability in cattle and sheep it greatly reduced when dietary sulfur (S) exceeds approximately 0.25% of the diet DM (Ward, 1978; Underwood and Suttle, 1999). Therefore, the Cu:Mo ratios below 3:1 can induce a Cu deficiency (Miltimore and Mason, 1971) and if diets also contain > 0.25% S, further impairment of Cu absorption may result (Dias et al., 2014).

Few experiments have been conducted investigating the bioavailability of Mo contained in water consumed by cattle and even fewer have investigated the impact of prolonged Mo exposure in water consumed by cattle. Thorndyke et al. (2020) determined Mo consumed in feed may have a greater impact on Cu absorption than Mo from drinking water. A portion of drinking water bypasses the highly reducing environment of the rumen and enters the abomasum via the esophageal groove (Garza and Owens, 1989; Zorrilla-Rios et al., 1990; Garza et al., 1990). Therefore, not all Mo consumed in drinking water would have the ability to interact with reduced S and ionized Cu in the rumen. Whereas Mo consumed in the diet would have a greater chance to interact with S and Cu in the rumen (Thorndyke et al. 2020). Therefore, the objective of this experiment was to investigate the influence of prolonged exposure to elevated Mo water concentrations on apparent absorption and retention of both Cu and Mo in pregnant multiparous beef cows. We hypothesized that long-term Mo supplementation at moderate concentrations of Mo supplied in the feed or water would not impact apparent absorption or retention of Cu or Mo in pregnant beef cows consuming a low quality forage diet with adequate S.

## MATERIALS AND METHODS

Prior to the initiation of the experiment, all animal use, handling, and sampling techniques described herein were approved by the Colorado State University Animal Care and Use Committee (IACUC approval #18-7819A).

Twelve multiparous beef cows were selected across six treatments from a long-term Mo supplementation experiment (n=2 cows/treatment; Thorndyke et al., 2023). Within this larger study, cows with calves (N = 54, n=9 cow-calf pairs per treatment) were assigned to one of six treatments where they remained for the duration of the study to examine life cycle health and production implications of long-term Mo exposure (Thorndyke et al., 2023). Animals chosen for this study were of similar body weight and days to calving. Cows were housed in individual pens and fed a low-quality grass hay diet (DM basis: 6.27% CP, 0.15% S, 6.84 mg Cu/kg, 2.58 mg Mo/kg) for 14 days to determine DMI. Cows received the same low-quality grass hay diet and the following treatments for 301 d prior to fecal and urine collection: 1) No supplemental Mo or Cu (Negative Control or Control), 2) Control + 3 mg Cu/kg DM from $CuSO_4 \cdot 5H_2O$ added to the basal diet (Positive Control or Control + Cu 3.0-diet); 3) Control + 500 µg Mo/L from $MoNa_2O_4 \cdot 2H_2O$ in drinking water (Mo 500-water), 4) Control + 1000 µg Mo/L of $MoNa_2O_4 \cdot 2H_2O$ in drinking water (Mo 1000-water), 5) Mo 1000-water + 3 mg Cu/kg DM from $CuSO_4 \cdot 5H_2O$ added to the basal diet (Mo 1000-water + Cu 3.0-diet), and 6) Control plus 3 mg Mo/kg DM from $MoNa_2O_4 \cdot 2H_2O$ added to the basal diet (Mo 3.0-diet). These treatments were maintained throughout the fecal and urine collection period.

Diets were fed once daily at 90% of the average intake for all animals to ensure total feed consumption. Water for all animals was supplied in individual 1,100 L water tanks. The same basal water (7.42 pH, 985.7 mg/L Total Dissolved Solids, >10 µg/L Cu, >10 µg/L Mo) was supplied in amounts that would allow all animals *ad libitum* access to water throughout the day. Molybdenum was added to the appropriate water tanks to supply the appropriate amount of Mo for each Mo-water treatment. Daily (24 h) water consumption was determined throughout the experiment for all animals as described by Kistner et al. (2017). Dried distiller’s grains (200 g) were used as the carrier for treatments receiving dietary Mo and/or Cu. Dried distiller’s grain (200 g) containing no Mo or Cu was also added to the appropriate treatments to balance dried distillers grain intake across all treatments. The basal DDG added to diets on DM basis: 28.9% CP, 0.54% S, 3.1 mg Cu/kg, 1.8 mg Mo/kg. Immediately after feeding the basal diet, the appropriate treatments were top-dressed and mixed thoroughly by hand for each feeding within a day.

***Fecal and Urine Collection***

Total fecal and urine output were measured daily for individual cows during the 3-d collection period. Urine was collected using catch containers and fecal material was allowed to be deposited on the concrete floor and immediately removed manually from the pen. The collected urine was placed in containers containing 100 mL of 6N HCl to prevent $NH_3$ volatilization. Urine volume was determined daily, and a 10% aliquot was retained and stored at -20ºC for each cow, daily. Feces collected each day (over a 24-h period) were handled as previously described. In the event that urine samples were inadvertently missed, pre-weighed paper towels were used to absorb the urine and then weighed. This allowed for quantification of the volume of the missed urine sample.

***Internal Feed Marker***

Ten grams of titanium dioxide ($TiO_2$) were added to the dried distillers grain supplement 5 days prior and during total collections days for all twelve animals and served as an internal feed marker to determine the amount of each treatment dose consumed daily. Samples were collected and apparent total tract digestibility was determined as described by Ebert et al. (2016).

***Analytical Procedures***

Feed, fecal and urine samples were analyzed for moisture using the AOAC (2006) Official Method 950.46 moisture removal process; crude protein (CP) using the AOAC (2006) Official Method 992.15 (TruSpec CN, 2004); ash using the ash oven method described in the AOAC (2006) Official Method 920.153; and ADF and NDF (ANKOM Technology, 2015). Feed, fecal and urine, and water, samples were analyzed for Mo and Cu concentrations using inductively coupled plasma mass spectrometry (EPA 200.8, rev. 5.4, 1994; PerkinElmer;

NexION 2000 B). Titanium dioxide was analyzed as described by Meyers et al., (2004). Water quality was analyzed using standard analytical techniques (EPA, 1983, 1986).

## RESULTS

The Cu:Mo ratio for each treatment was calculated based on the consumption of water, hay, and DDG. Based on all diet components the Cu:Mo ratios for all treatment groups Control, Control + Cu 3.0-diet, Mo 500-water, Mo 1000-water, Mo 1000-water + Cu 3.0-diet, and Mo 3.0-diet were 2.63:1, 3.79:1, 1.56:1, 1:14:1, 1.59:1, and 1.21:1 respectively. The effects of long-term Mo exposure in drinking water or diet on apparent absorption and retention of Cu and Mo in multiparous cows are shown in Table 4.1. By design, DMI was similar across all treatments. Dry matter digestibility and water intake were similar across treatments. Copper intake and apparent absorption and retention of Cu were greater ($P < 0.05$) in cows receiving supplemental Cu when compared to non-Cu supplemented cows. Apparent absorption of Cu was less ($P < 0.05$) in cows receiving 3 mg of Mo/kg DM (Control plus 3 mg Mo/kg DM added to the basal diet) and cows receiving Control + 1000 ug Mo/L when compared to all other treatments. By design, Mo intake was different ($P < 0.05$) across treatments. Apparent absorption of Mo was similar across all treatments. Apparent retention of Mo was greater ($P < 0.05$) in cows receiving Mo 3.0-diet and Control + 1000 μg Mo/L treatments compared to all other treatments. Titanium dioxide recovery rates ranged from 98.2 – 101.8%.

## DISCUSSION

The antagonistic effects of Mo and Mo and S have on Cu absorption are of high importance (Suttle, 1991; Gould and Kendall, 2011). This is especially true in locations where Cu concentrations in feeds are marginal, where S consumption is elevated (> 0.25% DM; Mills et al., 1977; Dias et al., 2013), and/or in situations where the Cu:Mo are less than 3:1 (Miltimore and Mason, 1971).

Previous studies from Kistner et al. in 2017 and Thorndyke et al. in 2020 indicate that elevated Mo consumed in drinking water impacts Cu metabolism differently compared to Mo consumed in the diet. Kistner et al., (2017) conducted an experiment exposing 30 growing beef steers to varying doses of Mo (0.0 mg/L, 0.16 mg/L, 0.32 mg/L, 0.48 mg/L, and 0.96 mg/L) as $Na_2MoO_4 \cdot 2H_2O$ in drinking water for 112 to 151 days. No adverse effects were observed during the experiment and Kistner et al., (2017) suggested water bypassing the rumen may have led to a lower impact of Mo consumed in the drinking water on Cu status in cattle. To test this proposed hypothesis, Thorndyke et al., (2020) investigated the influence of Mo sourced from feed vs water on the apparent absorption and retention of Cu and Mo in beef steers over a short duration study (2020). In this experiment, 12 beef steers received one of three treatments: 1) a control with no supplemental Mo, 2) a Mo-diet treatment with 5.0 mg Mo/kg DM from $Na_2MoO_4 \cdot 2H_2O$, and 3) a Mo-water treatment with 1.5 mg Mo/L from $Na_2MoO_4 \cdot 2H_2O$ with control steers consuming 25 mg Mo/d while Mo-diet steers consumed 79.7 mg Mo/d and Mo-water steers consumed 72.3 mg Mo/d for 5 days (Thorndyke et al. 2020). Apparent absorption and retention of Cu (mg/d) were greater in control steers when compared to Mo-diet steers, with apparent absorption and retention of Cu in steers on the Mo-water treatment not differing from controls or those receiving Mo-diet (Thorndyke et al., 2020). These results continued the bypass water theory and raised questions on length of exposure and animal mineral status.

To our knowledge, the current experiment is the first to investigate the influence of long-term Mo supplementation in water on apparent absorption and retention of Mo and Cu in multiparous beef cows. Animals in this study were exposed to treatments 301 d prior to individual DMI determination, $TiO_2$ administrations, and 3 d urine and fecal collections. Apparent absorption of Cu was less in cows receiving the control plus 3 mg Mo/kg DM added to the basal diet and cows receiving Control + 1000 μg Mo/L when compared to all other treatments. These data indicate that animals with the Cu:Mo ratios of 1.3:1 had reduced Cu absorption when S concentrations in the diet were 0.158% ± 0.06.

Apparent absorption of Mo was similar across all treatments indicating that the doses used in this experiment did not induce paracellular transport in the small intestine. However, apparent retention of Mo was greater in cows receiving Mo 3.0-diet and Mo 1000-water treatments compared to all other treatments. It's important to recognize that both treatments, Mo 3.0-diet and Mo 1000-water, that exhibited these results, had Cu:Mo ratios of 1.21:1 and 1.14:1, respectively. Whereas treatment groups, Mo 500-water and Mo 1000-water + Cu 3.0-diet, had lesser apparent retention of Mo yet still exposed to elevated Mo had Cu:Mo ratios of 1.56:1 and 1.59:1, respectively. Greater apparent retention of Mo when Mo is consumed in the diet is consistent with previous research suggesting that the formation thiomolybdates, a stepwise dehydrolysis of molybdate and S, in the highly reducing environment of the rumen can be poorly excreted from the body after absorption (Grace and Suttle, 1979). In this study the greater apparent retention of Mo from water Mo exposure is somewhat surprising but a similar finding to that of Thorndyke et al. 2020 and may ultimately be related to Cu:Mo ratio, rather than the source (feed or water) of Mo exposure.

Finally, these data indicate that long term Mo exposure in drinking water within the concentrations tested in this experiment appear to have less of an impact on Cu metabolism than Mo consumed in the diet. Therefore, we reject our null hypothesis that the source of long-term Mo exposure (water or feed) does not influence apparent absorption or retention of Cu and Mo.

It is important to note that Cu:Mo ratio appears to be a better predictor of the impact of Mo on Cu absorption especially if S concentrations are within adequate concentrations recommended by the NASEM (2016). In the current study S concentrations in the feed and water would not be considered elevated which allowed for researchers to assess Mo impact specifically on Cu metabolism, however, future research is warranted investigating the impact of Mo in the water with the presence of elevated dietary or water S concentrations.

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Table 4.1. The effects of long term molybdenum exposure in drinking water or diet on apparent absorption and retention of copper and molybdenum in multiparous cows.

| Item | Treatment | | | | | | | *P* < |
|---|---|---|---|---|---|---|---|---|
| | Negative Control[a] | Positive Control[b] | 500 µg Mo/L $H_2O$[c] | 1000 µg Mo/L $H_2O$[d] | 1000 µg Mo/L $H_2O$ + dietary Cu[e] | Mo diet[f] | SEM | Trt |
| DM intake, kg/d | 15.3 | 15.3 | 15.3 | 15.3 | 15.3 | 15.3 | --- | --- |
| Fecal DM, kg/d | 4.2 | 4.3 | 4.2 | 3.8 | 4.5 | 3.4 | 0.56 | 0.68 |
| DM digestion, % | 61.4 | 63.5 | 60.6 | 62.4 | 62.2 | 61.3 | 2.3 | 0.42 |
| Water intake, L/d | 36.8 | 39.6 | 38.3 | 39.5 | 37.4 | 38.2 | 1.53 | 0.76 |
| Copper | | | | | | | | |
| Intake, mg/d | 78.1[b] | 115.6[a] | 78.1[b] | 78.1[b] | 114.3[a] | 78.1[b] | 13.9 | 0.05 |
| Excretion, mg/d | | | | | | | | |
| Fecal copper | 76.1[b] | 112.3[a] | 76.2[b] | 75.7[b] | 111.5[a] | 76.5[b] | 13.2 | 0.05 |
| Urinary copper | 0.53 | 0.48 | 0.50 | 0.43 | 0.37 | 0.64 | 0.58 | 0.36 |
| Apparent absorption, mg/d[d] | 2.0[a] | 3.3[b] | 1.9[a] | 2.0[a] | 2.8[b] | 1.6[a] | 0.34 | 0.05 |
| Apparent retention, mg/d[e] | 1.47[b] | 2.82[a] | 1.40[b] | 1.23[b,c] | 2.43[a] | 0.96[c] | 0.20 | 0.05 |
| Molybdenum | | | | | | | | |
| Intake, mg/d | 33.7[c] | 31.5[c] | 52.9[b] | 71.0[a] | 71.1[a] | 74.4[a] | 7.01 | 0.05 |
| Excretion, mg/d | | | | | | | | |
| Fecal molybdenum | 31.0[c] | 30.0[c] | 50.9[b] | 68.9[a] | 69.4[a] | 71.2[a] | 6.9 | 0.05 |
| Urinary molybdenum | 1.62[a] | 0.53[b] | 1.03[a] | 0.62[b] | 0.56[b] | 1.49[a] | 0.35 | 0.05 |
| Apparent absorption, mg/d[d] | 2.69 | 1.49 | 2.05 | 2.15 | 1.70 | 3.22 | 0.48 | 0.15 |
| Apparent retention, mg/d[e] | 1.07[a] | 0.96[a] | 1.02[a] | 1.53[b] | 1.14[a] | 1.73[b] | 0.05 | 0.05 |

[a]Negative Control: no supplemental Mo or Cu added to the diet or water.
[b]Positive Control: 3.0 mg Cu/kg DM from $CuSO_4 \cdot 5H_2O$ added to the basal diet.
[c]500 µg Mo/L $H_2O$: Negative Control diet + 500 µg Mo/L from $MoNa_2O_4 \cdot 2H_2O$ supplied in the drinking water.
[d]1000 µg Mo/L $H_2O$: Negative Control diet + 1000 µg Mo/L of $MoNa_2O_4 \cdot 2H_2O$ supplied in the drinking water.
[e]Mo 1000-water plus 3 mg Cu/kg DM from $CuSO_4 \cdot 5H_2O$ added to the basal diet.
[f]Mo Diet: Negative Control diet plus 3 mg Mo/kg DM from $MoNa_2O_4 \cdot 2H_2O$ added to the basal diet.

# CHAPTER 5 – AN ASSESSMENT OF MOLYBDENUM AND COPPER STATUS OF COW-CALF PRODUCERS IN COLORADO OVER A 2-YEAR PERIOD

## SUMMARY

Commercial, multiparous, crossbred beef cows from 3 independent cow-calf production operations were selected to assess the molybdenum (Mo) and copper (Cu) status of cattle raised in CO. Fifteen cows from each operation were selected at random, during early summer and late fall processing. At the time of cattle processing, both jugular venipuncture blood samples and liver biopsies were obtained from each cow. Furthermore, all diet components, forage, water, and supplement samples were obtained from each location. Plasma, liver, water, and feed samples were analyzed for Mo and Cu concentrations via inductively coupled plasma mass spectrometry. Feed samples were also analyzed for moisture, crude protein (CP), ash, ADF and NDF. Water samples were sent to an established laboratory for general water quality analysis. On average, in the current survey of central Rocky Mountain Colorado forages samples contained 55.61% DM, 8.37% CP, 34.91% ADF, and 54.98 NDF with a Cu:Mo ratio of 2.8:1. Additionally, water quality of the samples obtained were well within the "safe and should pose no health problems" category for beef cattle. Plasma Mo concentrations of 0.22 (±0.10) mg/kg DM were considered to be elevated in 64% of all samples obtained, likely a result of the elevated Mo forage concentrations in the grazed plant material. Plasma Cu of 0.83 (±0.11) concentrations were within normal ranges for all samples obtained. Molybdenum and Cu liver concentrations of 3.74 (±1.29) and 82.54 (±22.76) respectively, were within ranges considered to be normal for beef cattle for all samples collected. Based on the results of this survey, Mo and Cu plasma and liver concentrations of cow-calf operations in the central Rocky Mountains Colorado were similar to

Mo and Cu plasma and liver concentrations in Cu supplemented cows in the previously described 2-year Mo supplementation cow-calf experiment. Furthermore, these data suggest that Cu supplementation at NASEM (2016) recommended concentrations of 10 mg Cu/kg DM total diet (or greater) meets the animals dietary Cu requirement for cattle consuming forages sampled.

## INTRODUCTION

Antagonist mineral interactions can reduce impair reproductive efficiency, negatively impact health, and reduce animal performance (Arthington and Ranches, 2021) which ultimately influence sustainability and producer profitability. Notably the interaction between Cu and Mo within the rumen of cattle can cause reduce systemic Cu status of the animal through the production of thiomolybdates in the rumen and result in a secondary Cu deficiency (Gould, and Kendall, 2011; Spears et al., 2022). In the Rocky Mountains of Colorado, mineral concentrations of Cu antagonists in grazed forages and drinking water for ruminants have the potential to create Cu deficiency. Livestock producers must be aware of the mineral concentrations in feedstuff and water to guarantee the best outcomes for their animals and improved profitability. Often mineral supplementation methods are implemented to ensure adequate consumption of nutrients required by the animal (Greene, 2000).

To our knowledge, little information is available assessing Cu status in livestock raised in the Rocky Mountains. Therefore, the objective of this evaluation was to understand the mineral consumption within the forage and water as well as Cu and Mo status of the grazing cattle in the Rocky Mountain's of Colorado.

## MATERIALS AND METHODS

Beginning in summer of 2018, commercial, multiparous, crossbred beef cows from 3 independent cow-calf production operations were selected to assess molybdenum (Mo) and copper (Cu) status of cattle raised in Colorado. Producer cow numbers ranged from 150-900 cows and all cows were maintained in the same location throughout the survey time period. All producers supplied typical mineral and protein supplementation to cows at the appropriate times of the year.

Fifteen cows from each operation were selected at random, during normal processing times: early summer and late summer/fall of 2018 and 2019. At the time of cattle processing, blood samples were obtained via jugular venipuncture and collected into a 7 ml heparinized trace-mineral-free vacutainer tubes (Becton Dickinson Co., Franklin Lakes, NJ). Once collected, blood samples were placed on ice and transported back to the laboratory (approximate time from collection until processing was 5 h). Vacutainer tubes were centrifuged at 2,000 × *g* for 15 min at room temperature and plasma was then transferred to acid-washed storage vials and stored at -20°C until analyzed for Mo and Cu concentrations.

Liver biopsies (approximately 150 mg wet weight) were also obtained from each cow at the time of blood sampling. Liver biopsies were obtained using the true-cut technique described by Pearson and Craig (1980) as modified by Engle and Spears (2000). Briefly, hair was clipped from a 10 cm x 10 cm area on the right side of the animal between the $11^{th}$ and $12^{th}$ ribs. The area was then scrubbed three times with betadine alternating with 70% alcohol. Five milliliters of a 2% lidocaine hydrochloride solution (Abbott Laboratories, Chicago, IL) were injected via a 20 gauge x 2.5 cm needle between the $11^{th}$ and $12^{th}$ rib on a line from the tubercoxae to the tip of the shoulder. A small incision (approximately 1.0 cm) was made using a #11 scalpel blade. A core sample of liver was collected using a modified Jam Shide bone marrow punch (0.5 cm x 14 cm; Sherwood Medical, St. Louis, MO). Following collection, samples were immediately rinsed with a buffered saline solution (pH 7.4), placed into acid-washed polyethylene tubes, capped, stored on ice and transported to the laboratory. Samples were then stored at –20°C until analyzed for Mo and Cu concentrations.

Forage and water samples were obtained at each location. Forage samples collected (primarily meadow grasses, grama grass (*Bouteloua sp.*), and mountain brome (*Bromus marginatus*)) were obtained from the pasture where the cattle were housed just prior to processing, as described by Holden et al. (1994). Also, water samples were obtained from the water supply within the pasture where the cows were housed prior to processing.

***Analytical procedures***

Plasma, liver, water, and feed samples were analyzed for Mo and Cu concentrations via inductively coupled plasma mass spectrometry (EPA 200.8, rev. 5.4, 1994; PerkinElmer; NexION 2000 B). For analysis, plasma samples were allowed to thaw at room temperature. One mL of 10% trichloroacetic acid was added to 1.0 mL of plasma or standard and then mixed vigorously. The mixture was placed in a -20°C freezer for 30 minutes to aid in precipitation and then centrifuged at 1,200 x g for 10 minutes. The supernatant was removed, placed into a clean, acid-washed test tube and then subjected to ICP analysis. Liver samples were thawed, dried for 24 h at 95°C in a forced air drying oven, and then allowed to cool to room temperature in a desiccator. Samples were weighed and then combined with 2 mL of 3.6 N nitric acid. The mixture was allowed to digest overnight in a water bath maintained at 95°C and then cooled to room temperature. Samples were then diluted in deionized $H_2O$ and subjected to ICP analysis.

Feed samples were analyzed for moisture using the AOAC (2006) Official Method 950.46 moisture removal process; crude protein (CP) using the AOAC (2006) Official Method 992.15 (TruSpec CN, 2004); ash using the ash oven method described in the AOAC (2006) Official Method 920.153; and ADF and NDF (ANKOM Technology, 2015). Water samples were also sent to an established laboratory (SDK Laboratories, Hutchinson, KS) for general water quality analysis (EPA, 1983).

## RESULTS AND DISCUSSION

### *Forage Nutrient Composition*

Table 5.1 describes the nutrient composition of sampled standing forage obtained from three cow-calf producers in located the central Rocky Mountains of Colorado over a two year period. Nutrient composition of forage samples collected in this survey were similar to those reported for the hay nutrient composition used in the previously described 2-year Mo supplementation cow-calf experiment. This was expected as the hay used in the cow-calf experiment was grown in the same geographical location. Furthermore, nutrient values of the forages collected in this survey were similar to those reported in the 1996 NAHMS Cow-Calf Health and Productivity Audit (Corah and Dargatz, 1996) for common grasses sampled in the Great Plains area (states included in the Great Plains sampling area were: Wyoming, Nebraska, Colorado, Kansas, New Mexico, Oklahoma, and Texas; native grasses: CP 9.2% DM; ADF 40.2% DM).

Although not directly comparable, nutrient composition of forage samples collected in this survey were generally similar to the nutrient composition of meadow hay in the NASEM (2016) Composition of Selected Feeds for Beef Cattle (Chapter 18; Meadow hay mean ± SD: 9.79±2.64 % CP; 60.85±4.57 % NDF; and 35.79±3.31%ADF).

Molybdenum concentrations reported in this survey (2.51 mg Mo/kg DM±0.72) were similar to Mo concentrations of grass samples (plant species not given) obtained near the Blue River and Tenmile Creek in Colorado (2.1mg Mo/kg DM± no SD reported; Kubota, 1975) and those for meadow hay reported in the Composition of Selected Feeds for Beef Cattle (NASEM, 2016; 1.81±0.85 mg Mo/kg DM; and 4.48 ±1.25 mg Cu/kg DM). Copper concentrations of grasses collected in the Blue River and Tenmile Creek area were extremely low [range 1.1 – 1.7 mg Cu/kg DM; Kubota (1975)]. However, Cu concentrations of forage collected in the present survey were greater (7.03 mg Cu/kg DM±1.47) than those reported by Kubota (1975).

The ratio of Cu:Mo is important as well as the total concentration of Mo in the diet. Molybdenum toxicity due to excessive Mo intake in the feed has been studied. Underwood (1999) reported that cattle grazed on pastures containing between 20 and 100 mg Mo/kg DM (< 1:1 Cu:Mo ratio) in forage experienced mild to extreme forms of scouring. Ward (1978) reported that cattle fed more than approximately 100 mg Mo/kg DM or fed a Cu:Mo ratio of 2:1 or less can experience Cu deficiencies. In the current survey of western Colorado forages, the Cu:Mo ratio of the forage samples collected was 2.8:1.

***Water quality and nutrient composition of water***

Water quality and mineral concentrations of water samples obtained from three local cow-calf producers are shown in Table 5.2. Overall, water quality of the samples obtained was well within the "safe and should pose no health problems" category for beef cattle: total dissolved solids ≤ 1,000 mg/L; water hardness ≤ 60 mg/L; nitrate as $NO_3$-N ≤ 10 mg/L; and all elements were < upper-limit guidelines; NASEM, 2016). Water quality and nutrient composition of water samples collected in this survey were similar to those reported for the water quality and nutrient composition of water used in the previously described 2-year Mo supplementation cow-calf experiment that was obtained from the Williams Fork River.

***Plasma and Liver Mo and Cu Concentrations***

Molybdenum and Cu concentrations in plasma and liver samples obtained from cows from three local cow-calf producer operations are shown in Table 5.3. Plasma Mo concentrations were considered to be elevated in 64% of all samples obtained (Normal: 0.01-0.10 and elevated: 0.08-10.0 mg Mo/L; Puls, 1994). These elevated plasma Mo concentrations are most likely due to the elevated Mo forage concentrations in the grazed plant material. Plasma Cu concentrations were within normal ranges (0.6 mg Cu/L; Puls, 1994) for all samples collected.

Molybdenum and Cu liver concentrations were within ranges considered to be normal for beef cattle for all samples collected (Normal: 0.6-6.0 mg Mo/kg liver DM; CSU Veterinary Diagnostic Laboratory; Cu deficiency defined as less than 20 - 30 mg Cu/kg liver DM; Mills, 1987). Liver Mo concentrations were numerically similar in cows from this survey (3.74 mg Mo/kg DM±1.29) when compared to final liver Mo concentrations reported in cows used in the previously described 2-year Mo supplementation cow-calf experiment (3.93 mg/Mo/kg DM±1.20; regardless of Mo supplementation). Liver Cu concentrations were numerically greater (82.5 mg of Cu/kg DM±22.8) in cows sampled in the current survey relative to final liver Cu concentrations in Cu supplemented cows (PC and Mo 1000-water+Cu) used in the previously described experiment (66.1 mg Cu/kg DM ±1.0). The reason for this numerical difference is most likely due to the amount of Cu supplemented to survey cows relative to cows receiving Cu supplementation treatments in the previously described 2-year Mo supplementation cow-calf experiment. Cattle sampled in the current survey were supplemented with a typical mineral supplement that was formulated to supply approximately 10-15 mg of supplemental Cu/kg DM per animal per day in addition to the Cu content (7.03 mg Cu/kg DM±1.47) in the grazed forage, whereas cows in the Cu supplemented treatments in the previously described 2-year experiment received 3 mg of supplemental Cu/kg DM per animal per day in addition to the Cu contained in the forage (6.84 mg Cu/kg DM±2.91).

Based on the results of this survey, Mo and Cu plasma and liver concentrations in cow-calf operations in the central Rocky Mountains of Colorado were similar to Mo and Cu plasma and liver concentrations in Cu supplemented cows in the previously described 2-year Mo supplementation cow-calf experiment. Furthermore, these data suggest that Cu supplementation at NASEM (2016) recommended concentrations of 10 mg Cu/kg DM total diet (or greater) meets the animals dietary Cu requirement.

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Table 5.1. Nutrient composition of sampled standing forage obtained from three Colorado cow-calf producers over a two year period (n=12 samples; dry matter basis).

| Nutrient | Mean | Standard deviation |
|---|---|---|
| Dry matter, % | 55.61 | 7.39 |
| Crude Protein, % | 8.37 | 1.91 |
| Acid detergent fiber, % | 34.91 | 2.17 |
| Neutral detergent fiber | 54.98 | 3.74 |
| Net energy for lactation, Mcal/kg | 0.57 | 0.12 |
| Net Energy for gain, Mcal/kg | 1.34 | 0.29 |
| Net energy for maintenance, Mcal/kg | 1.19 | 0.17 |
| Total digestible nutrients, % | 54.37 | 3.21 |
| Digestible energy, Mcal/kg | 2.67 | 0.45 |
| Metabolizable energy, Mcal/kg | 2.25 | 0.32 |
| Calcium, % | 0.45 | 0.13 |
| Phosphorus, % | 0.15 | 0.08 |
| Potassium, % | 2.14 | 0.32 |
| Magnesium, % | 0.18 | 0.24 |
| Sodium, % | 0.04 | 0.02 |
| Sulfur, % | 0.16 | 0.09 |
| Aluminum, mg/kg | 172.24 | 25.47 |
| Arsenic, mg/kg | < 1.5 | --- |
| Barium, mg/kg | 16.39 | 4.52 |
| Beryllium, mg/kg | < 0.2 | --- |
| Cadmium, mg/kg | < 0.2 | --- |
| Chromium, mg/kg | 3.89 | 0.94 |
| Cobalt, mg/kg | 0.21 | 0.17 |
| Copper, mg/kg | 7.03 | 1.47 |
| Iron, mg/kg | 205.36 | 17.47 |
| Lead, mg/kg | < 1.5 | --- |
| Manganese, mg/kg | 138.49 | 31.25 |
| Molybdenum, mg/kg | 2.51 | 0.72 |
| Nickle, mg/kg | 1.92 | 0.25 |
| Selenium, mg/kg | < 1.50 | --- |
| Silver, mg/kg | < 0.4 | --- |
| Zinc, mg/kg | 20.89 | 1.28 |

Table 5.2. Water quality and mineral concentrations of water samples obtained from three Colorado cow-calf producers (n=12 samples; mean ±SD).

| Item | |
|---|---|
| pH, s.u. | 7.30 (±0.21) |
| Chloride, mg/L | 6.11 (±0.15) |
| Total Hardness, mg/L | 30.28 (±2.01) |
| Nitrate-Nitrogen, mg/L | <1.0 (±N/A) |
| Calcium, mg/L | 8.39 (±0.35) |
| Magnesium, mg/L | 2.14 (±0.10) |
| Phosphorous, mg/L | <0.1 (±N/A) |
| Potassium, mg/L | <5.0 (±N/A) |
| Sodium, mg/L | <5.0 (±N/A) |
| Sulfate, mg/L | 2.69 (±0.19) |
| Aluminum, mg/L | 0.27 (±0.04) |
| Cobalt, mg/L | <0.01 (±N/A) |
| Copper, µg/L | <10.0 (±N/A) |
| Iron, mg/L | 0.38 (±0.07) |
| Manganese, mg/L | <0.01 (±N/A) |
| Molybdenum, µg/L | <10.0 (±N/A) |
| Selenium, µg/L | <30.0 (±N/A) |
| Total Dissolved Solids, mg/L | 67.31 (±5.86) |

Table 5.3. Molybdenum and copper concentrations in plasma and liver samples obtained from cows from three Colorado cow-calf producer operations (n=164 total plasma and 154 total liver samples; mean ±SD).

| Item | |
|---|---|
| Plasma | |
| Molybdenum, mg/kg DM | 0.22 (±0.10) |
| Copper, mg/kg DM | 0.83 (±0.11) |
| Liver | |
| Molybdenum, mg/kg DM | 3.74 (±1.29) |
| Copper, mg/kg DM | 82.54 (±22.76) |