THESIS

MOLYBDENUM EXPOSURE IN DRINKING WATER VS FEED AFFECTS COPPER APPARENT ABSORPTION DIFFERENTLY IN BEEF CATTLE CONSUMING A HIGH FORAGE DIET

Submitted by

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In partial fulfillment of the requirements

For the Degree of Master of Science

Colorado State University

Fort Collins, Colorado

Fall 2020

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ABSTRACT

MOLYBDENUM EXPOSURE IN DRINKING WATER VS FEED AFFECTS COPPER APPARENT ABSORPTION DIFFERENTLY IN BEEF CATTLE CONSUMING A HIGH FORAGE DIET

Twelve Angus steers were utilized to investigate the influence of molybdenum (Mo) in drinking water or feed on apparent absorption and retention of copper (Cu) and Mo. Steers were fed a low-quality grass hay diet for 14 days. Steers were then housed in individual metabolism stalls for 5 days to determine dry matter intake (DMI). Steers were then blocked by body weight and DMI and randomly assigned within block to one of three treatments. Treatments consisted of: 1) control (no supplemental Mo); 2) 5.0 mg Mo/kg DM from sodium molybdate dihydrate (Mo-diet), and 3) 1.5 mg Mo/L from sodium molybdate dihydrate delivered in the drinking water (Mo-water). After the 5 day DMI determination period, total fecal and urine output was collected for 5 days. Dry matter intake, Cu and water intake, and DM digestibility were similar across treatments. As expected, Mo intake was greater ($P < 0.05$) in Mo-water and Mo-diet steers when compared to controls but similar between Mo-water and Mo-diet steers. Apparent absorption and retention of Cu (% of Cu intake) was greater ($P < 0.05$) in controls when compared to Mo-diet supplemented steers. Apparent absorption and retention of Cu (% of Cu intake) in steers in the Mo-water treatment did not differ from controls or those receiving the Mo-diet. Molybdenum-diet and Mo-water supplemented steers had similar apparent absorption and retention of Cu. Apparent absorption and retention of Mo (% of Mo intake) was greater in controls when compared to Mo supplemented steers. These data indicate that Mo consumed in

water may impact Cu absorption and retention to a lesser extent than Mo supplemented in the diet.

ACKNOWLEDGMENTS

I could not have asked for a better advisor, Terry Engle, Ph.D., you have helped me grow not only professionally but personally. Your knowledge, guidance, and patience have taught me how to balance academic and family life, thank you. To the remainder of my committee members, Jason Ahola, Ph.D., and Jim Ippolito, Ph.D., your insight and advice during graduate school has been irreplaceable.

To my mentor and friend, Octavio Guimaraes, thank you for everything. I cannot stress the integral part you had on my role as an animal scientist. I will forever be indebted to you. Nicole Tillquist, your knowledge, and support in the laboratory, writing process, and graduate school in general have made this experience pleasant and I truly appreciate that. Rebecca Barrington, Jonah Levenson, Briana Tangredi, and the rest of the animal nutrition team, without your help the project in this thesis would not have been possible.

I would also like to thank Ken, Kim, and Hannah Thorndyke, Andie Apple, Emily Beard, and the rest of my family and friends who kept me sane during this journey. Additionally, my deepest gratitude to Hunter Barlow, your love and unwavering support for all my goals does not go unnoticed. Thank you for never letting me quit.

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CHAPTER 1 – REVIEW OF LITERATURE

INTRODUCTION

Essential minerals are required for all living systems to help facilitate biological processes required for life. Essential minerals are classified as either macro- or micro/trace-minerals and this naming scheme is related to the quantity at which the animal or plant needs to consume a specific mineral. Macro minerals (calcium, magnesium, phosphorus, potassium, sodium, chlorine, and sulfur) are required by mammals in relatively large amounts (>100 mg/kg DM), and are often represented as a percent of the diet (Paterson, 2005; NASEM, 2016). Essential micro- or trace minerals (chromium, cobalt, copper, iodine, iron, manganese, molybdenum, nickel, selenium, and zinc) are required in concentrations of less than 100 mg/kg DM (Paterson, 2005; NASEM, 2016). Although the total amount of a particular mineral may vary drastically from macro minerals to trace minerals, the mineral amount has little to do with its significant role in a living system (McDowell, 1992). These essential minerals take part in four wide-ranging biological functions, such as 1) structural, 2) physiological, 3) catalytic, and 4) regulatory, promoting normal tissue growth, homeostasis, enzyme function and immune function, and cell regulation (Underwood and Suttle, 1999; Paterson, 2005).

In ruminants, trace minerals exist in cells and tissues in various chemical combinations, and in characteristic concentrations depending on the trace mineral consumed and the tissue in which it is metabolized (McDowell 1992; Underwood and Suttle, 1999; Baker 2006). Trace mineral concentrations must be maintained between narrow ranges if tissue functional and structural integrity is to be maintained, and animal growth, health, and productivity of the animal are to remain unhindered (Underwood and Suttle, 1999; Baker 2006) Trace mineral deficiencies, toxicities, and imbalances require ruminants to metabolically compensate for nutrient deviations, sacrificing important biological functions. In doing so, certain metabolic diseases can manifest, and overall animal production can be depressed, thus decreasing overall animal performance (especially growth and reproduction) and health (immune function; Baker, 2006). Thus, maintaining proper ruminant performance requires careful attention on essential dietary minerals.

Ruminants have traditionally received essential minerals through feed and/or feed supplements intake. Minerals present in the body function as organic complexes or chelates, therefore it is important to provide minerals that can be readily absorbed and converted to biologically active forms in the animal (Spears, 1996). Therefore, the total mineral amount provided in the diet or water is not as important as the ability of a mineral to be absorbed and utilized in biological systems (Underwood and Suttle, 1999). Most studies conducted dealing with these minerals have investigated mineral intake through feed and dietary mineral supplements. Significant data regarding ruminant mineral bioavailability in water is severely lacking.

MOLYBDENUM

Molybdenum (Mo) has a variety of industrial purposes. Molybdenum is used to manufacture special steels and alloys that are strong and temperature stable. Molybdenum is specifically used in tool-making, electrical contacts, x-ray tubes, high pressure boiler plates, high temperature furnaces, and even pigments (WHO, 1996). This hexavalent metal occurs naturally as lead and iron molybdates, but mainly as molybdenite (MoS_2), which physically resembles graphite (Fairhall, 1945; WHO, 1996). Molybdenum disulfide has unique properties such as a lubricant additive (WHO, 1996). Molybdenum compounds are used in agriculture either as direct seed treatment to decrease clogging in high speed planting implements, or in the formation of fertilizers to prevent Mo deficiency (WHO, 1996).

Molybdenum is an important element in the metal, chemical, and agricultural industries, as well as being an essential trace mineral required for optimal health and production in animals and plants (LeGendre and Runnells, 1975). Molybdenum is known to be present in metallic cofactors, in forms such as iron-Mo and pterin-based Mo cofactors, and is involved in several mammalian Mo dependent enzymes (e.g., nitrogenase, nitrate reductases, sulfite oxidase, and xanthine oxidoreductases; Schwarz et al. 2009). In plants, Mo is essential for nitrogen fixation and nitrate reduction (LeGendre and Runnells, 1975; Schwarz et al., 2009). As with most nutrients, Mo an excess or scarcity can be detrimental to biological processes (LeGendre and Runnells, 1975).

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 or if Mo was metabolized to a toxic product in the rumen. Although the direct effects of excess Mo have not been extensively evaluated in cattle, the indirect impact of Mo on Cu metabolism has been extensively studied. Ruminants are more sensitive to Mo induced Cu deficiency than are non-ruminant species, as determined via toxicological study comparisons conducted in mice, rabbits, sheep, and cattle (Comar et al. 1949; Ward 1978). It has been postulated that cattle are more sensitive to a Mo induced Cu deficiency than sheep when pasture plants containing elevated Mo concentrations are grazed (Cunningham et al., 1959; Ward, 1978).

Sources

Molybdenum does not exist in nature in a pure metallic state; but is always found associated with other elements such as sulfur, oxygen, tungsten, lead, uranium, iron, and various others (Jarrell et al., 1980). Excess Mo in soil from either natural (geological sources) or industrial contamination can influence Mo concentrations in plants and ground water (Ward, 1978). Molybdenosis has occurred in cattle grazing pastures adjacent to coal mine spoils or pastures near aluminum or steel alloy mills (Erdman, 1978). The major routes of Mo entry into grazing animals are through grazed forages and drinking water (Gould and Kendall, 2011).

Soil

Naturally occurring soil Mo typically found as sesquioxides, water soluble Mo, or organically-bound Mo, with Mo concentrations decreasing with soil depth (Albasel and Pratt, 1989). Soil Mo availability for plant depends on soil pH and phosphate concentrations (Barshad, 1948; Albasel and Pratt, 1989). In acidic soils, Mo can be bound in sesquioxides forms (e.g. iron oxides), creating strong associations and making Mo unavailable for plant uptake (Albasel and Pratt, 1989). In basic soils (e.g., pH above 7), sesquioxides dissolve, releasing Mo, and thus plant-available Mo concentrations increase (Kincaid, 1980). When pastures are top dressed with phosphate fertilizers (regardless of soil pH), plant Cu availability may decrease and result in Cu deficiency in grazing cattle (Cunningham et al., 1959). Plant-available Mo concentrations obviously vary depending on pH or management, thus why total soil Mo concentration is not a good indicator of plant-available Mo concentration. Plants Mo concentrations 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 (Barshad, 1948; LeGendre and Runnells, 1975; Albasel and Pratt, 1989).

Feed

Molybdenum is also naturally occurring in plants, as it is a necessary plant nutrient. In a three year plant study, Jensen and Lesperance (1971) compared several plant species and several 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, plant part, depth to water table, and agronomic management practices. Other researchers have confirmed these findings (1971; LeGendre and Runnells, 1975; Wittenberg and Boila, 1988). Overall, Mo accumulation in the aforementioned experiments varied significantly; legumes generally accumulated more Mo than grasses, likely due to the role Mo plays in nitrogen fixation in legumes (Lesperance and Bohman, 1963; Jensen and Lesperance, 1971; LeGendre and Runnells, 1975). However, plant Mo accumulation can vary between leguminous species (Kubota et al., 1961; LeGendre and Runnells, 1975). One commonality across plant species (e.g., grasses or legumes) is that Mo content appear to increase with plant age (Barshad, 1948; Kubota et al., 1961). Actively growing plants are able to absorb soluble Mo, with Mo concentrations typically greatest in plant blades and leaves (e.g., 1.5 to 5.0 mg Mo/kg DM which can be harmful to grazing ruminants; Barshad, 1948; Kubota et al., 1961). Forage grown where the water table was near the soil surface has been shown to contain more Mo than when water table depths were greater (Jensen and Lesperance, 1971). Plant Mo concentrations can range from concentrations indicative of deficiency (<0.10 mg Mo/kg DM) to values as high as 300-400 mg Mo/kg DM (LeGendre and Runnells, 1975; Kabata-Pendias, 2010; McGrath et al., 2010). Plants are more sensitive to Mo deficiency than elevated Mo concentrations (Barshad, 1948; LeGendre and Runnells, 1975). Therefore, plant species selection, where the environment is advantageous for possible Mo imbalances and cattle will be grazing, should focus on grasses adapted to the particular climate and soil conditions and that do not overly accumulate Mo.

Molybdenum has an important function in plant growth because it is associated with nitrogen metabolism (Albasel and Pratt, 1989; Kaiser et al., 2005). Molybdate (MoO_4^{2-}), which is the predominant soil form available to plants, is required by plants at very low concentrations, although legumes typically require 2 to 3 more Mo than grasses (Abasel and Pratt, 1989; Kaiser et al., 2005). Within plants, the role of Mo is primarily as an enzyme activator. Molybdenum is incorporated into the nitrate reductase enzyme which allows plants to convert nitrite to nitrate (Albasel and Pratt, 1989; Kaiser et al., 2005), this is essential for proper plant growth in photosynthesis. An exact Mo requirement for plants is difficult to determine because Cu and sulfur (S) soil concentrations can influence plant Mo metabolism (Albasel and Pratt, 1989; Kaiser et al., 2005).

Cattle and sheep that develop molybdenosis usually do so as a result of grazing vegetation enriched in Mo. Plant Mo concentrations ranging from 5.0 to 15.0 mg Mo/kg DM can cause toxic effects in grazing animals (LeGendre and Runnells, 1975; NASEM, 2016). However, animal Cu status tends to determine the critical Mo concentration at which toxicity symptoms first appear. Miltimore and Mason (1971) were the first to suggest that the ratio of Cu:Mo should be no less than 2:1 in forage plants grazed by ruminants, as increasing the Mo concentration could cause Mo induced Cu deficiency in the animal. Molybdenum supplied in the organic form in plant components appeared more toxic than intake of Mo through a mineral supplement (e.g., tub, lick), most likely due to the availability of the organic plant form Mo compared to that inorganic form found in the supplement (Jensen and Lesperance, 1971).

Water

Due to the toxicity induced in ruminants by ingestion of Mo-enriched vegetation, the federal water quality committee suggested a standard of 5 µg Mo/L in irrigation water for continuous use on soils, and 50 µg Mo/L for intermittent use on fine textured soils (LeGendre and Runnells, 1975; Albasel and Pratt, 1989); the current Colorado Mo agricultural water standard is 160 µg Mo/L (EPA, 2012). 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. Since cattle consume much larger quantities of water than dry matter, the expectation of Mo toxicity under lower water Mo concentrations would be logical (Ward, 1978). Chappell (1975) reported that many streams in the US contain molybdenum in concentrations above 50 µg Mo/L, and many of these are associated with industrial Mo sources from mining.

According to the National Animal Health Monitoring Service (NAHMS; 1997) quality water supply is essential to the production of healthy cattle, with drinking water being the main source for cattle. Water is an excellent solvent and frequently contains solutes and suspended particles, that can alter the smell, flavor, physical and chemical properties of water (WHO, 1996). Some of these impurities and imbalances in the water can result in poor performance, health, and possibly death in livestock (WHO, 1996; NAHMS 1997). Within the Rocky Mountains, areas with elevated Mo concentrations due to geology or human activity exist, which can result in Mo either naturally or anthropogenically entering ground or surface waters (Kistner et al. 2017). Chappell (1975) sampled the Ten Mile Creek in Colorado, finding Mo concentrations several orders of magnitude above background concentrations. Furthermore, Chappell (1975) also samples near Colorado mines and reported dissolved Mo concentrations as high as 25 mg Mo/L in aqueous discharge.

Few studies have investigated the impact of drinking water Mo in cattle. However, Kincaid (1980) and Kistner et al. (2017) both conducted studies to analyze animal performance, toxicity/deficiency and various mineral interactions when Mo was supplied at elevated concentrations in drinking water. Over a 21-d period, Kincaid (1980) supplied increasing inorganic Mo concentrations in drinking water to 12 calves, while supplying a basal diet containing <1 mg Mo/kg DM, 13 mg Cu/kg DM, and 0.29% S. Although Mo exposure was relatively acute, to our knowledge, this was the first experiment to investigate the impact of Mo in drinking water on cattle. In the Kincaid (1980) study, liver Cu concentrations were reduced in animals receiving 50 mg Mo/L in drinking water but not in the animals receiving 1-10 mg Mo/L; however, Cu in plasma was increased for high dosed Mo cattle while ceruloplasmin changes from 61% to 43%. Kincaid (1980) concluded that Mo in water may be less toxic to calves that Mo in forage, with a minimum Mo toxic concentration in drinking water is between 10 and 50 mg Mo/L for calves; the estimated the safe drinking water Cu:Mo could be 0.5:1. Kistner et al. (2017) supplied increasing Mo concentrations (up to 960 μg of inorganic Mo/L) in drinking water while feeding a high concentrate finishing diet to 30 conventional crossbred feedlot steers for 131 d, then studied the effects of Mo water on performance, carcass characteristics, and mineral status within the animal. Water Mo treatments were: 1) 0.0 μg/L, 2) 160 μg/L, 3) 320 μg/L, 4) 480 μg/L, and 5) 960 μg/L of supplemental Mo added as Na_2MoO_4 to the drinking water (Kistner, 2017). The overall conclusion was similar to that of Kincaid (1980), whereby water Mo concentrations between 160-960 μg/L had no impact on performance, mineral status, water intake, or carcass characteristics.

Kistner et al. (2017) hypothesized that the reason Mo drinking water was less toxic to cattle may be due to portion of drinking water bypassing the rumen when consumed. Therefore, Mo in a portion of the drinking water is not exposed to the rumen environment, unlike Mo from forage intake. Several researchers have estimated that 18 to 90% of consumed water can bypass the rumen and enter the abomasum via the esophageal groove in cattle and sheep (Warner, 1968; Woodford et al., 1984; Garza and Owens, 1989; Garza et al., 1990). Therefore, with such a large range interdependent on animal size, age, diet, and physiological state, it would be incorrect to assume drinking water would equilibrate with ruminal fluid (Woodford et al., 1984).

COPPER

Copper has a variety of different uses (industrial, mechanical, catalytic, pigment, superconductors, etc.). From an agriculture standpoint, Cu is used as a feed supplement, fungicide, and bactericide (Richardson, 2003). Copper is classified as an essential trace mineral (NASEM, 2016) for beef cattle. Copper serves as a functional component in many enzymes necessary for various physiological functions. Elvehjem (1935) was one of the first to categorize Cu as an essential trace mineral in ruminants and observed deficiency symptoms in animals when Cu intake was low.

Sources

Soil

Copper can be found in rock formations and tends to occur as sulfide deposits, although, many soil conditions can influence Cu availability to animals (McDowell, 1992). Similar to Mo, total soil Cu content is not a good indicator of Cu availability to plants or animals (McDowell, 1992). Soil Cu is most available to plants when grown in poor drainage, acidic, and under low concentrations of other metal antagonists. Soil Cu is least available to plants grown in high organic matter containing soils, as organic matter forms complexes with Cu and reduces its availability (Kabata-Pendias, 2010).

Feed

Plant Cu concentrations typically range from 1 to 50 mg Cu/ kg DM, with wide ranges indicative of environmental (e.g., soil, water) variations (McDowell, 1992). When assessing forage Cu concentrations, one should also be aware of Cu-antagonist elements such as, Mo, S, and Fe (NASEM, 2016). Plants need the right conditions for soil Cu optimization, with soil chemistry along with plant species and weather variations needing to be considered (McDowell, 1992). As plants mature and the soil becomes more alkaline which decreases soluble Cu concentration; grasses tend to contain approximately 5.0 mg Cu/kg DM and is lower than legumes averaging 15.0 mg Cu/kg DM when grazed with the majority of Cu found in the grains and leaves rather than the stems (McDowell, 1992).

MINERAL INTERACTIONS

There is a well-known interaction between Cu-Mo-S in ruminant animals. Early Cu and Mo investigations focused on ruminant animal intake was based on the observed reductions in productivity of animals grazing a particular pasture high in Mo (Ferguson, 1938). Neilands et al. (1948) hypothesized that Cu, Mo and an unknown mineral may have influenced mineral availability to grazing livestock.

Copper-Molybdenum Interaction

While studying the imbalances of Mo in the diet, researchers indicated animals would have imbalances appearing to resemble Cu deficiency. Dick and Bull (1945) conducted a six month study providing a consistent dietary Cu dose while altering the Mo intake between high and low dose groups altering the Cu:Mo, noting that Mo decreased liver Cu concentrations when compared to sheep not receiving dietary Mo. The authors concluded that relatively small dietary Mo doses can alter Cu absorption in an unknown chemical reaction; ultimately, indicating there could be a need for a safe forage Cu:Mo range when feeding ruminants (Dick and Bull, 1945). However, Dick and Bull (1945) did not analyze forages for S content.

The estimate of a safe dietary Cu:Mo ratio was set to 2:1, due to an apparent Cu deficiency in ruminant diets below a 2:1 ratio (Miltmore and Mason, 1971). Investigating various feed samples, as well as previous studies analyzing ruminant physical and biological status, determined the Cu:Mo in feeds can have profound impacts on Cu status in cattle. Cattle receiving diets of 4.3:1 (Cu:Mo) appeared healthy, diets of 2.3:1 resulted in individual animals scouring (diarrhea), and 1:1 diets were associated with emaciation, lethagia, changes in hair, and severe reductions in weight gain (Miltmore et al., 1964; Miltmore and Mason, 1971). The use of Cu injections in animals paired with dietary Cu supplements was implemented by Miltmore et al. (1964), resulting in decreased scouring incidences and improved cattle weight gains.

Molybdenum-Sulfur Interaction

A study conducted by Dick (1953a), investigated the impact of two different hay sources (chaffed Lucerne hay or chaffed Oaten hay) with excess Mo supplementation (10 mg/kg/day; as ammonium molybdate drench) to a small number of sheep. The authors reported that sheep fed the chaffed Lucerne hay had lower blood Mo concentrations, decreased liver Cu concentration, and increased Mo content in urine. The conclusion drawn was that sulfate in the Lucerne hay caused an antagonistic reaction with the supplemented Mo. In another study investigating the influence of forage type and Mo on Cu metabolism, Dick (1953b) reported that Mo retention and excretion was affected by S intake: when S intake was low, Mo accumulated in animal blood and liver; when S intake was high, Mo was excreted in the urine and blood accumulation did not occur. Thus, a relationship exists between Cu, Mo, and S in ruminant diets. Further hypothesis from Dick (1953b) indicated that it would follow suit that the inadequate Cu storage in the liver by ruminants consuming high Mo diets could also be dependent of the concentration of S in the diet.

Dietary Mo and S supplements have similar effects on Cu and Mo metabolism. Molybdenum supplemented exclusively at 4.0 mg/kg increased Mo concentrations in plasma but has no effect on dietary Cu availability; however, S supplemented exclusively at 3 g/kg caused reductions in Cu availability (Suttle, 1974b). When Mo and S were fed together, a 50% reduction in sheep Cu availability was realized (Suttle, 1974b).

Elevated S in drinking waters can be an issue where cattle feeding occurs. Elevated S in water can result in toxic hydrogen sulfide gas being produced in the reducing environment of the rumen. This can cause animal poor performance as well as S-induced polioencephalomalcia (PEM) that can result in death. Due to the antagonistic reaction occurring between Mo and S in the rumen that causes a Mo-S (e.g., thiomolybdate) complex to be formed, Kessler (2012) hypothesized that supplementing Mo when S in the water was high would help to alleviate PEM risk. However, this resulted in Mo toxicity and even greater H_2S production (Kessler, 2012).

Copper-Molybdenum-Sulfur Interaction

Most Mo studies conducted address this Cu-Mo-S interaction and thiomolybdate formation within the ruminant. A study using Jersey heifers investigated supplementation of Mo (ammonium molybdate), Cu (copper sulfate), S (sodium sulfate) in relation to the mineral status of the animal following roughly a 2 year-long study (Cunningham, 1959). When Mo was supplemented alone or with Cu, marked increases in Mo concentration will occur in the blood and liver. This is not the case when S was fed in conjunction with Mo, which yielded greater Mo excretion (Cunningham, 1959). Molybdenum by itself or fed with S (as SO_4) can cause impaired Cu storage in the liver and decreased blood Cu concentrations (Cunningham, 1959). Liver Cu status decreased in all treatment groups including those receiving supplemented Cu (54 mg/L in the drench and 9 mg Cu/kg DM in the diet), with the least marked decrease in Cu status observed in animals receiving Cu, Mo, and S (as $NaSO_4$) in the supplement (Cunningham, 1959). Animals supplemented with Mo without S had an increase in blood Mo concentration, although that increase was not observed when animals were supplemented with S simultaneously (Cunningham, 1959).

Copper-Mo-S interactions most likely occurs in the rumen. When occurring in the rumen, it is believed that interactions render most Cu and Mo unavailable for absorption (Suttle, 1974a). There have been other hypothesized mechanisms such as blocking of Cu transport across membranes, competition for a common carrier system, the formation of an insoluble Cu-Mo complex, and the formation of a Cu-sulfide complex which is unable for absorption (Dick et al., 1975). The principle mechanistic formation of thiomolybdates and its subsequent reactions are: a) SO_4 and partial degradation of S containing amino acids is reduced in the rumen to sulfide or hydrosulfide ions by the microbial populations; b) the sulfide product reacts with dietary molybdates to create oxythiomolybdates or tetrathiomolydates in the rumen; c) these thiomolybdates react with dietary Cu in the rumen to form cupric thiomolybdates rendering Cu unavailable for absorption (Dick et al., 1975; Mills and Davis, 1987).

In ruminant diets when Mo is found in excess of Cu in the presence of a S-rich environment, the production of tri- and tetra-thiomolybdates may also exert toxic effects resulting in impaired reproductive status (Suttle, 1991). In a study with feedlot cattle, Dias et al. (2013) reported that a low Cu:Mo paired with increased Mo and S intake in the diet resulted in changes plasma and liver concentrations and even reduced ADG and feed efficiency. Identifying these systemic sites may result in a way to better treat animals suffering from thiomolybdate production.

In geographical locations where Mo excess occurs, an induced Cu deficiency can be expected in cattle (Cunningham, 1959). As the Cu:Mo ratio decreases, S intake becomes more important in controlling the sites of interaction by increasing the rate of Mo excretion. This interaction could be a source of monitoring, treatment, and prevention for ruminants suffering from Cu toxicity if the correct proportions for supplementation can be identified (Suttle, 1974a).

DIGESTION, ABSORPTION, AND EXCRETION

In the rumen, it is thought that microorganisms reduce sulfates to sulfides which then react with Mo found in solid digesta, to form thiomolybdates. When thiomolybdates encounter Cu in the rumen, they can bind Cu and render it unavailable (Gould and Kendall, 2011). Stepwise dehydrolysis reactions are responsible for thiomolybdate formation. An oxygen from molybdate is displaced with S from a sulfide donor at one of four reaction steps, with water being created at each step; the reaction can be reversed if exposed to high temperatures and is pH dependent (Gould and Kendall, 2011). In a study with cultured rumen fluid, the main thiomolybdates present were tri- and tetra- thiomolybdates, with the formation of a particular thiomolybdate dependent on pH and sulfide:Mo concentration. At a pH of ≥ 8.0, di-thiomolybdates were formed whereas at a pH of 6.5 more tri- and tetra- thiomolybdates were produced (Clarke and Laurie, 1980). When thiomolybdates are located in the rumen digesta solid phase, thiomolybdate stability is created. If found in the liquid phase, thiomolybdates are considered unbound, making it easier for the reversal of the thiomolybdate compound (Gould and Kendall, 2011). Because available Cu is also typically found within the digesta, it is typically in the solid phase as well, thereby facilitating the formation of Cu-thiomolybdate complexes (Ward et al., 1993; Gould and Kendall, 2011).

Absorption

Most Mo is readily absorbed in the small intestine in hexavalent water soluble forms such as sodium and ammonium molybdate. Even insoluble forms such as molybdenum trioxide and calcium molybdate find their way past the gastro-intestinal tract of ruminants, but molybdenum sulfate will not be absorbed (Davies, 1972; Mills and Davis, 1987). Copper is primarily absorbed in the small intestine although it has the ability to be absorbed in all segments of the gastrointestinal tract and transported in the blood by transcuprein and albumin (Davis and Merts, 1987; Thomas, 2018). Once absorbed, Cu (<10% absorption rate) is transported to the liver and used for either metallo-protein synthesis, stored in lysosomes, excreted in the bile, or incorporated into ceruloplasmin for use and transport to other areas of the body (Dias et al. 2013; Thompson, 2018). It would be advantageous to find a Cu form that would not interact with thiomolybdates or S and would remain soluble in the digestive tract for absorption to take place (Suttle, 1974a).

Historically, it was thought that Mo was absorbed in the small intestine due to the delay in blood concentrations following oral administration of Mo. More recently, that delay is thought to be caused by the conversion of Mo to thiomolybdates in the rumen which have been shown to be absorbed through the rumen (Kelleher et al., 1983; Telfer et al, 2004; Hall; 2018). It does seem that thiomolybdates will be absorbed via an active carrier, and that absorption is efficient, as it does not appear to be regulated at the mucosal level. Increasing the Mo concentration will increase the absorption rate which could lead to the toxicity issues reported in ruminants (Hall, 2018). The hypothesized fates of thiomolybdates formed in the rumen are: 1) absorbed through the rumen wall; 2) passed with digesta through the abomasum; 3) bound to Cu in the rumen and then excreted; 4) bound to the solid phase digesta creating chemical stability; and 5) hydrolyzed in the liquid phase digesta (Gould and Kendall, 2011).

Water movement through the reticulorumen is an important component of total digestion. In addition to its metabolic functions, water also serves as a mode for digesta transport out of the rumen (Woodford et al., 1984; Garza and Owens, 1989). If all drinking water consumed entered the rumen there could be significant shifts in volume, osmotic pressure and potentially temperature that could alter microbial populations and possibly reduce the fermentation process (Garza and Owens, 1989).

Molybdenum can be found in the liver, kidney, and bone, but relatively little retention in these tissues as Mo has a short life in the body (Hall, 2018). Copper distribution in the body is vast. Most of the total Cu absorbed into the body is found in the liver (72-79%), muscle such as the heart (8-12%), skin and pelt (~9%), and skeleton (~2%) in sheep (Davis and Mertz, 1987). Tissues with low Cu concentrations are sex organs, and the pituitary and thyroid glands (Davis and Mertz, 1987).

Excretion

The main routes of mineral excretion are through the urinary system and the feces. Due to the fact that Mo has a relatively short retention in the body, the main excretion method appears to be in the urine followed by and fecal excretion (Ward, 1978; Hall, 2018). While studying the three-way interaction of Cu-Mo-S, researchers have noted that the urinary excretion of Mo and S are elevated as dietary Mo and S increase (Dick, 1953a). When Mo intake is in excess, Mo excretion will inevitably be increased and therefore the possibility of Mo becoming an environmental pollutant in confined/concentrated animal feeding operations is probable (Kessler et al., 2012). During the lactation phases of ruminant animals, it has been reported that large amounts of Mo are excreted through the milk (Phillippo, et al. 1987).

The main excretory route for ingested Cu is through the feces, most of which is unabsorbed Cu. Bile also serves as another site for Cu excretion from ruminants (Davis and Mertz, 1987). Minimal Cu amounts can be excreted in the urine and sweat of ruminants (Davis and Mertz, 1987). Milk excretion of Cu is homeostatically controlled at low levels and does not respond to dietary changes in Cu (Ward, 1978).

FUNCTION

In general, there are four functions of essential elements for cattle: structural; physiological; catalytic; and regulatory. Structural functions stem from minerals that help form structural components in body organs and tissues such as bones, muscle, and membranes (Underwood and Suttle, 1999). Minerals that occur in body fluids are responsible for maintaining osmotic pressure, acid base balance, and membrane permeability. (Underwood and Suttle, 1999). Minerals function with enzymes possible serving a component in the or possibility in the hormone systems are catalytic in nature and minerals involved in cell replication and differentiation are involved in regulatory mechanisms (Underwood and Suttle, 1999).

The main function of Mo is to serve as a component in a coenzyme required by many enzymes for normal biological functions. Molybdenum Coenzyme (MoCo) is a pterin (ring structure with specific stereochemistry) that, once Mo binds to, is ready to perform its enzymatic function(s) (Hall, 2018). The MoCo is required for oxygen transfer reactions associated with aldehyde oxidase, sulfite oxidase, and xanthine oxidase (Mills and Davis, 1987; Hall, 2018). Although, there has never been a documented Mo deficiency in cattle or other ruminants when fed practical diets, the production of thiomolybdates can create a Cu deficiency. Once Mo and S form thiomolybdates, the affinity for thiomolybdates to bind to other metals (such as Cu) increases, therefore causing a decrease in Cu availability for other biological functions.

Molybdenum is also thought to have some relationship with reproduction physiology. A delay in puberty (up to 8-12 weeks) in Mo supplemented cattle has been reported (Phillippo et al., 1987). During this study, a significant reduction in Luteinizing Hormone (LH) release was observed over the 11 week Mo supplementation period. Molybdenum supplementation also appeared to have a role in decreasing conception rates (Mo supplemented animals conception rate = 12-33% compared to 57-80% observed in animals not supplemented with Mo (Phillippo et al., 1987). Because the Cu status and rate of weight gain did not seem to accompany the symptoms of reproductive inefficiencies, it has been speculated that Mo alone could be associated with decreased LH release as a result of an altered ovarian steroid secretion and/or the feedback of the steroids of the hypothalamo-pituitary system (Phillippo et al, 1987). Similar issues have been reported in males in terms of impaired reproductive success, but the mechanism is unknown (Thomas and Moss, 1951).

The main function of Cu is to serve as a component in various copper metallo-enzymes such as lysyl oxidase, cytochrome oxidase, superoxide dismutase, ceruloplasmin, and tyrosinase (McDowell, 1992). Copper is second to zinc (Zn) in the number of enzymes it serves as a vital component, making Cu essential for normal biological system functionality; it is required for iron (Fe) metabolism, cellular respiration, cross-linking and connective tissue, Central Nervous System formation, reproduction, and immune function as well as many others (McDowell, 1992; Underwood and Suttle, 1999; Baker et al., 2006). Any and all of these systems that require Cu can be impaired when Cu availability is inhibited.

Ceruloplasmin is an oxidase enzyme that contains the major fraction of blood Cu (Ward, 1978). The activity of ceruloplasmin in the blood can be measured and is thought to be a relatively good estimate of available Cu in the blood. Using the difference in ceruloplasmin activity and the ceruloplasmin:plasma Cu ratio can provide insight into the impact of dietary Mo and S on Cu metabolism (Ward, 1978; Telfer et al., 2004). Cytochrome C oxidase is another Cu dependent enzyme of interest. Cytochrome C oxidase is involved in the oxidative phosphorylation and energy production of a cell by facilitating the conversion of oxygen to water during cellular respiration (Spears, 1999). Cytochrome C oxidase activity can be reduced by elevated dietary Mo and S (Telfer et al., 2004).

As previously discussed, the interactions between trace minerals and animal production are extremely complex. Many factors can affect an animal's response to trace mineral supplementation such as the duration and concentration of trace mineral supplementation, physiological status of an animal (pregnant vs. open), the absence or presence of dietary antagonists, environmental factors, and the influence of stress on trace mineral metabolism. The subsequent chapter will discuss research designed to determine the impact of long-term Mo supplementation in water and feed on cow-calf production over a two year period.

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CHAPTER 2 – MOLYBDENUM EXPOSURE IN DRINKING WATER VS FEED AFFECTS COPPER APPARENT ABSORPTION DIFFERENTLY IN BEEF CATTLE CONSUMING A HIGH FORAGE DIET

SUMMARY

Twelve Angus steers were utilized to investigate the influence of molybdenum (Mo) in drinking water or feed on apparent absorption and retention of copper (Cu) and Mo. Steers were fed a low-quality grass hay diet for 14 days. Steers were then housed in individual metabolism stalls for 5 days to determine dry matter intake (DMI). Steers were then blocked by body weight and DMI and randomly assigned within block to one of three treatments. Treatments consisted of: 1) control (no supplemental Mo); 2) 5.0 mg Mo/kg DM from sodium molybdate dihydrate (Mo-diet), and 3) 1.5 mg Mo/L from sodium molybdate dihydrate delivered in the drinking water (Mo-water). After the 5 day DMI determination period, total fecal and urine output was collected for 5 days. Dry matter intake, Cu and water intake, and DM digestibility were similar across treatments. As expected, Mo intake was greater ($P < 0.05$) in Mo-water and Mo-diet steers when compared to controls but similar between Mo-water and Mo-diet steers. Apparent absorption and retention of Cu (% of Cu intake) was greater ($P < 0.05$) in controls when compared to Mo-diet supplemented steers. Apparent absorption and retention of Cu (% of Cu intake) in steers in the Mo-water treatment did not differ from controls or those receiving the Mo-diet. Molybdenum-diet and Mo-water supplemented steers had similar apparent absorption and retention of Cu. Apparent absorption and retention of Mo (% of Mo intake) was greater in controls when compared to Mo supplemented steers. These data indicate that Mo consumed in

water may impact Cu absorption and retention to a lesser extent than Mo supplemented in the diet.

INTRODUCTION

Dietary requirements of molybdenum (Mo) for beef cattle are not well defined, most likely because, under practical feeding conditions, Mo deficiency in beef cattle has never been documented (NASEM, 2016). However, it has been well documented that elevated dietary Mo concentrations, (Dick and Bull, 1945; Dick, 1952) especially in combination with elevated dietary S can reduce Cu status in ruminants (Suttle, 1974; Price and Chesters, 1985; Ward and Spears, 1997). The reduction in ruminant Cu status is most likely due to formation of insoluble Cu-Mo-S complexes in the rumen (Suttle, 1991), or thiomolybdates (TM).

Formation of thiomolybdates (TM) occurs in the rumen and, can be absorbed through the rumen wall or small intestine if Cu is unavailable to bind to the TM (Smith and Akinbamijo, 2000; Telfer et al., 2004; Gould and Kendall, 2011). The stepwise dehydrolosis of molybdate whereby an oxygen ion is replaced by a S ion from sulfide at one of four reaction steps. This leads to the formation of TM compounds having a high Cu affinity and thus forming Cu-Mo-S complexes (Smith and Akinbamijo, 2000; Gould and Kendall, 2011). When Cu-TM are formed the complex becomes insoluble, and thus limits ruminant Cu absorption (Suttle, 1991; Gould and Kendall, 2011). Reduced Cu absorption can lead to ruminant health issues.The antagonistic TM effect occurs under moderate S dietary intake. Even with sufficient dietary Cu, with Cu:Mo ratios below 3:1 or with diets containing >2.5 g S/kg DM, a Cu deficiency may still occur (Dias et al. 2013).

Limited research has been conducted investigating the bioavailability of Mo contained in water consumed by cattle. Kistner et al. (2017) exposed steers to varying drinking water Mo doses for 112 to 151 days. Supplementing up to 960 µg Mo/L in drinking water did not affect performance, water intake, carcass characteristics, or Cu and Mo concentrations in liver or plasma, despite supplying a total dietary Mo and S intake that would approximate 3.4 mg Mo/kg DM and approximately 3.5 g S/kg DM. based on the aforementioned research, the experimental objective was to investigate the influence of Mo supplementation in drinking water or feed on apparent absorption and retention of Mo and Cu in beef cattle consuming a high forage diet. We hypothesized that Mo supplemented in water would have less of an impact on apparent absorption and retention of Cu than if Mo were supplemented in the diet.

MATERIALS AND METHODS

Prior to project initiation all animal care, handling, and procedures described herein were approved by the Colorado State University Animal Care and Use Committee (IACUC approval # A3572-01).

Twelve Angus steers (BW 694.1 ± 47.4 kg) fitted with ruminal cannulae were utilized. Steers were fed a low-quality grass hay diet (DM basis: 6.3% CP; 0.12% S, 3.4 mg Cu/kg, 2.4 mg Mo/kg) in a group pen for 14 d with *ad libitum* access to water (pH 7.60; <0.01 ug Cu/L, <0.01 ug Mo/L). After the 14 d diet adaptation period, steers were housed in individual metabolism stalls (3.0m x 1.1m pens equipped with automatic waterers, individual plastic feeders, and rubber matted floors) for 5d to allow steers to acclimate to the new surroundings. During the acclimation period, DMI for each steer was determined. At the end of the acclimation period, steers were blocked by DMI (n=3 steers per block with the closest mean DMI). Once animals were appropriately blocked by DMI, each steer within a block (n=3) was fed 90% of the steer within the triplicate with the lowest average DMI during the acclimation period. This method of blocking was used to ensure equal amounts of feed intake occurred for individual steers within each block during the 5 d total fecal and urine collection period.

Steers within a block were randomly assigned one of three treatments (n = 4 steers per treatment). Treatments consisted of: 1) control (no supplemental Mo); 2) 5.0 mg of supplemental Mo/kg DM from sodium molybdate dihydrate ($MoNa_2O_4 \cdot 2H_2O$; Mo-diet), and 3) 1.5 mg Mo/L of $MoNa_2O_4 \cdot 2H_2O$ delivered in the drinking water (Mo-water). After the 5 d DMI determination period, total fecal and urine output was collected every 24 h for 5 d.

Feeding, Fecal, and Urine Collection

Diets were fed twice daily (60% and 40% of the ration in the morning and afternoon, respectively). All steers received the same basal water containing no Mo. Water was supplied in amounts that would allow animals *ad libitum* access to water throughout the day. The Mo-water treatment was made fresh in carboys that supplied the Mo water treatment to the appropriate animals as needed. Daily (24 h) water consumption was determined throughout the experiment for all animals. Dried distillers grains (250 g/d) was used as the carrier for Mo for steers receiving the Mo-diet treatment. Dried distillers grains (250 g) containing no Mo was also added to the control and Mo-water treatment diets. Immediately after feeding the basal diet, the appropriate supplement amounts (again, 60% and 40% of the ration in the morning and afternoon, respectively) was top-dressed for each feeding period within a day. Total fecal and urine output was measured every 24 h for each steer during the 5 d collection period. Fecal bags and urine harnesses used for fecal and urine collection were similar to those described by Tolleson and Erlinger (1989) and Border et al. (1963), respectively. Urine was collected in carboys to determine daily (over a 24 h period) output, and a 10% aliquot was retained and stored at -20ºC for each steer, daily. Feces collected each day (over a 24 h period) were weighed, thoroughly mixed, and sub-sampled (10.0% of wet weight). Duplicate, individual fecal sub-samples were sealed in plastic bags, labeled, and stored at -20ºC. Prior to chemical analysis, samples were proportionally composited (10% of the total mass or volume/day within sample type) across all collection days for each animal.

Analytical Procedures

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). Urine, feces, feed, and water samples were analyzed for Cu and Mo using inductively coupled plasma mass spectrometry (EPA 200.8, rev. 5.4, 1994; PerkinElmer; NexION 2000 B).

Statistical Analyses

Dry matter, Mo, Cu and water intake, DM digestibility, and apparent absorption and retention of Mo and Cu were analyzed using a mixed effects model (PROC MIXED, SAS Inst. Inc., Cary, NC) for a completely randomized block design. Individual animal was considered the experimental unit. Multiple covariance structures were compared using AIC to determine the most appropriate covariance structure for data analysis. For all response variables, significance was determined at $P \leq 0.05$ and tendencies were determined at $P > 0.05$ and ≤ 0.10.

RESULTS

Dietary and water nutrient data are shown in Tables 2.1 and 2.2, respectively. The impact of Mo exposure in feed or water on apparent Cu and Mo absorption and retention in beef steers is shown in Table 2.3. The average Mo intake by steers receiving on the Mo-diet treatment was 79.7 mg Mo/d with approximately 4.82 mg Mo/kg DM coming from supplemental Mo. Steers on the Mo-water treatment had an average intake of 72.3 mg Mo/d with approximately 1.51 mg Mo/L supplied in the water. Dry matter intake, water intake, and DM digestibility were similar across treatments. Apparent absorption (mg/d) and urinary excretion of Cu were greater ($P < 0.05$) in control and Mo-water supplemented steers when compared to Mo-diet steers. When expressed as a percentage of Cu intake, apparent absorption of Cu was greater ($P < 0.05$) in control vs. Mo-diet supplemented steers. Percent apparent Absorption of Cu tended ($P < 0.08$) to differ between Mo-water and Mo-diet steers. Apparent retention of Cu expressed as a percentage of intake or mg/d was greater ($P < 0.05$) in control compared to Mo-diet supplemented steers. Copper apparent retention in Mo-water steers was similar ($P < 0.13$) to controls.

Apparent absorption of Mo expressed as a percent of total Mo intake was greater ($P < 0.05$) in controls when compared to Mo supplemented steers (Table 3). Molybdenum retention expressed as mg/d was greater (P < 0.05) in Mo-diet supplemented steers compared to those fed the control or Mo-water treatments. When expressed as a percentage of Mo intake, apparent retention of Mo was greater ($P < 0.05$) in control vs. Mo-water and Mo-diet supplemented steers. Apparent retention of Mo expressed a mg/d was different ($P < 0.05$) across all treatments. Steers receiving Mo-diet had the greatest apparent retention of Mo (mg/d) followed by Mo-water, and control steers.

DISCUSSION

The best known mineral interaction that can cause a reduction in Cu absorption and retention in cattle and sheep is the Cu-Mo-S interaction (Suttle, 1991). Earlier research indicated that elevated dietary Mo concentrations (ranging from 5.0 – 50.0 mg Mo/kg DM; Dick and Bull, 1945; Dick, 1952) reduced Cu status in ruminants. However, in these earlier experiments, dietary S concentration were not measured. Later research indicated that although dietary Mo can slightly reduce Cu status in ruminants, dietary S in combination with Mo has a much more profound impact on reducing Cu status in ruminants (Suttle, 1974; Price and Chesters, 1985; Ward and Spears, 1997). Suttle (1975) demonstrated that Cu deficient ewes supplemented with 6.0 mg Cu/kg DM and fed a control diet low in S and Mo (0.1% S; 0.5 mg Mo/kg DM) exhibited a faster Cu repletion rate than sheep supplemented with 0.3% S or 0.3% S plus 4.0 mg Mo/kg. Mills et al. (1977) evaluated the effects of increasing dietary Mo concentration (from 0.05 to 5.0 mg Mo/kg DM) at either moderate (0.18% S) or high (0.33% S) dietary concentrations of S on liver Cu concentrations in calves fed 11.9 mg Cu/kg DM for 112 d. Calves receiving low dietary Mo (0.05 mg Mo/kg DM) with moderate or high dietary S accumulated in excess of 110 mg of Cu/kg DM in liver over the 112-d experiment. Whereas calves receiving high Mo (5.0 mg Mo/kg DM) and moderate S accumulated 66% less liver Cu over the 112-d experiment.

Limited research has been conducted investigating the impact of Mo contained in water consumed by cattle on Cu status. Kincaid (1980) determined the impacts of 0.0, 1.0, 10.0, and 50.0 mg of Mo/L of drinking water on performance and Cu status of calves. Calves had *ad libitum* access to water treatment and feed (basal diet contained 13 mg Cu/kg DM) for 21 days. Calf performance was not affected by Mo dose. At the highest Mo water concentrations an increase in plasma Cu concentrations and a numeric decrease in liver Cu concentrations was observed (Kincaid, 1980). 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) postulated that Mo in water could be less toxic than Mo in forage, and that the minimum toxic concentration of Mo in water for calves is between 10 and 50 mg of Mo/L.

Recently, Kistner et al. (2017) exposed steers to varying doses of Mo in drinking water. Water treatments consisted of: 1) 0.0 μg/L, 2) 160 μg/L, 3) 320 μg/L, 4) 480 μg/L, and 5) 960 μg/L of supplemental Mo added as Na_2MoO_4 to the drinking water. Molybdenum level did not affect gain, feed intake, water intake or plasma and liver Cu concentrations during the 112 to 151-d study (Kistner et al., 2017). Total dietary Cu concentrations ranged from 9.7 to 11.1 mg of Cu/kg DM in this study.

To our knowledge, the current experiment is the first to investigate the influence of short term Mo supplementation in water and feed on apparent Mo and Cu absorption and retention in beef cattle. Molybdenum concentrations used in this experiment were fed for a short period of time and averaged 76.0 mg of Mo consumed per steer per day for Mo- supplemented steers (Mo-water and Mo-diet). Apparent Cu absorption, as a function of % intake and mg/d, was lower in steers receiving Mo-diet when compared to Mo-water and controls (Table 2.3). As discussed by many researchers a major portion (18 - 80%) of water consumed by mature cattle and sheep can bypass the rumen and enter the abomasum via the esophageal groove (Warner and Stacy, 1968; Woodford et al., 1984; Garza and Owens, 1989; Zorrilla-Rios et al., 1990, Garza et al., 1990). Although the amount of water bypassing the rumen was not determined in the current experiment, this could explain why the Mo-water treatment had no impact on apparent absorption of Cu. Any Mo bypassing the rumen would not interact with rumen S and Cu.

Apparent retention of Cu (mg/d) for Mo-water supplemented steers did not differ from control or Mo-diet steers. The intermediate impact of Mo supplemented in water on Cu retention (mg/d) can be explained by greater urinary Cu excretion in Mo-water vs. Mo-diet steers. Alternatively, if thiomolybdates were absorbed in steers fed Mo-diet, this may have reduced urinary excretion of Cu.

Apparent absorption of Mo expressed as a percent of Mo intake was greater in controls when compared to both Mo supplemented treatments. However, when expressed on a mg of Mo absorbed per day, apparent absorption of Mo was greater in Mo-diet supplemented steers when compared to control and Mo-water supplemented steers. Furthermore, apparent retention of Mo (mg/d) differed across all treatments (Mo-diet > Mo-water > controls). The greater retention (mg/d of Mo in Mo-diet supplemented steers when compared to controls is consistent with previous research (Grace and Suttle, 1979) suggesting that TM formed from S and Mo in the rumen are poorly excreted following absorption. The greater apparent absorption (mg/d) in Mo-diet steers compared to control and Mo-water supplemented steers was surprising. We anticipated that if Mo from Mo-diet steers were to form insoluble Mo-S-Cu complexes in the rumen, that the apparent absorption of Mo would be lesser in Mo-diet vs control and Mo-water steers. The absorption of Mo is greatly affected by S intake (Grace and Suttle, 1979). Hay used in the present study only contained 0.12% S. However, water consumed was high in sulfate (432.6 mg/L). Considering the S derived from water, the total concentration of S in the present study was 0.16% of DM consumed. While total S intake was slightly above NASEM (2016) recommendation of 0.15% S, this level would be considered moderate, and may have limited the formation of TM in the rumen (Grace and Suttle, 1979; Suttle, 1999).

Research using radio labeled ^{99}Mo, indicated that when ruminants consume diets high in roughage, 90-95% of Mo is excreted in the feces with approximately 2-4% of Mo excreted in the urine (Miller et al., 1993). As concentrates in the diet increase, the urine becomes a larger proportion of Mo excretion by the animal (Miller et al., 1993) indicating that diet type can influence apparent absorption and retention of Mo. Apparent absorption and apparent retention of Mo in the current experiment are similar to those reported by Miller et al. (1993) in ruminants fed a high roughage diet.

Overall, these data indicate that Mo in drinking water, within the concentrations examined, appears to have less impact on Cu metabolism than if Mo is supplemented in the diet. Therefore, we fail to reject our hypothesis that Mo supplemented in water would have less of an impact on apparent absorption and retention of Cu than if Mo were supplemented in the diet. Further research is warranted investigating long term Mo supplementation on apparent absorption and retention of Cu and Mo in beef cattle.

Table 2.1. Ingredient and nutrient composition of the forage and dried distillers grain supplement basal hay diet (dry matter basis).

Ingredient	%
Grass Hay	99.8
Dried distillers grains	0.20

Analyzed chemical composition	Ingredient	
Nutrient	Grass hay	Dried Distiller's grains
Dry matter, %	85.23	92.1
Crude Protein, %	6.3	28.9
Acid detergent fiber, %	34.9	15.9
Neutral detergent fiber	57.8	34.2
Net Energy for gain, Mcal/kg	1.14	1.48
Net energy for maintenance, Mcal/kg	1.08	2.24
Total digestible nutrients, %	54.0	87.3
Digestible energy, Mcal/kg	2.5	4.0
Metabolizable energy, Mcal/kg	2.0	3.3
Calcium, %	0.43	0.04
Phosphorus, %	0.15	0.84
Potassium, %	1.91	0.98
Magnesium, %	0.14	0.35
Sodium, %	0.02	0.21
Sulfur, %	0.14	0.54
Cobalt, mg/kg	0.25	0.68
Copper, mg/kg	6.8	3.1
Iron, mg/kg	90.2	112.3
Manganese, mg/kg	182.3	21.9
Molybdenum, mg/kg	2.5	1.8

Table 2.2. Chemical composition of basal water.

Item	Result
pH (s.u.)	7.41
Chloride (mg/L)	28.4
Total Hardness (mg/L)	725.3
Nitrate-Nitrogen (mg/L)	<1.0
Calcium (mg/L)	174.3
Magnesium (mg/L)	54.2
Phosphorous (mg/L)	<0.1
Potassium (mg/L)	<5.0
Sodium (mg/L)	56.9
Sulfate (mg/L)	432.6
Aluminum (mg/L)	0.05
Cobalt (mg/L)	<0.01
Copper (µg/L)	<10.0
Iron (mg/L)	0.10
Manganese (mg/L)	0.07
Molybdenum (µg/L)	<10.0
Selenium (µg/L)	<30.0
Total Dissolved Solids (mg/L)	985.4

Table 2.3. The effects of molybdenum exposure on apparent absorption and retention of copper and molybdenum in fistulated beef steers.

Item	Control	5.0 mg Mo/kg diet DM	1.5 mg Mo/L drinking water	SEM	P<
DM intake, kg/d	11.4	11.4	11.4	1.01	---
Fecal DM, kg/d	5.4	5.0	5.1	0.51	0.82
DM digestion, %	52.3	55.6	54.6	2.79	0.71
Water intake, L/d	31.9	32.4	31.5	0.86	0.76
Copper					
Intake, mg/d	68.7	68.7	68.7	6.10	---
Excretion, mg/d					
Fecal copper	65.4	66.4	65.7	6.02	0.99
Urinary copper	0.67[a]	0.51[b]	0.72[a]	0.05	0.03
Apparent absorption, % of intake	4.9[a]	3.3[b]	4.4[a,b]	0.39	0.05
Apparent absorption, mg/d	3.3[a]	2.3[b]	3.0[a]	0.18	0.01
Apparent retention, % of intake	3.9[a]	2.5[b]	3.3[a,b]	0.33	0.05
Apparent retention, mg/d	2.6[a]	1.8[b]	2.3[a,b]	0.17	0.02
Molybdenum					
Intake, mg/d	25.0[b]	79.7[a]	72.3[a]	4.26	0.001
Excretion, mg/d					
Fecal molybdenum	21.7[a]	75.5[b]	69.0[b]	4.20	0.01
Urinary molybdenum	2.3	2.6	1.9	0.23	0.18
Apparent absorption, % of intake	13.4[a]	5.4[b]	4.6[b]	0.60	0.01
Apparent absorption, mg/d	3.4[b]	4.2[a]	3.3[b]	0.21	0.03
Apparent retention, % of intake	4.5[a]	2.0[b]	1.9[b]	0.37	0.01
Apparent retention, mg/d	1.1[b]	1.6[a]	1.4[c]	0.04	0.01

[a,b,c]Means within a row with differing superscripts differ $P < 0.05$.

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