THESIS

THE EFFECTS OF MOLYBDENUM WATER CONCENTRATION ON FEEDLOT PERFORMANCE, TISSUE MINERAL CONCENTRATION, AND CARCASS QUALITY OF FEEDLOT STEERS

Submitted by

Madison Kistner

Department of Animal Sciences

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Master's Committee:

Advisor: Terry Engle
Co-Advisor: John J. Wagner

Timothy N. Holt

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ABSTRACT

THE EFFECTS OF MOLYBDENUM WATER CONCENTRATION ON FEEDLOT PERFORMANCE, TISSUE MINERAL CONCENTRATION, AND CARCASS QUALITY OF FEEDLOT STEERS

Thirty cross-bred steers (initial BW 375 ±37.2, replicate 1; and 535.0 ± 39.4 kg, replicate 2) were utilized to investigate the effects of Mo water concentration on performance, carcass characteristics, and mineral status of feedlot steers fed a growing and finishing diet for 151 and 112 d for replicate 1 and replicate 2, respectively. The experimental design was a randomized complete block design. Steers were blocked by weight and then divided into 2 weight block replicates each consisting of 15 steers. Steers were randomly assigned within block to one of 5 treatments (3 pens/treatment; 1 steer/ pen; 2 replicates/treatment). Water treatments consisted of: 1) 0.0 µg, 2) 160 µg, 3) 320 µg 4) 480 µg Mo/L, and 5) 960 µg of supplemental Mo/L added as Na_2MoO_4 to the drinking water. Steers were housed in individual pens that contained individual 265 L water tanks for monitoring water intake. Daily water intake was recorded for each steer. Steers were individually weighed on 2 consecutive days at the beginning and end of the experiment and interim weights and jugular blood samples were obtained every 28 d. Liver biopsies were obtained on d0 and 84 from each steers. Steers were transported to a commercial abattoir, slaughtered, and individual carcass data and liver samples were collected. Initial BW was used as a covariate for statistical analysis of the data and significance was determined at $P \leq 0.05$. No differences were observed for final BW ($P \leq 0.98$). Overall ADG, DMI, feed efficiency and water intake were similar across treatments. Hot carcass weight, dressing percentage, yield

grade, LMA, adjusted fat thickness, KPH, and marbling scores were similar across treatments. Liver and plasma Cu, Mo, and Zn concentrations were similar across treatments. These data indicate that water Mo concentration had no impact on performance, mineral status, water intake, and carcass characteristics in feedlot steers fed a high concentrate diet.

Key Words: Water intake, molybdenum, beef cattle performance, mineral status

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Introduction of Molybdenum, Sulfur and Copper

Minerals can be divided into two main categories, 1) macro-minerals and 2) micro or trace minerals. Macro minerals are minerals that are needed/required in relatively large amounts in comparison to micro or trace elements, which are needed/required in relatively small amounts. In general macro minerals are required at concentrations greater than 100 mg/kg of the diet and are often expressed as a percentage of the diet while trace minerals are required at concentrations less than 100 mg/kg of the diet (McDowell, 1992; NRC, 1996).

The general functions of minerals can be broken down into four categories: 1) structural- minerals that play a role as components of tissues; 2) physiological- minerals that are involved in acid-base balance; 3) catalytic- minerals that are components of enzyme and hormone systems; and 4) regulatory- minerals that are involved in cell replication processes, (Underwood and Suttle, 1999).

Trace minerals are better described as an element present in animal tissues and the amounts are typically reported in parts per million (Church, 1988), but due to the importance of trace minerals supplementation; it's postulated that the amount of information known is small compared to the true impact that minerals have on metabolic functions. Reasons for continuous function fluxes are largely due to mineral absorption and/or function being affected by interactions with antagonist compounds (Spears, 2013). The understanding of the dynamic relationship between minerals and their interactions with antagonistic compounds has created a need for alternative forms of mineral sources such as organic and hydroxy mineral sources. To attempt to accurately estimate the bioavailability of trace minerals, both the interaction of

minerals with antagonistic compounds and the specific function of the mineral need to be understood. A well-known mineral interaction is the three-way interaction of Molybdenum (Mo), Sulfur (S) and Copper (Cu) (Spears, 2013). This relationship came to be understood once Cu deficiencies became more prominent in ruminants (Dick, 1953). It was discovered that Cu deficiencies are most common in cattle grazing in areas high in Mo and S (Goonerate et al., 1989). In these specific cases the reason for Cu deficiency can sometimes be due to the formation of thiomolybdates from Mo and S (Gould, 2011). The source, absorption, metabolism and interaction of these three minerals will be discussed later in this chapter.

Molybdenum and Mining

***Molybdenum*:** Molybdenum serves as a functional component of the substrate binding site for sulfate oxidase, xanthine oxidase, and aldehyde oxidase which are enzymes in the body that help to catalyze a wide variety of reactions such as purine degradation, stress response and detoxification of sulfite (Coughlan et al., 1968; Schwarz and Mendel, 2006). According to the NRC (2000) there is not a specific dietary requirement of Mo (NRC, 2000). Due to it's availability in forages, Mo toxicity (Molybdenosis) can be of concern. Underwood (1999) reported that cattle grazed on pastures containing between 20 and 100 mg Mo/kg DM, experience mild to extreme forms of scouring. Cattle can recover quickly when changed to pastures with normal Mo concentrations in the forage (3-5 mg Mo/kg DM; Underwood, 1999).

Molybdenum Mining in CO: In the early 1900's large mineral deposits of Mo were discovered in Bartlett Mountain CO, which later became recognized as Lake County, CO (Butler and Vanderwilt, 1993). Climax Molybdenum Company© (Leadville, CO) was established as a mining operation for Mo, which was heavily used during World War I for its ability to strengthen steel (Butler and Vanderwilt, 1993). Soil, legume and grass samples that were taken

along the Blue River, which runs through Lake County, CO have been reported to have elevated concentrations of Mo (9.2, 33.3 and 2.1 mg Mo/kg DM, respectively; Kubota, 1975). Aside from this area having naturally high Mo deposits, areas with high water tables in this location have been reported to have high amount of Mo in nearby forages as well (Umesh and Lipsett,1982). The Ten-mile creek lies 18 miles northeast of the Climax mine and is highly mineralized (Northwest Colorado Council of Governors, 2012) showing as high as 4,536 kg/day mass flow rate of Mo 9 mg Mo/L (Chappell, 1973). Limited research has been conducted investigating the impact of water Mo concentrations on ruminant animal performance. Kincaid (1980) studied the effects of supplemented Mo through water ingestion on 5-week old Holsteins. Kincaid (1980) reported a safe ratio of Cu to Mo in this experiment of 0.5:1.0. Kincaid also postulated that Mo in water could be less toxic than that in forage, and that the minimum toxicity level of Mo in water for calves is between 10 and 50 mg of Mo/L (10,000 and 50,000 ug of Mo/L; Kincaid, 1980). Studying the effects of controlled amounts of ingested Mo in cattle is important due to the influence of Mo on Cu status in ruminants.

Sources

Molybdenum*:* Molybdenum is found to be high in soils that consist of granite rock, shales and slates. (Kubota, 1972). Molybdenum absorption in plants is mostly from soluble forms in the soil, predominantly molybdate (MoO_4^{-2}) and soil colloids (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, 1972). Molybdenum also works in nitrate reduction as en electron carrier in the enzyme nitrate reductase that reduces nitrate to nitrite in plants and in some animals (Nicholas and Nason, 1955). It has been reported that application of Mo to the soil will increase crude protein in plants (Reddy, 1964). Due to Mo's

role in nitrogen fixation in plants, a Mo deficiency can cause a reduction in plant nitrogen metabolism and overall nitrogen fixation in plants (Gupta and Lipsett, 1982). Legume plants such as ladino clover and birdsfoot trefoil have a higher Mo requirement especially during the wet season. Legumes with alkaline soils (pH 7.0-7.5) are most efficient in absorbing Mo (Cameron and Goss, 1948). It's hypothesized that this is due to the MoO_4^{2-} anion being exchangeable in soil ($2\ OH^- \leftrightarrow MoO_4^{2-}$; Gupta and Lipsett, 1982). Although there is little data available as to what is deemed normal for Mo concentrations in soil, normal soil Mo concentrations are suggested to range from 0.5 – 5 mg Mo/kg DM (Gupta and Lipsett, 1982). Legumes grown in areas with naturally high deposits of Mo in the soil have been found to contain between 20 – 40 mg Mo/kg DM (Kubota, 1975).

Copper*:* Copper is an essential trace mineral for beef cattle and is typically fed in supplemental form to maintain normal Cu status (Smart, et al., 1992). Copper is typically supplemented in a free-choice mineral salt mix to grazing beef cattle (Peters, 2011) and is incorporated in the total mixed rations for cattle fed in confinement. Feeds shown to be the highest in Cu are leafy brassicas (12.8 % availability) cereals (9.1 %) and hay (7.3%) (Suttle, 2010). Availability of Cu in soils can range from 1-22 mg Cu/kg DM and the solubility is dependent on pH and soil texture. Typically, mineral soils that are finer in texture will contain the highest amounts of Cu (University of Minnesota Extension, 2002). Unlike Mo, as soil pH increases the availability of Cu to the plant decreases. Gupta and Mehla (1979) found that when applying Mo at levels of 0, 0.5, 1 and 2 mg Mo/kg DM to sandy loam soil containing 1.8 ppm Cu, the Cu: Mo ratio decreased from 2.5 to 0.34 at the highest concentration of Mo application (2 mg Mo/kg DM).

Sulfur: Sulfur is an easily accessible mineral in feedstuffs and is also supplemented to beef cattle. The recommended concentration of S in beef cattle diets is 0.15% according to the NRC requirements (NRC, 2000). Sulfur concentration in forages can range from 0.5 – 5.0 kg DM (Suttle, 2010). Legumes show an accessible amount of S with around 2 g/kg DM in the plant tissue (Suttle, 2010). Sulfur can be supplemented as sodium sulfate, calcium sulfate, ammonium sulfate, or elemental S (NRC, 2000). Sulfur amino acids are important in ruminant nutrition as they can be used as an energy source for rumen microorganisms as well as amino acid synthesis. Nitrogen (N) to S ratios should be maintained at a 13.5-15:1 ratio for maintaining microbial health and function (Kandylis, 1984). Distiller's grain, which is a by-product of ethanol production, can contain high concentrations of S. A study conducted by Vanness (2009) found S concentrations in distiller's grains averaging at 0.79% DM which was averaged from 1,200 samples from 6 ethanol plants (Vanness et al., 2009). Feeding distillers grains requires close monitoring so that S concentrations are not fed in concentrations that may induce polioencephalomalcia (Vanness et. al, 2009). Regions high in natural deposits of S tend to have higher concentrations of sulfate S in the water sources.

Absorption

Molybdenum: Trace mineral absorption is dictated by the homeostatic mechanisms that regulate absorption and storage within tissues (Spears, 2002). Trace mineral absorption is therefore dependent on the mineral status of the animal (Spears, 2002). Molybdenum is absorbed through the rumen epithelium as well as the small intestine as (MoO4) through active carrier-mediated transport mechanisms. There is little current data examining these mechanisms in detail (Nielsen, 1999). The specific Mo carrier mediated pathway for absorption is the same pathway for sulfate (SO_4^{2-}). Dick (1953) reported that increasing dietary sulfate decreased absorption of

Mo from the rumen. Once absorbed, Mo is transported by either albumin or alpha-macroglobulins in the blood (Hayes and Kruger, 2014). ModA, ModB and ModC are three genes that code for the transporter ModABC. This transporter is responsible for extracellular uptake of Mo (Perinet, 2016). Supporting studies show that the ModABC plays an important role in transporting the mineral to Mo containing enzymes (Perinet, 2016).

Copper: When Cu is in an available form, it is primarily absorbed through the small intestine and partly in the rumen by chelating and attaching to soluble ligands (Suttle, 1991). Zinc (Zn) has been shown to be an antagonistic mineral that can inhibit the absorption of Cu. Therefore higher levels of Zn can decrease Cu absorption (Cousins, 1985). Copper is absorbed through the mucosal cells of the lumen of the small intestine as cuprous ions by Ctr1 transporters (Prohaska, 2008). Very few cupric ions leave the intestine and are ultimately excreted if not oxidized for transport (Prohaska, 2008). Once the Ctr1 protein has transported Cu into the cells, metallochaperones transport Cu to cuproenzymes (Prohaska, Gybina, 2004). The metallochaperone, Atox1 is required for the incorporation of Cu into bile and for the delivery of Cu to holoceruplasmin, which is a protein in ceruloplasmin (CP) that helps to regulate Iron (Fe) homeostasis (Hellman and Gitlin 2002; Prohaska, 2008). super oxide dismutase (SOD) is an enzyme that converts radicals into hydrogen peroxide and oxygen. Copper chaperone for SOD (CCS) is a Cu metalochaperone that facilitates the transport of Cu into (SOD). Cox17 is a copper chaperone for cytochrome C oxidase and a protein in the mitochondria (Palumaa et al., 2004). Lastly two P-type ATPases, CopA and CopB membrane proteins have been shown to help increase Cu uptake by cells (Pena, 1999). Aside from Cu's use in enzyme function, most Cu is transported to the liver bound to albumin and stored (Laurie and Pratt, 1986)

Sulfur: Sulfur is important to provide in the diet because it is necessary for utilization by rumen microorganisms for S-amino acid synthesis (Richter, 2011). It is also necessary for thiamine production and for maintaining an optimum ratio of N: S so that microbes can incorporate both elements into microbial proteins (Richter, 2011). It is important to understand that certain classes of microbes in the rumen have different actions to degrade and utilize S. Inorganic S enters the rumen as sulfate (SO_4^{2-}) and is converted to sulfide (S^{2-}) by the dissimilatory rumen microbes (Kandylis, 1984). The sulfide is then used by a sulfide-oxidizing group of microbes, which use the S to synthesize S-amino acids. There is a small population of bacteria in the rumen that cannot utilize inorganic S and therefore utilize methionine as their S source. Assimilatory microbes can only utilize organic sources of S and only convert enough organic S to meet their energy needs (Kandylis, 1984). The hydrogen sulfide circulating in the rumen can either be absorbed through the rumen wall, re-used by microbes to be incorporated into bacterial protein or belched causing Polioencephalomalacia (PEM) (Kandylis, 1984). Microbes can be efficient in utilizing sulfide as long as there is enough fermentable energy supplied in the diet (Suttle, 2012). An ideal N:S ratio is approximately 13.5-15.1 (Richter, 2011). An interesting interaction is S's role in thiamine production. Thiamine is a S containing B vitamin that is required in the diet at 0.2 % DM of feed for the efficient synthesis of thiamine by the rumen microbial organisms (Oliverira et al., 1995).

Polioencephalomalacia is diagnosed as being caused by dietary S exceeding 0.4% DM resulting in hydrogen sulfide formation in the ruminal gas cap (Oliverira et al., 1995). It's postulated that S induces PEM either by hydrogen sulfide production or in some cases by the degradation of thiamin triphosphate (TPP) and therefore causing a thiamin deficiency (Amat, et al., 2013). Clinical signs of thiamin deficiency are reduced growth rates, scouring or weight loss,

central nervous system disorders are not shown until later on possibly due to the apoenzyme-thiamine complexes that help buffer the marked decrease in thiamine enzyme activity (Rammell and Hill, 1986) ultimately resulting in an impairment in energy metabolism in the brain (Amat, et al., 2013). Loneragan though, found that in a case of 16 calves all diagnosed with Polioencephalomalacia, ruminal hydrogen sulfide levels were high, while blood thiamine levels were within reference range (Loneragan et.al, 1998). Sulfide has a short half-life in the rumen. Bray (1969) found that in sheep 40-90% of sulfide was absorbed within 60 min, where only about 25% of sulfate was absorbed within 60 min. It is estimated that the half-life of sulfides within the rumen is between 10-22 min Bray, 1969). Sulfur is absorbed mainly in the small intestine by active transport in the form of dietary, microbial or fungal protein (Suttle, 1999). Methionine is an essential S-amino acid that needs to be supplied in the diet. Once absorbed methionine provides the methyl group for the formation of cysteine and cystine (Suttle, 1999).

Transport

Molybdenum: Molybdenum is a part of several metaloenzymes such as nitrate reductase, aldehyde oxidase, sulfite oxidase, and xanthine oxidase. Molybdenum also functions as a structural component of the co-enzyme Moco (Mendel, 2012) . Moco is a pterin based Mo cofactor (Mendel, 2012). The synthesis of Moco is a four-step process that involves the use of ATP, Fe and Cu (Mendel, 2012). Moco is sensitive to oxidation and is formed by Mo covalently binding to two sulfur atoms (Mendel, 2012). Molybdenum can then carry out its signaled enzymatic function once bound to Moco and transported (Mendel, 2012).

Copper: Absorbed Cu is transported to the liver where it can either be stored as a Cu-metallothionine complex or transported to other tissues that require this element (Suttle, 2012; See Figure 1).

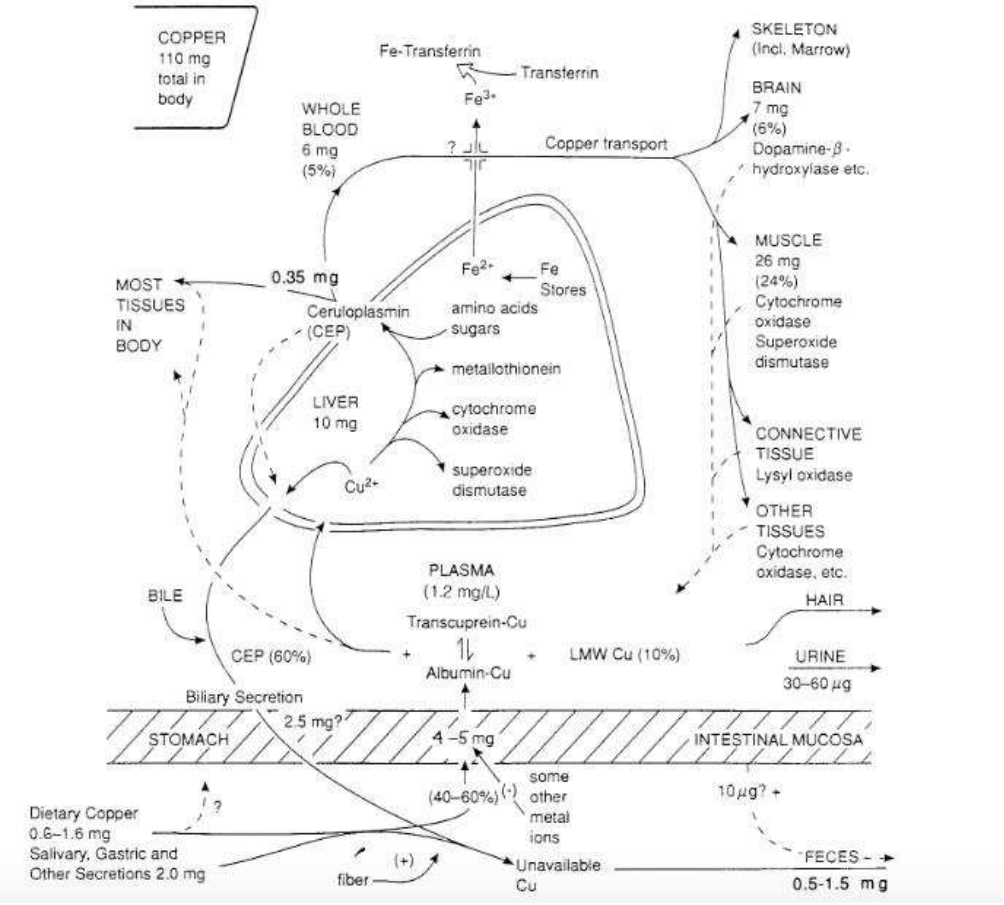

Figure 1. Summary of the biochemistry and metabolism of copper in the human (Linder and Hazegh-Azam. 1996)

If Cu is in Cu2+ form, the liver can incorporate Cu2+ into SOD and cytochrome c oxidase to help prevent free radical activity (Linder and Hazegh-Azam, 1996). If Cu accumulates in the liver in excessive amounts, the production of metallothionine, which regulates absorption of metals in the body, will increase to signal the excretion of Cu in the bile (Suzuki et al., 2001). Under normal liver Cu concentrations, Cu is incorporated into ceruloplasmin (CP) (Linder and Hazegh-Azam, 1996). Once synthesized in the liver, CP transports Cu to cells for the incorporation into Cu dependent enzymes (Kies, 2012). Once in contact with the cell, reducing equivalents are transferred to CP. This transforms copper into its cuprous form by the acceptance of an electron, which makes it unstable, causing it to disassociate from the protein (Kies, 2012).

Ascorbic acid facilitates the transmembrane transfer of the free Cu into the cell (Kies, 2012). Once in the cell Cu is incorporated into cellular enzymes with the help of different chaperones such as ascorbic acid, glutathione, CU-ATPases, ATP7A and ATP7B (Kies, 2012) (Lutsenko, et al., 2008).

Sulfur: Post absorptive metabolism of elemental S and S containing amino acids is similar in ruminants and non-ruminants. The metabolism of the essential amino acid methionine is important to provide a foundation for new S-amino acid synthesis. Serine sulfhydrase catalyzes the synthesis of cysteine through the condensation f H2S and the amino acid L-serine to form L-cysteine (Allison, 1969). These S-amino acids (Cysteine and Cystine) are transported to the circulatory system through sodium dependent exchanges. Methionine and cysteine are metabolized through multiple pathways resulting in many different products and uses (Griffith, 1987). Both of these amino acids play important catalytic roles and therefore serve as intermediaries for further protein synthesis and protein structure (Griffith, 1987). Methionine is metabolized to S-adenosylmethionine by the enzyme Methionine adenosyltransferase using ATP (Griffith, 1987). This compound is responsible for most methylation reactions by adding a methyl group to substrates to modify gene expression or protein synthesis (Griffith, 1987). Cysteine plays a substantial role in tissue protein synthesis. At the tissue level, cysteine is a part of myosin and casein, which are tissue fibers contributing to muscle and mammary tissue as well as wool and hair. (Suttle, 2012). Cysteine also plays an important role through as a component of glutathione (Griffith, 1987). The cysteine in glutathione works as an active site for nucleophiles (Griffith, 1987). This allows the bond between S and Hydrogen (H) on glutathione to scavenge free radicals of Oxygen (O^2) and carbon (C) as well as electrophiles (Griffith, 1987).

Functions

Molybdenum: The ultimate function of Mo is to act as a structural component for enzymes (Coughlan, 2014). As previously mentioned Mo is a crucial for the function of four main enzymes; nitrate reductase, aldehyde oxidase, sulfite oxidase and xanthine oxidase (Coughlan, 2014). Nitrate reductase functions by converting inorganic nitrogen into nitrite for proper nitrogen turnover in algae, fungi and plants (Morozkina and Zvyagilskaya, 2007; Coughlan, 2014). Aldehyde oxidase is a metabolizing enzyme located in the tissues that oxidizes aldehydes into carboxylic acid and requires Moco in animals (Coughlan, 2014). Sulfate oxidase in animals is located in the mitochondria and oxidizes sulfite to sulfate and generates ATP in the process by transferring electrons and utilizes Moco (Kisker, 1997). Xanthine oxidase catalyzes the oxidation of hypoxanthine to xanthine, which is ultimately converted to uric acid (Rashidi and Pashaei-Asl, 2012).

Copper: Copper is apart of multiple enzymes, with Zn being the only mineral incorporated into more enzymes than Cu. The two main enzymatic functions that are highly dependent on Cu are pigmentation and connective tissue development (Suttle, 2012). A common sign of Cu deficiency is when cattle start to experience hair de-pigmentation (Suttle, 2012). This is controlled by the Cu dependent enzyme tyrosinase. Tyrosinase produces melanocytes that make the pigment melanin (Riley, 1997). Lysyl oxidase in involved in the cross linking of connective tissues (i.e. collagen and elastin; Smith-Mungo and Kagan, 1998). The enzyme is only active with its Cu cofactor tightly bound to the enzyme (Smith-Mungo and Kagan, 1998). Copper deficiency can result in a decrease in connective tissue cross-linkage, however, it has been reported that this enzyme can in fact function at a Cu deficient state but not at full potential (Smith-Mungo and Kagan, 1998). Copper also plays in important role in cellular respiration

through cytochrome C oxidase (Berg et al., 2002). This enzyme is the located in the final section of the electron transport chain (Berg et al., 2002). Lastly, Cu along with Zn functions as a structural component of SOD that catalyzes the conversion of the superoxide radical O^{-}_{2} into oxygen and hydrogen peroxide (Trainer, et al., 1983; Suttle, 2012).

Sulfur: Sulfur has many functions within the body and plays an even bigger role in cattle. On a cellular level, S is a part of disulfide bonds, which provide structural configuration of polypeptide chains and the location for attachment of substrates to active sites within enzymes (Suttle, 2012). As previously mentioned, S is a part of the vitamin thiamin as well as hormones such as insulin (insulin interaction with tissues is through S bonds; Fong, et al., 1961) and oxytocin. Sulfur in the form of sulfate is a part of chondroitin in connective tissue, cartilage, hair, wool, feathers, hoofs and horns (Suttle, 2012). Dietary S is also used in combination with Chloride (Cl) to balance cation–anion loads in dairy cattle diets to assist with Ca metabolism (Tucker et al., 1991).

Sulfur, Copper, and Molybdenum Interactions

Mineral Relationship: The rumen is the site for the dynamic interaction between S, Cu and Mo. Copper availability can be greatly affected dietary S and/or Mo concentrations (Underwood, 1999). Miltemore and Mason (1970) found that (2:1) Cu:Mo ratios were deemed as sufficient, when Cu dropped below this concentration, symptoms of Cu deficiency were seen. Understanding the symbiotic relationship of these two minerals has proven to be helpful in the treatment of Mo toxicity. Underwood (1999) concluded that scours in cattle grazing on legume pastures high in Mo content, was alleviated by supplementing elevated doses of Cu. Sulfur alone can affect the availability of Cu as well. As rumen protozoa digest S-amino acids in the rumen, free sulfide from this breakdown is prone to react with Cu, creating Copper-sulfide (Cu_2S; Ivan

1989). The rumen microbes do not as efficiently use copper-sulfide. Furthermore, S can decrease the absorption of Mo, and Mo can decrease the amount of S available ATP-sulfurase activity (Grace and Suttle, 1979). In the absence of Cu in the rumen two main reactions can occur. In the scenario of pre-thiomolybdate formation, S can act on Mo inhibiting absorption. There are two possible scenarios: the first is through the idea that both minerals compete for the same absorptive pathway. Therefore when S is in high concentrations, Mo will not be absorbed. The second is through renal tubular re-absorption where a higher mount of Mo is excreted in the urine instead of re-entering the bloodstream from the kidneys (Suttle, 1974).

Thiomolybdate Formation: Together S and Mo create an antagonistic effect on Cu through the formation of thyomolybdates in the rumen. This interaction has been well studied. However, this three-way interaction can be influenced by dietary energy, protein and other mineral concentrations. The process of thiomolybdate formation is as follows: Organic or inorganic S is reduced to sulfide by rumen microorganisms. Molybdenum starts in the form of MoO_4 and sulfide as H_2S (Gould and Kendall, 2011). Molybdenum is then dehydrolized of its hydrogen, which is released as a water molecule, and the hydrogen is then replaced with sulfide (See Figure 2).

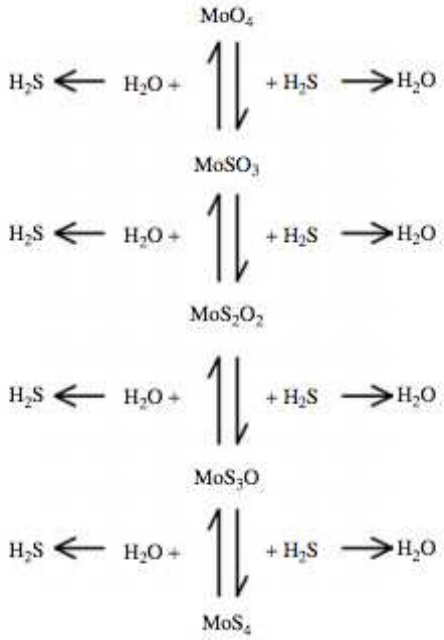

Figure 2. The chemical formation of thiomolybdates in the rumen (Gould and Kendall, 2011).

This process is repeated four times to form MoO_4 (Gould and Kendall, 2011). The ratio of S:Mo available in the rumen at the time of thiomolybdate formation is indicative of the chemical stability of the thiomolybdate. In studies conducted by Clark and Laurie (1980) they reported that monomolybdates form at a ratio of 3:1, dimolybdates form at 5-10:1, trithiomolybdates form at >10:1 and tetramolybdates which attract the highest amount of Cu occur at 300:1 in the rumen. The accumulation of tetramolybdates in the rumen depends on the availability of sulfide at the time. The half-life of sulfides in the rumen is approximately 10 to 22 minutes, therefore the occurrence of tetramolybdates forming are not as common (D R´emond, 1996). Certain thiomolybdates have different actions of binding Cu (Gould and Kendall, 2011). Tetramolybdates have a high affinity for Cu and will be the first to react with Cu rendering the Cu unavailable for absorption (Gould and Kendall, 2011). This allows di and trithiomolybdates to be more available for absorption (Gould and Kendall, 2011). Thiomolybdates are absorbed

mainly in the small intestine once leaving the rumen. Once absorbed thiomolybdates that have already attached to Cu from the rumen or have bound to circulating Cu in the plasma will bind to albumin with a higher affinity for the binding site than Cu alone (Mason, 1986). The Cu containing thiomolybdates do not attach to albumin on the specific Cu binding site but on a separate histidine-binding molecule (Mason, 1986). In this situation non-ceruloplasmin Cu tends to increase, with this it can be postulated that Cu bound to albumin by thiomolybdates stays bound due to its high binding affinity to albumin and therefore uptake by the liver is inhibited preventing the synthesis of ceruloplasmin-Cu (Mason, 1986). The decrease in ceruloplasmin results in decreased activity of the Cu-dependent enzymes cytochrome-c oxidase and SOD (Mason, 1986). Mason reported that a decrease in diamine oxidase activity is closely related to thiomolybdates being present, and once metabolized, activity increases back to normal function (Mason, 1986). Mason also reported that when 25-50 mg of Mo was given to 400-450 kg steers, Cu stores in the cytosol declined and metallothionine Cu declined but at 40 h post infusion, metalothionine increased significantly. Mason hypothesized that this is due to increased albumin bound Cu and increased excretion of Cu bound thiomolybdates that caused the overall depletion of Cu reserved in the animal (Mason, 1986).

Evaluating Molybdenosis Through Feed and Water Ingestion: Molybdenum toxicity (Molybdenosis) can be of concern due to the formation of thiomolybdates, resulting in possible depleted Cu stores. A physiological Cu deficiency in the animal from molybdenosis can lead to a multitude of issues affecting growth and overall animal health. 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 closely studied. Underwood (1999) reported that cattle grazed on pastures containing between 20 and 100 ppm on dry matter basis experience mild to extreme

forms of scouring. Ward, (1978) reported that cattle with Mo intake generally over about 100 mg Mo/kg DM or Cu:Mo ratio of 2:1 or less can experience Cu deficiencies. A Cu:Mo ratio of 6:1 is recommended (Erdman et al., 1978). Kubota (1975) reported that cattle grazing on forages with 10-20 mg Mo/kg DM exhibited Mo toxicity symptoms. Limited controlled research has been conducted investigating the impact of Mo water concentrations on beef cattle performance. 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). The basal diet contained 0.29% sulfur (50% above normal), 13 mg of Cu/kg diet dry matter (DM), and less than 1 mg of Mo/kg diet dry mater (DM). There was no difference in body weight gain (0.71 kg/d) or water intake (5.1 L/d) across all water treatments. At the greatest Mo water concentrations (50.0 mg Mo/L), Kincaid (1980) reported an increase ($P < 0.05$) 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 Cu and ceruloplasmin concentrations and liver Cu concentrations. Kincaid (1980) indicated that the safe ratio of Cu to Mo in this experiment was 0.5:1.0. Kincaid (1980) also postulated that Mo in water could be less toxic than that in forage, and that the minimum toxic level of Mo in water for calves is between 10 and 50 mg of Mo/L (10,000 and 50,000 µg of Mo/L).

The lowest Mo dose (1.0 mg Mo/L) used by Kincaid (1980) was greater than the current Mo agricultural water standard of 160 µg/L (Environmental Protection Agency, 2012). However, the Kincaid (1980) experiment had a small sample size and short exposure period. Therefore, it is difficult to determine the impact of prolonged exposure to elevated Mo water concentrations. Based on the above information, our working hypothesis was that longer

exposure to lower doses of Mo in the water at greater cattle growth rates and water intake would reduce Cu status of the animal. Therefore, the objective of this experiment was to examine the effects of long term exposure of Mo in drinking water consumed by rapidly growing finishing cattle at relevant test concentrations of 0.0, 160, 320, 480, and 960 µg of Mo/L, which bracket the current Mo agricultural water standard standards.

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Chapter II: The Effects of Molybdenum Water Concentration on Feedlot Performance, Tissue Mineral Concentrations, and Carcass Quality of Feedlot Steers.

Introduction

Molybdenum (Mo) functions as a component of several oxidase enzymes (xanthine oxidase, sulfite oxidase, and aldehyde oxidase; Coughlan, 2014). But dietary requirements for Mo are not well defined for beef cattle (NRC, 2016). High concentrations of dietary Mo and S have been reported to induce Cu deficiency in beef cattle through the formation of dietary thiomolybdates in the rumen (Underwood and Suttle, 1999), which is arguably the most heavily studied mineral interaction in beef cattle. However, even Mo alone can have antagonistic effects on Cu absorption. Ward (1978) investigated the independent effect of molybdenum on Cu absorption and concluded that elevated Mo intake reduces Cu availability and can lead to a physiological Cu deficiency. This was attributed to a Cu-Mo complex which forms in the rumen and cannot be broken down and absorbed. Based on this and previous experiments, it appears that the ratio of the antagonistic elements seems to be more important than the actual amounts. Miltimore and Mason (1971) reported that if copper: molybdenum ratios fall below 2:1, copper deficiency can be produced. Therefore, feeding additional copper has been recommended in areas where a molybdenum interaction is suspected.

In 2010, the Colorado Water Quality Control Commission adopted a molybdenum standard of 300 ug of Mo/L in Regulation No. 31, (5CCR 1002-31) to protect agriculture uses in Colorado. The EPA disapproved this standard in 2011 because it included an assumption that livestock producers supplement their cattle's diet with copper, which offsets the toxic effects of molybdenum. The Commission subsequently adopted a value of 160 ug/L (derived without

assumed copper supplementation) in Regulation Nos. 32 – 37 (5CCR 1002 32-37) to address EPA's concerns. The approach for calculating the standard for cattle was changed in the 2015 Regulation No. 38 hearing, when a value of 150 ug/L was derived based on dietary and water intake rates for various life stages of cattle. (5CCR 1002-38).

Limited controlled research has been conducted investigating the impact of Mo water concentrations on beef cattle performance. 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). The basal diet contained 0.29% sulfur (50% above normal), 13 mg of Cu/kg diet dry matter (DM), and less than 1 mg of Mo/kg diet dry mater (DM). There was no difference in body weight gain (0.71 kg/d) or water intake (5.1 L/d) across all water treatments. At the greatest Mo water concentrations (50.0 mg Mo/L), Kincaid (1980) reported an increase ($P < 0.05$) 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 Cu and ceruloplasmin concentrations and liver Cu concentrations. Kincaid (1980) indicated that the safe ratio of Cu to Mo in this experiment was 0.5:1.0. Kincaid (1980) also postulated that Mo in water could be less toxic than that in forage, and that the minimum toxic level of Mo in water for calves is between 10 and 50 mg of Mo/L (10,000 and 50,000 µg of Mo/L).

The lowest Mo dose (1.0 mg Mo/L) used by Kincaid (1980) was greater than the current Colorado Mo agricultural water standard of 160 µg/L (Environmental Protection Agency, 2012) However, the Kincaid (1980) experiment had a small sample size and short exposure period. Therefore, it is difficult to determine the impact of prolonged exposure to elevated Mo water

concentrations. Based on the above information, our working hypothesis was that longer exposure to lower doses of Mo in the water at greater cattle growth rates and water intake would reduce Cu status of the animal. Therefore, the objective of this experiment was to examine the effects of long term exposure of Mo in drinking water consumed by rapidly growing finishing cattle at relevant test concentrations of 0.0, 160, 320, 480, and 960 µg of Mo/L, which bracket the current Mo agricultural water standard standards and Cu:Mo ratios.

Materials and Methods

Prior to the initiation of this experiment all animal care, handling, and procedures described herein were approved by the Colorado State University Animal Care and Use Committee.

Cattle: Seventy commercial cross-bred steers with an initial body weight of 457±17.1 kg were utilized in this experiment. Steers were housed at the Agricultural, Research, Development, and Education Center (ARDEC) in Fort Collins, CO. Prior to initiating the experiment, all cattle were processed. Processing procedures included obtaining an individual BW, assigning a breed type code, and the application of an electronic identification tag. Each steer was vaccinated for viral (Bovi-Shield Gold®, IBR-BVD, Zoetis Animal Health) and clostridial (Ultra Choice 7, Bacterin-Toxoid, Zoetis Animal Health) diseases and treated for parasites (Noromectin®, Injectable Ivermectin, Norbrook Laboratories Limited and Safe-Guard®, Fenbendazole, Merck Animal Health). Steers were implanted with Revalor® XS (Merck Animal Health) administered in their right ear on the day of processing.

The next morning steers were weighed and then ranked by BW. Individuals that were beyond ± 2 SD from the mean BW were eliminated from further consideration for the experiment. Steers exhibiting excessive Brahman, Longhorn, or dairy breed type or if they were

found to be bulls, heifers, or displaying symptoms of health problems were eliminated from consideration. The remaining steers were assigned a random number from 1 to 1000 using the random number function in Excel 2007® (Microsoft Corporation, Redmond, WA). Steers with the lowest random numbers were eliminated from the experiment reducing the number of remaining steers to 30. The 30 eligible steers were ranked by BW and divided into 2 weight block replicates, each one consisting of 15 steers. Each successive weight block replicate was labeled as replicates 1 and 2 with the heaviest group of 15 steers considered as replicate 1 and the lightest group of 15 steers considered as replicate 2. Replicate 2 steers were group housed in one pen and fed a corn silage-based growing diet growing diet for 160 d until replicate 1 steers were slaughtered.

Steers in replicate 1 were ranked by initial BW and stratified by BW and breed to one of 5 water treatments so that BW and breed were equally represented within each treatment. Steers were then sorted into their respective individual pens and the experiment initiated. Water treatments consisted of: 1) 0.0 µg, 2) 160 µg, 3) 320 µg 4) 480 µg Mo/L, and 5) 960 µg of supplemental Mo/L added as Na_2MoO_4 (Acros Organics, Geel, Belgium; Purity:99%) to the water. The sodium contribution from Na_2MoO_4 was balanced across water treatments by using NaCl. Each pen was 2.5m x 20.0m and equipped with an individual water tank, individual feed bunk, and a 3.0 m concrete bunk apron. The feed bunks, water tanks, and concrete aprons were covered by a metal roof to supply shade and protection from inclement weather. Initial BW used for the experiment was the average of the 2 weights obtained on d -1 and 0. The same randomization procedures were used for replicate 2 steers after replicate 1 steers were slaughtered.

Diets and Animal Care: Steers were fed a corn silage-based growing diet for 28 d and then transitioned to a corn based finishing diet (Table 1). Diet changes during the step-up program were simultaneous for all treatments. Steers reached the corn based finishing diet by d 47 of the experiment.

Rations were formulated to meet or exceed National Research Council (2000) requirements for growing-finishing beef cattle. The basal growing, step-up, and finishing diets contained 5.4, 4.1, and 3.7 mg Cu/kg DM, respectively. Supplemental Cu as $CuSO_4 \cdot 5H_20$ was added to the supplement to supply a total mixed ration that contained NRC (2000) recommended concentrations of Cu (10.0 mg Cu/kg DM) and S (0.15 %; Table 1). Monensin feeding was initiated on d 1 and Tylan® was introduced into the step-1 diet. Monensin was fed at 28.0, 36.5 and 44.4 g/metric ton, on a dry matter (DM) basis in the growing, step 1, and corn based finisher diets, respectively. Tylan was fed at 90 $mg \cdot head^{-1} \cdot day^{-1}$ beginning with the step 1 diet. Optaflexx was fed to all treatments the final 28 d of the finishing period at 27.3 g/metric ton (DM basis), providing approximately 300 $mg \cdot head^{-1} \cdot day^{-1}$.

Cattle feed bunks were observed each morning to determine the daily total feed delivery. Cattle were fed in amounts that allowed *ad libitum* access to feed throughout the day. Feed was delivered to pens once daily. Feed amounts delivered to each pen were recorded manually by feedlot personnel. Diet samples were collected weekly for DM and nutrient content determination. A weekly DM determination of feed was conducted by drying duplicate 100 g samples for 48 h using a 60ºC forced air drying oven. Weekly samples were composited by month and analyzed for DM, crude protein (CP), neutral detergent fiber (NDF), and nutrient elements Ca, P, K, S, Mg, Cu, Mo, Zn, Fe, Co, Mn and ether extract (crude fat).

Pens were checked daily by trained feedlot animal care personnel to monitor cattle for health and locomotion problems and to ensure proper functioning and cleanliness of the water tanks, structural integrity of fences, and cleanliness of the feed bunks. Steers exhibiting symptoms of health problems were assigned scores of 0 or 1 for each of the following symptoms: eye discharge, nasal discharge, diarrhea, reduced feed intake, coughing, rapid breathing, and depressed appearance. Rectal body temperatures were recorded for suspect steers that were removed from their pen. Two additional points were assigned to steers exhibiting body temperatures greater than 39.7°C. Steers with a total of 4 or more points were considered moribund. All moribund steers were treated according to the appropriate treatment protocol, immediately returned to the pen, and allowed a chance to recover. If problems persisted concerning the health status of a specific steer, the steer was examined by the attending veterinarian and was removed from the experiment if recovery in a timely fashion was unlikely.

Weighing, Sampling, and Carcass Data Collection: Steers were individually weighed on 2 consecutive days at the beginning and end of the experiment and interim BW were obtained every 28 d. Blood samples were collected from all steers, via jugular venipuncture, into heparinized, trace-mineral-free Vacutainer tubes (Becton Dickinson Co., Franklin Lakes, NJ) at the beginning, end, and every 28 d throughout the experiment. Liver biopsies were collected on d 0 and 84 of the experiment and a liver sample was obtained at the time of slaughter. Liver biopsies were obtained using the true-cut technique described by Pearson and Craig (1980), as modified by Engle and Spears (2000). Immediately post-collection, liver samples were rinsed with 0.01 *M* phosphate buffered saline (pH 7.4), placed into acid-washed polypropylene tubes, capped, placed on ice for approximately 1 h, transported to the laboratory, and stored at –20°C until analyzed.

Steers were slaughtered on d 151, and 112, for replicates 1 and 2, respectively. On the day of slaughter, steers were transported to a commercial abattoir, randomly presented for slaughter using standard U.S. beef industry practices and USDA/Food safety inspection service criteria 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. Carcass data were collected by Center for Meat Safety and Quality personnel at Colorado State University and included dressing percentage, longissimus muscle area (LMA), subcutaneous adipose tissue thickness, kidney, pelvic, and heart fat (KPH), marbling score, quality grade, and yield grade.

Liver samples were collected (approximately 200 g wet weight) on the day of slaughter from the left lobe of each liver after being inspected by USDA personnel. Following collection, liver samples were placed in Whirl Pak bags containing the slaughter order number, placed on ice, and transported to the laboratory. Samples were then stored at –20°C until analyzed.

Water Delivery and Monitoring: Each animal had access to an individual 265 L Rubbermaid® structural foam stock tank (102.9 cm x 61cm x 81cm, length, height, and width, respectively). Water intake was monitored daily at 0800h ± 30 min by measuring the disappearance of water over a 24 h period. Since the tank was not symmetrical, water volume for every 0.25cm on a plastic meter stick was correlated with the amount of water remaining in the tank. This calibration was accomplished by metering (TM Series Water Meter, Great Plains Industries, Inc. Wichita, KS; Accuracy ± 3.0%) 0.25 cm of water into each tank, recording the liters of water metered and then weighing the amount of water as a secondary validation of water volume. Water tank calibrations were conducted approximately every two months.

To account for evaporation, a separate water tank was placed in front of an empty pen and measured daily. Daily water disappearance was determined using the following equation: WD = [V1 – (V2 + evap.)] where: WD = water disappearance (assumed to be water intake), L/d; V1 = the previous day's water volume; V2 = the current day's volume; and evap. = the amount of water disappearance due to evaporation. As an internal check, a line was placed around the inside of all tanks that corresponded to the tank containing 265 liters of water. Water tanks were refilled every 3-4 d or when the tank was half full. During the refilling process, the water meter was attached to the end of the hose and the amount of water required to fill the tank up to the 265 l line was recorded. This was reconciled with the daily water measurement readings over the time between tank re-filling to ensure accurate water disappearance measurements. Prior to the experiment, the highest dose of Mo (960 µg/L) was thoroughly mixed and allowed to stand for 7 d without cattle access. Initial (d 0) and final (d 7) Mo concentrations were 968 ± SD 1.8 and 970 ± SD 1.2, respectively indicating that Na_2MoO_4 remained in solution over a 7-d period.

Sodium molybdate dehydrate (Na_2MoO_4) was added to each tank in concentrations appropriate for each treatment. A stock solution of 40,000 mg of Mo/L was made to generate the correct mg/L of Mo for each of the treatments through appropriate dilutions. When water tanks were refilled, the amount of water that disappeared from each tank was calculated and the appropriate amount of the Mo stock solution (adjusted for the increase of Mo concentration due to tank evaporative losses) was added with a calibrated Eppendorf® adjustable volume pipette (100-1000 µl). The water in the tank was then thoroughly mixed with a paddle mixer attached to a high speed cordless drill and sampled. Water samples were obtained from every tank at the time of refilling and analyzed for Mo concentrations. General water quality was analyzed three times throughout the experiment. At the time of basal water sampling, all water tanks were

emptied (prior to feed delivery), washed, refilled, reconstituted with the appropriate Mo treatments, and then sampled to confirm the actual Mo concentration.

Analytical Procedures: Feed, water, plasma, and liver samples were sent to an established laboratory (SDK Labs, Hutchinson, KS) for routine nutrient, mineral, and water quality analysis. Water samples were also sent to an established laboratory (Accutest Labs, Wheat Ridge, CO) for water Mo concentration analysis using inductively coupled plasma mass spectrometry (ACP-MS) where prepared samples are introduced into a radio frequency plasma by nebulization. Energy transfer processes cause dissolvation, atomization, and ionization. The ions are extracted from the plasma through a differentially pumped vacuum interface and separated on the basis of their mass to charge ratio by a quadrupole mass spectrometer. The ions transmitted through the quadrupole are detected by an electron multiplier, and the ion information is processed by a data handling system. (SGS Accutest, 2016).

Statistics: Feedlot performance, water intake, plasma and liver trace mineral concentrations, and continuous carcass data were analyzed on an individual animal basis as a randomized block design using a restricted maximum likelihood-based, mixed effects model repeated measures analysis (PROC MIXED, SAS Inst. Inc., Cary, NC) where appropriate. Treatment was included in the model as a fixed effect and replicate was included in the model as a random effect. Significance was determined at $P \leq 0.05$. In the event of a significant F test, treatment means were separated using the PDIFF option of the LSMEANS statement of SAS. Steers initial BW was used as a covariant in the analysis of all response variables.

Results and Discussion

One steer in the control group died from hemorrhagic bowel syndrome, which is a

disorder caused by hemorrhaging and obstruction of the small intestine, 14 d prior to slaughter. No other animal health issues were observed during this experiment. The average Mo water concentrations and basal water quality of all water treatments are shown in Figure 1 and Table 2, respectively. Overall mean Mo concentrations for each water treatment were 4.1, 184.6, 344.4, 512.3, and 1026.8 μg Mo/L for 0, 160, 320, 480, and 960, μg Mo/L treatments. Mean water quality measurements (Table 2) for all treatments indicated water was suitable for livestock consumption based on previous publications (NRC, 2001; NRC 2005; Wright, 2007; and National Academies of Sciences, Engineering, and Medicine, 2016).

The effects of water Mo concentration on performance and water intake of feedlot cattle are shown in Table 3. There were no replicate x treatment or treatment x time interactions for any of the response variables measured. Therefore, overall main effects are presented. Initial and final BW, DMI, ADG, and feed efficiency were similar across treatments. Previously published literature investigating the influence of water Mo concentration on cattle performance is limited. 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) from ammonium molybdate. The barley-based basal diet contained 0.29% sulfur, 13 mg of Cu/kg diet dry matter (DM), and less than 1 mg of Mo/kg diet dry mater (DM). Feed intake, body weight gain and water intake were similar across all water treatments. At the greatest Mo water concentration, Kincaid (1980) reported an increase ($P < 0.05$) 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 Cu and ceruloplasmin concentrations and liver Cu concentrations. Kincaid (1980) indicated that the safe ratio of Cu to Mo in this

experiment was 0.5:1.0. Kincaid (1980) also postulated that Mo in water could be less toxic than that in forage, and that the minimum toxic level of Mo in water for calves is between 10 and 50 mg of Mo/L.

The calves in the Kincaid (1980) experiment consumed < 1.0, 4.8, 50.0 and 270.0 mg of Mo/d (from diet and water combined) for treatments 0.0, 1.0, 10.0, and 50.0 mg of Mo/L, respectively (Cu:Mo ratios of: > 27:1, 4.6:1, 0.5:1, and 0.08:1, respectively). In the current experiment Mo intakes (total diet and water combined) were 7.2, 14.3, 19.3, 25.1, and 42.0 mg of Mo/d for treatments 0.0, 160, 320, 480, and 960, µg Mo/L, respectively (Cu:Mo ratios of: 15.9:1, 8.7:1, 5.9:1, 4.6:1, and 2.8:1, respectively). The greatest Mo intake and smallest Cu:Mo ratio in the current experiment were 42.0 mg of Mo/d and 2.8:1, respectively, for steers consuming the 960 µg Mo/L water treatment (measured at 1026.8 µg/L or 1.027 mg/L) which falls between the Mo water concentrations of 1.0 and 10.0 mg Mo/L reported by Kincaid (1980).

Numerous experiments have been conducted supplementing dietary Mo to beef cattle diets to induce a Cu deficiency. However, few experiments have been conducted where the effects of Mo can be separated from the effects of a Cu deficiency. Calves born to dams receiving either 5.0 mg Mo/kg DM or 600 mg Fe/kg DM supplementation during the last third of gestation had a similar reduction in plasma Cu concentrations prior to weaning (both groups were considered to be Cu deficient; plasma Cu concentrations < 0.6 mg of Cu/L; Mills, 1987). However, body weight gain was lower in calves receiving supplemental Mo compared to Fe supplemented calves. Humphries, et al. (1983) and Phillippo et al. (1987a) have also reported a decrease in weight gain in young growing, Cu-deficient calves where Cu deficiency was induced by Mo supplementation (5 mg Mo/kg DM) but not in calves where a similar Cu deficiency was induced with Fe supplementation. The reduction in weight gain was a function of reduced feed

intake and feed efficiency. Furthermore, Phillippo et al. (1987b) observed a lower ($P < 0.05$) peak LH amplitude in young (90 to 130 d old) Cu-deficient Hereford × Holstein heifers supplemented with 5 mg Mo/kg diet (6.55 ng LH/ml) vs. Cu-deficient heifers receiving either 500 mg Fe/kg diet (14.82 ng LH/ml) or Cu-adequate heifers receiving no supplemental trace minerals (18.20 ng LH/ml). These data indicate that the influence of dietary Mo on body weight gain and reproductive performance may be independent from Cu status.

The cattle used in the aforementioned experiments were relatively young. Experiments using older cattle have reported no impacts of high dietary Mo supplementation (5.0-10.0 mg Mo/kg DM) on body weight gain or reproductive hormone profile (Wittenberg and Boila, 1988; Xin et al., Ahola, et al., 2005). Dietary concentrations of Mo in excess of 100 mg Mo/kg DM caused clinical signs of Mo toxicity in growing heifers (Lesperance and Vohman, 1963) and yearling steers (Cook et al., 1966).

The influence of water Mo concentration on carcass characteristic is shown in Table 4. Hot carcass weight, dressing percentage, yield grade and marbling score were similar across treatments. These data are in agreement with Ward and Spears (1997) where supplementing 5 mg of Mo/kg DM to growing (Mo intake ≈ 37.5 mg/d) and finishing (Mo intake ≈ 47.5 mg/d) steer diets not supplemented with Cu (basal diets contained 6.9 mg of Cu/kg DM; Cu:Mo ≈ 1.38:1) had no impact on carcass quality.

Liver and plasma Zn, Cu, and Mo concentrations are presented in Table 5. There were no replicate x treatment or treatment x time interactions for any of the response variables measured. Therefore, overall main effects are presented. Liver and plasma Zn, Cu, and Mo concentrations were similar across treatments and were within adequate ranges for beef cattle (Mills, 1987; Puls, 1994). Our working hypothesis was that Cu status (liver and plasma), of rapidly growing

cattle with long term exposure to water containing different doses of Mo, would be reduced in a dose dependent manner. However, Cu status was not altered in this experiment. Numerous experiments have been conducted utilizing elevated dietary Mo concentrations (5.0-10.0 mg Mo/kg DM yielding 35-100 mg Mo/d intake) alone or in combination with elevated dietary S (0.3% or greater) to induce a Cu deficiency in ruminants (Suttle and Field, 1968; Suttle, 1974a; Suttle, 1974b Wittenberg and Bolia, 1988; Genglebach, et al., 1994; Ward et al., 1997; Suttle, 1991; Ahola, et al., 2005). The discrepancy between the current experiment and previously published experiments may be due the method of Mo delivery and/or diet type used in the current experiment.

In the current experiment, Mo was delivered in the water whereas Mo was included in the diet in the majority of previously published experiments. Water consumption contributed approximately 2.6, 83.3, 157.5, 250.8, and 456.2% of the total Mo consumed for cattle receiving water treatments containing 0, 160, 320, 480 and 960 µg Mo/L, respectively. Several researchers, using various methods to estimate ruminal bypass of consumed water have reported that between 18 and 80% of the 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, Graza et al., 1990). Furthermore, using two different markers (polyethylene glycol and chromium-EDTA) to estimate drinking water ruminal bypass, Garza et al. (1990) reported that drinking water bypassing the rumen was greater for cattle consuming a high concentrate diet compared to cattle consuming a prairie hay based diet (polyethylene glycol marker: 79 vs 49% water bypass for concentrate vs. forage based diets, respectively; chromium-EDTA marker: 66 vs 51% water bypass for concentrate vs. forage based diets, respectively). Although not measured in the

current experiment, if the majority of drinking water bypassed the rumen, the majority of the Mo in the drinking water would not be able to interact with Cu and/or S in the rumen to reduce the availability of Cu to the animal. This could explain why Cu status was not altered in this experiment.

Diet type has also been shown to influence the impact of Mo on Cu status in ruminants. The diet used in the current experiment was a high concentrate grain-based diet that contained 0.15% S and was supplemented with Cu in amounts to give a total dietary Cu concentration of 9.8 mg Cu/kg DM. Based on the Nutrient Requirements of Beef Cattle, Eighth Revised Edition (National Academies of Sciences, Engineering, and Medicine, 2016) recommendations, both S and Cu were adequate for finishing steers. Therefore, thiomolybdate formation was not expected to influence Cu status. However, Mo can reduce Cu availability independent of thiomolybdate formation by forming a Cu-Mo complex in the rumen, which cannot be digested and absorbed (Ward, 1978, Suttle, 1991). We still expected to observe a reduction in Cu status as Mo intake from drinking water increased. The reason for the lack of reduction in Cu status may also be due to diet composition. Diets that are high in digestibility and fermentable carbohydrates have been reported to improve the availability of Cu when dietary Mo concentrations are elevated (COSAC, 1982; Wang et al., 1988; Suttle, 1991). The improvement in Cu availability with a high concentrate diet may be due to having a: 1) lower indigestible fiber content therefore reducing the negative impact of fiber on Cu absorption; 2) lower ruminal pH which could increase Cu solubility, and 3) more rapid removal of sulfide into the blood stream that may prevent thiomolybdate formation (Suttle, 1991). Furthermore, ionophores have been reported to improve plasma Cu and rumen soluble Cu concentrations in steers (Starnes et al., 1984, Spears and Harvey, 1985, Stabel, et al., 1989). The improvement in Cu availability in

steers fed ionophores is possibly due to a reduction in protozoal numbers in the rumen which ultimately decrease sulfide production and thiomolybdate formation (Spears, 1990).

Results of this experiment indicate that Mo addition to drinking water up to 960 µg Mo/L for cattle had no impact on performance, mineral status, water intake, and carcass characteristics in rapidly growing feedlot steers fed a high concentrate diet. However, factors that influence ruminal bypass of drinking water and diet type (forage vs concentrate) need further investigation.

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Appendix A: Tables and Figures

Table 1. Dry matter ingredient composition diets[a]

Ingredient	Grower	Step-1	Finish
Cracked Corn	25.0	39.0	64.0
Whole Corn	10.1	----	---
Corn silage	30.2	54.0	30.0
Alfalfa Hay	27.8	----	---
Supplement[b]	6.9	7.0	6.0
Chemical Analysis			
Dry Matter, %	61.0	53.9	62.3
CP, %	14.1	12.2	12.7
ADF, %	18.1	12.8	7.3
NDF, %	28.6	24.8	14.4
NEg, Mcal/kg	1.11	1.30	1.40
NEm, Mcal/kg	1.72	1.93	2.01
Ca, %	0.84	0.61	0.62
P, %	0.31	0.30	0.29
Mg, %	0.20	0.15	0.14
S, %	0.17	0.16	0.15
Zn, mg/kg	48.3	38.7	39.1
Cu, mg/kg	11.1	9.7	9.8
Mn, mg/kg	36.8	26.9	23.3
Mo, mg/kg	1.1	0.50	0.60
Co, mg/kg	0.25	0.18	0.18

[a]Monensin feeding initiated on day 1 and Tylan® was introduced into the step-1 diet. Monensin was fed at 28.0, 36.5 and 44.4 g/metric ton, on a dry matter (DM) basis in the growing, step 1, and finisher diets, respectively. Tylan was fed at 90 mg·head^{-1}·day^{1} beginning with step 1. Optaflexx was fed to all treatments the final 28 d of the finishing period at 27.3 g/metric ton DM basis, 300 mg·head^{-1}·day^{1}.

[b]Combination of a protein supplement containing Monensin and Tylan and a vitamin/mineral premix.

Table 2. Water quality and mineral concentrations of experimental water treatments (mean ±SD).

	Treatment, µg Mo/L of Drinking Water				
Item	0	160	320	480	960
pH	7.37±0.19	7.25±0.10	7.31±0.14	7.40±0.27	7.47±0.30
Chloride mg/ L	30.0±4.1	30.0±3.5	26.3±3.4	26.7±4.2	28.3±3.0
Total hardness, mg/L	750.3±63.1	769.5±48.8	732.3±50.0	741.8±58.1	736.8±64.2
Calcium, mg/L	196.3±14.7	200.7±11.3	191.8±11.7	193.3±14.4	193.0±16.0
Magnesium, mg/L	63.1±6.4	65.2±5.2	61.6±5.2	62.9±6.0	61.9±5.9
Sodium, mg/L	70.3±10.1	73.0±8.6	68.9±9.2	71.7±13.3	72.7±16.6
Sulfate, mg/L	482.7±35.8	485.5±42.2	474.0±58.0	470.2±28.4	483.7±39.2
Iron, mg/L	0.06±0.03	0.07±0.02	0.07±0.05	0.07±0.04	0.06±0.05
Manganese, mg/L	0.05±0.03	0.06±0.03	0.05±0.03	0.05±0.03	0.05±0.02
Electrical conductivity, umhos/cm	1543.3±103.3	1556.7±76.1	1511.7±76.8	1525.0±99.9	1503.3±98.5
Total dissolved solids, mg/L	1094.3±73.1	1104.0±53.7	1072.2±54.6	1081.5±70.8	1066±69.8

Table 3. Effect of water molybdenum concentration on performance and water intake of feedlot cattle[ab].

Item	Treatment, µg Mo/L of Drinking Water					SEM	*P* <		
	0	160	320	480	960		Dose	Time	Dose x Time
Body Wt., kg									
Initial[b]	462.0	444.0	456.0	445.1	452.6	12.1	0.99	---	---
Final[b]	710.3	711.2	706.0	701.9	712.2	16.8	0.98	---	---
Dry matter intake, kg/d									
d0- Final	11.7	12.6	11.7	11.8	12.2	0.62	0.92	0.001	0.50
Average daily gain, kg/d									
d0-Final	1.93	2.0	1.93	1.94	1.96	0.11	0.91	0.001	0.29
Gain: Feed									
d0-Final	0.174	0.165	0.169	0.169	0.167	0.006	0.94	0.001	0.25
Avg. Daily Water Intake L/d	30.66	35.2	34.1	31.5	33.2	5.3	0.40	0.0001	0.99

[a]Initial BW was used as a covariate for all statistical analysis.
[b]No Time (.0001) significance or Dose x Time (0.23) interaction was observed, therefore initial and final values shown.

Table 4. Effect of supplemental molybdenum concentration and source on carcass characteristics of feedlot cattle.

	Treatment, µg Mo/L Drinking Water						*P* <
Item	0	160	320	480	960	SEM	Dose
Hot carcass weight, kg	433.2	434.2	422.7	428.2	430.7	15.5	0.98
Dressing percentage[a], %	63.2	63.7	62.7	63.0	63.0	0.70	0.98
Yield Grade	3.55	3.52	3.28	3.41	3.17	0.34	0.91
Marbling Score[b]	546.9	585.7	537.4	518.5	496.9	55.4	0.29

[a]A 4% pencil shrink was applied to all final live weights used to calculate dressing percentage.
[b]Marbling score; 300 = Slight0, 400 = Small0, 500 = Modest0.

Table 5. Effect supplemental molybdenum concentration and source on mineral status of feedlot cattle.

Item	Treatment, µg Mo/L Drinking Water						*P* <		
	0	160	320	480	960	SEM	Dose	Time	Dose x Time
Liver									
Zn, mg/kg DM	70.9	82.5	77.3	87	73.6	8.5	0.68	0.94	0.54
Cu, mg/kg DM	115.5	139.5	119.9	132.2	132.4	30	0.93	0.001	0.51
Mo, mg/kg DM	2.3	2.2	2.3	2.1	2.2	0.17	0.90	0.002	0.93
Plasma, d 112									
Zn, mg/L	1.2	1.4	1.4	1.4	1.3	0.1	0.62	0.60	0.19
Cu, mg/L	1.3	0.97	1.0	1.1	1.0	0.15	0.42	0.79	0.69
Mo, µg/L	0.13	0.10	0.10	0.10	0.10	0.01	0.43	0.33	0.43

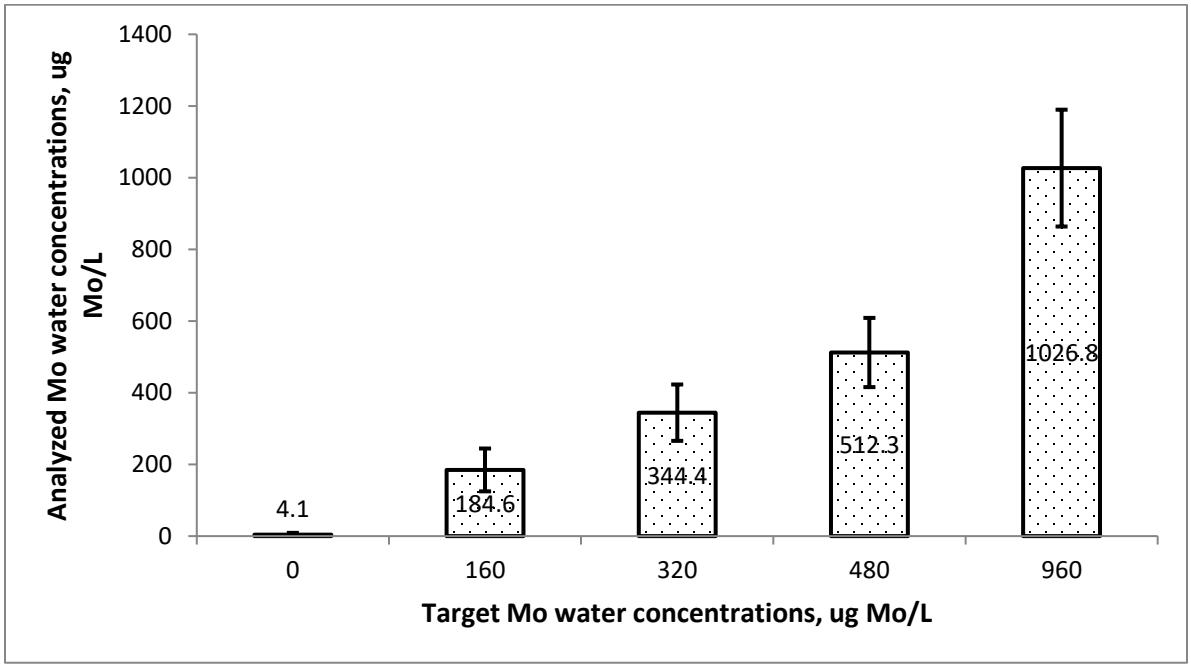

Figure 3. Average Mo concentrations of water throughout the experiment. The x-axis denotes the target Mo concentrations (treatments) and the y-axis denotes the actual measured Mo concentration. Bars in the figure indicate the mean value for all samples within a treatment and the errors bars are the standard deviation of all samples obtained within a treatment. Each water tank was sampled 111 (approximately every 3-4 days when water tanks were refilled).

Appendix B: Code for statistical analysis (SAS

```
dm'output; clear; log; clear;
options ls=100 ps=150;
data BW;
input month monthr set rep tag pen dose period time bw oadg dmip dmihp dmiho fep feo
ibw fbw;
cards;
;
 proc mixed data=bw ;
class monthr set tag dose ;
model fep = BW dose|monthr/ ddfm=kr residual;
random set tag(dose*set);
*repeated monthr / type=ar(1) subject=tag(dose*set) r rcorr;
lsmeans dose|monthr/ adjust=tukey pdiff;
*slice dose*monthr / sliceby = monthr pdiff adjust=tukey;
run;

dm'output; clear; log; clear; ';
options ls=100 ps=150;
data BW;
input month monthr set rep tag pen dose period time bw oadg dmip dmihp dmiho fep feo
ibw fbw;
cards;
;
proc mixed data=bw ;
class monthr set tag dose;
model fbw = dose|monthr/ ddfm=kr residual;
random set tag(dose*set);
*repeated monthr / type=ar(1) subject=tag(dose*set) r rcorr;
lsmeans dose|monthr/ adjust=tukey pdiff;
*slice dose*monthr / sliceby = monthr pdiff adjust=tukey;
run;

dm'output; clear; log; clear; ';
options ls=100 ps=150;
data BW;
input month monthr set rep tag pen dose time DMI DMIkg ADMIkg;
cards;
;
 proc mixed data=bw ;
class monthr set tag dose ;
model DMIkg = dose|monthr/ ddfm=kr residual;
```

```
random set tag(dose*set);
*repeated monthr / type=ar(1) subject=tag(dose*set) r rcorr;
lsmeans dose|monthr/ adjust=tukey pdiff;
*slice dose*monthr / sliceby = monthr pdiff adjust=tukey;
run;

dm'output; clear; log; clear; ';
options ls=100 ps=150;
data BW;
input month monthr set rep tag pen dose period time bw oadg dmip dmihp dmiho fep feo
ibw fbw;
cards;
;
 proc mixed data=bw ;
class monthr set tag dose ;
model oadg = dose|monthr/ ddfm=kr residual;
random set tag(dose*set);
*repeated monthr / type=ar(1) subject=tag(dose*set) r rcorr;
lsmeans dose|monthr/ adjust=tukey pdiff;
*slice dose*monthr / sliceby = monthr pdiff adjust=tukey;
run;

dm'output; clear; log; clear; ';
options ls=100 ps=150;
data BW;
input month monthr set rep tag pen dose period time bw oadg dmip dmihp dmiho fep feo
ibw fbw;
cards;
;
proc mixed data=bw ;
class monthr set tag dose ;
model feo = ibw dose/ ddfm=kr residual;
random set tag(dose*set);
*repeated monthr / type=ar(1) subject=tag(dose*set) r rcorr;
lsmeans dose/adjust=tukey pdiff;
*slice dose*monthr / sliceby = monthr pdiff adjust=tukey;
run;

dm'output; clear; log; clear; ';
options ls=100 ps=150;
data water;
input month monthr set rep tag pen dose period time dwi adwi ibw day;
cards;
;
proc mixed data=water;
class monthr set tag day dose ;
```

```
model adwi = ibw dose|day/ ddfm=kr residual;
random set tag(dose*set);
repeated day / type=ar(1) subject=tag(day*dose*set) r rcorr;
lsmeans dose|day/ adjust=tukey pdiff;
*slice dose*monthr / sliceby = monthr pdiff adjust=tukey;
run;

dm'output; clear; log; clear; ';
options ls=100 ps=150;
data week;
input month monthr set rep tag pen dose period time DWIL ADWI week ibw Nott day;
cards;
;
proc mixed data=week ;
class monthr set tag dose ;
model week = dose|monthr/ ddfm=kr residual;
random set tag(dose*set);
repeated monthr / type=ar(1) subject=tag(dose*set) r rcorr;
lsmeans dose|monthr/ adjust=tukey pdiff;
*slice dose*monthr / sliceby = monthr pdiff adjust=tukey;
run;

dm'output; clear; log; clear; ';
options ls=100 ps=150;
data cd;
input monthr set rep pen dose tag hw lw dp adp ms msc ccri ccrc artc ibw;
cards;
;
proc mixed data = cd ;
class monthr set tag dose ;
model hw = ibw dose|monthr/ ddfm=kr residual;
random set tag(dose*set);
*repeated monthr / type=ar(1) subject=tag(dose*set) r rcorr;
lsmeans dose|monthr/ adjust=tukey pdiff;
*slice dose*monthr / sliceby = monthr pdiff adjust=tukey;
run;

dm'output; clear; log; clear; ';
options ls=100 ps=150;
data cd;
input monthr set rep pen dose tag hw lw dp adp ms msc ccri ccrc artc ibw;
cards;
;
proc mixed data = cd ;
class monthr set tag dose ;
model dp = ibw dose|monthr/ ddfm=kr residual;
```

```
random set tag(dose*set);
*repeated monthr / type=ar(1) subject=tag(dose*set) r rcorr;
lsmeans dose|monthr/ adjust=tukey pdiff;
*slice dose*monthr / sliceby = monthr pdiff adjust=tukey;
run;

dm'output; clear; log; clear; ';
options ls=100 ps=150;
data cd;
input monthr set rep pen dose tag hw lw dp adp ms msc ccri ccrc artc ibw;
cards;
;
proc mixed data = cd ;
class monthr set tag dose ;
model yg = ibw dose|monthr/ ddfm=kr residual;
random set tag(dose*set);
*repeated monthr / type=ar(1) subject=tag(dose*set) r rcorr;
lsmeans dose|monthr/ adjust=tukey pdiff;
*slice dose*monthr / sliceby = monthr pdiff adjust=tukey;
run;

dm'output; clear; log; clear; ';
options ls=100 ps=150;
data cd;
input monthr set rep pen dose tag hw lw dp adp ms msc ccri ccrc artc ibw;
cards;
;
proc mixed data = cd ;
class monthr set tag dose ;
model ms = dose|monthr/ ddfm=kr residual;
random set tag(dose*set);
*repeated monthr / type=ar(1) subject=tag(dose*set) r rcorr;
lsmeans dose|monthr/ adjust=tukey pdiff;
*slice dose*monthr / sliceby = monthr pdiff adjust=tukey;
run;

dm'output; clear; log; clear; ';
options ls=100 ps=150;
data Liver;
input Month MonthR set rep tag Pen Dose Period Time IBW Calcium Phosphorus
Magnesium Sodium Sulfur Cobalt Copper Iron Manganese Molybdenum Selenium Zin;
cards;
;
proc mixed data =Liver ;
class monthr set tag dose ;
model Molybdenum = ibw dose|monthr/ ddfm=kr residual;
```

```
random set tag(dose*set);
*repeated monthr / type=ar(1) subject=tag(dose*set) r rcorr;
lsmeans dose|monthr/ adjust=tukey pdiff;
*slice dose*monthr / sliceby = monthr pdiff adjust=tukey;
run;

dm'output; clear; log; clear; ';
options ls=100 ps=150;
data Liver;
input Month MonthR set rep tag Pen Dose Period Time IBW Calcium Phosphorus
Magnesium Sodium Sulfur Cobalt Copper Iron Manganese Molybdenum Selenium Zin;
cards;
;
proc mixed data =Liver ;
class monthr set tag dose ;
model Zin = dose|monthr/ ddfm=kr residual;
random set tag(dose*set);
*repeated monthr / type=ar(1) subject=tag(dose*set) r rcorr;
lsmeans dose|monthr/ adjust=tukey pdiff;
*slice dose*monthr / sliceby = monthr pdiff adjust=tukey;
run;

dm'output; clear; log; clear; ';
options ls=100 ps=150;
data Liver;
input Month MonthR set rep tag Pen Dose Period Time IBW Calcium Phosphorus
Magnesium Sodium Sulfur Cobalt Copper Iron Manganese Molybdenum Selenium Zin;
cards;
;
proc mixed data =Liver ;
class monthr set tag dose ;
model Copper = ibw dose|monthr/ ddfm=kr residual;
random set tag(dose*set);
*repeated monthr / type=ar(1) subject=tag(dose*set) r rcorr;
lsmeans dose|monthr/ adjust=tukey pdiff;
*slice dose*monthr / sliceby = monthr pdiff adjust=tukey;
run;

dm'output; clear; log; clear; ';
options ls=100 ps=150;
data Plasma;
input Month MonthR set rep tag Pen Dose Period Time IBW Calcium Phosphorus
Magnesium Sodium Sulfur Cobalt Copper Iron Manganese Molybdenum Selenium Zin; ;
cards;
;
proc mixed data =Plasma ;
```

```
class monthr set tag dose ;
model Molybdenum = dose|monthr/ ddfm=kr residual;
random set tag(dose*set);
*repeated monthr / type=ar(1) subject=tag(dose*set) r rcorr;
lsmeans dose|monthr/ adjust=tukey pdiff;
*slice dose*monthr / sliceby = monthr pdiff adjust=tukey;
run;

dm'output; clear; log; clear; ';
options ls=100 ps=150;
data Plasma;
input Month MonthR set rep tag Pen Dose Period Time IBW Calcium Phosphorus
Magnesium Sodium Sulfur Cobalt Copper Iron Manganese Molybdenum Selenium Zin; ;
cards;
;
proc mixed data =Plasma;
class monthr set tag dose ;
model Copper = ibw dose|monthr/ ddfm=kr residual;
random set tag(dose*set);
*repeated monthr / type=ar(1) subject=tag(dose*set) r rcorr;
lsmeans dose|monthr/ adjust=tukey pdiff;
*slice dose*monthr / sliceby = monthr pdiff adjust=tukey;
run;

dm'output; clear; log; clear; ';
options ls=100 ps=150;
data Plasma;
input Month MonthR set rep tag Pen Dose Period Time IBW Calcium Phosphorus
Magnesium Sodium Sulfur Cobalt Copper Iron Manganese Molybdenum Selenium Zin; ;
cards;
;
proc mixed data =Plasma ;
class monthr set tag dose ;
model Zin = dose|monthr/ ddfm=kr residual;
random set tag(dose*set);
*repeated monthr / type=ar(1) subject=tag(dose*set) r rcorr;
lsmeans dose|monthr/ adjust=tukey pdiff;
*slice dose*monthr / sliceby = monthr pdiff adjust=tukey;
run;
```