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The effects of molybdenum water concentration on feedlot performance, tissue mineral concentrations, and carcass quality of feedlot steers[1,2,3]

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ABSTRACT: Thirty cross-bred steers (initial BW 452.0 ± 12.1 kg) were used to investigate the effects of Mo water concentration on performance, carcass characteristics, and mineral status of feedlot steers. The experimental design was a randomized complete block design. Steers were blocked by weight and then divided into 2 weight blocks each consisting of 15 steers. Steers were randomly assigned within block to one of 5 treatments (3 steers/treatment per block). 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 Na2MoO4 to the drinking water. Steers were housed in individual pens (steer = experimental unit) that contained individual 265 L water tanks for monitoring water intake. Steers were fed a growing diet for 28 d and then transitioned to a finishing diet. Block 1 steers were fed for a total of 151 d and block 2 steers were fed for a total of 112 d. 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 d 0 and 84 from each steer within each block. 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 data and significance was determined at $P \leq 0.05$. No differences were observed for final BW ($P > 0.98$). Overall ADG ($P > 0.91$), DMI ($P > 0.92$), feed efficiency ($P > 0.94$), water intake ($P > 0.40$), hot carcass weight ($P > 0.98$), dressing percentage ($P > 0.98$), yield grade ($P > 0.91$), and marbling score ($P > 0.29$) did not differ across treatments. Lastly, no treatment differences were observed for liver concentrations of Cu ($P > 0.93$), Mo ($P > 0.90$) and Zn ($P > 0.86$) or plasma concentrations of Cu ($P > 0.42$), Mo ($P > 0.43$) and Zn ($P > 0.62$). These data indicate that water Mo concentration, within the range studied, had no impact on performance, mineral status, water intake, and carcass characteristics in feedlot steers.

Key words: beef cattle performance, mineral status, molybdenum, water intake

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INTRODUCTION

Dietary requirements for Mo are not well defined for beef cattle (NASEM, 2016). However, elevated concentrations of dietary Mo and S have been reported to induce Cu deficiency in beef cattle through the formation of thiomolybdates in the rumen (Underwood and Suttle, 1999). Molybdenum alone can also have antagonistic effects on Cu absorption (Ward 1978). Miltimore and Mason (1971) reported that if Cu:Mo ratios fall below 2:1, Cu deficiency can be produced. Therefore, feeding additional Cu has been recommended in areas where a Mo interaction is suspected.

[1]Use of trade names in this publication does not imply endorsement by Colorado State University or criticism of similar products not mentioned.

[2]Mention of a proprietary product does not constitute a guarantee or warranty of the products by Colorado State University or the authors and does not imply its approval to the exclusion of other products that may also be suitable.

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The majority of research investigating the impact of Mo on Cu metabolism in ruminants has been conducted by supplementing varying concentrations of Mo to a basal diet. However, certain areas within the Rocky Mountains contain rock deposits with high concentrations of Mo. Due to natural events or human activity, Mo deposits can become soluble and enter ground or river water systems increasing Mo water concentrations that exceed the Colorado Mo agricultural water standard of 160 μg/L (Environmental Protection Agency, 2012). Limited controlled research has been conducted investigating the impact of Mo water concentrations on beef cattle performance. In 1980, Kincaid conducted an experiment utilizing 5 wk old Holstein calves. Calves were allowed ad libitum access to drinking water containing targeted concentrations of 0.0, 1,000, 10,000, and 50,000 μg of Mo/L for 21 d. There were no differences in BW gain or water intake across all water treatments. Kincaid (1980) concluded that the safe ratio of Cu to Mo in this experiment was 0.5:1.0 and postulated that Mo in water could be less toxic than Mo in forages.

The lowest Mo dose used by Kincaid (1980) was greater than the current Colorado Mo agricultural water standard of 160 μg/L (Environmental Protection Agency, 2012). However, Kincaid (1980) utilized a small sample size and short exposure period making it difficult to determine the impact of prolonged exposure to elevated Mo water concentrations. Based on this information, our working hypothesis was that longer exposure to Mo in the drinking water 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 (Protocol #14–5386A).

Cattle

Seventy single sourced commercial cross-bred steers with an initial BW of 457 ± 17.1 kg were housed at the Agricultural, Research, Development, and Education Center in Fort Collins, CO from which 30 steers were selected for this experiment. Before 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, Parsippany, NJ) 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, Madison, NJ). 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 leaving 30 Angus, Hereford, and Angus × Hereford steers for the experiment. The 30 eligible steers were ranked by BW and divided into 2 weight blocks, each one consisting of 15 steers. Each successive weight block was labeled as block 1 and 2 with the heaviest group of 15 steers considered as block 1 and the lightest group of 15 steers considered as block 2. Block 2 steers were group housed in 1 pen and fed a corn silage-based diet for 160 d until block 1 steers were slaughtered.

Steers in block 1 were ranked by initial BW (average of d -1 and 0 body weights) and stratified by BW and breed to 1 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, 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 2.5 m × 20 m and equipped with an individual water tank, individual feed bunk, and a 3 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 block 2 steers after block 1 steers were slaughtered.

Diets and Animal Care

Steers were fed a corn silage-based growing diet for 28 d and then transitioned to the step-1 diet and finishing diet over an 18 d period (Table 1). Steers reached the corn based finishing diet by d 47 of the experiment.

Rations were formulated to meet or exceed the NASEM (2016) nutrient requirements for growing-finishing beef cattle. The basal growing, step-1, and finishing

Table 1. Dry matter ingredient composition of diets[1]

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[2]	6.9	7.0	6.0
Chemical Analysis			
DM, %	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

[1]Monensin feeding was 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/t, on a DM basis in the growing, step 1, and finisher diets, respectively. Tylan was fed at 90 mg × head^{-1} × d^{-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, providing approximately 300 mg × head^{-1} × day1.

[2]Combination of a molasses-condensed corn distillers with solubles based liquid protein supplement (contained on a DM basis: 92.9% CP, 1.28% CF, 14.6% Ca, 0.22 P, 35,640 IU of Vitamin A, 761.4 g/t monensin; and 228.6 g/t tylosin) and a wheat midds-soy fiber based dry pellet supplement (contained, on a dry matter basis: 19.9% CP, 3.5% crude fat; 0.23% calcium; 0.77% phosphorus; 403 mg Cu/kg DM; 2,273 mg zinc/kg DM; 2.9 mg Co/kg DM; 13.3 mg I/kg DM and 1,267 mg manganese/kg DM. Different proportions of the supplements were added to supply the correct amount of nutrients for each diet.

diets contained 5.4, 4.1, and 3.7 mg Cu/kg DM, respectively. Copper as $CuSO_4 \times 5H_20$ was added to the supplement to supply a total diet that contained the NASEM (2016) recommended concentration of Cu (10.0 mg Cu/kg DM; 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/t, on a DM basis in the growing, step 1, and corn based finisher diets, respectively. Tylan was fed at 90 mg × head^{-1} × d^{-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/t (DM basis), providing approximately 300 mg × head^{-1} × d^{-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 and orts were collected weekly for DM and nutrient content determination. Dry matter intake was calculated by subtracting the DM mass of orts from the total DM mass of feed delivered over a given time period then dividing by the days from the last ort collection. A weekly DM determination of feed was conducted by drying duplicate 100-g samples for 48 h using a 60°C forced air drying oven. Weekly samples were composited by month and analyzed for DM, CP, 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 feed bunks. Steers exhibiting symptoms of health problems were assigned a score 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 morbid. All moribund steers were treated according to the appropriate treatment protocol, immediately returned to their appropriate home 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 blocks 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. Standard carcass data measurements were collected by Center for Meat Safety and Quality personnel at Colorado State University.

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 × 61cm × 81cm, length, height, and width, respectively). Water intake was monitored daily at 0800 h ± 30 min by measuring the disappearance of water over a 24 h period. Since the tank was not symmetrical, water volume for every 0.25 cm 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 2 mo.

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 L of water. Water tanks were refilled every 3 to 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 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 concentration 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 to 1,000 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 3 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 Laboratories, Hutchinson, KS) for routine nutrient analysis: DM (Shreve et al., 2006), CP (AOAC, 2012: Method 976.06), ADF and NDF (ANKOM Technology, 2015) , Ca, K, Na (AOAC, 2012: Method 956.01), P (AOAC, 2012: Method 965.17), Mg (AOAC, 2012: Method 968.08) and S, Co, Cu, Fe, Mn, Mo, Zn (AOAC, 2012: Method 985.01), and water quality analysis (EPA, 1983, 1986). 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; SGS Accutest Labs).

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). Treatment, time, and treatment × time were included in the model as fixed effects and replicate within block was included in the model as a random effect. Significance was determined at $P \leq 0.05$ for dose, time and dose × time interactions. 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 covariate in the analysis of all response variables.

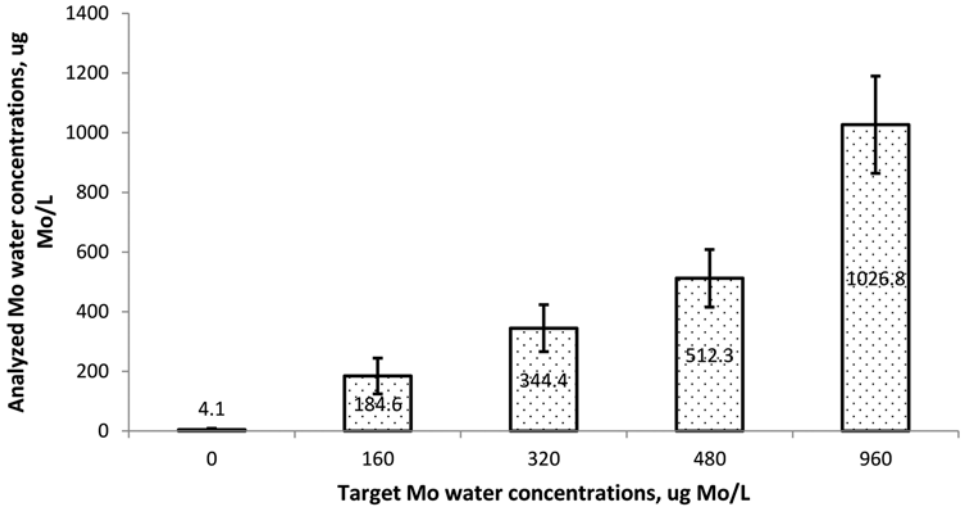

Figure 1. Average Mo concentrations of water throughout the experiment. The *x*-axis denotes the target Mo concentrations (treatments) and the *y*-axis denotes the actual measured Mo concentration. Bars in the figure indicate the mean value for all samples within a treatment and the errors bars are the standard deviation of all samples obtained within a treatment. Each water tank was sampled every 3 to 4 d when water tanks were refilled (n = 111 samples per water tank over the entire experiment).

RESULTS AND DISCUSSION

One steer in the control group died from hemorrhagic bowel syndrome 14 d before 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 Fig. 1 and Table 2, respectively. Overall mean Mo concentrations for each water treatment were 4.1, 184.6, 344.4, 512.3, and 1,026.8 µg Mo/L for 0, 160, 320, 480, and 960, µg Mo/L treatments, respectively. 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 NASEM, 2016).

The effects of water Mo concentration on performance and water intake of feedlot cattle are shown in Table 3. There were no treatment × time interactions for any of the response variables measured. Therefore, overall treatment main effects are presented. Initial and final BW, DMI, ADG, and feed efficiency did not differ ($P > 0.10$) 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 wk old, Holstein calves. Calves were allowed ad libitum access to drinking water containing targeted concentrations of 0.0, 1,000, 10,000, and 50,000 µg of Mo/L (analyzed Mo concentrations were < 1.0, 1,000, 8,000, and 53,000 µg Mo/L, respectively) from ammonium molybdate. The barley-based basal diet contained 0.29% sulfur, 13 mg of Cu/kg diet DM, and less than 1.0 mg of Mo/kg diet DM. Feed intake, body weight gain, and water intake over the 21 d experiment did not differ 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, 1,000, and 10,000 µg of Mo/L in drinking water had similar plasma Cu and ceruloplasmin concentrations and liver Cu concentrations. Kincaid

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, water intake and total molybdenum intake per day of feedlot cattle[1,2]

	Treatment, µg Mo/L of Drinking Water						*P* <		
Item	0	160	320	480	960	SEM	Dose	Time	Dose × Time
BW, kg									
Initial	462.0	444.0	456.0	445.1	452.6	12.1	0.99	–	–
Final[2]	710.3	711.2	706.0	701.9	712.2	16.8	0.98	–	–
DM intake, kg/d									
d0- Final	11.7	12.6	11.7	11.8	12.2	0.62	0.92	0.001	0.50
ADG, 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
Total Mo intake[3], mg × hd^{-1}× d^{-1}	7.2	14.3	19.3	25.1	42.0	–	–	–	–

[1]Initial BW (average of d -1 and 0 bodyweights) was used as a covariate for all statistical analysis.

[2]No dose × time ($P < 0.23$) interaction was observed, therefore initial and final values shown.

[3]Average mg of Mo consumed × hd–1 × d–1 from feed and water combined.

(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 10,000 and 50,000 µg 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, 1,000, 10,000, and 50,000 µg 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 (Table 3; 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 which falls between the Mo water concentrations of 1,000 and 10,000 µg Mo/L reported by Kincaid (1980).

Numerous experiments have been conducted supplementing 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 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 excess dietary Mo supplementation (5.0 to 10.0 mg Mo/kg DM) on body weight gain or reproductive hormone profile (Xin et al., 1991; Ahola et al., 2005). However, experiments with bovine reproductive cells indicate that thiomolybdates can depress reproductive hormone production (Kendall et al., 2003; Kendall et al., 2006).

Dietary concentrations of Mo in excess of 100 mg Mo/kg DM (490.0 to 528.0 mg of Mo consumed × hd^{-1} × d^{-1}) have been reported to cause clinical signs of Mo toxicity in growing heifers (Lesperance and Bohman, 1963) and yearling steers (Cook et al., 1966). The greatest intake of Mo achieved in the Kincaid (1980) experiment was 270 mg/d and in the current experiment the greatest Mo intake was 42 mg/d both of which are substantially lower than concentrations causing Mo toxicity in cattle as reported by Lesperance and Bohman (1963) and Cook et al. (1966).

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[1], %	63.4	63.5	62.5	63.4	63.5	0.70	0.98
Yield Grade	3.55	3.52	3.28	3.41	3.17	0.34	0.91
Marbling Score[2]	546.9	585.7	537.4	518.5	496.9	55.4	0.29

[1]A 4% pencil shrink was applied to all final live weights used to calculate dressing percentage.

[2]Marbling score; 300 = Slight0, 400 = Small0, 500 = Modest0.

The influence of water Mo concentration on carcass characteristics is shown in Table 4. Hot carcass weight, dressing percentage, yield grade and marbling score did not differ ($P > 0.10$) 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 block × treatment or treatment × time interactions for any of the response variables measured. Therefore, overall treatment main effects are presented. Liver and plasma Zn, Cu, and Mo concentrations did not differ ($P > 0.10$) 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 to 10.0 mg Mo/kg DM yielding 35 to 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, 1968a,b; Suttle, 1974a; Suttle, 1974b Wittenberg and Boila, 1988, Wittenberg and Devlin, 1987; Gengelbach et al., 1994; Ward et al., 1997; Suttle, 1991; Ahola et al., 2005). The diet used in the current experiment was formulated to contain 0.15% dietary S as recommended by the NASEM (2016). Intake of S from feed or water containing elevated concentrations of S has been documented to induce Cu deficiency and polioencephalomalacia in feedlot cattle (Gould, 1998; Nichols et al., 2013; Drewnoski et al., 2014). The discrepancy between the current experiment and previously published experiments may be due the method of Mo delivery, S content of the diet, 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 2.0, 42.7, 59.6, 68.4 and 80.0% 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, Garza et al., 1990). Furthermore, using 2 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 by-

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

	Treatment, µg Mo/L Drinking Water						*P* <		
Item	0	160	320	480	960	SEM	Dose	Time	Dose × 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

pass 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 moderately 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 NASEM (2016) nutrient recommendations, dietary 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).

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, Reffett-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). Although not directly comparable to the current experiment, elevated soil pH (> 6.0) has been reported to increase Mo concentrations in certain plants (Mitchell, 1957). Little is known about the influence of rumen and small intestinal pH on Mo absorption kinetics.

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 of rapidly growing feedlot steers fed a moderately high concentrate diet. However, factors that influence ruminal bypass of drinking water and diet type (forage vs. concentrate) need further investigation.

LITERATURE CITED

Ahola, J. K., T. E. Engle, and P. D. Burns. 2005. Effect of copper status, supplementation, and source on pituitary responsiveness to exogenous gonadotropin-releasing hormone in ovariectomized beef cows. J. Anim. Sci. 83:1812–1823. doi:10.2527/2005.8381812x

ANKOM Technology. 2015. Filter bag technique, Method 5. AOCS Approved Procedure: Ba 6a-05.

AOAC. 2012. Official methods of analysis. 19th Ed. Assoc. Off. Anal. Chem. Gaithersburg, MD.

Cook, G. A., A. L. Lesperance, V. R. Bohman, and E. H. Jensen. 1966. Interrelationship of molybdenum and certain factors to the development of the molybdenum toxicity syndrome. J. Anim. Sci. 25:96–101. doi:10.2527/jas1966.25196x

COSAC. 1982. Trace element deficiency in ruminants. Report of a study group commissioned by the Scottish Agricultural Colleges and Research Institutes. East of Scotland Coli. Agric., Edinburgh.

Drewnoski, M. E., D. J. Pogge, and S. L. Hansen. 2014. High-sulfur in beef cattle diets: A review. J. Anim. Sci. 92:3763–3780. doi:10.2527/jas.2013-7242

Engle, T. E., and J. W. Spears. 2000. Effects of dietary copper concentration and source on performance and copper status of growing and finishing steers. J. Anim. Sci. 78:2446-2451.

Environmental Protection Agency (EPA). 1983. Methods for Chemical Analysis of Water and Wastes. EPA-600. Office of Research and Development Washington, DC.

Environmental Protection Agency (EPA). 1986. Test Methods for Evaluating Solid Waste: Physical/Chemical Methods. EPA publication SW-846, 3rd ed. Office of Research and Development Washington, DC.

Environmental Protection Agency (EPA). 2012. Colorado Water quality control commission. EPA Disapproval Action on Additional 2010 Revisions to the Basic Standards and Methodologies for Surface Water. Colorado Department of Public Health and Environment. Denver, CO.

Garza, F. J. D., and F. N. Owens. 1989. Quantitative origin of ruminal liquid with various diets and feed intake. Okla. Agr. Exp. Sta. Res. Rep. MP-127:84–88.

Garza, F. J. D., J. Zorrilla-Rios, and F. N. Owens. 1990. Ruminal water evasion and steady state. Okla. Agr. Exp. Sta. Res. Rep. MP-129:114–121.

Gengelbach, G. P., J. D. Ward, and J. W. Spears. 1994. Effect of dietary copper, iron, and molybdenum on growth and copper status of beef cows and calves. J. Anim. Sci. 72:2722–2727.

Gould, D. H. 1998. Polioencephalomalacia. J. Anim. Sci. 76:309–314. doi:10.2527/1998.761309x

Humphries, W. R., M. Phillippo, B. W. Young, and I. Bremner. 1983. The influence of dietary iron and molybdenum on copper metabolism in calves. Br. J. Nutr. 49:77–86. doi:10.1079/BJN19830013

Kendall, N. R., P. Marsters, R. J. Scaramuzzi, and B. K. Campbell. 2003. Expression of lysyl oxidase and effect of copper chloride and ammonium tetrathiomolybdate on bovine ovarian follicle granulosa cells cultured in serum-free media. Reproduction 125:657–665. doi:10.1530/rep.0.1250657

Kendall, N. R., P. Marsters, L. Guo, R. J. Scaramuzzi, and B. K. Campbell. 2006. Effect of copper and thiomolybdates on bovine theca call differentiation in vitro. J. Endocrinol. 189:455–463. doi:10.1677/joe.1.06278

Kincaid, R. L. 1980. Toxicity of Ammonium Molybdate Added to Drinking Water of Calves. J. Dairy Sci. 63:608–610. doi:10.3168/jds.S0022-0302(80)82978-X

Lesperance, A. L., and V. R. Bohman. 1963. Effect of inorganic molybdenum and type of roughage on the bovine. J. Anim. Sci. 22:686–694. doi:10.2527/jas1963.223686x

Mills, C. F. 1987. Biochemical and physiological indicators of mineral status in animals: Copper, cobalt, and zinc. J. Anim. Sci. 65:1702–1711. doi:10.2527/jas1987.6561702x

Mitchell, R. L. 1957. Trace element contents of plants. Research, London. 10:357–362.

Miltimore, J. E., and J. J. Mason. 1971. Copper to Molybdenum and Molybdenum and Copper Concentrations in Ruminant Feeds. Can. J. Anim. Sci. 51:193–200. doi:10.4141/cjas71-026

NASEM (National Academies of Sciences, Engineering, and Medicine). 2016. Nutrient Requirements of Beef Cattle, 8th Revised ed. Washington, DC: The National Academies Press.

National Research Council. 2001. Nutrient Requirements of Dairy Cattle. Washington, DC: National Academies Press.

National Research Council. 2005. Mineral Tolerance of Domestic Animals. Washington, DC: National Academies Press.

Nichols, C. A., V. R. Bremer, A. K. Watson, C. D. Buckner, J. L. Harding, and G. E. Erickson. 2013. The effect of sulfur and use of ruminal available sulfur as a model to predict incidence of polioencephalomalacia in feedlot cattle. Bov. Pract. 47:47–53.

Phillippo, M., W. R. Humphries, and P. H. Garthwaite. 1987a. The effect of dietary molybdenum and iron on copper status and growth in cattle. J. Agric. Sci. 109:315–320. doi:10.1017/S0021859600080746

Phillippo, M., W. R. Humphries, T. Atkinson, G. D. Henderson, and P. H. Garthwaite. 1987b. The effect of dietary molybdenum and iron on copper status, puberty, fertility, and oestrous cycles in cattle. J. Agric. Sci. 109:321–336.

Puls, R. 1994. Mineral levels in animal health: Diagnostic data In: Minerals in Animal Nutrition. 2nd ed. Sherpa International, Clearbrook, BC.

Pearson, E. G., and A. M. Craig. 1980. The diagnosis of liver disease in equine and food animals. Mod. Vet. Pract. 61:233–237.

Reffett-Stabel, J., J. W. Spears, R. W. Harvey, and D. M. Lucas. 1989. Salinomycin and lasalocid effects on growth performance, mineral metabolism and ruminal fermentation in steers. J. Anim. Sci. 67:2735–2742. doi:10.2527/jas1989.67102735x

Shreve, B., N. Thiex, and M. Wolf. 2006. NFTA method 2.1.4—Dry matter by oven drying for 3 hours at 105°C. NFTA Reference Methods. National Forage Testing Association, Omaha, NE.

Spears, J. W. 1990. Ionophores and nutrient digestion and absorption in ruminants. J. Nutr. 120:632–638.

Spears, J. W., and R. W. Harvey. 1985. Dietary sodium and potassium for steers fed lasalocid. J. Anim. Sci. 61(Suppl. 1):473 (Abstr.).

Starnes, S. R., J. W. Spears, M. A. Froetschel, and W. J. Croom, Jr. 1984. Influence of monensin and lasalocid on mineral metabolism and ruminal urease activity in steers. J. Nutr. 114:518–525.

Suttle, N. F. 1974a. Effects of organic and inorganic sulphur on the availability of dietary copper to sheep. Br. J. Nutr. 32:559–568. doi:10.1079/BJN19740109

Suttle, N. F. 1974b. Recent studies on the copper-molybdenum-sulphur interrelationship. Proc. Nutr. Soc. 33:299–305. doi:10.1079/PNS19740053

Suttle, N. F., and A. C. Field. 1968a. Effect of intake of copper, molybdenum and sulphate on copper metabolism in sheep I. Clinical condition and distribution of copper in the blood of the pregnant ewe. J. Comp. Path. 78:351–362. doi:10.1016/0021-9975(68)90013-3

Suttle, N. F., and A. C. Field. 1968b. Effect of intake of copper, molybdenum and sulphate on copper metabolism in sheep. Copper status of the newborn lamb. J. Comp. Pathol. 78:363–370. doi:10.1016/0021-9975(68)90014-5

Suttle, N. F. 1991. The interactions between copper, molybdenum, and sulphur in ruminant nutrition. Annu. Rev. Nutr. 11:121–140. doi:10.1146/annurev.nu.11.070191.001005

Underwood, E. J., and N. F. Suttle. 1999. The Mineral Nutrition of Livestock (3rd ed.). CABI Publishing, Wallingford, UK. doi:10.1079/9780851991283.0000

Ward, G. M. 1978. Molybdenum toxicity and hypocuprosis in ruminants: A review. J. Anim. Sci. 46:1078–1085. doi:10.2527/jas1978.4641078x

Ward, J., G. Genglebach, and J. Spears. 1997. The effects of copper deficiency with or without high dietary iron or molybdenum on immune function of cattle. J. Anim. Sci. 75:1400–1408. doi:10.2527/1997.7551400x

Ward, J., and J. Spears. 1997. Long-term effects of consumption of low-copper diets with or without supplemental molybdenum on copper status, performance, and carcass characteristics of cattle. J. Anim. Sci. 75:3057–3065. doi:10.2527/1997.75113057x

Warner, A. C. I., and B. D. Stacy. 1968. The fate of water in the rumen. Br. J. Nutr. 22:389–410. doi:10.1079/BJN19680047

Wittenberg, K. M., and T. J. Devlin. 1987. Effects of dietary molybdenum on productivity and metabolic parameters of lactating beef cows and their offspring. Can. J. Anim. Sci. 67:1055–1066. doi:10.4141/cjas87-111

Wittenberg, K. M., and R. J. Boila. 1988. Supplementary copper for growing cattle consuming diets high in molybdenum or molybdenum plus sulfur. Can. J. Anim. Sci. 68:1143–1154. doi:10.4141/cjas88-130

Wang, Z. Y., Poole, D. B. R., Mason, J. 1988. The effects of supplementation of the diet of young steers with Mo and S on the intracellular distribution of copper in liver and on copper fractions in blood. Br. Vet. J. 144:543–551. doi:10.1016/0007-1935(88)90024-3

Woodford, S. T., M. R. Murphy, C. L. Davis, and K. R. Holmes. 1984. Ruminal bypass of drinking water in lactating cows. J. Dairy Sci. 67:2471–2474. doi:10.3168/jds.S0022-0302(84)81599-4

Wright, C. L. 2007. Management of water quality for beef cattle. Vet. Clin. Anim. 23:91–103.

Xin, Z., D. F. Waterman, R. W. Hemken, and R. J. Harmon. 1991. Effects of copper status on neutrophil function, superoxide dismutase, and copper distribution in steers. J. Dairy Sci. 74:3078–3085. doi:10.3168/jds.S0022-0302(91)78493-2

Zorrilla-Rios, J., J. D. Garza, and F. N. Owens. 1990. Fate of drinking water in ruminants: Simultaneous comparison of two methods to estimate rumen evasion. Okla. Agr. Exp. Sta. Res. Rep. 129:167–169.