Treatment of High-Sulfate Water used for Livestock Production Systems
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Abstract: Reliable drinking water sources that meet minimum quality standards are essential for successful livestock production. Recent surveys have shown that water sources throughout the semi-arid rangelands of the U.S. are not of sufficient quality to support optimum herd/flock health and performance. Water sources high in sulfur (S) concentrations, usually in the form of sulfate (SO_4^{2-}), are problematic in many western regions. High SO_4^{2-} concentrations in water sources can arise from several factors. First, water sources can be naturally high in SO_4^{2-}. Second, drought conditions can cause SO_4^{2-} to be concentrated within the water source. Third, conventional oil and gas production can also increase SO_4^{2-} content within the water source. Many of these water sources are used for livestock production systems, especially throughout the western states. High-SO_4^{2-} water has been shown to reduce performance and cause secondary health and immunity complications in exposed livestock. Additionally, high SO_4^{2-} levels in drinking water are a primary cause of polioencephalomalacia (PEM) in ruminant livestock. Sulfur-induced PEM (sPEM) is a disease state in ruminant animals that can cause 25% morbidity and 25-50% mortality in affected populations, resulting in substantial economic losses to the livestock producer. Currently, there are no available treatments for affected livestock, and frequent and stringent testing of drinking water sources for levels of SO_4^{2-} and other S compounds, a costly and time-consuming process, is the best prevention strategy. In addition, methods for SO_4^{2-} removal from the water source are neither cost-effective nor practical. Ferrous carbonate ($FeCO_3$) is a soluble iron salt that is routinely used in water treatment plants to bind S. We hypothesize that treatment of high-SO_4^{2-} water with $FeCO_3$ would bind excess S, enabling such sources to be used for livestock production. Our objectives were to 1) determine the effectiveness of $FeCO_3$ treatment in binding S in high-SO_4^{2-} water, and 2) determine if treatment of high-SO_4^{2-} water with $FeCO_3$ prevents the reduced performance and poor health normally observed in livestock consuming high-SO_4^{2-} water. Briefly, wether lambs (n = 80) were assigned to one of four treatments in a randomized complete block design with 20 wethers per treatment replicated over 2 pens per treatment. Treatments included: 1) control feed and low-S water (57 mg SO_4^{2-}/L; LS); 2) control feed and high-S water (2,250 mg SO_4^{2-}/L; HS); 3) low-Fe (250 ppm $FeCO_3$) feed and high-S water (2,250 mg SO_4^{2-}/L; HSLI); and 4) high-Fe (500 ppm $FeCO_3$) feed and high-S water (2,250 mg SO_4^{2-}/L; HSHI) for a 50 d trial period. All wethers received *ad libitum* access to feed and water. Body weights and blood samples were taken on d -1, 25, and 50, and rumen H_2S gas was measured on d -1 and 50. Lambs were slaughtered after the trial period for liver and muscle sample collections. There were no differences in ADG between treatment groups ($P = 0.351$). Daily water intake was also not different ($P = 0.305$) between treatment groups, nor was daily feed intake ($P = 0.116$). Mineral analyses showed no treatment effects ($P \geq 0.214$) on plasma concentrations of Fe or Zn, or on hepatic concentrations of Se, Fe, Mo, Mn, or Zn ($P \geq 0.094$). However, both plasma and hepatic Cu concentrations were different ($P \leq 0.023$) between the LS and HSLI groups, with LS wethers having greater concentrations of Cu than HSLI wethers. Production of H_2S gas was increased ($P < 0.001$) during the trial, with the LS group having lower ($P \leq 0.012$) H_2S concentrations in the rumen than the HS, HSHI, and HSLI groups. Real-time RT-PCR was performed on 23 genes to assess potential changes at the molecular level. Genes identified as differentially expressed ($P \leq 0.05$) in response to treatment were *SLC26A2*, *GNMT*, *ITGβ2*, *PRKACA*, *APEX1*, *EPB41*, *TG2*, *PCNA*, and *PIPOX*. Genes identified as having a tendency to be differentially expressed ($P \leq 0.10$) in response to treatment included *TGFβ1*, *SOD1*, *TUSC3*, and *TJP1*. Genes that were differentially expressed between treatments were consistently upregulated in the HSHI group compared to the other treatment groups. These differentially expressed genes are involved in immune function, oxidative damage, apoptosis, tumor suppression, and tight junctions, suggesting that high dietary Fe, perhaps in combination with high dietary S, influences cellular functions in wethers consuming high-S water. In summary, results of this study suggest that supplementation of an Fe compound to ruminant animals exposed to high-S drinking water was not effective at preventing H_2S gas accumulation in the rumen, and would likely not prevent the negative effects associated with high dietary S.

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Statement of Critical Regional or State Water Problem: ***Need for Project.*** Reliable drinking water sources that meet minimum quality standards are essential for successful livestock production. However, recent surveys have shown many water sources, especially throughout the semi-arid rangelands of the U.S. and Wyoming, are not of sufficient quality to support optimum herd/flock health and performance [1]. Many of these low quality sources are dangerously high in S and S compounds, especially SO_4^{2-}, due to underlying soil conditions, drought conditions, and(or) manmade contaminants (e.g. conventional gas and oil water; CBM water). However, because of limited water resources throughout the western regions, many of these high-SO_4^{2-} water sources are still used in livestock production systems. Ruminant livestock consuming high-SO_4^{2-} water are prone to poor growth and performance and health complications, including polioencephalomalacia (sPEM), a neurological disorder typically terminating in death. Outbreaks of sPEM can cause 25% morbidity and 25-50% mortality in affected populations. This in combination with the losses in growth and performance results in significant economic losses to producers. Currently, there are no available treatments for livestock affected by high-SO_4^{2-} water consumption (including sPEM), and frequent and stringent testing of drinking water sources for levels of SO_4^{2-} and other S-compounds, a costly and time-consuming process, is the best prevention strategy. Methods for removal of SO_4^{2-} from the water source include reverse osmosis, distillation, and ion exchange, none of which are cost-effective or practical for livestock producers.

Who Would Benefit and Why. Many water sources high in SO_4^{2-} are still used for livestock production due to lack of alternative available sources. Additionally, in many of these areas it is neither feasible nor practical to haul in water low in SO_4^{2-}. Therefore, identification of an effective treatment for high-SO_4^{2-} water sources would 1) prevent the health and performance problems associated with livestock consuming high-SO_4^{2-} water, and 2) allow producers to use available water resources despite high SO_4^{2-} concentrations.

Statement of Results or Benefits: ***Information to be Gained.*** Ferrous carbonate ($FeCO_3$) is a soluble iron salt with potential to bind S. We hypothesized that treatment of high-SO_4^{2-} water with $FeCO_3$ will bind excess S and ultimately prevent the poor performance and health (i.e. sPEM) of livestock consuming high-SO_4^{2-} water. We expected to determine 1) if $FeCO_3$ treatment of high-SO_4^{2-} water sufficiently binds S and reduces it to levels within the recommendations for livestock production, and 2) if $FeCO_3$ treatment of high-SO_4^{2-} water prevents the decreased performance and poor health that livestock typically experience when administered high-SO_4^{2-} water. Identification of a practical, water-applied treatment for high-SO_4^{2-} water would offer livestock producers a means by which such sources could be used for livestock production without compromising herd/flock health and performance.

How Information Was Used. The information garnered from this research was used to 1) determine if treatment of high-SO_4^{2-} water with $FeCO_3$ lowers S concentrations to within the recommended levels for livestock production, and 2) determine if the $FeCl_2$ water treatment prevents the reduced performance and poor health normally observed in livestock consuming high-SO_4^{2-} water. Unfortunately, our results suggested that Fe does not effectively lower S concentrations, nor did it positively affect animal performance. Further research is needed to identify other potential treatments for high-SO_4^{2-} water. *Our long-term goal is to develop a practical, water-applied treatment for high-SO_4^{2-} water sources that will bind S and reduce it to levels acceptable for livestock consumption, enabling such sources to be used in livestock production systems.*

Nature, Scope, and Objectives of the Project: The basic nature of the proposed research was to identify a water-applied treatment for high-SO_4^{2-} water that producers can affordably and practically apply to those high-SO_4^{2-} water sources used for livestock production. This research is especially important for livestock producers in the western regions of the U.S., including Wyoming, where high-SO_4^{2-} water sources are prevalent. The objectives of this project were to 1) determine if $FeCO_3$ treatment of high-SO_4^{2-} water reduces S to levels within the accepted recommendations for livestock production, and 2) determine if treatment of high-SO_4^{2-} water with $FeCO_3$ prevents the reduced performance and poor health normally observed in livestock consuming high-SO_4^{2-} water. Health was assessed by incidence of sPEM and changes in hepatic gene regulation; performance was assessed by measures of feed intake, feed efficiency, and gain. This project was completed over a two year period by one M.S. student (Amanda Jons).

Methods, Procedures, and Facilities: Cattle and sheep are both ruminant livestock affected by high-SO_4^{2-} water. Sheep are commonly used as a model species, as they are the most economical ruminant farm animal available. This study used ram lambs (n = 80; 6 months of age) maintained at the University of Wyoming's Stock Farm. The Stock Farm houses a GrowSafe Feed Intake and Behavior Monitoring system, the only system to-date specifically designed to collect feed intake and behavior data of individual sheep in group settings.

Rams were allowed a 10 d adjustment period to become accustomed to the GrowSafe system. Prior to treatment, drinking water was analyzed for concentrations of total dissolved solids, S, and S compounds. Ram lambs were randomly assigned to one of four treatments for the 60 d trial period: low-SO_4^{2-} water (57 mg SO_4^{2-}/L water; n = 20); high-SO_4^{2-} water (2,250 mg SO_4^{2-}/L water; n = 20); high-SO_4^{2-} water + low $FeCO_3$ (2,250 mg SO_4^{2-}/L + 250 mg $FeCO_3$/L; n = 20); or high-SO_4^{2-} water + high $FeCO_3$ (2,250 mg SO_4^{2-}/L + 500 mg $FeCO_3$/L; n = 20). This level of SO_4^{2-} administration has been shown to cause performance and health deficiencies in previous studies conducted by the PI and co-PIs. All lambs were administered the same diet throughout the experimental period. Lambs were allowed *ad libitum* access to feed and water. Weights were recorded on d -2 and -1, d 29 and 30, and d 60 and 61, and averaged for more precise estimates of initial, mid, and final BW, respectively. Blood samples were collected in conjunction with BW on d -2, d 29, and d 60 for serum Fe, copper (Cu^{2+}), and molybdenum (Mo) analyses. High SO_4^{2-} acts as a Cu^{2+} antagonist in ruminants, irreversibly binding Cu^{2+} and rendering it unavailable for utilization. Additionally, Mo is involved in the binding of Cu^{2+} through the formation of thiomolybdates in high-S environments. Because excess H_2S gas production is causal to sPEM, rumino-centesis was performed on d -2 and d 60 to obtain measures of, and changes in, ruminal H_2S gas production. Ruminal gas was collected via the rumen gas caps and aspirated through H_2S detector tubes.

Traits measured for each individual ram using the GrowSafe system included daily feed intake, residual feed intake (a measure of feed efficiency), daily feeding time, and rate of feed intake. In addition, average daily gain was estimated for each lamb. Lambs were closely monitored for clinical signs of sPEM. No lambs exhibited severe signs of sPEM and had to be removed from the trial. All lambs were euthanized at the end of the trial period for liver collection; muscle samples were also collected to determine S accumulation in muscle tissues. Multiple subsamples of each liver were collected for mineral and gene expression analyses.

Results indicated that there were no differences in ADG ($P = 0.351$), daily water intake ($P = 0.305$), or daily feed intake ($P = 0.116$) between treatments. Mineral analyses showed no treatment effects ($P \geq 0.214$) on plasma concentrations of Fe or Zn, or on hepatic concentrations of Se, Fe, Mo, Mn, or Zn ($P \geq 0.094$). Production of H_2S gas was less ($P \leq 0.012$) in low-S control lambs compared to lambs in the high-S treatment groups; no differences in H_2S gas production were detected between high-S treatment groups. These production results suggested that Fe was not effective in countering the effects of high dietary S (through the drinking water in this instance) in lambs.

Gene Expression Analyses. The liver plays a key role in sulfide (S_2) detoxification. Previous studies in our laboratory have shown chronic exposure to high-SO_4^{2-} water induces changes in hepatic gene regulation (Table 1). Many of these genes are integral to immune function, indicating that liver health is affected by high-S water. Assessment of those genes in the present study may determine any changes in health due to the $FeCO_3$ treatment at a molecular level. Assessment at the molecular level is essential to determine if the $FeCO_3$ treatment causes any changes not readily detectable at the phenotypic level. Real-time RT-PCR was performed to determine if treating high-SO_4^{2-} water with $FeCO_3$ causes molecular changes and alters liver function. Briefly, RNA was extracted from liver subsamples from each animal, reverse transcribed into cDNA, and quantified relative to a standard housekeeping gene. Genes identified as differentially expressed in response to treatment were *SLC26A2* ($P = 0.004$), *GNMT* ($P = 0.032$), *ITGβ2* ($P = 0.030$), *PRKACA* ($P = 0.016$), *APEX1* ($P = 0.024$), *EPB41* ($P < 0.001$), *TG2* ($P = 0.002$), *PCNA* ($P = 0.033$), and *PIPOX* ($P = 0.029$). Genes identified as having a tendency to be differentially expressed in response to treatment included *TGFβ1* ($P = 0.097$), *SOD1* ($P = 0.070$), *TUSC3* ($P = 0.077$), and *TJP1* ($P = 0.059$). Genes that were differentially expressed between treatments were consistently upregulated in the high-SO_4^{2-} water/high FeCO group compared to the other treatment groups. These differentially expressed genes are involved in immune function, oxidative damage, apoptosis, tumor suppression, and tight junctions, suggesting that high dietary Fe, perhaps in combination with high dietary SO_4^{2-}, influences cellular functions in wethers consuming high SO_4^{2-} water.

Table 1. Genes identified as differentially regulated in response to high-S water.

Gene(s)	**Function**
MHC Class I Heavy Chain	Immune system
MHC Class II, DQ alpha 5, DQ beta, and DRB3, TGF beta 1	Immune system; Stimulus response
Regakine 1 and Integrin, beta 2	Immune system; Stimulus response; Binding
Inhibin, Beta A	Immune system; Binding
Interleukin 8 Receptor, Beta	Response to stimulus
ZFP 385A, Interleukin 1 Rc, HOP Homeobox, Pyrroline-5 CR 1, Cys-rich EGF-like 2	Binding
Tubulin beta 4, Protein Kinase (cAMP-dependent, catalytic, alpha)	Cytoplasmic function; Binding
Transglutaminase 2	Cation binding
Lysosomal-associated Protein Transmembrane 4 Alpha	Cytoplasmic function
Glycine N-Methyltransferase	Hepatic S-adenosylmethionine function
Aldo-keto Reductase Family 1, Member B10	Oxidoreductase activity
Pyruvate Carboxylase, Uncoupling Protein 2	Cell/membrane function

Significance. Results suggested that Fe does not effectively lower S concentrations, nor did it positively affect animal performance or health. Further research is needed to identify other potential treatments for high-SO_4^{2-} water.

Student Training. This project served as the thesis project of a M.S. student (Amanda Jons) in the Department of Animal Science, with PI Cammack as the advisor for the student. The student was trained in the areas of animal production, toxicity, genomics, and water quality, and was responsible for carrying out all aspects of this research project, including both the animal and laboratory components. Together with the PIs, the student prepared manuscripts that will be submitted shortly. The M.S. student prepared two Annual Reports for the Department of Animal Science describing her experiment and presenting results. She presented her work at the American Society of Animal Science national meeting in the summer of 2011 in New Orleans; the abstracted was published in the meeting proceedings. Amanda also presented a poster summarizing her work at the Colorado Ruminant Nutrition Roundtable. Finally, Amanda presented her work at the Department of Animal Science's spring seminar series, and defended her thesis work successfully in the summer of 2011. Amanda continued to work in the PIs laboratory following her graduation, and is currently seeking a research position. This research was also an opportunity for undergraduate training. A number of undergraduate students assisted with this project, giving them the opportunity to gain hands-on animal care and laboratory experience. Two of the undergraduates helping with project will be starting graduate school themselves in the fall of 2012.

Publications.

Jons, A.M, K.L. Kessler, K.J. Austin, C. Wright, and K.M. Cammack. 2011. Iron carbonate supplementation of lambs administered high-sulfur water. J. Anim. Sci. 89(E-Suppl.):694.

Jons, A.M., K.J. Austin, K.L. Kessler and K.M. Cammack. 2011. Effects of $FeCO_3$ supplementation on hepatic gene expression of wethers administered high-S water. Colorado Nutrition Roundtable. Fort Collins, CO.

Jons, A.M., K.L. Kessler, K.J. Austin, C. Wright, and K.M. Cammack. 2012. Effects of iron carbonate supplementation on performance of lambs administered high-sulfur water. Small Ruminant Research. In preparation.

Jons, A.M., K.L. Kessler, K.J. Austin, C. Wright, and K.M. Cammack. 2012. Effects of iron carbonate supplementation on hepatic gene expression of lambs administered high-sulfur water. Small Ruminant Research. In preparation.

Related Research. The current NRC recommendation for dietary S is < 0.3% dry matter (DM), with the maximum tolerable concentration estimated at 0.4% DM [2]. Sulfur content in water, however, is typically reported in parts per million (ppm), and the most common form of S in water is SO_4^{2-}. Polioencephalmalacia is associated with water SO_4^{2-} concentrations of ≥ 2,000 mg/L, which when combined with a typical 0.2% DM S feedstuff results in 0.53% DM total dietary S [3]. Therefore, when S or SO_4^{2-} content of water is included in the estimation of dietary S, the total dietary S is often much higher than anticipated. *Mechanism:* Sulfate and S_2 together form a recycling system, as absorbed S_2 is oxidized into SO_4^{2-} in the liver. Levels of H_2S gas increase with greater dietary S. The excess production of H_2S inhibits cytochrome oxidase in the electron transport system, reduces ATP production, and ultimately causes necrosis in the brain [4]. The S_2 ion is also capable of binding to hemoglobin and forming sulfhemoglobin, reducing the ability of the blood to deliver oxygen to the body [5].

Sources of High-S Water: Survey and field data have consistently shown surface and subsurface water can be high in SO_4^{2-}, particularly throughout the western regions of the U.S. The Water Quality for Wyoming Livestock & Wildlife review [6] reported that of > 450 forage and water collection sites located throughout the U.S., 11.5% exceeded the dietary S concentrations considered safe for livestock. Of those sites, 37% were located in the western U.S., including Wyoming. Drought further exacerbates the high SO_4^{2-} problem, as SO_4^{2-} is concentrated in the water due to greater evaporation and reduced moisture recharge [7]. In addition, conventional gas and oil produced water discharge can be high in SO_4^{2-}, particularly in arid regions such as the Big Horn Basin (John Wagner, personal communication). Of five water discharge sites sampled in the Big Horn Basin, two exceeded 2,000 mg SO_4^{2-}/L [8], well above the limit considered safe for livestock consumption. Although many CBM water sources are low in SO_4^{2-}, including those in the Powder River Basin, there have been reports of high and variable SO_4^{2-} concentrations (hundreds to thousands of mg/L) in CBM waters from the Fort

Union Formation in Campbell County [9]. Because of the limited availability of water resources in those regions, many of those sources high in SO_4^{2-} are still used for livestock production.

***High-S Water and Performance*:** Poor performance of animals exposed to drinking water sources with high levels of SO_4^{2-} is common. Declines in average daily gain in cattle consuming high- SO_4^{2-} water have been reported in both grazing and confined environments [1,10]. Also, decreases in feed consumption and body weight gain in ruminant livestock exposed to high-SO_4^{2-} drinking water are consistently reported. *High-S Water and PEM:* Polioencephalomalacia is characterized by necrosis of the cerebral cortex and remains one of the most prevalent central nervous system diseases in cattle and sheep [2,11]. Clinical signs of PEM may include head pressing, blindness, incoordination, and recumbency accompanied by seizures [2], with young ruminants the most commonly affected [12]. The limited amount and availability of quality water is problematic for producers, especially when livestock consuming SO_4^{2-} contaminated water are also exposed to forages with moderately elevated S levels [3].

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