Use of manganese oxide to attenuate negative effects of high sulfur concentrations in distillers grains-based finishing rations

Forward

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The Agricultural Utilization Research Institute is pleased to present this study in collaboration with the MN Corn Growers Association and the MN Corn Research & Promotion Council. The goal of this study evaluate the optimal use of Distiller's Grains (DGs) in feedlot cattle diets. A limiting factor to DGs utilization has been sulfur toxicity. Manganese Oxide (MnO), an oxidizing agent, was proposed as a means to mitigate hydrogen sulfide production in the rumen of cattle consuming diets high in sulfur (S). This is the primary hypothesis of this study which includes *in vitro*, *in vivo*, and carcass evaluations. This report finds a significant decrease in the ruminal production and release of hydrogen sulfide from cattle fed a high sulfur diet with manganese supplementation (1000 ppm). This decrease in hydrogen sulfide production may allow greater inclusion of DGs while avoiding the negative impact of sulfur toxicity.

The observed result of MnO in attenuating hydrogen sulfide production in beef cattle during the first 28 days on high-S feed. This corresponds with the period in which feedlot cattle are being adapted to a high-concentrate, and in many cases, high-S diet. Implications from this experiment suggest that MnO should be included in feedlot diets that contain dietary S concentrations at or near 0.60% of DM, however further investigation is warranted. Researchers also suggest that MnO supplementation may need to be increased over time as cattle consume more feed, and therefore more dietary sulfur.

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FINAL REPORT

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Introduction

Production of hydrogen sulfide (H_2S) in the rumen of cattle consuming diets high in dietary sulfur (S) appears to reduce performance due to S toxicity. Hydrogen sulfide gas produced in the rumen enters the bloodstream via absorption through the rumen wall or inhalation of eructated H_2S. Oxidation of circulatory H_2S into sulfates occurs in the blood and liver and circulates to the brain. In severe toxicity cases, H_2S can cause polioencephalomalacia (necrosis of the brain) and may be fatal in cattle. Because feedlot cattle are typically fed concentrate diets that may contain high dietary S levels due to inclusion of distillers grains plus solubles (DGS), H_2S production may be increased and aided by lower ruminal pH in these cattle. Therefore, S toxicity and polioencephalomalacia (PEM) can be a significant concern to the feedlot industry, especially with an increased inclusion of DGS in finishing rations.

Manganese oxide (MnO) is a powerful oxidizing agent and is also used as a Mn source in mineral supplements fed to feedlot and range cattle. In aqueous solutions, MnO oxidized H_2S to sulfate and elemental S at pH of 5.0 at the rate of 1.49 g of MnO to 1 g of H_2S (Herszage and Afonso, 2003). Most cattle consuming feedlot diets have ruminal pH values that range from 5.25 to 6.0; therefore, MnO may oxidize H_2S in the rumen and reduce negative effects resulting from sulfur toxicity. The hypothesis of these experiments is feeding MnO within recommended levels of Mn will result in oxidation of H_2S into sulfates which are eliminated in the small intestine to reduce or prevent negative effects of H_2S on health and performance of feedlot cattle consuming high S diets.

Experiment 1. *In vitro e*valuation of hydrogen sulfide production in batch cultures containing manganese oxide and high substrate sulfur concentrations.

Exp. 1 Materials and Methods

An *in vitro* batch culture experiment was conducted in the ruminant nutrition laboratories at University of Minnesota-Saint Paul to determine the production of H_2S under various concentrations of Mn. All procedures involving animals were reviewed and approved by the University of Minnesota Institutional Care and Use Committee. Approximately 1-h prior to batch culture inoculation, ruminal fluid was collected prior to AM feeding from a Holstein-Montbéliarde crossbred lactating dairy cow fitted with a flexible, four-inch ruminal cannulae. The dairy cow was housed at the dairy facility on the University of Minnesota-Saint Paul campus and was fed a typical 60:40 forage:concentrate lactation ration containing monensin sodium (Rumensin®; Elanco Animal Health; Greenfield, IN). Ruminal grab samples were collected from cranial, caudal, left lateral, and right lateral areas of the rumen to obtain a representative sample and strained through four layers of cheesecloth into a pre-warmed (with hot water) insulated container to obtain ruminal fluid. The insulated container was completely filled prior to sealing to minimize oxygen exposure to the ruminal fluid. The sealed insulated container was immediately transported to the ruminant nutrition laboratory for processing of the ruminal fluid for inoculation of the batch culture.

Substrate and Treatments. A diet (DM-basis) of 81% dried DGS (0.77% S) and 19% ground corn (0.15% S) was formulated to contain 0.65% dietary S. The DGS was left intact, but the corn grain was ground to pass through a 1-mm screen (Wiley mill; Swedesboro, NJ) prior to mixing. Feed grade MnO was thoroughly mixed with the ground corn prior to addition of the DGS to attain appropriate concentration of Mn in the total diet for each treatment substrate. Concentrations of Mn evaluated were 0, 500, 1,000, 1,500, 2,000, and 2,500 ppm. Subsamples of each treatment substrate were dried at 100°C for 24 h in a laboratory oven (Thelco Laboratory Oven, model 130DM; Precision Scientific Inc.; Chicago, IL) to determine laboratory DM (AOAC, 1996) prior to measuring treatment amounts into serum bottles.

Gas Production and Hydrogen Sulfide Measurement. To obtain approximately 0.7 g DM of substrate, 0.71 g (as-is) of each substrate was measured into one of four, 125 mL glass serum bottles (Wheaton Science Products; Millville, NJ) per concentration of Mn, for a total of 24 bottles in the incubation. Fifty mL of a 1:1 solution of 25 mL McDougall's saliva (McDougall, 1948; pre-warmed to 39° C with pH of 7.0 and containing sodium bicarbonate, sodium phosphate, potassium chloride, sodium chloride, magnesium sulfate, urea, and calcium chloride) and 25 mL rumen fluid were measured into each 125 mL serum bottle in random order. Each bottle was purged with CO_2 for 10 s and crimp-sealed with a butyl-rubber stopper and metal retainer (Wheaton Science Products; Millville, NJ). All bottles were placed randomly and incubated at 39° C for 24 h in a reciprocal shaking water bath (Precision Scientific Model 50; Thermo Scientific Inc., Waltham, MA) set for constant agitation at 30 rpm. Initial pH (6.69) of the 1:1 solution of McDougall's saliva and ruminal fluid was measured using a hand-held pH probe (Thermo Orion model 710A pH meter; Cole-Parmer Instruments; Vernon Hills, IL).

At 2, 5, 10, and 24-h post-inoculation, bottles were briefly removed from the shaking water bath for gas measurement. Volume of gas produced at each time interval was measured in each serum bottle via water displacement in an inverted 250-mL glass buret by puncturing the butyl-rubber stopper with an 18 gauge x 25.4 mm hypodermic needle connected to a 5-mL syringe (Monoject; Covidien, Mansfield, MA) fitted with a 3-way valve that was connected on one end to rubber tubing that allowed gas to escape into the inverted buret. Following each gas measurement, an additional 5 mL of gas were extracted from the head space of each bottle using a 5 mL tuberculin syringe and 18 gauge x 25.4 mm needle (Monoject; Covidien, Mansfield, MA) for analysis of H_2S concentration. Following gas measurement and collection at 24 h, pH of the contents in each serum bottle was measured using a hand-held pH probe (Thermo Orion model 710A pH meter; Cole-Parmer Instruments; Vernon Hills, IL). Total gas volume of each bottle was corrected for 24-h incubation time (1,440 min), and rates of total gas produced per min of incubation and per mg of substrate DM incubated were calculated.

The method for analyzing H_2S concentration is similar to the procedure originally described by Siegel (1965) and also reported by Kung et al. (1998), Leibovich et al. (2009) and Quinn et al. (2009). Five mL of alkaline water (prepared with 0.1 *N* NaOH; pH 8) were injected into a 10 mL evacuated blood collection tube (Kendall Monoject Red Stopper sterile blood collection tube, 16 x 100 mm, 10 mL draw; Covidien, Mansfield, MA) using a disposable 5 mL tuberculin syringe and 18 gauge x 25.4 mm hypodermic needle (Monoject; Covidien, Mansfield, MA). The gas was

slowly bubbled through the alkaline water in the evacuated blood collection tube to capture the H_2S, and immediately following, 0.5 mL DPD (N, N dimethyl-p-phenylenediamine sulfate solution) and 0.5 mL of ferric chloride reagents were injected into each evacuated blood collection tube using separate 1 mL tuberculin syringes and 22 gauge x 25.4 mm hypodermic needles (Monoject; Covidien, Mansfield, MA).

Concentrations of H_2S released into the gas produced during *in vitro* incubation were estimated using the methylene blue method (Siegel, 1965). Calibration solutions were prepared using RAD171 (Radiello Methylene Blue Calibration Standard for H_2S; Supelco Product No. RAD171, Bellefonte, PA) to calculate a standard calibration curve (Leibovich et al., 2009). The first calibration solution was prepared by adding 0.5 mL of RAD171 to 24.5 mL of distilled water. This first solution has a concentration of 1.145 µg/mL of S^{-2}. The second calibration solution was prepared with 3.75 mL of the first solution and 1.25 mL of distilled water to make a solution with a concentration of 0.85875 µg/mL of S^{-2}. The third calibration solution included 2.5 mL of the first solution and 2.5 mL of distilled water and had a concentration of 0.5725 µg/mL of S^{-2}. The fourth calibration solution was prepared with 1.25 mL of the first solution and 3.75 mL of distilled water to make a solution containing 0.28625 µg/mL of S^{-2}. A blank solution was prepared by adding 0.5 mL of DPD reagent followed by 0.5 mL of ferric chloride reagent to 5 mL of alkaline water. After 30 min of reaction time in dim lighting, absorbance of each sample was read at a wavelength of 665 nm on a spectrophotometer equipped with a vacuum receiver and sipper cell (Gilford Response™; Gilford Systems, Oberlin, OH).

The known S_2 concentrations of the calibration solutions were divided by 0.9409 (H_2S has 94.09% S) to obtain H_2S concentration (µg/mL). The absorbance values and H_2S concentrations of the calibration solutions were regressed to obtain a standard curve and regression equation. Absorbance of the blank solution was subtracted from all sample absorbance values to determine true absorbance of gas samples. The regression equation of the standard curve and true absorbance values were used to calculate H_2S concentration in each gas sample. Total corrected gas volume of each bottle was multiplied by H_2S concentration to determine total µg H_2S released which was then converted to total µmol H_2S released for each Mn concentration by dividing by the molecular weight of H_2S (34.08 g/mol). Rates of H_2S released per mL gas produced, per min of incubation, and per mg of substrate DM incubated were calculated.

Statistical Analyses. Data were analyzed using the MIXED procedure of SAS (SAS 9.2, SAS Institute, Inc.; Cary, NC). *In vitro* gas production and H_2S release were analyzed as repeated measures using an autoregressive covariance structure. Fixed model effects included Mn concentration, hour, Mn concentration by hour interaction, and replicate. The random effect was flask.

Rates of gas production and H_2S release, final pH, and change in final pH were analyzed using the MIXED procedure of SAS (SAS 9.2, SAS Institute, Inc.; Cary, NC). Fixed model effects included Mn concentration and replicate.

Statistical significance was declared with P-values ≤ 0.05, and trends were discussed with 0.05 < P-values ≤ 0.10. The PDIFF option was used to separate least squares means when a significant F-test statistic was present, and the largest standard error of the mean is reported.

Exp. 1 Results and Discussion

Cumulative gas production (mL) over time for each treatment is reported in Table 1. There was a significant (P = 0.02) Mn concentration x h interaction; therefore, simple effects for gas production values are discussed at each h of collection. At 2-h post-inoculation, gas production was similar (P ≥ 0.09) across all Mn treatment concentrations and averaged 83.2 mL. At 5-h post-inoculation, gas production was similar (P ≥ 0.25) across all Mn concentrations and averaged 127.5 mL. However, by 10-h post-inoculation, gas production from 2,000 ppm Mn was greater (P = 0.04) than 500 ppm Mn (174.4 vs. 170.2 mL) but similar (P ≥ 0.14) to 0, 1,000, 1,500, and 2,500 ppm Mn (171.8, 171.4, 172.2, and 172.9 mL, respectively). At 24-h post-inoculation, gas production from 2,000 ppm Mn was greater (P = 0.03) than 0 and 500 ppm Mn (216.8 vs. 212.2 and 212.3 mL) but similar (P ≥ 0.15) to 1,000, 1,500, and 2,500 ppm Mn (214.2, 215.3, and 213.9 mL). It is unclear why 2,000 ppm Mn produced more gas than 0 or 500 ppm Mn concentrations. Although *in vitro* gas production is a reflection of microbial fermentation when incubating substrate with ruminal fluid (Getachew et al., 1998), differences observed in total gas production are small and may not be biologically significant.

The interaction of Mn concentration x h for H_2S release was not significant (P = 0.19); therefore, main effects of treatment for H_2S release are reported (Table 2). Concentration of 1,000 ppm Mn released less (P = 0.03) H_2S than 0, 500, and 2,500 ppm Mn (2.64 vs. 3.37, 3.21, and 3.33 ± 0.17 µmol), but 1,000 ppm Mn released similar (P ≥ 0.19) H_2S as 1,500 (2.96 µmol) and 2,000 ppm Mn (2.69 µmol). May et al. (2010) replaced portions of steam-flaked corn with 15 or 30% WDGS and reported increased *in vitro* H_2S release as substrate S concentration increased from 0.13% S (1.23 µmol H_2S), 0.19% S (1.72 µmol H_2S), to 0.29% S (2.45 µmol H_2S) for substrate containing 0, 15, or 30% WDGS, respectively. Compared to the control containing 0.29% dietary S, 1.09% dietary S in substrate increased total 24-h *in vitro* gas production (95.1 vs. 98.6 mL) and H_2S (2.33 vs. 3.00 µmol) but did not affect VFA (139.9 vs. 137.3 mM), NH_3-N concentrations (49.3 vs. 46.7 mg/dL), VFA proportions, pH (6.29 vs. 6.31), or methane and hydrogen production in an *in vitro* experiment (Kung et al., 1998). Others have reported increasing *in vitro* H_2S release as S concentrations increase in substrate (Alves de Oliveira et al., 1997; Kung et al., 2000; Quinn et al., 2009; Smith et al., 2010), so it is logical to expect greater H_2S release in the current experiment compared to other experiments due to high S concentrations in all substrates. However, concentrations of supplemental Mn ranging from 1,000 to 2,000 ppm appeared to reduce total H_2S release perhaps through oxidation of H_2S into sulfate, S, and small amounts of thiosulfate (Herszage and Afonso, 2003), but it is unclear why the effect seems to be absent at 2,500 ppm Mn.

Rates of total gas production and H_2S release, as well as final pH and pH change, are reported in Table 2. The rate of H_2S release per mL of gas produced for 2,000 ppm Mn tended to be lower (P = 0.09) than rates for 0, 500, and 2,500 ppm Mn (0.027 vs. 0.033, 0.035, and 0.034 ± 0.002 µmol/mL) but similar (P ≥ 0.15) to 1,000 ppm (0.028 µmol/mL) and 1,500 ppm (0.031 µmol/mL).

Rate of gas production per min of incubation was similar ($P = 0.28$) and averaged 0.149 ± 0.001 mL/min across treatments. Rate of H_2S release per min of incubation was similar ($P = 0.12$) and averaged 0.005, 0.005, 0.004, 0.005, 0.004, and 0.005 ± 0.000 µmol/min for 0, 500, 1,000, 1,500, 2,000, and 2,500 ppm Mn. Rate of gas production per mg of incubated substrate DM was similar ($P = 0.25$) and averaged 3.015, 3.015, 3.044, 3.063, 3.079, and 3.038 ± 0.022 mL gas/mg substrate DM for 0, 500, 1,000, 1,500, 2,000, and 2,500 ppm Mn. Rate of H_2S release per mg of incubated substrate DM was similar ($P = 0.12$) and averaged 0.100, 0.104, 0.086, 0.096, 0.082, and 0.103 ± 0.006 µmol H_2S /mg substrate DM. Initial pH of the 1:1 solution of McDougal's saliva and rumen fluid was 6.69 across all treatments. Following 24-h incubation, final pH tended to be lower ($P = 0.06$) for 0 ppm Mn compared to 500, 1,000, and 1,500 ppm Mn (5.70 vs. 5.74, 5.73, and 5.74 ± 0.01) but was similar ($P \geq 0.13$) to 2,000 (5.72) and 2,500 (5.71) ppm Mn. Subsequently, overall pH change tended to be greater ($P = 0.06$) for 0 ppm Mn compared to 500, 1,000, and 1,500 ppm Mn (-0.995 vs. -0.955, -0.960, and -0.950 ± 0.010) but was similar ($P \geq 0.13$) to 2,000 (-0.973) and 2,500 (-0.978).

Experiment 2. Effect of supplementing 1,000 ppm manganese oxide to feedlot cattle diets with high dietary S concentrations on ruminal fermentation.

Exp. 2 Materials and Methods

A metabolism experiment was conducted at the feed efficiency facility located at North Florida Research and Education Center, Marianna, FL, to determine if 1,000 ppm Mn in high-S, finishing diets containing DDGS affects DMI, ruminal pH, and concentrations of ruminal H_2S gas and ammonia-N (NH3-N) in feedlot cattle. All surgical and research procedures were reviewed and approved by the University of Florida Institutional Animal Care and Use Committee. Seven Angus-crossbred beef steers (964 ± 134 lb initial BW) fitted with flexible ruminal and proximal duodenal cannula were assigned randomly to one of two pens (n = 3 and 4 per pen) in a switchback experimental design. Treatment diets were assigned to pen and contained (as-is basis; Table 3) 65% cracked corn, 20% dried DGS (First United Ethanol, LLC.; Camilla, GA), 8.2% Bahia grass hay, 0.46% dietary S, and either 0 or 1,000 ppm Mn. Dietary S concentration was increased with feed grade $CaSO_4$ (Calcium sulfate dihydrate; Maximo Sulca - DH, Yesera Monterrey, S.A., Mexico), and Mn concentration was increased with feed grade MnO (600,000 ppm; Manganous Oxide 60 Feed Grade; Erachem, Baltimore, MD).

The feed efficiency facility was equipped with GrowSafe® 4000 technology (GrowSafe® Systems Ltd.; Airdrie, Alberta, Canada) to monitor daily feed intake of all steers. To mimic once daily feeding typically practiced in conventional feedlots, steers were allowed *ad libitum* access to feed bunks from 0730 to 1630 throughout the experimental period. The experimental period consisted of 14 d, with 10 d of diet adaptation and 4 d of sample collection. Individual wireless rumen sensors (KB1102 bolus; Kahne Limited, Auckland, NZ) were placed in the rumen of each steer more than 18 h prior (on d 10) to the first gas collection time and were programmed to measure ruminal pH every 5 min throughout the duration of the 4-d sample collection period. Following completion of experimental period 1, wireless rumen sensors were removed from individual steers, pH data were downloaded to a computer, steers were rotated to opposite pens and placed on new treatments, and the experimental period was repeated.

Ruminal Gas Collection and Hydrogen Sulfide Measurement. On d 11 and 12 of each experimental period, ruminal gas samples were collected for analysis of H_2S concentration. Ruminal gas was collected from each steer using a 60 mL syringe with a 15.2 cm, 18 gauge needle (Popper® Deflected Noncoring Septum Penetration Needle, Thermo Fisher Scientific, Inc.; Pittsburgh, PA) that was inserted directly through the ruminal fistula plug into the head space of the rumen at 1 h prior to and 1, 2, 3, 4, and 6 h post-feed access (0730). For each steer at each time point, 30 mL of ruminal gas were extracted and depressed back into the ruminal head space two times prior to final extraction of 30 mL, of which 5 mL gas were then slowly bubbled into 5 mL of prepared alkaline water (pH 8) in a 10 mL evacuated blood collection tube using a 18 gauge x 25.4 mm hypodermic needle. All evacuated blood collection tubes were stored in an insulated container containing ice until all ruminal gas collections were completed for each day. The method for measuring H_2S is similar to the procedure described for Exp. 1, and all samples were prepared at one time.

Immediately following the last ruminal gas collection at 6 h post-feeding, 0.5 mL DPD and 0.5 mL of ferric chloride solution were injected into each evacuated blood collection tube using separate 1 mL syringes and hypodermic needles. Calibration solutions were prepared using RAD171 to calculate a standard calibration curve (Leibovich et al., 2009). A blank solution was prepared by adding 4 mL of DPD reagent followed by 4 mL of ferric chloride reagent to 40 mL of alkaline (pH 8) water.

After 30 min of reaction time in a dark room, 200 µL of each calibration solution and sample were plated in triplicate into a 96-well plate, with the blank and calibration solutions plated in the first wells. Absorbance of each sample was read at a wavelength of 665 nm on a microplate reader (Beckman Coulter AD 340C Absorbance Detector; Beckman Coulter, Inc., Brea, CA). The known S^{-2} concentrations of the calibration solutions were divided by 0.9409 (H_2S has 94.09% S) to obtain H_2S concentration (µg/mL). Absorbance of the blank solution was subtracted from all sample absorbance values to determine true absorbance of gas samples. The absorbance values and H_2S concentrations (µg/mL) of the calibration solutions were regressed to obtain a standard curve and regression equation. The regression equation of the standard curve and true absorbance values were used to calculate H_2S concentration in each gas sample. Total µg/mL H_2S was then converted to total µmol/mL H_2S by dividing by the molecular weight of H_2S (34.08 g/mol).

Ruminal Fluid Collection and Ruminal Ammonia-Nitrogen Concentration. On d 13 and 14, ruminal fluid samples were collected via rumen fistulae for ruminal NH3-N concentration. Ruminal grab samples were collected from cranial, caudal, left lateral, and right lateral areas of the rumen of each steer at 1 h prior to and 1, 2, 3, 4, and 6 h post-feed access (0730) to obtain a representative sample, mixed, and strained through three layers of cheesecloth into a 1,000 mL beaker. Ruminal fluid pH was measured with a benchtop pH meter (Pinnacle M530 Benchtop pH meter; Corning Inc., Lowell, MA). One 20 mL ruminal fluid sample was retained in a plastic, screw-cap, 50 mL conical tube, stabilized with 5 mL $H_2S\ O_4$, and immediately stored at -20° C for analysis of ruminal NH_3-N concentration at the North Dakota State University Ruminant Nutrition Laboratory (Fargo, ND). Ruminal NH_3-N concentration was determined according to

the procedure reported in Sigma Technical Bulletin #640. The concentration of NH_3-N was determined colorimetrically with a spectrophotometer (Beckman Coulter DU 800; Beckman Instruments, Inc.; Fullerton, CA) set at a wavelength of 570 nm.

Statistical Analyses. Data were analyzed using the MIXED procedure of SAS (SAS 9.2, SAS Institute, Inc.; Cary, NC). Steer DMI and ruminal pH were analyzed as repeated measures using an autoregressive covariance structure. Fixed model effects included treatment, day, hour and treatment by hour interaction. Random effect was steer nested within treatment. Due to a better model fit, ruminal NH_3-N concentration and cumulative H_2S concentration were analyzed as repeated measures using compound symmetry. Fixed model effects included treatment, day, hour, and treatment by hour interaction. Random effect was steer nested within treatment.

The area under the curve for ruminal H_2S concentration was calculated for each treatment and was analyzed using the MIXED procedure of SAS (SAS 9.2, SAS Institute, Inc.; Cary, NC). Fixed model effect was treatment and random effect was steer nested within treatment.

Statistical significance was declared with P-values ≤ 0.05, and trends were discussed with 0.05 < P-values ≤ 0.10. The PDIFF option was used to separate least squares means when a significant F-test statistic was present, and the largest standard error of the mean is reported.

Exp. 2 Results and Discussion

Effects of supplementing 1,000 ppm Mn in high-S finishing diets containing DDGS on DMI, ruminal pH over time, and ruminal H_2S and NH_3-N concentrations in feedlot steers are reported in Table 4. Daily DMI was similar ($P = 0.22$) across treatments and averaged 19.0 vs. 19.6 ± 1.1 lb/d for steers consuming MNO and CON, respectively. High dietary S concentrations of finishing diets (Bolsen et al., 1973; Zinn et al., 1997; Uwituze et al., 2011) or sulfate concentrations of drinking water (Loneragan et al., 2001) have been reported to reduce performance and negatively affect some carcass characteristics of feedlot cattle. Ruminal pH was higher ($P = 0.02$) at 1 h prior to feeding in steers consuming MNO (6.29) compared to steers consuming CON (6.01). However, no pH differences were observed ($P \geq 0.18$) between treatments at other time points measured, and ruminal pH was equal in all steers by 6 h post-feeding (5.89 vs. 5.78, 5.81 vs. 5.66, 5.74 vs. 5.62, 5.69 vs. 5.63, and 5.62 vs. 5.62 ± 0.07 for MNO vs. CON at 1, 2, 3, 4, and 6 h post-feeding, respectively).

Average ruminal H_2S concentration tended to be lower ($P = 0.09$) in steers consuming diets containing MNO compared to the CON diet (0.190 vs. 0.227 ± 0.016 µmol/mL). This observation follows results observed in Exp. 1 and could be attributed to higher ruminal pH in steers consuming 1,000 ppm Mn at 1 h prior to access to high-S finishing diets, thus promoting an initial ruminal environment less conducive to H_2S formation. The formation of H_2S is widely accepted to be pH-dependent, with greater formation occurring as pH is reduced (Gould, 1998). In contrast, Alves de Oliveira et al. (1997) reported no differences in sulfate or sulfide concentrations in a semi-continuous fermenter system when pH was reduced by 0.62 units, which may be representative of a typical pH reduction observed during chronic acidosis in feedlot cattle. *In vivo* values of ruminal sulfide concentration ranging from 0.07 to 0.35

µmol/mL have been observed in growing goats consuming diets with S concentrations close to requirements of 0.22% DM (Qi et al., 1993).

Although the 0.46% dietary S concentration fed in the current experiment exceeds the maximum recommended S concentration for finishing diets (NRC, 2005), the values observed for ruminal H_2S concentration in the current experiment are within the range observed when dietary S concentrations met animal requirements (Qi et al., 1993). However, values reported (main effects) in the current experiment are average ruminal H_2S concentrations from 1 h prior to access to feed through 6 h following access to feed and do not necessarily reflect maximum cumulative concentrations. Although cumulative ruminal H_2S concentrations (simple effects, data not shown) exceeded the range reported by Qi et al. (1993) as post-feeding time increased, no indications of S toxicity or PEM were observed in steers during this experiment.

Cummings et al. (1995) reported the capacity to generate H_2S from sulfate in steers increased after 10 to 12 d of feeding a high-S diet; however, Alves de Oliveira et al. (1997) reported 7 d of adaptation to a high-S diet in an *in vitro* semi-continuous fermenter system were sufficient to reach high sulfide production capacity. Dietary inorganic S consumed in the form of sulfates is a greater threat to ruminant health than organic sources of S because sulfates are more readily reduced to S^{-2} by sulfate-reducing bacterial populations in the rumen. Sulfides then combine with H^+ through a dissimilatory reduction process to result in H_2S that may accumulate to toxic concentrations in the rumen (Gould et al., 2002; Kung, 2008). Calculated area under the curve for ruminal H_2S concentration in this experiment was similar (P = 0.26) between treatments and averaged 0.505 and 0.572 ± 0.041 µmol*h/mL for MNO and CON. It appears supplemental MnO had minimal effects on reducing the formation of H_2S from dietary sulfates in the rumen. One explanation of this result may be attributed to dietary phosphate reducing the rate of sulfide oxidation by occupying the surface sites of the MnO molecule and preventing surface-controlled reduction of H_2S (Yao and Millero, 1993).

Ruminal NH_3-N concentration was not different (P = 0.32) between treatments and averaged 10.4 and 8.9 mg/dL for MNO and CON. Ruminal NH_3-N concentrations in the current experiment were lower than values reported by Alves de Oliveira et al. (1997) when evaluating the effect of acidic conditions in a semi-continuous fermenter system on NH_3-N concentrations. Even when the pH of liquid outflow was reduced by 0.62 units (representative of chronic acidosis in feedlot cattle), the *in vitro* NH_3-N concentrations remained in the range of 15 to 21 mg/dL, which is considered to be adequate to satisfy microbial requirements (Alves de Oliveira et al., 1997). Wallace (1979) observed increased bacterial growth and subsequent increased *in situ* rates of DM and CP degradation of barley grain when ruminal fluid NH_3-N concentrations increased from 9.7 to 21.4 mg/dL, which is in agreement with Erdmann et al. (1986). In contrast, Satter and Slyter (1974) suggested *in vitro* ruminal NH_3-N concentrations greater than 5 mg/dL would meet requirements to allow for optimal microbial growth rates and fermentation. Differences in the reported optimal ruminal NH_3-N concentrations may be due to techniques employed, substrates evaluated, or criteria used for evaluation, particularly because N recycling back to the rumen can significantly alter the ruminal requirement for optimal NH_3-N concentrations and corresponding dietary CP concentration to meet these requirements

(Pritchard and Males, 1985). The observed similar ruminal NH_3-N concentrations and DMI in the current experiment suggest microbial requirements for N were met and fermentation was not limited in steers fed either treatment diet even though VFA production was not measured directly.

Experiment 3. Effect of supplementing 1,000 ppm manganese oxide to feedlot cattle diets with low and high dietary S concentrations on live performance and carcass characteristics.

Exp. 3 Materials and Methods

The feedlot experiment consisted of 182 head (107 crossbred heifers and 75 crossbred steers, initial BW = 728 ± 62 lbs) blocked by weight and sex and arranged into 16 pens. All experimental procedures were reviewed and approved by the University of Minnesota Institutional Animal Care and Usage Committee. Steer pens (n=8) contained 9-10 head/pen, while heifer pens (n=8) contained 13-14 head/pen. Cattle were fed one of four dietary treatments arranged in a 2 x 2 factorial. The two factors were dietary S concentration of 0.35 or 0.60% (DM basis), and dietary MnO supplementation of 0 or 1,000 ppm. The arrangement allowed for four pen replications per simple effect and eight pen replications per main effect. The basal diet contained 44% high moisture corn, 35% wet DGS, 9% corn silage, 3% straw, and 9% supplement. Wet DGS were sourced from Otter Tail Ag Enterprises in Fergus Falls, MN, and contained approximately 34% DM, 29.5% CP, and 0.85% S. Sulfur concentration of DGS ranged from 0.80 to 0.91% during the experiment. High S concentrations in the 0.60% S treatments were achieved through supplementation of a gypsum premix.

Cattle were fed for *ad libitum* intake once daily at 0800 and were weighed every 28 d. Cattle were marketed upon visual appraisal of approximately 0.40 in. of backfat. This resulted in the heavy block of steers and heifers being on feed for 173 d and the light block on feed for 201 d. Heavy block cattle were harvested at Tyson Fresh Meats in Dakota City, NE, and light block cattle were harvested at JBS Swift in Grand Island, NE. Upon harvest, carcasses were evaluated and for hot carcass weight (HCW), ribeye area (REA) at the 12th rib, backfat (BF) at the 12th rib, marbling score, and USDA quality grade (QG) and yield grades (YG).

Statistical Analyses. Feedlot growth and feed intake data, as well as BF, REA, HCW, marbling, and average USDA QG and YG were analyzed using the MIXED procedure of SAS (SAS 9.2, SAS Institute, Inc.; Cary, NC). Pen served as the experimental unit for all analyses. Fixed effects consisted of dietary S concentration, dietary MnO supplementation, and their interaction. Cattle sex and weight block were included in the model as random effects.

Categorical data (frequency of individual USDA YG and QG) were analyzed using the GENMOD procedure of SAS (SAS 9.2, SAS Institute, Inc., Cary, NC). Pen served as the experimental unit for analyses. Fixed effects consisted of dietary S concentration, dietary MnO supplementation, and their interaction. Cattle sex and weight block were included in the model as random effects.

Simple effects were evaluated when a significant ($P < 0.10$) F-test was observed for any variable. When a significant F-test was not observed for any interaction, main effects were evaluated. Differences were considered significant when $P < 0.05$, and trends were discussed when P was less than 0.10 but greater than 0.05.

Exp. 3. Results

Live performance data are shown in Table 5. After 28 d on feed, cattle consuming low-S treatments had a 12% greater DMI ($P < 0.01$) and a 25% greater ADG ($P < 0.01$) than those consuming high-S treatments. Though MnO supplementation did not affect DMI ($P = 0.31$), there was a trend ($P = 0.09$) for greater ADG with MnO supplementation through the first 28 d on feed. There was also a S x MnO interaction for feed conversion, where the-high S, no MnO treatment had a poorer feed conversion than all other treatments ($P < 0.01$; Figure 1).

These results suggest that MnO had an effect on feedlot performance when supplemented to high-S diets through 28 d. McAllister et al. (1997) reported the incidence of PEM cases peaked between 15 and 30 d on feed. The relationship to days on feed could be due to the changes in the ruminal environment that are associated with adapting cattle to a high-concentrate diet. Sager et al. (1990) observed clinical signs of PEM beginning on d15 after adaptation to a high-concentrate diet with excess S. During this time, ruminal pH becomes increasingly more acidic. The pKa for hydrogen sulfide is 7.2 (Kung et al., 1998), which indicates that as ruminal pH decreases, a greater amount of hydrogen sulfide will be in the more toxic protonated form. At a ruminal pH in the 5.0-5.5 range, which will occur with high-concentrate rations, nearly 100% of the hydrogen sulfide would be in the protonated form.

Beyond the MnO results observed at d 28, we did not observe any MnO effects or S x MnO interactions throughout the experiment, and by the end of the experiment the cattle consuming the 0 and 1,000 ppm MnO treatments had similar ($P \geq 0.35$) final BW, DMI, ADG, and feed conversion. This suggests that the inclusion of MnO may be useful when cattle are adapting to a high-S and high-concentrate diet, but loses its efficacy beyond that time.

Though no MnO effects were observed beyond d 28, high dietary S concentrations continued to suppress feedlot performance. Cattle consuming the low-S treatments had 10% greater final BW ($P < 0.01$), 19% greater DMI ($P < 0.01$), 25% greater ADG ($P < 0.01$), and a 5% improvement in feed conversion ($P = 0.02$).

Carcass characteristics are shown in Table 6. A S x MnO interaction ($P = 0.01$) was present for frequency of carcasses grading Choice or greater. Cattle that consumed the high-S, no MnO treatment had lower percentage Choice or greater carcasses than all other treatments (Figure 2). A S x MnO interaction ($P = 0.01$) was present for frequency of No Roll carcasses, where the high-S, no MnO treatment produced 12.2% No Roll carcasses, which was greater ($P < 0.04$) than either the low-S, no MnO treatment (0.0%) or the high-S, 1,000 ppm MnO treatment (3.6%). The low-S, 1,000 ppm MnO treatment produced 9.4% No Roll carcasses, which was greater ($P = 0.02$) than the low-S, no MnO treatment and not different ($P > 0.14$) from the other two treatments (data not shown).

A S x MnO interaction ($P = 0.03$) was also present for frequency of USDA yield grade (YG) 1 carcasses. The high-S, no MnO treatment produced 12.2% YG 1 carcasses, which was greater ($P < 0.02$) than all other treatments (data not shown). This result corresponds with the Choice and higher and No Roll results, which generally show the high-S, no MnO treatment to have produced a lower quality, less-finished carcass.

A surprising S x MnO interaction ($P < 0.01$) occurred for LM area, where the low-S, no MnO treatment produced a 14.3 sq in LM area, which was greater ($P < 0.02$) than all other treatments. The low-S, 1,000 ppm MnO and high-S, 1,000 ppm MnO treatments each produced 13.8 sq in. LM area, which were greater ($P < 0.02$) than the high-S, no MnO treatment (13.3 sq in; data not shown).

No other S x MnO interactions were present for any carcass characteristic ($P > 0.18$). Manganese oxide inclusion did not affect ($P > 0.17$) any measured carcass characteristic.

Overall, it appears that MnO may have a positive impact on beef cattle feedlot performance, particularly when supplemented to high-S feedlot diets during the first 28 d on feed. The effects of dietary S concentration were present throughout the experiment, and appeared to accumulate over time.

Overall Conclusions and Implications

Through this set of experiments it appears that MnO may have application in attenuating high S concentrations in beef cattle diets. The effect appears to be minimum concentration of MnO that appears to be effective is 1,000 ppm. It also appears that this concentration is most effective during the first 28 days on feed, which generally corresponds with the period in which feedlot cattle are being adapted to a high-concentrate, and in many cases, high-S diet. According to the *in vitro* and *in vivo* experiments, it appears that the response is due to a less acidic ruminal environment when MnO is supplemented, which leads to less H_2S production and therefore decreases the incidence of H_2S toxicity. The positive effect of MnO supplementation appears to disappear after 28 days on feed, which suggest that cattle adapt to high S concentrations over time.

Implications from this experiment suggest that MnO should be included in feedlot diets that contain dietary S concentrations at or near 0.60% of DM. However, in the feedlot experiment there were no treatments testing the response in intermediate dietary S diets between 0.35 and 0.60% dietary S. Therefore, the actual breakpoint where MnO supplementation is useful remains unknown, and warrants further investigation. In addition, because cattle consume more feed, and therefore more dietary S, as they grow, it is possible that the amount of MnO necessary to offset dietary S may change over time, and increased supplementation with increased dietary S intake could have a benefit.

Table 1. Cumulative gas production (mL) from *in vitro* fermentation of high-sulfur substrate containing dried distillers grains plus solubles and experimental concentrations of manganese supplied as manganese oxide (Exp. 1)[1].

	Concentration of Mn, ppm					
Timepoint[2]	0	500	1,000	1,500	2,000	2,500
0	---	---	---	---	---	---
2	83.5	81.8	82.1	82.7	85.2	84.0
5	127.2	127.5	127.5	126.3	128.1	128.5
10	171.8[ab]	170.2[a]	171.4[ab]	172.2[ab]	174.4[b]	172.9[ab]
24	212.2[a]	212.3[a]	214.2[ab]	215.3[ab]	216.8[b]	213.9[ab]
SEM[3]	1.2	1.2	1.2	1.5	1.5	1.2

[1]*P*-values for cumulative *in vitro* gas production included: Mn concentration $P = 0.57$, h $P < 0.01$, and interaction of Mn concentration by h $P = 0.02$.

[2]Number of hours post-inoculation of the *in vitro* batch culture.

[3]Standard error of the mean within Mn concentration.

[ab]Means within row with uncommon superscripts differ ($P \leq 0.05$).

Table 2. Effects of *in vitro* fermentation of high-sulfur substrate containing dried distillers grains plus solubles and experimental concentrations of manganese supplied as manganese oxide on total hydrogen sulfide released, total hydrogen sulfide released per mL of gas produced, rates of gas production, rates of hydrogen sulfide production, final pH, and pH change (Exp. 1).

	Concentration of Mn, ppm							
Item	0	500	1,000	1,500	2,000	2,500	SEM[1]	*P*-Value[2]
H_2S, µmol/24 h	3.37[a]	3.21[ab]	2.64[c]	2.96[ac]	2.69[bc]	3.33[a]	0.17	0.03
H_2S per mL Gas, µmol/mL	0.033[ac]	0.035[a]	0.028[bc]	0.031[ab]	0.027[b]	0.034[a]	0.002	0.09
Rate (Gas), mL/min	0.147	0.148	0.149	0.150	0.150	0.149	0.001	0.28
Rate (H_2S), µmol/min	0.005	0.005	0.004	0.005	0.004	0.005	0.000	0.12
Rate, mL gas/mg Diet DM	3.015	3.015	3.044	3.063	3.079	3.038	0.022	0.25
Rate, µmol H_2S/mg Diet DM	0.100	0.104	0.086	0.096	0.082	0.103	0.006	0.12
Final pH	5.70[b]	5.74[a]	5.73[a]	5.74[a]	5.72[ab]	5.71[ab]	0.01	0.06
pH Change	- 0.995[b]	- 0.955[a]	- 0.960[a]	- 0.950[a]	- 0.973[ab]	- 0.978[ab]	0.010	0.06

[1]Standard error of the mean.

[2]Main effect of the treatment.

[abc]Means within row with uncommon superscripts differ ($P \leq 0.05$).

Table 3. Formulated ingredient and analyzed chemical composition of the high-sulfur finishing diet fed to beef steers (Exp. 2).

Ingredient	% of DM
Cracked Corn	65.0
Dried DGS[1]	20.5
Bahia Grass Hay	8.4
Mineral Mix	0.2
White Salt	0.2
Liquid Supplement	4.0
$CaSO_4$[2]	1.5

Chemical[3]	Mean	SD
DM, %	88.9	0.4
CP, %	15.0	1.3
NDF, %	22.4	5.8
ADF, %	12.4	3.9
TDN, %	79.0	3.4
NEg, Mcal/lb	0.59	0.05
S, %	0.46	0.12
Ca, %	0.67	0.07
P, %	0.42	0.06
Mg, %	0.27	0.04
K, %	0.88	0.08
Na, %	0.10	0.04
Cu, ppm	14.3	5.7
Fe, ppm	133.5	34.9
Zn, ppm	47.5	6.5

[1]Dried distillers grains plus solubles were sourced from First United Ethanol, LLC, Camilla, GA. Manganous oxide (600,000 ppm; Manganous Oxide 60 Feed Grade; Erachem, Baltimore, MD) was mixed in with the DDGS in the 1,000 ppm MNO treatment diet only. The MNO diet contained 959 ppm Mn and the CON diet contained 60 ppm Mn (analyzed by Dairy One Forage Analysis Laboratory).
[2]Calcium sulfate (Calcium sulfate dihydrate; Maximo Sulca - DH, Yesera Monterrey, S.A., Mexico) was added to the diet to increase dietary S to a targeted concentration of 0.5% DM.
[3]Diets were analyzed for chemical analysis by Dairy One Forage Analysis Laboratory (Ithaca, NY). Mean and standard deviation (SD) values are derived from the average of four diet samples analyzed by Dairy One Forage Analysis Laboratory. Values are reported on a DM-basis.

Table 4. Effects of supplementing 1,000 ppm manganese in high-sulfur finishing diets on dry matter intake, ruminal pH, and ruminal hydrogen sulfide and ammonia-nitrogen concentrations in beef steers (Exp. 2).

	Treatment[1]			*P*-Value[2]		
Item	MNO	CON	SEM[3]	Treatment	Time	Treatment x Time
DMI, lb/d	19.0	19.6	1.1	0.46	< 0.01	0.22
Ruminal pH[4]						
-1 h	6.29[a]	6.01[b]	0.07	0.22	< 0.01	0.02
1 h	5.89	5.78	0.29			
2 h	5.81	5.66	0.18			
3 h	5.74	5.62	0.29			
4 h	5.69	5.63	0.53			
6 h	5.62	5.62	0.98			
H_2S, µmol/mL	0.190	0.227	0.016	0.09	< 0.01	0.31
H_2S AUC, µmol*h/mL	0.505	0.572	0.041	0.26	---	---
NH_3-N, mg/dL	10.4	8.9	1.0	0.32	< 0.01	0.09

[1]Treatments included finishing diets either containing 1,000 ppm supplemental Mn (MNO) or 0 ppm supplemental Mn (CON).
[2]Main effects of treatment are reported when Treatment x Time interaction *P*-values > 0.05; Simple effects are reported when Treatment x Time interaction *P*-values ≤ 0.05.
[3]Standard error of the mean.
[4]Time points listed are hours relative to time of access to feed by steers (0730 daily); thus, -1 h = average of pH values measured every five min between 0630 and 0730; 1 h = average of pH values measured every five min between 0830 and 0930; 2 h = average of pH values measured every five min between 0930 and 1030; 3 h = average of pH values measured every five min between 1030 and 1130; 4 h = average of pH values measured every five min between 1130 and 1230; and 6 h = average of pH values measured every five min between 1330 and 1430.
[ab]Means within row with uncommon superscripts differ (*P* ≤ 0.05).

Table 5. Main effects of manganese oxide inclusion in low and high-S feedlot diets on beef cattle feedlot performance (Exp. 3).

	Sulfur Concentration, %		Manganese Oxide, ppm			*P*-values		
Item	0.35	0.60	0	1,000	SEM[1]	Sulfur	MnO	SxMnO
Initial BW, lbs	728	727	727	728	27	0.76	0.40	0.43
Day 28 BW, lbs	829	808	812	824	25	<0.01	0.04	0.19
DMI d 0-28, lbs/d	18.7	17.3	17.8	18.1	0.2	<0.01	0.31	0.63
ADG d 0-28, lbs	3.60	2.88	3.05	3.43	0.20	<0.01	0.09	0.15
F:G[2] d 0-28	5.19	6.01	5.84	5.28	0.33	0.01	0.03	0.03
Final BW, lbs	1,357	1,230	1,295	1,292	17	<0.01	0.80	0.31
Final DMI, lbs/d	22.7	19.1	20.7	21.0	0.2	<0.01	0.35	0.31
Final ADG, lbs	3.37	2.69	3.05	3.02	0.09	<0.01	0.65	0.28
Final F:G[2]	6.73	7.10	6.79	6.95	0.17	0.02	0.35	0.10

[1] Standard error
[2] Feed:Gain ratio

Table 6. Main effects of manganese oxide inclusion in low and high-S feedlot diets on beef cattle carcass characteristics (Exp. 3).

	Sulfur Concentration, %		Manganese Oxide, ppm			*P*-values		
Item	0.35	0.60	0	1,000	SEM[1]	Sulfur	MnO	SxMnO
HCW, lbs	858	777	818	817	10	<0.01	0.81	0.31
Backfat, in	0.58	0.47	0.52	0.52	0.04	<0.01	0.74	0.91
REA[2], sq in	14.1	13.5	13.8	13.8	0.4	<0.01	0.97	<0.01
Marbling[3]	521	492	492	520	18	0.19	0.21	0.49
USDA YG[4]	3.14	2.79	2.94	2.98	0.20	<0.01	0.66	0.26
YG 1, %	1.39	8.39	6.11	2.67	1.57	0.01	0.28	0.03
YG 2, %	14.0	19.4	18.7	14.7	3.0	0.22	0.36	0.23
YG 3, %	28.2	22.1	22.8	27.5	3.4	0.22	0.33	0.33
YG 4, %	8.81	8.84	7.45	9.52	2.32	0.83	0.53	0.65
YG 5, %	1.00	0.00	1.00	0.00	0.51	0.19	0.19	0.19
Prime QG[5], %	6.46	5.10	4.89	6.66	2.30	0.67	0.59	0.90
Choice QG, %	80.0	74.1	75.7	78.4	2.6	0.12	0.47	0.01
Prime+Choice, %	86.5	79.2	80.6	85.1	2.5	0.06	0.23	0.01
Select QG, %	8.9	12.9	13.3	8.5	2.4	0.25	0.17	0.84
No Roll QG, %	4.70	7.90	6.11	6.49	2.01	0.27	0.90	0.01

[1] Standard error.
[2] Ribeye (longissimus muscle) area measured at the 12th rib.
[3] Marbling score assessed by USDA grader where 400 = Small^0, 500 = Modest^0, etc.
[4] USDA yield grade assessed by USDA grader.
[5] USDA quality grade assessed by USDA grader.

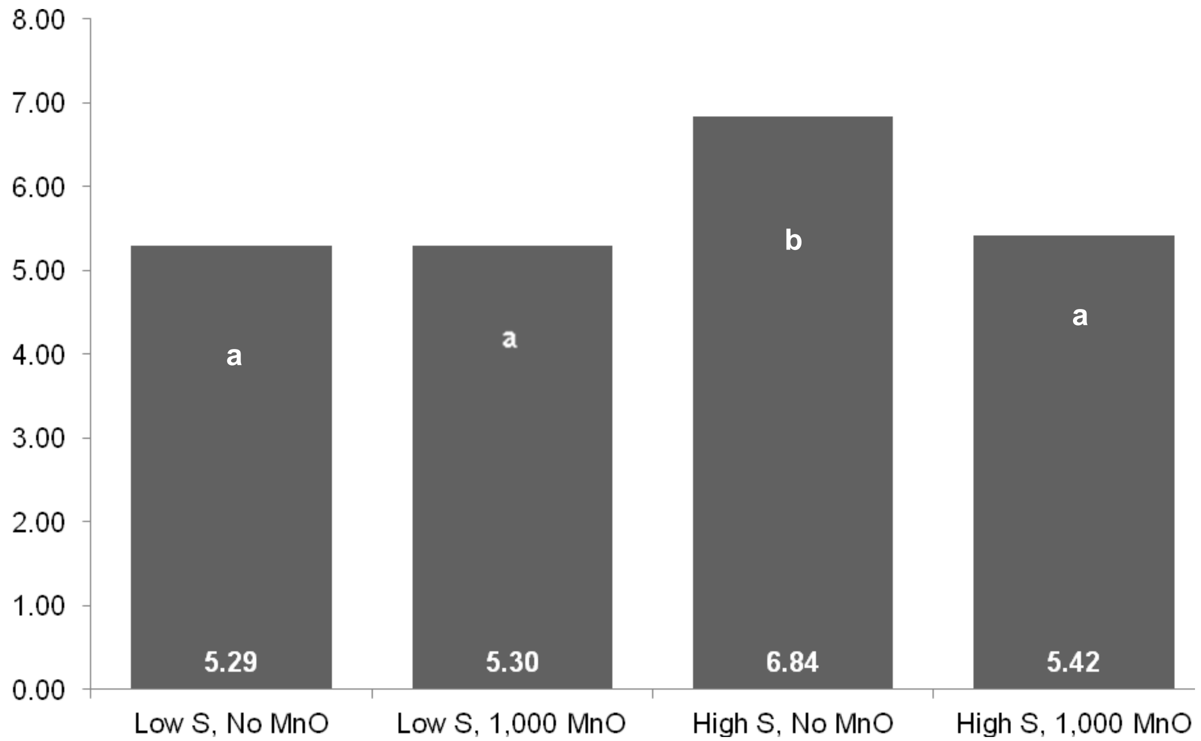

Figure 1. Simple effects of dietary S and MnO concentration on feed conversion during the first 28 days on feed (Exp. 3).
[ab] Uncommon scripts indicate difference ($P < 0.05$). Standard error = 0.38.

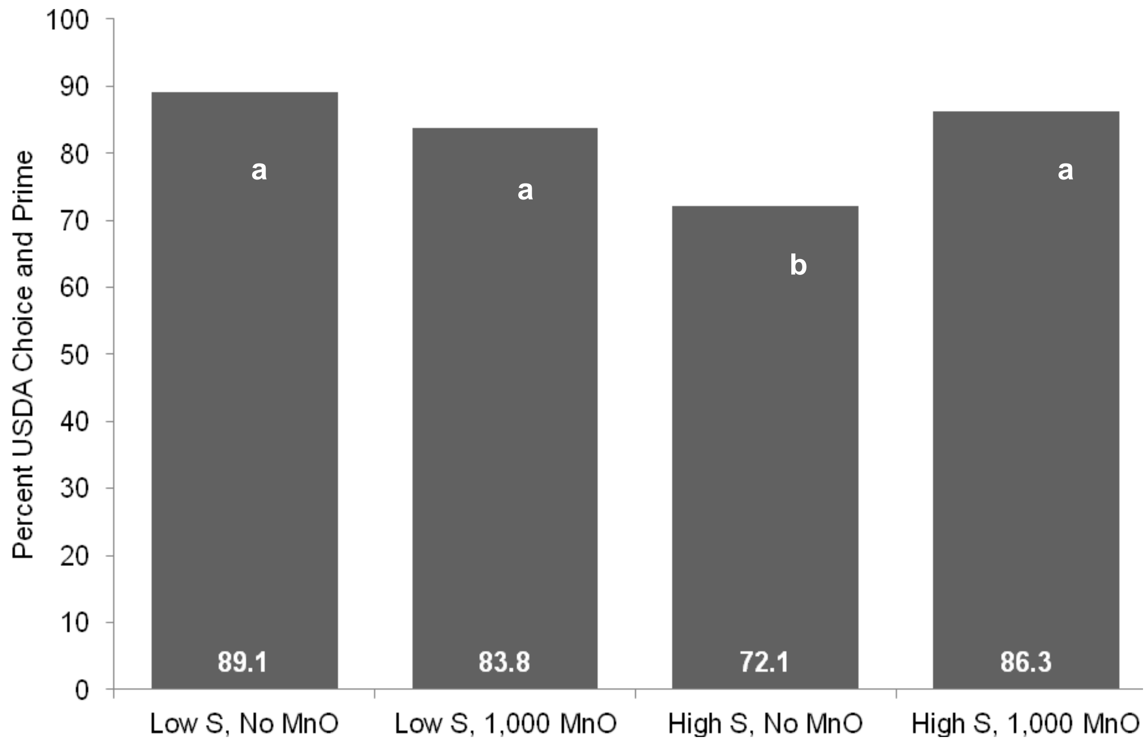

Figure 2. Simple effects of dietary S and MnO concentration on percentage of carcasses grading USDA Choice and Prime (Exp. 3).

[ab] Uncommon scripts indicate difference ($P < 0.05$). Standard error = 3.9.

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