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Establishing reference ranges for sulfur concentrations in bovine liver and kidney samples

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Abstract

Sulfur toxicosis is a common cause of polioencephalomalacia in cattle. Diagnosis requires comprehensive determination of dietary sulfur intake, which may not be possible if feedstuffs are no longer available. The goal of this study was to establish reference ranges for concentrations of sulfur in liver and kidney samples from cattle dying of causes unrelated to sulfur toxicosis. Samples were collected from 71 cattle and assessed for sulfur content using inductive coupled plasma atomic-emission spectroscopy. Cattle demonstrating neurologic signs or gross or histologic lesions of the brain were excluded from the study. Sulfur concentrations were calculated on a wet- and dry-matter basis. Values were examined for Gaussian distribution, and 95% confidence intervals were calculated using the mean for normally distributed values and the median for non-normally distributed values. The 95% confidence interval for liver sulfur was 4,522 to 9,982 ppm dry-matter (1,270 to 2,640 ppm wet-matter). The 95% CI for kidney sulfur was 5,070 to 19,017 ppm dry-matter (1,187 to 2,918 ppm wet-matter). Correlation assessments suggest that dry-matter liver analysis may be the best defined and most reliable measure of sulfur burden. Additional research is needed to assess concentrations of sulfur present in the liver and kidney of cattle with sulfur toxicosis, the impact of dietary intake on tissue concentrations, and to determine whether postmortem sulfur measurement is a viable method of diagnosing sulfur toxicosis.

Key words: polioencephalomalacia, PEM, sulfur, sulfate, cattle, distillers grains

Résumé

L'intoxication au soufre est une cause fréquente de la polio-encéphalomalacie chez les bovins. Le diagnostic requiert de bien connaître l'apport alimentaire en soufre ce qui n'est pas possible si les denrées ne sont plus disponibles. Le but de cette étude était d'établir des limites de référence pour la concentration de soufre dans des échantillons de foie et de rein prélevés chez des bovins morts pour d'autres raisons que l'intoxication au soufre. Des échantillons ont été recueillis à partir de 71 bovins et la concentration de soufre a été déterminée avec la spectroscopie de masse utilisant un plasma à couplage inductif. Les bovins montrant des signes neurologiques ou des lésions macroscopiques ou histologiques au cerveau ont été exclus de l'étude. La concentration de soufre a été établie sur la base de matière sèche ou humide. La normalité des données a été examinée et des intervalles de confiance à 95% ont été calculés en utilisant la moyenne pour les valeurs normalement distribuées ou la médiane pour les distributions non-normales. L'intervalle de confiance à 95% pour la concentration de soufre dans le foie était de 4,522-9,982 ppm de matière sèche (1,270-2,640 ppm de matière humide). L'intervalle de confiance à 95% pour la concentration de soufre dans le rein était de 5,070-19,017 ppm de matière sèche (1,187-2,918 ppm de matière humide). L'approche de la corrélation suggère que l'analyse au niveau du foie sur la base de la matière sèche serait la mesure la mieux définie et la plus fiable de la charge de soufre. Des travaux supplémentaires sont nécessaires afin de déterminer la concentration de soufre présent dans le foie et dans le rein chez les bovins avec toxicose de soufre, de mesurer

l'impact de l'apport alimentaire sur la concentration dans les tissus et d'établir si la mesure postmortem de soufre est une méthode fiable pour diagnostiquer l'intoxication au soufre.

Introduction

Sulfur toxicosis is a common cause of polioencephalomalacia (PEM) in cattle, a disease that can result in unexpected deaths.[16,18,22] PEM is grossly descriptive of the loss of cortical brain parenchyma that correlates with laminar cortical necrosis histologically, especially of neurons. These lesions can result from a number of different insults, including water deprivation/salt toxicosis, lead intoxication, thiamine deficiency, and sulfur toxicosis. In cases of peracute illness and death related to sulfur toxicosis, histologic lesions may be mild or absent, thereby hindering diagnosis.

Clinical diagnosis of PEM may be based upon clinical signs, response to thiamine administration, or cessation of additional cases following appropriate intervention. A variety of circumstances can suggest sulfur toxicosis as a cause or contributor to PEM, including historic information on sulfur content in feed and water, introduction of new feed or water with potentially elevated sulfur content, and cessation of additional cases following dietary changes. Attempts to diagnose sulfur toxicosis antemortem have been unrewarding,[7] and currently the only way to definitively diagnose sulfur toxicosis as the cause of PEM or unexpected death is through documentation of excessive dietary sulfur intake. Examination of all feed and water sources is necessary, as intake from various sources is additive. Feed or water with marginally high sulfur content may be safe if combined with low-sulfur water or feeds, but combining two or more marginally high sources can result in clinical disease.

Elevated sulfur has myriad sources, including plants, water, and processed feedstuffs. Water used for animal agriculture in some parts of the United States has a naturally elevated sulfur content.[27] Various plants, including those within the *Brassicaceae* and *Kochia* families, can accumulate sulfur.[10,11,14,18,22] While most grasses have low sulfur content, this can vary by circumstance, and high protein forages, such as alfalfa, can serve as a notable source of sulfur.[9] Feed can be secondarily contaminated with sulfur through the application of pesticides, or sulfur can be introduced into the diet directly through such things as medication or supplements.[12,22] Yet, the most concerning source of dietary sulfur for beef cattle is dried distillers grains with solubles (DDGS) and other corn co-products. With expanded North American ethanol production, these co-product feedstuffs have become readily available and frequently utilized as high-value feed sources.[4,7,25] Sulfuric acid is added during fermentation and is used to flush distillation columns, resulting in DDGS and other ethanol co-products frequently contributing excessive sulfur to diets. Both performance and health issues, including PEM, have been documented in cattle where DDGS composed a large percentage of the diet.[2,13,28] Most troubling, the amount of sulfur present in corn co-products is highly variable.[4,25] Thus, each new batch requires analysis to determine sulfur concentrations.

Dietary recommendations for sulfur intake have been made, with a maximum suggested intake of 0.4% of dietary sulfur (dry matter basis) for beef cattle.[21] However, cattle consuming high-forage diets are at less risk of sulfur-associated PEM than cattle on high-concentrate diets, and cattle on feed 15 to 30 days appear to be at greatest risk.[17,18,23] Feeding higher amounts (0.6 to 1.2% sulfur) does not necessarily induce PEM;[5,6] these variables may make interpretation of dietary sulfur values challenging. Moreover, assessment of dietary sulfur intake requires that all feed and water sources be evaluated to determine the total quantity of sulfur consumed per head. In some cases, the feed or water have been completely consumed prior to diagnosis and are unavailable for testing.

Though sulfur concentrations can be measured in liver and kidney tissue using inductive-coupled plasma analysis-atomic emissions spectroscopy (ICP-AES), the results cannot be interpreted due to lack of published normal values from cattle.[15] The objective of the study reported here was to use ICP-AES to develop reference ranges for sulfur in tissues from cattle with no clinical signs or histologic lesions of PEM. This could provide a basis for additional work examining tissues from cattle with PEM, as well as those consuming elevated amounts of sulfur, with the ultimate goal of enabling the diagnosis of sulfur toxicosis when complete information regarding sulfur concentrations in feed and water is unavailable.

Materials and Methods

Tissue Collection

Tissue samples were collected from 71 cattle presented to the Oklahoma Animal Disease Diagnostic Laboratory for necropsy between January 2010 and March 2012. Twelve cattle were euthanized as part of an unrelated study; the remainder were client-owned submissions. Cattle demonstrating neurologic signs or gross or histologic lesions of the brain were excluded from the study. The cattle ranged from <1 year to 10 years in age. Approximately 100 grams of kidney and liver tissue were collected from eligible animals and stored at -4°F (-20°C) until transported for analysis.

Determination of Sulfur Concentrations

Samples were shipped overnight on ice to the New York State Animal Health Diagnostic Center at

Cornell University, where each sample was processed using a modified microwave digestion procedure previously described,[a] and analyzed using ICP-AES. Instrument conditions were set as recommended by the manufacturer.[b] Briefly, samples were weighed into Teflon™ vessels which were closed prior to digestion using nitric acid, hydrochloric acid, and hydrogen peroxide at 410°F (210°C). ICP-AES was used to determine the concentrations of sulfur by monitoring the plasma emission at 181.975 nm. Due to the low wavelength, a high-flow nitrogen purge was used on the spectrometer to ensure the exclusion of atmospheric oxygen. The moisture content of the samples was determined by AOAC official method of analysis.[1] The resulting mineral concentrations were calculated on a dry-weight and wet-weight basis.

Statistical Analysis

Values for liver and kidney sulfur concentration were assessed for skewness, kurtosis and normality by the D'Agostino-Pearson test for normal distribution. Data determined to be normally distributed had a mean and standard deviation calculated, which was used to create a 95% confidence interval. For data with a non-Gaussian distribution, the median was determined and a 95% confidence interval created using the non-parametric percentile method.[3] Spearman's rho correlation coefficients were calculated to assess how well the values from the various tissue types correlated with each other. Statistical analysis was done using Microsoft Excel[c] and MedCalc.[d]

Results

Results are summarized in Table 1. Liver samples had a mean dry-matter of 27.5% (SD 5.1%). Liver sulfur concentrations calculated on both dry- and wet-matter basis demonstrated acceptable kertosis and skewness, and were found to be normally distributed by D'Agostino-Pearson normality test (P=0.62 for dry; P=0.48 for wet). Liver sulfur concentration on dry-matter basis ranged from 3,670 to 11,041 parts per million (ppm). The mean was 7,252 ppm, with a standard deviation of 1,393, creating a 95% confidence interval (CI) of 4,522 to 9,982 ppm. Liver concentrations on a wet-matter basis ranged from 966 to 2,763 ppm. The mean wet-matter basis liver sulfur concentration was 1,955, with a standard deviation of 350. This produces a 95% CI of 1,270 to 2,640 ppm.

Kidney samples had a mean dry-matter content of 21.3% (SD 4.0%). Kidney sulfur concentrations demonstrated notable skewness and kurtosis, with dry-basis values being more deranged than wet-basis values. Both dry- and wet-basis values failed to meet D'Agostino-Pearson criteria for normality (P<0.001). Kidney sulfur concentration on a dry-matter basis ranged from 4,353 ppm to 21,633. The median value was 7,869 ppm, with a 95% CI of 5,070 to 19,017 ppm. Kidney sulfur concentrations on a wet-matter basis ranged from 1,020 to 3,180 ppm. The median value was 1,622 ppm, with a 95% CI of 1,187 to 2,918 ppm.

Sulfur concentrations calculated on a dry-matter basis from liver samples correlated only moderately well with the wet-matter basis of those same samples (Spearman rho of 0.395). There was stronger correlation between dry- and wet-matter basis values for kidney samples (0.536). There was moderate correlation between liver and kidney values calculated on a wet-basis (rho =0.365), but strong correlation (0.609) between tissues when examined on a dry-matter basis. All of these correlations were significant at the 0.002 level (2-tailed).

Discussion

Sulfur toxicosis is a common cause of PEM in cattle.[8] Consumption of sulfur-containing compounds results in dissimilatory bacteria in the rumen converting much of the various forms of sulfur to hydrogen sulfide gas. Some of these sulfur compounds are absorbed into the bloodstream across the rumen wall, while the gas is eructated, inhaled into the lungs, and absorbed into the blood stream. Once in circulation, hydrogen sulfide can react with heme to produce sulfide radicals. The liver serves to oxidize sulfide radicals and hydrogen sulfide to sulfate, which is excreted in urine.[13,26] However, an excessive burden of hydrogen sulfide in the blood stream is believed to affect neuronal tissue in the brain, including the neuropil, resulting in neuronal necrosis.[24] The reactive nature of sulfur compounds in the circulation makes testing of blood or serum challenging. However, the metabolic pathways through the liver and excretion via the kidney would seem to make these tissues ideal for assessing sulfur burdens. No threshold value could be established for urine thiosulfate to be predictive of development of PEM, but concentrations were elevated by increased dietary sulfur intake.[7] This would seem to support investigation of kidney sulfur concentrations, particularly considering most testing would be indicated postmortem. Establishment of normal sulfur concentrations in liver and kidney tissue of cattle could greatly facilitate postmortem diagnosis of sulfur toxicosis.

The sulfur concentrations in both wet- and dry-matter kidney tissue in this study possessed non-normal distributions. Because of the wider range of values from kidney tissues, and the fact that confidence intervals were created for these tissues using non-parametric methodology, the CI for kidney values are much wider than the CI for liver concentrations. The distribution of values from kidney was greatly impacted by two individuals with remarkably high sulfur concentrations compared to the other animals (18,363 and 21,633 ppm).

Table 1. Summary of findings of analysis of bovine tissues for sulfur concentration.

Tissue	Mean dry matter (range)	Mean/median sulfur concentration, in ppm dry matter basis (range)	Mean/median sulfur concentration, in ppm wet basis (range)	95% confidence internal, in ppm dry matter basis	95% confidence internal, in ppm wet basis
Liver	27.5% (13.1%-37.1%)	7,252[a] (3,670-11,041)	1,955[a] (966-2,763)	4,522-9,982	1,270-2,640
Kidney	21.3% (13.4%-34.3%)	7,869[b] (4,353-21,633)	1,622[b] (1,020-3,180)	5,070-19,017	1,187-2,918

[a]Mean value
[b]Median value

Removal of these two outliers would have created a normal Gaussian distribution, but neither animal had historical or pathological justification for exclusion from the study. It is possible that sulfur concentrations in kidney tissue are more variable than those in the liver, and/or more readily altered by dietary intake. This may make assessment of renal sulfur content more meaningful for assessing toxicosis, although it may also make interpretation of such measurements more difficult; additional work would be justified to examine these possibilities. The current study created reference ranges from both tissues that should be beneficial for better defining normal concentrations for comparison to tissue concentrations present in cases of sulfur toxicosis as well as animals on high sulfur diets without evidence of sulfur toxicosis.

The correlation between dry- and wet-matter basis calculations of liver sulfur was lower than would be anticipated, and the best across-tissue correlations were found on a dry-matter basis. This may be explained by both antemortem and postmortem factors which would affect tissue moisture content, including venous congestion, hydration status, and desiccation of tissues after death. These findings, along with the non-normal distribution of kidney values, may suggest that liver sulfur concentrations reported on a dry-matter basis may be most amenable to yielding consistent results and producing a narrower range of normal sulfur content. However, further research is warranted to confirm the usefulness of such measurements, particularly including samples from cattle with historical and pathologic evidence consistent with sulfur toxicosis.

Sulfur concentrations in fresh liver of sheep are reported to range from 2,600 to 2,800 ppm and from 1,600 to 1,800 ppm in fresh kidney tissue from sheep.[20] *Mineral tolerance of animals*[20] states that other species are expected to have similar concentrations, and that tissue sulfur concentration is not significantly increased by addition of sulfur to the diet. However, no empiric data are provided in support of these assertions and it is unclear how these values were ascertained. The study reported here found concentrations generally lower than the reported sheep values, and a notably wider range. It remains to be determined if tissue concentrations vary significantly in cases of sulfur toxicosis, or are affected by dietary intake.

It has been recommended that reference intervals be constructed from at least 120 healthy individuals.[3] Yet it is important to note that this recommendation is made for establishing a reference range relevant to healthy individuals. It is unlikely that clinicians would choose to assess sulfur tissue concentrations in clinically normal cattle. Therefore, it is appropriate to establish a range for animals that presented for ill-thrift, unexpected death, or unexplained illness. As such, most samples used in this study were obtained from animals submitted for necropsy due to intractable disease or natural death; the most commonly encountered conditions in our population were clostridial myositis and pneumonia. It is unclear how these various conditions would alter sulfur metabolism, excretion, or tissue concentrations, and certainly warrants additional consideration and research. Ideally, additional cattle could have been enrolled, up to 120. An increase in the number of cattle would likely have reduced the breadth of the reference ranges rather than shifting them substantially. Assessment of tissue sulfur concentrations in cattle with confirmed diagnoses of sulfur toxicosis is necessary to determine if a smaller reference range would be beneficial.

Conclusions

This study confirms that ICP-AES analysis of liver and kidney tissues is a viable method of sulfur assessment and provides a basis for establishing baseline sulfur concentrations in cattle not suffering from sulfur toxicosis. We determined a 95% CI of 4,522 to 9,982 ppm for sulfur in the liver (dry-matter basis).

This information can serve as a basis for comparison with cases of confirmed or suspected sulfur toxicosis, and can provide guidance in examining samples from cattle likely suffering from PEM attributable to excessive sulfur consumption.

Endnotes

[a]Microwave Sample Preparation Note: 5BI-5 2, Bovine Liver (SRM 1577a), CEM Corp., 100 Smith Farm Road, Matthews, NC
[b]PerkinElmer, Shelton, CT
[c]Microsoft Corp., Redmond, WA
[d]MedCalc, Mariakerke, Belgium

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Declaration of Conflicting Interests

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