PEER REVIEWED

The effect of sulfur and use of ruminal available sulfur as a model to predict incidence of polioencephalomalacia in feedlot cattle

C. A. Nichols[1]; **V. R. Bremer,**[1] *PhD;* **A. K. Watson**[1]; **C. D. Buckner,**[1] *PhD;* **J. L. Harding**[1]; **G. E. Erickson,**[1] *PhD;* **T. J. Klopfenstein,**[1] *PhD;* **D. R. Smith,**[2] *DVM, PhD, DACVPM (Epid)*
[1]*Department of Animal Science, University of Nebraska-Lincoln, Lincoln, NE 68583-0908*
[2]*Department of Pathobiology and Population Medicine, Mississippi State University, College of Veterinary Medicine, Mississippi State, MS 39762*
Corresponding author: Dr. David R. Smith, PO Box 6100, Mississippi State, MS 39762; dsmith@cvm.msstate.edu

Abstract

A longitudinal analysis of health and nutritional data from 16,760 cattle within 80 beef feedlot finishing trials was conducted to evaluate the effect of dietary sulfur on polioencephalomalacia (PEM). Data were compiled from health and research records collected at the University of Nebraska Research Feedlot between 2002 and 2009. Poisson regression was used to test the relationship between health outcomes and dietary factors on PEM incidence. Because of the low incidence of PEM in these data, statistical significance was defined *a priori* at alpha ≤0.10. There was a significant interaction between concentration of dietary sulfur and forage NDF on the incidence of PEM cases. The dietary sulfur by forage neutral detergent fiber (NDF) interaction is probably due to forage fiber moderating H_2S production in the rumen. Rumen available sulfur (RAS) was estimated for feedstuffs. As level of RAS increased in the diet by 0.1%, the incidence of PEM increased nearly 3-fold. There was no interaction between RAS and forage NDF on PEM incidence. It may be that RAS is a better measure of PEM risk than dietary sulfur because it estimates the rumen available forms of sulfur which pose a greater risk of inducing PEM, and RAS accounts for PEM risk independent of the risk due to forage fiber. It may be possible to manage PEM incidence by using RAS measurements to formulate rations fed to feedlot cattle.

Key words: byproducts, feedlot cattle, neutral detergent fiber, NDF, polioencephalomalacia, PEM, sulfur

Résumé

Une étude longitudinale sur des données nutritionnelles et de santé, incluant 16 760 bovins en finition provenant de 80 essais dans des parcs d'engraissement, a été menée afin d'évaluer l'effet de l'apport de soufre des aliments sur la polioencéphalomalacie (PEM). Les données ont été compilées à partie des registres de santé et de recherche recueillis à *l'University of Nebraska Research Feedlot* entre 2002 et 2009. La régression de Poisson a été utilisée pour tester l'effet de la relation entre les déterminants de santé et les facteurs alimentaires sur l'incidence de PEM. En raison de la faible incidence de PEM dans cette étude, le seuil alpha de signification statistique a été défini a priori à ≤ 0.10. Il y avait un effet significatif de l'interaction entre l'apport de soufre des aliments et la teneur en fibre au détergent neutre de la ration sur l'incidence de PEM. Cette interaction entre l'apport de soufre des aliments et la teneur en fibre au détergent neutre reflète probablement l'effet modérateur des fibres alimentaires sur la production de H2S dans le rumen. La biodisponibilité du soufre dans le rumen a été estimée pour différents aliments. Lorsque la biodisponibilité du soufre dans le rumen augmentait de 0.1%, l'incidence de PEM augmentait par un facteur de près de trois. Il n'y avait pas d'effet significatif de l'interaction entre la biodisponibilité du soufre dans le rumen et la teneur en fibre au détergent neutre sur l'incidence de PEM. Il se peut que la biodisponibilité du soufre dans le rumen soit une meilleure mesure du risque de PEM que l'apport de soufre des aliments car elle permet de mieux estimer les formes de soufre disponibles dans le rumen qui engendrent un plus grand risque de PEM. De plus, la biodisponibilité du soufre dans le rumen explique le risque de PEM indépendamment du risque causé par les fibres de la ration. Il serait possible de gérer l'incidence de PEM en utilisant des mesures de biodisponibilité du soufre dans le rumen pour formuler les rations données aux bovins en parc d'engraissement.

© Copyright American Association of Bovine Practitioners; open access distribution.

Introduction

With expansion in the production of renewable fuels, availability of ethanol byproduct feeds has increased. Byproduct feeds, like distillers grains plus solubles (DGS), are utilized very efficiently by ruminants.[14] However, one of the challenges with using DGS at large rates of dietary inclusion is the potential for increased level of sulfur (S) in the diet.[18] The S in DGS is derived from two sources, S-containing amino acids and sulfuric acid. About half of the S in DGS is from S-containing amino acids, while the remaining S in DGS comes from sulfuric acid, assuming DGS is about 0.8% S.[1]

Polioencephalomalacia (PEM) is a central nervous system disease of ruminants that can occur sporadically or in outbreaks.[5] The disease is a pathological diagnosis not specific to any etiology. However, some of the risk factors for PEM are associated with feeding practices such as low amounts of dietary roughage and elevated dietary S above 0.3%[21] or 0.4%[20] in feedlot diets.

The National Research Council[20] suggests diets fed to feedlot cattle should not exceed 0.40% S. However, data supporting the threshold suggested by the National Research Council are limited. Vanness et al[23] evaluated the risk for PEM at increasing dietary S levels. Data were compiled from cattle on byproduct feeding experiments. They concluded that cumulative incidence of PEM was low (0.14%) in diets containing 0.46% or less S. The Vanness et al data were included in the current analysis. It has also been suggested that increasing roughage inclusion in high byproduct diets may reduce the level of hydrogen sulfide (H_2S) present in the rumen due to its ability to increase ruminal pH.[24]

The objectives of the current study were to determine the effect of dietary S, fiber, or rumen availability of sulfur on incidence of PEM in feedlot cattle.

Materials and Methods

All procedures involving animal care and management were approved by the University of Nebraska's Institutional Animal Care and Use Committee. The University of Nebraska-Lincoln feedlot research program utilized spring-born steers that were weaned in the fall. Upon arrival into the feedyard, cattle were vaccinated against common viral diseases (infectious bovine rhinotracheitis, bovine viral diarrhea types 1 and 2, parainfluenza-3, bovine respiratory syncytial virus), *Histophilus somni*, and treated for intestinal parasites. About 14 to 21 days after arrival, cattle were revaccinated for common viral diseases, pinkeye, *Clostridium chauvoei, C. septicum, C. novyi, C. sordellii, C. perfringens* types C and D, and *Histophilus somni*. After the initial receiving period, the larger cattle (i.e. greater than 600 lb; 272 kg) were fed high-grain finishing diets from approximately November to May. The lighter steers were fed forage-based diets or grazed crop residues through the winter. The following May, the heaviest 700 steers (i.e. 800 lb [363 kg] or greater) were fed high grain finishing rations to October. The lighter steers in May grazed summer pastures and were subsequently fed finishing rations from September to February as long yearlings.

Data were compiled from 80 finishing trials conducted at the University of Nebraska-Lincoln Agricultural Research and Development Center research feedlot (Mead, NE) from 2002-2009. Steers (n=16,760) in these studies consisted of crossbred steer calves or yearlings. Cattle included in the analysis were fed diets that ranged from 0.12% to 0.72% S (DM basis, Table 1). Feed ingredients utilized in studies were analyzed for S.[a] Feed samples were collected weekly and retained following DM analysis. For each individual experiment, monthly composites were analyzed for S. Sulfur undegradability of each feedstuff was calculated by estimating percent organic sulfur from S-containing amino acids (methionine and cysteine).[19] This value was multiplied by undegradable intake protein (UIP)[3,4,12] which yielded percent undegradable intake S. Undegradable intake S of the feedstuff was subtracted from total S to calculate rumen available sulfur (RAS).[22] Neutral detergent fiber was determined for forage using the procedure described by Van Soest and Wine.[25] At the UNL research feedlot, B-vitamin (thiamine) is routinely included in the supplement fed to cattle consuming diets containing wet or dry milling byproducts. Most of the trials that fed byproducts included in the current analysis were empirically supplemented with 140 mg/steer/day of thiamine as a PEM preventive.

Computerized health records were maintained on all cattle. Feedlot illnesses of particular interest to the current study included PEM, respiratory disease, infectious pododermatitis, and ruminal tympany. Cattle were visually appraised by feedlot personnel daily for signs of disease. Cattle were determined to be PEM cases if they exhibited central nervous system signs consistent with a diagnosis of PEM (poor coordination, disorientation, and blindness).

Statistical Analysis

Days at risk for PEM was calculated as the number of days elapsing from the start of the feeding period until: 1) a diagnosis of PEM; 2) removal from the study for other reasons; or 3) marketing at the completion of the study. Poisson regression was used to test factors associated with PEM incidence. A generalized estimating equations model[b] was defined by the Poisson distribution, log link, and an offset of the natural logarithm value of days at risk. A compound symmetry correlation structure was defined to account for clustering of pens of animals within experiments. Only PEM cases that occurred during the finishing period, including dietary

© Copyright American Association of Bovine Practitioners; open access distribution.

Table 1. Average nutrient composition (% of DM) of ingredients fed in UNL research feedlot trials summarized for 2002-2009. Values for total S have standard deviation provided ( ) when multiple samples were available, based on month fed across years.

Feed	Total sulfur, %	Rumen available sulfur, %	Neutral detergent fiber, %
Protein feeds			
WDGS	0.79 (0.13)	0.56	34.0
MDGS	0.77 (0.09)	0.53	34.0
DDGS	0.76 (0.12)	0.52	34.0
WDG	0.50 (0.08)	0.22	42.5
Dakota bran cake	0.41 (0.01)	0.39	30.3
CCDS	1.12	1.08	3.0
Sweet bran	0.50	0.44	37.8
ADM WCGF	0.47 (0.04)	0.41	37.8
Steep	0.90	0.70	2.0
Brewers grits	0.34	0.26	34.0
CGM	0.72	0.21	5.0
Energy feeds			
SFC	0.14	0.06	10.8
HMC	0.13	0.09	10.8
DRC	0.14	0.06	10.8
FGC	0.14	0.06	9.0
Whole corn	0.14	0.06	10.8
Reconstituted corn	0.14	0.10	10.8
Roughage sources			
Alfalfa hay	0.25 (0.04)	0.19	55.5
Brome hay	0.15 (0.004)	0.18	75.5
Corn bran	0.22	0.21	72.2
Corn stalks	0.20	0.18	81.3
Soyhulls	0.26	0.23	61.8
Sorghum silage	0.10	0.08	62.2
Corn silage	0.08	0.06	43.9
Wheat straw	0.13 (0.01)	0.11	80.4

WDGS=wet distillers grains plus solubles
MDGS=modified distillers grains plus solubles
DDGS=dry distillers grains plus solubles
WDG=wet distillers grains
Dakota bran cake=corn bran plus distillers solubles
CCDS=condensed corn distillers solubles
Sweet bran=corn bran, steep, distillers solubles and germ meal
ADM WCGF=corn bran, steep and distillers solubles
CGM=corn gluten meal
SFC=steam flaked corn
HMC=high moisture corn
DRC=dry rolled corn
FGC=fine ground corn
Reconstituted corn=ground corn with water added back (25% DM) and ensiled

step-up, were included in the analysis. Factors tested by univariable and subsequently by multivariable analysis were dietary sulfur, NDF, sulfur x NDF, RAS, RAS x NDF, respiratory disease, infectious pododermatitis, and ruminal tympany. Multivariable models (one including dietary S, another with RAS) were tested by manual forward selection. Models were evaluated by model fit (QIC) and score statistic type III *P*-value. Because of the low incidence of PEM, significance was defined *a priori* at alpha ≤ 0.10.

Results

Twenty-seven cattle were diagnosed with PEM from among 16,760 cattle contributing 2,370,213 days at risk for PEM; therefore, incidence density of PEM was

© Copyright American Association of Bovine Practitioners; open access distribution.

0.11 cases per 10^4 animal-days. The average number of days-on-feed at the time of PEM diagnosis was 72 (median=62). The range of days-on-feed at PEM diagnosis was 125 (23 to 148). Thirteen of the 27 animals diagnosed with PEM showed signs of PEM infection in the first 60 days of the feeding period.

Infectious pododermatitis was diagnosed in 477 steers contributing 2,318,076 days at risk (incidence density=2.1 cases per 10^4 animal-days). Respiratory disease was diagnosed in 221 steers contributing 2,346,882 days at risk (incidence density=0.94 cases per 10^4 animal-days). There were 21 cases of ruminal tympany over 2,370,397 days at risk (incidence density=0.09 cases per 10^4 animal-days).

The median S content of the rations was 0.27% DM with a range of 0.12% to 0.73%. The median RAS value was 0.20% DM with a range of 0.06% to 0.51%. Rations contained a median value of 4.16% DM forage NDF (DM basis) with a range of 0% to 8.78%. Water from 4 wells supplying the feedlot was tested for sulfur 11 times over the duration of the study period. The mean S content of the water was 88.4 mg/L (Std. dev.=36.2 mg/L) with a range of 41 to 150 mg/L. Because of these low levels, S from water was not included in the analysis.

Only dietary S and RAS were significantly associated with the incidence of PEM in univariable analysis. Each 0.1% increase in dietary sulfur increased the risk for PEM 2-fold (RR=2.03, P=0.006); also, each 0.1% increase in RAS increased PEM risk by a factor of 2.6 (P=0.007). However, there was a significant interaction between dietary S and forage NDF on PEM incidence (P=0.07; Figures 1 and 2). The nature of the interaction was that greater levels of dietary S increased the relative risk for PEM for each unit decrease in forage NDF; alternatively, greater levels of forage NDF reduced the relative risk for PEM for each unit increase in dietary sulfur. For example, the interaction indicates that at a dietary S level of 0.3% the relative risk for PEM is 1.1 for a ration with 2% forage NDF compared to a ration with 6% forage NDF; whereas, at a dietary S level of 0.6% the relative risk for PEM between the same forage NDF levels is 7.2.

Forage NDF became a significant variable in the full model with RAS, however there was no significant interaction between the 2 variables (Figure 3). Accounting for forage NDF, the incidence density for PEM increased approximately 3-fold for every 0.1% increase in RAS (RR=2.9, P=0.0072). Accounting for RAS, each 1% increase in forage NDF decreased the incidence of PEM by 19% (RR=0.81, P=0.10).

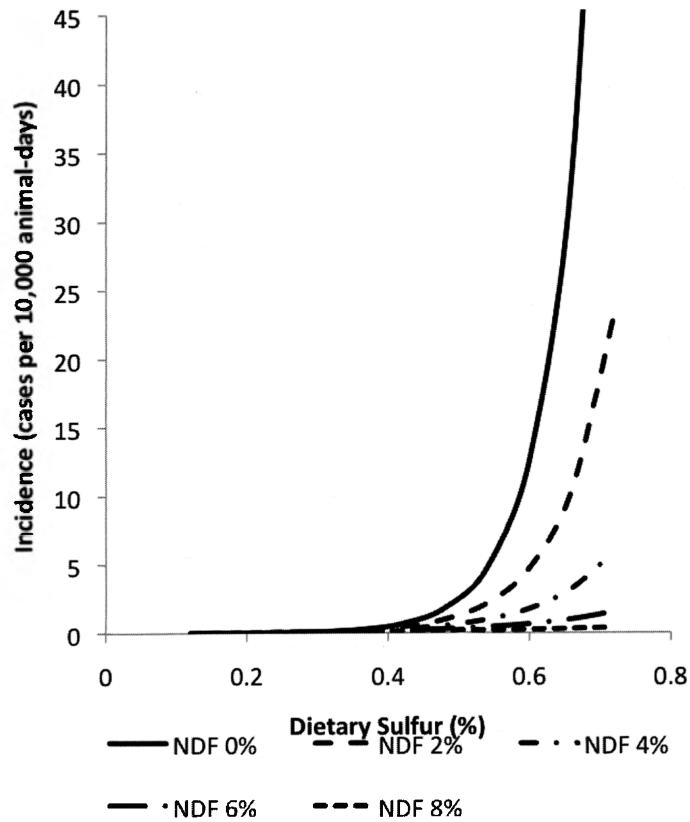

Figure 1. Effect of sulfur and forage NDF level on polioencephalomalacia (PEM) incidence (PEM cases per 10^4 animal-days). A dietary sulfur x forage NDF interaction (P=0.07) was observed based on a Poisson regression analysis of 27 PEM cases occurring during the finishing phase of 16,760 steers fed at the University of Nebraska-Lincoln research feedlot.

Discussion

In these data, PEM cases occurred fairly uniformly over the feeding period. Contrary to these results, McAllister et al[16] conducted a similar analysis using health records collected from a commercial feedlot (January 1990 to January 1996) to determine when feedlot cattle most frequently expressed signs of PEM. Of the 246 cattle that contracted PEM, 192 (78%) became ill between days 15 and 30. These authors reported the peak incidence occurring on day 19. Two hundred forty (98%) cases were detected within 59 days subsequent to entering the feedlot. Cattle in the McAllister study consumed diets that contained on average 0.20% S (2.4g of SO_4^{2-}/2.2 lb [1 kg] of DM) and consumed water containing on average 2,500 ppm SO_4^{2-} (2.5 g of SO_4^{2-}/L). Authors estimated that during the summer overall dietary S intake (both from diet and water) was 0.67% of DM (160 g/steer daily SO_4^{2-}). Incidence of PEM was greatest during the summer months and decreased during the winter months. Also, McAllister et al reported that prior to 1993 PEM cumulative incidence was only 0.07%; PEM cumulative incidence rose to 0.88% after 1993.[16]

© Copyright American Association of Bovine Practitioners; open access distribution.

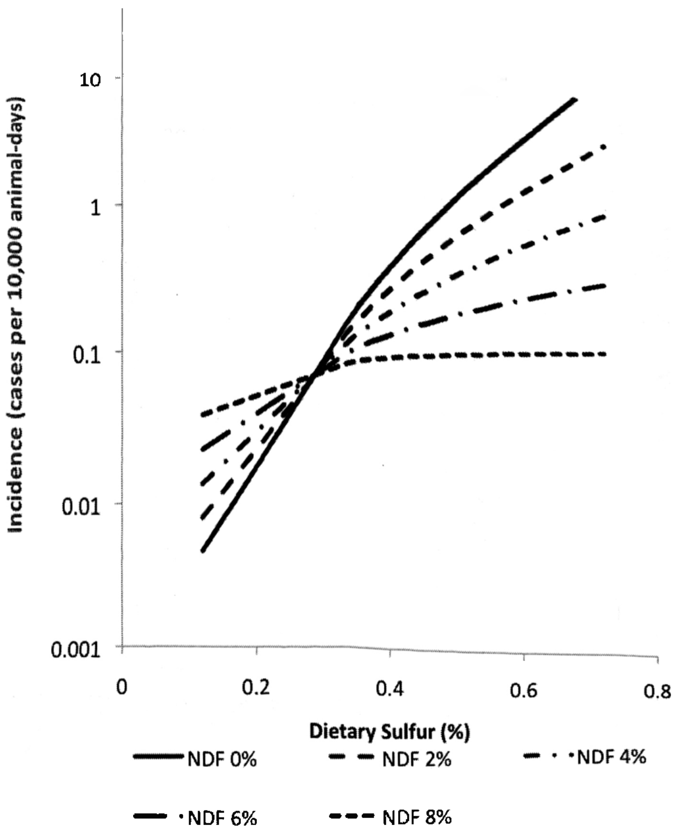

Figure 2. Data from Figure 1 presented on a log scale to illustrate the nature of the statistical interaction between dietary sulfur and forage fiber on incidence of PEM.

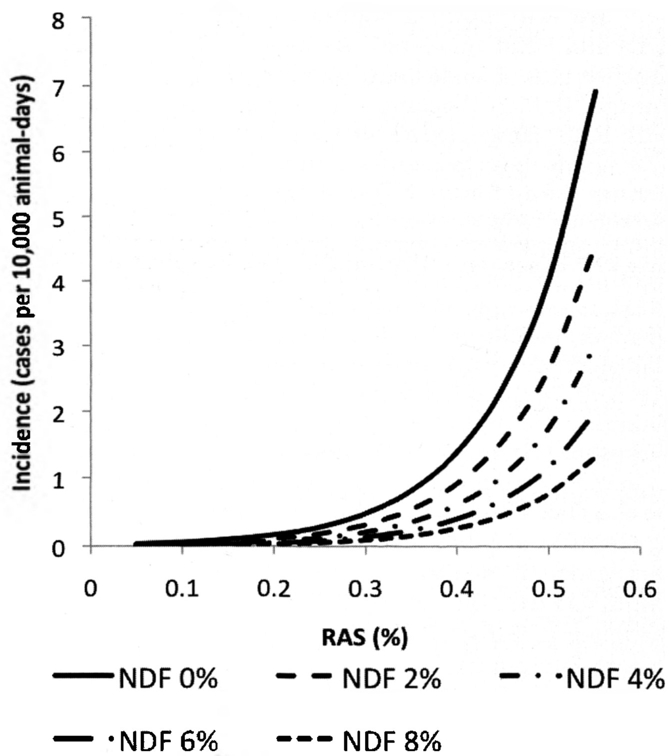

Figure 3. Effect of rumen available sulfur (RAS) and forage NDF level on polioencephalomalacia (PEM) incidence (PEM cases per 104 animal-days) based on a Poisson regression analysis of 27 PEM cases occurring during the finishing phase of 16,760 steers fed at the University of Nebraska-Lincoln research feedlot.

The commercial feedlot indicated that it had installed a new well that supplied water for the feedlot in late 1992. The new well, along with the increased incidence of PEM during the summer, suggest that S from the water supply was likely responsible for the PEM cases reported by McAllister.[16] The S source in most drinking water is sulfate, which is a highly rumen-available inorganic source of S. Dietary sulfur in the current analysis consisted of a mixture of organic and inorganic sources, and water sulfates were negligible.

In this analysis, dietary S and NDF levels interacted to influence the incidence of steers with PEM. The dietary S by forage NDF interaction is probably due to forage fiber moderating H_2S production in the rumen. The National Research Council[20] suggests that diets fed to feedlot cattle should not exceed 0.40% S because of the increased risk for PEM. However, Vanness et al[23] examined the risk for PEM at increasing dietary S levels. They reported that feedlot cattle could tolerate finishing diets containing 0.46% dietary S with relatively low risk of contracting PEM, with wet distillers grains plus solubles (WDGS) as the primary dietary S source. In a finishing study, cattle were fed diets with 3 levels of NDF (4, 7, and 10% DM) and 2 different forage sources (corn stalks and brome grass hay).[17] The authors reported an increase in ruminal pH as roughage (corn stalks or brome grass hay) replaced corn in the diet. Morine et al also reported a reduction in H_2S concentration as NDF level increased in the diet, which resulted in a negative correlation (r=-0.65) between ruminal pH and H_2S.[17] If H_2S is responsible for inducing PEM in feedlot cattle, these data support the reduction in PEM risk as dietary NDF from forage increased as observed in the current study. Morine et al conducted a second study that evaluated the effect of multiple levels (4, 6.5, 9, 11.5, and 13% NDF DM) of roughage NDF on rumen H_2S and pH.[17] Data indicated a linear decrease in ruminal H_2S along with a linear increase in ruminal pH with increasing concentration of NDF. Authors indicated that although H_2S continued to decrease as roughage level increased, magnitude of benefit was reduced when increasing roughage NDF inclusion from 9% to 13%, compared to increasing NDF from 4% to 9%.[17] These findings may indicate that a practical threshold exists for forage fiber level when attempting to prevent PEM.

© Copyright American Association of Bovine Practitioners; open access distribution.

We also found a significant association between RAS and PEM incidence. As level of RAS increased in the diet, risk of feedlot cattle contracting PEM increased. Forage NDF also became a significant factor in the model with RAS. However, no forage NDF level by RAS interaction was detected, unlike what was observed for total dietary S and forage NDF. This suggests that RAS and NDF both help to explain risk for PEM, but they do so independently. The RAS model examines the available fraction of S for finishing diets and its relationship with PEM, which may make it a better model for explaining PEM in feedlot cattle. Many studies indicate that the risk of PEM in ruminants increases as S intake increases via feed or drinking water.[6-8] As the sulfur level of a finishing diet increases, production of ruminal H_2S also increases. Hydrogen sulfide is a normal product of rumen microbial metabolism.[13] However, excess amounts pose a risk of inducing PEM. Feeding trials conducted by Gould et al[11] indicated a positive association between increased sulfide concentrations in rumen fluid and ruminal gas cap[9] and the onset of PEM in cattle fed diets designed to induce PEM. The supplemental source of S in both feeding trials consisted of sodium sulfate, which is an inorganic source of S.[9,11] Sodium sulfate is 100% rumen available, and therefore has a greater capacity for influencing the production of H_2S compared with a mixed-S source (rumen available and rumen undegradable S). High SO_4 water was used to produce the high S treatment in a trial comparing cattle fed high S (0.81% of DM) diets to steers fed low S (0.30% of DM) diets in which there was a greater frequency of PEM in the high-S-treated cattle.[2]

Sulfur values reported for feedstuffs are often presented on a total-sulfur basis, which does not take into account the rumen availability of the S. Sulfur can still pose an important PEM risk even if dietary sulfur levels are considered low if most of the S is rumen available. A study compared the health and performance of cattle consuming high S water (3651 ppm) compared to cattle given low S water (566 ppm).[2] Steers in this trial were fed a blend of 50% crested wheatgrass hay and 50% pellet (88% wheat middlings) on an as-fed basis. The dietary S was approximately 0.20% S (DM basis) for the crested wheatgrass plus pellet diet, which we estimate to be about 0.17% RAS (DM). Based on DM intake, steers consumed 16 to 20 g/day of S from the feed. However, water contributed an additional 10 g/day of S for low S water treatment or 54g/day of S for the high S water, which would be 100% RAS. Therefore, adding the water S intake results in an RAS of 0.27% for low S water treatment and 0.79% RAS for high S water treatment. Data also indicated frequency of PEM, confirmed by cortical lesions or exhibiting PEM signs, including anorexia, ataxia, blindness, lethargy, muscle tremors, diarrhea, opisthotonos (star-gazing), head pressing, repetitive chewing, and in severe cases recumbency or seizure or both, was greater ($P \leq 0.05$) for cattle consuming high S water (16 out of 72 steers) compared to steers given access to low S water (0 out of 24 steers).[2] However, the data from Cammack et al[2] were based on steers fed a forage-based diet that would contain much greater concentrations of NDF compared to our data set.

Conversely, high total dietary S may not pose a significant risk to cattle if rumen availability of S is low. Overall, there appear to be many sources of S that may be important in ruminal H_2S production and development of PEM.[10] Inorganic sources of S are highly rumen available, which pose a greater challenge for PEM in ruminants than undegradable sources of sulfur. Inorganic sources of sulfur are more prone to inducing PEM because inorganic sources like SO_4^{2-} are more readily reduced to S^{2-}.[10,15] Due to the reducing environment of the rumen, relatively large quantities of H^+ are present and the readily available H^+ bind with S^{2-} to create toxic H_2S gas. This gas can then enter the blood stream where it eventually may cause brain tissue damage and clinical signs of PEM.[8]

Conclusions

Results from this study confirm the role of dietary S in finishing diets in PEM incidence, and that the risk is dependent on forage fiber levels (forage NDF). It appears that roughage is an important factor in reducing PEM related illness in feedlot cattle, which may be due to its ability to regulate rumen pH. More importantly, RAS may be a better measure of PEM risk than dietary S because it estimates the rumen available forms of S which pose a greater risk of inducing PEM, and RAS accounts for PEM risk independent of the risk due to forage fiber. It may be possible to manage PEM incidence by using RAS measurements to formulate rations fed to feedlot cattle.

Endnotes

[a]TruSpec®S, Leco Corporation, St. Joseph, MI
[b]Proc GENMOD procedure of SAS, Version 9.3, SAS Institute Inc., Cary, NC

Acknowledgements

A contribution of the University of Nebraska Agricultural Research Division, supported in part by funds provided through the Hatch Act.

References

1. Buckner CD, Vanness SJ, Erickson GE, Klopfenstein TJ, Benton JR. Sampling wet distillers grains plus solubles to determine nutrient variability. University of Nebraska Extension Pub, MP91:113-114, 2008.

© Copyright American Association of Bovine Practitioners; open access distribution.

2. Cammack KM, Wright CL, Austin KJ, Johnson PS, Cockrum RR, Kessler KL, Olson KC. Effects of high-sulfur water and clinoptilolite on health and growth performance of steers fed forage-based diets. *J Anim Sci* 2010; 88:1777-1785.
3. Cooper RJ, Milton CT, Klopfenstein TJ, Scott TL, Wilson CB, Mass RA. Effect of corn processing on starch digestion and bacterial crude protein flow in finishing cattle. *J Anim Sci* 2002; 80:797-804.
4. Corrigan ME, Klopfenstein TJ, Erickson GE, Meyer NF, Vander Pol KJ, Greenquist MA, Luebbe MK, Karges KK, Gibson ML. Effects of level of condensed distillers solubles in corn dried distillers grains on intake, daily body weight gain, and digestibility in growing steers fed forage diets. *J Anim Sci* 2009; 87:4073-4081.
5. Fecteau G, George LW. Mentation, abnormality, depression, and cortical blindness. In: Anderson DE, Rings DM, eds. *Current veterinary therapy food animal practice*. 5th ed. St. Louis: Saunders Elsevier, 2009; 307.
6. Galyean ML, Rivera JD. Nutritionally related disorders affecting feedlot cattle. *Can J Anim Sci* 2003; 83:13-20.
7. Gooneratne SR, Olkowski AA, Christensen DA. Sulfur-induced polioencephalomalacia in sheep: Some biochemical changes. *Can J Vet Res* 1989; 53:462.
8. Gould DH. Polioencephalomalacia. *J Anim Sci* 1998; 76:309-314.
9. Gould DH, Cummings BA, Hamar DW. In vivo indicators of pathologic ruminal sulfide production in steers with diet-induced polioencephalomalacia. *J Vet Diagn Invest* 1997; 9:72.
10. Gould DH, Dargatz DA, Garry FB, Hamar DW. Potentially hazardous sulfur conditions on beef cattle ranches in the United States. *J Am Vet Med Assoc* 2002; 221:673-677.
11. Gould DH, McAllister MM, Savage JC, Hamar DW. High sulfide concentrations in rumen fluid associated with nutritionally induced polioencephalomalacia in calves. *Am J Vet Res* 1991; 52:1164.
12. Haugen HL, Lamothe MJ, Klopfenstein TJ, Adams DC, Ullerich MD. Estimation of undegradable intake protein in forages using neutral detergent insoluble nitrogen at a single in situ incubation time point. *J Anim Sci* 2006; 84:651-659.
13. Kandylis, K. Toxicology of sulfur in ruminants: Review. *J Dairy Sci* 1983; 67:2179.
14. Klopfenstein TJ, Erickson GE, Bremer VR. Board-invited review: Use of distillers by-products in the beef cattle feeding industry. *J Anim Sci* 2008; 86:1223-1231.
15. Kung L Jr. Metabolism of sulfur in the rumen. *J Anim Sci* 2008; 86 (E-Suppl. 3):57. (Abstr.)
16. McAllister MM, Gould DH, Raisbeck MF, Cummings BA, Loneragan GH. Evaluation of ruminal sulfide concentrations and seasonal outbreaks of polioencephalomalacia in beef cattle in a feedlot. *J Am Vet Med Assoc* 1997; 211:1275-1279.
17. Morine S, Drewnoski M, Hansen S. Determining the influence of dietary roughage concentration and source on rumen parameters related to sulfur toxicity. Iowa State University Animal Industry Report. A. S. Leaflet R2695, 2012.
18. Neville BW, Schauer CS, Karges K, Gibson ML, Thompson MM, Kirschten LA, Dyer NW, Berg PT, Lardy GP. Effect of thiamine concentration on animal health, feedlot performance, carcass characteristics, and ruminal hydrogen sulfide concentrations in lambs fed diets based on 60% distillers dried grains plus solubles. *J Anim Sci* 2010; 88:2444-2455.
19. Nutrient requirements of dairy cattle. 7th ed. Washington DC: National Academy Press, 2001.
20. Nutrient requirements of beef cattle, update of 7th ed. Washington DC: National Academy Press, 2000.
21. Nutrient requirements of beef cattle. Washington DC: National Academy Press, 2003.
22. Sarturi JO, Erickson GE, Klopfenstein TJ, Rolfe KM, Buckner CD, Luebbe MK. Impact of source of sulfur on ruminal hydrogen sulfide and logic for the ruminal available sulfur for reduction concept. *J Anim Sci* 2013; 91:3353-3359.
23. Vanness SJ, Klopfenstein TJ, Erickson GE, Karges KK. Sulfur in distillers grains. University of Nebraska Extension Pub. MP92:79-80, 2009.
24. Vanness SJ, Meyer NF, Klopfenstein TJ, Erickson GE. Ruminal sulfide levels in corn byproduct diets with varying roughage levels. University of Nebraska Extension Pub. MP92:81-83, 2009.
25. Van Soest PJ, Wine RH. Nutritional ecology of the ruminant. 2nd ed. *J Assoc Off Anal Chem* 1967; 50:50-55.

© Copyright American Association of Bovine Practitioners; open access distribution.