SULFUR INDUCED POLIOENCEPHALOMALACIA IN WEANED BEEF HEIFERS EATING CORN GLUTEN FEED

By

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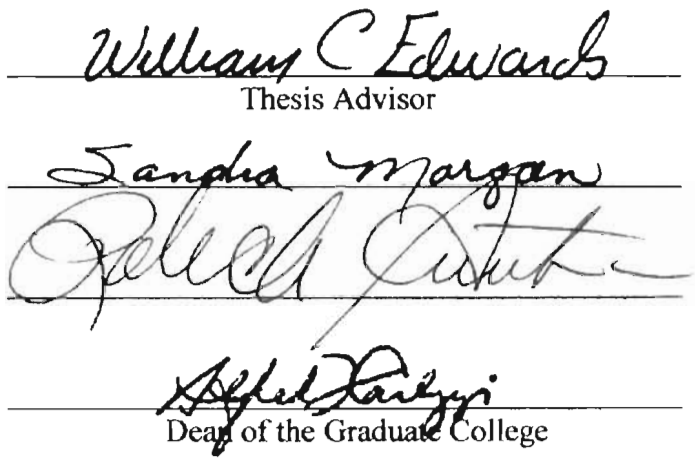

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PREFACE

This research was initiated due to the occurrence of clinical cases of polioencephalomalacia diagnosed by the Oklahoma Animal Disease Diagnostic Laboratory. The majority of these cases were caused by the consumption of corn gluten feed containing toxic levels of sulfur. Although there are numerous references to corn by-products causing polioencephalomalacia, no reports of controlled studies using high sulfur corn gluten feed to reproduce clinical disease were found.

The objective of this research was to evaluate the effect of feeding corn gluten feed with moderate to high levels of sulfur on the incidence of polioencephalomalacia and severity and distribution of brain lesions. Also evaluated was the effect different levels of sulfur has on copper, selenium, and zinc levels in 400 pound heifers.

Also, the use of hydrogen detector tubes to measure the hydrogen sulfide content of the rumen gas cap and analysis of breath samples after breathing filtered air for evidence of sub-clinical lung damage were examined.

I wish to thank Dr. W. C. Edwards for allowing me the opportunity to obtain further education in veterinary toxicology. His and Dr. Sandra Morgan's involvement and encouragement in this research made it infinitely easier to complete. Dr. Vickie Cooper's contribution of a great deal of personal time and her expertise in performing the postmortem examinations and histopathology analysis are greatly appreciated. Her evaluation of the extent and distribution of the brain lesions was a major objective of this

research. Without Dr. Morgan's advice and encouragement on meeting deadlines, this manuscript would not be on paper at this time.

I greatly appreciate Dr. Robert Smith for taking an extraordinary amount of time, especially with very short notice, to be a member of this committee. His help in the organization and editing of this manuscript was invaluable.

Without the assistance of Dr. David Lalman in experimental design, procurement and purchase of the research animals, and research facilities this research project would not have been possible. Thanks must be extended to Oklahoma State University's Department of Animal Science for accepting less than market value for the calves and for the use of the Bovine Nutrition and Physiology Research Facility to house this research. Also, special appreciation must be given to Steve Welty and his crew for tending these calves daily.

Dr. Michael Davis' experience and participation in the breath analysis explored a new avenue in the evaluation of sulfur toxicosis and will add useful information to the database of ethane analysis as an indicator of oxidative stress. His and Kaylin Brown's tolerance of my mistakes in learning to use the gas chromatography instrument is acknowledged and appreciated.

Dr. Larry Claypool's expertise with statistical analysis and interpretation of results of pilot studies using small numbers of observations allowed critical evaluation of the data collected during this study.

Dr. Fessessework Gaule, Max Whaley, and Brent Johnson's contributions involving toxicological analysis are also greatly appreciated.

Next, Emily Cooper's help in organizing and formatting this thesis is greatly appreciated. She and my daughter Lindsay are credited with preparing and placing tables and graphs in this manuscript in their proper places. I deeply appreciate the time and effort that my daughter, Nicole, and my wife Kathy have spent in proofreading and editing the text of this thesis.

Last, without the initial and continued encouragement and support of Kathy, Nicole, Lindsay, and my five-year-old granddaughter Hannah, I would not be pursuing this degree after 25 years of mixed animal practice. This degree is dedicated to them.

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NOMENCLATURE

ANOVA	analysis of variance
ATP	adenosine 5' triphosphate
BVD	bovine virus diarrhea
cc	cubic centimeter
CoA	coenzyme A
CSH	cottonseed hulls
GLM	general linear model
H	high treatment diet
HS^-	hydrosulfide ion
HSO_3^-	hydrogen sulfite ion
H_2S	hydrogen sulfide
M	moderate treatment diet
MH	moderately high treatment diet
NADH	nicotinamide adenine dinucleotide
PEM	polioencephalomalacia
ppm	part per million
SBH	soybean hulls
S^0	elemental sulfur
S_2	disulfide
TEME	thromboembolic meningoencephalitis

UV	ultra violet

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CHAPTER I

INTRODUCTION

Sulfur in the Middle Ages.

The first reports of the physical properties of sulfur occurred in the third century when sulfur known, as "Divine Water" or "Water of Sulphur", was reported to be able to change substances to gold. This was accepted by the alchemist of the Middle Ages as the basis for the process of transmutation. The medical books of Dioscorieds of Greece and Piny the Elder refer to sulfur as brimstone and describe its use in religion, medicine, and warfare during ancient times.

Sulfur's Biological Function

Sulfur is an essential mineral that is present in every cell in the body. It is found in three amino acids: methionine, cystine, and cysteine and is also a component of thiamine, biotin, insulin, conezyme A, and glutathione. It is important in energy, carbohydrate, and fat metabolism. Sulfhydryl groups bind coenzyme A to acyl groups to form acetyl CoA molecules. When acetyl CoA is hydrolyzed, energy rich thioesters are formed. The energy rich metabolites of this hydrolysis mediate a wide range of biochemical reactions throughout the body. Iron-sulfur proteins are a component of the NADA electron-transport chain of the citric acid cycle. Sulfur is also important in the detoxification of phenols and cresols.

Sulfur is a component of complex carbohydrates found in connective tissue and is present in hair, wool, and feathers. Sulfur constitutes 0.15% of the body's weight and 10% of the mineral composition of the body. Ruminants, horses, and possibly rabbits do not require organic sulfur in their diets. These species have the ability to convert

elemental and inorganic sulfur into sulfur containing compounds necessary for life. Other domestic livestock require organic sulfur in their diets.

History of Polioencephalomalacia

Reports of polioencephalomalacia (PEM), also known as cerebrocortical necrosis, first appeared in veterinary literature in the late 1950s and early 1960s.[1,2,3] The diseases "forage poisoning" and "blind staggers", diagnosed in Colorado and Wyoming at that time, were considered to be forms of PEM with different etiologies. Mercury and cobalt poisoning, spongiform-like infectious agents, *Clostridium welchii*, and selenium excess or deficiency have been proposed as possible etiologic agents.[1,3] By the early to mid 1970s, impaired thaimine metabolism in ruminants became entrenched in the literature as the cause of PEM. It was thought that thiaminase compounds in plants either destroyed thiamine or prevented its synthesis.[5-18]

Sulfur's Role in PEM

Sulfur was suggested as a cause of PEM in the early 1980s, while investigators were searching for the reason of the increase in cases of PEM in beef herds and feedlots in Missouri. Sulfate salts were being used to limit feed consumption in these herds. It was noted that when sulfates were removed from the ration the cases of PEM decreased, and when reintroduced into the ration clinical cases of PEM increased again.[4] Whether the increase in cases of PEM was a direct action of the sulfur on the animal or an indirect action by blocking thiamine production was not known at that time.[4] Several investigators have since shown that PEM can be caused by the direct action of excess dietary sulfur independent of factors affecting thiamine status.[19-32] A majority of the

previously reported cases of blind staggers have now been attributed to sulfur induced PEM.[33]

In the plains states through the intermountain region of the United States and into Canada, high sulfate water is frequently reported as a cause of PEM. In the cattle feeding states, PEM due to addition of gypsum to control feed intake occurs.[4,31-38] PEM also occurs in cattle grazing small grain pasture, especially if it is fertilized with sulfate containing fertilizers.[39]

The daily sulfur requirement of both growing and adult beef cattle is 1500 to 2000 ppm of the ration on a dry matter basis; 4000 ppm is considered the maximum tolerated dose.[39] Clinical cases of PEM occur at levels lower than 4000 ppm.[29,31,40]

The occurrence of PEM in two large groups of stocker cattle fed corn gluten feed, was the impetus for this research. Numerous reports list corn by-products as being high in sulfur and initiating PEM, but no reports of controlled research projects using CGF could be found. Efforts to find the reason for toxic levels of sulfur in corn by-products were met with resistance and denial by some parts of the corn industry. This project was designed to explore the relationship between CGF and sulfur toxicosis, and also to make cattlemen aware that the by-products of the wet milling of corn require increased management to be used efficiently and safely by cattle.

Case Reports

Case 1. This case involved 150 calves weighing 500 pounds. They were pastured on dry grass until wheat pasture became available. These calves were receiving grass hay and self-fed a mixture of 50% corn gluten feed (CGF) and 50% soybean hulls (SBH).

Within three weeks of first exposure to the bulk feed, several calves exhibited signs of central nervous system disease including blindness, circling, head pressing, and bruxism followed by lateral recumbency, opisthotonos, and death. Blood and tissue tested at Oklahoma Animal Disease Diagnostic Laboratory (OADDL) were negative for lead. Characteristic lesions associated with PEM and consisting of segmental laminar cortical edema and necrosis were confirmed by histopathology. A sample of the bulk CGF was found to contain 6800 ppm sulfates, which equates to 2300 ppm sulfur. The total sulfur content was not determined for this feed sample. The water these animals were consuming was negative for sulfates. In all, 11 animals died, 25 became blind and 40 head became "poor doers" that were still owned one year later.

Case 2. This case also involved the feeding of 50% CGF and 50% SBH to a group of stocker calves weighing 550 to 600 pounds. The calves were being held on pasture composed primarily of crabgrass until wheat pasture became available. This cattleman also had PEM occurring concurrently in another group of smaller calves weighing 280 pounds to 320 pounds. They were fed a commercial liquid feed consisting of 50% molasses and 50% whey while in the receiving pens.

Signs associated with neurological disease started occurring in the group of larger calves approximately two weeks after the self-feeders were filled with the CGF mixture. Blood and tissue lead levels were normal. Polioencephalomalacia was confirmed by histopathology. Two samples of the bulk CGF were tested for total sulfur and found to contain 6800[a] and 8200 ppm sulfur, respectively. The SBH's contained 1210 ppm sulfur and the pasture grass contained 3080 ppm sulfur. The water available to these calves was negative for sulfates. Although the incidence of PEM stopped in this group of larger

calves when the feeding of the CGF mixture was discontinued, clinical PEM was still occurring in the group of light-weight calves.

These light-weight calves were fed a molasses based liquid feed containing 50% molasses and 50% dehydrated whey. This feed was fed in open tanks without lick wheels per label directions to encourage maximum consumption. The calves also had access to bulk feeders containing 14% protein pellets and free choice bermuda grass hay. The rancher stated, "Some calves appeared to eat only the liquid feed while others only ate the creep feed." It was noted that only the calves that ate the liquid feed, as evidenced by the constant presence of molasses on their muzzles, exhibited PEM. Polioencephalomalacia was confirmed as the cause of illness in this group by histopathology. The molasses, whey mixture, creep feed, and hay contained 4450 ppm, 1210 ppm, and 2090 ppm total sulfur, respectively. The water source was negative for sulfate. When the liquid feed was discontinued, no further cases of PEM occurred.

This cattleman historically maintained a 1.0 to 1.5% death loss while handling 6000 head of stocker-cattle annually. While using high sulfur CGF over a four-month period, he treated 49 calves for PEM. Seventeen of 557 head died as a result of eating CGF. Also, there were 81 clinical cases of PEM and 49 deaths in the 1700 calves fed the liquid feed. Several animals from both groups had residual neurological impairment.

This rancher's neighbor, who also purchased lightweight calves, fed the same brand of liquid feed and had confirmed cases of PEM at the same time. The number of clinical cases and death loss were never made available.

Case 3. In this case, a cattleman offered a liquid molasses-based feed supplement to his calves that were grazing wheat pasture which was producing marginal forage due

to lack of rain. Two weeks later, the rancher moved these calves to better wheat pasture. The following day, he found three animals exhibiting signs of neurological disease. The rancher treated these animals himself with thiamine and antibiotics. Three weeks later one animal died and was presented to the OADDL for necropsy. This animal was confirmed to have PEM. Its brain sodium levels were normal as was the blood lead levels from all three calves. The sulfur content of the liquid feed was 8800 ppm. The two primary ingredients listed were condensed corn distillers solubles and molasses. Both are known to contain high levels of sulfur. The other two animals had permanent neurological impairment.

Definition of PEM

Polioencephalomalacia is a descriptive term that describes the deterioration of the gray matter of the cerebral cortex. Sulfur-induced PEM, lead poisoning, and water deprivation-sodium chloride toxicosis all produce brain lesions consistent with this description.[29,32,42-44] Grain overload also is reported to produce lesions of PEM.[45-54] Historically, this disease has been associated with the thiamine status of the animal and primarily linked with thiamine deficiency. It is thought that thiamine deficiency is caused by thiaminase compounds present in plants or formed as a result of decreased rumen pH.[1-18,45-54] Interference with thiamine production or usage by drugs, such as amprolium, or mineral excesses or deficiencies also are reported to induce PEM .[1-18] The association of thiamine with PEM was inferred by several authors that reported the presence of rumen thiaminases and the benefit of thiamine therapy on animals affected with PEM.[5-18,49-52]

Initially, three possible relationships were postulated as how sulfur could cause PEM. First, it was thought that sulfate might degrade thiamine in the rumen leading to thiamine deficiency. The second theory was that sulfate might interfere with the production of thiamine by the rumen microflora. The third possibility questioned whether a toxic contaminant was present in the sulfate salt used in the feed.[4]

Research data and clinical case reports published since then still question the significance of thiamine in sulfur-induced PEM.[16-20] However, it is now well documented that sulfur-induced PEM occurs independently of thiamine status, and that total sulfur intake both in the feed and water must be considered when assessing sulfur-induced PEM. [22,23,26,30,34,36,40] Table 1 lists the sulfur content of some common cattle feeds.

TABLE 1 SULFUR CONTENT OF COMMON FEEDS[39,41]

FEED	SULFUR%	FEED	SULFUR%
Alfalfa Hay	0.54	Corn Gluten Feed	0.47
Bermuda Hay	0.21	Corn Gluten Meal	0.60
Sudan Hay	0.06	Whey Dehydrated	1.15
Cottonseed Meal	0.42	Molasses	0.60
Soybean Meal	0.48	Barley Malt Sprouts	0.85
Cottonseed Hulls	0.08	Brewers Grain	0.58
Soybean Hulls	0.11	Wheat Oat Pasture	0.71[41]

The normal sulfur content of a ruminant ration is 1500 to 2000 ppm; therefore the addition of any high sulfate feed can easily exceed the maximum tolerated dose of 4000 ppm recommended by the National Research Council (NRC).[29]

Sulfur Metabolism by Rumen Bacteria

Rumen bacteria metabolize elemental, inorganic, and organic sulfur. Elemental and organic sulfur yield less free sulfur radicals than the metabolism of sulfate. Elemental sulfur's availability is 30 to 40% of methionine, and 45 to 50% of sodium sulfate. [45,46]

The reduction of sulfate in the rumen is accomplished by either assimilatory or dissimilatory bacteria.[30,31,57,58] Assimilatory bacteria reduce sulfate for their own metabolic needs and produce the amino acids that contain sulfur.[30] These bacteria include *Bacteroides*, *Butyvibrio*, and *Lachnospira* which produce less sulfide than dissimilatory bacteria.[58] Their sulfide production is limited by the presence of other organic sulfur compounds in the rumen.[58]

Dissimilatory bacteria also utilize sulfates for their energy needs, but produce much more sulfide than needed. *Desulfovibrio* and *Desulfotomaculum* species are the major bacteria in this group.[57,58] The reduction of sulfates by the dissimilatory bacteria account for the majority of sulfide production in the rumen.[58,59] These bacteria are limited by the amount of hydrogen sulfide present in the rumen gas cap.[58] It has been shown, *in vitro*, that an increase in the hydrogen sulfide content in the headspace of hydrogen sulfide generation tubes limited the production of sulfide. When the gas cap was replaced, as would occur with eructation, the ability of rumen bacteria to produce hydrogen sulfide also increased.[58] The number the dissimilatory bacteria were thought to increase in response to increased rumen sulfate, but current research has shown that colony numbers do not increase, but their capacity to produce sulfide does increase with

acclimation of 10 to 12 days.[17,58,59] Other research has shown *in vitro* acclimation within 7 days.[30]

Other non-sulfate reducing rumen bacteria contain cysteine desulfhydrase, an enzyme that allows them to metabolize sulfur-containing proteins liberating additional sulfide. These bacteria, like the dissimilatory bacteria, are anaerobes. Bacteria capable of enzymatic release of sulfide from sulfur containing amino acids include *Veillonella*, *Megasphaera, Wolinella, Selenomonas, Anaerovibrio*, and *Clostridium* spp.[58]

Interactions between these different types of bacteria involve dissimilatory bacteria utilizing inorganic sulfur for energy and liberating sulfide, which can be used by assimilatory bacteria for production of sulfur containing amino acids and liberation of additional sulfide. Other bacteria contain enzymes that metabolize these amino acids, liberating additional sulfide.[43] Hydrogen sulfide that enters the portal bloodstream first disassociates to $H^+ + HS^-$ due to increased pH of the blood. The HS^- then is oxidized by heme to H_2O and S, which is converted back to sulfate by sulfide oxidase in the liver, some of which is excreted in saliva, swallowed and reconverted to sulfide.[59,60] The role each particular group of bacteria play in contributing to the total amount of rumen hydrogen sulfide at any particular time is unknown.

The metabolism of hydrogen sulfide is presented in Figure 1.[61] This involves three different pathways: oxidation to sulfate, methylation, and reaction with metallo-proteins.[61] Sulfur's toxic effects are exerted primarily by disruption of the various enzyme complexes.

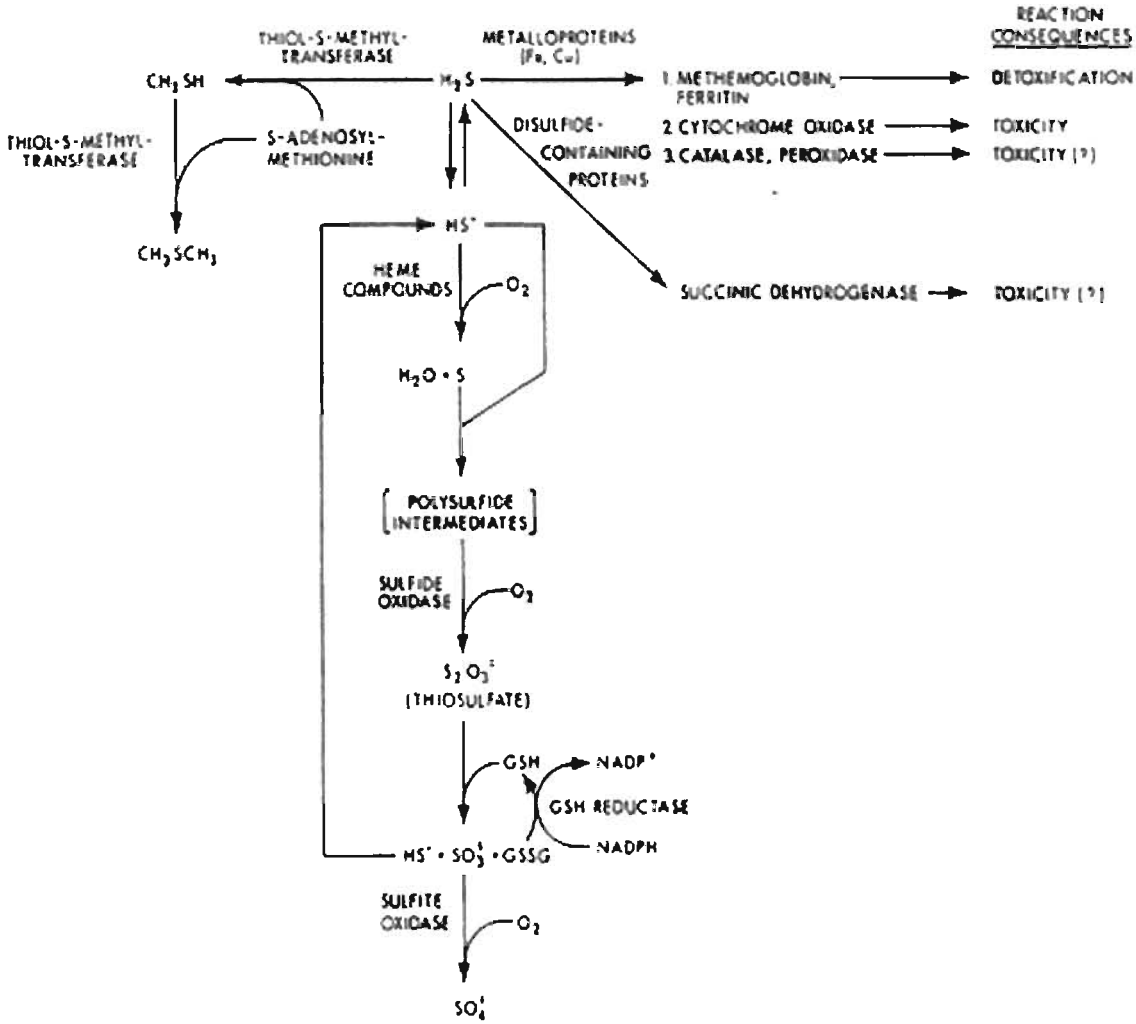

FIGURE 1. Metabolism of H_2S.

The major route of excretion of sulfide from the body is by conversion to sulfate in the liver, which is then eliminated in urine.[60,61]

Hydrogen Sulfide in the Rumen Gas Cap

The concentration of the sulfur metabolites, HS^-, HSO_3^-, S_2, S^0 within the rumen fluid and gas is not static and is greatly affected by rumen pH.[30,31,42] The neutral to acidic

nature of the rumen pH favors the formation of H_2S, which has pKa values for first and second dissociation steps of 7.04 and 11.96, respectively. One third of H_2S exists undissociated at a pH of 7.4, with 66% in the form of the hydrosulfide ion.[61] When rumen acidity increases, the amount of hydrogen sulfide present in the rumen also increases. With a change of pH from 6.8 to 5.2, the percent of hydrogen sulfide in the rumen gas cap increased from 46.8% to 97.2%.[32] From this increase, it is apparent that the amount of readily available carbohydrate in the animal's ration is an important factor in the initiation of PEM. Hydrogen sulfide and sulfide radicals are extremely toxic to tissue and both are readily absorbed through the rumen wall into the portal bloodstream.

Mechanism of Action of H_2S Toxicity

Hydrogen sulfide and free sulfide radicals inhibit the electron transport chain by reacting with essential proteins similar to, but much more potent than cyanide.[61] It damages the oxidative processes within the mitochondria by blocking cytochrome aa_3, which depletes ATP.[22,43,60,61] This blockage can paralyze the carotid body causing acute respiratory failure.[22,61]

The affinity of H_2S for brain tissue is thought to be due to the brain's high lipid content, which is associated with its numerous oxidative processes and the low level of antioxidants found in brain tissue.[20] The fluorescence, commonly observed when the brain is grossly examined under UV light (365 nm), is thought to be attributed to ceroid lipofuscin, a product of lipid peroxidation found in macrophages.[22] Auto-fluorescence is

not specific to brain tissue and is not always present with PEM. The liver, in addition to brain tissue, exhibited fluorescence in research lambs dosed with sulfide solution.[22]

Sulfides also interfere with the antioxidant enzymes superoxide dismutase, and glutathione peroxidase, which are present in blood. These enzymes protect the body from oxidative injuries by acting as scavengers of free radicals. The reaction of H_2S with metalo-proteins can have beneficial effects in detoxification processes within the animal.[61]

Inhalation of sub-lethal doses of hydrogen sulfide cause lung damage leading to atypical interstitial pneumonia.[62-64] Poison gas wells and manure gases are sources for direct inhalation exposure for livestock. If acute death does not occur, PEM can occur later.[32]

Clinical Signs Related to Sulfur Toxicity

Reduced feed intake and diarrhea were thought to be the most significant effects of high dietary sulfates. This concept led to the use of gypsum (calcium sulfate) in feedlot rations to limit feed intake.

Two syndromes are recognized with sulfur-induced PEM.[1,43] In the first syndrome, signs occur acutely with the animals found recumbent and comatose. These animals generally do not respond to treatment and die due to irreparable brain damage.

A second syndrome is also recognized when animals show symptoms typical of central nervous disease over a longer period of time. Signs include ataxia, fine muscle tremors of the face and head, bruxism, circling, head pressing, stupor, and cortical blindness. The menace reflex is absent but the palpebral reflex is present and the pupils

respond to light. Nystagmus with medial-dorsal strabismus of the eyeball may be present. These symptoms may be followed by lateral recumbence, opisthotonos, clonic-tonic convulsions with paddling motion and death. Animals that have recovered may be unproductive because of permanent brain damage.[1,29]

Lesions Associated with PEM

Gross brain lesions that occur with PEM, in addition to fluorescence, include gross swelling and edema, which can cause herniation of the medulla and cerebellum into the foramen magnum. The brain loses its turgidity and becomes soft to the touch. Flattening of the gyri of the cerebral hemispheres and yellowish brown discoloration is seen. Bilateral laminar cortical malacia, with occasional hemorrhage, and varying degrees of cavitation are often visible.[65]

Microscopic lesions reveal the neurons in the affected areas to be smaller than normal or they may be missing. The brain's astrocytes become acidophilic, swollen, and lose their processes creating increased space between neurons. Spongiform degeneration is present with dead neurons replaced by eosinophilic globules. The blood vessels increase in size and the density of the macrophages increases. Astrogliosis is evident with healing.[65]

It has been reported that the location and severity of brain lesions present in the thalamus without significant lesions present in the cerebellum may be diagnostic for sulfur-induced PEM. This author examined 40 previous cases diagnosed as thiamine-dependent PEM and compared the lesions with those observed with sheep exposed to high dietary sulfur. It was concluded that severe lesions involving the thalmus were not

present in cases of PEM considered thiamine-dependent.[33] Other authors in separate clinical investigations of sulfur-induced PEM found extensive thalmus and midbrain lesions, and reported their diagnostic significance for sulfur toxicosis.[26,36] These lesions had been considered nonspecific, resulting from any condition that causes the brain to swell and constrict the blood supply to the affected areas of the brain.[24,65,66]

The location and extent of sulfur-induced brain lesions was investigated in lambs that were fed one of four diets with two levels of sulfur and two levels of thiamine.[24] This research project involved 56 lambs (Table 2) and categorized the severity and distribution of brain lesions in lambs fed either 1900 or 6300 ppm dietary sulfur. Each treatment was subdivided into either low or high supplemental thiamine. Twenty-eight sites from nine sequential brain sections of each brain were analyzed and ranked according to type and severity of the brain lesions. Lesions were also present in some of the low sulfur treatments, although no cases of clinical PEM were noted in these treatments.

The results of this study suggest that lesions associated with sulfur-induced PEM occur due to brain swelling, and result from herniation of the midbrain and brainstem into the foramen magnum.[24] Cortical lesions occur first because the cortex has the greatest demand for oxygen and is the part of the brain most sensitive to temporary ischemia.[24] These researchers discounted thiamine deficiency as the primary cause of PEM and considered it more correctly labeled as thiamine-responsive polioencephalomalacia. Table 2 lists the location and score for severity of lesions found in this study. Seven lambs developed clinical PEM throughout this trial, but brain lesions characteristic of PEM also occurred in lambs on the low sulfur diet that did not show clinical signs of central nervous system disease.

Table 2. Median Lesion Scores for Sites of Central System that Showed Significant Treatment Associated Lesions[24]

Site (N)	$LSLB_1$[d]	$LSHB_1$	$HSLB_1$	$HSHB_1$
Post Rostral Supra-sylvia gryrus	0(4)[a]	0(9)[a]	3(11)[b]	2(7)[ab]
Marginal Gyrus	0(8)[a]	0(9)[a]	2(7)[b]	2(8)[b]
Sylvian Gyrus	0(9)[a]	0(7)[a]	3(7)[b]	1(13)[ab]
Rostral Sylvia Gyrus	0(7)[a]	0(7)[a]	2(10)[b]	1(13)[ab]
Short Gyrus of Insula	0(7)[a]	0(8)[a]	1(20)[b]	1(15)[ab]
Caudal Sylvian Gyrus	0(7)[a]	0(9)[a]	3(4)[b]	2(1)[b]
Caudal Supraslyvian Sulcus	0(6)[a]	0(6)[a]	0(1)[a]	2.5(2)[b]
Endomarginal gyrus	0(8)[a]	0(5)[a]	2.5(8)[b]	1(3)[ab]
Ectomarginal gyrus	0(8)[a]	0(9)[a]	2(11)[b]	2(9)[b]
Rostral Sylvian Sulcus	0(6)[a]	2(5)[a]	2(14)[b]	2(7)[b]
Presylvian Sulcus	0(1)[a]	0(3)[a]	2(10)[b]	1(14[b])
Lateral Rhinal Sulcus	0(4)[a]	0(5)[a]	2(11)[b]	2(8)[b]
Anterior Rhinal Sulcus	0(4)[a]	0(5)[a]	2(12)[b]	1(6)[ab]
Sylvian Sulcus	2(1)[a]	0(5)[a]	2(8)[b]	2(7)[b]
Ectosylvian Sulcus	0(8)[a]	0(4)[a]	2(18)[b]	2(9)[b]
Oblique Sulcus	0(3)[a]	2(5)[a]	3(11)[b]	2.5(4)[b]
Ectomarginal sulcus	0(5)[a]	0(6)[a]	3(11)b	2(8)[ab]
Endomarginal Sulcus	0(5)[a]	0(6)a	3(5)[b]	2(8)[b]
Marginal Sulcus	0(3)[a]	0(7)[a]	3(9)[b]	2(5)[b]
Splenial Sulcus	0(5)[a]	0(7)[a]	3(7)[b]	1(6)[b]
Caudate Nucleus	0(7)[a]	0(9)[a]	3(7)[b]	1(6)[b]
Substantia Nigra	0(6)[a]	0(8)[a]	1(14)[b]	1(14)[b]
Lateral Geniculate Body	0(8)[a]	0(8)[a]	0(12)[b]	1(5)[b]
Medial Geniculate Body	0(8)[a]	0(8)[a]	0(14)[b]	0(9)[a]
Anterior Dorsal	0(6)[a]	0(7)[a]	1(12)[b]	1(5)[b]
Ventrolateral Nucleus	0(6)[a]	0(9)[a]	1(16)[b]	1(12)[b]
Amygdaloid Body[c]	0(6)[a]	0(8)[a]	2(9)[b]	0(10)a
Hippocampus	0(6)[a]	0(8)[a]	1(12)[b]	0(13)[b]

[ab]Means differ ($P<0.05$) using Tukey's multiple range test following a significant ($P<0.05$) F value from a one-way GLM procedure: [c] all effects were attributed to sulphate administration (0.63%) when analyzed using Friedman two-way analysis of variance. Only the amyloid body showed a B_1 effect when the higher level of B_1 was assicuated with less sever lesions in the 0.63 %S treated group; [d] LS treatment = diet containing 0.19 % S, HS treatment = diet containing B_1 13.7 mg/kg, HB_1 = treatment containing B_1 243 mg/kg; LB_1 treatment = diet containing B_1 13.7 mg/kg; Numbers within parentheses refer to the number of sections examined.

It has also been suggested that the presence of lesions in the deeper brain structures are related to the very high levels of sulfur in the diet and length of illness.[42]

Differential Diagnosis, Treatment, and Prevention of PEM

Diseases to be considered in a list of differential diagnosis of PEM in stocker cattle should include: TEME, rabies, *Listeria*, and magnesium deficiency (grass tetany), lead toxicity, and water deprivation-sodium ion toxicity. Grain overload is also reported to cause PEM. Lead, water deprivation, and sulfur-induced PEM can be indistinguishable clinically and microscopically, and are differentiated by analysis of tissue for lead and sodium. Blood, liver, and kidney are routinely analyzed for lead content. Water deprivation is diagnosed by brain sodium content. Magnesium analysis can be done on serum from live animals or aqueous humor can be used postmortem. TEME, rabies and *Listeria* have characteristic postmortem lesions. Fluorescent antibody tests are also available. Rumen acidosis is characterized by rumen pH values of <5.2 and the presence of lesions in the rumen and liver.

Treatment of sulfur-induced PEM is symptomatic. In animals that are still ambulatory and able to eat and drink, removal from the source of the sulfur allows for partial to full recovery of a significant proportion of the affected calves.[67] Research lambs that developed clinical PEM have fully recovered without therapy.[24] Therapy for moderately to severely affected animals includes thiamine at 10 mg/kg IV followed by 10 mg/kg IM *bid* for 2 to 3 days. If no improvement is noted during that time, permanent brain damage is probable, and if applicable, salvage should be considered.[68] Furosemide

is indicated to reduce brain edema. Dexamethasone aids in reducing brain swelling and also decreases inflammation.[67]

Thiamine's Relationship to Central Nervous System Disease

Thiamine (vitamin B_1) is an essential, sulfur containing, water-soluble vitamin. Although it is synthesized in the stomach and intestines of all animals, non-ruminants require dietary vitamin B_1. Rumen bacteria are capable of synthesizing adequate amounts of thiamine, even with less than optimal nutrition.[69] Whole grains and liver contain large amounts of thiamine. Beriberi, a nutritional disease of humans, is common in countries that consume large amounts of polished rice. Polishing removes the hull, which is rich in vitamin B_1. This disease is characterized by muscle weakness and mental confusion. Administration of thiamine to individuals with beriberi gives rapid improvement.

A thiamine containing coenzyme thiamine pyrophosphate (TPP), shown in Figure 2, is essential in the first step of the citric acid cycle. This coenzyme is one of five coenzymes that are necessary to the pyruvate dehydrogenase complex. This multienzyme complex is the catalyst in converting pyruvate to acetyl CoA, which reacts with oxaloacetate in the first reaction of the citric acid cycle in aerobic respiration (Figure 3).[70] TPP is a coenzyme in six different decarboxylation reactions in aerobic respiration.[70]

During a multi-enzyme complex reaction each intermediate in the reaction is immediately converted to the next intermediate without diffusing from the reaction site. Thiamine pyrophosphate, lipoic acid, CoASH, FAD, and NAD^+ are all necessary to perform these reactions. Thiamine pyrophosphae is formed by the transfer of a

pyrophosphoryl-group from ATP to thiamine. The intermediate of this first reaction undergoes decarboxylation, forming an acetaldehyde unit which is bound to hydroxyethyethylthiamine pyrophosphate (HETPP). This coenzyme then releases an acetyl group to coenzyme A yielding acetyl CoA.[70,71] Thiamine's importance in these reactions explain its role in the health of the central nervous system and allows an understanding of why the administration of supplemental thiamine benefits patients with CNS disease.

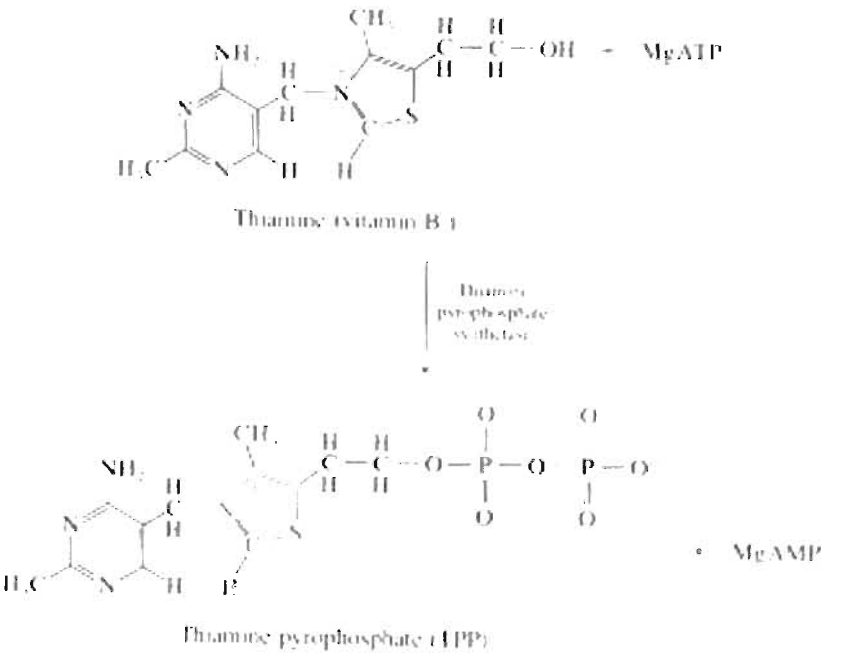

Figure 2 TPP's Formation[70]

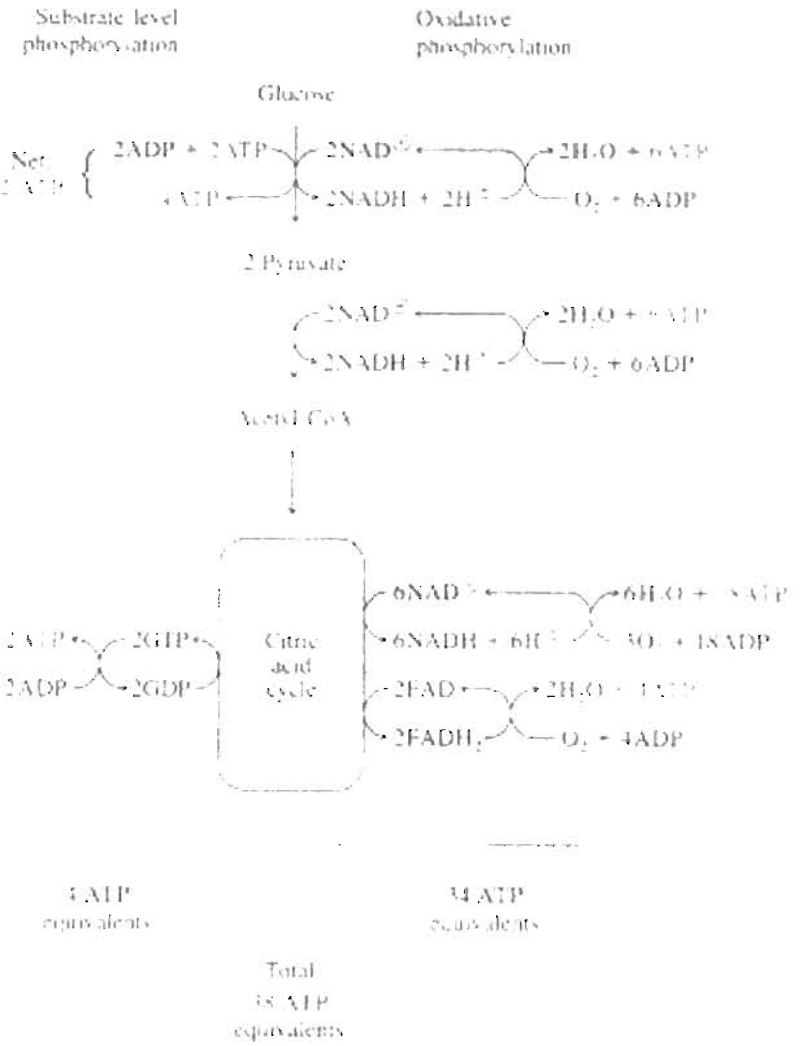

Figure 3 Glycolysis and the Citric Acid Cycle[70]

Thiamine, PEM, and Lead Poisoning

Treatment with vitamin B_1 is very important in lead poisoning. Better remission of the clinical signs of lead poisoning has been reported to occur using only thiamine therapy compared to combined thiamine and chelation therapy using Na_2,CaEDTA or Na_2,CaEDTA therapy only.[73] The addition of increased dietary thiamine has also been shown to eliminate or lessen clinical signs of PEM, even when characteristic brain lesions of PEM were present on histopathology.[24]

Thiamine also decreases the deposition of lead in tissue and lowers blood lead values. [72,74] In a group of dairy cows with experimental lead poisoning, thiamine significantly decreased lead deposition in liver, kidney, and brain, and to a lesser extent in bone.[74] Thiamine also influences cellular membrane function and the conduction of nerve impulses.[72]

The mechanism of the protective and preventative actions of thiamine following exposure to toxic levels of lead is not completely understood. Since excess lead and excess sulfur produce similar brain lesions and clinical signs, the possibility exists that thiamine's beneficial effects on PEM are the same or very similar. It has been postulated that thiamine may form insoluble complexes with lead, resulting in decreased absorption and increased elimination of bound lead.[74] The formation of similar insoluble sulfur-thiamine complexes may also occur, which could decrease the level of sulfur exposure in toxic situations.

Thiamine deficiency was incriminated as the etiology of PEM by numerous English veterinarians following significant improvement in clinical cases of cerebrocortical necrosis in sheep and calves treated with thiamine.[10-15] Research conducted by these veterinarians led to the discovery of the presence of thiaminase compounds in the rumen fluid of affected animals and also decreased blood levels of thiamine based coenzymes.[10-15]

Another research project using amprolium, a structural analog of thiamine, concluded thiamine deficiency leads to PEM. In this research, amprolium was used as a model to induce thiamine deficiency.[6] The results of this research are confusing to determine whether thiamine deficiency did occur in these sheep. According to the

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author, all seven sheep used in this experiment died from either amprolium toxicity or thiamine-deficient PEM. The classification to ascertain whether the deaths were due to thiamine deficiency or amprolium toxicity was determined by the length of time on trial and the brain lesions present. Four sheep died within three weeks of the beginning of the trial, and their deaths were attributed to amprolium toxicity. The three sheep that were classified as having thiamine-deficient PEM developed symptoms at six weeks and had decreased TPP levels. However, these values were not as low as previously reported TPP values for sheep considered to have naturally occurring thiamine-deficient PEM.[6]

In the early seventies several reports linked PEM to thiaminase compounds in *Kochia scoparia*.[7,8] The fact that *Kochia* is a sulfur accumulator and that the water source for these animals contained greater than 3000 ppm sulfate was not considered significant at that time.[7,8] The association of PEM caused by thiaminase production secondary to rumen acidosis was also reported at this time. Currently there are numerous reports in the literature of PEM associated with the ingestion of high levels of sulfur while levels of dietary, blood, and tissue thiamine were normal.[19,20,24,29-32] No literature linking PEM to a strict thiamine deficiency was found using available computer search procedures.

Prevention of sulfur-induced PEM involves eliminating exposure to high sulfur feed or water. The availability of adequate roughage is important in preventing rumen acidosis, which leads to an increase in hydrogen sulfide production. The addition of copper or molybdate has been reported to bind with sulfur to form insoluble sulfates, which are eliminated unchanged in the feces.[75,76] Adding 9,10 anthraquinone to the feed

inhibits sulfate reduction by the rumen bacteria.[31,77,78] Removing rumensin from the diet could reduce PEM as rumensin increases sulfate reduction by rumen bacteria.[31]

When water is the source of sulfur, removal from the water is necessary. If the sulfate level of the water is marginal, the amount of sulfur in the pasture and supplemental feeds must be known to prevent exceeding toxic levels. Grass samples tested for OADDL at Michigan State University have contained over 3000 ppm total sulfur. A sulfate level of 1000 ppm in water is the recommended maximum level when high environmental temperature or elevated sulfur levels in the feed are present.[76]

Although the nutrient content of corn by-products are comparable to conventional cattle feeds, the occurrence of toxic levels of sulfur in sporadic batches of CGF adds a high level of risk to their use.[79-84] To prevent PEM when feeding CGF, it is recommended that the animals receive no more than one-half percent of their body weight as CGF per day. Limiting the use of CGF in this manner simply dilutes total sulfur intake when the sulfur content of the CGF is unknown. If CGF is fed at rates above this level, the sulfur content of each new purchase of CGF should be determined prior to use. If high sulfur CGF is used, the sulfur content of other feedstuffs and available water should be known. Sulfur levels as low as 2500 ppm in the feed and water sulfate levels of 2000 ppm have been reported to decrease feedlot performance and carcass quality.[86,87]

Liquid feed supplements containing molasses and corn distiller's products also need to be fed with caution, especially if adequate forage is not available. The availability of adequate forage helps decrease the consumption of molasses based

supplements and aids in maintaining normal rumen pH. Molasses containing feeds have been reported to induce sulfur related PEM.[88]

CHAPTER II

Material and Methods

Fourteen weaned beef heifers with an average weight of 174.4 kg ±17.7 kg were used in a completely randomized design to evaluate the effects of three levels of dietary sulfur. The calves were assigned to one of three treatments designated M, MH, and H, which contained 3860, 5540, and 7010 ppm sulfur, respectively. The base ration consisted of 70 % corn gluten feed that contained 4450 ppm sulfur and 30 % CSH. This mixture comprised the ration for the 4 calves in M (moderate) treatment and contained 3860 ppm sulfur. Sodium sulfate was added to this ration to make treatments for MH (moderately high) and H (high), containing 5540 and 7010 ppm sulfur, respectively. There were 5 calves each in treatments MH and H. Community water was available to all treatments and contained 56 ppm sulfur. The nutrient content of these three diets is presented in Table 3.

Table 3. Nutrient Analysis of Feeds

	Treatments		
	M	MH	H
CP %	14.88	15.02	15.03
Avail P %	14	14.15	14.27
AD Fiber %	16.47	16.41	17.84
ND Fiber %	35.45	37.82	37.95
Sulfur %	0.386	0.554	0.701
Cu PPM]	9.51	7.63	6.04
Zn PPM	63.7	63.2	63
Se PPM	<10.0	<10.0	<10.0
Vit A* mg/ml	2070	3500	2266
Vit E PPM	12.08	16.54	16.09

* measured as Retinol

This research was co-sponsored by the Oklahoma State University College of Veterinary Medicine and the Oklahoma State University Department of Animal Science, and was conducted at the Animal Science Nutrition and Physiology Research Center. The calves were placed in individual pens and fed treatment M for three days, after which the calves in MH and H were switched to their respective diets. Feed intake was monitored twice daily during the 37 day study.

Blood samples were collected via jugular puncture from all calves prior to the introduction of the base ration, and at seven-day intervals or at euthanasia thereafter. Serum copper and zinc levels and whole blood selenium concentrations were determined from these samples. Also pretrial and final plasma samples were analyzed for vitamin A and vitamin E/cholesterol ratios.

Rumen hydrogen sulfide samples were obtained and analyzed using the procedure developed by researchers at Colorado State University.[6] The left paralumbar fossa was clipped and prepared for aseptic surgery. A sterile 3 inch 18 gauge needle was fitted with a 3-way stopcock and inserted into the rumen gas cap using sterile technique. A measured volume of rumen gas was drawn through a hydrogen sulfide detector tube placed between the stopcock and an airtight sampling pump. The hydrogen sulfide content of the rumen gas was read directly from the calibrated tubes.[b]

Two different calibrated H_2S detector tubes were used; one measured from 1–150 ppm and the other measured 100–2000 ppm hydrogen sulfide. The volumetric pump allowed samples to be measured in 5, 50, 60, 70, 95, and 100 ml units.[c] Hydrogen sulfide was not present in one pretrial sample even after multiple 100 ml samples were drawn through the 1-15 ppm detector tube. When hydrogen sulfide levels exceeded the 2000

ppm maximum detection limit, another tube was attached to the stopcock and a smaller fraction of rumen gas was drawn through the tube and the value adjusted to 100 ml.

Figure 4 - Rumen Gas Sample Taken From Paralumbar Fossa

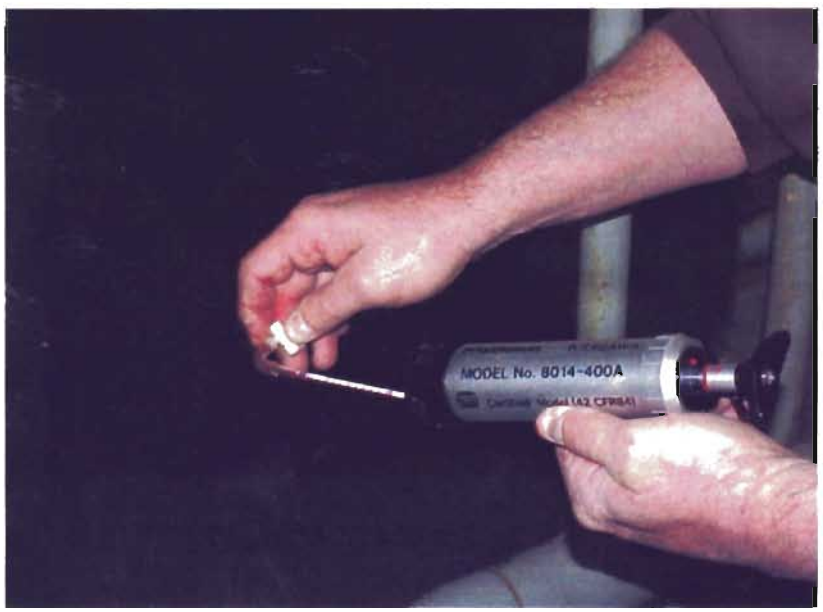
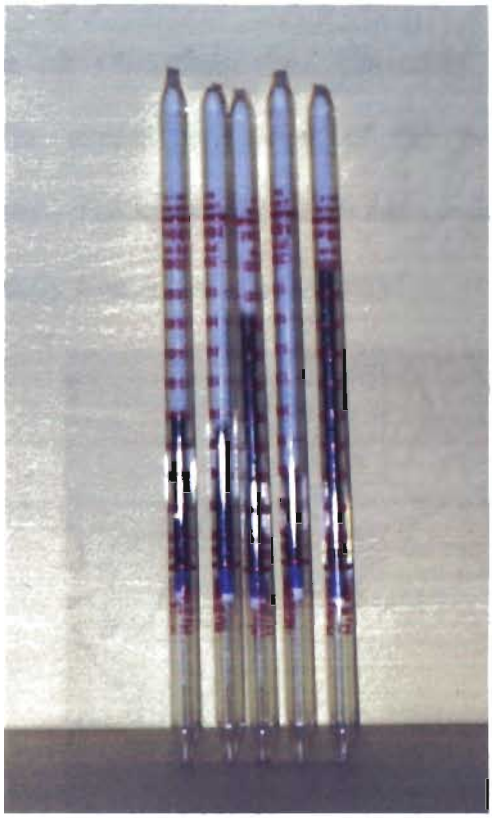
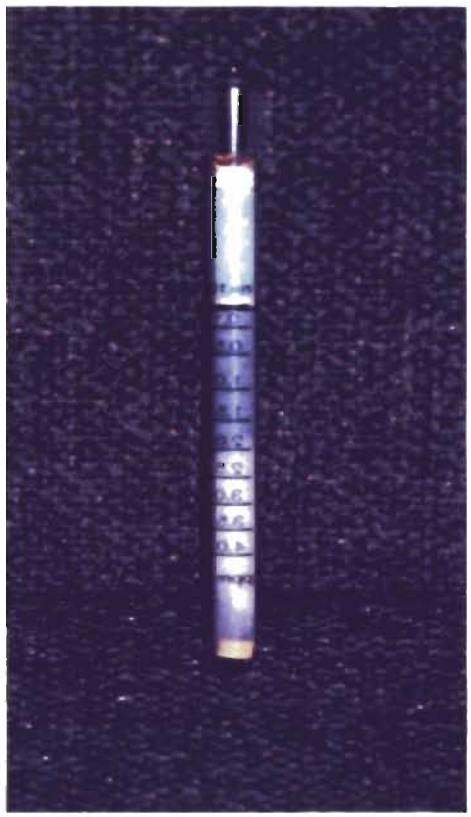


Figure 5 - Volumetric Air Pump





Figures 6 and 7 Hydrogen Sulfide Detector Tubes

Filtered breath samples were obtained by allowing the calf to breathe into a mask for two minutes before collecting the sample to be analyzed. This filter removed 99.7 % of the ethane, nitrous oxide and hydrogen from atmospheric air.[d] Then breath samples were collected in airtight containers and transported to the Respiratory Research Laboratory at the Oklahoma State University College of Veterinary Medicine for analysis. The ethane component of the breath sample was analyzed by gas chromatography. The hydrogen sulfide and nitrous oxide content of the breath samples were read directly from a toxic gas monitor.[e]

Figure 8 – Collection of Breath Samples

Observation

Animals were observed for illness twice daily. Animals that exhibited signs of central nervous system disease, such as cortical blindness, circling, head-pressing, lateral recumbancy, or coma, were examined and then humanely euthanized by administration of intravenous sodium pentobarbital.

Postmortem Evaluation

Postmortem examination of all calves was done at the OADDL. Lung, liver, spleen, thymus, lymph node, heart, kidney. rumen, reticulum, omasum, abomasum, ileum, and colon were fixed in 10 % buffered formalin. Stained tissue was analyzed microscopically. Immunohistochemistry and virus isolation were performed for BVD. Lung and intestine samples were submitted for viral and bacterial culture.

In addition to being examined for gross lesions, brains were weighed and their volumes measured. Brain weight and volume-to-body-weight ratios were calculated for each animal. One-half of the brain was fixed in buffered 10% formalin while the other half was examined under ultraviolet light for fluorescence. The sodium content of each brain was determined by flame atomic absorption spectrophotometery.

Toxicology

Fresh samples of liver were analyzed for copper, selenium, zinc, and lead content by atomic absorption spectrophotometery. The pH of each rumen sample was determined.

Statistical Analysis

General linear models and analysis by variance (GLM ANOVA) procedures were used for the analysis of these data. These procedures included variable analysis by group, variable change within group in relation to time periods, and a split plot analysis (mixed) to compare changes over time.

CHAPTER III

Results

Death Loss and Feeding Performance

Ten calves exhibited clinical PEM over the course of this research project, includeing all calves in treatments MH and H. Results of feeding performance are shown in Table 4. Mean days on trial were 37 (M), 31 (MH), and 21 (H). Although high dietary sulfur is reported to reduce feed intake, there was no statistical difference in feed intake (P>0.3) or final body weight (P>0.17) between treatments .[7]

Table 4 Feed Intake and Performance by Treatment

Trt	N	Mean Days	Daily intake (kg)	Total gain (kg)	ADG (kg)	Final Wt.(kg)
M	4	37	5.6165[a]	24.318[a]	0.6513[a]	196.61[a]
MH	5	31	5.5224[a]	20.766[a]	0.5608[ab]	184.98[a]
H	5	21	4.7314[a]	-0.454[b]	-0.07[b]	165.18[a]
LSD			0.9868	20.163	0.6313	34.31

[a,b] Means with the same letter in a column are not statistically significant P>0.05

Compared to treatments MH and H, calves in treatment M had higher total gain (P<0.04) and also a higher daily gain (P<0.04) than calves in treatment H. An

explanation for this is the difference in average days on trial, the H calves were on trial 10 and 26 days less than the MH and M calves, respectively.

The first two cases of PEM occurred in calves in the H treatment group, and occurred on days 15 and 17. The first calf was found down, semi-comatose and unable to stand on its own. This was the only calf to develop acute symptoms. The second calf exhibited head pressing, circling and ran into the fence when removed from the corner. It was also blind and lacked a menace reflex. The remaining cases of PEM developed between days 21 and 37. Since 4 calves were diagnosed with PEM on day 35 and one calf was diagnosed on day 37, acclimation to the increased sulfur in the diet apparently did not occur during the 37 day trial period. Table 5 shows individual feed intake and gain.

Table 5 Individual Feed and Performance Data

ID	Treatment	Polio	Days/ Trial	Feed (kg)	Total Sulfur (kg)	Sulfur Kg/day	Daily Consumption	Gain (kg)	ADG (kg)
702	H	yes	13	60.00	0.420	0.0323	4.615	0	0.000
690	H	yes	15	59.32	0.415	0.0277	3.955	-0.91	-0.061
529	H	yes	21	111.14	0.778	0.0371	5.292	-0.91	-0.043
725	H	yes	21	81.87	0.573	0.0273	3.899	-12.27	-0.584
576	H	yes	35	206.36	1.445	0.0413	5.896	11.82	0.338
253	MH	yes	15	74.32	0.412	0.0275	4.955	-2.73	-0.182
647	MH	yes	35	201.12	1.094	0.0313	5.746	55.00	1.571
739	MH	yes	37	195.46	1.063	0.0288	5.283	16.82	0.455
881	MH	yes	35	206.82	1.125	0.0314	5.909	17.27	0.493
942	MH	yes	37	211.59	1.151	0.0311	5.719	17.27	0.467
429	M	no	37	219.09	0.846	0.0229	5.921	25.00	0.676
626	M	no	37	230.80	0.891	0.0241	6.238	17.27	0.467
689	M	no	37	210.91	0.814	0.0220	5.700	29.09	0.786
982	M	no	37	170.46	0.658	0.0178	4.607	25.91	0.700

Mineral Analysis

There were no statistical differences between treatments in pre-trial and post-trial serum or blood values for copper, selenium, zinc, vitamin A, or vitamin E (Table 6).

There was a trend among the total population of calves towards an increase in the post-trial selenium levels as compared to the pre-trial values

Blood selenium values trended to increase in all calves during the course of this trial. The pre-trial blood selenium values were marginally deficient, with values between 0.073 - 0.12 ppm (adequate values are 0.2-1.2 ppm). The post-trial selenium values ranged from 0.11-0.217 ppm. Serum zinc values also increased for all calves during the study. All pre-and post-trial zinc values were in the normal range. Vitamin A and vitamin E/cholesterol ratios (normal 125-250; 1.5-2.5, respectively) declined for all treatments during the study. Although these values declined, all post-treatment levels for both vitamin A and vitamin E levels remained within normal limits. All of the rations for the 3 treatments were deficient in both vitamin A and E. The vitamin A and vitamin E blood concentrations were analyzed to eliminate these vitamins as the cause of disease in these calves.

Table 6 Mean Group Changes in Copper, Selenium, Zinc and Vitamins A and E

Trt	N	Copper	Selenium	Zinc	Vitamin A	Vit E/Chol*
M	4	0.0218	0.089	0.875	-510.50	-3.875
MH	5	-0.1746	0.070	0.70	-557.2	-3.930
H	5	-0.0160	0.053	0.42	-486.8	-4.060
	LSD	0.335	0.0401	0.3583	180.58	2.0679

No statistical differences were detected among these values
* vitamin E/cholesterol ratio

Copper, selenium, and zinc content in the liver samples were within normal limits for all the calves. All liver samples were all negative for lead, and the sodium content of the brain tissue was within the normal range for all calves.

Rumen Hydrogen Sulfide

Mean rumen hydrogen sulfide levels for each treatment are presented in Table 7. The baseline rumen hydrogen sulfide levels ranged from 1 ppm to 60 ppm, with a mean of 19.22 ± 18.99 ppm. No significant differences occurred in M but significant differences did occur between time periods for MH and H.

Table 7 Least Squares Mean Rumen H_2S Levels (ppm) in Relation to Sample Period

	Treatments		
Day	M	MH	H
0	13.25[a]	27.2[a]	15.8[a]
7	812.50[a]	1840.00[a]	8000.00[c]
14	4920.00[a]	2930.00[b]	6060.00[c]
21	3120.00[a]	13521.00[c]	12123.00[c]
28	1700.00[a]	4096.27[a]	18642.00[b]
37	2623.29[a]	13521[c]	1142.09[a]

Samples within a column with the same superscript are not statistically different P>0.05

Breath Analysis

No correlation was found between sulfur intake or rumen hydrogen sulfide levels and the ethane concentration in the breath samples. The ethane levels did not suggest sub-clinical oxidative injury to the lungs of the calves in any treatment. No clinical signs of respiratory illness were observed throughout the trial.

Brain Analysis

Gross brain lesions were seen in 12 of the 14 calves, while microscopic lesions were present in all calves. Gross lesions included cerebral edema with flattened cerebral gyri, malacia, and focal hemorrhage with cavitation occurring in the cerebral cortices and

cerebellum. Microscopic lesions were indicative of severe polioencephalomalacia, including multifocal pyogranulomatous vasculitis, meningitis, and marked menigeal edema. Increased perineuronal, periaxonal, and perivascular clear space within the neuropil was evident. Hemorrhages were present within the neuropil along with localized neuronal degeneration of the brainstem. Segmental degeneration of Purkinje cells was also evident. Microscopic lesions present in the calves that did not develop clinical PEM included congestion and edema within the cerebral corticies, with the presence of microcavitations in the brainstem.

The brain to body weight ratio was significantly less for treatment H calves compared to calves in treatments M and MH. Brain weight and brain volume were similar among all treatments.

Miscellaneous Clinical Observations

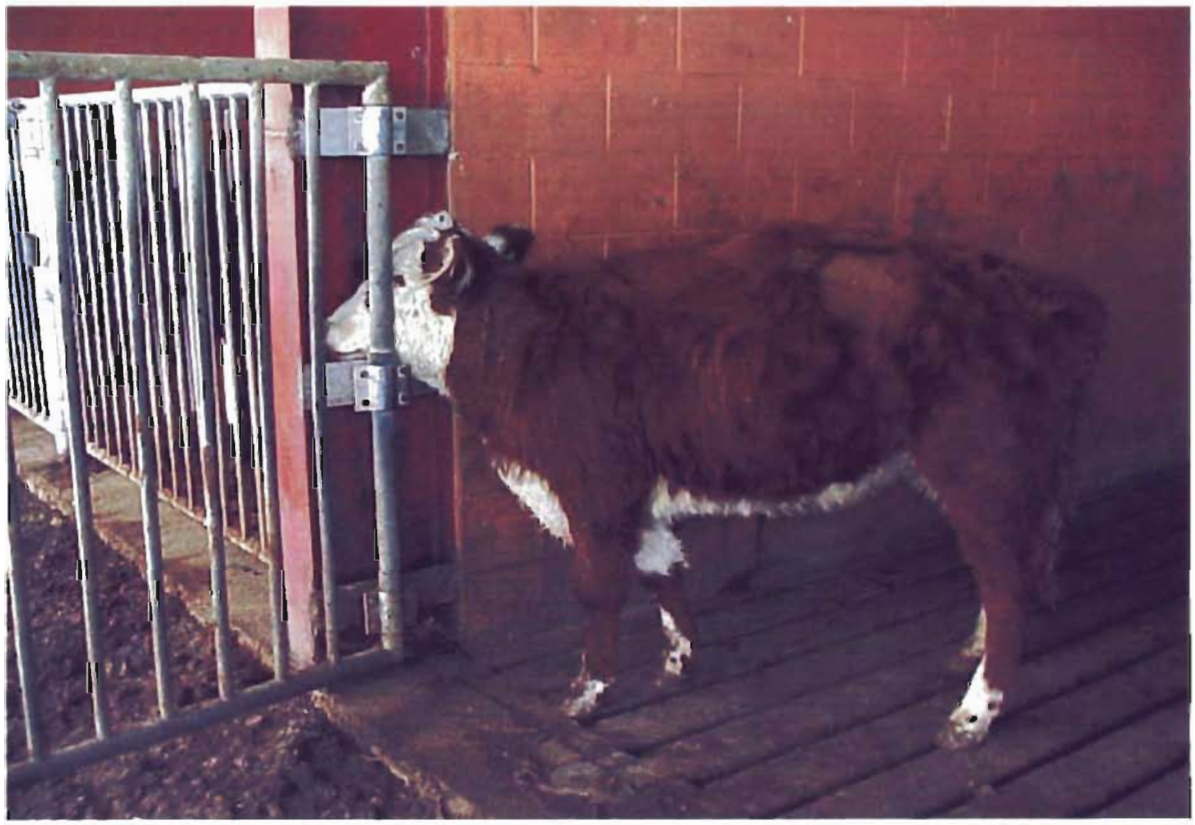
All calves periodically had self-limiting thin, soft stools. Calf #626 in M was treated with a probiotic paste,[f] on day one of the trial. Rumen pH values determined at postmortem examination ranged from 4.93 to 6.77. The rumen contents was liquid and no motile protozoa were present in any of the samples. The stomach and intestinal content of all the calves in the study had a pungent odor.

Table 8 Individual Calf - Brain Data

Calf ID	Treatment	Polio	Micro Lesions	Gross Lesions	Days /trial	Brain wt (gm)	Brain-Body wt ratio	Brain vol ml
702	H	Yes	Yes	No	13	370	0.0025	375
690	H	Yes	Yes	Yes	15	380	0.0025	375
529	H	Yes	Yes	Yes	21	385	0.0023	375
725	H	Yes	Yes	Yes	21	340	0.0024	324
576	H	Yes	Yes	Yes	35	400	0.0019	400
253	MH	Yes	Yes	Yes	15	365	0.0021	400
647	MH	Yes	Yes	Yes	35	335	0.0016	375
739	MH	Yes	Yes	Yes	37	370	0.0019	375
881	MH	Yes	Yes	Yes	35	350	0.0022	375
942	MH	Yes	Yes	Yes	37	330	0.0018	375
429	M	No	Yes	No	37	350	0.0018	375
626	M	No	Yes	Yes	37	395	0.0019	375
689	M	No	Yes	Yes	37	350	0.0017	350
982	M	No	Yes	Yes	37	330	0.0019	350

Calf 253 – Head Pressing

Figure 9



Calf 253 Trapped in Corner

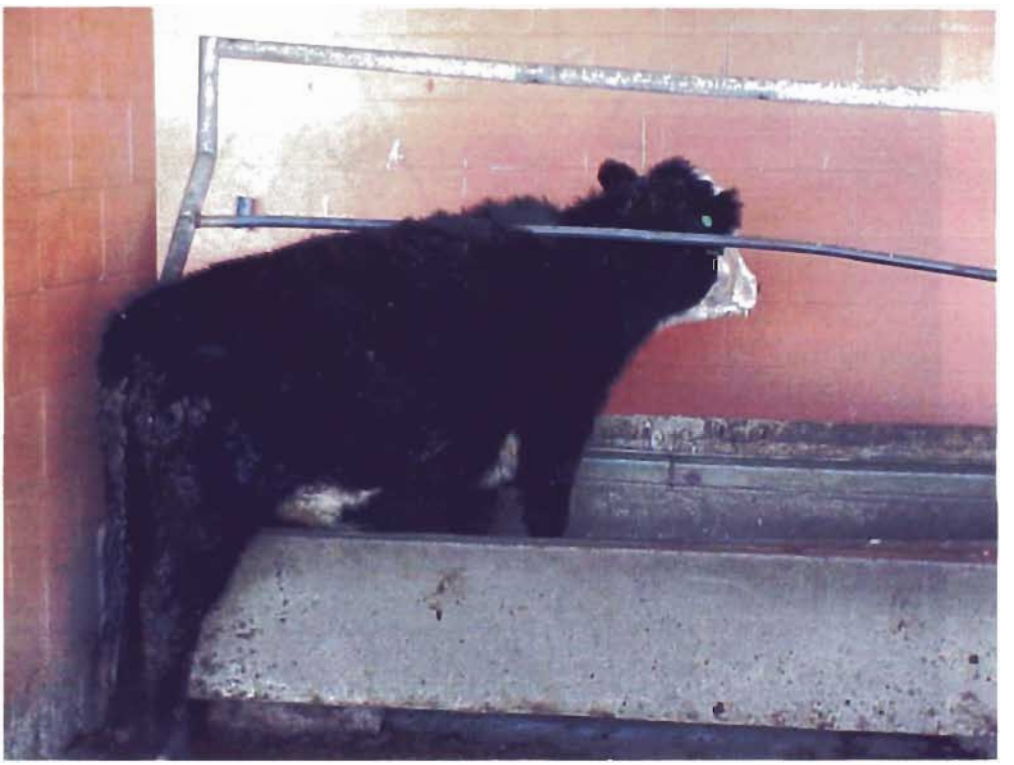
Figure 10

Calf 253 – Crossed Front Legs

Figure 11

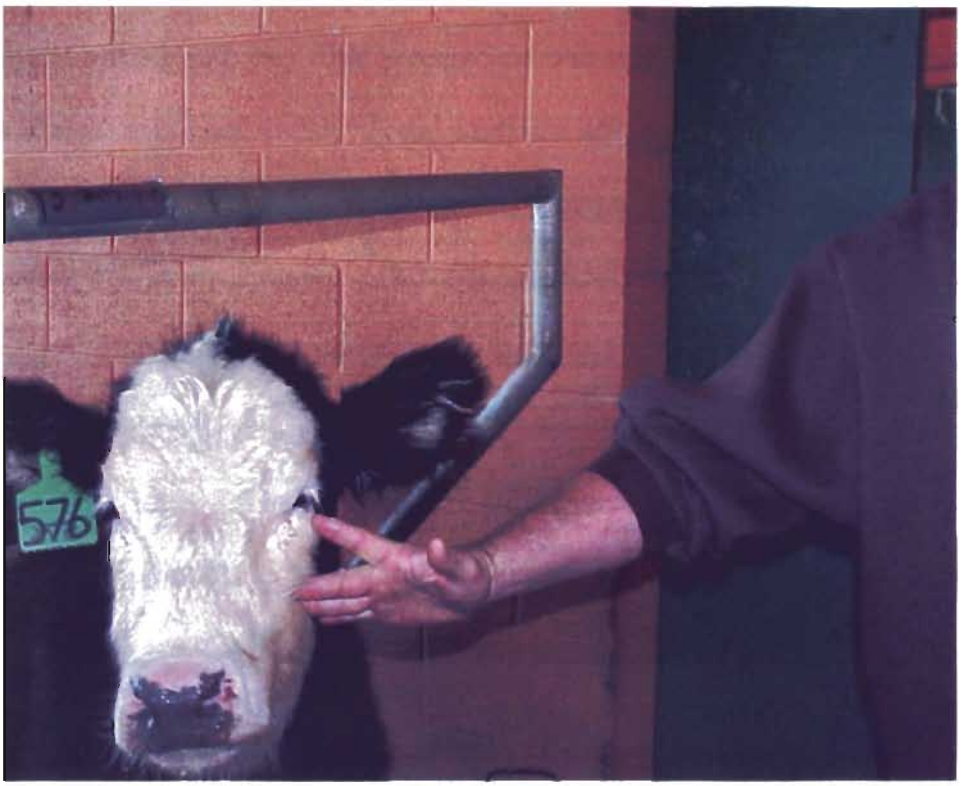
Calf - 690 Blank Stare and Drool

Figure 12



Calf 576 – Trapped in Feed Bunk

Figure 13



Calf 576 – Cortical Blindness and Absence of Menace Reflex

Figure 14

CHAPTER IV

DISCUSSION

Corn gluten feed was used in this study because it has been frequently associated with episodes of PEM in Oklahoma. Corn gluten feed is what remains of the corn seed after products such as ethanol, corn syrup, corn starch, corn gluten meal, etc. have been removed by the wet milling of corn. This process involves steeping corn in water for a variable period of time. Exogenous sulfur compounds are added in the steeping process, to stabilize the pH, carbohydrate, and protein fractions in order to separate them from the husk. This protein-rich and occasionally sulfur-rich steep water is then either added back to the husk to make corn gluten feed, or the steep water can be sold separately as corn distillers solubles, which is used as a protein source in various liquid cattle supplements.

The base CGF used in this study contained 4450 ppm sulfur. The NRC suggests that 1500 to 2000 ppm sulfur is the daily requirement for all classes of cattle; 4000 ppm is the maximum tolerated dose.[5] Although no clinical cases of PEM were diagnosed in the calves consuming less than 4000 ppm sulfur, gross and microscopic brain lesions were found. Brain lesions have occurred in sheep with sub-clinical PEM.[9] Sulfur levels greater than 2000 ppm in the feed or levels of 2000 ppm sulfate in water are reported to decrease feedlot performance and carcass value.[10,11]

Ten of 14 calves developed clinical polioencephalomalacia in this study. These calves were forced to consume CGF with toxic levels of sulfur similar to those seen in clinical cases diagnosed at OADDL. Colorado State University researchers have suggested that sulfur-induced PEM is more likely to occur if the feed contains highly

fermentable carbohydrates and has short fiber length. Both of these properties are present in corn gluten feed, even when mixed with cotton or soybean hulls.

The rumen and intestinal content of all the calves in this study were liquid and foul smelling with no live protozoa present. The pH levels of 4.9 to 6.2 present at necropsy favor increased hydrogen sulfide production by rumen bacteria. This in turn leads to an increased incidence of PEM.

Polioencephalomalacia is reported to occur as a result of grain overload, also called rumen acidosis or simply acidosis.[45-49] Although blood pH values of less than 7.35 are necessary for a clinical acidosis, rumen pH and the presence or absence of clinical signs are commonly used as indicators of acidosis.[49] Rumen pH values of cattle exhibiting acidosis that are below 5.2 are classified as acute, and values between 5.2 and 5.6 considered subclinical acidosis.[49,89,90] Acute acidosis is associated with clinical disease consisting of lethargy, variable feed intake, anorexia, rumen stasis, dehydration, diarrhea, metabolic acidosis, depression, and shock while subclinical acidosis is associated with reduced feed intake and poor performance without evidence of other metabolic disturbance.

As previously stated, rumen pH is extremely important in the pathogenesis of sulfur-induced PEM. The pK_a values for the disassociation of the first proton of hydrogen sulfide is 7.04.[61] At a pH of 7.4, approximately one third of the hydrogen sulfide is undissociated and exists as H_2S with the other two thirds disassociated as various sulfur and hydrosulfide ions.[61] As the rumen content becomes more acidic the amount of H_2S produced increases. When the rumen pH is less than 7, greater than 95%

of the metabolites of sulfur are present as undissociaterd hydrogen sulfide in the rumen gas cap. The remaining sulfur ions, which are less toxic, remain in the rumen fluid.

The association of clinical signs of CNS disease occurring as a result of the disruption of normal rumen function dates back to at least the early 1900s.[91-95] Brain lesions characteristic of PEM, including gliosis, demyelination, and neuronal degeneration of the gray matter of the cerebrum, thalamus, and medulla, have been associated with digestive disturbances and were described by Strafuss and Monlux in 1965.[96] They concluded, "The mechanism of action of toxic factors in ruminant indigestion and the relationship to central nervous system changes is open to speculation. It is known that altered rumen microflora and accumulation of toxic factors in rumen fluids result in biochemical alterations in blood that would suggest interference with critical metabolites. The fact that interference with critical metabolites or nutrients might cause cellular anoxia could explain the lesions we observed in the CNS associated with ruminant indigestion."

Studies of rumen acidosis in the early 1970s utilizing a purified liquid diet to induce acidosis in lambs, found an association between acidosis, CNS disease and PEM.[50] These researchers reported on the benefits of using thiamine to treat PEM, and suggested that adding supplemental thiamine may help prevent PEM.[50] This diet was subsequently used as a model to study both acidosis and PEM. The diet was comprised of corn starch, cane sugar, urea, purified casein, and sulfate containing minerals. Another diet also used for similar research at this time replaced corn starch and cane sugar with corn sugar.[54] The lambs fed these diets ingested approximately 10 grams of sulfur per day from the mineral and casein components of these diets. Since these lambs weighted

36 kg the level of sulfur exposure from this diet alone would have been >.28 grams per kilogram of body weight. By comparison, the heifers used in our research had an average daily intake of sulfur of intake of .124, .178 and .19 grams per kilogram for M, MH and H treatments. respectively. Based on this comparison and our current knowledge of the toxic effects of sulfur on ruminants, it is likely that the occurrence of PEM in these lambs was due to sulfur toxicity.

Additional research in the 1970s that used these liquid diets to study both acidosis and PEM described brain lesions characteristic of PEM, and also attributed the brain lesions to the presence of thiaminases in the rumen fluid.[51,52,54] Citing references from English literature concerning thiaminases, it was concluded that thiaminases were produced in response to the disruption of the rumen microorganisms because of increased rumen acidity. With our present understanding of the relationship between sulfur and PEM, it is probable that the occurrence of clinical PEM and brain lesions found in this early acidosis research was due to sulfur toxicosis, and not disruption of normal thiamine synthesis or metabolism.

Two MH treatment calves, 881 and 739, had postmortem rumen pH values < 5.5. Calf 881 developed clinical signs of PEM on day 35 of the trial. On the day of euthanasia, the heifer was anorexic, showed an apprehensive stare, and was ataxic. She could see straight ahead but would collide with objects slightly out of her central line of vision. Gross lesions present at postmortem examination included congested, dilated cerebral vessels with prominent edema and flattening of the cereberal gyri. Multifocal hemorrhages were also present. Malacia of the gray matter was seen during histopathological examination. Rumen pH was 4.93.

On day 32, calf number 739 was found standing over her water trough and attempting to drink with her muzzle halfway submerged and lapping water. Large amounts of water ran from her mouth when she moved from the trough. The feed-bunk was covered with water and the feed had been pushed to both sides with very little of it eaten. This calf exhibited bruxism on several previous occasions prior to becoming unable to eat or drink. The heifer had an anxious expression, was moderately depressed and circled, but she continued attempts to eat and drink throughout the day. The following morning the bottom of the feed bunk and the pen were dry, and the heifer was able to eat and drink normally, although she was still ataxic. She was euthanized the next morning. During gross examination of the brain, congested and dilated vessels were seen, moderate edema and multifocal minute hemorrhages in the cerebral cortices were observed . Malacia of the cerebral cortices was noted on histopathology. Her rumen pH was 5.18.

Although both of these calves had rumen pH values and clinical signs compatible with a diagnosis of acidosis, there were no post mortem lesions of the rumen or liver to support the diagnosis. When clinical signs of PEM occur in calves fed a diet prone to cause rumen acidosis, the rations should be critically evaluated for other possible causes of CNS disease. Critical evaluation of previous research linking PEM with thiamine deficiency resulting from the presence of thiaminases suggests that the high sulfur content of the feeds used in these studies was the most probable cause of PEM. An extensive literature search did not reveal any reports where thiaminase was found in the rumen contents of animals exhibiting PEM, and subsequently transferred to test animals that developed PEM. The inference from previous research is that since the

administration of exogenous thiamine helps prevent or reduce clinical symptoms of PEM, and because various thiaminases are found in the rumen fluid of the test animals exhibiting PEM a connection must exist between the two. More recent understanding of sulfur-toxicosis suggests that sulfur is at least one of the toxic factors of altered rumen metabolism that initiates CNS disease postulated by Strafuss and Monlux in 1965.

The absence of live rumen protozoa in our test animals was an unexpected but significant finding. Rumen protozoa function to improve both carbohydrate and protein digestion in ruminants.[97-99] Reports suggest that a lack of rumen protozoa only improves performance in young pre-ruminants, such as veal calves on a high energy diet containing a low amount of nondegradable protein.[97] Lack of rumen protozoa in other classes of ruminants is considered detrimental to production. Rumen protozoa also have a significant impact on the digestion, amount, and availability of sulfur, copper, zinc, iron and magnesium present within the rumen.[100,101]

The reason for the defaunation of the calves used in this research is not known, but it is possibly related to the high rumen hydrogen sulfide levels. Further research is needed in this area to determine if rumen hydrogen sulfide levels affect the number of rumen protozoa of calves consuming low to moderate levels of dietary sulfur.

Not all CGF contains toxic levels of sulfur, but it is rumored that some processing plants consistently produce products with high sulfur levels. This concern needs to be addressed by the producers of corn by-products. Unless the sulfur levels are consistant, feeding of CGF should be considered risky, especially when adequate long-stemmed roughage is not available.

There are numerous reports of feeding various forms of sulfur at levels greater than those in this study. Interestingly, in some studies scientists fed rations with sulfur levels up to 17,000 ppm and did not see clinical PEM. In other studies, feeding diets containing 2600 ppm sulfur resulted in clinical PEM. Whether the sulfur is elemental, organic or inorganic is important, but the type and length of fiber being fed may be of greater importance. In trials where high levels of sulfur did not result in PEM, long stem fiber was fed. In the clinical cases discussed in our study, fiber was provided to the affected calves in the form of dry grass and hay. If the hay or grass was limited or if the calves simply chose the grain mixture over the long stem fiber, they would be much more susceptible to decreases in rumen pH, which would predispose the animal to increased rumen hydrogen sulfide concentrations, frequently resulting in sulfur-induced PEM. Additional research is needed to explore feeding high sulfur diets with differing levels of long stemmed fiber.

Although the overall incidence of PEM is low, cases presented to OADDL have been associated with high morbidity and mortality and also significant numbers of nonproductive calves that survived clinical illness. To avoid this high morbidity and mortality, cattlemen need to know the sulfur content of the CGF and other dietary ingredients that they are feeding.

As expected, copper levels decreased as levels of sulfur in the diet were increased. There was no associated decrease in selenium or zinc with increased dietary sulfur. Selenium levels tended to increase for all calves from pretest values. Although vitamin A and E levels decreased, post-trial values were within normal ranges. These values were determined in order to eliminate vitamin A or E as a cause of disease.

The mean baseline level of 19.2 ppm for rumen hydrogen sulfide of in this study was much lower than the 450 ppm previously reported.[4] Further research using this procedure is needed to continue to define reliable baseline values for hydrogen sulfide levels in the rumen gas cap. This procedure was a quick and minimally invasive way to access sulfur consumption on a group level. Its use for individual animal diagnosis can be quite misleading as rumen hydrogen sulfide levels in the rumen gas cap decline rapidly during periods of anorexia.

Breath analysis to evaluate the effect of hydrogen sulfide on lung tissue did not suggest damage at the cellular level. Although it is widely reported that ruminants inhale up to 60% of eructated gas, it is interesting that detectable levels of H_2S were not present when breath samples were analyzed.[29,32,43,100] From this observation it would appear that direct absorption of HS^- and H_2S through the rumen wall is the major route of exposure. Whether eructated gas is inhaled and results in direct lung exposure or is a major route of exposure to H_2S needs further research. This test is currently impractical for clinical use, but when it is refined it will be useful in studying lung pathology.

The presence of lesions consistent with PEM in deeper brain structures other than the cerebral cortices has been reported to be diagnostic for sulfur-induced PEM, and is suggested as a way to differentiate it from what is conventionally called "thiamine-dependent" PEM.[23,26,36] The reason for this was the finding of extensive malacic lesions and hemorrhage in the thalamus and brainstem, without evidence of herniation of these structures into the foramen magnum. It was assumed that lesions present with "thiamine-dependent" forms of PEM were the result of pressure necrosis that occurs in the neurons resulting from brain swelling. Since brain swelling was not evident in the animals

exposed to toxic levels of sulfur, the authors concluded that the damage in these areas were a direct result of and specific for toxic levels of sulfur. Others have attributed the presence of lesions in the mid and hindbrain to ischemia caused by either impaired blood flow to these areas due to brain edema, or tissue hypoxia due to temporary reduced blood pressure.

In our study, lesions consistent with polioencephalomalacia were present in the cerebral cortices, thalamus, brainstem and cerebellum, with moderate brain swelling observed upon gross examination. It was concluded that the lesions were secondary to impaired blood flow and not diagnostic for sulfur-induced PEM.

A major objective of this research was to determine the relationship of specific brain lesions to various causes of PEM; as it turns out this is probably irrelevant. The current literature does not support thiamine deficiency as a primary cause of PEM. The association between PEM and plants containing thiaminases cited, in earlier US literature has been recently attributed to the sulfur content of the plants, and not thiaminases. In the early literature from England, thiaminase was considered the cause of the PEM based upon the animal's response to supplemental thiamine. Although thiaminases have been linked to PEM associated with acidosis for these same reasons, current literature review does not support this premise.

CHAPTER V

SUMMARY

Polioencephalomalacia is a descriptive term used to describe softening of the gray matter of the outer layers of the cerebral cortex. This term has become synonymous with

thiamine deficiency or impaired thiamine metabolism, which is unfortunate as the current literature does not support thiamine deficiency as a cause of PEM in animals with a functional rumen. Thiamine-responsive or sulfur-induced prefixes for PEM is a more accurate description of the pathogensis of PEM and should be considered for future use with sulfur toxicosis. Controlled studies are needed to evaluate the relationship, if any, between thiamine status and PEM. Sulfur toxicosis, lead poisoning, and sodium ion toxicois-water deprivation produce polioencephalomalacic lesions.

Sulfur has been proven to be at least one of the toxic factors that produces CNS disease as postulated by Strafuss and Monlux in 1965. Sulfur-induced polioencephalomalacia, without reference to thiamine status, has become a recognized form of PEM. Sources of sulfur include both water and feedstuffs, including pasture grasses. All sources of sulfur need to be considered when PEM is diagnosed in a group of calves. Sulfates in water are reported most commonly in the plains and intermountain states and Canada. At the OADDL, feed supplements containing CGF are the sulfur source most often associated with PEM.

Corn gluten feed can be high in sulfur, as exogenous sulfur compounds are used in wet milling of corn. The sulfur content of CGF tested by OADDL commonly exceeds 4000 ppm, which is considered by the NRC to be the maximum tolerated dose. When the sulfur content of CGF is unknown, calves should not be fed more than 0.5% of their body weight of CGF daily. Other common sulfur sources include molasses based liquid feeds, containing either dehydrated whey or corn distiller's solubles.

When PEM occurs in stocker cattle, excess inorganic and organic sulfur in the diet should be considered in the differential diagnosis. Further research is needed to

evaluate whether dietary sulfur fed to stocker calves at levels less than used in this research have an impact on future feedlot performance and carcass value. It also appears from this and other research that the National Research Council's recommended maximum tolerated level of 4000 ppm sulfur should be reevaluated, as levels less than 4000 ppm have been shown to be detrimental to animal performance.

Additional research using copper and or molybdenum supplementation, the addition of buffers to feed, or other supplements such as 9,10-anthraquinone to prevent PEM is needed. Cases of sulfur-induced PEM diagnosed at OADDL have increased dramatically during this past year. This increase is associated with not only the use of by-products of the corn industry, but increased water sulfate levels associated with the prolonged drought conditions occurring in the plains states of the United States.

Notations

A Inductively Coupled Plasma Analysis. Michigan State University, East Lansing Mich.

B Kitagawa Precision Gas Detector Tubes. 120SA and 120SB, Matheson Gas Products 166 Keystone Road, Montgomeryville, Pa. 18936

C Matheson-Kitagawa Model No. 8014-400A. Matheson Gas Products 166 Keystone Road, Montgomeryville, Pa. 18936

D Organic Vapor P100-105110, Survivair Inc. 3001 S Susan, Santa Anna, Calif. 92704

E Matheson-Kitagawa Model Toxic Gas Monitor No. 1000TGM. Matheson Gas Products 166 Keystone Road, Montgomeryville, Pa. 18936

F Probiocin Paste. CH Biosystems. Milwaukee, Wis.

References

1. Jensen R, Griner LA, Adams OR: Polioencephalomalacia of cattle and sheep. J Am Vet Med Assoc 129:311-321,1956.
2. Terlecki S, Markson LM: Cerebrocortical necrosis in cattle and sheep. Vet Rec 70:23-27,1961.
3. Howell JMcC: Polioencephalomalacia in calves. Vet Rec 73:1165-1179,1961.
4. Raisbeck MF: Is polioencephalomalacia associated with high-sulfate diets. J Am Vet Med Assoc180:1303-1305,1982.
5. Dickie CW, Nelson RJ, Frazee DG: Polioencephalomalacia in range cattle. J Am Vet Med Assoc 175:460-462,1979.
6. Loew FM, Dunlop RH: Induction of thiamine inadequacy and polioencephalomalacia in adult sheep with amprolium. Am J Vet Res 332:195-2205,1972.
7. Dickie CW, Berryman JR: Polioencephalomalacia and photosensitization associated with *Kochia scoparia* consumption in range cattle. J Am Vet Med Assoc 175:463-465, 1979.
8. Dickie CW, James LF: *Kochia scoparia* poisoning in cattle. J Am Vet Med Assoc 183:765-768,1983.
9. Pierson RE, Jensen R: Polioencephalomalacia in feedlot lambs. J Am Vet Med Assoc166:257-259,1975.
10. Pill AH, Davies ET, Collings DF: The experimental reproduction of lesions of cerebrocortical necrosis in a calf. Vet Rec 78:737-738, 1966.
11. Pill AH: Evidence of thiamine deficiency in calves affected with cerebrocortical necrosis.Vet Rec. 81:177-180, 1967.

12. Davies E T, Pill AH, Austwick PKC: The possible involvement of thiamine in the aetiology of cerebro-cortical necrosis. Vet Rec 83:681-682,1968.

13. Markson LM, Terlecki S, Lewis G: Cerebrocortical necrosis in calves. Vet Rec 79:578-579, 1966.

14. Edwin EE, Lewis G, Allcroft R: Cerebrocortical necrosis: a hyposthesis for the possible role of thiaminases in its pathogenesis. Vet Rec 83:176-177, 1968.

15. Edwin EE, Jackman R: Ruminal thiaminase and tissue thiamine in cerebrocortical necrosis. Vet Rec 92:640-641, 1973.

16. Brent BE, Bartley EE: Thiamin and niacin in the rumen. J Anim Sci 59:813-821,1983.

17. Haven TR, Caldwell DR, Jensen R: Role of predominant rumen bacteria in the cause of polioencephalomalacia (cerebrocortical necrosis) in cattle. Am J Vet Res 44:1451-1455, 1983.

18. Gooneratne SR, Andrzej AA, Klemmer RG: High sulfur related thaimine deficiency in cattle: a field study. Can Vet J 30:139-146, 1989.

19. Gooneratne SR, Olkowski AA, Christensen DA: Sulfur-induced polioencephalomalacia in sheep: some biochemical changes. Can J Vet Res 53:462-467, 1989.

20. Olkowski AA, Gooneratne SR, Rousseaux CG: Role of thiamine status in sulphur induced polioencephalomalacia in sheep. Res Vet Sci 52:78-85, 1992.

21. Short SB, Edwards WC: Sulfur (hydrogen sulfide) toxicosis in cattle. Vet Hum Toxixol 31:451-453, 1989.

22. McAllister MM, Fould DH, Hamar DW: Sulphide-induced polioencephalomalacia in lambs. J Comp Path 106:267-278, 1992.

23. Jeffrey M, Higgins RJ, Simpson VR: Polioencephalomalacia associated with the ingestion of ammonium sulphate by sheep and cattle. Vet Rec 134:343-348, 1994.

24. Rousseaux CG, Olkowski AA, Chauvet A, et al: Ovine polioencephalomalacia associated with dietary sulphur intake. J Vet Med A 38:229-239, 1991.

25. Bulgin MS, Lincoln SD, Mather G: Elemental sulfur toxicosis in a flock of sheep. J Am Vet Med Assoc 208:1063-1065, 1996.

26. Low JC, Scott PR, Howie F: Sulphur-induced polioencephalomalacia in lambs. Vet Rec 38:327-329, 1996.

27. Beck C, Dart AJ, Collins MB: Polioencephalomalacia in two alpacas. Aust Vet J 74:350-352, 1996.

28. Hill FI, Ebbett PC: Polioencephalomalacia in cattle in New Zealand fed chou moellier (*Brassica oleracea*). New Zealand Vet J 45:51-59, 1997.

29. Olkowski AA: Neurotoxicity and secondary metabolic problems associated with low to moderate levels of exposure to excess dietary sulphur in ruminants: a review. Vet Hum Toxicol 39:359-360, 1997.

30. De Oliverira L A, Jean-Blain C, Komisarczuk-Bony S: Microbial thiamin metabolism in the rumen simulating fermenter (rusitec); the effect of acidogenic conditions, a high sulfur level and added thiamin. Brit J Nut 78:599-613, 1997.

31. Kung L, Bracht JP, Hession AO: High-sulfate induced PEM in cattle examined. Feedstuffs Nov 16:12-17, 1998.

32. Gould DH: Polioencephalomalacia. J Anim Sci 76:309-314, 1998.

33. O'Toole D, Raisbeck M, Case JC: Selenium-induced "blind staggers" and related myths. A commentary on the extent of historical livestock losses attributed to selenosis on western US rangelands. Vet Pathol 116:104-116, 1996.

34. Hibbs CM, Thilsted JP: Toxicosis in cattle from contaminated well water. Vet Hum Toxico 25:253-254, 1983.

35. Veenhuizen MF, Shurson CG: Effects of sulfate in drinking water for livestock. J Am Vet Med Assoc 201:487-492, 1992.

36. Hamlen H, Clark E, Janzen E: Polioencephalomalacia in cattle consuming water with elevated sodium sulfate levels: a herd investigation. Can Vet J 34:153-158, 1993.

37. Weeth HJ, Hunter JE: Drinking of sulfate-water by cattle. J An Sci 32:277-281,1971.

38. Harries WN: Polioencephalomalacia in feedlot cattle drinking water high in sodium sulfate. Can Vet J 28:717, 1987.

39. Hardt PF, Ocumpaugh WR, Freene LW: Forage mineral concentration, animal performance, and mineral status of heifers grazing cereal pastures fertilized with sulfur. J Anim Sci 69:2310-2320, 1991.

40. National Research Council, Nutrient Requirements of Beef Cattle. National Academy Press Washington DC. 204-213, 1196

41. Sager FL, Hamar DW, Gould DH: Clinical and biochemical alterations in calves with nutritionally induced polioencephalomalacia. Am J Vet Res 51:1969-1973, 1990.

42. Gould DH, McAllister MM, Savage JC: High sulfide concentration in rumen fluid associated with nutritionally induced polioencephalomalacia in calves. Am J Vet Res 52:1164-1169, 1991.

43. Lonergan GH, Gould DH, Callan RJ: Association of excess sulfur intake and an increase in hydrogen sulfide concentrations in the ruminal gas cap of recently weaned beef calves with polioencephalomalacia. J Am Vet Med Assoc 213:1599-1604, 1998.

44. Padovan D: Polioencephalomalacia associated with water deprivation in cattle. Cornell Vet 70:153-159, 1980.

45. Kersting KW, Thompson JR: Diseases of the ruminant forestomach. In: Howard and Smith ed. Philadelphia: *Current Veterinary Therapy 4 Food Animal Practice.* W B Saunders 1999:507-509

46. Garry FB: Diseases of the alimentary system. In: Bradford P. Smith ed: St. Louis, *Large Animal Internal Medicine.*Mosby 1990:747-781.

47. Underwood W. Rumen lactic acidosis.part I.epidemiology and pathophysiology. *The Compendium* 14:1127-1134,1992.

48. Underwood W. Rumen lactic acidosis. Part II. Clinical signs,diagnosis, teatment, and prevention. *The Compendium* 14:1265-1269,1992.

49. Owens FN, Secrist DS, Hill WJ, et.al: Acidosis in cattle: a review. *J Anim Sci* 76:275-286, 1998.

50. Lusby KS, Brent BE: An experimental model for polioencephalomalacia. *J Anim Sci* 35:270 (abstr.) 1972.

51. Spaienza DA, Brent BE: Rumen thiaminase and polioencephalomalacia. *J Anim Sci* 35:1134 (abstr) 1972.

52. Spaienza DA, Brent BE: Ruminal thiaminase vs. concentrate adaptation. *J Anim Sci* 39:251 (abstr) 1974.

53. Brent BE: Relationship of acidosis to other feedlot ailments. *J Anim Sci* 43:930-935, 1973

54. Vestweber JGE, Leipold HW: Induced ovine ruminal acidosis. Pathologic changes. *Am J Vet Res* 35:1537-1540, 1974.

55. Kahlon TS, Meiske JC, Goodrich RD: Sulfur metabolism in ruminants. In vitro availability of various chemical forms of sulfur. J Anim Sci 41:1147-1152. 1975.

56. Slyter LL, Chalupa W, Oltjen RR: Response to elemental sulfur by calves and sheep fed purified diets. J Anim Sci 66:1016-1027, 1988.

57. Cummings BA, Gould D H, Caldwell DR: Ruminal microbial alterations associated with sulfide generation in steers with dietary sulfate-induced polioencephalomalacia. Am J Vet Res 56:1390-1394, 1995.

58. Cummings B A, Caldwell DR, Gould DH: Identity and interactions of rumen microbes associated with dietary sulfate-induced polioencephalomalacia in cattle. Am J Vet Res 56:1384-1389, 1995.

59. Slyter LL, Chalupa W, Oltjen RR: Sulfur influences on rumen microorganisms in vitro and in sheep and calves. J Anim Sci 63:1949-1959, 1986.

60. Kandylis K: Toxicology of sulfur in ruminants: review. J Dairy Sci 67:2179-2187,1983.

61. Beauchamp RO, Bus JS, Popp JA: A critical review of the literature on hydrogen sulfide toxicity. CRC Critical Reviews in Toxicology 13:25-56, 1984.

62. Gould DH, Cummings BA, Hamar DW: In vivo indicators of pathologic ruminal sulfide production in steers with diet-induced polioencephalomalacia. J Vet Diagn Invest 9:72-76, 1997.

63. Loneragan GH, Gould DH, Wagner JJ: Patterns of ruminal H_2S generation in feedlot cattle. The Bov Proceed 30:136, 1997.

64. Kerr LA, Linnabary RD: A review of interstitial pneumonia in cattle. *Vet Hum Toxicol* 31:247-254, 1989.

65. Jubb KVF, Huxtable CR: The nervous system, polioencephalomalacia in ruminants In:Jubb, Kenney and Palmer eds. *Pathology of Domestic Animals* 3rd ed. San Diego: Academic Press, Inc. 1993:342-343.

66. Lindenberg R: Compression of brain arteries as a pathogenic factor for tissue necrosis and their arteries of predilection. *J Neuropathol Exp Neurol* 14:223-288, 1955.

67. Divers T J: Neurologic diseases,toxic and metabolic encephalopathies. In: Howard and Smith ed. Philadelphia: *Current Veterinary Therapy 4 Food Animal Practice*. W B Saunders 1999, 660-661.

68. Plumb DC: Thiamine HCl In: *Veterinary Drug Handbook*. Ames: Iowa State University Press 1999:605-607.

69. Breves G, Hoeller H, Harmeyer J, et al: Thiamin balance in the gastrointestinal tract of sheep. *J Anim Sci* 51:1177-1181, 1980.

70. Rawn JD: The citric acid cycle. In: *Biochemistry*. Burlington Neil Patterson Pub 1989, 329-337.

71. Phillips RW: Water-soluble vitamins. In: Booth and McDonald ed. *Veterinary Pharmacology and Therapeutics* 6th Ames Iowa State University Press. 698-702, 1988a.

72. Kim JS, Crichlow EC, Blakley BR, et al: The effects of thiamine on the neurophysiological alterations induced by lead. *Vet Hum Toxicol* 32:101-105, 1990.

73. Coppock RW, Wagnor WC, Reynolds JD. et al: Evaluation of edetate and thiamine for treatment of experimental lead poisoning in cattle. *Am J Vet Res* 52:1860-1865, 1991.

74. Bratton GR, Zmudzki J, Bell MC, et al: Thiamin (vitamin B_1) effects on lead intoxication and deposition of lead in tissues: therapeutic potential. *Toxicol Appl Pharacol* 58:164-172, 1981.

75. Ivan M, Veira DM: Effects of copper sulfate supplement of growth, tissue concentration and ruminal silubilities of molybdenum and copper in sheep fed low and high molybdenum diets. *J Dairy Sci* 68:891-896, 1985.

76. Puls R: Sulfur. *Mineral Levels in Animal Heath.* Clearbrook, Canada Sherpa International: 264-266, 1994.

77. Kung L Jr, Smith KA, Ranjit NK, et al: The effect of 9,10 anthraquinone (aq) ruminal fermentation in lambs. J *Anim Sci* 74(suppl. 1):96 (abstr), 1996.

78. Kung L Jr., Hession AO, Bracht JP: Inhibition of sulfate reduction to sulfide by 9,10-anthraquinone in vitro ruminal fermentations. *J Dairy Sci 81*:2251-2256, 1998.

79. Wagner JJ, Lusby KS, Horn GW: Condensed molasses solubles, corn steep liquor and fermented ammoniated condensed whey as protein sources for beef cattle grazing dormant native range. J Anim Sci 57:542-552, 1983.

80. Firkins JL, Berger L, Fahey GC: Evaluation of wet and dry distillers grains and wet and dry corn gluten feeds for ruminants. J Anim Sci 60:847-860, 1985.

81. Green DA, Stock RA, Goedeken FK: Energy value of corn wet milling by-product feeds for finishing ruminants. J Anim Sci. 65:1655-1664, 1987.

82. Berger LL: Corn gluten feed, sulfur nutrition of beef cattle discussed. Feedstuffs March 20:12-13, 2000.

83. Larson EM, Stock RA, Klopfenstein TJ, et al: Feeding value of wet distillers byproducts for finishing ruminants. *J Anim Sci* 71 22208-2236, 1993.

84. Krehbiel CR, Stock RA, Herold DW. et al: Feeding wet corn gluten to reduce subacute acidosis in cattle. *J Anim Sci* 73:2931-2939, 1995.

85. Zinn RA, Alverez E, Mendez M: Influence of dietary sulfur level on growth performance and digestive function in feedlot cattle. *J Anim Sci* 75:1723-1728,1997.

86. Wagner JJ, Loneragan GH, Gould DH: The effects of varying water sulfate concentration on feedyard performance and water intake of steers. *J Anim Sci* 75(suppl. 1):272, 1997.

87. Mella CM, Perez-Oliva O, Loew FM: Induction of bovine polioencephalomalacia with a feeding system based on molasses and urea. *Can J comp Med* 40:104-110, 1976.

88. Nocek JE: Bovine acidosis: implications on laminitis. *J Dairy Sci* 80:1005-10028, 1997.

89. Ortolani EL. Induction of lactic acidosis in cattle with sucrose: relationship between dose, rumen fluid pH and animal size. Vet Human Toxicol 37:462-464, 1995.

90. Udall DH, Cushing HR, Fincher: Interpretation of diseases of the nervous system. *Cornell Vet* 12:101, 1922.

91. Mettler FA: Some neurologic derangements of animals. *Cornell Vet* 36:192, 1946.

92. Fincher MG: Diseasses of the digestive tract in bovines. *J Am Vet Med Assoc* 96:466, 1940.

93. McIntosh RA: Digestive disturbances of cattle. *J Am Vet Med Assoc* 98:441, 1941.

94. Hoflund S, Hedstrom H: Disturbances in rumen digestion as a predisposing factor in the appearance of acetonemia. Cornell Vet 38:405, 1948.

95. Strafuss AC, Monlux WS: A central-nervous-system reaction to disturbances in ruminant digestion. *Cornell Vet* 56:128-141, 1966.

96. Veira DM: The role of ciliate protozoa in nutrition of the ruminant. *J Anim Sci* 63:1547-1560, 1986.

97. Katz MP, Nagaraja TG, Fina LR: Ruminal changes in monensin and lasalocid-fed cattle grazing bloat-provocative alfalfa pasture. *J Anim Sci* 63:1246-1257, 1986.

98. Ivan M, Verira DM, Kelleher CA: The alleviation of chronic copper toxicity by ciliate protozoa. *Brit J Nutrition* 55:361-367, 1986.

99. Hume ID, Bird PR: Synthesis of microbial protein in the rumen iv. The influence of the level and form of dietary sulphur. *Aust J Agric Res* 21:315-322, 1970.

100. Doughterty RW, Stewart WE, Nold MM: Pulmonary absorption of eructated gas in ruminants. Am J Vet Res 23: 205-211, 1962.

101. Ivan M, Effects on faunation and type of dietary protein on gastric solubility and liver content of copper in sheep. *J Anim Sci* 67:3028-3035, 1989

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