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The Relationship Between Sulfur, Thiamine and Polioencephalomalacia–A Review

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Abstract

Thiamine deficiency has been classically described as the cause of ruminant polioencephalomalacia (PEM). More recently excess dietary sulfur has been shown to be a major cause of PEM. This paper reviews the relationship between PEM and thiamine metabolism in mature cattle, thiaminase in plants, rumen acidosis and excess dietary sulfur.

Signs of PEM caused by lead poisoning, water deprivation-sodium ion toxicity and sulfur toxicosis are clinically and microscopically indistinguishable. Diagnosis of sulfur-induced PEM relies on ruling out other diseases, and demonstrating elevated dietary sulfur intake. Total sulfur intake from both feed and water must be determined when investigating an outbreak of PEM in cattle.

Résumé

L'insuffisance en thiamine est normalement considérée comme la cause de la polio-encéphalomalacie (PEM) chez les ruminants. Plus récemment, un excès de soufre alimentaire a aussi été impliqué comme cause importante de la PEM. Cet article revoit la relation entre la PEM et le métabolisme de la thiamine chez le bétail adulte, la thiaminase chez les plantes, l'acidose du rumen et l'excédent de soufre alimentaire.

Les signes associés à la PEM qui résultent soit de l'intoxication au plomb, de la toxicité des ions sodium entraînée par le manque d'eau ou soit de l'intoxication au soufre sont identiques tant au niveau clinique que microscopique. Le diagnostic de la PEM associé à l'intoxication au soufre nécessite l'élimination des autres causes potentielles et la démonstration d'une prise alimentaire élevée en soufre. La quantité totale de soufre provenant de la nourriture et de l'eau doit être déterminée lors de l'examen des causes associées à la PEM chez le bétail.

History of Polioencephalomalacia

Polioencephalomalacia (PEM) is a term that describes necrosis of the gray matter of the brain, commonly called "polio". This disease has historically been associated with thiamine deficiency. This is unfortunate as lead poisoning, water deprivation-sodium ion toxicity and consumption of excess dietary sulfur all produce similar lesions.

Reports of central nervous system (CNS) disease resulting from impaired rumen function first appeared during the early 1900's.[20,33,56,58,83,84] In 1966 Strafuss and Monlux described brain lesions characteristic of PEM including gliosis, demyelination, and neuronal degeneration of the gray matter in the cerebrum, thalamus and medulla that were associated with digestive disturbances in ruminants.[82] 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 fluid 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."[82]

Forage poisoning and blind staggers diagnosed in Colorado and Wyoming during the late 1950's were considered to be forms of PEM associated with thiaminase compounds found in selenium accumulating plants.[38,82] Mercury and cobalt poisoning, in addition to infectious agents, have been suggested as possible etiologic agents.[35,38] During the 1970's and 1980's, numerous scientists reported that thiaminase compounds found in plants and rumen fluid either destroyed thiamine or prevented its synthesis, therefore initiating thiamine-deficient PEM.[5,11,13-15,18,19,22,29,35,47,53,67-69,82] Rumen acidosis

was also reported to cause thiamine-deficient PEM.[4,21,44,52,66,80,81,85,86] Based on this literature, thiamine deficiency became widely accepted as the cause of ruminant PEM.

The Role of Sulfur in PEM

Sulfur was first linked to PEM in the 1980's when investigators determined that gypsum (calcium sulfate) added to cattle rations to control feed intake caused PEM. When gypsum was removed from rations, the incidence of PEM decreased.[73] These investigators questioned whether the added sulfate salts altered thiamine metabolism or if an unknown toxin was present in sulfates.[73] It is now known that sulfur-induced PEM is caused by the direct action of excess dietary sulfur, independent of factors affecting thiamine status.[2,7,12,23-28,30-32,37,45,48-51,54,57,60-65,75-77,87]

Sources of dietary sulfur are widespread. High sulfate water, commonly called gyp water, is a common cause of PEM throughout the plains and intermountain regions of the United States and Canada.[87] Also, feeds containing grain, dairy and sugar by-products can contain high levels of sulfate.[26,57,60,61,63] Small grain forages have been found to contain high sulfur levels, especially if fertilized with sulfate fertilizers.[30] Exposure to elemental sulfur can also cause PEM.[7]

The National Research Council (NRC) recommends that rations for growing and adult beef cattle contain 1500 to 2000 ppm sulfur (0.15-0.20% of the ration on a dry matter basis), a requirement easily met by most ration formulations.[59,62] Dormant bermuda grass can contain over 3000 ppm sulfur,[61] which illustrates that many common feedstuffs can contain high levels of sulfur. Inclusion of ingredients with high sulfur content can easily exceed the recommended maximum level of 4000 ppm (0.40%) sulfur.[59] Clinical cases of PEM occur at levels lower than that.[45,63,75,76] Sulfur levels as low as 2500 ppm in feed and sulfate levels of 2000 ppm in water have been reported to decrease feedlot performance and carcass quality.[89,90] Table 1 lists the sulfur content of some common feeds.

Table 1. Sulfur content of common feeds.[30,76]

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

Sulfur Metabolism by Rumen Bacteria

Rumen bacteria metabolize elemental, inorganic and organic sulfur, therefore the total sulfur content of feeds must be determined when establishing sulfur intake levels. Elemental and organic sulfur compounds yield fewer free sulfur radicals when metabolized than does sulfate. The availability of elemental sulfur is 30% to 40% of methionine, and 45% to 50% of sodium sulfate.[40,78]

The reduction of sulfate in the rumen is accomplished by both assimilatory and dissimilatory bacteria.[9,10,12,45,79] Assimilatory bacteria, which includes *Bacteroides*, *Butyvibrio*, and *Lachnospira spp*, reduce sulfate for their own metabolic needs and produce sulfur containing amino acids.[9,10] These bacteria produce less sulfide than dissimilatory bacteria since sulfide production is limited by the presence of other organic sulfur compounds in the rumen.[9]

Dissimilatory bacteria, predominantly *Desulfovibrio* and *Desulfotomaculum spp,* also utilize sulfates for their energy needs, but produce much more sulfide than needed.[9,10] The reduction of sulfates by dissimilatory bacteria account for the majority of sulfide production in the rumen.[9,10] Production of hydrogen sulfide by dissimilatory bacteria is limited by the amount of hydrogen sulfide present in the rumen gas cap while colony numbers remain constant.[9,10] These bacteria increase hydrogen sulfide production during an acclimation period of seven days *in vitro,* or 10-12 days *in vivo*.[9,10,12]

Rumen bacteria that contain cysteine desulfhydrase liberate additional sulfide via enzymatic reactions with sulfur-containing proteins.[9,10,49] These bacteria, like dissimilatory bacteria, are anaerobes. Bacteria capable of enzymatic release of sulfide include *Veillonella*, *Megasphaera, Wolinella, Selenomonas, Anaerovibrio* and *Clostridium spp.*[9,10]

Hydrogen sulfide present in the rumen gas cap readily diffuses into the portal bloodstream where it disassociates to $H^+ + HS^-$ because of the increased pH of blood. In blood HS^- is oxidized by heme to H_2O and S, which is reconverted to sulfate by sulfide oxidase present in the liver.[1,25,41,79] Hydrogen sulfide also undergoes methylation reactions and conjugation with metallo-proteins.[1] Sulfur's toxic effects are exerted by disruption of cytochrome oxidase enzyme complexes. The major route of excretion of sulfate from the body is through urine.[1,41] Some sulfate is excreted in saliva, which when swallowed re-enters the rumen sulfur pool.[41,78]

Hydrogen Sulfide in the Rumen Gas Cap

The concentration of the sulfur metabolites HS^-, HSO_3^-, S_2 and S^0 within the rumen fluid and gas are not static and are greatly affected by rumen pH.[1,12,26,45] The acidic nature of the rumen pH favors the formation of

H_2S, which has a pKa value 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 two-thirds in the form of the hydrosulfide ion.[1] 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 hydrogen sulfide in the rumen gas cap increased from 46.8 to 97.2%.[26] From this, it is apparent that the amount of readily available carbohydrate in the ration is an important factor in the initiation of sulfur-induced PEM.

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.[1,45] These radicals damage oxidative processes within the mitochondria by blocking cytochrome C, which leads to depletion of ATP with cellular anoxia and death.[1,25,41,49,77] Acute respiratory failure can occur due to paralysis of the carotid body.[1,54]

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.[63] The preponderance of lesions in the cerebral cortices are related to the high demand for oxygen in this area of the brain.[75] Grey matter has a higher demand for oxygen then white matter because of its many synapses.[65] Fluorescence of brain tissue can occur with ultraviolet illumination (365 nm). Its presence is attributed to ceroid lipofuscin, a product of lipid peroxidation found in macrophages.[54] Auto-fluorescence is not specific to brain tissue and is not always present with PEM.[60] The liver, in addition to brain tissue, has exhibited fluorescence in research lambs dosed with sulfide solution.[54]

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 scavenging free radicals. The reaction of H_2S with metallo-proteins can have beneficial effects in detoxification of xenobiotics within the animal.[1]

Although inhalation of eructated hydrogen sulfide is reported to damage lung tissue,[17,24,43,50] calves consuming high sulfate diets with rumen hydrogen sulfide levels reaching 24,000 ppm did not exhibit clinical or postmortem signs of lung damage.[60] Breath analysis of expired air was performed on calves in that study and indicators (ethane, nitrous oxide and hydrogen sulfide) of oxidative damage to lung tissues were not detected.[60] Whether significant amounts of hydrogen sulfide gain entry into the bloodstream via eructation is questionable and merits future research.[60] Poison gas wells and manure slurry pits are sources for direct inhalation exposure of hydrogen sulfide for livestock.[34] When acute death does not occur, PEM can result.[45]

Clinical Signs Related to Sulfur Toxicity

Two PEM syndromes are recognized.[38,48] In one syndrome, signs occur acutely and the animals are usually recumbent and comatose. These animals generally do not respond to treatment and die because of irreparable brain damage.

A second syndrome is recognized where animals show clinical signs of central nervous disease over varying periods 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 signs may be followed by lateral recumbency, opisthotonos, clonic-tonic convulsions with paddling motion and death. Animals that have recovered may be unproductive because of permanent brain damage.[22,62]

Lesions Associated with PEM

Gross brain lesions characteristic of PEM include gross swelling and edema, which can cause herniation of the medulla and cerebellum into the foramen magnum. The brain loses its turgidity or tone. Flattening of the gyri of the cerebral hemispheres and yellowish brown discoloration is often seen. Bilateral laminar cortical malacia, with occasional hemorrhage and varying degrees of cavitation, are frequently visible.[39]

When the brain is examined microscopically, neurons in the affected areas are smaller than normal or completely absent. Astrocytes become acidophilic, swollen and lose their processes, creating increased space between neurons. Spongiform degeneration is present with dead neurons replaced by eosinophilic globules. Blood vessels increase in size and the density of the macrophages increases. Astrogliosis is evident with healing.[39]

The distribution and severity of brain lesions associated with PEM are reported to be diagnostically significant for distinguishing sulfur-induced PEM from thiamine-deficient PEM.[26,27,37] Other authors attribute the pattern and degree of brain lesions seen with PEM to progressive ischemia and edema.[39,46,74] This discussion is irrelevant, however, as controlled studies establishing that thiamine deficiency causes PEM are lacking.[60,62,75]

Causes of PEM

Thiamine deficiency was incriminated as the cause of PEM by numerous English veterinarians following significant improvement of affected sheep and calves

treated with thiamine.[11,18,19,53,69,70] Research conducted by these veterinarians led to the discovery of thiaminase compounds in rumen fluid of affected animals, and also decreased blood levels of thiamine-based coenzymes.[11,18,19,53,69,70] Based on these findings, coupled with positive responses to thiamine therapy, it was concluded that functional thiamine deficiency caused clinical PEM in these animals. This concept was supported by research that used amprolium, a structural analog of thiamine, as a model for thiamine deficiency.[47] Whether the sheep used in this research actually developed thiamine deficiency is unclear, as all the sheep died after receiving the same treatment, but only three of seven had lesions characteristic of PEM.[23,60]

Thiaminase compounds in plants, such as *Kochia scoparia,* are also reported to cause PEM.[13,14] Animals consuming these plants were also drinking water containing greater than 3000 ppm sulfate. Since these reports were published, *Kochia spp,* has been recognized as a sulfur accumulator. Total sulfur intake by these cattle from both water and *Kochia spp* plants was most likely well above the suggested toxic dose of 4000 ppm.

Many cases of blind staggers and forage staggers attributed to the thiaminase content of selenium accumulating plants are now considered to have been caused by high sulfate levels, either in the plants or water the animals were consuming.[64]

Rumen acidosis has also been reported to cause thiamine-dependent PEM.[5,21,44,52,66,79,80,81,85,88] Researchers using experimental diets to investigate rumen acidosis reported thiamine-induced PEM. Experimental diets used in these studies were comprised of corn and dairy products supplemented with minerals in the form of sulfate salts. After finding thiaminase compounds in rumen fluid, it was concluded that PEM resulted from thiamine deficiency, which was in agreement with the accepted theory at that time. With a better understanding of the role of excessive dietary sulfur in the pathogenesis of PEM, it is more likely that the induced acidosis, with the lowered rumen pH, increased the toxicity of sulfur compounds in the experimental ration.[60]

Current literature strongly suggests that PEM is caused by ingestion of high levels of sulfur while dietary, blood and tissue thiamine levels are normal.[23,49,54,55,75,76] Numerous reports in veterinary literature refer to thiaminase compounds and decreased thiamine containing coenzymes as causes of PEM, but no controlled research trials have been performed to establish that functional thiamine deficiency exists.[60,62]

Differential Diagnosis, Treatment and Prevention of PEM

When ruminants are exhibiting CNS signs, the differential diagnosis should include PEM, thrombolic meningoencephalitis (TEM), rabies, listeriosis, magnesium deficiency (grass tetany), lead toxicity and water deprivation-sodium ion toxicity. The clinical signs and microscopic lesions caused by lead toxicity, water deprivation, and sulfur-induced PEM are indistinguishable. Lead poisoning and water deprivation-sodium ion toxicity are differentiated from sulfur toxicity by analysis of blood and tissue. Blood samples from live animals and liver and kidney from dead animals should be routinely analyzed for lead. Sodium levels in serum and CSF aid in diagnosing water deprivation-sodium ion toxicity in live animals, while brain tissue is used for postmortem sodium analysis. Magnesium levels can be determined from serum from live animals, while aqueous humor remains the postmortem sample of choice. Thrombolic meningoencephalitis, rabies and listeriosis have characteristic microscopic lesions. Fluorescent antibody testing is also used to diagnose these diseases.

Ruling out other diseases and documenting intake of feed or water containing high levels of sulfur supports a diagnosis of sulfur-induced PEM. Currently there is no diagnostic test to confirm sulfur toxicosis, but measuring rumen hydrogen sulfide levels of herd mates is a tool to determine exposure to potentially toxic levels of sulfur.[24,50,55,60] Herdmates selected for rumen hydrogen sulfide measurement should be healthy as rumen sulfur levels in anorectic, sick animals can decline rapidly.[49,60]

Treatment of sulfur-induced PEM is symptomatic. If animals are still ambulatory and able to eat and drink, removal from the sulfur source results in partial to full recovery of most affected animals.[16] Research lambs with clinical PEM have fully recovered without therapy.[75] Moderate to severely affected animals should be treated with thiamine at 4.54-9.1 mg/lb (10-20 mg/kg) IV *bid* or *tid,* followed by the same dose administered IM *bid* for 2 to 3 more days.[48] If no improvement is noted during that time, permanent brain damage is probable, and salvage or euthanasia should be considered.[71]

Administration of dexamethasone at 0.45-0.92 mg/lb IV (1-2 mg/kg) aids in the reduction of cerebral edema.[16,48,71] Additional symptomatic therapy for brain edema and seizure control should be considered when economically justified. Mannitol and furosemide aid in reducing brain swelling.[16,48] Diazepam, phenobarbital or pentobarbital are recommended to control seizures.[48] Mannitol and the medications used to control seizures are not approved by the Federal Food and Drug Administration for use in food producing animals. To avoid tissue residue of these drugs, the Food Animal Residue Avoidance Databank (FARAD) should be consulted before their use at 888-873-2723.

To prevent sulfur-induced PEM, ruminants must not have access to feed or water containing potentially toxic levels of sulfur. If water is the primary source of

sulfur, water with lower levels must be provided. If the sulfate level in the water is marginal, the sulfur content in forages and supplemental feeds must be determined in order to calculate total sulfur intake from all sources. Water sulfate levels less than 500 ppm are recommended, and 1000 ppm is the maximum recommended level when the environmental temperature is high or sulfur levels are elevated in feed.[72] The taste discrimination threshold for sulfur in livestock drinking water is 2000 ppm sulfate.[72]

When feeding high concentrate diets to ruminants, ration changes must be done gradually to minimize acidosis as a lower rumen pH will promote increased hydrogen sulfide production. The addition of copper or molybdenum has been reported to bind with sulfur to form insoluble sulfates, which are eliminated unchanged in the feces.[36] Removing monensin from the diet could reduce the risk of PEM as monensin increases sulfate reduction by rumen bacteria,[45] however, this is unpractical because of the beneficial effects monensin has on improved feed conversion, bloat prevention and reduced cost of gain.

The Relationship of Thiamine to Central Nervous System Disease

Thiamine (vitamin B_1) is a sulfur containing, water-soluble vitamin. It is synthesized in the stomach and intestines of all animals, but non-ruminants also require dietary sources. Rumen bacteria are capable of synthesizing adequate amounts of thiamine, even with less than optimal nutrition.[6]

Thiamine pyrophosphate (TPP) is an essential coenzyme in six different decarboxylation reactions in aerobic respiration.[41,67,74] This coenzyme is one of five necessary for the pyruvate dehydrogenase complex. This multienzyme complex is the catalyst to convert pyruvate to acetyl CoA, which reacts with oxaloacetate in the first reaction of the citric acid cycle in aerobic respiration.[74] Thiamine is essential for normal cellular membrane function and the conduction of nerve impulses.[41] Because of the high energy demands of the central nervous system, adequate thiamine is important to maintain CNS health, which explains why supplemental thiamine benefits patients with CNS dysfunction. The importance of thiamine is illustrated by its usefulness to treat lead poisoning. Cattle with lead poisoning respond better to thiamine alone than combined thiamine and CaEDTA therapy or CaEDTA by itself.[3,8,42] The addition of thiamine to the diet has been shown to eliminate or reduce the severity of clinical signs of PEM, even when characteristic brain lesions of PEM were present microscopically.[75]

The exact reason that thiamine is effective for treatment of lead toxicity is not completely understood. Because lead and sulfur toxicity produce similar clinical signs and brain lesions, it is reasonable to assume that the beneficial effects from treatment of sulfur-induced PEM with thiamine are similar.

Conclusions

Polioencephalomalacia is an economically important disease of ruminants. The relationship between PEM and diet is well established. Sulfur toxicity is now recognized as a major cause of PEM and evaluation of sulfur intake from both feed and water should be undertaken when characteristic clinical signs and postmortem lesions occur. Sulfur-induced PEM is differentiated from infectious CNS diseases, lead poisoning and water deprivation-sodium ion toxicity by utilizing antemortem and postmortem diagnostic tests that eliminate the latter diseases. At this time, specific postmortem diagnostic tests to confirm sulfur toxicosis are not available. Rumen hydrogen sulfide samples from penmates of sick or dead animals can be analyzed for hydrogen sulfide levels to support exposure to excess dietary sulfur and assess risks of exposed animals.

Thiamine is the drug of choice for treatment of sulfur-induced PEM. Additional therapy to control cerebral edema and seizures may be considered for valuable animals. Limiting access to feed or water containing high levels of sulfur and preventing sudden and steep decreases in rumen pH levels should aid in reducing PEM. Although some agents are known to bind dietary sulfur, most are unproven or impractical. Research is needed to find safe, effective agents to prevent sulfur-induced PEM.

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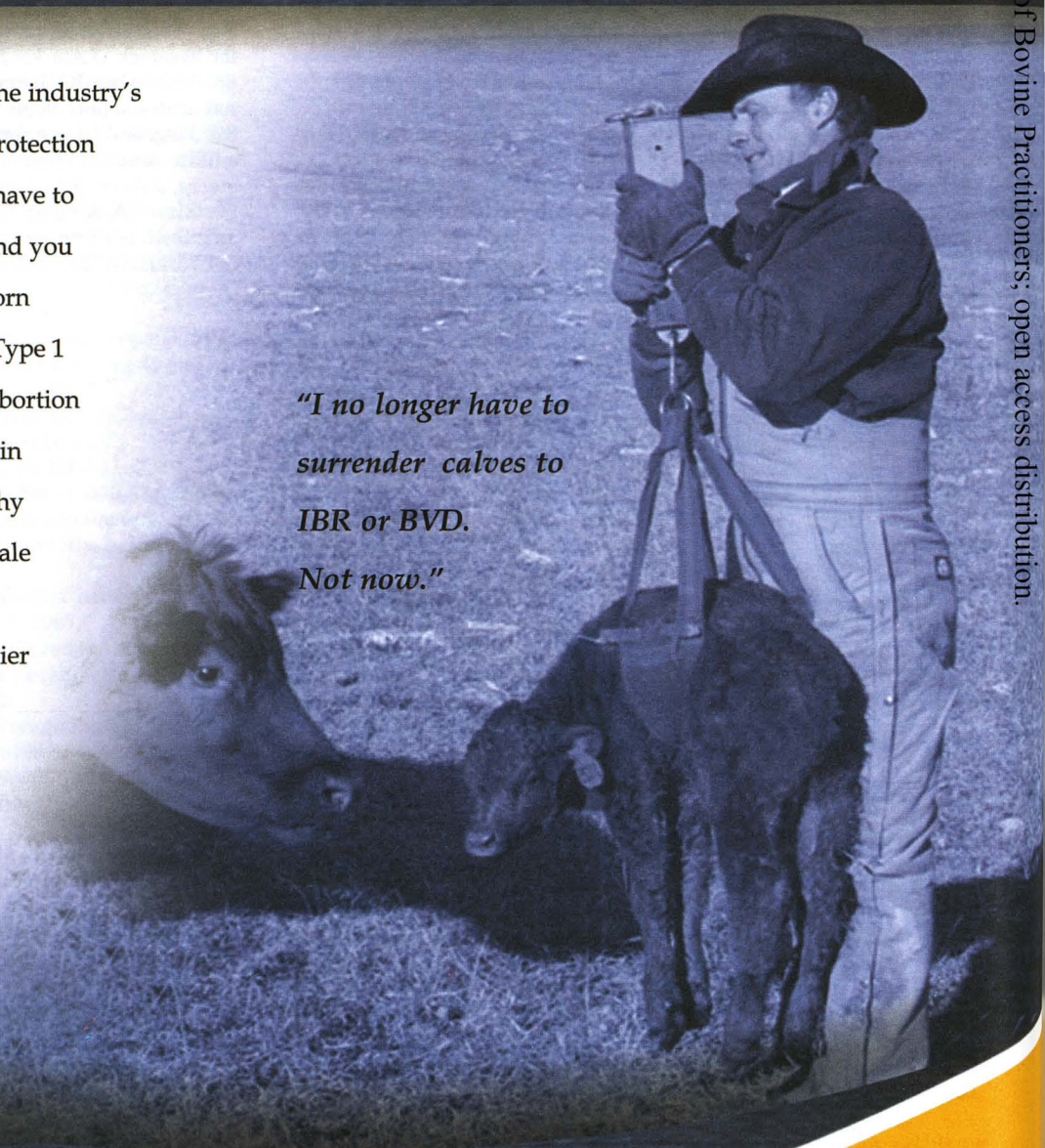

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