Describing post-mortem characteristics of bovine congestive heart failure and evaluating variability of ionophore concentrations in feed delivered to feedyard cattle

by

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Abstract

Bovine congestive heart disease (CHF) has garnered the attention of both veterinarians and beef cattle producers, raising concerns regarding animal health, welfare, and economics. The exact cause of non-infectious right-sided CHF is not well known. Published data suggest a variety of factors such as genetics, concurrent pulmonary diseases, and other demographics play a role in the onset of CHF. To further complicate matters, CHF clinical case definition varies among feedyards, leading to potential misclassifications. Progressive right-ventricular remodeling leading to cardiomegaly, is a hallmark of CHF. Cardiac eccentric hypertrophy occurs following increased pre-load volume, leading to a dilated ventricle, increased myocardial mass, and increased end-diastolic volume. These anatomical changes result in decreased cardiopulmonary functionality. The objective of this research was to refine bovine right-sided CHF diagnosis and improve post-mortem diagnostic approaches utilizing objective methods in combination with subjective methods, as well as evaluate variability of ionophore concentrations in feed.

This thesis delves into associations of objective and subjective post-mortem heart evaluations with the presence of CHF. Digital photographs and CHF diagnoses were acquired from an observational cross-sectional study on feedyard cattle mortalities. Digital cardiac photographs were measured in several locations to determine potential changes in heart size. Systemic post-mortem evaluations of 346 feedyard mortalities resulted in 106 cases being classified with cardiac enlargement/misshapen ventricle (CEMV). The CEMV cases were determined based on gross evaluation of heart size and shape with no signs of infectious heart disease present. A subset of CEMV cases were determined to be CHF when cases included congested (nutmeg) liver and at least two of the following lesions: serous or serosanguineous

pleural, peritoneal, or pericardial effusion. Eleven of the 346 mortalities were classified as CHF. Descriptive statistics and multivariate models were used to identify associations between objective heart measurements and subjective heart scores with the prevalence of CEMV or CHF. The CEMV model showed heart width, left ventricular thickness, and right ventricular lumen area significantly associated with the prevalence of CEMV cases ($p<0.05$). Heart scores were also associated with the prevalence of CEMV cases ($p<0.01$). In contrast, identifying associations between CHF and objective heart measurements and subjective heart was not possible because the CHF model did not converge. Heart measurements and scores can be used to improve diagnosis of heart disease post-mortem and provide further insight into subclinical heart disease.

An additional retrospective case-control study was conducted to determine potential differences in cardiac and hepatic histopathology between CHF cases and feedyard mortalities from other causes. Post-mortem examination was performed and samples retrieved from animals with grossly abnormal and normal hearts. Case-definition for CHF included abnormal heart shape, congested liver, and presences of ancillary signs; all other cases were considered as non-CHF for comparison. Cardiac and hepatic histopathology was performed categorizing fibrosis and necrosis into either none/minimal or moderate/severe changes. A generalized linear model was used to evaluate potential associations between the probability of CHF with histopathological lesions. Of the 87 cases included for analysis, 11 met the case definition for CHF. Cardiac histopathologic changes were identified in grossly normal and abnormal hearts with moderate/severe necrosis in 14.9% of mortalities and 16.1% moderate/severe fibrosis of mortalities. Hepatic necrosis was moderate/severe in 33.3% of mortalities and hepatic fibrosis was moderate/severe in 16.1% of mortalities. Liver fibrosis was the only factor associated with

CHF probability and cattle with moderate/severe liver fibrosis were more ($P < 0.01$) likely (0.57 +/- 0.13) to have CHF compared to cattle with minimal liver fibrosis (0.04 +/- 0.02). These results illustrate cardiac histopathologic changes were relatively rare and not associated with the probability of abnormal heart shape; however, hepatic histopathology may be useful in confirming CHF.

This thesis further investigated potential external contributors to CHF in cattle, such as the potential role of ionophore toxicosis. Ionophores are a class of antibiotics commonly added to feed in commercial cattle feeding operations to act as a coccidiostat and increase feed efficiency. In high concentrations, ionophores can cause acute cardiotoxic effects in cattle, resulting in clinical signs similar to CHF. The first objective of this study was to evaluate the differences in the mean and variation of monensin concentrations between bunk location (at-delivery and 2 hours post-delivery), and between the batch location (beginning and end of batch as delivered from feed truck) to evaluate finisher ration samples from feedyards. One truck load was considered a batch and consisted of four feed samples (A, B, C, and D) from one central Kansas feedyard on eight different days. Sample points A and B were collected from the feed bunk at the initial time of feed delivery from the point where the truck began unloading (A) and where the truck finished unloading (B). Sample points C and D were collected from the same feed bunk 2 hours after-delivery from the point where the truck began unloading (C) and where the truck finished unloading (D). The second objective was to evaluate associations of feedyard diet particle size with monensin concentrations. Particle size distribution of starter and finisher ration samples were determined using a Penn State particle separator with four sieves (S1 = 0.75, S2 = 0.31, S3 = 0.16, S4 = <0.16). All samples were analyzed for monensin concentrations using Ultra Performance Liquid Chromatography (UPLC). Descriptive statistics were conducted on

monensin concentrations and variability between samples and two multivariate models were used to identify associations between monensin concentrations in 1) batch or bunk location and 2) sieve location or ration type. There was no interaction between delivery timing and start or end of the batch; however, batch location affected monensin concentration in that the start of the batch had a higher (32,934 ± 18,982; mean ± SD) monensin concentration compared to the end of the batch (28,185 ± 17,560). In the second model, there was an interaction between sieve and ration type ($P<0.01$). The monensin concentrations in both starter (7,122 ± 3,477) and finisher (9,638 ± 4,705) rations were lower in S1. The monensin concentrations in both starter (25,837 ± 12,613) and finisher (18,384 ± 8,975) rations were highest in S4. These results show that monensin concentrations in feed samples can have important variability.

These projects demonstrated the utility of objective heart measurements, subjective scoring systems, and liver histopathology in further refining the bovine right-sided CHF definition for feedlot cattle. Utilizing gross pathological findings, histopathologic findings, and monensin concentration variability, these useful approaches provide data that help fill the information gaps related to bovine right-sided CHF. This work warrants further research and lays a foundation for future hypotheses like evaluating objective post-mortem diagnosis and identification of risk factors for CHF.

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Finally, I would like to acknowledge the generous financial support that made this research possible from the American Angus Association Foundation, the Foundation for Food and Agriculture Resources, Legacy Animal Nutrition, and Innovative Livestock Services. Thank you for your contributions and continual support.

Dedication

This thesis is affectionately dedicated to my family—Momma, Michael, Chloe, and Ian. Thank you for your unwavering love and support in all my endeavors; I owe every accomplishment to you.

To my Momma, Paula Wadley, thank you for being my best friend through all the challenges and victories of graduate school. You instilled in me the belief that I can do anything I put my mind to and giving up is never an option. I can only hope to be half the woman you are someday.

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Finally, I lovingly dedicate this thesis to my late grandfather, Dr. George Robert Wadley, for sharing with me his passion for veterinary medicine and agriculture. His encouragement to embrace the unknown has been one of the driving forces behind my journey thus far.

Above all else, I attribute every accomplishment to my faith in Jesus Christ, who apart from Him, I can do nothing.

Chapter 1 - A review of literature: bovine congestive heart failure in feedyard cattle

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Abstract

Bovine right-sided congestive heart failure (CHF) is a mechanical, non-infectious heart disease characterized by myocardial remodeling and venous congestion. This purpose of this review was to evaluate the literature regarding bovine CHF and its clinical signs, gross features, histopathologic features, and risk factors. This review will not cover infectious or congenital heart diseases.

Introduction

Bovine right-sided congestive heart failure (CHF) is a mechanical, non-infectious heart disease that has begun to garner the attention of veterinarians and beef producers. Historically, CHF in cattle was considered a high-altitude issue, commonly referred to as “high-mountain disease”, “high-altitude disease”, or “brisket disease”.[1] As more research on this topic has been published, the syndrome has also been described at moderate- to low-altitudes.[2] Right-sided CHF is an untreatable and often fatal disease that is characterized by cardiac remodeling that results in a sequelae of pathologic events. The right side of the heart receives deoxygenated blood from the inferior and superior vena cava. In CHF, the pre-load volume becomes excessive and stretches the myocardium, causing cardiomegaly and mechanical damage to the cardiac tissue. The

volume overload also causes venous congestion, thus leading to liver congestion or nutmeg liver[3,4,5,6].

Materials and Methods

The search was conducted in December of 2024 using the PubMed database. The search string included: bovine, or cattle, or feedlot cattle, or *bos taurus*, and congestive heart failure, or right-sided heart failure, or right sided heart failure. The search was limited to studies specifically related to non-infectious, mechanical, right-sided heart failure in feedyard cattle published in English between the years 2004-2024. This review includes case reports and research articles.

Results and Discussion

CHF Clinical Diagnostics

Anti-mortem clinical signs of CHF in cattle can manifest in a multitude of ways, some of which may overlap with other disease syndromes, especially bovine respiratory disease (BRD) and infectious heart diseases. This overlap of similar clinical signs complicates the diagnostic decision for CHF. According to Reef and McGuirk (2002), CHF can be differentiated from other heart diseases by accounting for signs, like effusion and edema, that result from exorbitant amounts of hydrostatic pressure[7]. Finding edema in the brisket, ventral abdomen, or submandibular areas would indicate ascites that accumulated in the veins and surrounding tissues because the malfunctioning right ventricle cannot efficiently pump blood[8]. According to a study observing 17 clinical cases of right-sided CHF by Moxley et al., 2019, all cases had brisket and ventral abdominal edema[4]. It is important to note that edema is also a common finding in brisket disease, also known as bovine high-mountain disease (BHMD), which is a disease syndrome

with an entirely different etiology compared to CHF. However, despite differing etiologies, BHMD ultimately manifests in the right ventricle of the heart which causes the edema.

BHMD occurs when cattle are raised at high altitudes (> 2130 m) and experience altitude-related pulmonary hypoxia and hypertension[9]. According to Johnson et al., 2023, right-sided CHF occurs at all levels of elevation and is not specific to high-altitude, indicating that the pathology of CHF is different than that of BHMD[2]. The focus of this review is specific to right-sided CHF in cattle and will not discuss BHMD, which is a distinguishably different disease than CHF.

Other non-specific CHF signs include dyspnea, tachycardia, lethargy, anorexia, abducted elbows, decreased pulse strength, and a distended jugular vein[8,5]. In a study that identified associations between clinical findings and those relationships to the presence of CHF in 67 cows, the most common CHF signs were jugular distention (88.1%), brisket and/or submandibular edema (77.6%), muffled heart sounds (76.1%), tachycardia (67.2%), elbow abduction (67.2%), and dyspnea (61.2%)[10]. Clinical signs of BRD usually include depression, anorexia, dyspnea, abducted elbows, and nasal and/or ocular discharge[11]. Effusion and edema are the most specific clinical signs indicating presence of CHF in cattle and can be used in conjunction with these non-specific clinical signs to differentially diagnosis CHF in cattle.

CHF Post-mortem Diagnosis

While CHF is primarily related to the heart, the repercussions of a malfunctioning heart can often be seen in other organ systems throughout the body. Because of the ancillary problems throughout the body system that are caused by CHF, full systematic post-mortem evaluations provide important information to discerningly make a final diagnosis. At post-mortem evaluation, gross signs of CHF can include cardiomegaly, liver congestion (nutmeg liver), and

serous or serosanguineous effusion of the pleural, pericardial, and/or peritoneal cavities. Signs of myo-, endo-, or pericarditis would indicate inflammatory heart disease rather than mechanical right-sided CHF.

Cardiomegaly can be identified at various levels of severity post-mortem. Pre-load volume overload causes a dilated chamber that stretches the myocardium and causes an enlarged heart[12,3]. A heart scoring system developed by Dr. Tim Holt from Colorado State University is a useful approach that provides some objectivity to an otherwise very subjective observation[13]. The heart scoring system categorizes hearts post-mortem on a 1-5 scale, with a heart score of 1 being "normal" and a heart score of 5 being "severely remodeled"[13].

Liver congestion, or nutmeg liver, is a common ancillary finding in CHF cases. Congestion of the liver occurs when the right-sided heart dysfunction leads to increased intravenous pressure and impairs blood flow, engorging the hepatic veins with blood. The hepatic venous congestion results in blood congestion around the central veins. Due to the lack of blood flow and oxygen being delivered to the parenchyma, dark centrilobular necrosis will cause a mottled nutmeg appearance throughout the liver (Hilscher and Sanchez, 2016). Reported by a study by Moxley et al., 2019, all 17 cattle diagnosed with CHF had severe chronic passive congestion[4].

Effusion and edema accumulate in cattle with CHF due to increased hydrostatic pressure[7,14]. In a healthy animal, there are normal small amounts of pleural, peritoneal, and pericardial fluids to lubricate organs and promote functionality. When the right side of the heart begins to fail and venous pressure increases, the restricted blood flow increases filtration pressure and exaggerates the formation of transudate lymphatic fluid[15].

Infectious heart diseases, such as endo-, myo-, and/or pericarditis, may exhibit some similar clinical signs to right-sided CHF. However, inflammatory heart diseases are caused by bacterial or viral infections and do not have the same etiology as mechanical CHF. Septic myocarditis is commonly caused by bacterial organisms Histophilus somni or Trueperella pyogenes infiltrating the bloodstream and landing on the aortic or mitral valves. Bacterial endocarditis is one of the most common valvular endocardial diseases and can be caused by T. pyogenes, Streptococcus spp., and Staphylococcus spp. Septic pericarditis is most commonly caused by infection introduced by traumatic reticuloperitonitis, or "hardware disease"[8].

CHF Histopathologic Findings

Utilizing cardiac histopathology from post-mortem tissue samples can provide more insight into the disease process, helping with the final diagnosis. In cattle with dilated cardiomyopathy, morphological changes can be observed in the cardiac tissue. These changes include interstitial fibrosis throughout the myocardium and increased cardiomyocyte size. The connective tissue in the hearts of cattle with dilated cardiomyopathy can increase by 6.7 times, and the total number of cardiomyocytes can decrease. Using cardiac histopathology to detect severity of fibrosis may be a useful indicator of dilated cardiomyopathy[16].

Histopathology of the hepatic tissue can indicate when hepatic injury has occurred from chromic congestion. In hepatic tissue adversely affected by CHF, changes in sinusoidal dilation, centrilobular hepatocyte atrophy, and portal fibrosis can be observed. When right atrial and right ventricular dilation occurs, more severe liver fibrosis can be observed in tissue samples[17]. Using liver histopathology to detect severity of fibrosis can be useful to identify liver congestion, which is associated with CHF.

Risk Factors of CHF

Ionophores are safe and effective drugs when used according to the label that act as growth promotants and anticoccidials. In high enough amounts, ionophores can induce toxic effects with CHF-like clinical signs. These signs include lethargy, ataxia, tachycardia, dyspnea, distended jugular vein, and brisket edema. Grossly, cattle may have pericardial, peritoneal, or pleural effusion, and congested liver. Ionophores are labeled to be fed anywhere from 5 to 40 g/ton, or no less than 50 and no more than 480 mg/head/day. The LD1 and LD50 (lethal dose for 1% of the population and lethal dose for 50% of the population, respectively) for ionophores in cattle is 5.5 mg/kg and 26.5 mg/kg, respectively. There have been no studies to evaluate the effects of chronic sublethal ionophore exposure[18,19].

Recent research has indicated that genetics may be associated with CHF in cattle. According to Heaton et al., 2019, the EPAS1 gene was not associated with CHF, indicating that genomic testing for EPAS1 is not useful in identifying CHF[20]. In 2022, Heaton et al. reported that cattle with the genes for ARRDC3 and NIFA had a much higher risk for CHF[21]. Cattle with two copies of risk alleles in either gene increased their likelihood of CHF by 8-fold, and cattle with two copies of risk alleles in both genes increased their likelihood of CHF by 28-fold[21]. This data is useful for understanding CHF risk factors, but does not provide any information regarding the pathology of bovine CHF. The exact etiology of bovine right-sided CHF is still unknown, but these risk factors can help in better understanding the disease process and possible management practices for detecting CHF.

Literature Cited

1. Jensen R, Pierson RE, Braddy PM, et al. Brisket disease in yearling feedlot cattle. 1976;169:515-517.

2. Johnson BT, White BJ, Dahlman DA, Rademacher RyanD, Amrine DE, Larson RL. Retrospective analysis of risk and timing of death from right heart failure in United States and Canadian feedlot necropsied cattle. javma. Published online April 28, 2023:1-7. doi:10.2460/javma.22.11.0517

3. Selvetella G, Lembo G. Mechanisms of Cardiac Hypertrophy. Heart Failure Clinics. 2005;1(2):263-273.

4. Alexander A, Jensen R. Pulmonary Vascular Pathology of High Altitude-Induced Pulmonary Hypertension in Cattle. American Journal of Veterinary Research. 1963;24:1112-1122.

5. Moxley RA, Smith DR, Grotelueschen DM, Edwards T, Steffen DJ. Investigation of congestive heart failure in beef cattle in a feedyard at a moderate altitude in western Nebraska. J VET Diagn Invest. 2019;31(4):509-522. doi:10.1177/1040638719855108

6. Buczinski S, Francoz D, Fecteau G, DiFruscia R. Heart disease in cattle with clinical signs of heart failure: 59 cases. 2010;51.

7. Krafsur GM, Neary JM, Garry F, et al. Cardiopulmonary remodeling in fattened beef cattle: a naturally occurring large animal model of obesity-associated pulmonary hypertension with left heart disease. Pulm circ. 2019;9(1):1-13. doi:10.1177/2045894018796804

8. Reef VB, Whittier M, Allam LG. Thoracic ultrasonography. Clinical Techniques in Equine Practice. 2005;3(3):284-293. doi:10.1053/j.ctep.2005.02.006

9. Peek SF, Buczinski S. Examination of the Cardiovascular System. Published online 2018. doi:10.1016/B978-0-323-39055-2.00003-6

10. Glover GH, Newson IE. Brisket Disease (Dropsy of High Altitudes). The Agricultural Experiment Station Bulletin. 1914;(201):1-24.

11. Abd El Raouf M, Elgioushy M, Ezzeldein SA. Congestive heart failure in cattle; etiology, clinical, and ultrasonographic findings in 67 cases. Vet World. 2020;13(6):1145-1152. doi:10.14202/vetworld.2020.1145-1152

12. Griffin D, Chengappa MM, Kuszak J, McVey DS. Bacterial Pathogens of the Bovine Respiratory Disease Complex. Veterinary Clinics of North America: Food Animal Practice. 2010;26(2):381-394. doi:10.1016/j.cvfa.2010.04.004

13. Heffernan KR, Thomas MG, Enns RM, Holt T, Speidel SE. Phenotypic relationships between heart score and feed efficiency, carcass, and pulmonary arterial pressure traits1. Translational Animal Science. 2020;4(Supplement_1):S103-S107. doi:10.1093/tas/txaa114

14. Singh CK, Kumar A, Singh N. An overview on the diagnostic and therapeutic aspects of cardiac diseases in bovine. Journal of Entomology and Zoology Studies.

15. Witte CL, Witte MH, Dumont AE, Cole WR, Smith JR. Protein Content in Lymph and Edema Fluids in Congestive Heart Failure. Circulation. 1969;40(5):623-630. doi:10.1161/01.CIR.40.5.623

16. Nart P, Williams A, Thompson H, Innocent GT. Morphometry of Bovine Dilated Cardiomyopathy. Journal of Comparative Pathology. 2004;130(4):235-245. doi:10.1016/j.jcpa.2003.11.002

17. Dai DF, Swanson PE, Krieger EV, Liou IW, Carithers RL, Yeh MM. Congestive hepatic fibrosis score: a novel histologic assessment of clinical severity. Modern Pathology. 2014;27(12):1552-1558. doi:10.1038/modpathol.2014.79

18. Ensley S. Ionophore Use and Toxicosis in Cattle. Veterinary Clinics of North America: Food Animal Practice. 2020;36(3):641-652. doi:10.1016/j.cvfa.2020.07.001

19. Hall JO. Ionophore Use and Toxicosis in Cattle. Veterinary Clinics of North America: Food Animal Practice. 2000;16(3):497-509. doi:10.1016/S0749-0720(15)30083-9

20. Heaton MP, Bassett AS, Whitman KJ, et al. Evaluation of EPAS1 variants for association with bovine congestive heart failure. F1000Res. 2019;8:1189. doi:10.12688/f1000research.19951.1

21. Heaton MP, Harhay GP, Bassett AS, et al. Association of ARRDC3 and NFIA variants with bovine. F1000Research. 2024;11(385). doi:10.12688/f1000research.109488.2

Chapter 2 - Relationships of heart scores and postmortem cardiac measurements in congestive heart disease in feedyard cattle

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Abstract

Congestive heart failure (CHF) in feedyard cattle is of increasing concern among producers and can be difficult to diagnose definitively postmortem. In a cross-sectional observational study, we evaluated gross pathology findings, various heart measurements, and subjective heart scores (1-5 scale; 1 = normal, 5 = severely remodeled) to identify heart disease postmortem. In postmortem examinations of 346 feedyard deaths, we classified 106 (30.6%) cases as cardiac enlargement or misshapen ventricle (CEMV) when there was an abnormal heart shape or dilated ventricle(s), and no signs of infectious heart disease. CHF was defined as a CEMV case with chronic passive congestion of the liver (i.e., nutmeg liver) and ≥2 of the following lesions: serous or serosanguineous pleural, pericardial, or peritoneal effusion. Eleven of the 346 autopsied cattle were classified as having CHF. Descriptive statistics and multivariate models were used to identify statistical associations between objective heart measurements or

subjective heart scores and the prevalence of CEMV or CHF. CEMV cases had significantly increased heart widths, thinner left ventricular free walls, and expanded right ventricular lumen areas ($p < 0.05$). The CHF model did not converge because we had too few cases to be able to evaluate associations between CHF and variables of interest.

Key words: congestive heart failure, cattle, heart disease, post-mortem evaluation

Abbreviations: CHF = congestive heart failure, CEMV = cardiac enlargement/misshapen ventricle, BRD = bovine respiratory disease, AIP = acute interstitial pneumonia

Introduction

Bovine congestive heart failure (CHF), continues to be an animal health and economic concern for feedyard producers, reported mostly in the United States and Canada [14]. CHF in cattle is a non-infectious progressive disease that can occur when the right ventricle endures injury from pulmonary hypertension, thus hindering its ability to efficiently pump blood to the lungs for reoxygenation [1,3,11,13]. Clinical signs of CHF in live cattle can include brisket and submandibular edema, ascites, distended jugular vein with a pulse, tachycardia, and overall weakness [3]. There have been conflicting reports regarding the prevalence of feedlot bovine CHF in North America; one study reported the risk of CHF doubling between 2000 and 2012,[14] and another study reported that CHF risk does not appear to be increasing [9]. This can be partially explained by regional differences in production practices and breed types as well as the accuracy of clinical diagnosis by feedyard personnel [9,14]. Early heart disease is difficult to differentiate from other diseases with similar clinical signs, such as the bovine respiratory disease complex (BRDC) and acute interstitial pneumonia (AIP). Some of these similar clinical signs include

coughing, extending the neck, dyspnea, lethargy, and poor body condition [20]. Hence, CHF oftentimes remains undiagnosed and affected cattle have a poor prognosis [3].

The prevalence of CHF has been estimated to be ~4% of cattle deaths: over 3 y in 22 U.S. commercial feedyards with a total population of 4,596,205 cattle, 3,282 of 75,963 (4%) deaths were deemed to be from CHF.7 In another study, the prevalence of heart scores at slaughter (heart scores of 4 or 5 on a 5-point ordinal scale; 1 = normal, 5 = severely misshapen) and found that 1,357 of 32,763 (4.14%) slaughtered feedyard cattle had a heart score of 4 or 5 [5].

Additionally, the underlying morbidity and cardiopulmonary remodeling resulting in CHF has negative associations with growth, performance, and other health risks. Cattle treated ≥2 times for BRDC were more likely to not finish their feeding phase due to CHF compared to cattle never treated, or treated once, for BRDC [8]. If CHF could be diagnosed earlier, economic losses and welfare concerns could be mitigated by culling animals unlikely to respond to BRDC treatment. The cardiac health of feedyard cattle has a significant genetic component, with heritability estimates as high as 0.356 [2,4,11,15]. Average daily gain (the last 80-120 d on feed) and carcass traits, such as hot carcass weight, are negatively associated with heart scores of 4 or 5 [5]. Another risk factor reported was that the percentage of Angus cattle estimated from genomic breed composition are positively associated with more severe heart scores [2]. Much of the CHF research has been done at slaughter, but little research has investigated CHF postmortems throughout the feeding period.

Data obtained from autopsies provide useful information to further improve animal health and treatment protocols. Systematic postmortem examinations may give insight into disease prevalence, whether other diseases are concurrent with CHF, and provide definitive diagnoses to support or refute the clinical diagnoses (which can be used to train personnel on how to properly

diagnose the cause of death) [19,21]. Although research has evaluated objective cardiac measurements (including heart weights, ratios, and comparisons), little research has evaluated the value of digital photographs and post-hoc measurements to support a diagnosis of CHF based on gross postmortem findings. Although CHF is thought of as "true" non-infectious, congestive, mechanical heart failure, cardiac enlargement or misshapen ventricles (CEMV) can be observed when there is any lesion causing the heart to not function properly. Identifying CEMV during a postmortem examination is typically done subjectively by an observer, without objective cardiac measurements. However, when used in conjunction with heart scoring, digital images could be used to objectively measure hearts to remove some of the subjectivity in the diagnostic process.

Our objective was to determine if objective cardiac measurements, subjective heart scores, and gross postmortem diagnoses were associated with the probability of CEMV and CHF cases to determine which of the measures is useful for accurate diagnosis. We hypothesized that cardiac measurements would be associated with the probability of CEMV and CHF cases.

Materials and Methods

Our study was considered exempt by the Institutional Animal Care and Use Committee at Kansas State University as all evaluations were conducted postmortem following naturally occurring (non-study-induced) deaths. In our cross-sectional observational study, we investigated associations of cardiac measurements from postmortem examinations to the probability of bovine CEMV and CHF based on gross pathology findings and heart scores. Cattle enrolled in our study were autopsied from June 1, 2022, to July 29, 2022, at 6 Kansas feedyards. We used data gathered from cattle that died spontaneously and were autopsied within 18 h of death and/or had minimal-to-no gross autolysis based on the assessment of tissue color, appearance, odor, and texture by the technician who performed the postmortem examination.

Cattle demographics provided by the feedyard after the postmortem examination included sex, arrival weight, and days on feed. Additionally, to avoid any linearity assumptions, arrival weight was categorized in 90.7-kg increments and days on feed were categorized in 50-d increments for statistical analysis.

Postmortem protocol

Research personnel (P Schmidt, M Mancke, R Champagne, M Jensen) were trained by veterinarians and pathologists and performed all postmortem evaluations and documented gross lesions for each case. A veterinarian (BJ White) confirmed each gross diagnosis. All dead cattle received a postmortem examination, except for the brain, similar to previous work [16]. Examination of the heart included initial visual assessment within the thoracic cavity and pericardial sac. Pericarditis or effusion was noted. The heart was severed above the atria from the major vessels, and removed from the thoracic cavity. From the lateral view of the unopened heart, a heart score was assigned and any external abnormalities noted (petechiae, ecchymoses, pericarditis). A cross-sectional cut was made one-third of the way from the apex to the base to evaluate the ventricles and endocardium. Septal defects or gross evidence of infectious heart disease (cutting into the papillary muscle to assess myocarditis and examination of the heart valves) were noted. Each postmortem evaluation was documented, and various photographs were collected.

CEMV cases were cattle with an abnormally shaped heart and/or a dilated ventricle without signs of infectious heart disease. CHF cases were a subset of CEMV cases with a congested liver (i.e., nutmeg liver) and ≥2 ancillary findings of heart failure, such as serous or serosanguineous pericardial, pleural, or peritoneal effusion.

Heart photographs and objective measurements

Two digital photographs were taken of the heart for each case: a view of the right lateral side of the heart with the right coronary artery toward the camera (Fig. 1A), and a cross-sectional view of the right and left ventricles (Fig. 1B). Each photo included the case number and a ruler for standardizing computer measurements. If hearts were too autolyzed and/or flaccid (n = 49), or if photographic quality was not sufficient for taking measurements (n = 1), then we excluded the case. Digital measurements were made with computer software that determined the number of pixels per inch (ImageJ, https://imagej.net/develop/)[17]; 11 measurements per heart included 4 of the lateral view and 7 of the cross-sectional view.

The lateral-view photograph had the left ventricle on the right, and right ventricle on the left of the image (Fig. 1A). The heart was not cut before taking the lateral-view photograph. Digital measurements included whole heart area, circumference, length, and width (Fig. 2A). Heart area and circumference were measured by outlining the whole uncut heart area 3 times in pixels squared and pixels, then averaging those measurements. This average was then converted to cm2 for heart area. The circumference was measured using this same method, including conversion to cm. Length was measured from the tip of the apex vertically to the center of the base of the heart. Width was measured at the 2 widest portions of the heart and averaged.

From the cross-sectional photo of the heart, left ventricle (LV) and right ventricle (RV) lumen area, LV and RV lumen perimeter (Fig. 2A), LV and RV wall thickness, and septum thickness were measured (Fig.2B). LV and RV lumen area were measured as previously described for whole heart area and circumference. While tracing the lumen area, papillary muscles were excluded by drawing a line from one side of the base of the papillary muscle to the other side to transect the papillary muscle. This method was also used for LV and RV lumen circumference. LV wall thickness was determined by measuring a representative area from the

ventricular free wall to the lumen. RV free wall thickness was determined by measuring from the exterior to the lumen at the 2 widest points of the ventricle and averaged. Septum thickness was measured across the center of the septum. If the thickness of the septum varied greatly at different points, then 3 measurements were collected and averaged for final analysis.

Subjective heart scores

Randomized lateral-view heart images were sent for heart score evaluation to 3 professionals (J Buchanan, R Raymond, T Cook) with field experience in this area. These 3 observers were trained using the same methods within the same facilities with an available photo key of example heart scores and were anonymized to the case history. Inter- and intra-observer consistency was not assessed. Heart scores were on a 1-5 scale: 1 = a grossly normal heart (firm ventricles, prominent interventricular sulcus, prominent apex), 2 = mild changes (slightly flaccid ventricles, prominent interventricular sulcus, apex still prominent), 3 = moderate changes (right ventricle begins bulging, interventricular sulcus migrates towards midline , apex begins to round out), 4 = severe changes (enlarged right ventricle equal to the size of the left ventricle, interventricular sulcus through the midline, W-shaped apex), 5 = a severely enlarged or flaccid heart with a U-shaped apex (Fig. 3) [5]. Using the mode (or median if no mode was identified) of the 3 observers' scores, a condensed binomial classification of "normal" (heart scores 1, 2, or 3) or "abnormal" (heart scores 4 and 5) was used for statistical analysis. Cases were removed from the analysis if only 2 scores were received, and a mode could not be calculated.

Statistical analysis

To evaluate the statistical association between heart measurements and the probability of CEMV cases, a generalized linear mixed-effect model was fit using the 'glmer' function from the 'lme4' package in R Studio (https://cran.r-project.org/web/packages/lme4/). The dependent

variable for each model was the CEMV case. In the CEMV model, the independent variables included all 11 heart measurements, sex, arrival weight, and days on feed. Heart measurements included whole heart area, circumference, length, width, LV and RV lumen area, LV and RV lumen circumference, LV and RV wall thickness, and septal thickness. Three variables (sex, arrival weight, and days on feed) were considered experimental design factors based on previous research showing associations with heart disease [7]. The experimental design factors were included in all models regardless of significance. Feedyard identifier was included in each model as a random effect to account for lack of independence in animals from the same feedyard. Using a variance inflation factor (VIF) of > 2.5, multicollinearity among variables was assessed using the "varIMP" package in R Studio [10]. If 2 variables were above this cutoff, 1 was removed from the model. Model estimated means were calculated for the significant variables using the "emmeans" function from the "emmeans" package in RStudio. A second multinomial model was conducted with CEMV as the outcome and heart scores as the independent variable, with the same experimental design factors and random effect used in the previously mentioned models.

A generalized linear model was used to evaluate the association between heart measurements and CHF cases using the "glm" function in R Studio. The dependent variable for this model was the CHF case. In the CHF model, the independent variables included all 11 heart measurements, sex, arrival weight, and days on feed. Heart measurements included whole heart area, circumference, length, width, LV and RV lumen area, LV and RV lumen circumference, LV and RV wall thickness, and septum thickness. Three variables (sex, arrival weight, and days on feed) were considered experimental design factors based on previous research showing associations with heart disease [7]. The experimental design factors were included in all models regardless of significance. This model did not include feedyard identifier as a random effect due

to the lack of variance in this variable within the 11 CHF cases. Using a VIF of > 2.5, multicollinearity among variables was assessed using the "varIMP" package in R Studio [10]. If a variable was above this cutoff, one was removed from the model.

Results

Our project began with 417 postmortem cases in which 71 cases were excluded (measurement data not obtained, n = 50; missing heart scores n = 21). We included 346 cases (244 heifers, 102 steers) in the final analysis, with heifers making up 70.5% of total necropsied cattle—similar to the percentage of heifers in the total feedyard population.

Cardiac enlargement or misshapen ventricle cases and measurements

Of the 346 total cases, 106 (30.6%) were classified as CEMV cases based on gross diagnoses; 70 of the 106 were heifers and 36 were steers. Increased heart width, decreased LV thickness, and increased RV lumen area were associated with the probability of CEMV cases ($p < 0.01$).

Measurements of the heart width from the lateral view of the heart were significantly associated with CEMV prevalence. Hearts in CEMV cases were wider (23.1 ± 2.3 cm) than hearts in non-CEMV cases (21.6 ± 2.3 cm; $p < 0.05$; Table 1). Measurements from the cross-sectional view of the heart significantly associated with CEMV included LV thickness and RV lumen area. The average LV wall thickness was lower in CEMV cases (3.3 ± 0.8 cm) than in non-CEMV cases (3.6 ± 0.8 cm). The average RV lumen area was higher in CEMV cases (38.0 ± 22.6 cm^2) than in non-CEMV cases (22.6 ± 11.6 cm^2; Table 1).

Cardiac enlargement or misshapen ventricle cases and scores

There were 103 CEMV cases that had some degree of abnormal heart shape (scored 2, 3, 4, or 5; Table 2). The heart scores were significantly associated with the prevalence of CEMV

cases ($p < 0.01$). Heart scores of 1 or 2 had lower probability of CEMV being present compared to heart scores of 3, 4, or 5 (Fig. 4). There was no statistical difference of probability of CEMV among scores 3, 4, or 5.

There were 6 CHF cases that had severe changes in heart shape (scored a 4 or 5; Table 3). Due to the low number of cases, a model evaluating association between CHF and heart scores was not evaluated.

Congestive heart failure and measurements

Of the 346 total cases, 11 (3.2%) were considered CHF cases based on their gross diagnoses. Within the 11 CHF cases, 7 were heifers and 4 were steers. Due to the small population size ($n = 11$), the model did not converge and is not reported in this analysis.

Discussion

The 4% prevalence of CHF in our study was comparable to previous reports [5,7]. Objective heart measurements from digital photographs were associated with the likelihood of CEMV and CHF. Subjective heart scores were also associated with the probability of CEMV.

The CHF and CEMV prevalence that we found in our population of feedyard cattle is similar to the prevalences reported previously [5,7]. We identified non-infectious CHF in 3% of the deaths in our study.

The number of abnormal heart scores at slaughter may vary by region, breed type, or production system; a study found 22% of older animals with heart scores of 3-5 [5]. In feedyard cattle at typical slaughter age (12-18 months), 4.14% of animals had some degree of cardiac remodeling (heart score: 4 or 5) [2]. We evaluated feedyard deaths, which is a population prior to slaughter, and found that the overall prevalence of CEMV in deaths was similar (30.6%) to published studies [2]. We found that, although CHF accounts for a low proportion of total deaths, a

much higher percentage of deaths had some cardiac remodeling. As found in previous research[2], the total impact of heart disease is likely more than just the impact from fatal CHF cases.

From the external view of the heart, the width was larger in CEMV cases than in non-CEMV cases, as expected in right heart failure [13]. Expanded RV lumen area and diminished LV wall thickness were associated with CEMV in measurements from the cross-sectional digital photographs. The larger RV lumen area in CEMV cases aligns with disease causing right heart failure. One of the hallmarks of right heart failure is changes in heart shape due to ventricular expansion [6]. Cardiac eccentric hypertrophy occurs when volume overload leads to a dilated chamber, thus increasing the myocardial mass and increasing the end-diastolic volume [18]. As heart disease progresses and cardiomegaly occurs, the myocardium stretches and requires more effort to pump the larger amounts of blood [6,13]. The LV wall was thinner in CEMV cases than in non-CEMV cases, which may be due to increased overall heart size without addition to muscle mass in the ventricular wall. The ability to differentiate CEMV based solely on digital pictures was not the objective; however, these results indicate that some digital measurements change in CEMV.

Most of the cattle with CEMV in our study had a heart score of 3, 4, or 5 (81 of 106, 76%). The CHF diagnosis was based on not only visual evaluation of the heart but also adjunct pathology findings including nutmeg liver and effusions. This gross diagnosis of CHF should be indicative of true heart dysfunction, and most (9 of 11, 81%) of the CHF cases were classified with a heart score of 3, 4, or 5. Interestingly, 2 CHF cases had a heart score of 2, but no CHF cases had a heart score of 1. In the model of the relationship between CEMV and heart score, individuals with heart scores 1 and 2 were less likely to have CEMV compared to individuals

with heart scores of 3, 4, or 5. Cases with heart scores of 1 and 2 were less likely to have CEMV and CHF indicating that our heart scoring system aligned with clinical findings.

One of the primary limitations of our study is that a variety of concurrent disease processes were present. While this population differs from work done at slaughter, the population is comparable to other work done in feedyard deaths [7]. It should also be noted that the overall population at the feedyards contained more heifers (70.5%) than steers (29.5%). Both CEMV and heart scores are subjective assessments of the degree of cardiac changes; therefore, disagreements within individual cases between the scoring systems may occur. Moreover, there were only 11 CHF cases in our 106 CEMV cases. Due to the small sample size, the model did not converge and therefore was not included in our analysis.

Conflicts of Interest

The authors declare no conflicts of interest.

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Literature Cited

1. Alexander A, Jensen R. Pulmonary vascular pathology of high altitude-induced pulmonary hypertension in cattle. Am J Vet Res 1963;24:1112–1122.

2. Buchanan JW, et al. Variance component estimates, phenotypic characterization, and genetic evaluation of bovine congestive heart failure in commercial feeder cattle. Genetics 2023;14:1148301.

3. Buczinski S, et al. A study of heart diseases without clinical signs of heart failure in 47 cattle. Can Vet J 2010;51:1239–1246.

4. Heaton MP, et al. Association of ARRDC3 and NFIA variants with bovine congestive heart failure in feedlot cattle. F1000Research 2024;11:385.

5. Heffernan KR, et al. Phenotypic relationships between heart score and feed efficiency, carcass, and pulmonary arterial pressure traits1. Transl Anim Sci 2020;4(Suppl 1):S103–S107.

6. Holt T. Bovine high-mountain disease [Internet]. Bovine high-mountain disease. 2019.[cited 2024 Apr 19]. Available from: https://www.merckvetmanual.com/circulatory-system/bovine-high-mountain-disease/bovine-high-mountain-disease.

7. Johnson BT, et al. Retrospective analysis of cohort risk factors and feeding phase timing associated with noninfectious heart disease deaths in U.S. feedlot cattle. Transl Anim Sci 2021;5:txab220.

8. Johnson BT, et al. Association of feedlot disease treatments on the probability of heart disease syndrome in U.S. feedlot cattle. Bov Pract 2022;56:1–12.

9. Johnson BT, et al. Retrospective analysis of risk and timing of death from right heart failure in United States and Canadian feedlot necropsied cattle. J Am Vet Med Assoc 2023;261:1–7.

10. Johnston R, et al. Confounding and collinearity in regression analysis: a cautionary tale and an alternative procedure, illustrated by studies of British voting behaviour. Qual Quant 2018;52:1957–1976.

11. Krafsur GM, et al. Cardiopulmonary remodeling in fattened beef cattle: a naturally occurring large animal model of obesity-associated pulmonary hypertension with left heart disease. Pulm Circ 2019;9:1–13.

12. Kukor IM, et al. Sire differences within heart and heart fat score in beef cattle. Transl Anim Sci 2021;5:S149–S153.

13. Moxley RA, et al. Investigation of congestive heart failure in beef cattle in a feedyard at a moderate altitude in western Nebraska. J Vet Diagn Invest 2019;31:509–522.

14. Neary JM, et al. Right-sided congestive heart failure in North American feedlot cattle. J Vet Intern Med 2016;30:326–334.

15. Newman JH, et al. High-altitude pulmonary hypertension in cattle (brisket disease): candidate genes and gene expression profiling of peripheral blood mononuclear cells. Pulm Circ 2011;1:462–469.

16. Schmidt PH, et al. Determining frequency of common pulmonary gross and histopathological findings in feedyard fatalities. Vet Sci 2023;10:228.

17. Schneider CA, et al. NIH image to ImageJ: 25 years of image analysis. Nat Methods 2012;9:671–675.

18. Selvetella G, Lembo G. Mechanisms of cardiac hypertrophy. Heart Fail Clin 2005;1:263–273.

19. Wäsle K, et al. The post-mortem examination in ruminants and its possible benefit to ruminant clinical medicine. J Comp Pathol 2017;156:202–216.

20. White B. Monitoring systems for identifying bovine respiratory disease. Proc Am Assoc Bov Pract 2015;48:62–64.

21. Zühlke I, et al. Factors associated with cattle necropsy submissions in Switzerland, and their importance for surveillance. Prev Vet Med 2021;187:105235.

Figure 2-1. Example photos taken of the two different perspectives of the heart. During each post-mortem evaluation, two different images of the heart were captured, (A) lateral view and (B) cross-sectional view. These images were used to take measurements using the computer software ImageJ. A. Lateral view with coronary artery facing the camera. The left ventricle is on the right side, and the right ventricle is on the left side. No cuts have been made to the heart. B. Cross-sectional view evaluating the ventricles. A cut was made 1/3 of the way from the apex of the heart.

Figure 2-2. Examples of how the eleven digital cardiac measurements were measured in ImageJ software. A. Blue dashed line = circumference; yellow lines = length, width; black grid = whole heart area. B. Green dashed line = right ventricular lumen circumference; blue dashed line = left ventricular lumen circumference; black grid = left and right ventricular lumen areas. C. Blue line = right ventricular thickness; green line = left ventricular thickness; yellow line = septum thickness.

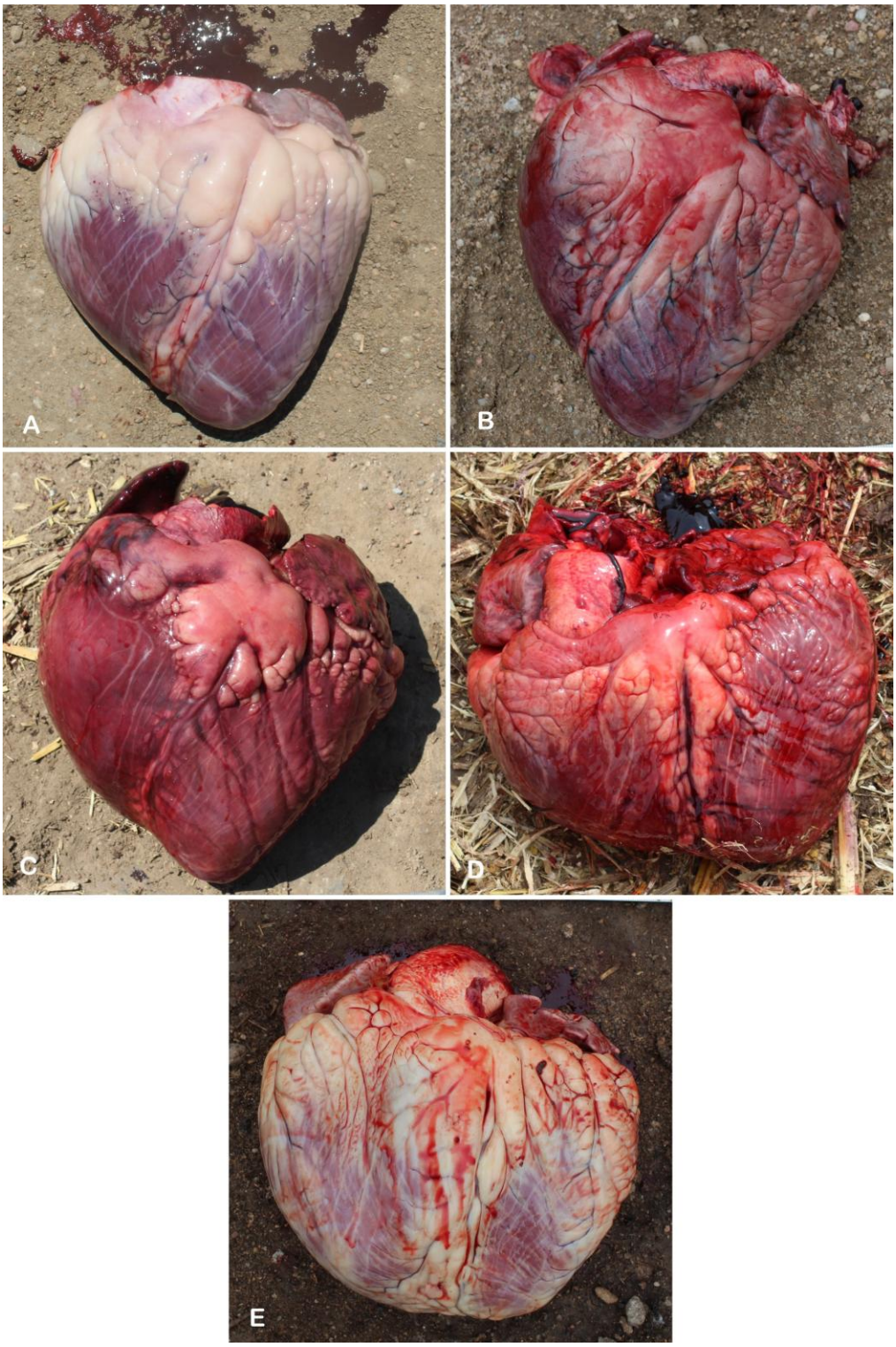

Figure 2-3. Heart scoring system used to administer a scale of cardiac remodeling to each necropsied heart. A score of 1 represents a physiologically normal heart, and a score of 5 represents severe cardiac remodeling.

Table 2-1. The means and standard deviation of CEMV case heart measurements (*n*=106) in centimeters.

	Width		Left Ventricular Thickness		Right Ventricular Lumen Area	
	Mean	SD	Mean	SD	Mean	SD
Non-CEMV	21.6	± 2.3	3.6	± 0.8	22.6	± 11.6
CEMV	23.1	± 2.3	3.3	± 0.8	38.0	± 22.6

Table 2-2. Number of heart scores for CEMV cases (n=106) and non-CEMV cases (n=240) administered during a trial with full systemic necropsies performed on each animal (n=346). Heart scores were allocated on a 1-5 scale, with 1 being physiologically normal and 5 being severely damaged with signs of significant remodeling.

	CEMV Scores					
Heart Score	***1***	***2***	***3***	***4***	***5***	**Total**
Non-CEMV	68	126	41	4	1	240
CEMV	3	22	51	21	9	106
Total	71	148	92	25	10	346

Table 2-3. Number of heart scores for Congestive Heart Failure cases (n=11) and Non-Congestive Heart Failure cases (n=335) administered during a trial with full systemic necropsies performed on each animal (n=346). Heart scores were allocated on a 1-5 scale, with 1 being physiologically normal and 5 being severely damaged with signs of significant remodeling.

	CHF Heart Scores					
Heart Score	***1***	***2***	***3***	***4***	***5***	**Total**
CHF	0	2	3	3	3	11
No CHF	71	146	89	22	7	335
Total	71	148	92	25	10	346

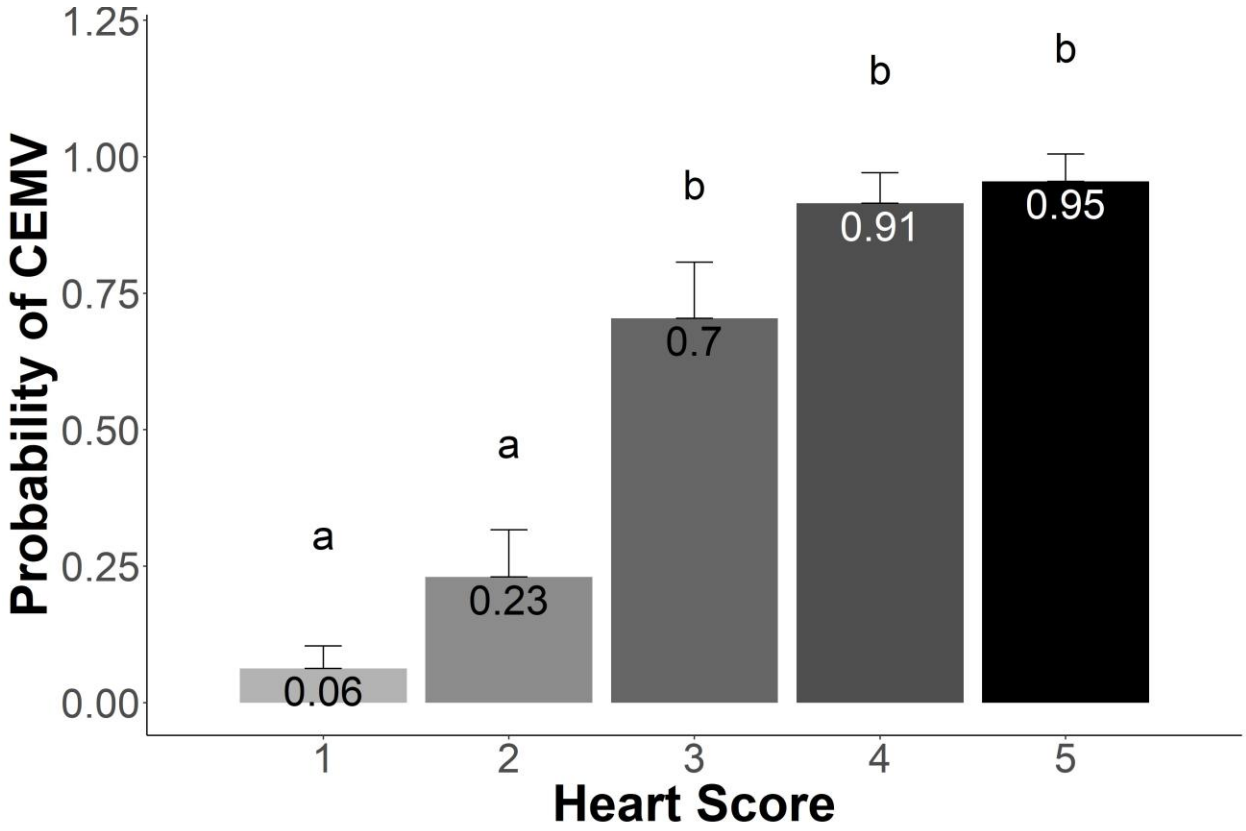

Figure 2-4. Graph displaying the probability of having a CEMV for each heart score. Each feedyard mortality in this trial received a heart score (n=346), and a subset of feedyard mortalities was classified as CEMV cases (n=106). Heart scores were administered on a 1-5 scale, with 1 being physiologically normal and 5 being severely damaged with signs of significant remodeling.

Table 2-4. Lesions present within total cases (n=346), CEMV cases (n=106), and CHF cases (n=11). Animals could have more than one lesion present.

Lesion	Total		CEMV		CHF	
			n	Percent	n	Percent
	346		106		11	
AIP	34	9.8%	11	10.4%	0	0%
BIP	134	38.7%	46	43.4%	4	36.4%
BP	123	35.5%	35	33.0%	5	45.5%
Other Pulmonary	75	21.7%	23	21.7%	3	27.3%
Gastrointestinal	237	68.5%	72	67.9%	9	81.8%
Bloat	30	8.7%	7	6.6%	0	0%
Cardiovascular	107	30.9%	94	88.7%	11	100.0%
Other Cardiovascular	34	9.8%	18	17.0%	7	63.6%
Other	75	21.7%	14	13.2%	1	9.1%

Other Pulmonary = embolic pneumonia, fibrinonecrotic larynx; Gastrointestinal = abdominal adhesions, colon ulcer, diaphragmatic hernia, gastrointestinal hemorrhage, hemoperitoneum, hemorrhagic gastrointestinal lesions (abomasum, small intestine, large intestine), liver adhesions, liver flukes, liver scars, intestinal strangulation; Other Cardiovascular = myocarditis, endocarditis, pericarditis, left ventricular hypertrophy, myocardial ecchymosis, ruptured aorta, anemia; Other = ascites, musculoskeletal, buller, calving, choke, kidney stones, neurologic, pyometra, reproductive.

Chapter 3 - Determining potential cardiac and hepatic histopathology differences between bovine congestive heart failure cases and feedyard mortalities from other causes

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Abstract

Non-infectious bovine congestive heart failure (CHF) is an important cause of feedyard mortality; however, underlying etiology and pathological process of disease development are unclear. The objective of this retrospective cross-sectional study was to determine potential differences in cardiac and hepatic histopathology between CHF cases and feedyard mortalities from other causes. Post-mortem examination was performed and samples retrieved from animals with grossly abnormal and normal hearts. Cases definition for CHF included abnormal heart shape, congested liver and presences of ancillary signs; all other cases were considered as controls (CTRL) for comparison. Cardiac and hepatic histopathology was performed scoring fibrosis and necrosis categorized into either none/minimal or moderate/severe changes. A

generalized linear model was used to evaluate potential associations between the probability of CHF with histopathological lesions. Of the 87 cases included for analysis 11 met the case definition for CHF. Cardiac histopathologic changes were identified in grossly normal and abnormal hearts with moderate/severe necrosis and fibrosis in 14.9% and 16.1% of all cases, respectively. Hepatic necrosis and fibrosis were moderate/severe in 33.3% and 16.1% of cases respectively. Liver fibrosis was the only factor associated ($P < 0.05$) with CHF probability and cattle with moderate/severe liver fibrosis were more ($P < 0.01$) likely (0.57 +/- 0.13) to have CHF compared to cattle with minimal liver fibrosis (0.04 +/- 0.02). These results illustrate cardiac histopathologic changes were relatively rare and not associated with the probability of CHF; however, hepatic histopathology may be useful in confirming CHF.

Key words: congestive heart failure, feedyard cattle, histopathology, necropsy

Abbreviations: CHF = congestive heart failure, DOF = days on feed

Introduction

Non-infectious congestive heart failure in cattle has historically been reported in feedyard cattle specifically transitioning from low to high altitudes resulting in pulmonary hypertension with subsequent right sided heart failure (high-altitude disease) [1,2]. More recently, congestive heart failure has been described in cattle housed in commercial feedyards at lower elevations [3,4,5]. The pathogenesis and cause of high-altitude disease has been well documented; however, the development of non-infectious bovine congestive heart failure (CHF) in feedyard cattle not exposed to high or changing altitudes remains poorly understood. Several risk factors associated with development of CHF nominal altitudes have been identified including sex, feedyard

location, and time of year arrived at feedyard [4,6]. Specific etiologic factors have not been defined, though genetics has been proposed to play a significant role in disease development with several genetic markers for heart disease recently identified [4,6,7,8].

Right-sided CHF is an untreatable non-infectious disease characterized by cardiac remodeling due to chronic increased pre-load, ventricular eccentric hypertrophy, poorly understood mechanical trauma, and eventual cardiomegaly which results in overall reduced efficiency and efficacy of cardiac function, persistently elevated hydrostatic pressure, and body cavity effusions (ascites) or chronic passive congestion of the liver [5,9—11]. Clinical signs of CHF include dyspnea, tachycardia, tachypnea, depression, decreased appetite, congested liver, serous and/or serosanguineous effusion (peritoneal, pleural, pericardial), ascites, edema of the brisket and ventral mandible, distention of the jugular vein, and exophthalmia. Many of these, especially early in the course of disease, are clinically difficult to distinguish from other common causes of feedyard mortalities. Diagnosis of congestive heart failure early in the course of disease is very challenging; in fact, definitive diagnosis of congestive heart failure (CHF) in feedyard cattle during postmortem examination is inconsistent, and this has contributed to difficulty comparing reports, cases, and further study and understanding of this disease in the industry [3,12]. Improved understanding of the underlying pathologic process could lead to improved and more specific diagnostic methods.

Death attributed to CHF represent an estimate of approximately 4% of feedyard cattle mortalities, and the risk of heart disease varies by individual feedyard with operations ranging from 0.7 deaths to 20.5 deaths per 10,000 head received [3,6]. Economic consequences from death loss are substantial, but subclinical impacts on performance are difficult to detect, measure, and

likely underreported [7]. Research has indicated that cattle with enlarged or misshapen hearts identified at harvest have decreased live animal and carcass performance [8,13].

Currently there is limited data describing histopathologic changes in heart and liver tissues in feedyard mortalities attributed to CHF. A previous study of CHF cases revealed several cardiac histopathologic changes; however, no comparison was available to mortalities attributed to other causes [5]. Evaluation and identification of significant differences in histologic lesions of heart and liver from CHF cases compared to these tissues from cattle mortalities of other causes may provide a deeper understanding of the CHF disease process. The objective of this retrospective cross-sectional study was to determine potential differences in cardiac and hepatic lesions by histopathologic examination between CHF cases and feedyard mortalities from other (non-CHF) causes.

Materials and Methods

2.1. Ethical Approval

All tissue samples were collected during post-mortem examination following natural death, and was thus exempt from review for approval by the Institutional Care and Use Committee at Kansas State University.

2.2 Study Design

This retrospective case-control study took place with feedlot cattle necropsied over two summers: June 1, 2022 – July 29, 2022, and June 1, 2023 – July 28, 2023, at six central high plains U.S. feedyards. Tissue samples were collected from cattle necropsied within eighteen hours of death to minimize the effect of postmortem autolysis.

2.3. Operational Data

Historical data including sex, days on feed (DOF) at the time of death, and estimated weight at death were collected for each animal from each feedyard's database. Sex was determined at necropsy and included heifers and steers. DOF at death and estimated weight at death were categorized to avoid assumption of linear relationship between each variable and the outcome of interest (CHF status). Fifty-day increments were used to categorize DOF at death into: 0-50 days, 51-100 days, 101-150, and greater than 150. Estimated weight at death was categorized in pounds and converted to kg resulting in the following categories: list categories 134.6-272.8, 272.7-409.1, 409.1-545.5, 545.5-636.4 kg.

2.4. CHF Cases and Controls

Full post-mortem evaluations were performed on each prospective case, and gross lesions were documented with photographs [14]. Veterinarian [BJW]-trained and supervised research technicians performed all post-mortem evaluations, recorded gross lesions, captured digital pictures, and collected formalinized tissue samples. Gross photographs, gross diagnoses, and the cause of death for each case were reviewed and confirmed by a veterinarian [BJW].

As part of the postmortem examination in each animal, the heart was externalized and removed from the thoracic cavity for full visualization. The lateral aspect of the heart was evaluated to determine size and shape, the heart was then transected longitudinally through left and right ventricular free walls to visualize right and left ventricle lumen and right and left ventricular free wall and septal thickness. Each heart was evaluated for gross evidence of congenital cardiac disease, and evidence of endo-, myo-, or pericarditis; Two digital pictures were recorded from each heart, including a lateral view of the in situ thoracic viscera including heart from the right side of the body, and a cross-sectional view through the left and right

ventricles at a level just below the atrioventricular valve. The initial subset of CHF cases was selected for sample collection based on field evaluation of heart size and shape; when an abnormal heart size or shape was identified, heart and liver tissue samples were collected into formalin. For every CHF case identified, identical samples were collected as a control animal from the next non-CHF death at the same feedyard (after heart size and shape were determined to be normal).

All cases hearts were then assigned scores by a single veterinarian [BJW] using a previously published 0 to 5 ordinal scale, with a heart score of 1 being physiologically normal and 5 exhibiting severe cardiac remodeling [13] (Figure 3-1). If evidence of inflammation were present including epicarditis, pericarditis (usually of traumatic reticulopericarditis), a score of 0 was assigned and the case was removed from the study.

A diagnosis of CHF case (CHFC) was confirmed when an animal had a heart score of 3, 4, or 5; chronic passive congestion was present in the liver; and serous or serosanguineous effusion in at least two of the following cavities: pleural, peritoneal, and/or pericardial. Cases with evidence of infectious or inflammatory heart lesions, such as traumatic reticulopericarditis, Histophilus somni-induced myocarditis, or con-genital abnormalities were excluded from the analysis. Animals were confirmed as a control (CTRL) if they did not meet the criteria for a CHFC.

2.5. Histopathology

Representative cardiac and hepatic tissue samples were collected from CHF and CTRL cases in 1x1 cm^2 cubes and placed immediately into 10% neutral buffered formalin. Cardiac tissue was harvested from the papillary muscle of the heart. Hepatic tissue was harvested from affected tissue if chronic passive congestion was present, but if the liver was grossly normal,

samples were collected from the diaphragmatic surface of the right lateral liver lobe. After at least 72 hours formalin fixation, tissue samples were routinely trimmed (heart to facilitate visualization of the endocardial and/or epicardial surfaces; the liver to facilitate visualization of the capsular surface), embedded in paraffin, and then routinely sectioned by technicians in the histopathology laboratory at the Kansas State University Veterinary Diagnostic Laboratory for histologic analysis. Embedded tissues were sectioned at 4µm, then stained with hematoxylin and eosion (H&E) using the Leica ST5010 Autostainer XL (Leica Biosystems, Deer Park, IL, USA). Slides were then digitized by optical scanning using a brightfield Leica Aperio AT2 scanner at 400x magnification (Leica Biosystems). Morphologic changes (necrosis, fibrosis, inflammation) were assessed and photographed using the Aperio eSlide Manager software (Version 12.4.3.5008, Leica Biosystems).

Tissue samples were scored by a board-certified anatomic pathologist (BLP) blinded to the gross diagnosis and gross lesions described during field postmortem examination. Postmortem autolysis, necrosis, and fibrosis scores were assigned as normal tissue/minimal changes (score = 0/1, respectively) or moderate/severe changes (score = 2/3, respectively), and as illustrated in Figure 3-2. For postmortem autolysis, a score of 0/1 indicated mild changes including some mild loss of cellular or nuclear detail, increased bacteria but with retention of tissue architecture; a score of 2/3 indicated moderate to severe changes including significant loss of cellular and nuclear detail, many bacteria, and moderate to severe loss of tissue architecture. Heart or liver tissues with severe autolysis that occluded ability to confidently establish morphologic diagnoses were excluded from the study. For fibrosis, a score of normal tissue/minimal change indicated normal tissue or mild subendocardial or interstitial patchy (heart) or centrilobular (liver) deposition of loose connective tissue. A score of moderate/severe

fibroplasia indicated moderate to severe subendocardial to interstitial coalescing deposition of compact connective tissue that resulted in loss and replacement of myocardiofibers (heart), or centrilobular, portal, and bridging deposition of compact connective tissue that resulted in loss and replacement of hepatocytes (liver). For necrosis, a score of normal/minimal change indicated normal tissue or mild individual scattered coagulative necrosis of hepatocytes or myocardiofibers. A score of moderate/severe necrosis indicated moderate to severe patchy and coalescing (heart) or centrilobular and bridging (liver) coagulative necrosis.

2.6. Statistical Analysis

A generalized linear model was fit using the 'glm' function from the 'lme4' package in R Studio (https://cran.r-project.org/web/packages/lme4/) to analyze the association between histopathology findings and the probability of a CHFC or CTRL [15]. The dependent variable was CHF case status (CHFC or CTRL). The independent variables included liver necrosis, liver fibrosis, heart necrosis, heart fibrosis, sex, days on feed, and estimated current weight. Backwards elimination was used to select significant variables in the model using $P < 0.05$ as the cutoff.

Results

Using the initial selection criteria of cattle with CHFC or CTRL, cardiac and hepatic histopathology was collected on 118 necropsy cases. There were 9 animals re-moved from analysis due to no liver sample or gross autolysis. A further 12 samples were removed due to histopathologic autolysis. Finally, 10 animals were removed from analysis due to signs of infectious heart disease (heart score 0) resulting in 87 total animals for evaluation. The 87 total cases (56 heifers and 31 steers) were retrospectively classified, with 11 being CHFC and 76 being CTRL (Table 3-1). At the time of death DOF ranged from 6-216 days, and the median

DOF was 77 days. The estimated weight at death ranged from 152.7 kg to 625.9 kg (336 lbs. to 1377 lbs.), with the median being 412.3 kg (907 lbs.). Descriptive statistics for gross and histopathologic lesions are presented in Table 3-1.

Most cattle (43.6%) received a heart score of 1 with 17.2% and 21.8% scored as 2 or 3. Heart scores 4 and 5 represented 6.8% and 10.3% of the population respectively. The percentage of cases within each heart score group that also had significant histopathologic lesions of either cardiac necrosis or fibrosis (Figure 3-3), or hepatic necrosis or fibrosis (Figure 3-4) shows that moderate/severe histopathologic lesions were present in tissues from animals at each gross heart score.

The only variable identified to be significantly ($P<0.05$) associated with a diagnosis of congestive heart failure (CHFC) in this study was liver fibrosis; animals with moderate/severe liver fibrosis had a 57% (SE = 13%) model-adjusted probability of being diagnosed with congestive heart failure (CHFC), while cases with minimal liver fibrosis had a 4% (SE=2%) model-adjusted probability of being diagnosed with congestive heart failure (CHFC, Figure 5).

Discussion

This study evaluated feedyard mortalities to determine associations between histologic cardiac and hepatic lesions and the likelihood of a clinical CHF diagnosis. In the study population, 11/87 (12.7%) of animals were identified as CHFC; the proportion of affected animals is influenced by case selection and sampling strategy. Moderate/severe cardiac and hepatic necrosis and fibrosis were present in animals with all gross heart scores (1-5). Hepatic fibrosis score was the only variable significantly associated with a high likelihood of a diagnosis of congestive heart failure (CHFC). Results from this study illustrate that of the presence of

moderate/severe cardiac necrosis and fibrosis, and hepatic necrosis were not specific to CHF cases, and these changes occurred in multiple non-cardiac feedyard mortalities.

The study population was intentionally selected to include cases with abnormally shaped hearts (heart score 3, 4, 5) matched with cases with heart score [1,2]. The primary research interest was comparing CHF cases to non-CHF cases; therefore, a specific case definition for CHF was applied incorporating enlarged hearts (heart score 3, 4, 5), congested liver and at least two ancillary signs while excluding grossly diagnosed infectious or congenital heart disease. The resulting CHF cases represented a small proportion of the data, but a higher proportion of the population than has been re-ported in previous studies [6]. Most (23/34) of the cattle with enlarged / abnormally shaped hearts (heart score 3, 4, 5) did not qualify for the case definition of CHF based on the gross liver changes and signs of effusion, per the definition of CHFC. One possibility is these cattle were early in the disease process and though they had altered hearts grossly, they had not yet developed functional cardiac abnormalities or adjunct lesions associated with end-stage CHF. Another possibility is heart remodeling occurred as a result of pulmonary disease resulting in increased pressures and heart re-modeling as reported in other literature [16]. Gross heart scores are a useful tool for evaluating gross changes in heart size and shape, but abnormal heart scores were not associated with the ancillary signs of CHF in many cases indicating these scores should not likely be used as the sole measure to determine heart disease in feedyard cattle.

Descriptive analysis comparing gross heart scores in animals with moder-ate/severe cardiac necrosis and fibrosis revealed that both necrosis and fibrosis were identified in samples from cattle in every heart score (except heart score 5 which had no cases with cardiac fibrosis). Identifying necrosis and fibrosis in tissues from animals across the range of gross heart scores

was surprising, and a clear association between the presence or severity of cardiac or hepatic necrosis, or cardiac fibrosis with the gross heart scores was not present. Histopathology in any organ as a tool for diagnosticians to detect lesions in tissues is very sensitive; however, as a mechanism to identify pathogenesis for the observed changes of necrosis and fibrosis is nonspecific, and our data do not indicate that gross heart score is a useful predictor of cardiac necrosis or fibrosis (or vice versa). The majority of animals with high gross heart scores were presumed to be the most severely affected hearts in cases of CHF, yet these often showed normal tissue, or minimal cardiac necrosis or fibrosis. As these lesions were not frequently identified in our subset, it is possible that the clinical disease of chronic congestive heart failure in cattle may not have consistent gross or histologic lesion correlates, and the disease may be due to idiopathic cardiomyopathy (similar to other species including dogs and cats), which does not often correlate with specific cardiac histopathologic changes.

Descriptive analysis comparing gross heart scores in animals with moder-ate/severe hepatic necrosis and fibrosis revealed that hepatic fibrosis and necrosis were also identified in animals from each gross heart score; however, in gross heart score 1 the majority of cases had no/minimal hepatic changes while the majority of gross heart score 5 cases illustrated moderate/severe hepatic necrosis and fibrosis. The presence of hepatic necrosis or fibrosis is more likely a sequel to congestive heart disease and is not indicative if the cause or pathogenesis of cardiac insufficiency.

We identified only moderate/severe hepatic fibrosis score as a significant association with a confirmed diagnosis of CHF in feedyard mortalities; we found that though other lesions (cardiac necrosis, cardiac fibrosis, hepatic necrosis) were all observed, none of these were strongly associated with CHFC. This finding was unexpected as previous research highlighted

the importance (and abundance) of histologic cardiac fibrosis in cases with end-stage CHF [5]. A few key differences from the previous work include the types of cases selected and the comparison of CHF cases with non-CHF cases. In the previous study cattle were selected with clinical end-stage CHF, and had been in the feedyard for a minimum of 120 days; in contrast for our study, mortalities may have occurred at any point and so cattle may have died earlier in the disease process due to cardiac or non-cardiac causes (metabolic anomalies, arrhythmias associated with minimal histologic lesions, pneumonias, bloat, lameness, etc.), and before the formation of cardiac fibrous tissue. Additionally, the current study compared CHF cases to non-CHF mortalities and identified both cardiac necrosis and fibrosis in the non-CHF cases. This finding indicates cardiac necrosis and fibrosis are not specific to CHF and likely have other causes.

Previous research evaluating bovine cardiac and hepatic histopathology found left ventricular and right ventricular fibrosis in animals diagnosed with CHF [17]; however, this study did not specifically exclude infectious heart disease (i.e. pericarditis, myocarditis, endocarditis) or congenital defects (ex. ventricular septal defect, atrial septal defect, aortic stenosis) from the analysis. Infectious and congenital heart diseases are common cardiac pathologies observed in cattle and are very different diseases when compared to non-infectious mechanical right-sided CHF [10]. Additionally, the objective was to create a naturally occurring animal model for human heart disease; the objective of our study was to examine cardiac and hepatic lesions, and to assess how those lesions are associated with a CHF diagnosis for cattle [11]. Heart histopathology was not associated with CHF in the current study; this could indicate not all cases had primary cardiac lesions.

Another limitation of this study is that heart tissue samples were always collected from the papillary muscle to improve consistency and because this specific location is an easily repeatable sampling area. Anecdotally we have evaluated numerous locations from grossly enlarged and normal feedyard cattle hearts and have not observed significant differences in necrosis or fibrosis in various anatomic regions of the hearts; however, it is possible that changes are not evenly distributed, and statistically similar in papillary muscle compared to other anatomic regions of the heart. The papillary muscle may not be the ideal spot to sample heart histopathology in cattle, and further research would be beneficial to identify other spots in the heart that may provide a more representative sample. Another noteworthy limitation is this study population is specific to feedyard cattle mortalities that did not finish the feeding period. Full systemic postmortem evaluations were performed on all mortalities, but inconspicuous findings that were not grossly detectable may not have been fully evaluated.

Conclusions

Postmortem examinations on feedyard mortalities are a standard practice throughout the industry and this study aimed to operate within a protocol comparable to industry practices; however, there is significant value in evaluation of lesions in CHF deaths as part of the normal feedyard production phase, in light of the fact that a significant proportion of bovine CHF research has been restricted to data collected at harvest. These conclusions should only be extrapolated to populations comparable to this study's population.

Conflicts of Interest

The authors declare no conflicts of interest.

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Literature Cited

1. Glover GH, Newson IE. Brisket Disease (Dropsy of High Altitudes). The Agricultural Experiment Station Bulletin. 1914;(201):1-24.

2. Jensen R, Pierson RE, Braddy PM, et al. Brisket disease in yearling feedlot cattle. 1976;169:515-517.

3. Johnson BT, White BJ, Dahlman DA, Rademacher RyanD, Amrine DE, Larson RL. Retrospective analysis of risk and timing of death from right heart failure in United States and Canadian feedlot necropsied cattle. javma. Published online April 28, 2023:1-7. doi:10.2460/javma.22.11.0517

4. Neary JM, Booker CW, Wildman BK, Morley PS. Right-Sided Congestive Heart Failure in North American Feedlot Cattle. Journal of Veterinary Internal Medicine. 2016;30(1):326-334. doi:10.1093/tas/txab220

5. Moxley RA, Smith DR, Grotelueschen DM, Edwards T, Steffen DJ. Investigation of congestive heart failure in beef cattle in a feedyard at a moderate altitude in western Nebraska. J VET Diagn Invest. 2019;31(4):509-522. doi:10.1177/1040638719855108

6. Johnson BT, Amrine DE, Larson RL, Weaber RL, White BJ. Retrospective analysis of cohort risk factors and feeding phase timing associated with noninfectious heart disease deaths in U.S. feedlot cattle. Translational Animal Science. 2021;5(4):txab220. doi:10.1093/tas/txab220

7. Heaton MP, Bassett AS, Whitman KJ, et al. Evaluation of EPAS1 variants for association with bovine congestive heart failure. F1000Res. 2019;8:1189. doi:10.12688/f1000research.19951.1

8. Buchanan JW, Flagel LE, MacNeil MD, et al. Variance Component Estimates, Phenotypic Characterization, and Genetic Evaluation of Bovine Congestive Heart Failure in Commercial Feeder Cattle. Genetics; 2023. doi:10.1101/2023.01.19.524739

9. Alexander A, Jensen R. Pulmonary Vascular Pathology of High Altitude-Induced Pulmonary Hypertension in Cattle. American Journal of Veterinary Research. 1963;24:1112-1122.

10. Buczinski S, Francoz D, Fecteau G, DiFruscia R. A study of heart diseases without clinical signs of heart failure in 47 cattle. Can Vet J. 2010;51(11):1239-1246.

11. Krafsur GM, Neary JM, Garry F, et al. Cardiopulmonary remodeling in fattened beef cattle: a naturally occurring large animal model of obesity-associated pulmonary hypertension with left heart disease. Pulm circ. 2019;9(1):1-13. doi:10.1177/2045894018796804

12. Corbin MJ, Griffin D. Assessing Performance of Feedlot Operations Using Epidemiology. Veterinary Clinics of North America: Food Animal Practice. 2006;22(1):35-51. doi:10.1016/j.cvfa.2005.11.003

13. Heffernan KR, Thomas MG, Enns RM, Holt T, Speidel SE. Phenotypic relationships between heart score and feed efficiency, carcass, and pulmonary arterial pressure traits1. Translational Animal Science. 2020;4(Supplement_1):S103-S107. doi:10.1093/tas/txaa114

14. Schmidt PH, White BJ, Finley A, et al. Determining Frequency of Common Pulmonary Gross and Histopathological Findings in Feedyard Fatalities. Veterinary Sciences. 2023;10(3):228. doi:10.3390/vetsci10030228

15. R Core Team. R: A Language and Environment for Statistical Computing. Published online 2023. <https://www.R-project.org/>

16. Peek SF, Buczinski S. Examination of the Cardiovascular System.

17. Nart P, Williams A, Thompson H, Innocent GT. Morphometry of Bovine Dilated Cardiomyopathy. Journal of Comparative Pathology. 2004;130(4):235-245. doi:10.1016/j.jcpa.2003.11.002

Figure 3-1. Examples of heart scoring method used to determine level of cardiac changes at necropsy. Hearts were scored on a 0-5 scale with 0 (A) illustrating signs of infection, 1 (B) as normal heart shape progressing to 5 (F) with severe changes. Hearts scored 3, 4, or 5 were considered abnormal.

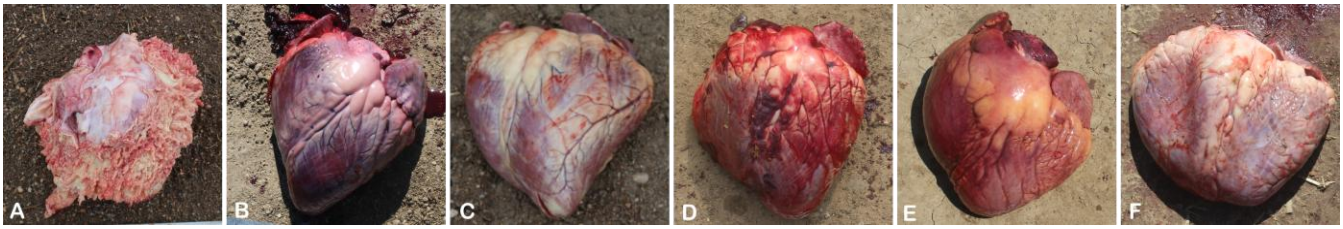
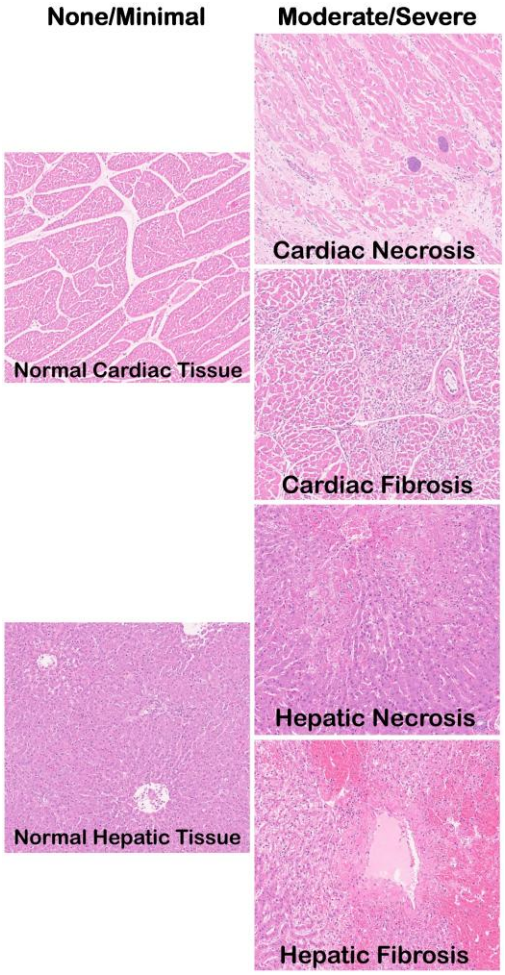


Figure 3-2. Histopathology examples from scoring system used to determine level of necrosis and fibrosis in cardiac and hepatic histopathology samples. Tissues were scored as none/minimal or moderate/severe*.



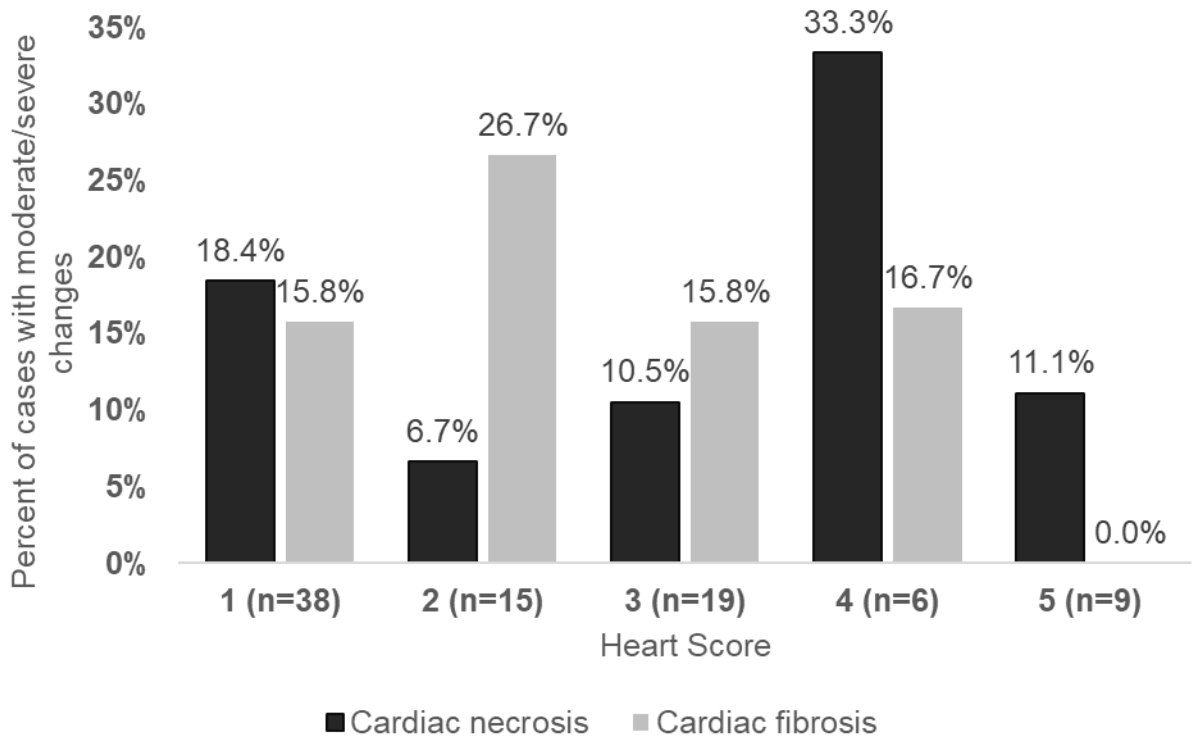
* For fibrosis, a score of normal tissue/minimal change indicated normal tissue or mild subendocardial or interstitial patchy (heart) or centrilobular (liver) deposition of loose connective tissue. A score of moderate/severe fibroplasia indicated moderate to severe subendocardial to interstitial coalescing deposition of compact connective tissue that resulted in loss and replacement of myocardiofibers (heart), or centrilobular, portal, and bridging deposition of compact connective tissue that resulted in loss and replacement of hepatocytes (liver). For necrosis, a score of normal/minimal change indicated normal tissue or mild individual scattered coagulative necrosis of hepatocytes or myocardiofibers. A score of moderate/severe necrosis indicated moderate to severe patchy and coalescing (heart) or centrilobular and bridging (liver) coagulative necrosis.

Table 3-1. Descriptive distribution of animal characteristics, gross lesions, and histopathological liver and heart lesions by CTRL (animals without congestive heart failure, CHF) and CHFC (cases with enlarged heart, congested liver and at least two of the following signs: pericardial, pleural, or peritoneal effusion) from selected feedyard mortalities necropsied at multiple central U.S. high plains operations (total n = 87). Histopathology samples were scored on a subjective ordinal scale by an experienced pathologist and categorized as mild or moderate/severe changes.

	CTRL	CHFC
Total enrolled (hd)	76	11
Animal Characteristics		
Sex		
Heifer	48	8
Steer	28	3
Days on feed		
0-50	35	6
51-100	15	1
101-150	11	4
> 150	15	0
Estimated current weight		
300-600	8	0
601-900	28	6
901-1200	31	5
1201-1400	9	0
Gross Lesions		
Heart score		
1-2	53	0
3,4,5	23	11
Congested Liver	7	11
Pericardial effusion	35	10
Pleural effusion	26	9
Peritoneal effusion	9	9
Histopathology		
Liver Necrosis		
None/Minimal	58	0
Moderate/Severe	18	11
Liver Fibrosis		
None/Minimal	70	3
Moderate/Severe	6	8
Heart Necrosis		
None/Minimal	66	8
Moderate/Severe	10	3
Heart Fibrosis		
None/Minimal	65	8

Moderate/Severe	11	3

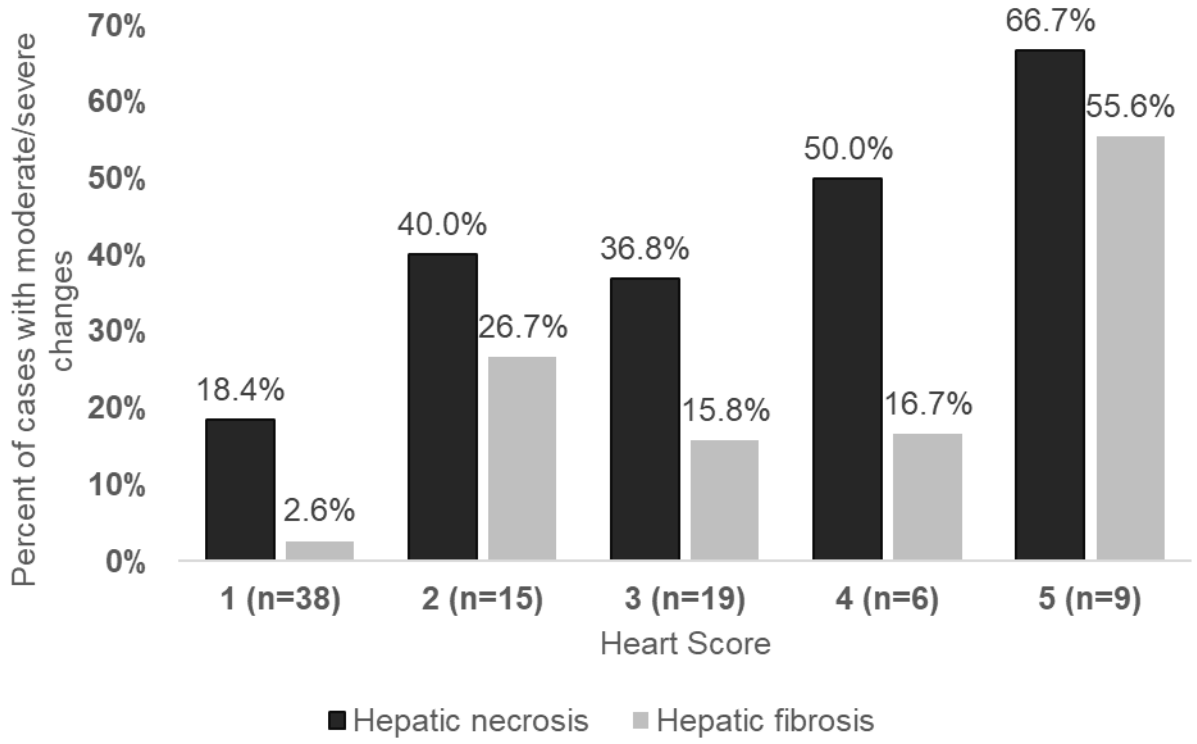
Figure 3-3. Cardiac necrosis and fibrosis in animals within each gross heart score* from 87 feedlot mortalities.



*Hearts were scored on a 0-5 scale where a score of 0 indicates inflammation, a score of 1 indicates a grossly normal heart shape and a score of 5 indicates severely enlarged or misshapen heart.

** * For fibrosis, a score of normal tissue/minimal change indicated normal tissue or mild suben-docardial or interstitial patchy (heart) or centrilobular (liver) deposition of loose connective tissue. A score of moderate/severe fibroplasia indicated moderate to severe subendocardial to interstitial coalescing deposition of compact connective tissue that resulted in loss and replacement of myo-cardiofibers (heart), or centrilobular, portal, and bridging deposition of compact connective tissue that resulted in loss and replacement of hepatocytes (liver). For necrosis, a score of nor-mal/minimal change indicated normal tissue or mild individual scattered coagulative necrosis of hepatocytes or myocardiofibers. A score of moderate/severe necrosis indicated moderate to severe patchy and coalescing (heart) or centrilobular and bridging (liver) coagulative necrosis.

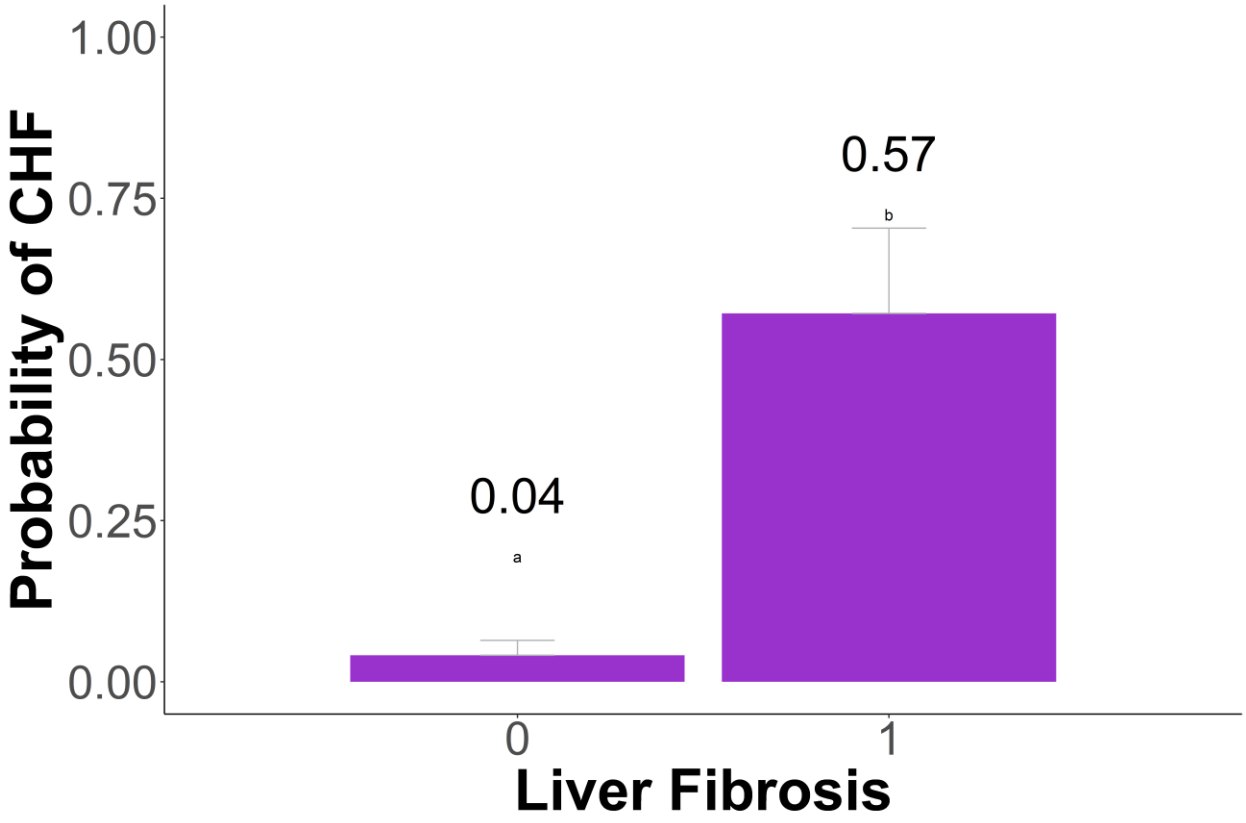
Figure 3-4. Hepatic necrosis and fibrosis in animals within each gross heart score* from 87 feedlot mortalities.



*Hearts were scored on a 0-5 scale with 0 illustrating signs of infection, 1 as normal heart shape progressing to 5 with severe changes.

*** For fibrosis, a score of normal tissue/minimal change indicated normal tissue or mild subendo-cardial or interstitial patchy (heart) or centrilobular (liver) deposition of loose connective tissue. A score of moderate/severe fibroplasia indicated moderate to severe subendocardial to interstitial coalescing deposition of compact connective tissue that resulted in loss and replacement of myo-cardiofibers (heart), or centrilobular, portal, and bridging deposition of compact connective tissue that resulted in loss and replacement of hepatocytes (liver). For necrosis, a score of nor-mal/minimal change indicated normal tissue or mild individual scattered coagulative necrosis of hepatocytes or myocardiofibers. A score of moderate/severe necrosis indicated moderate to severe patchy and coalescing (heart) or centrilobular and bridging (liver) coagulative necrosis.

Figure 3-5. Model estimated probability of being a congestive heart failure case (CHF) based on liver fibrosis score calculated from 87 feedlot mortalities (11 with CHF). Liver fibrosis was categorized as none/minimal (0) or moderate/severe (1). Cases were defined as CHF when heart was enlarged/misshapen, a congested liver was identified, and at least two of the following ancillary signs were identified: pleural, pericardial, or peritoneal effusion.



Chapter 4 - Determining variability and potential differences in commercial beef feedyard ionophore concentrations based on location within feeding batch and particle size

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Abstract

Ionophores are a class of antibiotics commonly added to feed in commercial cattle feeding operations to act as a coccidiostat and increase feed efficiency. In high concentrations, ionophores can cause acute cardiotoxic effects in cattle, resulting in clinical signs similar to CHF. The first objective of this study was to evaluate variability and potential differences in monensin concentrations between delivery timing (at-delivery and ~2 hours post-delivery samples), and batch location (the beginning and end of batches) in a central US High Plains commercial feedyard. One truck load was considered a batch and four feed samples (A, B, C, and D) were collected on eight different days. Samples A and B were collected from the feed bunk at initial feed delivery where the truck began unloading (A) and finished unloading (B).

Samples C and D were collected from the same feed bunk 2 hours after-delivery (post-cattle consumption) where the truck began unloading (C) and finished unloading (D). The second objective was to determine potential differences and variability in monensin concentrations based on feedyard ration type (starter/finisher) and particle size. Particle size distribution of each ration type were determined using a Penn State particle separator with four sieves (S1 = 0.75, S2 = 0.31, S3 = 0.16, S4 = <0.16). All samples were analyzed for monensin concentrations using Ultra Performance Liquid Chromatography (UPLC). Descriptive statistics were conducted on monensin concentrations and variability between samples and two multivariate models were used to identify associations between monensin concentrations in 1) delivery timing and batch location and 2) sieve location or ration type. There were no interactions between delivery timing and batch location; however, batch location was associated ($P<0.05$) with monensin concentration (ppb) in that the start of the batch had a higher (32,934 ± 18,982; mean ± SD) monensin concentration compared to the end of the batch (28,185 ± 17,560). In the second model, there was an interaction between sieve and ration type ($P<0.01$). Monensin concentrations in both starter (7,122 ± 3,477) and finisher (9,638 ± 4,705) rations were lower in S1. The monensin concentrations in both starter (25,837 ± 12,613) and finisher (18,384 ± 8,975) rations were highest in S4. These results show that monensin concentrations have important differences in batch location.

Key words: ionophore, monensin, congestive heart failure, toxicity, cattle

Abbreviations: CHF = bovine congestive heart failure, BRD = bovine respiratory disease, ppb = parts per billion

Introduction

Monensin is one of seven different ionophores first discovered in 1951 that has become an important additive to beef cattle feedyard rations. After 1975, ionophores were commonly used as both anticoccidials and growth promotants in beef cattle and are routinely fed throughout the feeding phase [1,2,3]. In general, ionophores inhibit inefficient acetate-producing ruminal bacteria, thus allowing other bacteria to produce more efficient energy sources from propionate and butyrate resulting in improved feed efficiency [4]. In a 2016 survey of the cattle feeding industry, 76% of feedyards with 1,000 head or more fed any form of ionophore, with monensin being the most common ionophore fed [5].

Monensin is a carboxylic polyether antibiotic that is produced by fermenting a strain of Streptomyces cinnamonensis and is one of the most commonly fed ionophores in feedyards [4]. According to monensin feeding labels, dosages in feed rations for improved feed efficiency in beef cattle are indicated to be fed anywhere from 5 to 40 g/ton (5,511 to 44,092 ppb, respectively), or no less than 50 and no more than 480 mg/head/day. Toxicologically, lethal dose metrics—LD1, LD10, and LD50—refer to the dose of a substance required to kill 1%, 10%, and 50% of a population, respectively. For monensin in cattle, the LD1, LD10, and LD50 is 5.5 mg/kg, 11.2 mg/kg, and 26.4 mg/kg of body weight, respectively [6]. For perspective, a 400 kg calf receiving the highest recommended dose would receive 1.2 mg/kg of body weight, which is 4.6 times lower than the LD1 (5.5mg/kg) threshold, or 22 times lower than the LD50 (5.5 mg/kg) threshold. The difference between a safe and toxic dose, or the therapeutic window, is relatively narrow with monensin, leaving little room for error. Minor inconsistencies in feed mixing, dosing calculations, or ration formulations could make the difference between a safe, therapeutic and effective dose, or a dose that approaches toxic levels. It is also important to note that these

calculations were done utilizing the lethal doses high enough to kill 1% and 50% of the population; the effects of chronic exposure to a sublethal dose (a dose less than the LD1, but still higher than expected) are unknown.

The molecular mechanism of action of monensin involves transportation of cations across plasma membranes. However, this mechanism can lead to monensin toxicity if cations from Ca^{2+} buildup within the cells. Particularly in muscle cells, Ca^{2+} release from the sarcoplasmic reticulum is important for stimulating muscle contraction. Cation accumulation promotes lipid peroxidation, thus creating an ion concentration imbalance, inhibiting phosphorylation, and negatively impairing cellular functions. Among affected tissues, myocardial and skeletal muscles are particularly susceptible to damage due to their high metabolic activity [7]. Monensin toxicosis in cattle can manifest with clinical signs and gross findings similar to bovine right-sided congestive heart failure (CHF) and bovine respiratory disease (BRD). Affected animals may exhibit signs of anorexia, diarrhea, weakness, ataxia, hyperpnea, tachycardia, hyperventilation, dilated jugular vein, brisket edema, and in severe cases, death [2]. Gross findings in chronic cases at post-mortem examination often include lesions persistent with CHF, such as cardiomegaly, congested (nutmeg) liver, petechia, ecchymosis, pericardial and peritoneal effusion, subcutaneous edema. However, in cases of acute death, it is possible that gross lesions may be absent.2,8 When fed in recommended dosages, monensin is a safe and effective drug for promoting growth and acting as an anticoccidial. The risk of monensin toxicity emphasizes the importance of accurate dosing and monitoring feed for proper mixing to avoid adverse effects. The exact frequency of ionophore-induced morbidities and mortalities and the risk of ionophore toxicosis-induced heart disease in feedyards is unknown. This may be due to incidents being underreported in the literature or completely unrecognized by feedyards. However, deaths due to

ionophore toxicosis from supplementation errors or contaminated feed have been reported in cattle [9,10].

Given that monensin toxicosis can cause cardiac damage and gross CHF-like signs at necropsy, the potential remains for some cases of CHF in cattle to be influenced by ionophore exposure, possibly from chronic sublethal exposure or acute dosing errors [8,10]. Bovine CHF is recognized as a significant cause of mortality in feedyard cattle, estimated to account for 4% of mortalities (3,282 out of 75,963 mortalities) in a study conducted with twenty-two U.S. commercial feedyards [11]. Similarly, another report that observed cardiomegaly at slaughter used a heart scoring system (heart scores that scored a 4 or 5 based on a 5-point ordinal scale with a score of 1 classified as normal and a score of 5 being classified as severely misshapen) and found that 1,357 out of 32,763 slaughtered feedyard cattle (4.14%) had a heart score 4 or 5 [12]. These data describe CHF in two different populations from feedyards (feedyard mortalities and animals that lived to be shipped to slaughter). This shows that CHF is present in both mortalities and in animals that lived to be shipped, impacting enough of the cattle population to warrant being an animal health and economic concern for the industry in the U.S. and Canada [13,14].

The potential for ionophore exposure may be influenced by cattle's intrinsic behavior to preferentially consume, or "sort" through feed particles. In finishing rations with minimal roughage, cattle may sort for medium- to longer-sized forage particles that might be more palatable or nutritious, thus leaving behind finer-sized particles that may contain higher concentrations of monensin [15]. Even with proper feed preparation and truck mixing, sorting behavior could potentially leave one calf consuming more of some particles in the ration, altering the particle distribution and monensin concentration of the feed leftover in the bunk [15]. Cattle that are sick or smaller than pen mates (and possibly more susceptible to adverse effects from

excessive monensin intake) may be less aggressive to push to the feed bunk, consuming the sorted leftover feed from cattle that were more aggressive and ate before them. If cattle ingest toxic amounts of monensin, clinical signs of toxicity can align with clinical signs of CHF. Commercial feedyards strive to provide uniform rations; however, little work has been done evaluating potential differences in monensin concentration based on delivery timing and batch location. Exploring variability of monensin concentrations in commercial feeding rations provides better information to understand the potential for toxic effects.

Despite thorough mixing variability in monensin concentrations will be present in a batch of feed; however, the amount of variability and upper range of expected concentrations is not well defined. Understanding the range of monensin variability can provide insight into the potential for a calf to consume more monensin than expected leading to acute or subacute monensin toxicosis. The objectives of this study were 1) to evaluate variability and potential differences in monensin concentrations between delivery timing (at-delivery and ~2 hours post-delivery samples), and batch location (the beginning and end of batches), and 2) to determine potential differences and variability in monensin concentrations based on feedyard ration type (starter/finisher) and particle size.

Materials and Methods

Batch Feed Samples Experiment

This was a prospective observational study designed to evaluate the means and variability of monensin concentrations between start and end of the batch before and after cattle feed consumption in finisher ration samples from one central Kansas feedyard. Feed samples were collected on eight different dates throughout December of 2023 to account for variability in feed

mixing. No animals were used in this study, and no Institutional Animal Care and Use Committee approval was required.

Samples were collected from a finisher ration in quart-sized plastic bags on eight different days throughout December 2023. A batch is defined as one feed truck's load of mixed feed. In a batch, four different samples would be collected. These four samples include Samples A, B, C, and D (Figure 4-1). Samples were collected as follows:

Sample A: beginning of the batch at-delivery,

Sample B: end of the batch at-delivery,

Sample C: beginning of the batch ~2 hours post-delivery,

Sample D: end of the batch ~2 hours post-delivery.

Each batch had a unique individual batch identification number that all four samples were assigned. This batch identification will be referred to as "batch number". Samples A and B were collected immediately upon dispensing of feed from the truck and were taken at time of feed delivery, meaning the cattle did not have time to begin eating and sort for preferred feed particles. Samples C and D were collected ~2 hours after initial feed delivery from the truck, allowing cattle time to sort preferred feed particles. The beginning of the bunk was determined by evaluating where the first drop of feed landed in the initial bunk upon truck delivery, and the end of the bunk was determined by evaluating where the last drop of feed landed in the last bunk upon truck delivery. At-delivery or 2 hours post-delivery will be referred to as "bunk location". The collection location for the beginning of the bunk for samples A and C and the end of the bunk for samples B and C was kept consistent between those samples within a batch. The beginning of the first bunk or the end of the last bunk will be referred to as "batch location". Individual truck identification for each sample was also recorded.

Particle Size Feed Samples Experiment

This was a prospective observational study designed to evaluate the mean differences of monensin concentrations between particle size in feedyard diets. Feed samples were collected from five central Kansas feedyards on two dates in June of 2023. No animals were used in this study, and no Institutional Animal Care and Use Committee approval was required.

Samples were collected from starter and finisher rations in quart-sized plastic bags from 5 different feedyards in central Kansas throughout June of 2023. One starter ration sample and one finisher ration sample were collected from each feedyard. Particle size distribution was determined using a Penn State Particle Separator with 3 sieves (4 layers). The upper sieve pore size was 19.05 mm, middle sieve 7.87 mm, lower sieve 4.06 mm, and the bottom pan collected any particles <4.06 mm. To sort samples, the particle separator was shaken vigorously five times in one direction on each side. This process was repeated seven more times. After shaking, Sieve 1 (S1) contained the largest feed particles, Sieve 2 (S2) contained medium- to large-sized feed particles, Sieve 3 (S3) contained small- to medium-sized feed particles, and Sieve 4 (S4) contained the smallest feed particles (Figure 4-2). Feed from all four layers were collected in separate plastic bags and analyzed for monensin concentrations. Each feed sample was assigned a unique “sample group” identification number that linked the four sorted samples back to its feedyard, collection date, and ration type.

Monensin Concentration Testing

Batch feed samples and particle size feed samples were both analyzed for monensin concentrations using the same laboratory methods. Feed samples were analyzed via LC/MS-MS at the Kansas State Veterinary Diagnostic Laboratory. Briefly, 1g of each collected feed sample was weighed into a 50 mL conical tube (Thermo Fisher Scientific, Waltham, MA). A method

blank and a quality control (QC) sample were included in each run. The QC sample was spiked with 100 ng/g of a monensin standard. All samples, including method blank and QC, were extracted with 90:10 Methanol:18 Mohm (Ω) water. The samples were then vortexed 30 minutes, then centrifuged for 10 minutes at 4500 RPM at 20 ◦C. The supernatants were then diluted 0.5x with 18 Ω water and centrifuged for another 5 minutes. The QC and method blank were put into a 2 mL polypropylene screw top vial (Thermo Fisher Scientific, Waltham, MA) for analysis and the feed samples were diluted 10x using 1:1 Methanol: 18Ω Water to ensure the detected values fell within the calibration curve before they were put in a 2 mL polypropylene screw top vials. An eight-point calibration curve was built by serial dilution of a 1 µg/mL monensin standard with 1:1 Methanol: 18 Ω water to give concentrations of 1, 2.5, 5, 10, 25, 50, 100, and 250 ng/mL. All samples including QC and method blank were loaded onto the liquid chromatography system (Waters Ultra Performance LC, Xevo TQ Absolute MS/MS; Waters Co., Milford, MA, USA). Samples were analyzed using a method validated for diagnostic use at Kansas State University Veterinary Diagnostic Laboratory. The QC sample was retested every 10 samples with a second analysis of the calibration curve at the end of each analytical run to check for monensin degradation. Results were calculated by instrument software (MassLynx, Waters Co., Milford, MA) and entered into Microsoft Excel (Microsoft Co., Redmond, WA) for reporting.

Batch Feed Samples Statistical Analysis

Evaluation of the associations between monensin concentrations with bunk and batch locations was conducted using a “lme” function from the “lme4” package in R Studio [14]. The dependent variable for the model was monensin concentration in ppb. To address skewness in the monensin concentration data distribution and reduce heteroskedasticity, the square root of

monensin concentration in ppb was used in this model. The independent variables included bunk location (at-delivery or ~2 hours post-delivery), batch location (beginning or end), and bunk by batch location effect. Sample group number and truck identification were included as random effects to account for the lack of independence among samples. Estimated marginal means of monensin concentration in ppb were calculated for the batch location and bunk location using the "emmeans" function from the "emmeans" package in R Studio. The estimated marginal means and standard errors were calculated on the square root-transformed monensin concentration scale to align with the model, then back-transformed to the original scale by squaring the values for data presentation.

Particle Size Feed Samples Statistical Analysis

To evaluate associations of monensin concentrations with particle size amongst starter and finisher ration samples, a multivariate model was conducted using "glmer" function from the "lme4" package in R Studio [14]. The dependent variable for the model was monensin concentration in ppb. To address skewness in the monensin concentration data distribution and reduce heteroskedasticity, the log of monensin concentration in ppb was used in this model. The independent variables included sieve (S1, S2, S3, S4), ration type (starter or finisher), and the interaction effect between sieve and ration type. The random effects included sample group identification to account for collection date and feedyard. Estimated marginal means were calculated using the "emmeans" function from the "emmeans" package in R Studio. The estimated marginal means and standard errors were calculated on the log-transformed monensin concentration to meet the assumptions of the model, then back-transformed to the original values by exponentiating the log-transformed estimated values.

Monensin Intake Based on Feed Intake Relative to Calf Weights

A table was used to display the amount of monensin (mg/kg) a calf would consume based on the proportion of feed intake relative to the calf's body weight. To determine this calculation, the monensin concentration (mg/kg of feed as fed) was multiplied by the feed consumption percentage (as a proportion of body weight). The range of possible monensin concentrations (mg/kg of feed as fed) was determined based on the minimum and maximum monensin concentrations detected in the batch sample study. This formula expresses feed consumption as a ratio of the calf's body weight, standardizing monensin intake across animals of varying sizes. This scaled formula allowed for monitoring the monensin intake in relation to both body weight and feed consumption, and for comparison to subclinical levels and the LD1 toxicity threshold.

Results

Batch Feed Samples Descriptives

A total of 244 feed samples were collected and analyzed for monensin concentrations (ppb). The mean monensin concentration was 31,966 ppb, minimum was 69 ppb, and maximum was 116,002 (Figure 4-3). A cumulative frequency plot showing the distribution of monensin concentrations (ppb) was utilized to show quartiles distinguishing the 25th, 50th, and 75th percentiles, which were 24,094 ppb, 28,027 ppb, and 33,671 ppb, respectively (Figure 4-4). Within the 0 to 25th, 26 to 50th, 51 to 75th, and 76 to 100th percentile, samples from the start of the batch location made up 47.5%, 42.6%, 45.9%, and 63.9% of samples, respectively, and samples from the end of the batch location made up 52.5%, 57.4%, 54.1%, and 36.1% of samples, respectively (Table 4-1).

Batch Feed Samples Model

The final model assessing the effects of batch location, bunk location, and their interaction on monensin concentrations (ppb) showed batch location to be significant ($P<0.01$). Bunk location and the interaction between batch and bunk location were not significant ($P > 0.05$). The model estimated marginal means from samples obtained from the end of the batch were 28,185 (SE = 1,590) and samples obtained from the start of the batch were 32,934 (SE = 1,719).

Particle Size Feed Sample Descriptives

A total of 10 starter and finisher ration samples were collected. After particle sizing, there were a total of 20 sorted starter ration samples and 20 sorted finisher ration samples. The percentage of the total amount of feed that fell on each respective sieve was calculated. For the starter ration samples S1, S2, S3, and S4, the percentage of total feed that fell on each sieve was 12.8%, 32.2%, 17.6%, and 37.4%, respectively. For the finisher ration samples S1, S2, S3, and S4, the percentage of total feed that fell on each sieve was 1.7%, 30.6%, 26.3%, and 41.4%, respectively. The mean monensin concentration was 14,088 ppb, minimum was 3,815 ppb, and maximum was 60,234 ppb. Within the starter ration samples, the mean monensin concentration was 16,326 ppb, minimum was 3,815 ppb, and maximum was 60,235 ppb. Within the finisher ration samples, the mean monensin concentration was 11,850 ppb, minimum was 4,932 ppb, and maximum was 26,206 ppb.

Particle Size Feed Samples Model

The final model assessing the effects of sieve, ration type, and their interaction on monensin concentrations (ppb) showed sieve and the sieve by ration type interaction to be significant ($P<0.01$). Ration type alone was not significant). The monensin concentrations in

both starter (7,122 ± 3,477; mean, SD) and finisher (9,638 ± 4,705) rations were lower in S1. The monensin concentrations in both starter (25,837 ± 12,613) and finisher (18,384 ± 8,975) rations were highest in S4 (Figure 4-5).

Monensin Intake Based on Feed Intake Relative to Calf Weights

A table showing the amount of monensin a calf would consume based on their feed consumption (as a percent of body weight) (Table 4-2). The recommended dose of monensin in feed concentrations is 30 mg/kg and the LD1 is 5.5 mg/kg in cattle. This table showed that even if a calf's feed consumption rate was 3% of their body weight with monensin concentrations at 120 mg/kg, the calf would consume 3.6 mg/kg of monensin. This dose is 1.9 mg/kg under the LD1 for cattle.

Discussion

The purpose of both objectives in this study was to evaluate potential differences in the mean and variability of monensin concentrations amongst batch and bunk locations, and the differences of monensin concentrations amongst means for starter and finisher ration particle size samples. Toxic concentrations of monensin in feed can induce CHF in cattle. Bovine CHF is an emerging disease syndrome with multiple risk factors that has garnered the attention of veterinarians and beef producers in recent years.

The batch feed sample analysis showed substantial variation in monensin concentrations among samples and demonstrated a difference in batch locations, with the end of the batch having lower monensin concentrations than the beginning of the batch. The model did not show that bunk location (at-delivery or ~2 hours post-delivery) or the interaction between batch location and bunk location were significantly associated with monensin concentrations. This may suggest that variability of monensin concentrations could be attributed to truck mixing rather

than cattle sorting. The particle size feed sample analysis showed significant variation between monensin concentrations and sieve location, with the smallest particles in S4 having the highest concentrations and the largest particles in S1 having the lowest concentrations. This finding would suggest that if cattle preferentially sort larger, more palatable particles, the potential risk of uneven monensin exposure for an animal that consumed the leftover feed in the bunk may increase. However, as previously noted with the batch feed sample analysis, the bunk location (which would have accounted for cattle sorting) was not significantly associated with monensin concentration variability. Regardless, this analysis still shows that monensin will be found mostly in smaller particles.

Interestingly, the bunk location did not show significant levels of variation in the batch feed sample analysis. The particle size feed sample analysis showed that the highest concentrations of monensin were found in the smallest feed particles in the S4 pan. The leftover feed in the bunk collected ~2 hours post-delivery did not have significantly different monensin concentrations than the feed collected at-delivery, indicating that the monensin concentrations within the ~2 hours post-delivery samples were as homogeneous as the at-delivery feed samples. Feed preparation accounts for cattle sorting behavior and ensure the ration has uniform particle size by grinding grains and other medium- to longer-sized feed particles [17]. This finding indicates that this management practice may help mitigate the effects of cattle sorting behavior.

While the monensin is generally safe if fed as labeled, it has a relatively narrow therapeutic index. This means that monensin has a steep dose-response relationship, and there is a small margin of error between a safe, therapeutic dose and a toxic, ineffective dose. Because of this, small deviations in monensin concentrations in feed could push levels into the toxic range. In the context of a feedyard setting, monensin variability in feed preparation or feed mixing is

extremely important to closely monitor. Any mixing inconsistencies or errors can greatly impact the actual monensin dose consumed by cattle. This particular feedyard mixed rations in a batch box—a hydraulically lifted box attached to the side of the feed trucks. Monensin can be fed in a liquid or pelleted form, and this feedyard added liquid monensin to the ration. Although 50% of samples were within the 24,094—33,671 ppb range, 5.3% of samples were two times above the target concentration at 60,000 ppb (Figure 4). Mixing protocols seem to homogenize feed well, but still leaves some room for error.

In this study, the batch feed sample analysis revealed notable variation in monensin concentrations from a feedyard, indicating that some cattle may inadvertently consume higher doses than intended. Based on the monensin concentration distribution observed in this study, a table displaying the range of possible monensin concentrations that a calf could consume based on the amount of feed consumption as a percent of body weight was determined (Table 1). This table monitors the possible monensin intake in relation to both food consumption and body weight to compare to the LD1 threshold (5.5 mg/kg). Among all the possible monensin concentrations ranging from 20 mg/kg to 120 mg/kg and the percentages of feed consumption, the highest possible monensin intake was 3.6 mg. This dose is 1.9 mg/kg below the LD1 threshold. Acute monensin toxicosis appears to be unlikely but cannot be completely ruled out due to the wide range in concentration variability based on the concentrations observed in this study. The variability in concentrations indicate that occasional “hot spots” of monensin in feed bunks could occur. Additionally, it is critical to note that the subacute dose for cumulative effects of sublethal doses of monensin is not well defined in the literature. The prolonged exposure of sublethal doses could increase the chances of a calf experiencing cumulative oxidative stress, especially in the myocardium [7]. This may increase the chances of a calf succumbing to chronic

cardiac damage that otherwise may not have been lethal at expected doses. Considering that current research indicates genetics play a role in CHF onset, this risk may be especially heightened in predisposed cattle [18,19].

Although the subacute dose needed for cumulative toxicity is not known, a study was conducted to determine the onset and degree of monensin toxicity in cattle [20]. This multiple dose study was conducted with thirty steers ranging from 210 to 308 kg body weight. Monensin doses were gavaged in a water slurry with 0, 400, 600, 1,000, 2,000, or 4,000 mg of monensin per animal per day for 7 days. As the dose increased, the feed consumption rate decreased. Cattle fed 1,000 mg or higher experienced complete anorexia after 2 days. Cattle on 400-, 600-, and 1,000 mg monensin treatments showed mild depression or diarrhea and no signs of dyspnea or ataxia. Cattle on 2,000- and 4,000 mg treatments showed anorexia, diarrhea, depression, dyspnea, and ataxia. Death loss occurred in the 2,000 mg group on day 5 (two deaths) and 7 (1 death) and 4,000 mg groups on days 4 (two deaths), 5 (one death), and 6 (one death). The LD1 and LD50 for monensin toxicosis in cattle is 5.5 mg/kg and 26.4 mg/kg [6]. This study demonstrates the monensin dosages required to see clinical effects in cattle, but it does not explore the dose of monensin required in actual feed to see clinical effects. Cattle in this dose titration study did not have the option to refuse the monensin doses, whereas feedyard cattle that begin to decrease feed intake or exhibit anorexia will inherently reduce their monensin intake. A multi-dose study monitoring the onset and degree of monensin toxicity in cattle by feeding sub-lethal and lethal doses in typical feed rations would provide external validity.

One of the limitations of this study is that the feed samples analyzed were collected in quart-sized plastic bags, then weighed to one gram for UPLC analysis. Samples were prepared

according to protocols and blended to homogenize, but the sample may still not be representative of the entire daily intake of a calf that can be 2-3% of their total body weight.

The purpose of this study was to evaluate monensin concentration variability in feedyard rations occurs and the chance of a calf consuming toxic doses, while not highly likely, is still a possibility. Results from this study showed that the beginning and end of a bunk had significant levels of variability, but there was no significance between concentrations of at-delivery and 2-hours post-delivery. Additionally, the S4 sieves for both starter and finisher rations had the highest concentrations of monensin. Monensin concentrations in this study were widely variable. Although toxic concentrations were not commonly observed, the effects of sublethal cumulative doses over time are unknown. More research is needed to determine the effects of sublethal doses of monensin in feed. Further research to directly link monensin variability of feedyard rations to the onset of bovine CHF is necessary to establish any causal relationships.

Conflicts of Interest

The authors declare no conflict of interest.

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Literature Cited

1. McDougald LR. The Biology of Coccidia. Edward Arnold; 1982.

2. Hall JO. Ionophore Use and Toxicosis in Cattle. Veterinary Clinics of North America: Food Animal Practice. 2000;16(3):497-509. doi:10.1016/S0749-0720(15)30083-9

3. Berger J, Rachlin AI, Scott WE, Sternbach LH, Goldberg MW. The Isolation of Three New Crystalline Antibiotics from Streptomyces1. J Am Chem Soc. 1951;73(11):5295-5298. doi:10.1021/ja01155a084

4. Novilla MN. Ionophores. In: Veterinary Toxicology. Elsevier; 2018:1073-1092. doi:10.1016/B978-0-12-811410-0.00078-7

5. USDA. Antimicrobial Use and Stewardship on U.S. Feedlots, 2017. USDA-APHIS-VS-CEAH-NAHMS; 2019. http://www.aphis.usda.gov/nahms

6. Potter EL, VanDuyn RL, Cooley CO. Monensin Toxicity in Cattle. Journal of Animal Science. 1984;58(6):1499-1511. doi:10.2527/jas1984.5861499x

7. Ekinci İB, Chłodowska A, Olejnik M. Ionophore Toxicity in Animals: A Review of Clinical and Molecular Aspects. IJMS. 2023;24(2):1696. doi:10.3390/ijms24021696

8. Ensley S. Ionophore Use and Toxicosis in Cattle. Veterinary Clinics of North America: Food Animal Practice. 2020;36(3):641-652. doi:10.1016/j.cvfa.2020.07.001

9. Brito ESA, Andrade TG, Oliveira CHSD, Moura VMBDD. Outbreak of monensin poisoning in cattle due to supplementation error. Cienc Rural. 2020;50(11):e20190996. doi:10.1590/0103-8478cr20190996

10. Basaraba RJ, Oehme FW, Vorhies MW, Stokka GL. Toxicosis in Cattle from Concurrent Feeding of Monensin and Dried Distiller's Grains Contaminated with Macrolide Antibiotics. J VET Diagn Invest. 1999;11(1):79-86. doi:10.1177/104063879901100113

11. Johnson BT, Amrine DE, Larson RL, Weaber RL, White BJ. Retrospective analysis of cohort risk factors and feeding phase timing associated with noninfectious heart disease deaths in U.S. feedlot cattle. Translational Animal Science. 2021;5(4):txab220. doi:10.1093/tas/txab220

12. Heffernan KR, Thomas MG, Enns RM, Holt T, Speidel SE. Phenotypic relationships between heart score and feed efficiency, carcass, and pulmonary arterial pressure traits1. Translational Animal Science. 2020;4(Supplement_1):S103-S107. doi:10.1093/tas/txaa114

13. Neary JM, Booker CW, Wildman BK, Morley PS. Right-Sided Congestive Heart Failure in North American Feedlot Cattle. Journal of Veterinary Internal Medicine. 2016;30(1):326-334. doi:10.1093/tas/txab220

14. Johnson BT, White BJ, Dahlman DA, Rademacher RyanD, Amrine DE, Larson RL. Retrospective analysis of risk and timing of death from right heart failure in United States and Canadian feedlot necropsied cattle. javma. Published online April 28, 2023:1-7. doi:10.2460/javma.22.11.0517

15. Silva Rodrigues M, Teixeira Dos Santos R, Martins Oliveira L, De Carvalho E. Intake pattern and feed sorting of beef cattle fed different sources of corn in a feedlot. Rev Colombiana Cienc Anim RECIA. 2024;16(2):e1075. doi:10.24188/recia.v16.n2.2024.1075

16. R Core Team. R: A Language and Environment for Statistical Computing. Published online 2023. <https://www.R-project.org/>

17. Heinrichs J. Evaluating particle size of forages and TMRs using the Penn State Particle Size Separator.

18. Heaton MP, Harhay GP, Bassett AS, et al. Association of ARRDC3 and NFIA variants with bovine. F1000Research. 2024;11(385). doi:10.12688/f1000research.109488.2

19. Buchanan JW, Flagel LE, MacNeil MD, et al. Variance Component Estimates, Phenotypic Characterization, and Genetic Evaluation of Bovine Congestive Heart Failure in Commercial Feeder Cattle. Genetics; 2023. doi:10.1101/2023.01.19.524739

20. Elanco Products Company. Rumensin Technical Manual for Pasture and Range Cattle. Division of Eli Lilly and Co.; 1978.

Figure 4-1. Sampling strategy for batch feed samples. One batch consisted of four samples collected from a full truck's complete feed route. Sample A was collected at delivery to the first bunk, before cattle began feeding. Sample B was collected at delivery to the last bunk, before cattle began feeding. Sample C was collected from the same location as Sample A ~2 hours post-delivery, after cattle consumed feed. Sample D was collected from the same location as Sample B ~2 hours post-delivery, after cattle consumed feed.

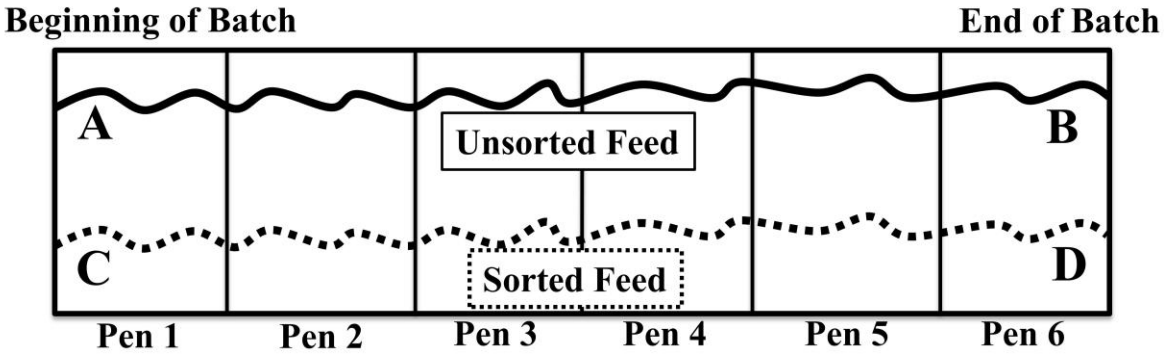

Figure 4-2. Representation of the 4 sieves of the Penn State particle separator and sampling strategy for particle size feed samples. The upper sieve pore size was 19.05 mm (S1), middle sieve 7.87 mm (S2), lower sieve 4.06 mm (S3), and the bottom pan collected any particles <4.06 mm (S4).

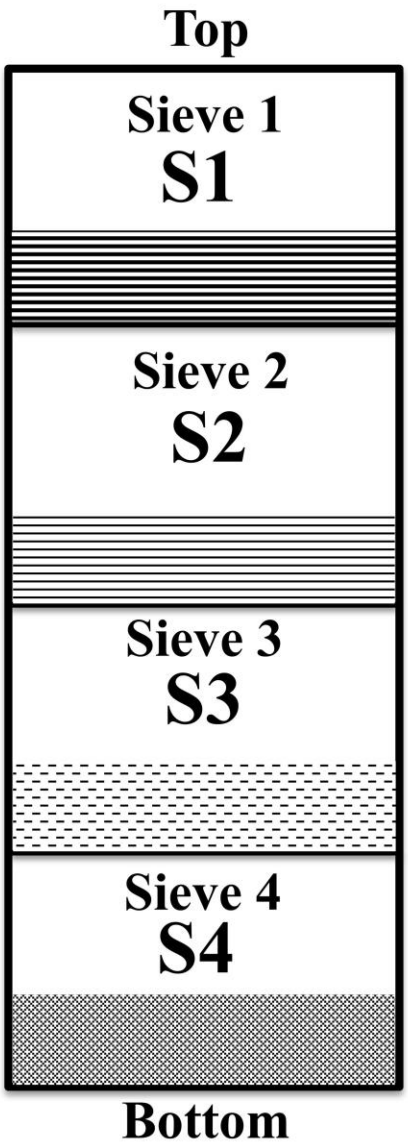

Figure 4-3. Box and whisker plot showing the raw distribution of monensin concentration (ppb) for 244 batch feed samples.

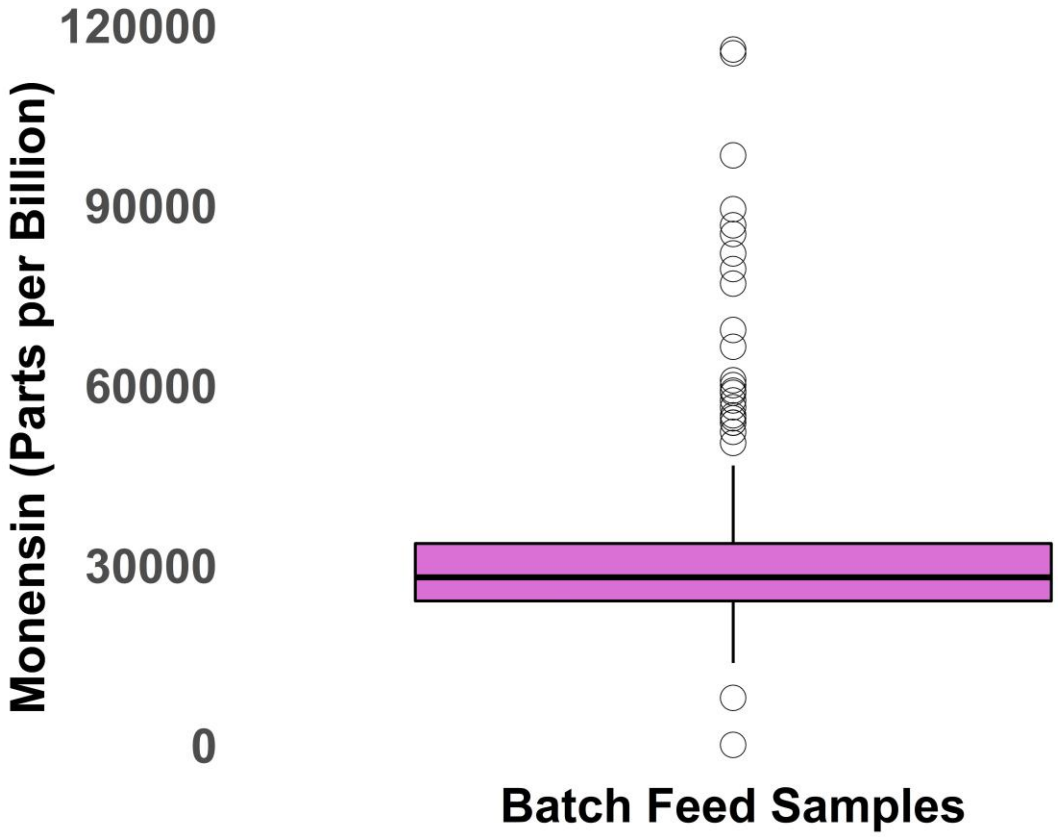

Figure 4-4. Cumulative frequency displaying the distribution of monensin concentration (ppb) and quartiles distinguishing the 25th, 50th (median), and 75th percentiles for batch feed samples. The 25th, 50th, and 75th percentile monensin concentrations were 24,094 ppb, 28,027 ppb, and 33,671 ppb, respectively.

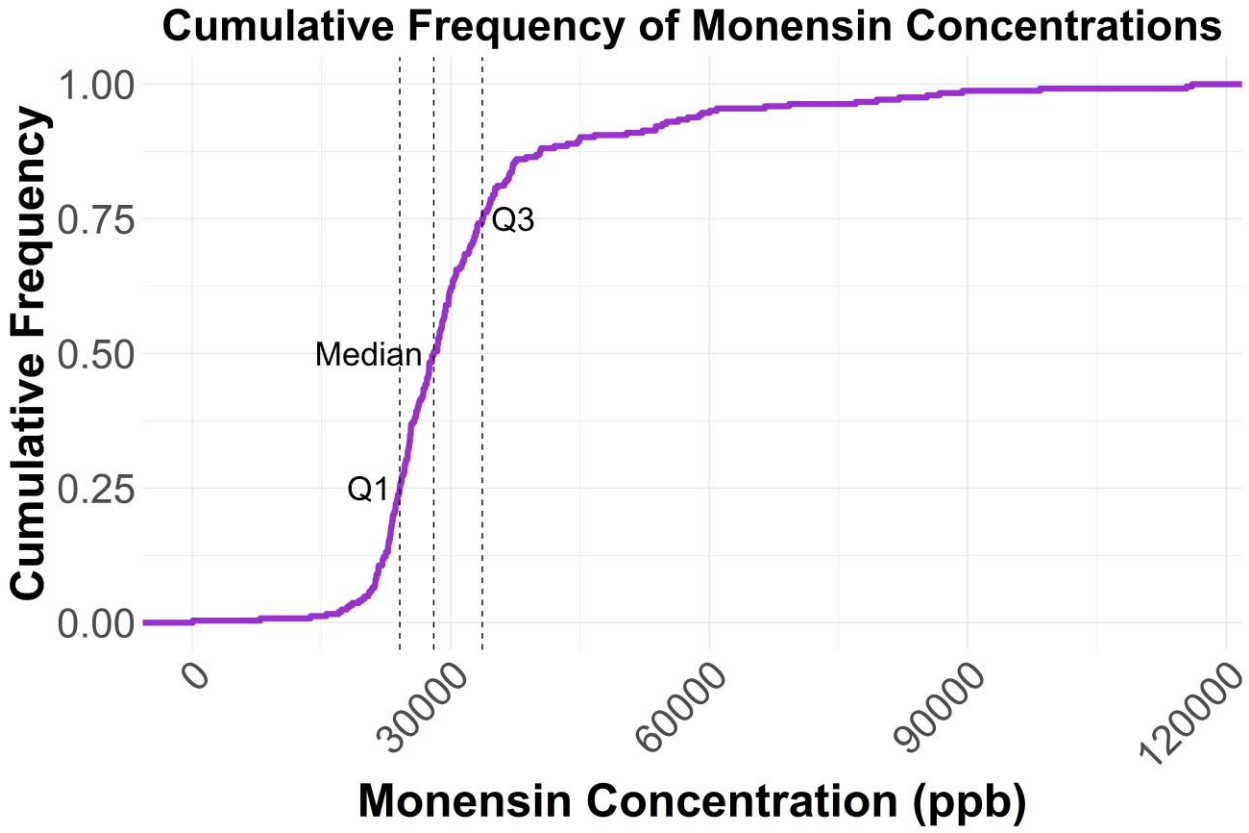

Table 4-1. Table showing the percentage of feed samples within each quartile of monensin concentrations (ppb) based on the cumulative frequency.

	Monensin Concentration (ppb) Quantile				
Sample Characteristics	**Q1 (25th)**	**Q2 (50th)**	**Q3 (75th)**	**Q4 (100th)**	**Total**
Bunk Location					
At-delivery	52.5%	42.6%	48.2%	55.7%	50%
2 hrs post-delivery	47.5%	57.4%	50.8%	44.3%	50%
Batch Location					
Beginning	47.5%	42.6%	45.9%	63.9%	50%
End	52.5%	57.4%	54.1%	36.0%	50%
Truck ID					
6	2.1%	3.3%	4.1%	2.1%	11.5%
7	10.3%	11.9%	7.8%	7.8%	37.7%
8	12.7%	9.8%	13.1%	15.2%	50.8%

Figure 4-5. The model estimated means for the sieve by ration type interaction showed that the S4 samples for both starter and finisher rations had the highest concentrations. Particle size distribution was determined using a Penn State Particle Separator with 3 sieves (4 layers). The upper sieve pore size was 19.05 mm (S1), middle sieve 7.87 mm (S2), lower sieve 4.06 mm (S3), and the bottom pan collected any particles <4.06 mm (S4). The S1 and S2 samples had the lowest concentrations of monensin, increasing to S4 having the largest concentration of monensin. The S4 starter samples were 25,837 ppb (SE = 5,641) and the S4 finisher samples were 18,384 ppb (SE = 4,013).

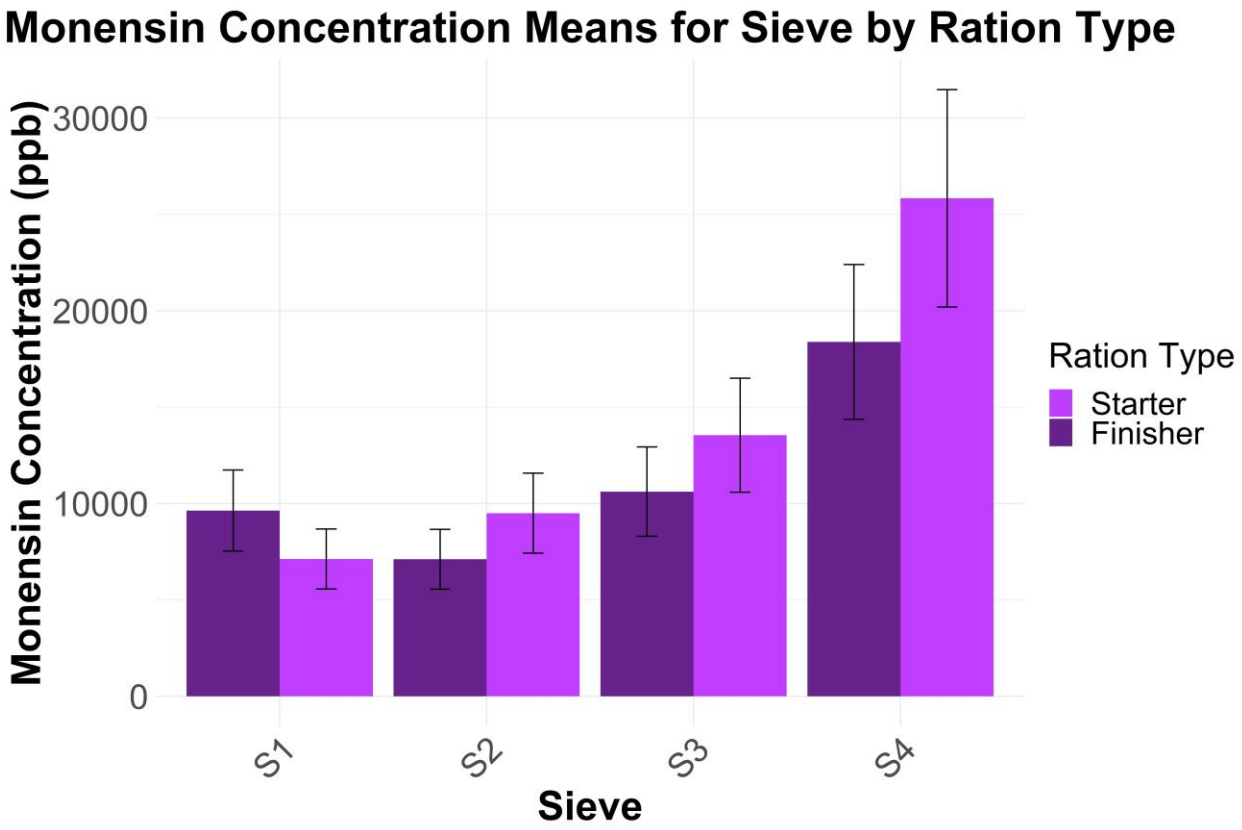

Table 4-2. Table showing the amount of monensin (mg/kg of body weight) a calf would consume based on the proportion of feed intake relative to the calf's body weight. Monensin concentration (mg/kg of feed as fed) was multiplied by feed consumption (as a proportion of body weight). The LD1 for monensin toxicosis in cattle is 5.5 mg/kg of body weight, and the LD50 is 26.4 mg/kg of body weight.

	Monensin Concentration (mg/kg of feed as fed)					
Feed Consumption (% body weight as fed)	***20***	***40***	***60***	***80***	***100***	***120***
1.00%	0.20	0.40	0.60	0.80	1.00	1.20
1.50%	0.30	0.60	0.90	1.20	1.50	1.80
2.00%	0.40	0.80	1.20	1.60	2.00	2.40
2.50%	0.50	1.00	1.50	2.00	2.50	3.00
3.00%	0.60	1.20	1.80	2.40	3.00	3.60

References

Abd El Raouf M, Elgioushy M, Ezzeldein SA. Congestive heart failure in cattle; etiology, clinical, and ultrasonographic findings in 67 cases. Vet World. 2020;13(6):1145-1152. doi:10.14202/vetworld.2020.1145-1152

Alexander A, Jensen R. Pulmonary Vascular Pathology of High Altitude-Induced Pulmonary Hypertension in Cattle. American Journal of Veterinary Research. 1963;24:1112-1122.

Berger J, Rachlin AI, Scott WE, Sternbach LH, Goldberg MW. The Isolation of Three New Crystalline Antibiotics from Streptomyces1. J Am Chem Soc. 1951;73(11):5295-5298. doi:10.1021/ja01155a084

Buchanan JW, et al. Variance component estimates, phenotypic characterization, and genetic evaluation of bovine congestive heart failure in commercial feeder cattle. : Genetics, 2023 doi:10.1101/2023.01.19.524739.

Buchanan JW, Flagel LE, MacNeil MD, et al. Variance Component Estimates, Phenotypic Characterization, and Genetic Evaluation of Bovine Congestive Heart Failure in Commercial Feeder Cattle. Genetics; 2023. doi:10.1101/2023.01.19.524739

Buczinski S, Francoz D, Fecteau G, DiFruscia R. A study of heart diseases without clinical signs of heart failure in 47 cattle. Can Vet J. 2010;51(11):1239-1246.

Buczinski S, Francoz D, Fecteau G, DiFruscia R. Heart disease in cattle with clinical signs of heart failure: 59 cases. 2010;51.

Corbin MJ, Griffin D. Assessing Performance of Feedlot Operations Using Epidemiology. Veterinary Clinics of North America: Food Animal Practice. 2006;22(1):35-51. doi:10.1016/j.cvfa.2005.11.003

Dai DF, Swanson PE, Krieger EV, Liou IW, Carithers RL, Yeh MM. Congestive hepatic fibrosis score: a novel histologic assessment of clinical severity. Modern Pathology. 2014;27(12):1552-1558. doi:10.1038/modpathol.2014.79

Ekinci İB, Chłodowska A, Olejnik M. Ionophore Toxicity in Animals: A Review of Clinical and Molecular Aspects. IJMS. 2023;24(2):1696. doi:10.3390/ijms24021696

Elanco Products Company. Rumensin Technical Manual for Pasture and Range Cattle. Division of Eli Lilly and Co.; 1978.

Ensley S. Ionophore Use and Toxicosis in Cattle. Veterinary Clinics of North America: Food Animal Practice. 2020;36(3):641-652. doi:10.1016/j.cvfa.2020.07.001

Glover GH, Newson IE. Brisket Disease (Dropsy of High Altitudes). The Agricultural Experiment Station Bulletin. 1914;(201):1-24.

Griffin D, Chengappa MM, Kuszak J, McVey DS. Bacterial Pathogens of the Bovine Respiratory Disease Complex. Veterinary Clinics of North America: Food Animal Practice. 2010;26(2):381-394. doi:10.1016/j.cvfa.2010.04.004

Hall JO. Ionophore Use and Toxicosis in Cattle. Veterinary Clinics of North America: Food Animal Practice. 2000;16(3):497-509. doi:10.1016/S0749-0720(15)30083-9

Heaton MP, Bassett AS, Whitman KJ, et al. Evaluation of EPAS1 variants for association with bovine congestive heart failure. F1000Res. 2019;8:1189. doi:10.12688/f1000research.19951.1

Heaton MP, Harhay GP, Bassett AS, et al. Association of ARRDC3 and NFIA variants with bovine. F1000Research. 2024;11(385). doi:10.12688/f1000research.109488.2

Heffernan KR, Thomas MG, Enns RM, Holt T, Speidel SE. Phenotypic relationships between heart score and feed efficiency, carcass, and pulmonary arterial pressure traits1. Translational Animal Science. 2020;4(Supplement_1):S103-S107. doi:10.1093/tas/txaa114

Heinrichs J. Evaluating particle size of forages and TMRs using the Penn State Particle Size Separator.

Holt T. Bovine High-mountain Disease [Internet]. Bovine High-mountain Disease. 2019.[cited 2024 Apr 19]. Available from: https://www.merckvetmanual.com/circulatory-system/bovine-high-mountain-disease/bovine-high-mountain-disease.

Jensen R, Pierson RE, Braddy PM, et al. Brisket disease in yearling feedlot cattle. 1976;169:515-517.

Johnson BT, Amrine DE, Larson RL, Weaber RL, White BJ. Retrospective analysis of cohort risk factors and feeding phase timing associated with noninfectious heart disease deaths in U.S. feedlot cattle. Translational Animal Science. 2021;5(4):txab220. doi:10.1093/tas/txab220

Johnson BT, et al. Association of feedlot disease treatments on the probability of heart disease syndrome in U.S. feedlot cattle. The Bovine Practitioner 2022;56:1–12.

Johnson BT, et al. Retrospective analysis of cohort risk factors and feeding phase timing associated with noninfectious heart disease deaths in U.S. feedlot cattle. Translational Animal Science 2021;5:txab220.

Johnson BT, White BJ, Dahlman DA, Rademacher RyanD, Amrine DE, Larson RL. Retrospective analysis of risk and timing of death from right heart failure in United States and Canadian feedlot necropsied cattle. javma. Published online April 28, 2023:1-7. doi:10.2460/javma.22.11.0517

Johnston R, et al. Confounding and collinearity in regression analysis: a cautionary tale and an alternative procedure, illustrated by studies of British voting behaviour. Qual Quant 2018;52:1957–1976.

Krafsur GM, Neary JM, Garry F, et al. Cardiopulmonary remodeling in fattened beef cattle: a naturally occurring large animal model of obesity-associated pulmonary hypertension with left heart disease. Pulm circ. 2019;9(1):1-13. doi:10.1177/2045894018796804

Kukor IM, et al. Sire differences within heart and heart fat score in beef cattle. Translational Animal Science 2021;5:S149–S153.

McDougald LR. The Biology of Coccidia. Edward Arnold; 1982.

Moxley RA, Smith DR, Grotelueschen DM, Edwards T, Steffen DJ. Investigation of congestive heart failure in beef cattle in a feedyard at a moderate altitude in western Nebraska. J VET Diagn Invest. 2019;31(4):509-522. doi:10.1177/1040638719855108

Nart P, Williams A, Thompson H, Innocent GT. Morphometry of Bovine Dilated Cardiomyopathy. Journal of Comparative Pathology. 2004;130(4):235-245. doi:10.1016/j.jcpa.2003.11.002

Neary JM, Booker CW, Wildman BK, Morley PS. Right-Sided Congestive Heart Failure in North American Feedlot Cattle. Journal of Veterinary Internal Medicine. 2016;30(1):326-334. doi:10.1093/tas/txab220

Newman JH, et al. High-Altitude Pulmonary Hypertension in Cattle (Brisket Disease): Candidate Genes and Gene Expression Profiling of Peripheral Blood Mononuclear Cells. Pulm circ 2011;1:462–469.

Novilla MN. Ionophores. In: Veterinary Toxicology. Elsevier; 2018:1073-1092. doi:10.1016/B978-0-12-811410-0.00078-7

Peek SF, Buczinski S. Examination of the Cardiovascular System. Published online 2018. doi:10.1016/B978-0-323-39055-2.00003-6

Potter EL, VanDuyn RL, Cooley CO. Monensin Toxicity in Cattle. Journal of Animal Science. 1984;58(6):1499-1511. doi:10.2527/jas1984.5861499x

R Core Team. R: A language and environment for statistical computing. 2024.Available from: https://www.r-project.org.

Reef VB, Whittier M, Allam LG. Thoracic ultrasonography. Clinical Techniques in Equine Practice. 2005;3(3):284-293. doi:10.1053/j.ctep.2005.02.006

Schmidt PH, White BJ, Finley A, et al. Determining Frequency of Common Pulmonary Gross and Histopathological Findings in Feedyard Fatalities. Veterinary Sciences. 2023;10(3):228. doi:10.3390/vetsci10030228

Schneider CA, et al. NIH Image to ImageJ: 25 years of image analysis. Nat Methods 2012;9:671–675.

Selvetella G, Lembo G. Mechanisms of Cardiac Hypertrophy. Heart Failure Clinics. 2005;1(2):263-273.

Silva Rodrigues M, Teixeira Dos Santos R, Martins Oliveira L, De Carvalho E. Intake pattern and feed sorting of beef cattle fed different sources of corn in a feedlot. Rev Colombiana Cienc Anim RECIA. 2024;16(2):e1075. doi:10.24188/recia.v16.n2.2024.1075

Singh CK, Kumar A, Singh N. An overview on the diagnostic and therapeutic aspects of cardiac diseases in bovine. Journal of Entomology and Zoology Studies.

USDA. Antimicrobial Use and Stewardship on U.S. Feedlots, 2017. USDA-APHIS-VS-CEAH-NAHMS; 2019. http://www.aphis.usda.gov/nahms

Wäsle K, et al. The Post-mortem Examination in Ruminants and its Possible Benefit to Ruminant Clinical Medicine. Journal of Comparative Pathology 2017;156:202–216.

White B. Monitoring systems for identifying bovine respiratory disease. AABP Proceedings 2015;:62–64.

Witte CL, Witte MH, Dumont AE, Cole WR, Smith JR. Protein Content in Lymph and Edema Fluids in Congestive Heart Failure. Circulation. 1969;40(5):623-630. doi:10.1161/01.CIR.40.5.623

Zühlke I, et al. Factors associated with cattle necropsy submissions in Switzerland, and their importance for surveillance. Preventive Veterinary Medicine 2021;187:105235.