Ultrasonography and management practices to understand pathogenesis and improve mitigation of fescue toxicosis and liver abscesses, and assess chute side diagnostics and prognostics of respiratory diseases in beef cattle

by

Luis Felipe Barbosa Braga Feitoza

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Abstract

The objectives of the studies were to investigate three major ailments in the cattle industry: fescue toxicosis, liver abscesses, and respiratory diseases. A total of five studies were designed and conducted to determine utility of ultrasound and preventive measures to understand pathogenesis and prevention of fescue toxicity, prevention of liver abscesses, and evaluate chute side diagnostics and prognostics of respiratory diseases in cattle.

In the first study, vasoactive compounds administered in low moisture, cooked molasses-based blocks were evaluated to mitigate fescue toxicosis and utilized ultrasonography to measure vascular diameter and assess blood perfusion to the periphery. Molasses-based block supplements with vasoactive compounds positively affected peripheral blood perfusion, improved thermoregulation and improved dry matter intake and average daily gain in cattle consuming ergot-infested tall fescue. In the second prospective study, strategic targeted durations of feeding tylosin phosphate at 60 mg animal per day for the first 30 or 60 days of the finishing phase on the incidence and severity of liver abscesses, performance and carcass characteristics were evaluated in feedlot cattle. Incidence of liver abscesses, overall cattle performance and carcass characteristics were not significantly affected by tylosin phosphate, however, a trend towards reduced severity of liver abscesses and increased body weight was observed.

Three observational studies were conducted to assess the utility of targeted thoracic ultrasonography for diagnosis of interstitial pneumonia, prognosis with respiratory disease treatments, and feedyard outcomes. In a cross-sectional study that targeted thoracic point-of-care ultrasound of caudodorsal right lung in feedlot cattle, a significant associations of ultrasound scores and days on feed at treatment with interstitial pneumonia were observed. The second study aimed to determine associations between negative outcomes (culled or died) and point-of-

care evaluations of feedlot cattle with chronic respiratory disease. Among the parameters evaluated, only ultrasound variables were significantly associated with negative outcomes of mortality or culling. The third was a cross sectional observational study that evaluated point-of-care chut side diagnostics at the time of respiratory disease treatment to assess likelihood of first treatment failure and negative outcomes. Variables significantly associated with did not finish (culled or died) and first treatment failure include ultrasound scores, days on feeding and body weights. The study identified key pulmonary and cattle demographic factors at chute side that valuable potential prognostic parameters when cattle are treated for respiratory disease.

In conclusion, the current research on beef cattle collectively advances our understanding of cattle health management by highlighting preventive and management practices and use of chute side diagnostic tools that can impact cattle outcomes. The integration of targeted diagnostics, such as thoracic ultrasonography, into routine management practices can significantly enhance prognosis accuracy and help with respiratory disease differentiation, optimize treatment decisions, and improve overall animal welfare. These findings not only offer practical applications for beef cattle operations but also provide a foundation for future research aimed at further refining cattle health management strategies and improving economic outcomes and animal welfare in the industry.

Keywords: Beef cattle, fescue toxicity, liver abscesses, respiratory diseases, ultrasonography, animal health

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Major Professor
Dr. B. J. White

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Table of Contents

List of Figures xii
List of Tables xvi
Acknowledgements xviii
Dedication xix
Chapter 1 - A review of literature: Thoracic ultrasound in cattle: methods, diagnostics, and prognostics. 1
Abstract 1
Introduction 2
Materials and Methods 4
Results and Discussion 4
History 4
Thoracic ultrasound method 5
Respiratory disease diagnosis 8
Respiratory disease prognosis 10
Thoracic ultrasonography training 11
Conclusions 12
References 13
Chapter 2 - Molasses-based block supplements for cattle fed endophyte-infected tall fescue (Festuca arundinacea) seed; Effects on growing performance, circulating biomarkers, heat stress, and coccygeal artery diameter. 23
Abstract 23
Introduction 26
Materials and methods 27
Experimental design 27
Cattle processing and data collection 28
Diet preparation and delivery 31
Statistical analyses 32
Results and Discussion 33
Molasses-based block intake 33

Cattle growth performance 33
Coccygeal artery diameter imaging 34
Thermography 35
Plasma glucose and L-lactate 35
Somatotropin and insulin-like growth factor I 37
Conclusion 37
References 39
Chapter 3 - Targeted durations of feeding tylosin phosphate on the incidence and severity of liver abscess in feedyard cattle 57
Abstract 57
Introduction 59
Materials and methods 60
Study Design 61
Cattle Processing 61
Diet Preparation 62
Harvest 64
Statistical Analyses 65
Results and Discussion 66
Liver Abscess Incidence and Severity 66
Steer Performance 68
Carcass Characteristics 69
Conclusions 70
Acknowledgments 70
References 71
Chapter 4 - Evaluation of targeted thoracic point-of-care ultrasonography for identification of interstitial pneumonia in feedyard cattle 86
Abstract 86
Introduction 88
Materials and Methods 89
Experimental Design & Enrollment Criteria 89
TT-POCUS method: 90

Histopathology 93
Statistical Analysis 93
Results 94
Discussion 96
Conclusions 100
Acknowledgements: 101
Disclosures: 101
Funding: 101
Conflict of interest statement: 102
References 103
Chapter 5 - Utilization of point-of-care evaluation to determine prognosis in feedyard cattle with multiple previous respiratory disease treatments. 119
Abstract 119
Introduction 121
Materials and Methods 123
Experimental Design & Enrollment Criteria 123
TT-POCUS 124
Pulse oximetry 125
Pulmonary auscultation score 125
Statistical Analysis 126
Results 128
Descriptive statistics 128
Logistic regression 129
Discussion 130
Conflict of interest statement: 133
Funding: 133
Author contributions: 133
Endnotes: 133
Acknowledgements: 134
References 135

Chapter 6 - Associations Between Chuteside Evaluations and 60-day Outcomes in Feedyard Cattle at Time of First Treatment for Respiratory Disease 147
Abstract 147
Introduction 149
Materials and Methods 150
Experimental Design & Enrollment Criteria 150
Pulse oximetry 152
Pulmonary auscultation score 152
Targeted Thoracic Point-of-Care Ultrasound 152
Statistical Analysis 153
Results 155
Descriptive statistics 155
Logistic regression 158
Discussion 160
Associations with first treatment failure and did not finish outcomes 160
Days on feed and body weight as prognostic indicators 161
Ultrasound lung score, B-line count and moth sign 161
Pulse oximetry and pulmonary auscultation 162
Conclusions 163
Acknowledgements: 163
References 166
Chapter 7 - Conclusion and future research 187
Summary of findings 187
Implications for the cattle industry 188
Limitations and future research 189
References 191

List of Figures

Figure 2.1 Overall intake of molasses-based block supplements by treatment. All cattle had *ad libitum* access to ground prairie hay, salt block, and their respective block supplements. CB = Control Block; AB = Block containing a proprietary blend of capsaicin and mannan oligosaccharide; MB = Block containing 0.3% crystalline menthol. 52

Figure 2.2 Effect of molasses-based block supplements on temperature differential (ΔT; °C). All cattle had *ad libitum* access to ground prairie hay and salt block. NC = negative control treatment with no fescue seed; other treatments were supplemented daily with ergot-infested tall fescue seed (TFS). PC = positive control treatment; CB = Control Block; AB = Block containing a proprietary blend of capsaicin and mannan oligosaccharide; MB = Block containing 0.3% crystalline menthol. All block supplements were provided *ad libitum*. 53

Figure 2.3 Serum somatotropin (GH) concentrations on day 84. All cattle had *ad libitum* access to ground prairie hay and salt block. Treatments are as follows: NC = negative control treatment with no fescue seed; other treatments were supplemented daily with ergot-infested tall fescue seed (TFS). PC = positive control treatment; CB = Control Block; AB = Block containing a proprietary blend of capsaicin and mannan oligosaccharide; MB = Block containing 0.3% crystalline menthol. All block supplements were provided *ad libitum*. 55

Figure 2.4 Serum insulin growth factor type 1 (IGF-1) concentrations on day 84. All cattle had *ad libitum* access to ground prairie hay and salt block. Treatments are as follows: NC = negative control treatment with no fescue seed; other treatments were supplemented daily with ergot-infested tall fescue seed (TFS). PC = positive control treatment; CB = Control Block; AB = Block containing a proprietary blend of capsaicin and mannan

oligosaccharide; MB = Block containing 0.3% crystalline menthol. All block supplements were provided *ad libitum*. 56

Figure 3.1 Study design for targeted duration of administration of tylosin phosphate included in the feed to provide at 60 mg/animal daily. Control treatment did not receive tylosin phosphate throughout the feeding period. Tylosin-30 treatment received tylosin phosphate for the first 30 days on feed, and Tylosin-60 treatment received tylosin phosphate for the first 60 days on feed and then changed to the control diet without tylosin. 84

Figure 3.2 Effects of targeted durations of administration of tylosin phosphate on averaged individual body weights progression. Control treatment (white bars) received no tylosin phosphate, Tylosin-30 treatment (gray bars) received tylosin phosphate in the feed to provide 60 mg/animal daily for the first 30 days of feeding period, and Tylosin-60 treatment (black bars) received tylosin phosphate for the first 60 days of the feeding period and then changed to the control diet without tylosin. 85

Figure 4.1 Right lateral view of adult bovine anatomy. The polygon trace in yellow represents the area scanned with the targeted thoracic ultrasonography, 8^{th} to 11^{th} intercostal space. Caudal to the lungs is the liver, displaying close proximity with the caudodorsal area of the right lung. 113

Figure 4.2 Model adjusted probability of interstitial pneumonia (IP) by ultrasound lung score (ULS)* from a population of 62 head evaluated with targeted thoracic point of care ultrasound (TT-POCUS) with subsequent histopathologic diagnosis of IP or non-IP. 114

Figure 4.3 Raincloud-boxplot displays temporal distribution of the 62 cases grouped by histopathological diagnoses by days on feed on individuals enrolled in this study (n= 62). Histopathological diagnosis of interstitial pneumonia (IP) = interstitial pneumonia; Non-IP

= Other respiratory syndromes without IP. Box and whisker represent the upper and lower quartiles, and the heavy line within the box represents the median..... 115

Figure 4.4 Raincloud-boxplot displays temporal distribution of the day intervals between mortalities and their chuteside evaluations with targeted thoracic ultrasonography (TT-POCUS) on individuals enrolled in this study (n= 62). Histopathological diagnosis of interstitial pneumonia (IP) = interstitial pneumonia; Non-IP = Other respiratory syndromes without IP. Box and whisker represent the upper and lower quartiles, and the heavy line within the box represents the median..... 116

Figure 4.5 (A-D) Histologic photomicrographs from an animal (#453) diagnosed with interstitial pneumonia. Figure 5A (4x): shows alveolar filling by protein, fibrin, and inflammatory cells; Figure 5B (20x) shows interstitial fibroplasia and bronchiolitis obliterans; Figure 5C (20x) shows alveolar hyaline membranes and pneumocyte type II hypertrophy; Figure 5D (case #453) is a transthoracic right lung point-of-care ultrasonography-obtained image showing absence of A-lines, merged B-lines, diffuse hyperechoic lung, with an overall ultrasound lung score (ULS) 3; (E-H) Histologic photomicrographs from an animal (#767) diagnosed with bronchopneumonia. Figure 5E (4x) shows bronchial, bronchiolar, and alveolar filling by inflammatory cells, necrotic debris, and fibrin; Figure 5F (20x) shows necrotizing bronchitis, bronchiolitis, bronchiectasis and bronchiolectasis; Figure 5G (20x) shows neutrophilic bronchitis, bronchiolitis, bronchiectasis and bronchiolectasis. Figure 5H (case #767) is a transthoracic right lung point-of-care ultrasonography-obtained image showing lung consolidation, absence of A-lines, and an overall ultrasound lung score (ULS) 5..... 117

Figure 5.1 Raincloud-boxplot displays temporal distribution of the days on feed at time of chuteside evaluations (POC) on individuals enrolled in this study (n= 98). 60-day outcome of DNF = did not finish (cull/death), recovered = animals that were alive at the 60-day post-evaluation. Box-plot represent the upper and lower quartiles, and the line within the box represents the median. 146

Figure 6.1 Raincloud-boxplot displays temporal distribution of the days on feed at time of chuteside evaluations (POC) on individuals enrolled in this study (n= 819). 60-day outcome of DNF = did not finish (cull/death), recovered = animals that were alive at the 60-day post-evaluation. Box-plot represent the upper and lower quartiles, and the line within the box represents the median. 183

Figure 6.2 Raincloud-boxplot displays temporal distribution of the days on feed at time of chuteside evaluations (POC) on individuals enrolled in this study (n= 819). 60-day outcome of FTF = first treatment failure (re-treat/cull/death), FTS = first treatment success, animals that were alive at the 60-day post-evaluation and were not re-treated. Box-plot represent the upper and lower quartiles, and the line within the box represents the median. 184

Figure 6.3 Model adjusted probability of interstitial pneumonia (IP) by ultrasound lung score (ULS)* from a population of 62 head evaluated with targeted thoracic point of care ultrasound (TT-POCUS) with subsequent histopathologic diagnosis of IP or non-IP. 185

Figure 6.4 Model adjusted probability of interstitial pneumonia (IP) by ultrasound lung score (ULS)* from a population of 62 head evaluated with targeted thoracic point of care ultrasound (TT-POCUS) with subsequent histopathologic diagnosis of IP or non-IP. 186

List of Tables

Table 2-1 Composition of basal forage and tall fescue seed (TFS) mixture (dry matter basis) ... 46

Table 2-2 Ergopeptine and ergovaline contents of tall fescue seed (TFS) 47

Table 2-3 Composition of molasses-based block supplements (dry matter basis) 48

Table 2-4 Effects of ergot-infested tall fescue seed and molasses-based block supplements on cattle growth performance 49

Table 2-5 Effects of ergot-infested tall fescue seed and molasses-based block supplements on coccygeal artery diameter (CAD; cm) measured between the 4th and 5th coccygeal vertebrae 50

Table 2-6 Effects of ergot-infested tall fescue seed and molasses-based block supplements on plasma concentrations of D-glucose (mg/dL) 51

Table 3-1 Diet ingredients and feed formulated nutrient compositions, DM basis. 77

Table 3-2 Steers removed from the study in the three treatments before harvest: Cause of mortality 79

Table 3-3 Incidence and severity of liver abscesses in steers fed finishing diets without tylosin phosphate (control) or with 60 mg/animal daily of tylosin phosphate fed for the first 30 (Tylosin-30) or 60 (Tylosin-60) days on feed 80

Table 3-4 Performance of steers fed diets without tylosin phosphate (control) or with 60 mg/animal daily of tylosin phosphate fed for the first 30 (Tylosin-30) or 60 (Tylosin-60) days on feed 81

Table 3-5 Carcass characteristics of steers fed finishing diets without tylosin phosphate (control) or with 60 mg/animal daily of tylosin phosphate fed for the first 30 (Tylosin-30) or 60 (Tylosin-60) days on feed 82

Table 4-1 Descriptive statistics of animals according to their demographics (sex, weight, days on feed categories, and treatment count), and thoracic ultrasonography variables (ultrasound lung score, A-line count categories, B-line count categories. 111

Table 5-1 Descriptive statistics of animals according to their demographics (sex, weight, and days on feed categories), real-time chute-side evaluations (ultrasound lung score, blood oxygen saturation, pulse, auscultation scores, A-line count categories, B-line count categories, pleura effusion, moth sign; and *post-hoc* evaluations (B-line area, gray value, intensity density). 135

Table 6-1 Descriptive statistics of did not finish (DNF) outcome model, cattle according to their demographics (sex, weight, and days on feed categories), real-time chute-side evaluations (ultrasound lung score, blood oxygen saturation, pulse, auscultation scores, A-line count categories, B-line count categories, pleura effusion, moth sign); and *post-hoc* measurement (B-line area). 174

Table 6-2 Descriptive statistics of first treatment outcome model, cattle according to their demographics (sex, weight, and days on feed categories), real-time chute-side evaluations (ultrasound lung score, blood oxygen saturation, pulse, auscultation scores, A-line count categories, B-line count categories, pleura effusion, moth sign); and *post-hoc* measurement (B-line area). 177

Table 6-3. Probabilities of variables that were associated with outcome of interest (did not finish or first treatment failure) converted from log-odds generated from multivariate logistic regression models grouped by its specific model. 180

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"Rock bottom became the solid foundation in which I rebuilt my life." - J.K. Rowling.

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Chapter 1 - A review of literature: Thoracic ultrasound in cattle: methods, diagnostics, and prognostics.

L. F. B. B. Feitoza[1], B. J. White[1]

[1]Beef Cattle Institute, Kansas State University, Manhattan, KS 66506, USA

Abstract

Thoracic ultrasonography (TUS) has emerged as a critical tool in the diagnosis and management of respiratory diseases in cattle, particularly bovine respiratory disease (BRD), which is one of the most economically significant health issues in feedyard operations. The objective of this review is to explore TUS in veterinary medicine including the historical development, methodologies, and clinical applications for diagnosing and prognosing respiratory diseases. The review also emphasizes the importance of operator training, noting that even novice operators can achieve diagnostic consistency with proper instruction. Ultrasound was introduced in the mid-20th century for back-fat thickness measurements, TUS has evolved to offer a non-invasive, real-time imaging modality that allows for the detection of lung and pleural abnormalities such as consolidations, pleural effusions, and B-lines. These features are vital indicators of respiratory disease, and their early identification through TUS can significantly improve clinical outcomes. Compared to traditional diagnostic methods like auscultation or radiography, TUS provides superior accuracy in detecting both subclinical and advanced respiratory conditions, particularly in high-risk populations. Furthermore, TUS has demonstrated strong prognostic value, with studies showing that the extent of lung consolidation correlates with higher relapse rates, reduced growth performance, and increased mortality. As TUS

continues to gain traction in cattle health management, its role in both individual animal care and broader herd health strategies is expected to expand, offering veterinarians a powerful tool for improving disease detection, treatment efficacy, and long-term outcomes. Continued research into refining TUS techniques and implementing effective training programs will further enhance the utility in bovine respiratory disease management.

Introduction

Thoracic ultrasound in cattle has emerged as a pivotal diagnostic tool, particularly in the assessment of respiratory diseases. This imaging method offers several advantages over traditional BRD assessment tools, including its non-invasive nature, lack of ionizing radiation, and ability to provide real-time imaging of thoracic structures.[1–6] The application of thoracic ultrasound (TUS) in cattle improves, diagnostics and prognostics value which can be critical for accurate management and treatment of bovine health issues.[7]

The methodology of thoracic ultrasound in cattle involves the use of low to medium frequency sound waves to allow for thoracic cavity penetration, including the lungs, pleura, and surrounding structures. This technique has been shown to be particularly effective in diagnosing conditions such as bovine respiratory disease (BRD), pleural effusion, and diaphragmatic hernias.[6,8–10] Thus, TUS serves as a rapid and practical calf-side diagnostic tool for assessing BRD prevalence and severity, demonstrating a significant correlation with clinical respiratory scores.[11,12] The ability to visualize lung pathology through ultrasound allows for the identification of specific conditions such as pulmonary emphysema and interstitial pulmonary syndrome, with distinct ultrasound artifacts associated with these diseases.[9,13,14]

In terms of diagnostics methods, thoracic ultrasound has proven to be superior to traditional radiographic techniques in certain scenarios. For example, Partlow et al., (2017) indicated TUS is more sensitive than chest radiography for detecting conditions like pneumothorax but direct comparisons in cattle are less established.[15] Evidence has shown clinical scores are very limited detecting and assessing severity of respiratory diseases, and an additional diagnostic method like TUS can be applied in combination to improve the accuracy.[7,16,17] Increased diagnostic sensitivity is crucial in chuteside settings where timely diagnosis can significantly impact treatment intervention selection and efficacy. The integration of ultrasound into routine veterinary practice has been advocated as a means to improve diagnostic accuracy and facilitate better clinical decision-making.[2,9,10]

Prognostically, thoracic ultrasound provides valuable information that can influence treatment strategies and management decisions. For instance, specific ultrasound findings, such as B-lines, can indicate pulmonary edema and correlate with the severity of respiratory conditions.[2,9] This association allows professionals to assess the prognosis of affected animals more accurately and determine treatment plans accordingly.

The TUS method is non-invasive and combined with the ability to provide real-time imaging, increases value for veterinarians. The ongoing development of thoracic ultrasound techniques and training programs will likely enhance application in bovine medicine, leading to improved health outcomes and management strategies for cattle. The objective of this review of literature is consolidate reports that describe the current understanding of methodology for thoracic ultrasonography for respiratory disease detection, and prognosis evaluation.

Materials and Methods

Data bases utilized for the presented literature include PubMed and Agricola. Key terms used for searching were: ((Ultrasound OR Sonography) AND (Cattle OR Bovine) AND (Thoracic OR Pulmonary) AND (Respiratory OR Pneumonia)). This initial literature search, yielded to 193 results in PubMed, 56 results in Agricola. The current literature review included original research articles, abstracts, and case reports. Articles evaluation for inclusion by one author, by reviewing title, abstract and full manuscript. Articles were excluded if did not meet study objectives. Upon screening, 44 documents were deemed fit for our objectives, an additional 10 articles were included to elaborate the discussion about TUS and BRD impact in the industry.

Results and Discussion

History

The use of ultrasonography technology in cattle dates back to 1956, when it was utilized for estimating back-fat thickness in live cattle.[18] In 1988, Lamb and associates worked on an extensive literature review to gather all the veterinary diagnostic ultrasound published work from 1966 to 1986, which yielded to 492 published articles. Digging deeper in these reports, only five were thoracic ultrasonography-related work and they were all in equine, ranging from 1981 to 1986.[19] The earliest identified TUS in cattle manuscript was (1991) published at the Journal of the American Veterinary Medical Association, used TUS to detect respiratory disease.[1] The application of TUS in cattle is recent compared to other species. Pivotal published works in the late 1990s and 2000s began to demonstrate the potential of TUS for BRD detection.[3,4,20–22]

The TUS in cattle progression in articles raw numbers over the decades through published articles have increased dramatically. In the 1990s in PubMed alone yielded on 20 publications, 2000s had 25 publications, 2010s 67 publications, and finally 2020s with only 5 years have produced 88 publications. Interest on the use of TUS in cattle is rapidly growing.

The early 2000s brought further advancements, with Jung and Bostedt (2004) exploring thoracic ultrasonography in neonatal calves, identifying lung consolidations and pleural pathologies at stages that traditional methods like auscultation could not detect.[21] Studies began to emerge in the early 2010s that highlighted the efficacy of thoracic ultrasound in diagnosing pulmonary diseases, such as bovine respiratory disease (BRD).[5,6,23–26] For instance, a prospective cohort study demonstrated associations between TUS findings and survival rates of dairy heifers, underscoring the prognostic value of thoracic ultrasonography.[27] In recent years, TUS for respiratory disease evaluation is being used routinely in dairy operations, specifically in young dairy calves.[11–13,16,28,29]

The role of thoracic ultrasound in herd health management has evolved alongside advancements in technology. The ability to monitor respiratory health on an individual animal basis has become increasingly important, particularly in high-production cattle operations. This shift towards data-driven veterinary practices to allow for better decisions reflects a broader trend in the industry towards precision livestock farming.

Thoracic ultrasound method

Ultrasonography serves as a reliable objective method for diagnosing lung damage, and TUS findings are well correlated with post-mortem examination.[3,4,7,30] Using TUS is achievable in a farm setting, with calf-side evaluations being feasible and consistently accurate following

proper training.[25,26,31,32] Diagnostic accuracy of TUS for BRD in calves, with a sensitivity of 79.4% and specificity of 93.9% for this specific study technique.[26]

Cattle TUS techniques have evolved significantly over time, with key advancements in probe selection, scanning protocols, and standardization of anatomical landmarks. Typically, a 3.5 to 5 MHz linear or convex probe is used, allowing adequate penetration for imaging lung structures while maintaining sufficient resolution.[24] Several studies highlight the use of intercostal spaces from the 4th to 11th ribs, which offer the best likelihood for detecting lung and pleural abnormalities.[5,9,21] These spaces are examined from dorsal to ventral regions to ensure comprehensive coverage of the lung fields.

Probe orientation is critical; it is typically held parallel to the ribs, with the ultrasound beam perpendicular to the pleural surface to maximize the detection of abnormalities such as consolidation or pleural fluid.[14,22] Buczinski et al. (2015) and Flock (2004) elaborated on the importance of visualizing the pleural line, which provides a key diagnostic marker for lung pathologies, including consolidations and pleuritis.[26,30] Additionally, ultrasonographic methods have been adapted for rapid chute-side assessments, as demonstrated in studies, where quick evaluations of lung consolidation were performed in high-risk feedyard cattle,[14] dairy calves,[6,11,25,28,32] and veal calves.[12,16]

One key advancement in ultrasonographic technique has been the refinement of pleural space assessment. Techniques such as “fanning,” where the probe angle is dynamically altered to capture different planes of lung tissue, are commonly used to identify lesions between ribs.[20] Additionally, coupling agents like isopropyl alcohol are often used in field conditions to optimize probe-skin contact, especially when time constraints prevent extensive preparation, like hair shaving or clipping.[6,9,25,31–33]

The use of TUS is limited due to logistics at the time and location of examination. Young calves are typically easier to restrain and manipulate the animal position to allow for a thorough examination.[25,34] On the other hand, TUS in adult cattle is more challenging.[9,35] The challenge with ultrasound in adult bovine starts with restraining, that you need at least a head-gate to restrain the animal, and at times a squeeze chute, where sometimes can cover the area of interest.[14] In addition, adult animals are harder to manipulate due to weight and strength, which at times might not allow for a full lung evaluation.

Time constraint is a major limiting factor for application of thoracic ultrasonography. Published reports have shown that a full TUS evaluation can take from 7 to 45 minutes.[6,9] Hence, the need to develop new methods to strategically evaluate specific areas of the lung and also specific populations at risk of bovine respiratory disease. Studies such as those by Timsit et al. (2019) highlight the efficiency of chute-side ultrasonography when used strategically, where rapid region-targeted assessments can be completed within minutes (4th to 6th mid to ventral intercostal spaces), particularly in high-throughput feedyard operations. Another study from Adams and Buczinski (2016) evaluated if 2-minute fixed time thoracic ultrasound assessment, would yield to valuable information about dairy calves, and the results showed that single timepoint 2-minute evaluation was associated with negative outcomes in these heifers.[34] Another strategic TUS method is reported by Pardon (2019), which the simplified scan start from caudodorsal tip of the lung, and move cranially in a single motion diagonally up to the 4th intercostal space, this strategic method reduces evaluation time and also can provide valuable information about pulmonary health.[32]

The balance between speed and accuracy is critical, and in most cases, a complete and focused examination can be accomplished swiftly without compromising diagnostic

accuracy.[6,28,36] The advancements in portable ultrasonography devices and improved operator training have contributed to reducing the time needed for comprehensive examinations, making ultrasound a practical tool for both clinical and field settings.

Respiratory disease diagnosis

Normal lung tissue is challenging to visualize via ultrasonography due to air content. Ultrasound waves do not propagate through air, hence the image of a properly aerated lung is usually dark (low echogenicity), with several pleural line reverberations (artifacts), called A-lines. A-lines appears as echogenic bands parallel to the pleural surface, and are consistently observed in a healthy lung. In normal lungs, parietal and visceral pleura often appear as a single, smooth hyperechoic line between the lung tissue and thoracic wall muscles. Lung movement, synchronous with respiration, is normal, and the lack of movement specially in the pleural line can indicate pathology (pneumothorax).[22,30]

As respiratory disease develops there are findings that indicate lung and pleura are undergoing injury. The most reported finding is parenchymal consolidation, which can also be called “hepatization” and that is due to the liver-like echogenicity appearance.[2,9,22] Consolidation is the highest degree of lung injury, a consolidated parenchyma is not capable to be aerated and the severity of the respiratory disease is associated with amount of consolidated lung tissue. Consolidation can appear as wedges from the pleural line, nodules, or affect the entire lung lobe.[14,37,38] Another common abnormal finding is pleural effusion, which is easy to identify as it shows fluid accumulation between the parietal pleura and visceral pleura.[22,30] One artifact usually observed in respiratory disease is the B-line, also called “comet tail”. This artifact is a vertical beam that often starts at the pleural line and prolongates across the image field.[2] B-lines are usually perceived as a normal artifact if they are rare (2 or less per field). Greater number and

width of B-lines is associated with more severe injury.[2,39–42] Studies consistently show that TUS can detect diseases such as bronchopneumonia, which often manifests as areas of lung consolidation. For example, Buczinski et al. (2014) demonstrated that ultrasound identified lung lesions in 29% of cattle that appeared healthy based on clinical signs. Furthermore, consolidation depths of more than 3 cm were commonly associated with bronchopneumonia, particularly in cattle suffering from *Mannheimia haemolytica*-induced infections.[7] In addition, bronchopneumonia findings are often found with more severe or developed injury on the cranioventral lobe.[43]

The pleura can also present abnormalities. Most common is pleural thickening, that is usually associated with pleuritis. Another pleural abnormality is called “moth sign”, this abnormality shows an irregular pleural line, with indentations (moth eaten), associated with sub-pleural consolidation.[2,3,13,40] These possible TUS findings or combinations can support respiratory disease detection and prognosis. The use of ultrasound to detect pleural effusion, another common manifestation of BRD, has also been widely documented. Studies by Tharwat et al. (2011), Babkine and Blond (2009), and Masset et al. (2022) confirm the sensitivity of ultrasound in detecting fluid accumulation in the pleural cavity, a feature that is not easily identified using traditional auscultation methods.[5,22,29] In more severe cases of pleuropneumonia, ultrasonography has been instrumental in visualizing both pleural fluid and consolidations that extend deep into the lung tissue.[2,3,22] This is a crucial advancement, as these conditions are typically difficult to diagnose without invasive procedures.

Accurate diagnosis of bovine respiratory disease (BRD) is critical to the effective management and health of cattle, particularly in feedyard operations where BRD is one of the most prevalent and economically significant diseases.[44] Early and precise identification of BRD

allows for timely intervention, reducing the risk of disease progression, relapse, and mortality.[37,45] Accurate diagnosis not only improves animal welfare by preventing chronic illness but also optimizes treatment protocols, reducing the overuse of antimicrobials and enhancing antimicrobial stewardship.[45,46] Additionally, studies like those of Buczinski et al. (2014) have highlighted that ultrasonography can reduce the reliance on empirical treatments by offering a clear picture of the severity of lung involvement.[36] Overall, accurate BRD diagnosis is vital for improving herd health, productivity, and the sustainability of livestock operations.[47,48]

The comparative accuracy of ultrasonography versus other diagnostic methods has been a key focus in the literature. For instance, studies like by Scott et al. (2013) and Buczinski et al. (2014) indicate that ultrasonography significantly outperforms auscultation and clinical scoring in identifying both subclinical and advanced lung pathology. Auscultation, while a cornerstone of veterinary physical examination, has been shown to miss substantial pulmonary abnormalities, particularly in early disease stages.[7,24,36,49] Furthermore, Buczinski et al. (2015) revealed that ultrasonography offers a higher diagnostic specificity than rectal temperature or respiratory scoring alone, emphasizing its role in confirming suspected BRD cases.[26]

Respiratory disease prognosis

One of the most promising aspects of thoracic ultrasonography in cattle is its ability to predict outcomes in cattle with respiratory diseases. Numerous studies have highlighted TUS prognostic utility, particularly in identifying cattle that are likely to relapse or experience poor growth performance after treatment.[9,14,23,34,37,50,51] Timsit et al. (2019) showed cattle with greater lung consolidation depths (≥5 cm) at the first diagnosis of bronchopneumonia had a significantly higher risk of relapse and lower average daily gain (ADG) compared to cattle with less severe lung lesions.[14] This highlights the potential for ultrasonography to guide not only diagnostic

decisions but also treatment plans, by identifying animals that may require more aggressive or prolonged treatment.

Rademacher et al. (2014) demonstrated TUS findings can predict mortality risk with significant accuracy. In these studies, large areas of lung consolidation were associated with poor outcomes, including death or culling. Cattle with consolidations exceeding 5 cm were more likely to die or be culled, emphasizing the importance of early detection and the potential to alter treatment protocols based on ultrasound findings.[9]

Furthermore, TUS has been shown to be a valuable tool for monitoring treatment efficacy.[52] Serial assessments of lung consolidations during the treatment period provide real-time feedback on whether lesions are resolving or worsening.[53] Study by Wolfger et al. (2015) have demonstrated that ultrasound can track the progression or regression of lung lesions, offering an objective measure of treatment success.[37] This capability is particularly useful in field settings, where traditional follow-up methods may be limited by logistical constraints. The ability to monitor animals through non-invasive imaging allows veterinarians to make informed decisions about treatment interventions. This not only optimizes animal health outcomes but also contributes to antimicrobial stewardship by reducing the overuse of antibiotics in cases where improvement is not occurring.

Thoracic ultrasonography training

The literature reviewed emphasize the importance of proper training for veterinarians and technicians performing thoracic ultrasonography in cattle, noting that while the technique can be highly accurate, operator experience plays a crucial role in ensuring diagnostic consistency.[6,38,54] Buczinski et al. (2013) highlight that with adequate training, even novice operators can perform thoracic ultrasonography with consistency and accuracy in detecting lung consolidations and

pleural pathologies in calves.[4,6,38] The basic instruction in probe handling and recognition of key anatomical landmarks can allow less experienced individuals to perform thoracic ultrasound evaluations.[30,32,38]

Similarly, Ollivett et al. (2011), Scott (2013), Ollivet and Buczinski (2016), and Pardon (2019) stress that thoracic ultrasound is not only highly accessible but can be performed efficiently in field conditions, given proper training. The training involved focuses on understanding the placement of the probe in intercostal spaces and interpreting common findings like pleural effusions and lung consolidation, which are critical for diagnosing bovine respiratory disease (BRD).[24,25,32,38] These studies collectively highlight the necessity of training to maximize the diagnostic potential of TUS, making it a viable and effective tool for diagnosing and managing BRD in cattle.

Conclusions

In conclusion, thoracic ultrasonography has emerged as an important tool for both diagnosing and prognosis in cattle with respiratory diseases. Its ability to detect subclinical disease, monitor treatment efficacy, and predict long-term outcomes can revolutionize bovine respiratory disease management, particularly in high-producing cattle operations settings where early and accurate diagnosis is critical. The advancements in probe technology, scanning techniques, and point-of-care assessments have made ultrasonography a cornerstone of veterinary diagnostics. As research continues to expand, the use of ultrasonography in bovine respiratory disease will likely become even more practical, offering veterinarians a powerful tool for improving animal health and welfare.

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Chapter 2 - Molasses-based block supplements for cattle fed endophyte-infected tall fescue (Festuca arundinacea) seed; Effects on growing performance, circulating biomarkers, heat stress, and coccygeal artery diameter.

L. F. B. B. Feitoza[1], B. J. White[2], and J. S. Drouillard[1*]

[1]Department of Animal Sciences and Industry, Kansas State University, Manhattan KS 66506.

[2]Beef Cattle Institute, Kansas State University, Manhattan, KS 66506, USA

[*]Corresponding author: James S. Drouillard

Highlights

- Supplementation of low moisture, cooked molasses-based blocks improved animal growth;
- Supplementation has a potential positive effect on peripheral perfusion;
- Results suggest that molasses-based block supplementation can induce vasodilation and improve thermoregulation.

Abstract

Toxicosis with ergot alkaloids from tall fescue induces persistent vasoconstriction in cattle, thereby decreasing peripheral blood flow and adversely affecting thermoregulation. Our objective was to evaluate the impact of cooked molasses-based block supplements (MBS) on performance and physiological measurements in cattle fed ergot-containing tall fescue seed

(TFS). Crossbred steers (n = 100; 287 ± 10.35 kg) were blocked by initial body weight and randomly assigned, within block to individual indoor feeding pens. Treatments consisted of: negative control (NC; prairie hay only); positive control (PC; hay plus TFS); and molasses block treatments fed hay and TFS with ad libitum access to a 34% protein block (CB); a 34% protein block containing 0.3% crystalline menthol (MB); or a 34% protein block containing a proprietary blend of mannan oligosaccharide and capsaicin (AB). The TFS was mixed with molasses (9:1), and the amount fed was increased gradually at 3-day intervals, starting at 45 g/animal daily and increasing to 520 g/animal by day 42. Temperature of the ocular conjunctiva (OC) and distal ear were assessed weekly by thermographic imaging, and temperature differential (ΔT) was determined (ΔT = Temperature of OC – Temperature of Distal Ear Tip). Molasses block supplements were weighed weekly, and coccygeal artery diameter (CAD) was measured with color-flow doppler ultrasound imaging (CFDU) on d 63 and d 84. Data were analyzed as mixed models with block as a random effect and treatment, time, and treatment by time interaction as fixed effects. Cattle fed MBS had greater dry matter intake (DMI) compared to NC and PC ($P < 0.01$), but DMI did not differ among MBS treatments ($P > 0.05$). Cattle fed cooked molasses-based block supplements (MBS) had greater average daily gain (ADG), DMI, and ADG:DMI ($P < 0.01$) compared to NC and PC. For the second half of the study, MBS treatments had lower ΔT ($P < 0.05$) than NC and PC, suggesting that peripheral blood perfusion was enhanced with supplementation. Treatments CB, AB and MB had greater overall CAD compared with NC and PC ($P < 0.05$). Supplementing with molasses-based block supplements can improve performance and potentially enhance peripheral blood perfusion in cattle consuming ergot-infested feeds.

Keywords: Heat stress, molasses-based blocks, tall fescue, thermography, vasoconstriction.

Abbreviations: AB, 34% protein block containing a proprietary blend of mannan oligosaccharide and capsaicin; ADG, average daily gain; Average environmental temperature (AET), °C; BW, body weight; CAD, coccygeal artery diameter; CB, 34% protein block; CFDU, color-flow doppler ultrasound imaging; CV, coefficient of variation; DE, temperature of the distal ear; DM, dry matter; DMI, dry matter intake; DOF, days on feed; eNOS, endothelium nitric oxide synthase; G:F, gain to feed; GH-1, bovine somatotropin; IGF-1, bovine insulin-like growth factor–1; MB, 34% protein block containing 0.3% crystalline menthol; MBS, cooked molasses-based block supplements; NC, negative control; OC, temperature of ocular conjunctiva; PC, positive control; TFS, tall fescue seed; TRP, transient receptor potential channel class; TRPM8, transient receptor potential cation channel subfamily Melastatin-related member 8; TRPV1, transient receptor potential channel class subfamily identified as vanilloid 1; VFA, volatile fatty acids; ΔT, temperature differential.

Introduction

The occurrence of ergot toxicosis (i.e., fescue foot or gangrenous ergotism) in grazing animals has been reported throughout the world (Klotz, 2015). Ergot alkaloid mycotoxins are secondary metabolites of fungi of the genera Claviceps and Epichloe spp. (Klotz, 2015; Klotz et al., 2016; Klotz et al., 2017). These fungi can infect grazed forages such as tall fescue (Festuca arundinacea), leading to the clinical condition of fescue toxicosis, characterized by reduced blood flow to peripheral tissues. Altered blood flow is the consequence of ergot alkaloid-induced vasoconstriction by adrenergic and serotonin agonistic effects. Accompanying symptoms of toxicosis include abdominal fat necrosis (Rumsey et al., 1979); hyperthermia; decreases in feed intake, body weight gain, and milk production (Schmidt et al., 1993); lameness, ear tip and tail tip necrosis (Walls, 1970); thus compromising animal well-being and performance. Moreover, the lack of alternative intervention methods besides removing the animals from the source of ergot-alkaloids urges for alternative methods to mitigate this problem.

Menthol is a multi-purpose compound that can be used as a flavoring in food and feed products or as an analgesic, antiseptic, and vasoactive agonist (Kamatou et al., 2013). Menthol has the ability to bind as an agonist to the transient receptor potential cation channel subfamily Melastin-related member 8 (TRPM8, Andersen et al., 2014). The TRPM8 receptors are found in various tissues, including arterial smooth muscle.

Capsaicin is a compound that induces the warm-hot sensation associated with ingestion of hot peppers. Transient receptor potential channel class 1 (TRP) is a binding target for the capsaicin compound, TRP is within a subfamily identified as vanilloid 1 (TRPV1) receptors (Mccarty et al., 2015; Szolcsányi, 2015; Craighead et al., 2017). Its vasoactive response is

explained by an increase in expression and activation of endothelium nitric oxide synthase (eNOS), as reported by McCarty et al. (2015) and Craighead et al. (2017).

We hypothesized that the addition of vasoactive compounds to molasses-based block supplements could be useful for mitigating vascular ergot responses such as vasoconstriction, thereby enhancing peripheral blood flow and alleviating heat stress, feed intake depression, and associated body weight losses. Overall objective of this study is to evaluate the effect of MBS supplementation on mitigating ergot-alkaloid intake's negative effects on blood perfusion and growth performance.

Materials and methods

Procedures involving live animals were conducted and approved within the guidelines of the Kansas State University Institutional Animal Care and Use Committee (Protocol # 4113).

Experimental design

The study trial was conducted from July 28, 2018, to October 19, 2018, and was designed as a randomized block design with five treatments using 100 crossbred steers (287 ± 10.35 kg) housed in individual indoor feeding pens. Steers were selected from a larger population of 121 animals, excluding animals with known health issues, poor temperament, or excessively heavy or light body weight. Selected candidates were then stratified based on initial body weight and assigned randomly, within strata (i.e., blocks), to one of 5 treatments. Cattle in all treatments had ad libitum access to salt blocks and ground prairie hay, referred as the common diet (Table 2-1). Treatments consisted of 1) Negative control (NC) for which animals received only common diet; 2) Positive control (PC) for which steers also were fed common diet and a daily aliquot of

endophyte-infected tall fescue seed (TFS; Table 2-1 and Table 2-2); 3) a control block (CB) treatment consisting of common diet and ad libitum access to a molasses-based block supplement containing approximately 35% crude protein; 4) A similarly formulated block that also contained 0.3% crystalline menthol (MB; manufactured by New Generation Supplements, Belle Fourche, SD); a similarly formulated block that contained a proprietary blend of mannan oligosaccharide and capsaicin (AB; manufactured by New Generation Supplements, Belle Fourche, SD). Cattle in the block supplement treatments all were given ad libitum access to prairie hay, salt blocks and the same daily aliquot of TFS. Tall fescue seed was presented to cattle as a mixture with molasses (90% tall fescue seed, 10% molasses), and the amount fed was gradually increased to avoid acute ergot toxicosis, starting with 45 g/animal daily on day 1 of the study and increasing progressively at 3-day intervals to achieve an intake of 520 g/animal daily by day 42.

Animals were housed in five barns, each containing 20 individual feeding pens, where each barn had 4 individuals from each treatment. Pens were 1.5 m wide by 7.0 m long, constructed of pipe fencing, and were open on the south fence. Automatic waterers were located approximately 3.5 m from feed bunks and were shared by animals in adjacent pens. Feed bunks were situated at the front of each pen.

Cattle processing and data collection

One hundred and twenty-one crossbred steers were purchased in Joplin, Missouri, and transported to the Kansas State University Beef Cattle Research Center. Animals were placed in holding pens and provided ad libitum access to ground alfalfa and water on arrival. Due to extremely hot weather, initial processing was delayed by 96 hours to avoid excessive handling

623 stress. For initial processing, steers were individually weighed, identified with numbered ear tags
624 in the right ear, vaccinated against clostridial (Ultrabac7 Somnubac, Zoetis Animal Health) and
625 viral (Bovishield Gold 5, Zoetis Animal Health) pathogens, treated for internal (Safeguard,
626 Merck Animal Health, De Soto, KS) and external (Standguard, Elanco Animal Health,
627 Greenfield, IN) parasites, and implanted with Component TE-200 with Tylan (Elanco Animal
628 Health, Greenfield, IN). Cattle then were placed into their designated treatment pens.
629 Individual body weights were collected at 21-d intervals and at the end of the 84-d
630 feeding period using an individual animal scale validated immediately before use. Average daily
631 gain was determined by subtracting the previous 21-d interval BW (body weight) from the
632 current BW and dividing the value by days on feed (DOF). Efficiency (gain:feed) was
633 determined by taking ADG and dividing it by dry matter intake (DMI). Infrared thermal imaging
634 data were collected by a trained technician, using the FLIR E40 high performance camera.
635 Temperature range of the camera was −20°C to 650°C with thermal sensitivity of ± 0.07°C and
636 accuracy of ± 1% of reading in this restricted range. The wide-angle lens was 25° × 19° (f = 0.4
637 m). Infrared resolution was 160 × 120 pixels. Emissivity was set to 0.98, distance from object 1
638 m. All images were collected at 7-day intervals by the same technician.
639 Images were collected for each animal from the ocular conjunctiva and the distal ear. If
640 more than one image was taken, the image with the best view of the area of interest was used.
641 These images were then analyzed in the FLIR Tools (Wilsonville, OR) software. The
642 temperature of the ocular conjunctiva (OC) was used as a proxy for core body temperature. The
643 temperature of the distal ear (DE) was determined as the mean of curvilinear points along the
644 periphery of the ear by using the selection tool from the software of FLIR Tools (Wilsonville,
645 OR) thermal imaging analysis, selecting the periphery of the distal third of one ear. The

646 temperature differential (ΔT = OC - DE) was used as an indicator of peripheral blood flow and
647 temperature homeostasis capacity.
648 Color flow doppler ultrasound imaging was used to identify and measure coccygeal
649 artery diameter. The usage of function color on the ultrasound machines facilitates identification
650 of blood flow, and it has been widely applied in the livestock reproduction field (Bollwein et al.,
651 2016; Beltrame et al., 2017; Arashiro et al., 2018). The coccygeal artery was assessed at the 4th
652 coccygeal vertebrae with a high-frequency array transducer (10 MHz, depth of the field was 2.5
653 cm), a technique reported previously by Klotz et al., (2016). The first measurement was taken 21
654 days after the amount of TFS mixture delivered reached its peak. The second measurement was
655 performed 21 days following the first measurement, or 42 days after achieving peak consumption
656 of TFS.
657 Blood samples were harvested on days 63 and 84 from ninety-five animals via jugular
658 vein using 18G × 25.4-mm needles. One sample from each animal was collected in fluoride
659 oxalate anti-coagulant vacuum tubes for plasma, and another in serum tubes (BD Vacutainer,
660 Franklin Lakes, NJ). Plasma and serum were harvested by centrifuging in a swinging bucket
661 rotor at 2000 × g and 4°C for 15 min, and transferred by pipette to storage tubes, which were
662 then capped and frozen at -20°C until analysis. Plasma was used to analyze concentrations
663 (mg/dL) of glucose and L-lactate using a biochemistry analyzer (YSI 2700 STAT Plus, Yellow
664 Springs, OH). Blood serum from day 84 was used with enzyme-linked immunosorbent assays
665 (ELISA) to quantify concentrations (ng/mL) of bovine somatotropin (bovine GH-1,
666 MyBiosource, Inc., San Diego, CA) and bovine insulin-like growth factor-1 (bovine IGF-1,
667 Sigma-Aldrich, Saint Louis, MO).

668 On days 63 and 84, coccygeal artery diameter (CAD) was measured between the 4th and
669 5th coccygeal vertebrae by color doppler ultrasonography using a portable ultrasound system
670 (Sonosite 180 Plus, Fujifilm, Bothell, WA) equipped with a linear array 10–5 MHz transducer.
671 Weather data were recorded daily at a local weather station (KKSMANHA69) located
672 1.4 miles from the Kansas State University Beef Cattle Research Center, Manhattan, KS.
673 Temperature data collected were used to calculate average environmental temperature (AET).
674

675 **Diet preparation and delivery**

676 Steers were presumed to be naive to MBS and thus were given ad libitum access as a
677 group to hay and the control molasses-based block for one week prior to initiating the study to
678 acclimate animals to use of the block supplements.
679 All treatment groups received a daily basal diet consisting of ad libitum prairie hay
680 (Table 2-1) and white salt blocks. Treatments that received TFS (PC, CB, AB, MB) were
681 initially fed 45 g daily. The amount gradually increased in 3-d intervals thereafter until achieving
682 a daily intake of 520 g/animal, which was accomplished by day 42. Intake of the TFS was
683 progressively increased to avoid acute ergot toxicosis. The TFS was mixed with sugarcane
684 molasses in a ratio of 9 parts TFS to 1 part molasses (Table 2-1). Tall fescue seeds were analyzed
685 for ergopeptine alkaloids and ergovaline, concentrations of which are shown in Table 2-2. By d
686 42 of the study, daily ingestion of ergopeptine alkaloids and ergovaline were 21.24 and 0.90
687 mg/animal, respectively.
688 Cooked molasses-based blocks were provided ad libitum for three treatment groups CB,
689 AB, and MB, and included a 34% protein block (Table 2-3); a 34% protein block containing a

690 proprietary blend of mannan oligosaccharide and capsaicin (Table 2-3); and a 34% protein block
691 containing 0.3% crystalline menthol (Table 2-3), respectively.
692 The basal diet was weighed for each pen daily and delivered to the steers at
693 approximately 1300 h. Fescue seed was ground through a 0.8 mm screen and mixed with
694 sugarcane molasses 9:1 in a horizontal segmented ribbon mixer for 30 minutes. The TFS mixture
695 was weighed and placed into labeled containers for each pen daily for top-dressing. Bunk
696 management was visually determined and adjusted daily to allow for ad libitum intake. Orts were
697 collected at 7-day intervals. To determine dry matter (DM) content, a subsample of the
698 unconsumed feed was dried for 48 h in a 55°C oven. Dry matter intake was determined for each
699 7-day interval and at the end of the experiment as follows: DMI = [(total feed accessible ×
700 %DM) – (unconsumed feed × %DM)]/interval length in days.
701

702 **Statistical analyses**

703 Analyses were performed using SAS version 9.4 (SAS Institute Inc., NC). The PROC
704 MIXED procedure was used for cattle growth performance, MBS intake, CAD, ΔT, plasma
705 glucose concentrations, and plasma L-lactate concentrations. Data for serum somatotropin (GH)
706 and insulin-like growth factor I (IGF- 1) were determined to be non-normally distributed when
707 evaluated using Shapiro-Wilk and Cramer-von Mises tests, and thus were transformed to natural
708 logarithms and analyzed using PROC UNIVARIATE.
709 Data were analyzed as mixed models with block as a random effect and treatment, time,
710 and treatment by time interaction as fixed effects.
711 Statistical models included treatment, time, and treatment by time as a fixed effect,
712 weight block and barn as a random effect, and animal as the experimental unit. Treatment effects

were declared significant at a level of $P < 0.05$. Least-squares means were compared between the treatment groups using the PDIFF function of SAS.

Results and Discussion

Five steers were removed from the study. One steer from the PC group was euthanized due to a bladder rupture, one from CB was removed due to a nasal bone fracture, two from NC were removed due to severe bovine respiratory disease, and one steer from MB was removed due to severe bovine respiratory disease.

Molasses-based block intake

Overall treatment effect of molasses-based block intakes did differ among MBS treatments ($P < 0.05$), presented in Figure 2.1. The MBS intake also differed by week ($P < 0.05$), when the lowest week's intake was on week 3 with average intake of 0.68 kg, and highest intake of 1.25 kg was on week 7. No evidence of treatment by day interaction was identified ($P > 0.05$). Intakes were greater than expected, and may have been impacted by maintaining the animals in continuous close proximity to blocks.

Cattle growth performance

Growth performance is summarized in Table 2-4. There were overall treatment effects for overall ADG, DMI, and G:F ($P < 0.01$). Improvements in performance due to supplementation of cooked molasses-based block supplements are consistent with observations reported by Greenwood et al. (2000) and Ciriaco et al. (2016). Supplementation of protein to cattle fed low quality forages has been observed to improve fiber digestibility, increase volatile fatty acid

(VFA) production, and improve DMI and ADG, as reported by Beaty et al. (1994) and Olson et al. (1999).

No difference was observed among MBS groups, suggesting that the proprietary blend of mannan oligosaccharide with capsaicin and crystalline menthol compounds did not have a significant effect on cattle growth. Van Bibber-Krueger et al. (2016) reported no impact related to the menthol supplemented at varying concentrations, including the same concentration used in this study. Previous studies have shown that capsaicin can improve DMI in beef cattle (Cardozo et al., 2006; Rodríguez-Prado et al., 2012) and also increase appetite in humans (Calixto et al., 2000). Contrary to these reports, we did not observe an effect of AB compared to other MBS groups. Interestingly, no difference was detected between NC and PC ($P > 0.05$) on any of the growth performance variables, suggesting that in this study the administration of TFS did not affect growth performance.

Coccygeal artery diameter imaging

No evidence of effect of measurement day or interaction of treatment by day was detected ($P > 0.05$). Overall treatment effect detected greater luminal area ($P < 0.05$) for the CB, AB, and MB groups compared to NC and PC, represented on Table 2-5. The results suggested those treatments positively influenced the coccygeal artery diameter but did not differ from each other ($P > 0.05$). While menthol and capsaicin are reportedly capable of causing vasodilation (McCarty et al., 2015; Melanaphy et al., 2016), the results did not differ from CB. In this study, intake of TFS appeared not to have caused a detectable effect on CAD, hence NC and PC were not statistically different ($P > 0.05$).

Thermography

Statistical analyses detected significant evidence of interaction of treatment by day ($P < 0.05$). A more consistent detection of significant difference among treatment groups was evident for the second half (day 42 and greater) of the study. The MBS treatments had lower averages for ΔT ($P < 0.05$) compared to the NC and PC groups, suggesting that peripheral blood perfusion was enhanced with MBS supplementation, as shown in Figure 2.2. The figure highlights the result on the measurements taken at day 77 and 84, where PC displayed a higher temperature differential compared to NC, and MBS. This result suggests that the effect of TFS administration negatively impacted blood perfusion. Menthol and capsaicin are known for their vasoactive effects, which was previously reported by Mccarty et al. (2015), Melanaphy et al. (2016), and Craighead et al. (2017), hence, the lower ΔT results in this study are consistent with that effect. Fortney et al. (1984) and Nose et al. (2018) reported that plasma composition is trivial for efficient thermoregulation, and the results suggest that protein supplementation could have an effect on thermoregulation. In this study, intake of TFS appears to have caused a negative detectable effect on thermoregulation on days 77 and 84, hence PC had the highest differential temperature ($P > 0.05$).

Plasma glucose and L-lactate

Plasma L-lactate concentrations did not differ among treatments for the effect of day, treatment, and treatment by day ($P > 0.05$). Our results suggested that MBS supplementation or TFS intake did not alter plasma L-lactate concentrations ($P > 0.05$).

Plasma L-lactate is a biomarker that is easily sampled and has been attracting interest in bovine medicine due to its potential to predict mortality outcomes and assess diseases including bovine respiratory disease, also known as BRD (Buczinski et al., 2015).

Plasma L-lactate can also be used to measure general metabolic stress, gluconeogenesis, muscle metabolism, and lipid metabolism. Greater L-lactate concentrations can occur with metabolic insults, and this biomarker is also related to gastrointestinal tract inflammation, and acidosis (Owens et al., 1998).

Another widely assessed biomarker is plasma glucose concentration, though complex inter-relationships between multiple tissues and organs can yield variable outcomes (Sébédio et al., 2009). In our study, it was not detected an effect on glucose concentration by day of collection and treatment by day interaction measurements. An overall effect of treatment was detected ($P < 0.05$) where the MB group had greater plasma glucose concentrations among PC and NC ($P < 0.05$), but did not differ among AB and CB ($P > 0.05$), as shown in the data represented in Table 2-6. Van Bibber-Krueger et al. (2016) reported no effect on glucose with menthol supplementation. Interestingly, menthol is said to have an antidiabetogenic effect by regulating insulin expression and enhancing pancreatic β-cells (Muruganathan et al., 2017). Overall, administration of TFS and MBS supplementation did not alter plasma concentrations of L-Lactate. In the other hand, an effect of treatment was detected when measuring plasma concentrations of glucose, where the MB group showed the highest levels of the compound.

Somatotropin and insulin-like growth factor I

Somatotropin (GH) intra- and inter-assay coefficients of variation (CV) were, respectively, 5.2% and 5.1%. Insulin-like growth factor 1 (IGF-1) had 3.7% intra-assay CV and 3.9% for inter-assay CV.

The goal for measuring those blood metabolites is that they are very closely related to growth performance, being directly proportional, meaning that greater concentrations of those two metabolites could have a positive effect on mass growth and cell proliferation (Houseknecht et al., 1988; Micke et al., 2010; Van Bibber-Krueger et al., 2016).

In this study, contrary to the hypothesis, no differences among treatment groups were observed. Serum somatotropin ($P > 0.05$), is shown in Figure 2.3. Serum IGF- 1 concentrations also did not differ among treatments ($P > 0.05$), Figure 2.4.

Our hypothesis was that supplementation of MBS would provide to the animal a greater amount of energy, protein, and minerals that would up-regulate the synthesis and secretion of IGF-I, which would ultimately increase the growth hormone levels, which was observed in studies by Elsasser et al. (1989). Van Bibber-Krueger et al. (2016) reported more specifically, related to supplementing crystalline menthol, that no significant difference was found on IGF-1 with the addition of crystalline menthol in a diet. Literature pertaining to capsaicin's effect on animal growth performance was not found.

Conclusion

In summary, supplementing cattle with low-moisture cooked molasses-based block supplements can improve ADG, DMI, and feed conversion. Differences among treatments from day 42 to day 84 in differential temperature (ΔT) points out the potential for thermoregulatory

823 enhancement by using the MBS, in particular for days 77 and 84. Also, MBS supplementation
824 displayed greater coccygeal artery diameter compared to NC and PC, suggesting that the
825 supplementation of MBS potentially enhanced peripheral blood perfusion in cattle compared to
826 animals not supplemented.

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967 **Table 2-1** Composition of basal forage and tall fescue seed (TFS) mixture (dry matter basis)

Item, %	**Prairie hay**	**TFS** †
Dry matter	93.09	90.46
Crude protein	5.84	12.60
Acid detergent fiber	47.44	17.02
Neutral detergent fiber	66.52	30.12
Calcium	0.50	0.39
Phosphorus	0.15	0.35
Potassium	1.13	0.18
Magnesium	0.18	-

†A mixture consisting of 90% ergot-infested tall fescue seed and 10% cane molasses.

968

969 **Table 2-2** Ergopeptine and ergovaline contents of tall fescue seed (TFS)

970

Item	Concentration, parts per billion
Ergosine	18,700
Ergotamine	10,560
Ergocornine	4,560
Ergocryptine	7,600
Ergocristine	5,800
Ergovaline	2,010
Total	49,230

Samples analyzed by University of Missouri Veterinary Medical Diagnostic Laboratory, Columbia, MO.

Concentration of compounds were measured using high performance liquid chromatography.

971 **Table 2-3** Composition of molasses-based block supplements (dry matter basis)

	Block supplement[†]		
Item, %	**CB**	**AB**	**MB**
Dry matter	94.76	94.78	94.53
Crude protein	34.21	34.44	34.35
Crude protein equivalent as non-protein nitrogen	15.22	14.75	15.85
Ash	15.10	15.93	15.21
Calcium	1.57	1.61	1.55
Phosphorus	1.00	0.97	0.99
Potassium	3.19	3.13	3.07

[†]CB = Control Block; AB = Block containing a proprietary blend of capsaicin and mannan oligosaccharide; MB = Block containing 0.3% crystalline menthol.

972

973 **Table 2-4** Effects of ergot-infested tall fescue seed and molasses-based block supplements on cattle growth performance

974

	Treatment						
Item	**NC**	**PC**	**CB**	**AB**	**MB**	**SEM**	***P*-value**
Initial BW, kg	286.3	288.1	288.0	286.2	287.5	6.38	0.40
Final BW, kg	284.9[a]	287.4[a]	326.6[b]	325.9[b]	328.4[b]	9.01	<0.01
ADG, kg	-0.15[a]	-0.03[a]	0.46[b]	0.43[b]	0.60[b]	0.117	<0.01
DMI, kg/d	4.98[a]	5.24[a]	7.19[b]	7.05[b]	7.29[b]	0.247	<0.01
gain:feed	-0.017[a]	-0.003[a]	0.029[b]	0.027[b]	0.037[b]	0.009	<0.01

[†]All cattle had *ad libitum* access to ground prairie hay and salt block. NC = negative control treatment with no fescue seed; other treatments were supplemented daily with ergot-infested tall fescue seed (TFS). PC = positive control treatment; CB = Control Block; AB = Block containing a proprietary blend of capsaicin and mannan oligosaccharide; MB = Block containing 0.3% crystalline menthol. All block supplements were provided *ad libitum*.

[a, b]Means without a common superscript letter displays overall treatment effect, $P < 0.05$.

Table 2-5 Effects of ergot-infested tall fescue seed and molasses-based block supplements on coccygeal artery diameter (CAD; cm) measured between the 4th and 5th coccygeal vertebrae

	Treatment[†]						*P*-value		
Day of study	NC	PC	CB	AB	MB	SEM	Trt	Day	Trt × Day
Overall	0.299[a]	0.317[a]	0.326[b]	0.338[b]	0.329[b]	0.009	0.04	0.10	0.67

[†]All cattle had *ad libitum* access to ground prairie hay and salt block. NC = negative control treatment with no fescue seed; other treatments were supplemented daily with ergot-infested tall fescue seed (TFS). PC = positive control treatment; CB = Control Block; AB = Block containing a proprietary blend of capsaicin and mannan oligosaccharide; MB = Block containing 0.3% crystalline menthol. All block supplements were provided *ad libitum*.

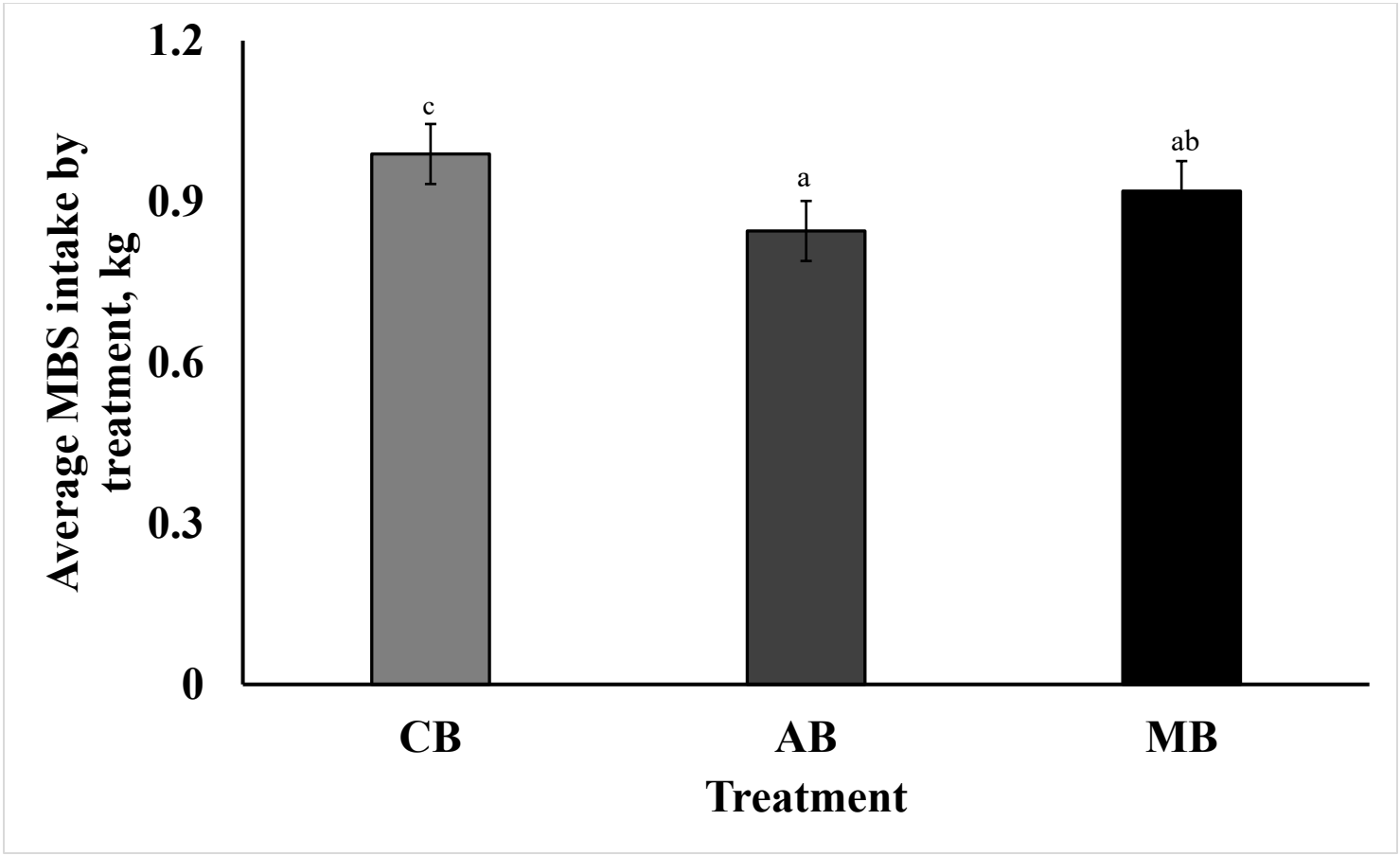
[a,b]Means without a common superscript letter displays overall treatment effect, $P < 0.05$.

Table 2-6 Effects of ergot-infested tall fescue seed and molasses-based block supplements on plasma concentrations of D-glucose (mg/dL)

	Treatment[†]						***P*-value**		
Item	NC	PC	CB	AB	MB	SEM	Treatment	Day	Trt × Day
D-glucose									
Overall	61.05[a]	60.90[a]	62.63[ab]	64.72[ab]	66.04[b]	2.437	0.04	0.91	0.27

[†]All cattle had *ad libitum* access to ground prairie hay and salt block. NC = negative control treatment with no fescue seed; other treatments were supplemented daily with ergot-infested tall fescue seed (TFS). PC = positive control treatment; CB = Control Block; AB = Block containing a proprietary blend of capsaicin and mannan oligosaccharide; MB = Block containing 0.3% crystalline menthol. All block supplements were provided *ad libitum*.

[a,b]Means without a common superscript letter displays overall treatment effect, $P < 0.05$.



982

983 **Figure 2.1** Overall intake of molasses-based block supplements by treatment. All cattle had *ad libitum* access to ground prairie hay,

984 salt block, and their respective block supplements. CB = Control Block; AB = Block containing a proprietary blend of capsaicin and

985 mannan oligosaccharide; MB = Block containing 0.3% crystalline menthol.

986 [a,b,c]Means without a common superscript letter displays overall treatment effect, $P < 0.05$, SEM = 0.055.

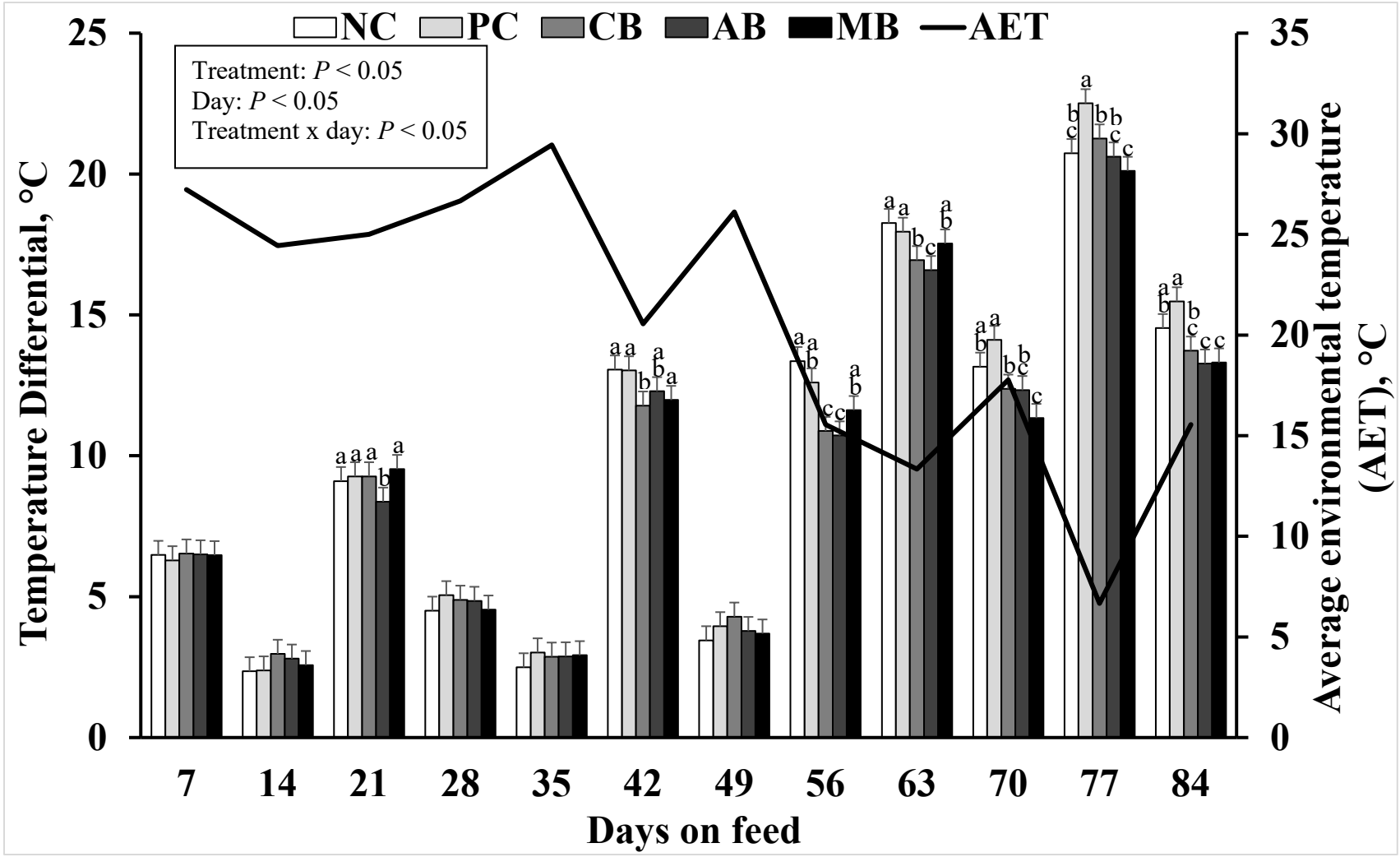


Figure 2.2 Effect of molasses-based block supplements on temperature differential (ΔT; °C). All cattle had *ad libitum* access to ground prairie hay and salt block. NC = negative control treatment with no fescue seed; other treatments were supplemented daily

990 with ergot-infested tall fescue seed (TFS). PC = positive control treatment; CB = Control Block; AB = Block containing a proprietary
991 blend of capsaicin and mannan oligosaccharide; MB = Block containing 0.3% crystalline menthol. All block supplements were
992 provided *ad libitum*.

993 [a,b,c]Means without a common superscript letter within day are different, treatment × day $P < 0.05$. SEM = 0.5016.

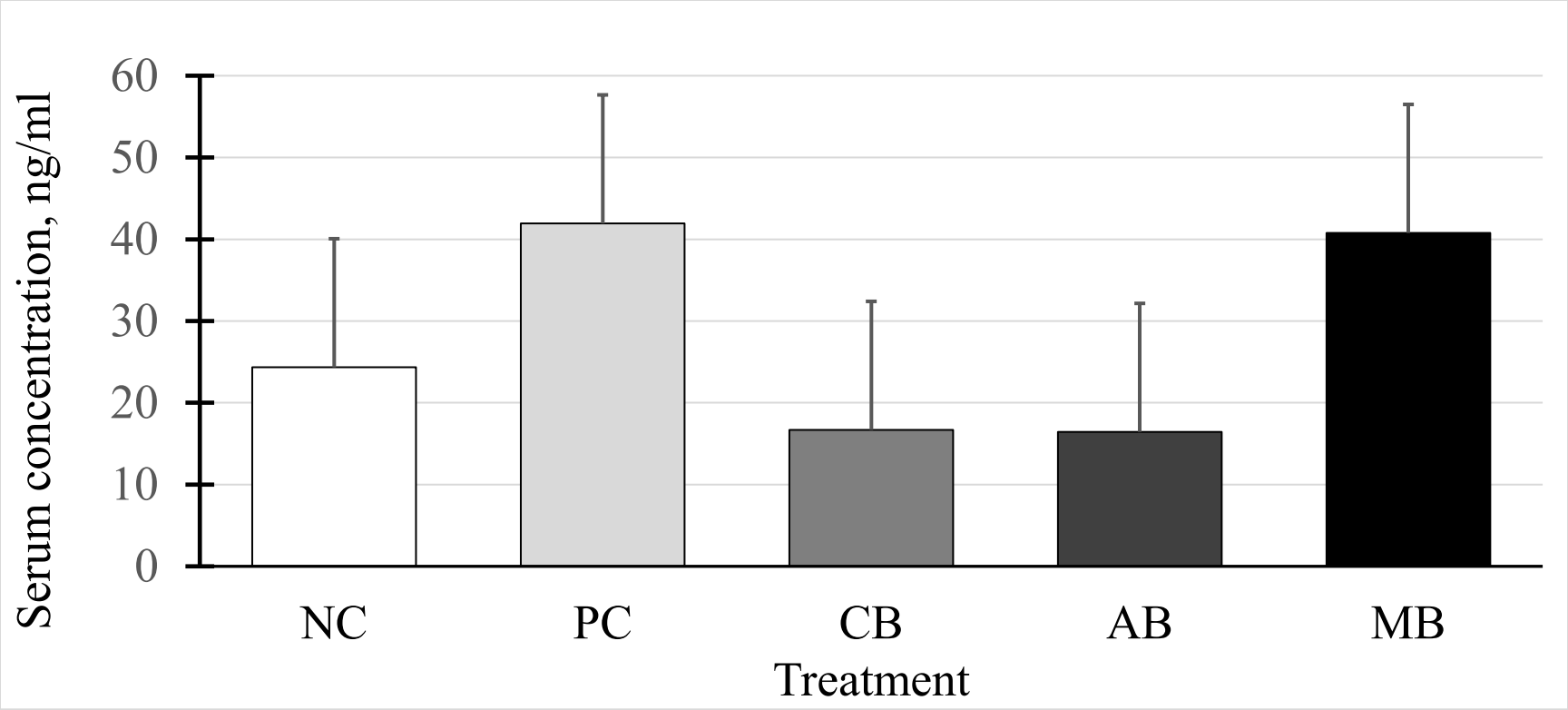


Figure 2.3 Serum somatotropin (GH) concentrations on day 84. All cattle had *ad libitum* access to ground prairie hay and salt block. Treatments are as follows: NC = negative control treatment with no fescue seed; other treatments were supplemented daily with ergot-infested tall fescue seed (TFS). PC = positive control treatment; CB = Control Block; AB = Block containing a proprietary blend of capsaicin and mannan oligosaccharide; MB = Block containing 0.3% crystalline menthol. All block supplements were provided *ad libitum*.

No treatment effect, $P = 0.29$. SEM = 15.718.

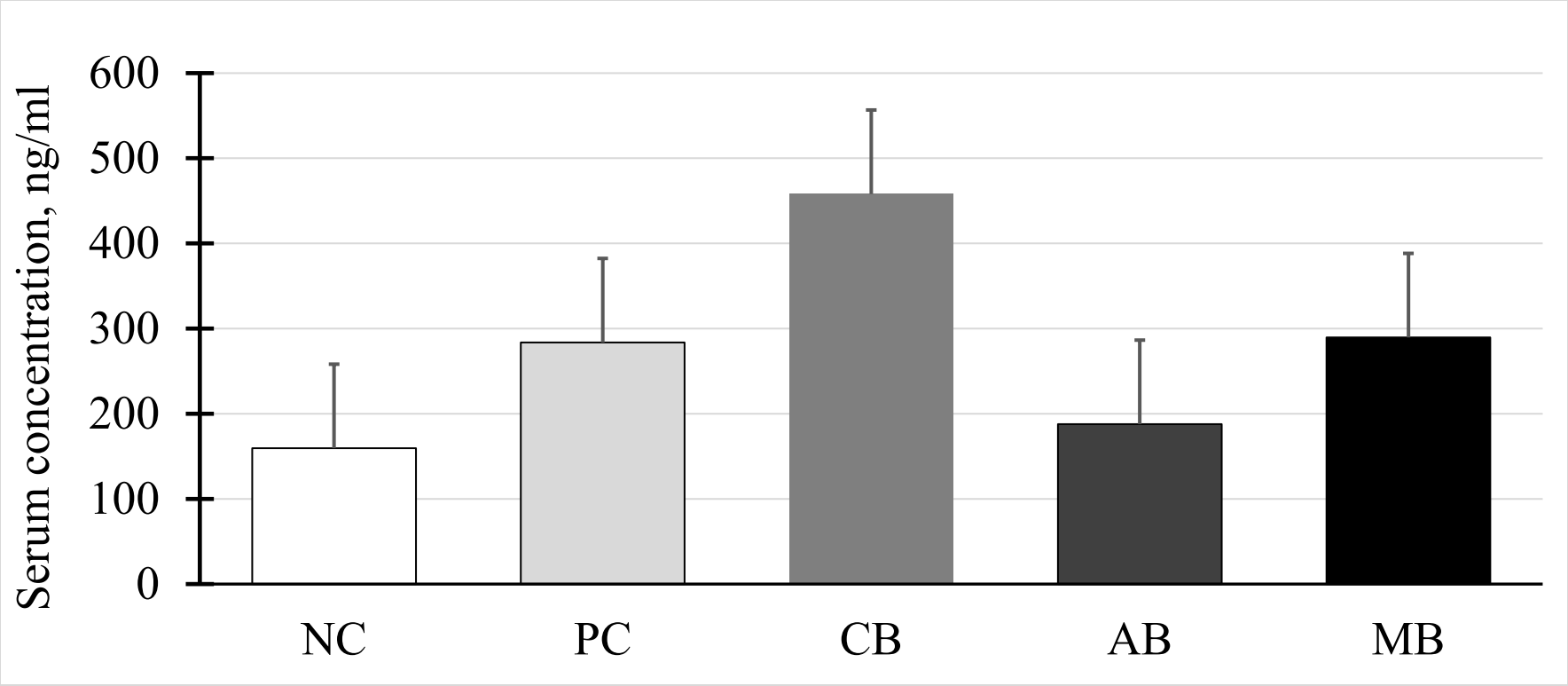


Figure 2.4 Serum insulin growth factor type 1 (IGF-1) concentrations on day 84. All cattle had *ad libitum* access to ground prairie hay and salt block. Treatments are as follows: NC = negative control treatment with no fescue seed; other treatments were supplemented daily with ergot-infested tall fescue seed (TFS). PC = positive control treatment; CB = Control Block; AB = Block containing a proprietary blend of capsaicin and mannan oligosaccharide; MB = Block containing 0.3% crystalline menthol. All block supplements were provided *ad libitum*.

No effect of treatment, $P = 0.09$. SEM = 98.7.

Chapter 3 - Targeted durations of feeding tylosin phosphate on the incidence and severity of liver abscess in feedyard cattle.

L. F. B. B. Feitoza[1], A. N. Baker[1], R. L. Thorn[1], L. S. Monteiro[1], F. Nasiu[1], T.G. Nagaraja[2], Q. Kang[3], and J. S. Drouillard[1*]

[1]Department of Animal Sciences & Industry;

[2]Department of Diagnostic Medicine & Pathobiology;

[3]Department of Statistics, Kansas State University, Manhattan KS 66506.

Abstract

Objective: To assess the impact of feeding tylosin phosphate at 60 mg/animal daily for the first 30 or 60 days of the finishing phase on the incidence and severity of liver abscesses, performance and carcass characteristics in feedyard steers.

Materials and Methods: Steers (n=462) were randomly assigned to one of three treatments: No tylosin phosphate (Control), tylosin phosphate in the feed to provide 60 mg/animal daily for the first 30 (Tylosin-30) or 60 days (Tylosin-60) of the finishing phase and then fed the control diet for the remaining days of the finishing phase. Steers were housed in pens with seven steers per pen and 22 pens per treatment. Steers were processed at a commercial abattoir and incidence and severity of liver abscesses and carcass characteristics were recorded.

Results and Discussion: Incidence of abscessed livers not affected by targeted durations of tylosin phosphate administration ($P = 0.93$). However, there was a trend for severe abscesses to be lower in Tylosin-30 and Tylosin-60 than the control ($P = 0.1$), likely because of inclusion of tylosin

phosphate during the diet transition phase when cattle are likely to experience ruminal acidosis. Steers that received tylosin phosphate had greater BW on day 30 or 60 compared to the control ($P < 0.05$). Steer performance and carcass characteristics were not affected ($P > 0.05$).

Implications and Applications: Administration of tylosin phosphate at 60 mg per animal/daily for the first 30 or 60 days of the finishing phase had no effect on the incidence of abscessed livers.

Keywords: feedyard steers, tylosin phosphate, liver abscesses, targeted duration, steer performance, carcass characteristics

Introduction

Liver abscesses (LA) are a source of economic loss in the feedyard cattle industry, with estimated annual impact of over $60 million in visceral losses alone (Herrick et al., 2022). Severe abscesses often extend into adjacent organs and tissues, requiring excision of the liver and trimming of the carcass during harvest, thus decreasing carcass weight and overall carcass value compared to cattle without liver abscesses (Grimes et al., 2024). Abscesses also can adversely affect abattoir line speeds, thereby increasing beef processing costs. Cattle with severe abscesses also have poorer performance compared to cattle that are abscess-free because of decreased dry-matter (**DM**) intake, average daily gain (**ADG**) and feed efficiency (Batista and Holland, 2022) Also, cattle with sever abscesses have reduced hot carcass weight and dressing percentage (Brink et al., 1990; Rezac et al., 2014; Grimes et al., 2024).

Development of liver abscesses in feedyard cattle is hypothesized to be a sequela to metabolic insult in the rumen, such as low ruminal pH, hence creating inflammation and ulcerations of the ruminal epithelial wall and providing the opportunity for bacteria to invade and create micro-abscesses (Amachawadi and Nagaraja, 2022). The primary etiologic agent, Fusobacterium necrophorum and other secondary bacterial species (Trueperella pyogenes, Salmonella enterica, etc.) translocate into portal circulation and subsequently infect hepatic tissue, generating abscesses (Smith, 1944; Jensen et al., 1954; Nagaraja and Chengappa, 1998; Nagaraja and Lechtenberg, 2007). In the finishing feeding period, the initial transition to a high-concentrate diet constitutes a period of elevated risk for metabolic insults and is believed to be one of the main risk factors that lead to abscess formation. Although, the prevailing theory of acidosis and rumenitis has existed for decades, the inability to detect liver abscesses in live cattle has hindered efforts to understand the timing of abscess development (Broadway et al., 2024).

Tylosin phosphate (Tylan™ 100; Elanco Animal Health, Greenfield, IN), included in the finishing diet, has been in use for decades to prevent liver abscesses in feedyard cattle. The approved use by the Food and Drug Administration Center for Veterinary Medicine (**FDA-CVM**) is for continuous feeding throughout the finishing phase at 8 to 10 g/ton of tylosin (90% DM basis) to provide 60 to 90 mg/animal/day (Feed Additive Compendium, 1998), under a valid Veterinary Feed Directive (**VFD**). Because tylosin is a macrolide and macrolides are categorized as medically important antibiotics with wide human therapeutic applications, the feedyard industry is seeking ways to reduce the use of tylosin. In addition to evaluation of antibiotic alternatives, such as postbiotics (Huebner et al., 2019), direct-fed microbials (Hoffman et al., 2024), essential oils (Meyer et al., 2009), and vaccines (Amachawadi and Nagaraja, 2016) to replace tylosin, efforts have focused on reducing the total amount of tylosin use by administering intermittently (Muller et al., 2018) or for the strategic duration of the finishing phase, instead of continuous feeding (Davedow et al., 2020; Linneen et al., 2023). Our objective was to evaluate the effects of the lower end of the approved dose range, 60 mg/animal daily, of tylosin phosphate administered during the initial 30 or 60 days of the finishing phase on the incidence and severity of LA, cattle growth performance, and carcass characteristics.

Materials and methods

Procedures involving live cattle were conducted and approved by the Kansas State University Institutional Animal Care and Use Committee guidelines (**IACUC**) protocol #4281.

Study Design

The study was conducted from March 3, 2021 to August 11, 2021, for cohort 1, and March 5, 2021, to August 13, 2021, for cohort 2. The experiment was designed as a randomized complete block design with three treatments using 462 crossbred beef steers with initial body weight (**BW**) of 377 ± 5.9 kg, selected from a larger population of 525 steers to have initial BW as similar as possible across treatments within block. Steers were blocked based on initial BW and assigned at random to one of three treatments within each block. Each weight block consisted of three pens, and each cohort consisted of 11 blocks with 231 steers. Steers were housed in 66 partially covered, concrete-surfaced pens, with each pen housing seven steers. Pens were 4.3 m × 8.5 m with pipe fencing, allowing approximately 5.3 m^2 of space per animal. Automatic waterers (Hoskins MFG, Hoskins, NE) were located approximately 0.5 m from feed bunks and were shared by animals in adjacent pens. Feed bunks allowed approximately 48 cm of linear bunk space per steer. Approximately 70% of the pen surface was covered with a corrugated steel roof. Treatments included no tylosin phosphate (**Control**), tylosin phosphate included to provide 60 mg/animal daily for the first 30 days on feed (**DOF; Tylosin-30**) or for the first 60 DOF (**Tylosin-60**), and then fed the control diet with no tylosin phosphate for the remainder of the study (Figure 3.1).

Cattle Processing

Five hundred and twenty-five crossbred steers were transported to the Kansas State University Beef Cattle Research Center (Manhattan, KS) from sale barns in Fall City, NE., Mankato, KS., and Salina, KS. No information was available pertaining to vaccination status or history of antibiotic administration. Upon arrival, steers were allocated into pens and provided *ad*

libitum access to ground alfalfa hay and water. Twenty-four to 48 h after each load was received, steers were individually weighed, identified with uniquely numbered with visual and radio frequency ear tags, injected with clostridial vaccine (Ultrabac 7 Somnubac, Zoetis Animal Health, Kalamazoo, MI) and a 5-way respiratory vaccine (Bovishield Gold 5 FP, Zoetis Animal Health); treated for endoparasites (Safeguard, Merck Animal Health, Rahway, NJ) and ectoparasites (Ultra-Saber, Merck Animal Health); and implanted with a subcutaneous long-acting trenbolone acetate/estradiol implant (Revalor XS, Merck Animal Health, Rahway, NJ).

On day -1, steers were divided by the order of processing into two cohorts (n = 231 steers per cohort) and subsequently blocked by BW and assigned to treatments within weight block. The two cohorts were staggered two days apart to allow for data collection. On day 0, steers assigned to the study were individually weighed before feed delivery and placed into their respective assigned pens. Individual BW were determined at 30-d intervals and at the end of the 161-d feeding period. Cattle in pens were weighed using a large platform pen scale (Central City Scale; Central City, NE) for pen weight data, and individual weights were collected using a scale-mounted hydraulic squeeze chute (Daniels Manufacturing Co., Ainsworth, NE). Both balances were certified before initiation of trial (WH Scale Co., Topeka, KS) and also were verified for accuracy before and after each use, using certified weights. Cattle weights were used to determine average daily gain (**ADG**) and Gain: feed (**G:F**).

Diet Preparation

Steers were transitioned to the finishing diet using three step-up diets over a 21-d period, each step-up diet transition occurred at 7-day interval, achieving final finishing diet on day 21. Diet was formulated based on the requirements for beef cattle in the National Academy of

Sciences Beef Cattle Nutrient Requirements (Nutrient Requirements of Beef Cattle, 8th Revised Edition, 2015). Ingredient and nutrient compositions of step-up diets and the final finishing diet are presented in Table 3-1. Monensin (Rumensin™, Elanco Animal Health) was include to provide 350 mg per animal per day throughout the feeding period, and ractopamine hydrochloride (Optaflexx™, Elanco Animal Health) was provided at 200 mg/steer/day for the last 42 days on feed.

The only difference among the three treatments was the inclusion of the premix supplement (ground corn, soybean oil and iron oxide) with no tylosin to the control or with tylosin to the Tylosin-30 and Tylosin-60 treatments. The premix for Tylosin-30 or Tylosin-60 was prepared with tylosin phosphate (Tylan™ 100; Elanco Animal Health, Greenfield, IN) added to provide 0 mg or 60 mg per 227 g of premix. The premix supplement was delivered in a fixed amount to the total mixed ration (**TMR**). based on the number of steers in the pen. Therefore, the amount of the premix supplement was not adjusted to the intake level during the 30 or 60 days of administration. Beginning on 31 or 61 DOF, both treatments received the control diet that included the premix without tylosin phosphate. All personnel involved in the feeding and data collection were blinded to treatments.

Diets were mixed twice daily in a truck-mounted rotary mixer (Model 490-14; Roto-Mix, Dodge City, KS) equipped with load bars and electronic scale system (Model 4610; Digi-Star, Fort Atkinson, WI). Diets were delivered to steers twice daily at approximately 0800 h and 1400 h using a wagon-mounted delivery box (Kelly-Ryan Equipment Company, Blair, NE) equipped with an electronic scale system (Model 4610; Digi-Star). To avoid cross contamination of diets, the truck-mounted mixer and feed wagon feed delivery box were thoroughly cleaned after each batch. Bunk scores for feed intake were visually determined and adjusted daily to allow *ad*

libitum intake with minimal feed residues remaining in the feed bunk. Unconsumed feed was removed as needed (e.g., due to excessive precipitation events), and also for each ration change (step-up regimen) and when the pens were weighed (to determine G:F). To determine daily DM content of diets, a subsample of the daily TMR was composited by week and dried for 48 h in a 55°C oven.

Harvest

Cattle were projected to be harvested at 161 DOF by design considering their initial BW and an expected 1.8 kg ADG. Pens of steers were weighed, loaded onto trucks, and transported to a commercial abattoir located 276 miles away from the study site, and travel time of 4:30 h. Final BW (**161 DOF**) was determined by taking the pen average and multiplying the value by 0.96 (pencil shrink), to account for 4% projected shrink. In abattoir, animals were identified following stunning and exsanguination and a unique carcass tag identifier was attached to the exposed brisket. Livers abscessed or not were assessed by a USDA FSIS inspector. Liver abscess severity scores were then assigned by a trained member of the research team using the scoring system described by Brown et al. (1975): 0 = no abscesses; A- = one or two small abscesses or abscess scars; A = 2 to 4 well-organized abscesses less than 2.54 cm diameter; and A+ = 1 or more large abscesses or multiple small to medium-sized abscesses, often with inflammation of surrounding the abscesses, sometimes with adhesion to the diaphragm and other adjacent tissues and organs. Following 36 h of carcass chilling, USDA quality grade; USDA yield grade; longissimus muscle (**LM**) area; 12th subcutaneous rib fat thickness; marbling score; percentage of kidney, pelvic and heart fat (**KPH**); and incidence of dark cutting beef were obtained through camera images (VBG 2000; E+V Technology GmbH & Co. KG, Oranienburg, Germany)

supplied by the abattoir and with compliance with USDA standards, calculated yield grade followed the equation: 2.50+(2.5 × adjusted fat thickness in inches)+(0.2 × percent kidney, heart and pelvic fat)+(0.0038 × hot carcass weight) - (0.32 × ribeye area in square inches), (USDA, 2017).

Statistical Analyses

Analyses were performed using SAS version 9.4 (SAS Institute Inc., NC). Pen was the experimental unit. Numerical endpoints were analyzed as linear mixed models using the GLIMMIX procedure of the Statistical Analysis System. The link function was identity, assuming direct relationship between predictors and response variable. Average daily gain, feed intake, and feed efficiency were calculated on a pen basis for the entire study period. The statistical model included treatment as the fixed effect and weight block as the random effect. In addition, the repeated statement was used to account for repeated measurements of the experimental unit (pen) variables.

Carcass characteristics, including hot carcass weight (**HCW**); calculated yield grade; longissimus muscle area; marbling score; subcutaneous fat thickness; and KPH were measured from individual carcasses. The statistical models for these endpoints included treatment as fixed effect, and weight block and pen as random effects. Least-square means (**LSM**) and their differences were used for treatment comparisons. The F-distribution approximated distribution of test statistics via the Kenward Roger method.

Categorical variables (liver abscess presence or absence, liver abscess score, yield grade, and quality grade) were analyzed using generalized linear mixed models with the SAS GLIMMIX procedure. Binary measures of liver abscess presence or absence, liver scores (A-, A,

and A+) were analyzed using the logit link function. Yield grade and quality grade are ordinal endpoints measured from individual animals and were analyzed using the cumulative logit link. Treatment was the fixed effect, and weight block and pen were random effects. Variances of random effects had a lower bound of 10^{-4}, ensuring that the model did not underestimate the variability in the random effects. Model fitness was assessed via the deviance-over-degrees-of-freedom ratio. The back transformed from logit scale LSMs (i.e., event rates) and LSM differences were used for treatment comparisons, the transformation allowed for a more interpretable data. Differences with significance level of $P \leq 0.05$ were considered statistically significant and tendency was defined as $P > 0.05$ to $P \leq 0.1$.

Results and Discussion

Fourteen steers died prior to harvest and DOF at death and the cause of death based on necropsy diagnosis are shown in Table 3-2. Of the 14 steers that died, five steers were in the control; three steers were in the Tylosin-30 treatment, and six steers were in the Tylosin-60 treatment. Eight of 14 steers (57%) were diagnosed as deaths due to bronchopneumonia.

Liver Abscess Incidence and Severity

Tylosin phosphate administered strategically in the first 30 or 60 DOF did not affect total liver abscess incidence (P = 0.93; 43.6, 41.7, and 43.2% for control, Tylosin-30, and Tylosin-60, respectively) (Table 3-3). Abscesses categorized as A- or A did not differ among the treatments (P > 0.10; Table 3-3). However, cattle fed tylosin phosphate for the first 30 or 60 DOF tended to have fewer severely (A+) abscessed livers (P = 0.10) compared to the control fed no tylosin phosphate. This effect could be attributed to the use of tylosin phosphate during the diet

adaptation phase (first 21 DOF) when cattle are likely to experience ruminal acidosis. A few other studies have evaluated different targeted durations of administration of tylosin phosphate at 8 to 10 g/ton of tylosin, on a 90% DM basis, to provide 60 to 90 mg/animal daily with the goal to reduce the overall use of tylosin phosphate in feedyard cattle (Müller et al., 2018; Walter et al., 2018; Davedow et al., 2020; Linneen et al., 2023). Walter et al. (2018) evaluated tylosin phosphate administration (9.7 g/ton on a 100% DM basis) during the first 42 of DOF, first 84 DOF, first 126 DOF, last 84 DOF, continuously throughout the entire feeding period, or not at all (control). Total percentage of abscessed livers decreased as days of tylosin phosphate feeding increased from the beginning of the feeding period (linear, $P < 0.05$) and were greatest in last 84 DOF and control, but least in first 126 and continuous administration. Müller et al. (2018) investigated an off-label duration where it was observed that intermittent feeding of tylosin phosphate (i.e., cycles of one week with tylosin phosphate followed by two weeks without) was comparable to continuous feeding in terms of the ability to prevent liver abscesses. In a study reported by Davedow et al. (2020), reduced duration of administration of tylosin phosphate for the first 125 of 161 of DOF had no effect on the incidence of total abscesses, but resulted in higher incidence of A+ liver abscesses compared to the no tylosin phosphate (23.5% vs. 19.8; $P = 0.05$). The overall prevalence of liver abscesses were similar among the treatments. A series of studies reported by Linneen et al. (2023) showed that strategic duration of administration of tylosin phosphate significantly impacted liver abscess incidence. In the first study, tylosin premix to provide 90 mg per steer daily was included for in the feed for targeted durations of the first 26%, 50%, or 75% or the last 50% of the 163 days of the finishing phase were evaluated. The administration of tylosin for the first 75% of DOF reduced incidence of total LA similar to continuously fed treatment. The second study targeted tylosin administration for different

durations, heifers that were fed tylosin phosphate for the last 83% and the control (no tylosin) exhibited the lowest total liver abscess incidence. Overall, the authors (Linneen et al., 2023) concluded that reducing tylosin phosphate to approximately 80% of the finishing period was effective in reducing the incidence, but the most effective control was obtained with continuous feeding of tylosin phosphate. The exact time of development of liver abscesses in feedyard cattle is not well defined, but the current study was designed to target the first 30 or 60 days because of the likely metabolic insult during transition to high-starch diets. Overall, the data on the impact of targeted duration of tylosin phosphate on the incidence of total or A+ liver abscesses have not been conclusive, and even conflicting. The reasons for the inconsistency and conflicting results can be because liver abscesses are a complex syndrome and incidence is influenced by cattle type, days on feed, feed type and intake patterns, behavior, and feedyard management. Although, a consistent finding has been that continuous feeding for the entire duration of the finishing phase was the most effective, with some studies showing that administering tylosin phosphate for the first half being more effective than the second half of the finishing phase.

Steer Performance

Steer performance is summarized in Table 3-4. No differences were noted between treatments for DMI ($P = 0.44$), ADG ($P = 0.53$), or G:F ($P = 0.48$). These observations are similar to studies reported previously that evaluated tylosin phosphate (Potter et al., 1985; Depenbusch et al., 2008; Müller et al., 2018). Body weights progression at 30-day intervals are shown in Figure 3.2. It is interesting that on 30 DOF, steers that received tylosin phosphate had greater body weights compared to the control treatment ($P < 0.01$), but DMI was not affected ($P = 0.30$). By day 60 (30 days after withdrawal of tylosin phosphate from the Tylosin-30), measures of cattle growth

performance (BW, ADG, DMI, and G:F) were not different between the control and Tylosin-30 (P = 0.14, P = 0.85, P = 0.93, and P = 0.94, respectively). In contrast, cattle in the Tylosin-60 treatment had greater body weights at 60 DOF (P = 0.04) compared to the control or Tylosin-30, but ADG, DMI, and G:F did not differ among treatments (P = 0.61). No differences in cattle performance were noted for subsequent DOF (90, 120, 150, and 161) between the three treatments (P > 0.05). Although, tylosin phosphate is not labeled as a growth promotant, during the period of tylosin phosphate administration (30 DOF and 60 DOF), steers in the tylosin phosphate treatments had significantly higher average body weights compared to the control treatment not receiving tylosin phosphate. Similar growth promotion response has been reported in cattle fed tylosin phosphate compared to cattle not fed tylosin phosphate (Potter et al., 1985).

Carcass Characteristics

No evidence of significant difference was detected across treatments when analyzing carcass characteristics, including HCW; dressing percentage; 12th-rib fat thickness; LM area; marbling score; USDA quality grade; KPH, and USDA yield grade (P = 0.40, P = 0.56, P = 0.11, P = 0.30, P = 0.99, P = 0.49, P = 0.91, and P = 0.31, respectively; Table 3-5). Lack of statistical differences among treatments with respect to HCW and dressing percentage are consistent with observations of Brink et al., (1990); Müller et al., (2018), Davedow et al. (2020), and Linneen et al. (2023). In contrast, Lawrence (2022) observed that cattle with severe liver abscesses (A+) have lighter and leaner carcasses. In the present study, no differences among treatments with respect to 12th-rib fat thickness or LM area were observed (P = 0.12 and P = 0.30, respectively). Results of previous studies have noted that carcasses from cattle with severe (A+) abscesses had less 12th-rib fat and smaller LM area compared to carcasses with normal livers (Brown and

Lawrence, 2010; Rezac et al., 2014). We observed no effect of tylosin phosphate on carcass quality grades (P = 0.49). Overall, targeted use of tylosin phosphate for the first 30 or 60 DOF did not affect carcass characteristics.

Conclusions

Strategically feeding tylosin phosphate at a targeted low dose of 60 mg/animal daily during the first 30 or 60 DOF did not affect liver abscess incidence, but there was a trend for reduced incidence of A+ abscesses. The overall cattle performance and carcass characteristics were not affected. A major limitation of the targeted duration of tylosin administration is lack of evidence for the timing of abscess development because of the inability to detect LA in live cattle. An evidence-based timing of LA development needs to be determined before designing studies that focus on strategic duration of tylosin administration to reduce the overall use of medically important antibiotic in feedyard cattle.

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Table 3-1 Diet ingredients and feed formulated nutrient compositions, DM basis. **Item**				
	Step-1	Step-2	Step-3	Final finishing diet
Ingredient (%)				
Steam-flaked corn	47.56	60.56	71.56	81.54
Condensed fermented corn extractives[1]	8.00	8.00	8.00	8.00
Alfalfa hay	41.00	28.00	17.00	7.20
Supplement[2]	3.44	3.44	3.44	3.44
Medicated premix, kg/animal daily,[3]	0.227	0.227	0.227	0.227
Nutrient composition				
Crude protein, %	16.70	16.00	15.41	14.88
Net energy maintenance, Mcal/kg	0.82	0.89	0.96	1.01
Net energy gain, Mcal/kg	0.54	0.60	0.65	0.70
Neutral detergent fiber, %	23.90	18.31	13.58	9.37
Calcium, %	1.25	1.06	0.90	0.75
Phosphorus, %	0.35	0.34	0.34	0.34

Salt, %	0.26	0.26	0.26	0.26

[1]Corn steepwater, ADM Animal Nutrition, Columbus, NE.

[2]Contained limestone, salt, urea, trace mineral/vitamin premix to provide (on a total diet DM basis) 0.15 mg/kg cobalt, 10 mg/kg copper, 0.50 mg/kg iodine, 20 mg/kg manganese, 0.10 mg/kg selenium, 30 mg/kg zinc, 2205 IU/kg vitamin A, 22 IU/kg vitamin E.

[3]Medicated premix to provide 0 mg for the control or 60 mg of tylosin phosphate (Tylan 100; Elanco Animal Health, Greenfield, IN) per animal daily for the first 30 or 60 days for the Tylosin and tylosin-60 treatments, respectively; 350 mg monensin USP (Rumensin 90; Elanco Animal Health) per animal daily throughout the finishing period; and for the final 28 to 42 days, 200 mg/animal daily of ractopamine hydrochloride (Optaflexx 45; Elanco Animal Health.

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Table 3-2 Steers removed from the study in the three treatments before harvest: Cause of mortality

Treatment[1]	Cause of death	No. of steers	Days on feed
Control	Bronchopneumonia	2	32, 147
	Septicemia	1	113
	Necrotizing fasciitis	1	119
	Heat stroke	1	147
Tylosin-30	Rumen tympany	1	25
	Bronchopneumonia	2	138, 147
Tylosin-60	Severe valvular endocarditis	1	96
	Bronchopneumonia	4	97, 147, 158, 160
	Heat stroke	1	125

[1]Control received no tylosin phosphate, Tylosin-30 received tylosin phosphate for the first 30 days, and Tylosin-60 received tylosin phosphate for the first 60 days of finishing phase.

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Table 3-3 Incidence and severity of liver abscesses in steers fed finishing diets without tylosin phosphate (control) or with 60 mg/animal daily of tylosin phosphate fed for the first 30 (Tylosin-30) or 60 (Tylosin-60) days on feed

	Treatment[1]				
Item	**Control**	**Tylosin-30**	**Tylosin-60**	**SEM**	***P*-value**
Abscessed livers, %	43.6	41.7	43.2	4.1	0.93
Liver abscess severity,[2] %					
A-	8.1	12.6	12.2	3.4	0.38
A	13.4	15.2	16.2	3.8	0.77
A+	22.1‡	13.8†	14.8†	3.6	0.10

[1]Control treatment did not receive tylosin phosphate throughout the feeding period; Tylosin-30 received 60 mg/animal daily for the first 30 DOF and on day 31 was transitioned to the control diet; and Tylosin-60 received 60 mg/animal daily for the first 60 DOF and on day 61 was transitioned to the control diet.

[2]Liver abscess scores were assessed as 0 = no abscesses; A- = one or two small abscesses; A = 2 to 4 abscesses. less than 2.54 cm diameter; and A+ = one or more large abscesses or multiple small or medium-sized abscesses with or without adhesions.

$^{†, ‡}$ Means without a common superscript tended to be different, $P < 0.10$.

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Table 3-4 Performance of steers fed diets without tylosin phosphate (control) or with 60 mg/animal daily of tylosin phosphate fed for the first 30 (Tylosin-30) or 60 (Tylosin-60) days on feed

	Treatment[1]				
Item	**Control**	**Tylosin-30**	**Tylosin-60**	**SEM**	***P*-value**
Initial BW, kg	377	377	377	5.89	0.86
Final BW, kg	664	671	670	5.44	0.47
ADG, kg/d	1.78	1.82	1.81	0.036	0.53
DMI, kg/d	10.01	10.06	10.24	0.140	0.44
G:F	0.1785	0.1816	0.1779	0.003	0.48

[1]Control treatment did not receive tylosin phosphate throughout the feeding period; Tylosin-30 received 60 mg/animal daily for the first 30 DOF and on day 31 was transitioned to control diet; and Tylosin-60 received 60 mg/animal daily for the first 60 DOF and on day 61 DOF was transitioned to the control diet.

Table 3-5 Carcass characteristics of steers fed finishing diets without tylosin phosphate (control) or with 60 mg/animal daily of tylosin phosphate fed for the first 30 (Tylosin-30) or 60 (Tylosin-60) days on feed

	Treatment[1]				
Item	**Control**	**Tylosin-30**	**Tylosin-60**	**SEM**	***P*-value**
HCW, kg	410	414	415	3.83	0.40
Dressed yield, %	61.7	61.7	61.9	0.31	0.56
12th-rib fat thickness, cm	1.36	1.37	1.45	0.03	0.11
LM area, cm2	90.4	91.9	91.4	0.96	0.30
Marbling score[2]	532	531	531	8.61	0.99
USDA Prime, %	12.8	8.7	5.4	2.20	0.38
USDA Choice, %	78.5	84.7	87.8	6.01	0.85
USDA Select, %	7.4	6.7	5.4	5.82	0.39
Sub-select,[3] %	1.3	0.0	1.4	1.12	0.06
KPH	1.82	1.83	1.84	0.02	0.91
Calculated yield grade[4]	3.16	3.15	3.25	0.07	0.31

Yield grade 1, %	4.0	2.6	1.4	1.88	0.36
Yield grade 2, %	32.1	38.1	33.3	5.50	0.51
Yield grade 3, %	54.4	49.3	49.0	5.81	0.58
Yield grade 4, %	9.4	10.0	15.6	3.71	0.18

[1]Control treatment did not receive tylosin phosphate throughout the feeding period; Tylosin-30 received 60 mg/animal daily for the first 30 DOF, at day 31 DOF was transitioned to control diet; and Tylosin-60 received 60 mg/animal daily for the first 60 DOF, at day 61 DOF was transitioned to control diet.

[2]Marbling score determined by computer imaging system (VBG 2000, E+V Technology GmbH & Co. KG, Oranienburg, Germany). Small = 400 to 499; Modest = 500 to 599, Moderate = 600 to 699, Slightly abundant = 700 to 799, and Moderately abundant > 800; Marbling score leading digit indicates score category.

[3]Consists of carcasses grading USDA Standard and USDA Commercial.

[4]Camera derived yield grade (BG 2000, E+V Technology GmbH & Co. KG, Oranienburg, Germany).

Yield grade 5 was not observed in any carcass in this study.

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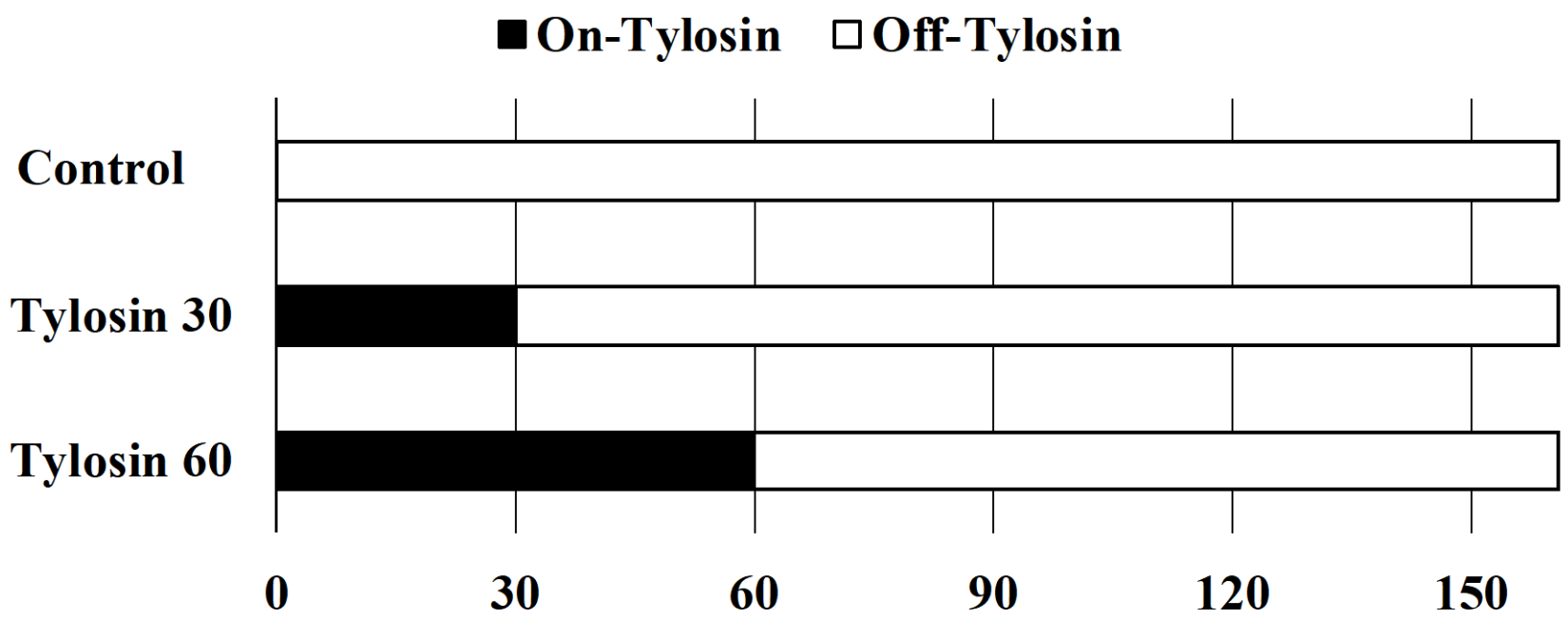

Figure 3.1 Study design for targeted duration of administration of tylosin phosphate included in the feed to provide at 60 mg/animal daily. Control treatment did not receive tylosin phosphate throughout the feeding period. Tylosin-30 treatment received tylosin phosphate for the first 30 days on feed, and Tylosin-60 treatment received tylosin phosphate for the first 60 days on feed and then changed to the control diet without tylosin.

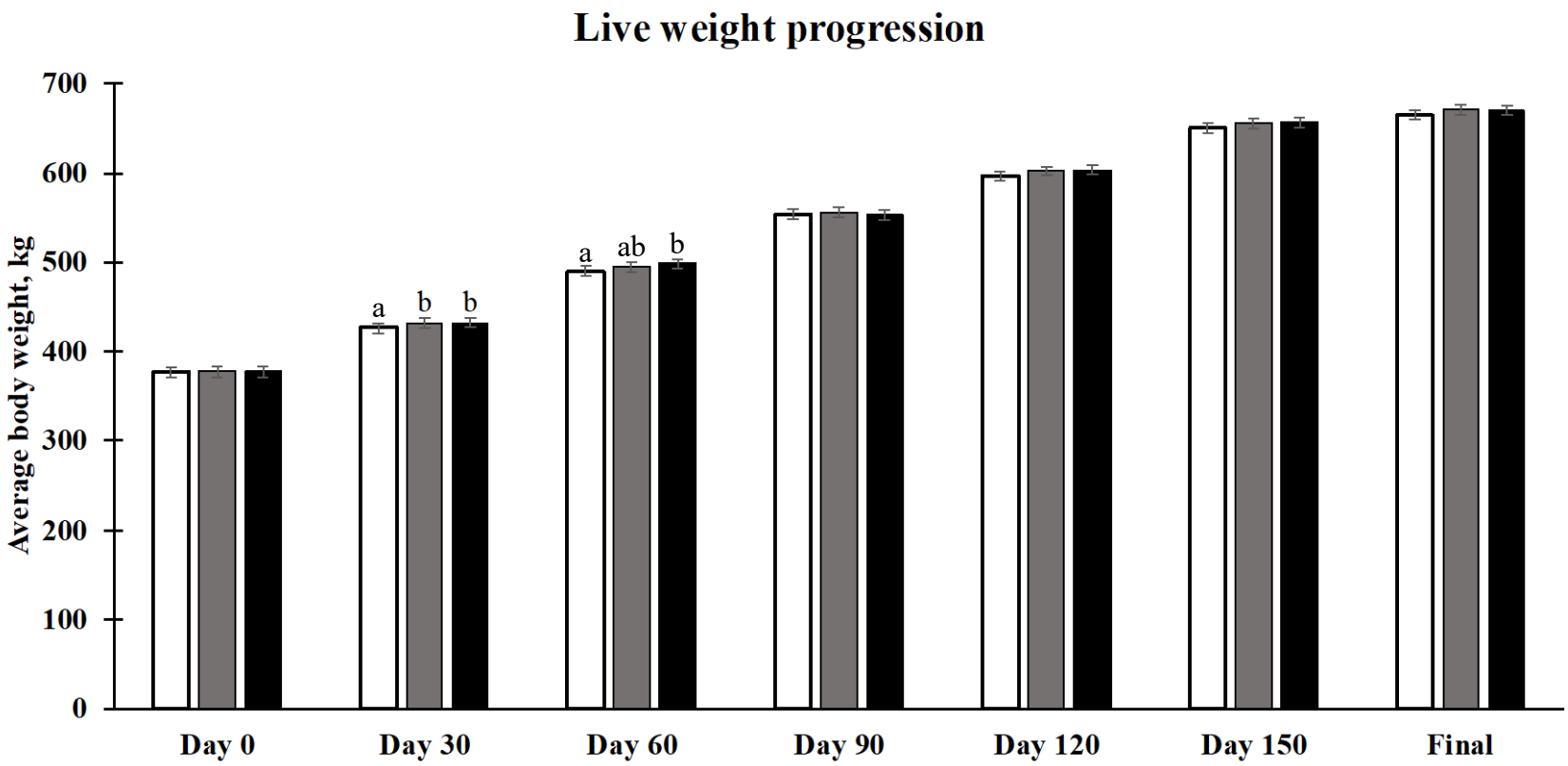

Figure 3.2 Effects of targeted durations of administration of tylosin phosphate on averaged individual body weights progression. Control treatment (white bars) received no tylosin phosphate, Tylosin-30 treatment (gray bars) received tylosin phosphate in the feed to provide 60 mg/animal daily for the first 30 days of feeding period, and Tylosin-60 treatment (black bars) received tylosin phosphate for the first 60 days of the feeding period and then changed to the control diet without tylosin.

[a,b] Means within a day and without a common superscript are different, Treatment × Day $P < 0.05$.

Chapter 4 - Evaluation of targeted thoracic point-of-care ultrasonography for identification of interstitial pneumonia in feedyard cattle

Luis F. B. B. Feitoza[1], Brad J. White[1*], Robert L. Larson[1], Makenna Jensen[1], Laura Carpenter[1], Brandon L. Plattner[2], Abigail Finley[3], Tyler J. Spore[4]

[1]Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, 1800 Denison Ave., Manhattan KS 66506, USA

[2]Kansas State Diagnostic Laboratory, Kansas State University College of Veterinary Medicine, Manhattan, Kansas, U.S.A.

[3]Texas A&M University – Veterinary Medicine & Biomedical Sciences, VERO, Canyon, Texas, USA.

[4]Innovative Livestock Services, 2006 Broadway Ave., Great Bend KS 67530, USA

Abstract

Objective: This study aims to use a novel approach of targeted thoracic point-of-care ultrasound (TT-POCUS) of caudodorsal right lung to determine potential associations between TT-POCUS measurements and presence of interstitial pneumonia (IP) in feedyard cattle.

Methods: Cross-sectional study evaluated feedyard animals (n= 62; 358.4±10.5 kg; steer, n= 24) using TT-POCUS at time of respiratory disease treatment and microscopic assessment of lesions at subsequent mortality. Evaluations took place from July to December 2023. Pulmonary

samples were collected at necropsy for histopathologic assessment, lesions were used to diagnose IP or non-IP. Statistical models determined potential associations between IP, cattle demographic, and TT-POCUS measured variables.

Results: Evaluations of animals enrolled (n= 62) displayed significant associations with IP including ultrasound lung score (ULS), B-line count, A-line count, and days on feed at treatment. Cattle with ULS of 5 had the lowest probability of IP (0.12±0.16) compared to cattle with ULS 2,3, or 4 (0.72±0.18, 0.93±0.06, 0.85±0.08, respectively). Cattle with B-line counts > 5 had higher probability of IP (0.86±0.11) compared to cattle with 3-5 B-lines (0.38±0.14). Probability of IP was higher in cattle with A-line counts < 3 (0.83±0.10) compared to cattle with ≥ 3 A-lines (0.51±0.15).

Conclusions: Strategic targeted thoracic ultrasonography has potential value of timely IP diagnosis in feedyard cattle; thereby offering important insights to therapeutic and management decisions.

Clinical Relevance: Targeted thoracic ultrasonography can be utilized as a method for IP diagnosis in feedyard cattle.

Key words: POCUS, chuteside, feedyard, interstitial pneumonia, thoracic ultrasound.

Introduction

Respiratory disease in cattle represents a significant and widespread health concern within the livestock industry, adversely affecting animal welfare and economic productivity.[1–3] Among the various respiratory conditions, interstitial pneumonia (IP) in cattle remains particularly challenging to diagnose early and treat successfully, due in part to limited comprehensive descriptions available in the literature.[4–8] Numerous potential causes, contributing factors, and even the basic pathologic processes underlying development of IP in feedyard cattle remain poorly understood, and this syndrome is often clinically indistinguishable from other respiratory diseases.[4,6,8–10] The three most common respiratory diagnoses in feedyard mortalities are bronchopneumonia (BP), bronchopneumonia with interstitial pneumonia (BIP), and acute interstitial pneumonia (AIP).[6,7,11] Combining AIP and BIP cases, total IP cases are reported to represent upwards 60% of feedyard mortalities.[7] A more detailed understanding of IP and development of effective and early diagnostic tools are crucial for enhancing disease management and treatment strategies.[9,12–14] The most reliable IP diagnostic method is histopathologic evaluation;[7,11,15] however, this is not feasible at the time of clinical diagnosis antemortem. Treatment decisions are made chuteside, so the inability to correctly differentiate pneumonias antemortem is a significant problem for which improved chuteside diagnostics are needed.

Differentiating IP from other respiratory illnesses is important due to the influence on treatment strategies and management decisions. Antimicrobial therapy is common in BP cases due to their infectious nature, whereas IP typically requires anti-inflammatory or supportive treatments to address the underlying pathogenic mechanisms.[4] Therefore, the ability to accurately identify and differentiate these conditions using TT-POCUS could lead to more

targeted and effective therapeutic interventions, ultimately improving animal health outcomes and reducing mortality rates.

Point-of-care ultrasound (POCUS) has emerged as a valuable diagnostic modality in human and veterinary medicine, offering accessible and non-invasive real-time imaging capabilities.[16–23] One limitation of POCUS applied to cattle respiratory disease is the systematic thoracic evaluation method is time consuming with previous literature reporting evaluations ranging from 7 - 45 minutes.[19,24] This study proposes a targeted thoracic POCUS (TT-POCUS) method specifically focusing on the right pulmonary caudodorsal region in cattle. The selected area for evaluation is commonly affected in cases of IP of feedyard cattle,[6] so targeted evaluation of only one region of the pulmonary area limits the overall time necessary to complete the evaluation. Overall, the objective of this study is to elucidate the potential associations between TT-POCUS imaging parameters observed at respiratory disease treatment and the subsequent presence of IP in cattle mortalities. By enhancing our understanding of IP and refining diagnostic techniques, this research aims to contribute to more effective disease management practices in feedyard operations.

Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee (IACUC) at Kansas State University (Protocol #4897).

Experimental Design & Enrollment Criteria

This experiment was designed as a cross-sectional observational study. Feedyard cattle respiratory morbidities were evaluated at time of treatment using TT-POCUS by a trained

86 veterinarian (LF) between July and December 2023, at one feedyard located in the high plains
87 region of United States. Systematic autopsies were performed by trained technicians (MJ, LC)
88 supervised by a veterinarian (BW) to collect pulmonary samples for histopathologic evaluation
89 when mortality occurred in cases previously evaluated by TT-POCUS. Study enrollment
90 required both TT-POCUS evaluation and a histopathologic diagnosis. Animals were not included
91 in the study if mortality did not occur following TT-POCUS or if pulmonary samples could not
92 be collected or were not evaluated histopathologically. Technicians were only present for
93 necropsy on specific days; therefore, all cattle that died following TT-POCUS may not have
94 been included.
95 The categories of DOF were determined based on previous reported work which
96 categorizes the temporal epidemiological characteristics of respiratory morbidities in feedyard
97 cattle, where 0-42 days are considered early DOF, 43-71 is mid DOF, and >71 is considered late
98 DOF.[27] Individual animal body weight (BW) was also recorded at time of treatment; animal
99 body weight was categorized in < 272, 272-362, 362-453, and ≥ 453 kg.
100
101 **TT-POCUS method:**
102 Commercial feedyard cattle were evaluated with TT-POCUS where animals were
103 restrained in a hydraulic squeeze chute and ultrasound lung scans were no longer than 60
104 seconds to maintain commercial feedyard work pace. Probe placement for TT-POCUS allowed
105 evaluation of only the right caudodorsal pulmonary lobe assessing between the 8^{th} and 11^{th} rib
106 (**Figure 4.1)**. The ultrasound probe unit utilized in this study was a Butterfly iQ+ Vet (1 MHz to
107 10 MHz), a point-of-care specialized probe, with 5 cm x 3 cm footprint, set on the optimized
108 preset for lung evaluation. Presets configured the probe array to linear, allowed for up to 30 cm

depth, and overall gain was set at 65%. Preparation of the scanning area was performed using 70% isopropyl alcohol without trimming/shaving the hair coat.

Scanning procedures involved probe placement placed parallel to the ribs, specifically starting at the 11th intercostal space (ICS) and moved from the dorsal aspect of the intercostal space (identified by visualization of the ventral part of longissimus dorsi) then sliding the probe ventrally until visualizing the liver, diaphragm, and caudal tip of the lung, also called as "curtain sign". Due to animal variability the "curtain sign" may not be visible at the 11^{th} ICS, requiring movement of the probe cranially to the next ICS (10^{th}). Each ICS from 11^{th} to 8^{th} was evaluated dorsally to ventrally similarly. Probe "fanning" was also performed, where the angle relative to the skin (side-to-side angle) is dynamically changed to facilitate the visualization of the pulmonary tissue between the bone structures (ribs).

Data collection from TT-POCUS evaluation was carried out in real-time and post-hoc analysis by a trained veterinarian. Real-time variables collected included: ultrasound lung score (ULS), B-line count, A-line count, presence of pleural effusion, and presence of pleural abnormalities (moth sign (pleural indentations) and pleural thickening). A 1 to 5 scale was used for ULS similar to previous research with ULS scores defined as: 1 - presence of < 3 thin B-lines (healthy lung); 2 - presence of 3 or more thin B-lines; 3 - presence of merged B-lines; 4 - presence of several wide/merged B-lines, abnormal pleural (moth sign); 5 - consolidated lung, might be accompanied with moth sign and effusion based on description of lung injury.[22,23,28] The B-line count was based on a representative count where B-lines were defined as hyperechoic vertical artifacts resembling comet tails. B-line count was categorized (0-2, 3-5, >5) based on theorized degree of severity, where more B-lines could translate to greater tissue injury. A-lines were defined as hyperechoic horizontal artifacts (reflections of the pleural line) and were

categorized in two groups: 0-2 A-lines and ≥ 3 A-lines. These groups were based on the goal of evenly dividing the population. The presence/absence of pleural effusion was determined by presence of hypoechoic/anechoic layer in pleural space, and pleural thickening/irregularity was recorded as present or not.

Other variables were generated post-hoc using the software ImageJ 1.54, measurements were taken from a single frame from the 60 second clip, where the frame of choice was the frame with most tissue abnormalities allowing to compare based in tissue injury within the caudodorsal region. The post-hoc variables included image brightness (pixel intensity density: average gray value x pixel count), average gray value (average of intensity units on image histogram) and B-line area, cm^2. Image brightness was based on pixel intensity density, this variable was measured by using a 7 cm linear selection, where the line would start on the pleural line, and go diagonally across the image where would go from the top to bottom of the image diagonally. Average gray value was also determined using the same 7 cm macro to evaluate image intensity unit. Total B-line area in cm^2 was calculated by the use of the polygon selection tool, where all the B-lines in the image would be measured, and the sum of these areas would equate to the total affected b-line area in cm^2, with the objective to account for potential differences in B-line width and merged B-lines. It is important to report that A-line evaluation has not being reported in the literature. On the other hand, B-line count has been reported extensively in the literature.[29,30] B-line count is often categorized into normal (0-2) versus abnormal (≥3).[23] In this present study, it was created the >5 category theorizing potential greater tissue injury.

Histopathology

Pulmonary tissue samples collected for histopathologic diagnosis were collected as a 1cm^2 tissue section from the right cranioventral lobe and right caudodorsal lung lobes. Samples were fixed by immersion in 10% neutral buffered formalin, paraffin embedded, and then processed routinely by the histology laboratory at the Kansas State Veterinary Diagnostic Laboratory (KSVDL). 4um thick tissue sections were cut to prepare slides, prior to routine staining with hematoxylin and eosin (H&E) using a Leica ST5010 Autostainer XL (Leica Biosystems, Deer Park, IL USA) and then digitized using a Leica Aperio AT2 digital slide scanner and evaluated by board-certified veterinary pathologists (AF and BLP) who were blinded to TT-POCUS evaluation and animal records. This study did not differentiate acute interstitial pneumonia (AIP) cases from bronchopneumonia with interstitial pneumonia (BIP) anatomically; for example, lesions of IP in either section of lung resulted in categorization as IP for any individual case. Key histopathologic lesions indicative of IP included: alveolar fibrin exudation, alveolar hyaline membranes, or septal necrosis supportive of (acute IP); type 2 pneumocyte hyperplasia and hypertrophy supportive of (subacute) IP; interstitial fibroplasia, and bronchiolitis obliterans supportive of (chronic) IP. Without these histopathologic features supportive of IP in either sample, cases were allocated to the Non-IP group.

Statistical Analysis

Excel (Microsoft Excel, Microsoft 365®) and R Studio (R Studio, version 2023.12.1, R Core Team, 2022) software were utilized for data handling. For descriptive purposes, a table comparing the outcome variables was built using the packages “dplyr” and “tidyr” in R Studio. A generalized linear model was utilized to analyze data using the “glm” with “logit” link function

of the native 'stats' package of R Studio. Histopathological diagnosis was the outcome variable of interest, where the diagnosis was classified binomially as IP (1) for interstitial pneumonia or Non-IP (0) for bronchopneumonia and all other respiratory syndromes without IP.

The model building process was carried by the use "scope" parameter to always include the fixed variables (Sex, and DOF) in the model iterations. The variables sex (heifer versus steer) and days on feed at time of chuteside evaluation (DOF) categories (0-42, 43-71, and > 71) were fixed in the model at the experimental design phase of the study, due to previous studies describing association of sex and late days on feed in acute interstitial pneumonia.[11,25,26] The stepwise selection with the backward direction using the "stepAIC" function in the "MASS" package of R Studio, where all the variables collected were included (BW, ULS, B-line categories, A-line categories, pleural effusion, pleural abnormality, image brightness, average gray value, B-line area) and least significant variable is removed at each selection step based on best model fitness assessed by lowest *Akaike Information Criterion* (AIC) coefficient. Multicollinearity amongst variables was assessed using the "car" package in R Studio, where calculates the variance inflation factor (VIF), variable was considered to have considerable collinearity VIF coefficient when > 2.5,[31] therefore removed from the model.

Results

A total of 1200 animals were evaluated using TT-POCUS at the time of respiratory treatment, with 66 animals resulting in mortalities on days technicians were present for necropsy and pulmonary sample collections. Four cases were excluded for incomplete data or missing histopathologic diagnosis resulting in 62 cases enrolled in the study. The population included 60% of the cases histopathologically diagnosed with IP and 40% diagnosed with Non-IP. There

were 38 heifers (61%), and 24 steers (39%) enrolled with descriptive statistics listed in **Table 4-1**.

The distribution of cases enrolled by DOF at time of treatment grouped by histopathologic diagnosis indicate that IP cases occurred dispersed throughout the feeding period (median of 50 DOF and interquartile range (IQR) 67 DOF). In contrast, Non-IP case distribution showed a tighter distribution of cases with a median of 28 DOF and IQR 16 DOF (**Figure 4.3**).

In addition to DOF, the interval in days from when the animal was evaluated at chuteside to the day of death is also displayed with the raincloud plot and box-plot combination (**Figure 4.4**), where IP cases had a shorter day interval window from chuteside evaluation to death, with a median of 4 days and IQR of 5 days. Non-IP cases displayed longer interval window from chuteside evaluation to death with median of 8 days and IQR of 6 days.

Multivariate logistic regression was utilized to identify potential associations between variables of interest (sex, days on feed (DOF), body weight (BW), ULS, B-line count category, A-line count category, pleural effusion, moth sign, pixel intensity density, average gray value and B-line area, cm^2) and an outcome response of a diagnosis of IP using a stepwise backward selection of variables. The fixed variable sex was not associated with the outcome IP in this study ($p = 0.59$), but DOF category was significantly associated with IP ($p = 0.01$). The final model included several variables significantly associated with probability of IP: ULS ($p = 0.02$), B-line count category ($p = 0.01$), and A-line count category ($p = 0.03$). The BW variable was removed from the model due to considerable high VIF (4.4).

Tukey-adjusted probabilities using the fit model showed the probability of IP was higher ($P < 0.05$) when animals were 43-71 DOF (87% ± 11) and >71 DOF (81% ± 16) compared to 0-42 DOF (29% ± 16). Ultrasound lung score of 1 was not present in any of the 62 cases, thus the

probabilities were only calculated for scores 2, 3, 4, and 5. An ULS of 5 was the least likely to be confirmed as IP by histopathology, with 12% ± 16% likelihood of a diagnosis of IP amongst all the scores ($p < 0.05$). Ultrasound lung scores of 2, 3, and 4 did not differ in the probabilities of IP ($p > 0.05$) (**Figure 4.2**). B-line count category of >5 B-lines in an ultrasound image was associated with an 86% ± 11% likelihood to be diagnosed with IP, while animals with 3 to 5 B-lines were associated with significantly lower probability of a confirmed diagnosis of IP (38% ± 14%, $p < 0.05$). B-line count category of 0 to 2 showed no statistical difference amongst the other two categories (75% ± 15% likelihood of IP; $p > 0.05$). The probabilities of A-line count categories 0-2 *versus* ≥ 3 A-lines were 83% ± 10% and 51% ± 15%, respectively ($p < 0.05$).

Discussion

Respiratory disease is a critical factor contributing to feedyard morbidity and mortality, and accurate classification of disease types at the time of treatment is essential to inform effective management and treatment strategies.[32] Results indicate TT-POCUS can be a valuable diagnostic tool for identifying IP in feedyard cattle morbidities, and similar promising results in identifying IP have been shown in human medicine during the COVID-19 pandemic, where bedside POCUS was utilized to evaluate IP.[16,33]

Lung evaluation using ultrasonography in veterinary medicine has been reported for respiratory disease detection.[17,23,28,34-40] However, this study proposed a novel evaluation method, where the evaluation time was ≤ 60 seconds, by targeting the caudodorsal lobe of the right lung and was able to decrease evaluation time drastically to find associations of real-time variables (ULS, B-line count, A-line). In contrast, to our study that developed the TT-POCUS, the traditional pulmonary ultrasound evaluation is more comprehensive and could take 7 – 45

minutes;[19,24] this significant time constraint is a limitation to deploying the method within a commercial operation, and also the accessibility to the cranioventral acoustic window can be challenging and time consuming.

Notably, our study demonstrated that real-time collected variables were the only ones significantly associated with histologic diagnosis of IP, suggesting that TT-POCUS can be effectively used chute-side for immediate diagnostic and therapeutic decision-making. Although we tested post-hoc calculated variables, none of them showed significant associations with IP, further highlighting the practical utility of real-time ultrasound evaluations in the field. In addition, performing TT-POCUS in a 60-second window increases the feasibility for application of this method in commercial operations where the time spent in diagnosis is a major determining factor for adoption. The ability of adopting TT-POCUS in a commercial operation can enhance diagnostic accuracy in real-time and improve treatment outcomes in feedyard cattle and aid informed management decisions.

Histopathologic analysis revealed that the majority of cases in our study were diagnosed with IP (60%), while 40% were diagnosed with Non-IP. Higher IP prevalence observed in this study may be best explained by our inclusion of both BIP and AIP, in contrast to other surveillance studies. Furthermore, another significant factor that could have contributed to higher than expected prevalence is the increased sensitivity due to histologic evaluation of two lung samples per animal. Previous epidemiologic publication reported prevalence of IP (AIP+BIP) mortalities in feedyard to be approximately 60.4% of the all the respiratory mortalities, while 40.4% of the respiratory mortalities were classified as Non-IP.[7,11]

Interstitial pneumonia cases were more dispersed across the feeding period, with a median DOF of 50 days and an interquartile range (IQR) of 67 days, indicating a wide

distribution of cases. In contrast, the Non-IP cases had a narrower distribution, with a median DOF of 28 days and an IQR of 16 days (Figure 4.3). Additionally, the interval from chuteside evaluation to death was shorter for IP cases (median of 4 days, IQR of 5 days) compared to Non-IP cases (median of 8 days, IQR of 6 days) (Figure 4.4), this shorter time interval from clinical diagnosis to death is the theorized quick development of interstitial pneumonia also described as acute.[8,25,41]

No epidemiologic association was identified in this study between IP and sex. The existing literature often reports a higher susceptibility of heifers to AIP and a higher proportion of steers presenting with BIP.[4,6,42] Our data did not show a statistically significant difference between heifers and steers in terms of IP probability, and the absence of a significant difference in our study may be attributed to specific management practices, sample size, or environmental factors unique to our study population.

The distribution of cases based on days on feed (DOF) showed that most animals diagnosed with Non-IP were in the 0-42 DOF category, which is considered the early-feeding phase. This temporal pattern was observed by previous studies, where Non-IP including bronchopneumonia had higher morbidity early in the feeding phase (< 42 DOF).[27,43] In contrast, animals diagnosed with IP were dispersed between the three time categories, having 40% prevalence in 0-42 DOF, and 60% in the 43-71 DOF and greater than 71 DOF combined. These DOF patterns for Non-IP (bronchopneumonia and other respiratory diseases not IP related) and IP (Figure 4.4) are similar to a recent epidemiologic study showing that IP occurred throughout the feeding phase.[11] Although, IP distribution was dispersed throughout the feeding period in the current study, the model-adjusted probabilities indicates that IP is more likely to happen mid and

290 late (> 42 DOF). These probabilities are corroborated by field data indicating that IP is often
291 described as a late-day disease.[4,25,42]

292 Ultrasound lung score indicated that animals with a score of 5 had higher prevalence
293 (83% of all ULS 5) in cattle in the Non-IP group. Hence, the probability of these animals with
294 confirmed IP were very low among those animals with ULS 5 from TT-POCUS (12% ± 16
295 likelihood), reinforcing that pulmonary tissue consolidation identified during ultrasound
296 evaluation is not likely to be associated with a confirmed diagnosis of IP.[33,44] (**Figure 4.5**). B-line
297 count category was also associated with IP outcome, where animals with more than 5 B-lines
298 identified at TT-POCUS displayed the greatest likelihood to be diagnosed with IP (86% ± 11%).
299 B-line is the artifact most evaluated in pulmonary ultrasonography, and its relevance in the
300 context of IP in our study is that more B-lines appear in the image when there are areas of
301 decreased air content.[23,29,45] The use of these real-time variables in differentiating respiratory
302 diseases have not been reported in other studies. In contrast, thoracic ultrasonography has been
303 used extensively in critical care for respiratory disease detection and assessment of disease
304 severity.[23] One limitation to B-line evaluation count is that as disease develops and pulmonary
305 injury worsen, B-lines have the potential to merge (**Figure 4.5**), making absolute quantification
306 difficult.[30] In an attempt to evaluate the potential association of merged B-lines, this study
307 analyzed the *post*-hoc variable B-line area to account for B-line merging. However, the statistical
308 analysis revealed no evidence of an association of B-line area with an ability to differentiate of
309 IP from Non-IP. We theorized that merging of B-lines was most likely accounted for in the
310 statistical model using ULS.

311 The A-line count category is a novel variable not described as useful for respiratory
312 disease differentiation. We found evidence of statistical association where A-line count category

lower than 3 displayed higher probability of IP diagnosis (< 3 *versus* ≥ 3 A-lines were 83% ± 10% and 51% ± 15% probability of IP). Normal lungs are expected to exhibit several A-lines due to the reflection of the ultrasound waves by the pleural line, which is caused by the air-filled lung. A-lines appear as hyperechoic horizontal artifacts, and their presence typically indicates a healthy lung with normal air content.[23] In contrast, lower A-line counts in IP cases can be attributed to the pathologic changes occurring in the lung tissue. IP involves infiltration of inflammatory cells into the interstitium and accumulation of fluid within the alveolar spaces, which disrupt the normal air-tissue interface. This disruption reduces the reflective surface area available to produce A-lines. As a result, the number of detectable A-lines decreases in association with decreased lung aeration.

Collectively several features including an ULS of 2,3, and 4, higher B-line counts, and lower A-line counts occur significantly more often in IP cases compared to Non-IP cases, suggesting that these markers could serve as useful diagnostic indicators in veterinary medicine for identifying severe interstitial lung disease. By incorporating ULS, B-line, and A-line counts into TT-POCUS evaluations, field veterinarians can improve their ability to differentiate IP from other respiratory diseases, leading to more targeted and early effective treatment strategies.

Conclusions

The findings of this study highlight the substantial potential of TT-POCUS as a diagnostic modality for the identification of IP in feedyard cattle. Significant associations were observed between histopathologic diagnosis of IP and imaging parameters, including B-line count, ULS, and A-line count. These associations show the potential diagnostic efficacy of TT-POCUS in differentiating interstitial lung disease from other respiratory diseases in a timely

manner. This diagnostic method can enhance management decisions, leading to improved health outcomes for feedyard cattle morbidities. Integrating TT-POCUS into routine sick animal diagnostics can streamline health monitoring and disease management processes.

The promising results from this study warrant further research and refinement of this diagnostic technique. Future studies should focus on developing this TT-POCUS protocol and validating these findings across diverse cattle populations and settings to fully realize the clinical benefits and ensure the widespread adoption of TT-POCUS in the field.

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Dr. White is a member of the scientific review board for the *American Veterinary Medical Association Journals (American Journal of Veterinary Research and Journal of the American Veterinary Medical Association*). He declares that he had no role in the editorial direction of this manuscript.

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Table 4-1 Descriptive statistics of animals according to their demographics (sex, weight, days on feed categories, and treatment count), and thoracic ultrasonography variables (ultrasound lung score, A-line count categories, B-line count categories.

	Histopathologic Diagnosis	
	Non-IP[1]	**IP[2]**
Sex		
Heifer	15	23
Steer	10	14
Days on feed categories		
0-42	20	15
43-71	3	9
>71	2	13
Body weight, average (SEM), kg	312 (± 11)	390 (± 14)
Body weight, categories, kg		
< 272	7	3
272-362	16	14
362-453	2	13
≥ 453	0	7
Treatment count, n		
1	11	18
2	8	15
3	6	4
Real-time measurements		

Ultrasound lung score, head*		
1	0	0
2	6	3
3	5	11
4	9	22
5	5	1
A-line count categories, head*		
0-2	10	22
≥ 3	15	15
B-line count categories, head*		
0-2	6	11
3-5	16	13
> 5	3	13
***Post-hoc* measurements†**		
B-line area, cm², average (SEM)	17 (± 2.2)	20 (± 2)
Gray value, average (SEM)	99 (± 9)	96 (± 6)
Intensity density, average (SEM)	14 (± 2)	14 (± 1)

[1]Cases diagnosed as non-interstitial pneumonia by histopathology; [2]Cases diagnosed with interstitial pneumonia by histopathologic evaluation; *Variables collected by real-time TT-POCUS. †*Post-hoc* measurements were performed by using ImageJ 1.54. Measurements were taken from a single frame from the 60 second clip, where the frame of choice was the frame with most tissue abnormalities allowing to compare based in tissue injury within the caudodorsal region.

Figure 4.1 Right lateral view of adult bovine anatomy. The polygon trace in yellow represents the area scanned with the targeted thoracic ultrasonography, 8th to 11th intercostal space. Caudal to the lungs is the liver, displaying close proximity with the caudodorsal area of the right lung.

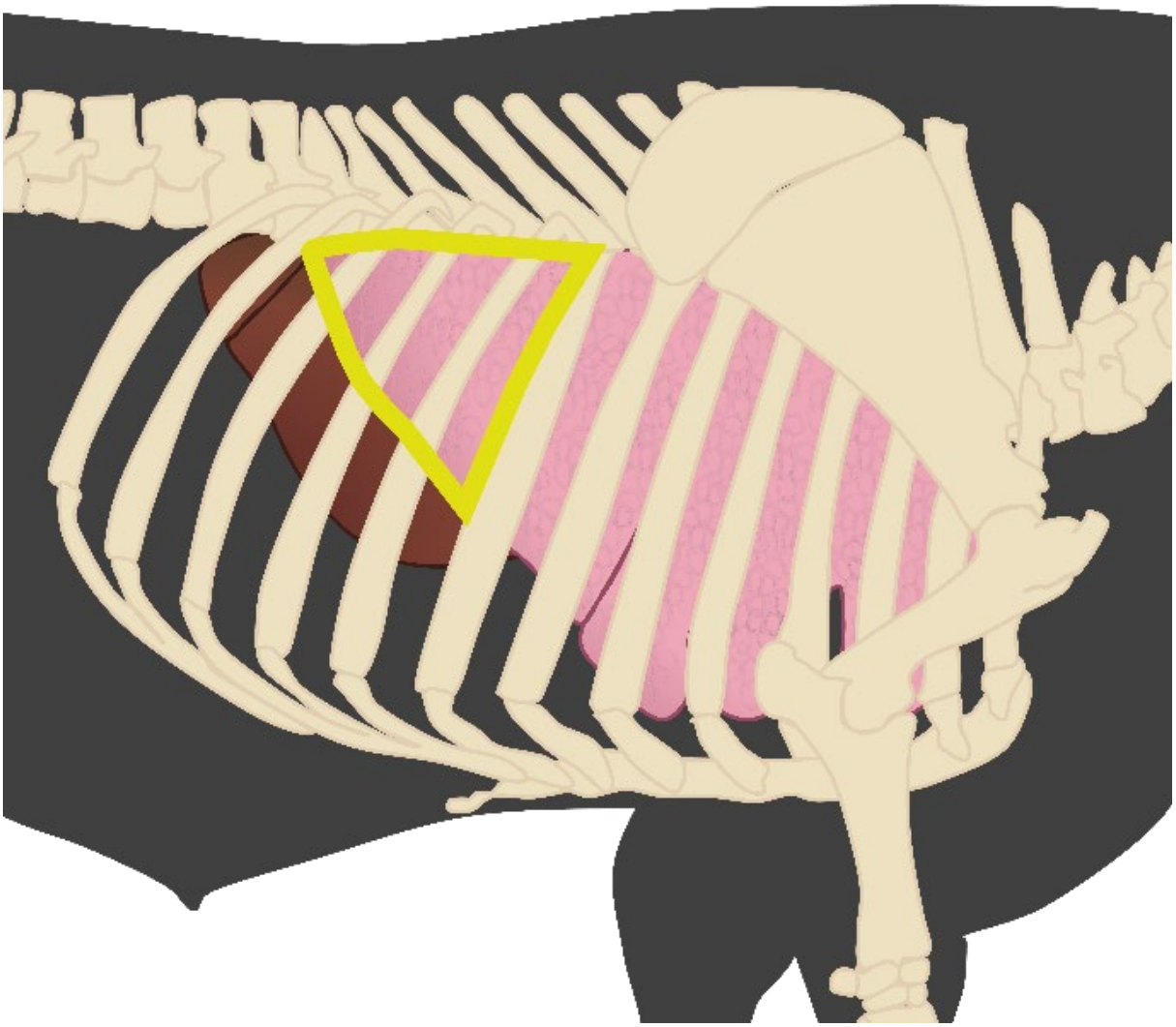
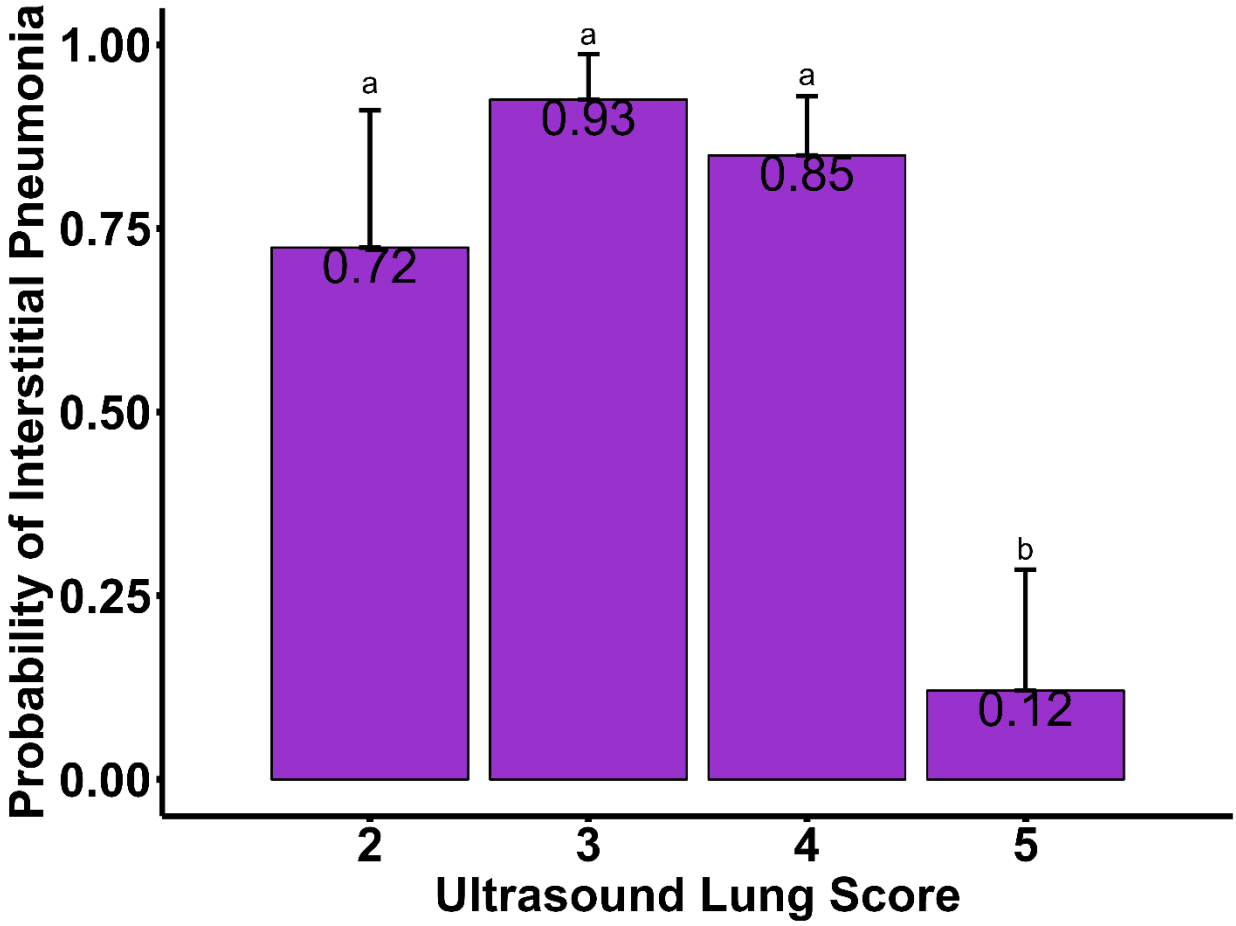


Figure 4.2 Model adjusted probability of interstitial pneumonia (IP) by ultrasound lung score (ULS)* from a population of 62 head evaluated with targeted thoracic point of care ultrasound (TT-POCUS) with subsequent histopathologic diagnosis of IP or non-IP.



*ULS scores defined as: 2 - presence of 3 or more thin B-lines; 3 - presence of merged B-lines; 4 - presence of several wide/merged B-lines, abnormal pleural (moth sign); 5 - consolidated lung, might be accompanied with moth sign and effusion based on description of lung injury.

Figure 4.3 Raincloud-boxplot displays temporal distribution of the 62 cases grouped by histopathological diagnoses by days on feed on individuals enrolled in this study (n= 62). Histopathological diagnosis of interstitial pneumonia (IP) = interstitial pneumonia; Non-IP = Other respiratory syndromes without IP. Box and whisker represent the upper and lower quartiles, and the heavy line within the box represents the median.

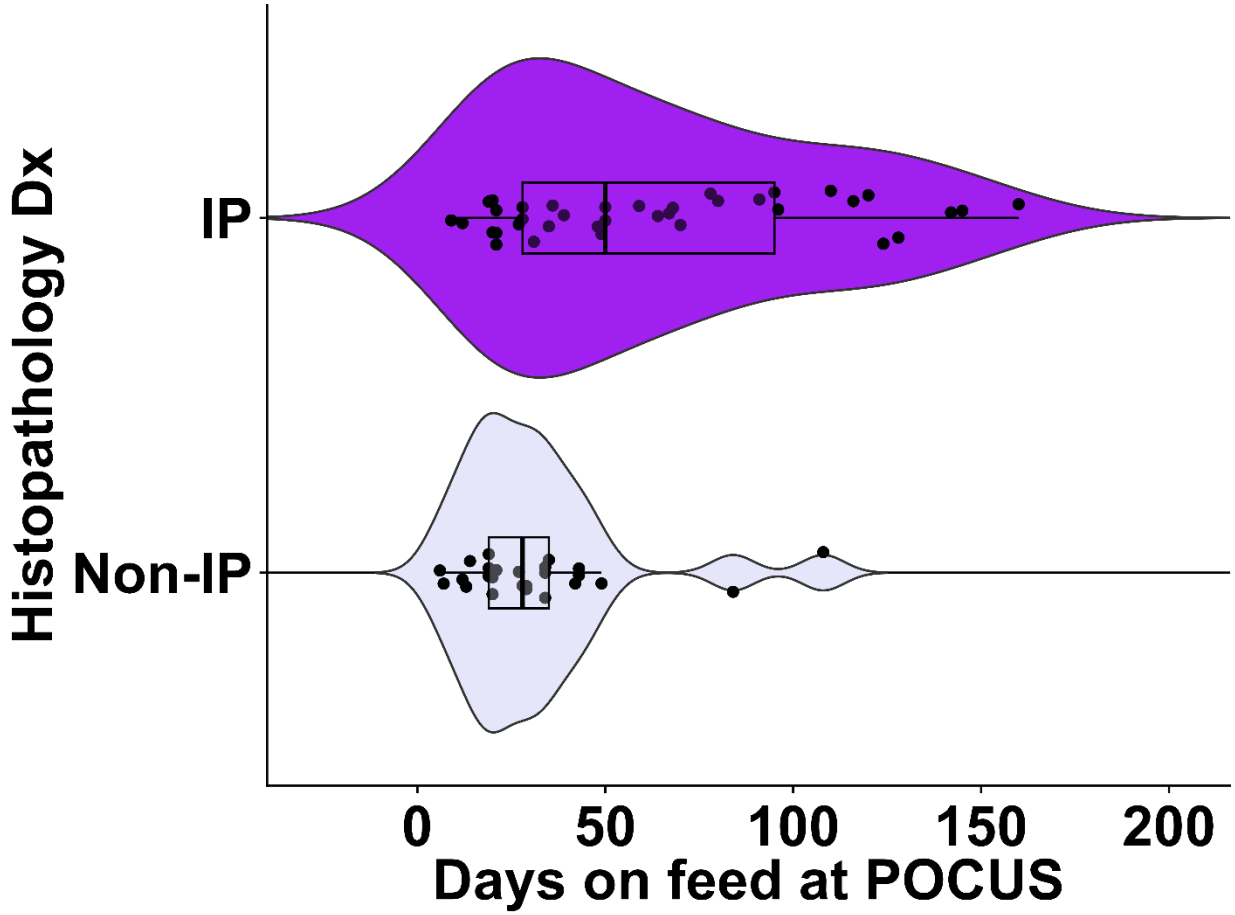


Figure 4.4 Raincloud-boxplot displays temporal distribution of the day intervals between mortalities and their chuteside evaluations with targeted thoracic ultrasonography (TT-POCUS) on individuals enrolled in this study (n= 62). Histopathological diagnosis of interstitial pneumonia (IP) = interstitial pneumonia; Non-IP = Other respiratory syndromes without IP. Box and whisker represent the upper and lower quartiles, and the heavy line within the box represents the median.

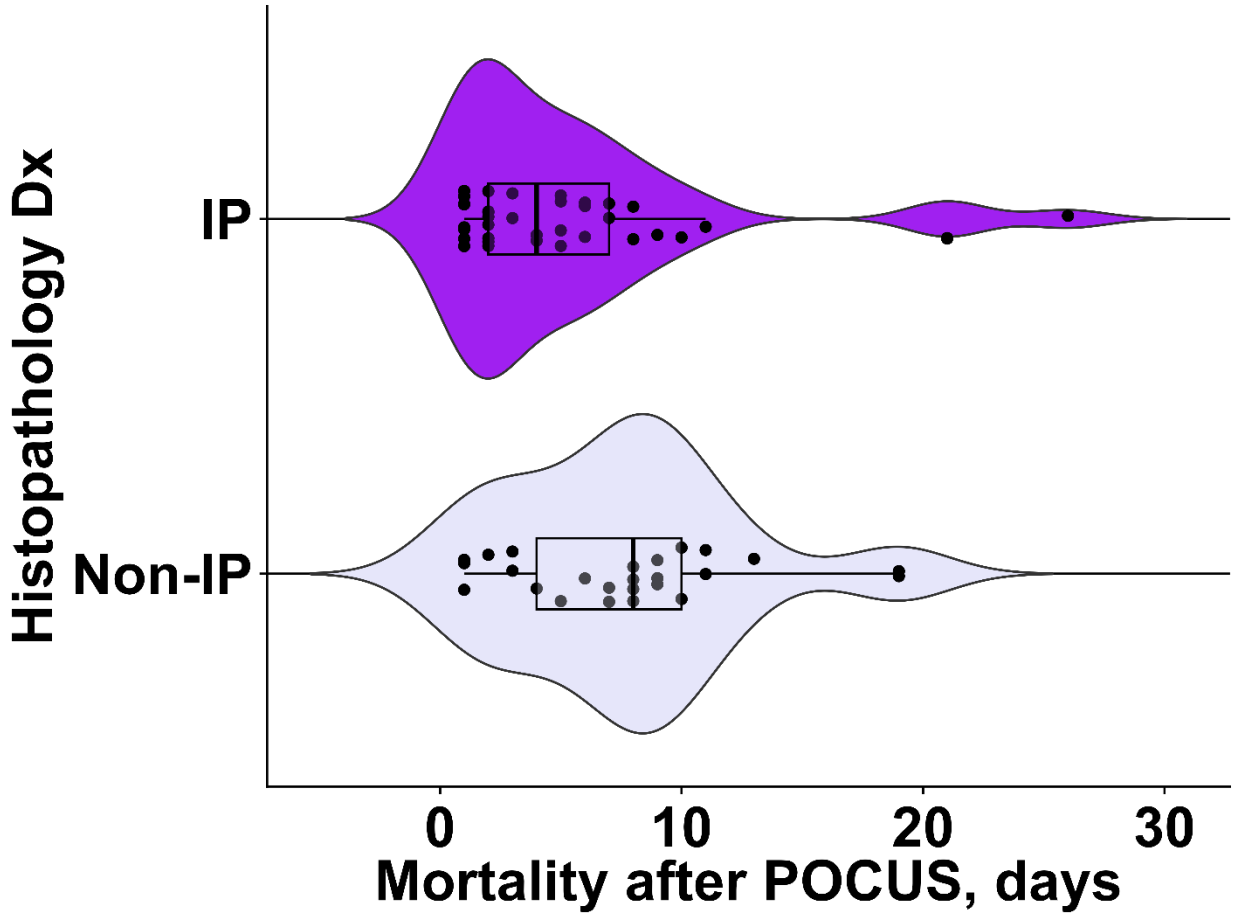
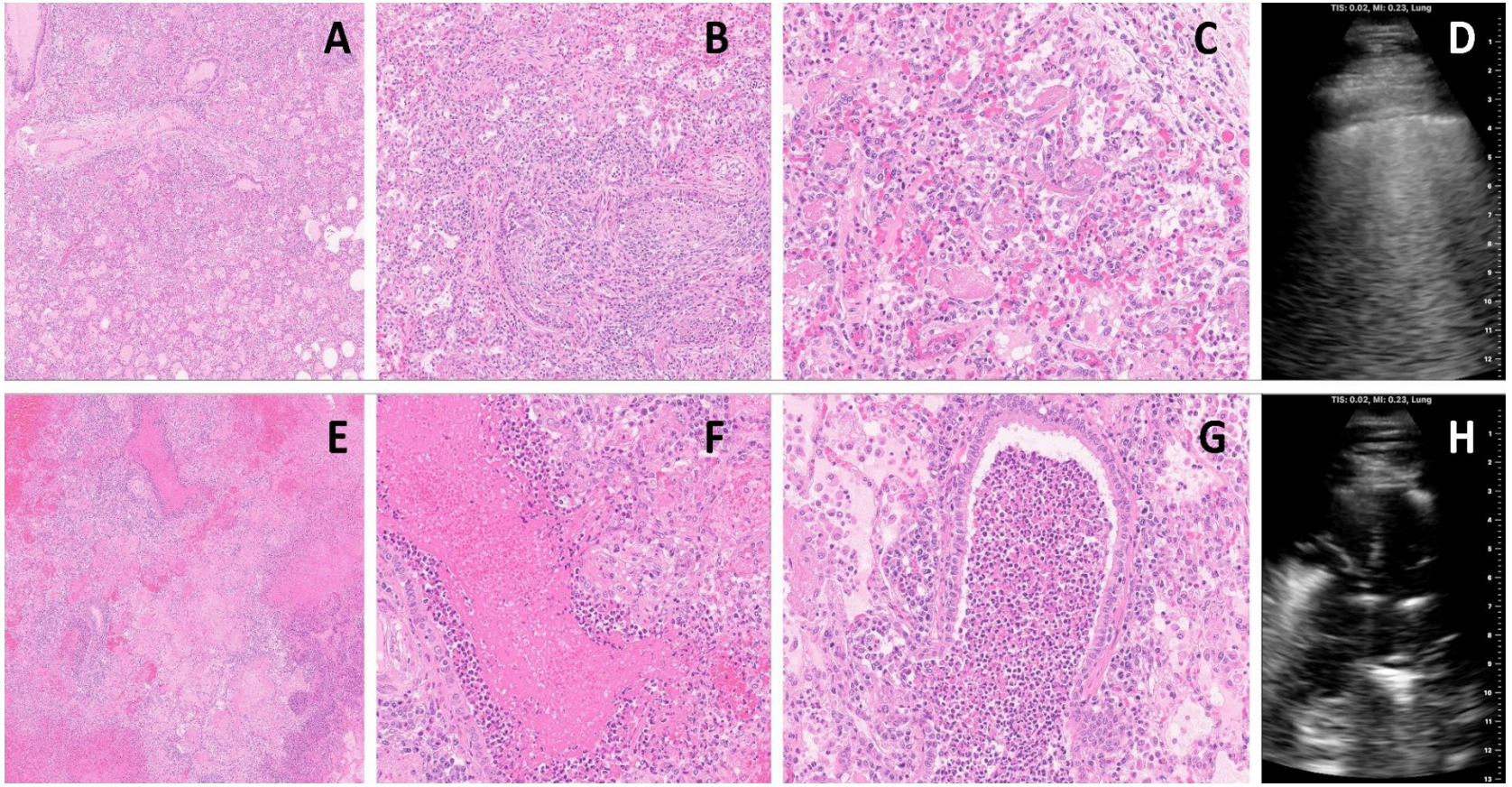


Figure 4.5 (A-D) Histologic photomicrographs from an animal (#453) diagnosed with interstitial pneumonia. Figure 5A (4x): shows alveolar filling by protein, fibrin, and inflammatory cells; Figure 5B (20x) shows interstitial fibroplasia and bronchiolitis obliterans; Figure 5C (20x) shows alveolar hyaline membranes and pneumocyte type II hypertrophy; Figure 5D (case #453) is a transthoracic right lung point-of-care ultrasonography-obtained image showing absence of A-lines, merged B-lines, diffuse hyperechoic lung, with an overall ultrasound lung score (ULS) 3; (E-H) Histologic photomicrographs from an animal (#767) diagnosed with bronchopneumonia. Figure 5E (4x) shows bronchial, bronchiolar, and alveolar filling by inflammatory cells, necrotic debris, and fibrin; Figure 5F (20x) shows necrotizing bronchitis, bronchiolitis, bronchiectasis and bronchiolectasis; Figure 5G (20x) shows neutrophilic bronchitis, bronchiolitis, bronchiectasis and bronchiolectasis. Figure 5H (case #767) is a transthoracic right lung point-of-care ultrasonography-obtained image showing lung consolidation, absence of A-lines, and an overall ultrasound lung score (ULS) 5.



47

Chapter 5 - Utilization of point-of-care evaluation to determine prognosis in feedyard cattle with multiple previous respiratory disease treatments.

Luis F. B. B. Feitoza[1], Brad J. White[1*], Robert L. Larson[1], and Tyler J. Spore[2]

[1]Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, 1800 Denison Ave., Manhattan KS 66506, USA

[2]Innovative Livestock Services, 2006 Broadway Ave., Great Bend KS 67530, USA

Abstract

Determining prognosis to differentiate recoverable and non-recoverable cattle is a critical knowledge gap impacting animal welfare and economic sustainability; and point-of-care (POC) diagnostics could provide prognostic insight. This study aims to determine associations between negative outcomes (mortality/culling) and POC evaluations of chronic respiratory cases. A cross-sectional study evaluated commercial beef feedyard cattle at their third or greater treatment for respiratory disease (n= 98, 799 ± 16 lb (362 ± 7 kg); Steer, n= 37; Heifer, n= 61). Post-evaluation outcomes were classified as did not finish (DNF = mortality / culling) or recovered (still present in feeding system) 60-days post-enrollment. Captured POC parameters were: sex, days on feed, body weight, pulse oximetry, pulmonary auscultation score at cranio-ventral lobe/caudo-dorsal lobe, and targeted thoracic lung ultrasound imaging. Multivariate logistic regression was used to evaluate potential associations between DNF and POC parameters. Only

ultrasound variables (B-line count, moth sign, and B-line area) were significantly ($P<0.05$) associated with DNF. Cases with B-line count ≥ 3 had higher probability of DNF (84% ± 5%) compared to cases with < 3 B-lines (34% ± 22%). Presence of moth sign was associated with higher DNF (78% ± 13%) compared to cases with no moth sign (43% ± 15%). Average B-line area was higher (18.7 ± 1.2 cm2) in DNF cases compared to recovered cases (9.6 ± 1.2 cm2). Strategic use of targeted thoracic POC ultrasound has potential usefulness for determining prognosis for chronically ill respiratory cases; offering valuable insights for treatment and management decisions in feedyard cattle health.

Keywords: Chuteside diagnostics, feedlot cattle, precision veterinary medicine.

Introduction

Respiratory diseases in feedyard cattle represent a significant challenge to both animal welfare and the economic sustainability of the livestock industry. Bovine respiratory disease (BRD) is the leading causes of morbidity and mortality in feedyard operations, resulting in substantial financial losses due to decreased productivity, increased veterinary costs, and mortality.[1–3] The complex etiology of respiratory conditions in cattle, often involving a combination of viral and bacterial pathogens, environmental stressors, and management practices, complicates both diagnosis and prognosis.[2,4–6] There is a need for estimating the degree of pulmonary injury to be able to choose the correct treatment strategy and management interventions. Treatment decisions are made at the point-of-care (chuteside), and the inability to provide an accurate prognosis for pneumonia is a problem.

The economic impact of cattle treated 3 or more times for BRD can be substantial. A study has shown that carcasses of cattle treated multiple times were worth $54.01 less compared to cattle that were never treated.[7] Another study calculated net return ($/animal) of -$227 for cattle treated ≥ 3 times for bovine respiratory disease, accounting for slaughter value, processing costs, treatment costs, feed costs, and other costs.[8] Booker discussed salvage slaughter of chronically diseased cattle and estimated a gross return of 25 to 50% of the original purchase cost, which would not cover feed costs, treatment cost, and any expense invested on that animal.[9] Economic losses from chronic illness can be substantial; however, identifying cattle as recoverable or non-recoverable allows operations to make informed decisions impacting both animal welfare and economic sustainability. The substantial economic impact of chronically ill

cattle shows the need for effective management, making the use of chuteside diagnostic tools crucial for improving prognostic accuracy and treatment decisions.

Diagnostic tools, such as rectal temperature,[10,11] facial infrared thermography,[10,11] pulse oximetry,[12] computer-aided lung auscultation,[13] traditional auscultation,[10,14] and point-of-care ultrasound, have shown promise in enhancing the accuracy of respiratory disease diagnosis in cattle.[15–21] Diagnostics at POC is a promising approach for real-time evaluation of diseased cattle, which facilitates the identification and improved prognostic accuracy for different respiratory conditions.[22–24] While research has been conducted in cattle at the first treatment for respiratory disease,[24] no work has been published describing prognostic factors for cattle with multiple previous BRD treatments. After feedyard cattle have received multiple treatments, the overall prognosis is worse than at initial treatment,[25,26] and evaluation of cattle being treated for the third or greater time is an important decision point to determine if the animal is recoverable or non-recoverable. While several POC tools are available, little work has been done evaluating potential relationships with specific cattle outcomes.

This study aims to evaluate prognostic ability of POC measures by determining potential associations between DNF outcome (mortality or culling) with POC parameters (pulmonary ultrasound, pulse oximetry, lung auscultations, and cattle demographics) observed at the time of treatment in feedlot cattle with chronic respiratory disease. More accurate prognosis enables strategic therapeutic and management decisions ultimately improving animal welfare and economic sustainability.

Materials and Methods

The Institutional Animal Care and Use Committee (IACUC) at Kansas State University approved this study protocol (Protocol #4897).

Experimental Design & Enrollment Criteria

This experiment was designed as a cross-sectional observational study with individual cattle as the experimental unit. Feedyard cattle respiratory morbidities were evaluated at the time of 3rd or greater BRD treatment by the use of various POC approaches by a trained veterinarian (LF). Data collection took place from July to December 2023, at one feedyard in the high plains region of the United States.

Cattle enrolled in the study were evaluated at the time of treatment for chronic respiratory disease. Chronic respiratory disease was defined as feedyard cattle that had been treated previously for any respiratory disease such that the cumulative treatment events at time of POC was >= 3. Variables collected were: targeted thoracic ultrasound (TT-POCUS) at the level of the right caudodorsal lung lobe, pulse oximetry (SPO2 and pulse rate per minute), pulmonary auscultation on the right cranioventral lung field and right caudodorsal lung field, and individual animal demographics (sex, body weight, and days on feed). Feedyard personnel were blinded to the POC evaluation, and any management decision regarding treatment and culling for the cattle observed in this cross-sectional study were made solely by feedyard personnel using existing protocols. Any animal that was culled or died for any non-respiratory disease related reason within the 60-day post-evaluation period were excluded from analysis.

TT-POCUS

In this study, enrolled commercial feedyard cattle underwent a targeted thoracic point of care ultrasound (TT-POCUS) method, with cattle restrained in a hydraulic squeeze chute. Due to the fast-paced environment of the commercial feedyard, each ultrasound examination was limited to 60 seconds. The evaluation focused on the right caudodorsal lung lobe, with the probe positioned between the 8th and 11th ribs. The ultrasound unit had a frequency range of 1 to 10 MHz and featured a specialized point-of-care probe with a 5 cm x 3 cm footprint, optimized for lung assessments. To prepare the scanning area, 70% isopropyl alcohol was applied to the animal in the area to be scanned, and no hair trimming or shaving was utilized.

The TT-POCUS procedure was accomplished with probe placement between the ribs (short-axis orientation), starting at the 11^{th} intercostal space. The probe was moved from the proximal aspect of the intercostal space, just ventral from the transverse processes, the proximal landmark is the *longissimus dorsi* muscle, sliding the probe ventrally to visualize the liver, diaphragm, and caudal portion of the lung. This process was repeated from the 11^{th} to 8^{th} intercostal space from dorsal to the ventral aspect of the lungs, repeating the process for each intercostal space. In addition to sliding the probe, a "fanning" technique was employed, adjusting the probe angle relative to the skin to enhance the visualization of pulmonary tissue between ribs.

Real-time data collection from the TT-POCUS evaluation was conducted by a trained veterinarian (LF). The collected variables included ultrasound lung scores (ULS), B-line count, A-line count, and the presence or absence of pleural effusion and pleural abnormalities (moth sign). The ULS were classified as follows: Score 1 - presence of fewer than 3 thin B-lines (indicative of healthy lung); Score 2 – presence of 3 or more thin B-lines; Score 3 - merged B-lines; Score 4 - multiple wide/merged B-lines with abnormal pleural findings (moth sign); Score

5 - consolidated lung. B-line count was determined by counting the number of B-lines on the lung field with the most apparent abnormalities; A-lines were counted using the same criteria as B-line count, with the only difference being A-lines are reverberation of the pleural line (horizontal artifacts), and B-line are vertical beams that goes across the lung field. Moth sign and pleural effusion were determined by having presence or absence of these abnormalities during scanning of 8^{th} to 11^{th} intercostal spaces. *Post-hoc* variables included measures of image brightness and B-line width, specifically pixel intensity density, average gray value, and B-line area (cm^2). These measurements were obtained from a single frame of the 60-second video clip using ImageJ 1.54b software, focusing on the frame with the most noticeable tissue abnormalities to assess injury within the caudodorsal lung region.

Pulse oximetry

Cattle restrained in the squeeze chute were evaluated with a pulse oximeter unit [c] equipped with a transflectance sensor [d] to collect blood oxygen saturation (SPO2) and pulse per minute (PPM) data. After cleaning debris and cerumen, the sensor was placed in between the ear ribs by a trained veterinarian (LF), within 15 seconds, the transflectance signal would stabilize to allow for data annotation.

Pulmonary auscultation score

Feedyard cattle were restrained in a squeeze chute, and auscultation scores for examinations lasting <30 seconds for each of two fields (caudodorsal and cranioventral) were recorded. Stethoscope e placement for these evaluations took place on the right caudodorsal lung lobe and right cranioventral lung field. Specifically, the cranioventral assessment took place

between the 4th and 5th rib on the right side of the animal, caudal to the radio-ulnar joint (CVPAS). The other auscultation field, the caudodorsal assessment, took place between the 8th and 9th rib on the right side of the animal at the level of the proximal third of the intercostal space (CDPAS). In this study, lung auscultation for both fields and all cattle were carried by the same trained veterinarian (LF). Pulmonary auscultation score (PAS), was defined in a modified score system based on previous published work,[10] where scores were condensed from the range 1 to 10, to 1 to 5 , where: 1 – normal lung sounds; 2 - presence of mild crackles/rales; 3 - presence of moderate crackles/rales ; 4 - presence of severe crackles/rales; 5 - presence of severe diffuse crackles/rales.

The primary outcome of this study was DNF, which was determined 60 days post-enrollment (POC day). The three possible outcomes at 60-day were: culled, died, or lived. The DNF outcome was a combination of cattle that failed to recover from respiratory disease in the 60-day interval (culled + died). If any case was culled or died for any non-respiratory disease related reason, this would result in removing the case from the statistical analysis. It is important to reiterate that any treatment intervention, or culling decisions were solely determined based on feedlot personnel assessment, which were blinded to the POC evaluations.

Statistical Analysis

Microsoft Excelf (Microsoft Excel, Microsoft 365) and RStudiog (RStudio, version 2023.12.1) were utilized for data management, processing and analyses. For descriptive analysis, a table comparing the outcome variables was constructed using the “dplyr” and “tidyr” packages in RStudio.

The variables body weight, days on feed, B-line count, and A-line count were categorized prior to data analysis to avoid assumption of linear relationships with the primary outcome (DNF). Body weight categories (BW) were: < 600, 600 to 799, 800 to 1000, > 1000 lb (< 272, 272 to 361, 362 to 453, and > 453 kg), and days on feed (DOF) were categorized similar to previous work27 into early (0 to 42), mid (43 to 71), and late (> 71) DOF. The B-line count was categorized into less than or greater than or equal to 3 B-lines similar to previous work.28 Finally, A-line count categories were categorized as < 3 and ≥ 3 similarly to B-line categories. Other categorical variables were: sex, pleural effusion, moth sign, ULS, and PAS. Variables analyzed as continuous were: Pixel intensity density, average gray value, B-line area, SPO2, and PPM.

The data analysis employed a generalized linear mixed-effects model implemented through the 'glmer' function from RStudio's "lme4" package. To appropriately address the binomial response variable, the 'logit' function in RStudio was applied, the degree of significance was set at $p < 0.05$. The primary outcome of interest was the 60-day status, specifically assessing the association of POC parameters with the odds of DNF. Cattle lot was used as a random effect in all models to account for a lack of independence in cattle managed and housed in similar fashions. Model development involved using the 'scope' parameter to ensure that fixed variables (Sex and Days on Feed) were consistently included across model iterations. Stepwise selection was performed in a backward direction using the 'stepAIC' function from the 'MASS' package in RStudio, iteratively removing the least significant variables based on the lowest AIC values. Multicollinearity (VIF) was also assessed when final model was determined using the "car" package in RStudio. Variables were considered to have

high collinearity when a coefficient was estimated to be > 2.5.[29] The log-odds from the logistic regression model was then converted to probabilities using the package "emmeans" in RStudio.

Results

Descriptive statistics

At the time of third or greater respiratory treatment event, 98 cattle were POC evaluated. Although, only 72 cattle were evaluated with pulse oximetry and pulmonary auscultation due to a protocol deviation where these parameters were added to the study after 26 cattle were already enrolled. There were 61 heifers (62%), and 37 steers (38%) enrolled with descriptive statistics listed in. There were 19 cases (19%) in the early feeding phase (0 to 42 DOF) category, 39 cases (40%) in the mid feeding phase (43 to 71 DOF) category , and 40 cases (41%) in the late feeding phase (>71 DOF) category. Seventy-five percent of the 98 cases were within the 600 to 799 lbs (272 to 361 kg) and 800 to 1000 lbs (362 to 453 kg) categories. Most cases DNF (n=63, 64%) within the 60-day post-evaluation period and 35 cases were deemed recovered (36%).

The distribution of cases enrolled by DOF at time of treatment grouped by 60-day outcome indicate that DNF cases occurred dispersed throughout the feeding period (median of 66 DOF and interquartile range (IQR) 19-113 DOF). In contrast, recovered cases displays an apparent narrower distribution with a median of 62 DOF and IQR 36-88 DOF (Figure 5.1).

Descriptive statistics for POC measures are listed in Table 5-1. Assessment by TT-POCUS revealed that most cases (73/98, 74%) were ULS 3,4, or 5. Most cases also had >= 3 A-lines (61, 62%) and B-lines (78, 79.5%). Blood oxygen saturation (%) averaged 86.5% ± 1.4% for cattle that recovered and DNF cases averaged 82.2% ± 1.5%. Pulse (PPM) averaged 78 ± 4 PPM for cattle that recovered and 72 ± 3 PPM for DNF. Cranioventral PAS indicated that 90%

of cattle placed in scores 1 and 2 recovered, whereas, 56% of cattle with scores 3 and 4 DNF; 86% of cattle with cranioventral PAS of 5 had an outcome of DNF. Caudodorsal PAS indicated that 40% of cattle placed in scores 1 and 2 DNF, while, 73% of cattle with scores 3 and 4 DNF; and 87% of cattle with caudodorsal PAS of 5 DNF.

Logistic regression

The multivariate logistic regression identified only three variables associated with DNF: B-line count category, moth sign, and B-line area ($p = 0.02$, $p = 0.03$, and $p = 0.04$, respectively). Although, included in the model as fixed variables, sex and DOF were not significantly associated with DNF ($p = 0.75$ and $p = 0.20$, respectively). On the other hand, variables that were not significantly associated with DNF outcome and therefore not selected by the study's multivariate logistic regression were: BW, ULS, A-line category, presence of pleural effusion, pixel intensity density, average gray value, SPO2, pulse rate, and both PAS scores. ($p > 0.05$). No variables were removed from the final model due to collinearity.

Tukey-adjusted probabilities using the fitted model showed the probability of DNF was lower when B-line < 3 (34% ± 22%) compared to when B-line ≥ 3 (84% ± 5%). Presence of moth sign displayed higher probability of DNF (78% ± 13%) compared to when moth sign was absent (43% ± 15 %). The *post-hoc* continuous variable B-line area showed an average of 18.7 ± 1.2 cm^2 for cattle that did not finish compared 9.6 ± 1.2 cm^2 for cattle that recovered.

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Discussion

Chronic respiratory disease represents a significant challenge in cattle management including the challenge to make appropriate decisions for differentiating between cattle likely to recover cattle likely to be unresponsive to further treatment.[30–32] This study evaluated multiple POC modalities in chronically diseased cattle to determine potential associations with the likelihood of DNF. While several POC metrics were not associated with clinical outcomes, some of the TT-POCUS variables could provide insight to improve prognostic accuracy. Accurate prognosis classification is crucial for improving animal welfare and economic sustainability.

An accurate description of or industry-wide acceptance of a chronically diseased respiratory case is lacking. Some reports define chronic cases based on extent of pulmonary lesion as identified by ultrasonography;[30,31] while other reports define chronic cases by the use of a computer-aided auscultation device,[13] and others define chronic cases as cattle with repeated unsuccessful treatment interventions.[7,33,34] In this study, chronic disease was defined as at least three treatments and at least one diagnosis of respiratory disease. A majority (64%) of outcomes for cattle in this study population were classified as DNF, meaning enrolled cattle had either died or were culled within the 60-day evaluation period. These numbers are similar to previous work: in 2020, Blakebrough-Hall et al observed 58% mortality in cattle deemed chronic (≥ 3 treatments).[8] Based on outcomes, the study population was representative of chronically diseased feedyard cattle.

The results of our multivariate logistic regression analysis identified key variables significantly associated with the DNF outcome. Among all the variables tested, the three variables associated with the model were derived from TT-POCUS: B-line category, presence of

moth sign, and B-line area. The presence of more than three B-lines was associated higher likelihood of DNF compared to cattle with fewer than 3 B-lines and the results illustrated a good distinction from the baseline overall DNF risk. Results agreed with several reports illustrating the importance of B-line and pleural observation in thoracic ultrasound evaluation in both human and veterinary medicine.[28,35–38] Studies in human medicine have similarly demonstrated that increased B-line counts are strongly correlated with severe lung diseases and poorer prognoses, which supports our findings in cattle.[38,39] Utilizing a POC B-line count could provide information on prognosis in chronically diseased cattle.

The presence of moth sign was another significant indicator of poor prognosis, with a 78% probability of DNF when the moth sign was present, compared to 43% when it was absent. Moth sign is often associated with advanced pulmonary pathology, including consolidation, sub-pleural fibrosis, and severe interstitial changes, which are difficult to reverse, particularly in chronically affected cases.[28,38,39] Finally, the post-hoc variable B-line area revealed a significant difference between cattle that did not finish (DNF) and those that recovered. Merged B-lines (coalescent or fused), could be indicative of extensive lung pathology thereby contributing to poor outcomes. Interestingly, the *post-hoc* B-line area did not display collinearity with B-line count categories, suggesting that B-line area can provide additional context, perhaps accounting for B-line merging. Both B-line count and B-line area provided separate pieces of information to the final multivariable model; however, B-line count remains the more feasible and easily interpretable metric for field application.

Pulse oximetry parameters, including blood oxygen saturation (SPO2) and pulse rate, were not associated with the DNF outcome despite being relevant indicators of respiratory function in clinical settings.[23,40] The lack of association in this study could also be attributed to

the site utilized to place the sensor (external ear). In 1999, Coghe et al. evaluated the accuracy of different sites for sensor placement in cattle, where he observed that nasal septum, tail and genital mucosa in females provided stable and strong signal.[40] In 2019, Baruck and associates also evaluated SPO2 to assess bovine respiratory disease progression; where it was observed that SPO2 could provide an objective measure of lung consolidation; their creative apparatus allowed for sensor placement intranasally to reach nasal folds.[23] Another potential limitation is that pigmented hide or skin has been often reported to be a contributing factor for inaccuracies, since majority of the cattle investigated were angus-cross cattle with predominately black hides, we theorize that could have been a limitation of this evaluation.[41–43] The type of measurement may not be the only factor influencing the relationship between SPO2 and outcomes as previous work that measured SPO2 from arterial blood also displayed no relationship between SPO2 and the level of lung lesions at necropsy.[44] Findings from this work did not illustrate a relationship between SPO2 or pulse and predicting accurate prognosis in chronically ill cattle.

Pulmonary auscultation scores (PAS) for both the cranioventral and caudodorsal lung areas did not demonstrate a significant association with DNF outcomes in our multivariate model. Buczinski et al. (2014), tested the ability of pulmonary auscultation to detect lung consolidation and observed sensitivity of < 6% and 97% specificity.[18] Previous research has also indicated that auscultation is valuable for detecting bovine respiratory disease,[10,14] although, it may lack the sensitivity needed to predict long-term outcomes in chronic cases.[18] One reason PAS may not have been included in the final multivariable model is the inclusion of findings from TT-POCUS captured similar information as PAS related to associations with DNF.

This study evaluated several potential POC technologies and only TT-POCUS findings were significantly associated with DNF. While other traditional metrics such as pulmonary

auscultation were not significantly associated with outcomes in this study, the strong associations observed with TT-POCUS variables suggest that these features should be prioritized in clinical evaluations. The ability to gather this valuable information at the time of disease treatment to accurately predict DNF outcomes could significantly enhance decision-making in feedyard management; potentially improving the overall health and productivity of the herd. Further research is warranted to refine these techniques and validate their applicability across diverse feedyard operations.

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Author contributions:

Conception and design, L.F., B.W., R.L., and T.S; data acquisition, L.F., B.W., and T.S.; data analysis, L.F., B.W., R.L.; interpretation, all authors; primary manuscript drafting, L.F.; critical review and revision of the manuscript, all authors; approval of the final published version, all authors.

Endnotes:

[a] Butterfly iQ+ Vet, Butterfly Network Inc., Burlington, MA

[b] ImageJ, U. S. National Institutes of Health, Bethesda, MD

[c] Masimo Rad 87, Masimo Corp., Irvine, CA

[d] Masimo Transflectance 3531 M-LNCS TF-I AH, Masimo Corp., Irvine, CA

[e] 3M Littmann Cardiology III, Maplewood, MN

[f] Excel 365, Microsoft, Redmond, WA

[g] RStudio, R Foundation, Vienna, Austria

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Table 5-1 Descriptive statistics of animals according to their demographics (sex, weight, and days on feed categories), real-time chute-side evaluations (ultrasound lung score, blood oxygen saturation, pulse, auscultation scores, A-line count categories, B-line count categories, pleura effusion, moth sign; and *post-hoc* evaluations (B-line area, gray value, intensity density).

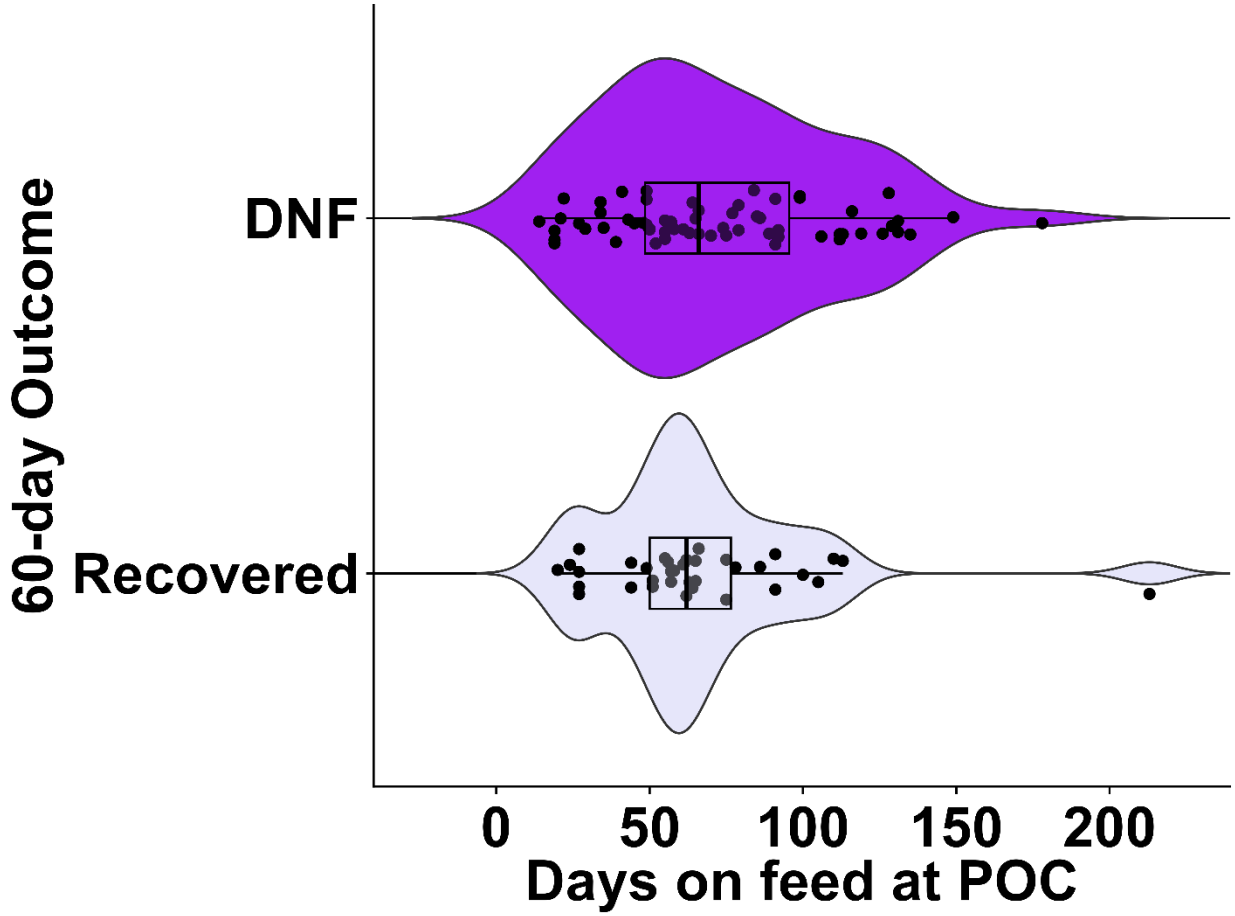
	60-day outcome	
	Recovered[1]	**DNF[2]**
Enrolled animals	35	63
Sex		
Heifer	22	39
Steer	13	24
Days on feed at enrollment categories		
0 to 42	6	13
43 to 71	18	21
> 71	11	29
Body weight, average (SEM), lb	831.48 (± 26.2)	779.68 (± 19.3)
Body weight, categories, lb		
< 600	4	9
600 to 799	9	26
800 to 1000	18	21
>1000	4	7
Real-time measurements		
Ultrasound lung score, head*		
1	5	0

2	11	9
3	13	22
4	5	27
5	1	5
Blood oxygen saturation (SEM), %*§	86.5 (± 1.4)	82.2 (± 1.5)
Pulse, average (SEM), PPM*§	78 (± 4)	72 (± 3)
Auscultation score (cranioventral), head*§		
1	2	0
2	7	1
3	11	13
4	7	10
5	3	18
Auscultation score (caudodorsal), head*§		
1	9	3
2	12	10
3	5	11
4	3	11
5	1	7
A-line count, head*		
0 to 2	6	31
≥ 3	29	32
B-line count, head*		
0 to 2	18	2

≥ 3	17	61
Pleural effusion, head*		
Absent	28	50
Present	7	13
Moth sign†, head*		
Absent	29	35
Present	6	28
***Post-hoc* measurements‡**		
B-line area, cm², average (SEM)	9.6 (± 1.2)	18.7 (± 1.2)
Gray value, average (SEM)	100.8 (± 8)	110 (± 5)
Intensity density, average (SEM)	16 (± 1)	18 (± 1)

[1]Animals that were live 60-day post-evaluation; [2]Animals that were culled or dead within 60-day post-evaluation; *Variables collected at chute-side real-time. †Moth sign is noted when the pleural line shows irregularities like pleural thickening and indentations; ‡*Post-hoc* measurements were performed by using the ImageJ 1.54. Measurements were taken from a still image, where the frame of choice was the frame with most ultrasonographic artifacts and abnormalities. §Only animals enrolled in the fall (n= 72) were evaluated with pulse oximetry and pulmonary auscultations.

Figure 5.1 Raincloud-boxplot displays temporal distribution of the days on feed at time of chuteside evaluations (POC) on individuals enrolled in this study (n= 98). 60-day outcome of DNF = did not finish (cull/death), recovered = animals that were alive at the 60-day post-evaluation. Box-plot represent the upper and lower quartiles, and the line within the box represents the median.



Chapter 6 - Associations Between Chuteside Evaluations and 60-day Outcomes in Feedyard Cattle at Time of First Treatment for Respiratory Disease

Luis F. B. B. Feitoza[1], Brad J. White[1*], Robert L. Larson[1], and Tyler J. Spore[2]

[1]Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, 1800 Denison Ave., Manhattan KS 66506, USA

[2]Innovative Livestock Services, 2006 Broadway Ave., Great Bend KS 67530, USA

Abstract

Point-of-care (POC) chuteside diagnostics may be valuable to enhance prognostic accuracy for diseased cattle. Accurate prognosis for cattle at time of first treatment with pneumonia facilitates informed management practices and impacts both animal welfare and antimicrobial stewardship. This study aimed to identify potential associations between chute-side parameters at the time of first treatment with the likelihood of first treatment failure (FTF) and negative outcomes (animal that did not finish due to mortality or culling, DNF).

A cross-sectional observational study evaluated commercial beef feedyard animals (n= 819, avg 797.9 ± 6.7 lb; Heifer, n= 475; Steer, n= 346) chute-side at time of first bovine respiratory disease (BRD) treatment. Multivariate logistic regression models were generated to evaluate potential associations between outcomes of interest (FTF and DNF) and sex, days-on-feed (DOF), bodyweight (BW), breed, pulse oximetry, lung auscultation score at cranio-ventral

lobe/caudo-dorsal lobe, and ultrasound lung score (ULS, scores 1-5) at caudo-dorsal lobe. Outcomes (FTF/DNF) were determined 60-days post-enrollment for each animal.

Variables significantly ($P< 0.05$) associated with DNF and FTF models included ULS, B-line count, moth sign, DOF, and BW. Cattle not finishing feeding phase were more likely to present a ULS of 5 (74%) compared to ULS of 1-4 (18%, 23%, 28%, 38%, respectively). Additionally those cattle with a ULS of 5 were more likely to be a treatment failure (74%) compared to those presenting a ULS of 1-3 (35%, 37%, 41%, respectively). B-line count >=3 an increased probability of negative outcomes was observed (61%, DNF; and 64%, FTF). Presence of moth sign increased negative outcomes probabilities (51%, DNF; and 65%, FTF).

This research identified key pulmonary and cattle demographic factors at chute side that are valuable potential prognostic parameters when cattle are first treated for BRD in a feedlot setting.

Key words: Chuteside diagnostics, feedyard cattle health, precision veterinary medicine

Introduction

Bovine respiratory disease (BRD) is a significant concern in the feedyard industry, representing one of the leading causes of morbidity and mortality in cattle.[1,2] The economic implications of BRD are substantial, encompassing not only direct costs related to treatment and mortality, but also indirect costs from reduced weight gain, poor carcass quality, and overall productivity.[2–5] The complexity of BRD in cattle often can be attributed to a combination of viral and bacterial pathogens, environmental stressors, and management practices, making diagnosis and prognosis difficult.[1,6,7] Beyond economic impacts, BRD affected cattle often experience severe distress and prolonged recovery periods, which is a significant animal welfare concern.[2]

Accurately prognosing cattle at time of first BRD treatment is a critical yet challenging aspect BRD management. Early and accurate prognosis is essential for informing management practices, guiding decisions on treatment interventions, and determining the appropriate allocation of resources.[8,9] Potentially, cattle welfare can be positively impacted by these decisions, as an accurate prognosis can prevent unnecessary suffering by identifying animals that may not benefit from further treatment, but will from early culling.[10]

Moreover, point-of-care (POC) diagnostics have potential to be adopted as valuable tool for enhancing prognostic accuracy at the chute-side. The POC tools such as pulse oximetry,[11–13] lung auscultation,[14,15] and thoracic ultrasound have been tested and utilized in veterinary medicine to provide real-time cattle pulmonary health insights.[11,16–21] These tools can be particularly relevant in feedyard operations, where rapid and accurate assessments are crucial for effective decision-making. Thoracic ultrasound, for instance, allows for the visualization of pulmonary abnormalities that may not be detectable through traditional methods,[22] while pulse

oximetry provides a non-invasive measure of blood oxygen saturation,[11] which can offer critical information about respiratory function.

Despite the potential benefits of POC diagnostics, there is a notable gap in the literature regarding their use for prognosing first treatment failure (FTF) and death/culling (DNF) in feedyard cattle at time of treatment.[23] Current research has primarily focused on the application of these tools in clinical settings, with limited exploration of their utility in chute-side scenarios. This lack of data underscores the need for studies that evaluate the associations between chute-side diagnostic parameters and critical outcomes such as FTF and DNF.

The objective of this study was to address this gap by assessing the associations between specific chute-side parameters including, cattle demographics, targeted thoracic ultrasonography, pulmonary auscultation, and pulse oximetry to the association with first treatment failure and not finishing the feeding phase (DNF) in a 60-day post-enrollment interval. By identifying valuable prognostic parameters for use at the chute-side, this research aims to contribute to the broader goal of improving feedyard cattle management, ultimately enhancing both animal welfare and economic sustainability in the industry.

Materials and Methods

The study protocol (Protocol #4897) was reviewed and approved by the Institutional Animal Care and Use Committee (IACUC) at Kansas State University.

Experimental Design & Enrollment Criteria

This research was designed as a cross-sectional observational study, with individual feedyard cattle serving as the experimental units. The study aimed to evaluate pulmonary health at the time of first treatment for bovine respiratory disease (BRD) using various point-of-care

(POC) diagnostic tools. Data collection occurred from Summer to Winter of 2023 at a commercial cattle operation located in the high plains of the United States by a trained veterinarian (LF).

Enrolled cattle were selected based on their first treatment for respiratory disease. Animals were selected from home pens to be treated and were treated by feedyard personnel, none of the POC evaluations or data were shared with feedyard personnel at time of treatment to not influence their management decisions.

Demographic data, including sex, breed, body weight (BW), and days on feed (DOF), were recorded for each animal at time of treatment. The study focused on identifying associations between POC diagnostic parameters and 60-day post-enrollment outcomes: First treatment failure (FTF) and did not finish (DNF). First treatment failure, refers to an animal that was re-treated, was culled, or died before the 60-day post-enrollment marker; DNF refers to animals that did not complete the feeding phase due to mortality or culling. Cattle that were deemed finished (decided by the commercial operation) and were sent to the abattoir before the 60-day evaluation period were excluded from the analysis.

Chuteside point-of-care evaluation parameters were collected by the use of pulse oximetry technology, which is capable to measure blood oxygen saturation (SPO2) and pulse per minute (PPM); lung auscultation targeted two thoracic locations on the right lung only (cranioventral and caudodorsal); and the last was targeted thoracic ultrasound (TT-POCUS) at caudodorsal level, this evaluation was also carried only on the right lung. Pulse oximetry and pulmonary auscultation were evaluated on a subset of 443 cattle due to a protocol deviation where these parameters were added late to the study, and after 376 cattle were enrolled already.

Pulse oximetry

Pulse oximetry [a] was performed by a trained veterinarian (LF) on a subset of cattle (n = 443), using a pulse oximeter unit equipped with a transflectance sensor [b]. The sensor was placed on the external ear (between the cartilage ribs) after cleaning to remove debris, and data were recorded once the signal stabilized, which would take around 15 seconds. The measurements included blood oxygen saturation (SPO2) and pulse per minute (PPM).

Pulmonary auscultation score

Pulmonary auscultation was conducted on the same subset of cattle (n = 443), with evaluations performed in less than 30 seconds per animal. The stethoscope[c] was placed on the right caudo-dorsal lung lobe and the cranio-ventral lung field. Specifically, the cranio-ventral assessment was conducted between the 4th and 5th ribs caudal to the olecranon (elbow), while the caudo-dorsal assessment took place on the 8th intercostal space (ICS). Pulmonary auscultation scores (PAS) were assigned on a scale from 1 to 5, based on the presence and severity of abnormal lung sounds, following a modified scoring system derived from DeDonder et al., (2010).[15] Score was defined as follows: 1 – normal lung sounds; 2 - presence of mild crackles/rales; 3 - presence of moderate crackles/rales ; 4 - presence of severe crackles/rales; 5 - presence of severe diffuse crackles/rales.

Targeted Thoracic Point-of-Care Ultrasound

A targeted thoracic ultrasonography (TT-POCUS) procedure was performed on all enrolled cattle (n = 819) while they were restrained in a hydraulic squeeze chute. The ultrasound device [d] of choice had a frequency range of 1 MHz to 10 MHz and was equipped with a

specialized POC probe featuring a 5 cm x 3 cm footprint, with an optimized preset for lung imaging and usage over hair coat, not requiring shaving or trimming. Isopropyl alcohol 70% was utilized to enhance probe to skin contact. Each ultrasound examination was limited to a 60-second duration to accommodate to the feedyard operation routine. The ultrasound area of interest was in the thoracic region correspondent to the right caudo-dorsal lung lobe. Ultrasound probe was placed in a short-axis orientation in the ICS between the 8^{th} and 11^{th} ribs on the right side to scan all areas of interest. The "fanning" technique (movement of the probe in a side-to-side fashion) was applied to maximize area scanned and enhance the visualization of lung tissue.

Ultrasound lung scores (ULS) were assigned based on the observed lung abnormalities, and were categorized as follows: Score 1 – fewer than 3 thin (< 7mm) B-lines; Score 2 – 3 or more thin (< 7mm) B-lines; Score 3 – merged (> 7mm)B-lines; Score 4 – multiple wide/merged (> 7mm) B-lines with abnormal pleural findings (e.g., moth sign); Score 5 – consolidated lung with pleural thickening/irregularities and effusion. In addition, B-line count category (0 to 2 and ≥ 3), A-line count category (0 to 2 and ≥ 3), and presence of pleural abnormalities were recorded (moth sign). *Post-hoc* analysis included measurement of B-line area (cm^2) using ImageJ software [e] from a still frame from the 60-second clip, where the image of choice was the frame with the most pulmonary abnormalities observed, this *post-hoc* measurement was carried on a sub-sample of 710 cases out of the 819 total collected for this study due to availability to saved ultrasound files.

Statistical Analysis

Data management and statistical analyses were conducted using Microsoft Excel [f] and RStudio [g] software. Descriptive statistics were summarized using the “dplyr” and “tidyr”

packages in RStudio. The variables, including bodyweight, days-on-feed, B-line count, and A-line count, were categorized for analysis. The BW as categorized as < 600, 600 to 799, 800 to 1000, > 1000 lb (< 272, 272 to 361, 362 to 453, and > 453 kg); Days-on-feed (DOF) were categorized based on recent published data.[25] Feeding phases were epidemiologically categorized as early-phase (0 to 42 days), mid-phase (43 to 71 days), and late-phase (> 71 days) DOF based on incidence of respiratory disease for each feeding phase. B-line count was categorized as <3, and ≥3, based on established thresholds indicative of pulmonary injury.[24] A-line count was categorized as <3, and ≥3, the rationale behind this cutoff was of exploratory nature. Other categorical variables included were sex (steer and heifers), ULS (1-5), PAS (1-5), and moth sign (yes or no). Continuous data included SPO2, pulse rate, and B-line area (cm^2).

Generalized linear mixed-effects model were carried using the 'glmer' function from the 'lme4' package in RStudio, with a binomial response variable (logit function). The model included fixed effects of sex, BW and DOF, and random effects of cattle lot. Stepwise model selection was conducted using the 'stepAIC' function from the 'MASS' package, with variables removed based on the least significance and model-fitness using AIC values. Multicollinearity was assessed using variance inflation factors (VIF), with a threshold of VIF > 2.5 considered indicative of high collinearity.[26] The significance level was set at $P < 0.05$.

Two outcomes of interest DNF (cull/dead) and FTF (re-treat/cull/dead) were tested to measure association with POC evaluation parameters data:. Each model was tested separately, and model-adjusted probabilities were calculated based final model for each outcome of interest. Following model estimation, the log-odds were transformed into probabilities to facilitate

interpretation. Transformation was performed using the 'emmeans' package in RStudio, enabling the computation of probabilities for each dependent variable included in the model.

Results

Descriptive statistics

Did not finish model

At the time of first respiratory treatment event, 819 cattle were POC evaluated, from those 474 were heifers (58%), and were345 steers (42%) (Table 6-1). There were 70% of cases in the early feeding phase (0 to 42 DOF; n = 573) category, 13% cases in the mid feeding phase (43 to 71 DOF; n = 104) category, and 17% cases in the late feeding phase (>71 DOF; n = 142) category. Seventy-five percent of cases were within 600 to 799 lbs (272 to 361 kg; n=XX) and 800 to 1000 lbs (362 to 453 kg; n =XX) categories. Within 60-day post-evaluation period, most cases were classified as recovered after first treatment (77%; n= 628) and 23% (n=191) cases were culled or died which accounts for the inherent basal prevalence of DNF in this studied population.

The distribution of cases enrolled by DOF at time of first treatment grouped by treatment success 60-day outcome indicate that DNF cases occurred later on the feeding phase and dispersed throughout the feeding period (median of 48 DOF and interquartile range (IQR) 91 DOF). In contrast, recovered cases displays an apparent narrower distribution with a median of 21 DOF and IQR 33 DOF (Figure 6.1).

Assessment by TT-POCUS revealed that most cases (82.4%; n=675/819) were classified as ULS 1, 2, or 3. Majority of the cases that displayed 0-2 B-line count (482, 88%) recovered after first treatment. Most cases also had >= 3 A-lines (459, 56%), were absent of effusion (664,

767 81%), and did not present moth sign (569, 69%). B-line area average was 19.7 ± 18.2 cm^2 for
768 cattle that DNF and 16.11 ± 13.1 cm^2 for cattle that recovered. Blood oxygen saturation averaged
769 83.1% ± 9.4% for cattle that DNF and 85.7% ± 8.5% for cattle that recovered. Pulse (PPM)
770 averaged 75.9 ± 25 PPM for cattle that DNF and 74.5 ± 22 PPM for cattle that recovered.
771 Cranioventral PAS indicated that 92% of cattle placed in scores 1, 2 and 3 recovered, whereas,
772 24.2% of cattle in scores 4 and 5 did not finish (DNF). Nevertheless, only 35% of cattle that
773 presented a cranioventral PAS of 5 did not finish. When caudodorsal PAS is considered, 9.5% of
774 cattle placed in scores 1, 2 and 3 DNF, while, 50% of cattle FTS with PAS 5.
775
776 ***First treatment failure model***
777 At time of first respiratory treatment event, 819 cattle were POC evaluated. Although,
778 only 443 cattle were evaluated with pulse oximetry and pulmonary auscultation due to a protocol
779 deviation where these parameters were added to the study after 376 cattle were already enrolled.
780 There were 474 heifers (58%), and 345 steers (42%) enrolled with descriptive statistics listed in
781 Table 6-2 Descriptive statistics of first treatment outcome model, cattle according to their
782 demographics (sex, weight, and days on feed categories), real-time chute-side evaluations
783 (ultrasound lung score, blood oxygen saturation, pulse, auscultation scores, A-line count
784 categories, B-line count categories, pleura effusion, moth sign); and post-hoc measurement (B-
785 line area).. There were 573 cases (70%) in the early feeding phase (0 to 42 DOF) category, 104
786 cases (13%) in the mid feeding phase (43 to 71 DOF) category , and 142 cases (17%) in the late
787 feeding phase (>71 DOF) category. Seventy-five percent of the 819 cases were within the 600 to
788 799 lbs (272 to 361 kg) and 800 to 1000 lbs (362 to 453 kg) categories. Most cases obtained
789 success after first treatment (n= 492, 60%) within the 60-day post-evaluation period and 327

cases were deemed first treatment failure (40%, inherent basal prevalence of FTF in this studied population).

The distribution of cases enrolled by DOF at time of first treatment grouped by treatment success 60-day outcome indicate that FTF cases occurred dispersed throughout the feeding period (median of 28 DOF and interquartile range (IQR) 57 DOF). In contrast, recovered cases displays an apparent narrower distribution with a median of 22 DOF and IQR 36 DOF (Figure 6.2).

Descriptive statistics for POC measures in the treatment success model are listed in Table 6-2. Assessment by TT-POCUS revealed that most cases (688/819, 84%) were classified as ULS 2, 3, or 4. Majority of the cases that displayed three or more B-line count (156, 58%) were a treatment failure. Most cases also had >= 3 A-lines (459, 56%), were absent of effusion (664, 81%), and did not present moth sign (569, 69%). B-line area average for DNF was 19.7 ± 18.2 cm^2 and 16.11 ± 13.1 cm^2 for the recovered outcome. Blood oxygen saturation (%) averaged 85.9% ± 8.3% for FTS cattle and FTF cases averaged 84.1% ± 9.2%. Pulse (PPM) averaged 74.6 ± 22 PPM for FTS cattle and 75.1 ± 23 PPM for FTF. Cranioventral PAS indicated that 87.4% of cattle placed in scores 1, 2 and 3 displayed first treatment success, whereas, 43.2% of cattle with scores 4 and 5 presented treatment failure; 51% of cattle with cranioventral PAS of 5 had a first treatment failure outcome. Caudodorsal PAS indicated that 29% of cattle placed in scores 1, 2 and 3 failed first treatment, while, 49.5% of cattle FTS with PAS scores 4 and 5.

Logistic regression

Did not finish model

The DNF multivariate logistic regression detected evidences of five variables to be associated with DNF, two variables related to cattle demographics, DOF and BW ($p = 0.001$, and $p = 0.007$, respectively), and three to TT-POCUS, B-line count category, moth sign, and ULS ($p = 0.001$, $p = 0.001$, and $p = 0.04$, respectively). Although, included in the model as fixed variable, sex was not significantly associated with DNF ($p = 0.22$). In contrast, variables that were not significantly associated with DNF outcome and therefore not selected by the study's multivariate logistic regression were: A-line category, presence of pleural effusion, B-line area, SPO2, pulse rate, and both PAS scores. ($p > 0.05$). No variables were necessary to be removed from the final model due to collinearity, VIF were: 1.1 (Sex), 1.9 (DOF), 1.5 (ULS), 2.0 (BW), 1.3 (B-line count), and.1.2 (moth sign).

In Table 6-3, Tukey-adjusted probabilities using the fitted model showed that cases that received first treatment for respiratory disease mid DOF (43 to 71 DOF) or late DOF (>71 DOF) were more likely to DNF (32% ± 7%, and 64% ± 6%, respectively). In contrast, cattle that were treated for respiratory disease at first time early on the feeding phase (0 to 42 DOF) were less likely to DNF (18% ± 3%). Probabilities of DNF by BW showed that cattle <600lb and >1000lb were more likely to DNF (61% ± 10% and 48% ± 8%, respectively), while 600 to 800 (34% ± 6%) and 800 to 1000 (25% ± 5%) were less likely to DNF. The probability of DNF was higher when B-line >= 3 (60% ± 6%) compared to when B-line 0 to 2 (24% ± 4%). Presence of moth sign displayed higher probability of DNF (51% ± 5%) compared to when moth sign was absent (24% ± 4 %). The probabilities to DNF by ULS scores are displayed on Figure 6.3. The probabilities of ULS 1 (18% ± 5%), 2 (24% ± 4%), 3 (28% ± 4%), and 4 (38% ± 6%) were not

different amongst them ($p > 0.05$). However, ULS 5 (74% ± 8%) showed evidence of significant difference from all other scores ($p = 0.001$), showing the greatest probability of DNF.

First treatment failure model

The FTF multivariate logistic regression also detected evidences of five variables to be associated with FTF, DOF and BW ($p = 0.007$, and $p = 0.01$, respectively), and three TT-POCUS variables, B-line count category, moth sign, and ULS ($p = 0.001$, $p = 0.001$, and $p = 0.03$, respectively). Although, included in the model as fixed variable, sex was not significantly associated with DNF ($p = 0.23$). In contrast, variables that were not significantly associated with FTF outcome and therefore not selected by the study's multivariate logistic regression were: A-line category, presence of pleural effusion, B-line area, SPO2, pulse rate, and both PAS scores. ($p > 0.05$). No variables were necessary to be removed from the final model due to collinearity, VIF were: 1.0 (Sex), 1.8 (DOF), 1.3 (ULS), 1.9 (BW), 1.2 (B-line count), and.1.1 (moth sign).

In Table 6-3, Tukey-adjusted probabilities using the fitted model showed that cases that received first treatment for respiratory disease late on feeding phase (>71 DOF) were more likely to FTF (66% ± 6%). In contrast, cattle that were treated for respiratory disease at first time early on the feeding phase (0 to 42 DOF) to mid DOF (43 to 71 DOF) were less likely to FTF (43% ± 4%, and 44% ± 7%, respectively). Probabilities of FTF by BW showed that cattle <600lb and >1000lb were more likely to FTF (60% ± 8% and 53% ± 6%, respectively), while 600 to 800 (53% ± 5%) and 800 to 1000 (38% ± 5%) were less likely to FTF. The probability of FTF was higher when B-line >= 3 (64% ± 5%) compared to when B-line 0 to 2 (43% ± 4%). Presence of moth sign displayed higher probability of DNF (65% ± 4%) compared to when moth sign was absent (37% ± 4 %). The probabilities of FTF by ULS scores are displayed on Figure 6.4. The

probabilities of ULS 1 (36% ± 6%), 2 (37% ± 4%), and 3 (41% ± 4%) were not different amongst them ($p > 0.05$). However, 4 (57% ± 6%) and ULS 5 (75% ± 7%) showed evidence of significant difference from all other scores ($p = 0.006$), showing a greater probability of FTF.

Discussion

This study aimed to investigate the potential prognostic value of various chute-side diagnostic tools, specifically targeted thoracic ultrasonography (TT-POCUS), pulse oximetry, and pulmonary auscultation, in predicting the outcomes of first treatment failure (FTF) and did not finish (DNF) in feedyard cattle with bovine respiratory disease (BRD) at first treatment. The findings shows that certain chute-side diagnostic parameters, in highlight, TT-POCUS variables, are significantly associated with the likelihood of these negative outcomes. These associations offer valuable information into improving the prognosis of cattle treated for BRD and contribute to better management practices.

Associations with first treatment failure and did not finish outcomes

The logistic regression models identified significant associations between both FTF and DNF outcomes and key parameters, such as days on feed (DOF), body weight (BW), B-line count category, ultrasound lung score (ULS), and the presence of the moth sign. The ULS and B-line count emerged as particularly important variables in both models, underscoring the value of TT-POCUS as a prognostic tool for BRD. Specifically, cattle with ULS scores of 5, indicating more severe lung pathology, had a significantly higher probability of FTF and DNF compared to those with lower ULS scores.

Days on feed and body weight as prognostic indicators

The association of DOF and BW with both FTF and DNF outcomes highlights the influence of feeding stage and cattle size on the prognosis of BRD. Similar results investigating epidemiological associations with mortality risk was also observed by Babcok and colleagues.[27]Cattle treated later in the feeding phase (mid DOF and late DOF) showed a greater probability of both FTF and DNF, suggesting that cattle in these stages may be at increased risk due to cumulative stressors and more advanced disease progression.

Similarly, cattle with BW < 600 lb also displayed a higher probability of DNF and FTF. This could be attributed to the fact that lighter cattle may be more vulnerable to BRD due to their transport and adaption stressors. Increased mortality risk in lighter cattle has been observed in multiple reports.[25,28,29]

Ultrasound lung score, B-line count and moth sign

The TT-POCUS variables were found to be strongly associated with both outcomes of interest. The probability of DNF and FTF was significantly higher in cattle with ULS scores of 5, with ULS 4 also demonstrating a greater probability for FTF compared to lower scores. B-line count was another important factor, with cattle showing ≥ 3 B-lines having an elevated likelihood of both DNF and FTF. The presence of the moth sign, a pleural abnormality observed during ultrasound, further increased the probability of poor outcomes. These results support the utility of TT-POCUS in assessing lung pathology and guiding prognosis in BRD cases.

Interestingly, other TT-POCUS variables, such as A-line count and pleural effusion, were not significantly associated with DNF or FTF. This suggests that while certain ultrasonographic findings are highly relevant for prognosis, others may have limited predictive value for these

902 specific outcomes. The use of ULS, moth sign and B-line count as prognostic markers in chute-
903 side settings provides a practical approach for veterinarians to make informed management
904 decisions, enabling more targeted treatment and potentially improving animal welfare and
905 feedyard productivity.

906 These findings aligns with previous research suggesting that severe lung consolidation is
907 indicative of poorer outcomes in cattle with respiratory disease.[20,30–33] In 2019, Timsit and
908 collaborators showed that cattle with lung consolidation (≥5 cm) at the first diagnosis of
909 bronchopneumonia had a significantly higher risk of treatment failure compared to cattle with
910 less severe lung lesions.[20] Another similar finding was reported by Rademacher and colleagues in
911 2014, where mortality risk was increased when lung consolidation was present.[21] B-line count
912 and moth sign (sub-pleural consolidation and pleural thickening) is often described in POC
913 evaluations in small animals, where the presence of moth sign and greater number of B-lines is
914 linked to greater pulmonary insult.[24] In human medicine, B-line evaluation is the core artifact
915 POC assessed for pulmonary diagnostics.[34–42]
916

917 **Pulse oximetry and pulmonary auscultation**

918 Pulse oximetry and pulmonary auscultation has been reported successfully in cattle for
919 viability and association with lung disease.[11–15] Although, pulse oximetry and pulmonary
920 auscultation were included as part of the chute-side evaluations, their parameters were not
921 significantly associated with either DNF or FTF in the final models. Blood oxygen saturation
922 (SPO2) and pulse per minute (PPM) values showed no clear differences between recovered and
923 DNF or FTF and FTS cases, and both cranioventral and caudodorsal pulmonary auscultation
924 scores (PAS) were similarly not associated with these outcomes. The lack of significant

associations may be attributed to several factors, including the variability in SPO2 readings based on sensor placement (external ear), which is different placement than previous research,[11–13] or the potential for subclinical lung pathology not detectable through auscultation.

Conclusions

The identification of TT-POCUS variables as significant prognostic indicators has practical implications for feedyard management. By providing real-time, actionable data at the time of first treatment, TT-POCUS allows for early identification of cattle at higher risk for FTF and DNF. This can support more informed decision-making, including treatment adjustments, selective culling, and resource allocation, ultimately enhancing animal welfare and reducing economic losses.

However, further research is warranted to refine the use of TT-POCUS and other POC diagnostics in feedyard settings. Future studies could explore the development of standardized scoring systems for ultrasound findings and evaluate the cost-effectiveness of implementing POC diagnostics as part of routine BRD management. Additionally, investigating the interplay between multiple POC parameters and their combined predictive power could enhance the accuracy of prognosis in BRD cases.

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954

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956 Authors declare no conflict of interest.
957

958 **Author contributions:**

959 Conception and design, L.F., B.W., R.L., and T.S; data acquisition, L.F., B.W., and T.S.;
960 data analysis, L.F., B.W., R.L.; interpretation, all authors; primary manuscript drafting, L.F.;
961 critical review and revision of the manuscript, all authors; approval of the final published
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963

964 **Endnotes:**

965 [a] Masimo Rad-87, Masimo Corp., Irvine, CA

966 [b] Masimo Transflectance 3531 M-LNCS TF-I AH, Masimo Corp., Irvine, CA

967 [c] 3M Littmann Cardiology III, Maplewood, MN

968 [d] Butterfly iQ+ Vet, Butterfly Network Inc., Burlington, MA

969 [e] ImageJ, U. S. National Institutes of Health, Bethesda, MD

[f] Excel 365, Microsoft, Redmond, WA

[g] RStudio, R Foundation, Vienna, Austria

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Table 6-1 Descriptive statistics of did not finish (DNF) outcome model, cattle according to their demographics (sex, weight, and days on feed categories), real-time chute-side evaluations (ultrasound lung score, blood oxygen saturation, pulse, auscultation scores, A-line count categories, B-line count categories, pleura effusion, moth sign); and *post-hoc* measurement (B-line area).

	60-day outcome	
	Recovered[1]	**DNF[2]**
Sex		
Heifer	348	126
Steer	280	65
Days on feed at enrollment categories		
0 to 42	481	92
43 to 71	77	27
> 71	70	72
Body weight, average (SD), lb	778.3 (± 163)	859.7 (± 254)
Body weight, categories, lb		
< 600	63	27
600 to 799	324	68
800 to 1000	188	36
>1000	53	60
Real-time measurements		
Ultrasound lung score, head*		
1	78	15

2	245	52
3	224	61
4	67	39
5	14	24
Blood oxygen saturation (SD), %*§	85.7 (± 8.5)	83.1 (± 9.4)
Pulse, average (SD), PPM*§	74.5 (± 22.2)	75.9 (± 25.1)
Auscultation score (cranioventral), head*§		
1	12	1
2	103	9
3	137	12
4	89	20
5	39	21
Auscultation score (caudodorsal), head*§		
1	103	6
2	129	13
3	83	14
4	54	19
5	11	11
A-line count, head*		
0 to 2	243	117
≥ 3	389	70
B-line count, head*		
0 to 2	482	67

≥ 3	150	120
Pleural effusion, head*		
Absent	530	134
Present	102	53
Moth sign†, head*		
Absent	491	77
Present	141	110
***Post-hoc* measurements‡**		
B-line area, cm², average (SEM)	16.11 (± 13.1)	19.7 (± 18.2)

[1]Animals that were live 60-day post-evaluation; [2]Animals that were culled or dead within 60-day post-evaluation; *Variables collected at chute-side real-time (n= 819). †Moth sign is noted when the pleural line shows irregularities like pleural thickening and sub-pleural consolidation (indentations); ‡*Post-hoc* measurement (n= 710) was performed by using the ImageJ 1.54. Measurement was taken from a still image, where the frame of choice was the frame with most ultrasonographic artifacts and abnormalities. §Only animals enrolled in the fall (n= 443) were evaluated with pulse oximetry and pulmonary auscultations.

Table 6-2 Descriptive statistics of first treatment outcome model, cattle according to their demographics (sex, weight, and days on feed categories), real-time chute-side evaluations (ultrasound lung score, blood oxygen saturation, pulse, auscultation scores, A-line count categories, B-line count categories, pleura effusion, moth sign); and *post-hoc* measurement (B-line area).

	First treatment 60-day outcome	
	Failure[1]	**Success[2]**
Sex		
Heifer	205	269
Steer	122	223
Days on feed at enrollment categories		
0 to 42	210	363
43 to 71	36	68
> 71	81	61
Body weight, average (SD), lb	802.4 (± 221.4)	793.8 (± 167.1)
Body weight, categories, lb		
< 600	43	47
600 to 799	156	236
800 to 1000	65	159
>1000	63	50
Real-time measurements		
Ultrasound lung score, head*		
1	30	62

2	101	197
3	108	177
4	61	45
5	27	11
Blood oxygen saturation (SD), %*§	84.1 (± 9.2)	85.9 (± 8.3)
Pulse, average (SD), PPM*§	75.1 (± 23.2)	74.6 (± 22.3)
Auscultation score (cranioventral), head*§		
1	4	9
2	34	78
3	39	110
4	42	67
5	31	29
Auscultation score (caudodorsal), head*§		
1	27	82
2	39	103
3	35	62
4	34	39
5	15	7
A-line count, head*		
0 to 2	181	179
≥ 3	137	322
B-line count, head*		
0 to 2	163	386

≥ 3	156	114
Pleural effusion, head*		
Absent	245	419
Present	74	81
Moth sign†, head*		
Absent	166	403
Present	152	98
***Post-hoc* measurements‡**		
B-line area, cm², average (SD)	18.9 (± 17.3)	(15.4 ± 12.2)

[1]Animals that were live 60-day post-evaluation; [2]Animals that were culled or dead within 60-day post-evaluation; *Variables collected at chute-side real-time (n= 819). †Moth sign is noted when the pleural line shows irregularities like pleural thickening and sub-pleural consolidation (indentations); ‡*Post-hoc* measurement (n= 710) was performed by using the ImageJ 1.54. Measurements were taken from a still image, where the frame of choice was the frame with most ultrasonographic artifacts and abnormalities. §Only animals enrolled in the fall (n= 443) were evaluated with pulse oximetry and pulmonary auscultations.

Table 6-3. Probabilities of variables that were associated with outcome of interest (did not finish or first treatment failure) converted from log-odds generated from multivariate logistic regression models grouped by its specific model.

	60-day outcome models*					
	Did not Finish			First treatment Failure		
Items	Probability	SE	*P*-value	Probability	SE	*P*-value
Sex						
Heifer	0.402	0.047		0.542	0.041	
Steer	0.322	0.052	0.223	0.483	0.051	0.235
Days on feed categories						
0 to 42	0.178	0.030		0.433	0.042	
43 to 71	0.319	0.069	0.018	0.437	0.068	0.983
>71	0.639	0.057	0.001	0.662	0.058	0.004
Body weight categories, lb						
<600	0.612	0.102		0.603	0.079	
600 to 800	0.337	0.062	0.005	0.535	0.053	0.397
800 to 1000	0.247	0.048	0.001	0.385	0.047	0.008

>1000	0.477	0.076	0.324	0.527	0.065	0.444
Ultrasound Lung Score						
1	0.185	0.053		0.355	0.060	
2	0.237	0.040	0.402	0.371	0.040	0.798
3	0.283	0.043	0.146	0.408	0.040	0.416
4	0.382	0.065	0.017	0.574	0.056	0.006
5	0.740	0.081	0.001	0.745	0.074	0.001
B-line categories						
0 to 2	0.242	0.040		0.430	0.042	
≥3	0.605	0.058	0.001	0.638	0.046	0.001
Moth Sign						
Absent	0.2375	0.037		0.371	0.040	
Presence	0.506	0.053	0.001	0.652	0.043	0.001

[1]Animals that were live 60-day post-evaluation; [2]Animals that were culled or dead within 60-day post-evaluation; [3]Animals that were live 60-day post-evaluation; [4]Animals that were culled or dead within 60-day post-evaluation ; [*]Two distinct logistic regression models were used to assess the two different outcomes: treatment success outcome (n= 819), and the later for did not finish outcome

(cull or dead) (n= 819). The log-odds from the logistic regression models were converted to probabilities using the package 'emmeans' in RStudio.

Figure 6.1 Raincloud-boxplot displays temporal distribution of did not finish outcome model on the days on feed at time of chuteside evaluations (POC) on individuals enrolled in this study (n= 819). 60-day outcome of DNF = did not finish (cull/death), recovered = animals that were alive at the 60-day post-evaluation. Box-plot represent the upper and lower quartiles, and the line within the box represents the median.

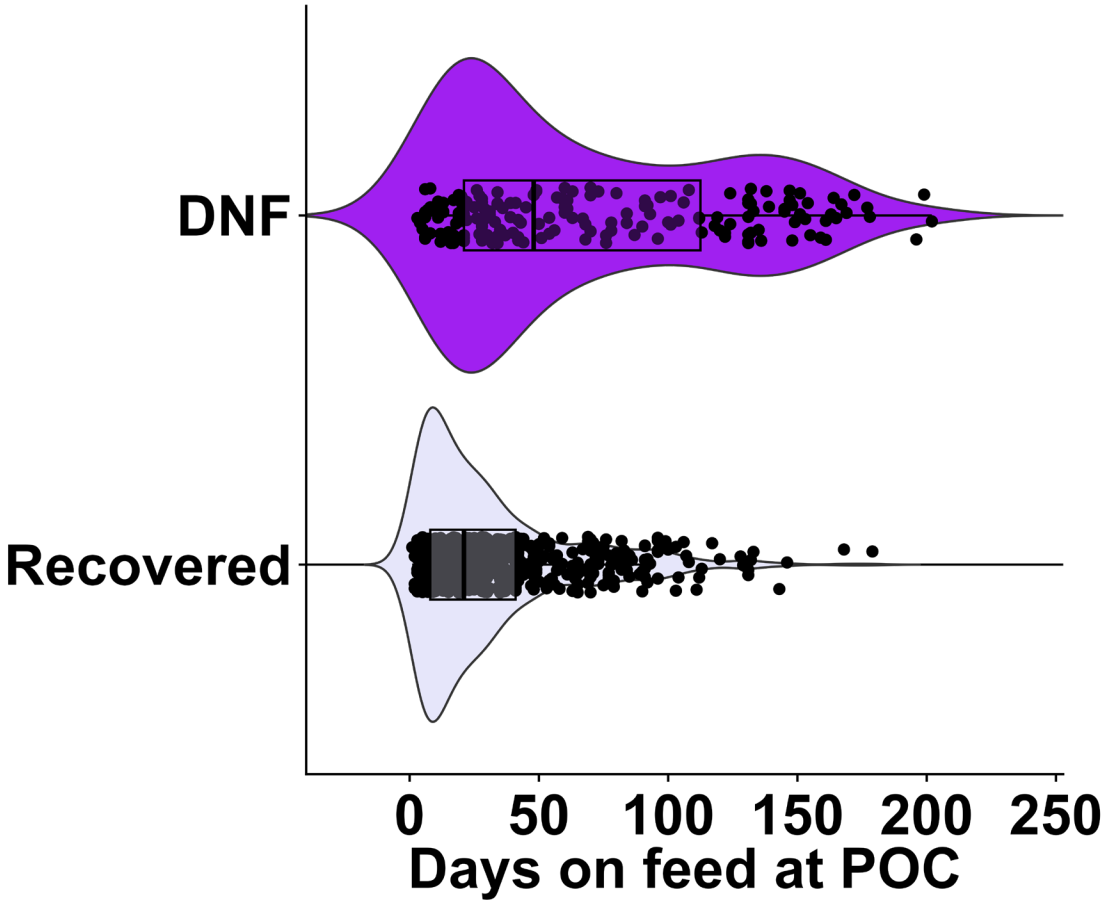


Figure 6.2 Raincloud-boxplot displays temporal distribution of treatment success outcomes model on the days on feed at time of chuteside evaluations (POC) on individuals enrolled in this study (n= 819). 60-day outcome of FTF = first treatment failure (re-treat/cull/death), FTS = first treatment success, animals that were alive at the 60-day post-evaluation and were not re-treated. Box-plot represent the upper and lower quartiles, and the line within the box represents the median.

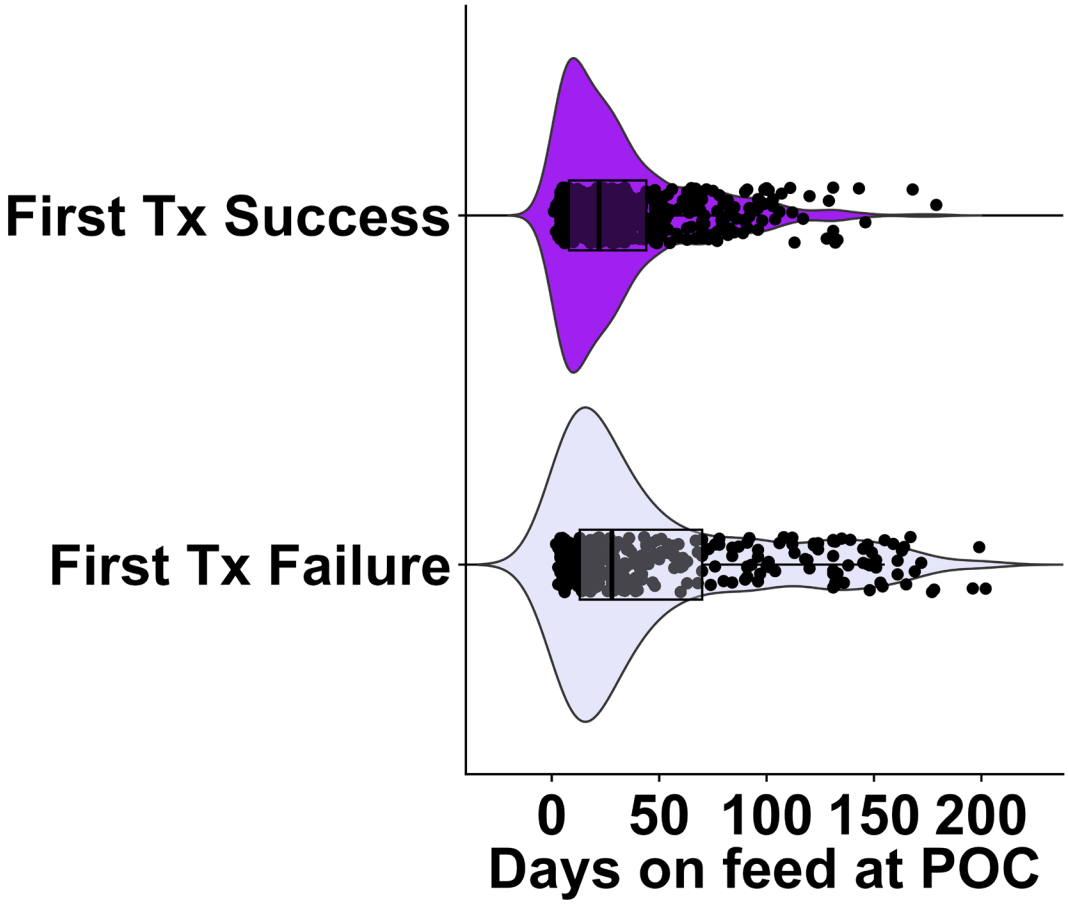
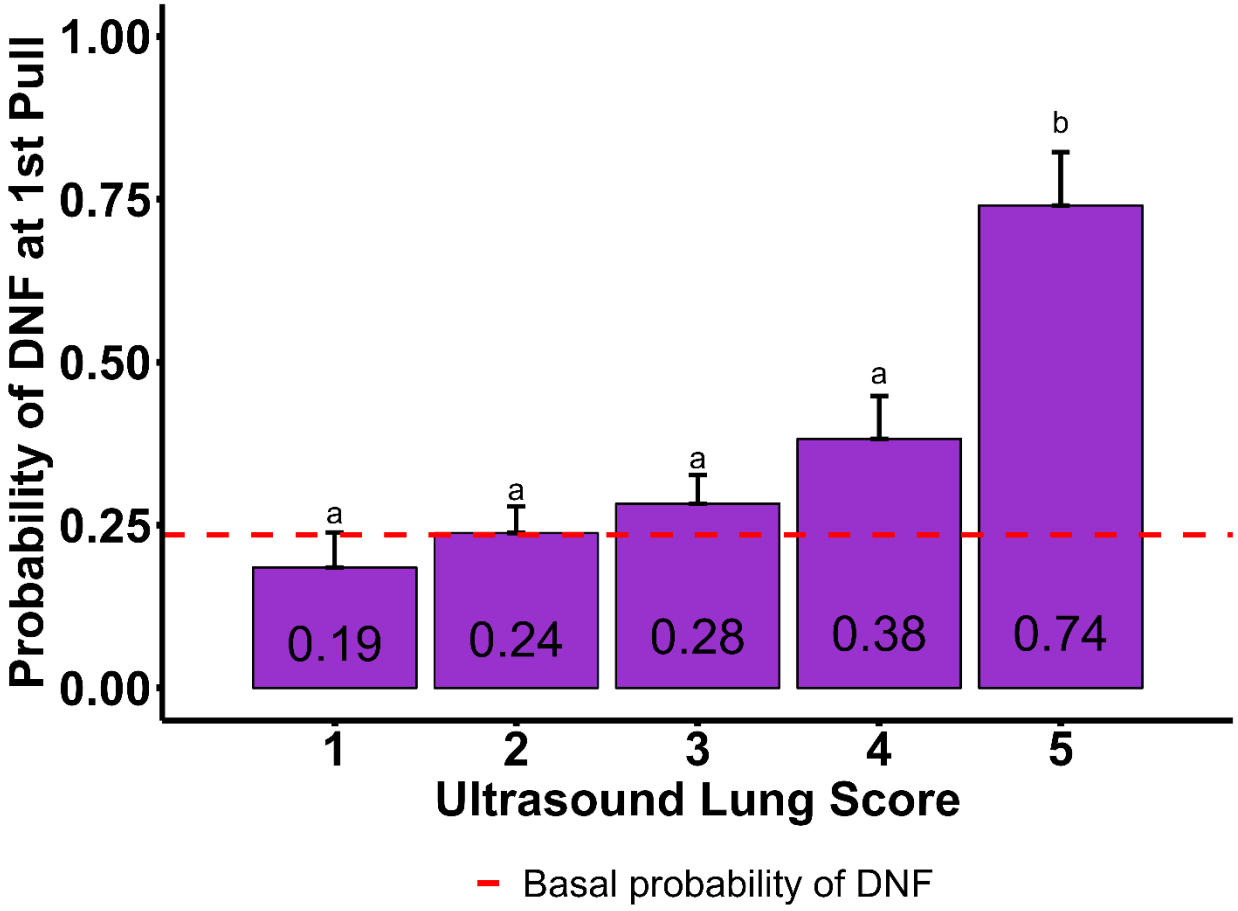


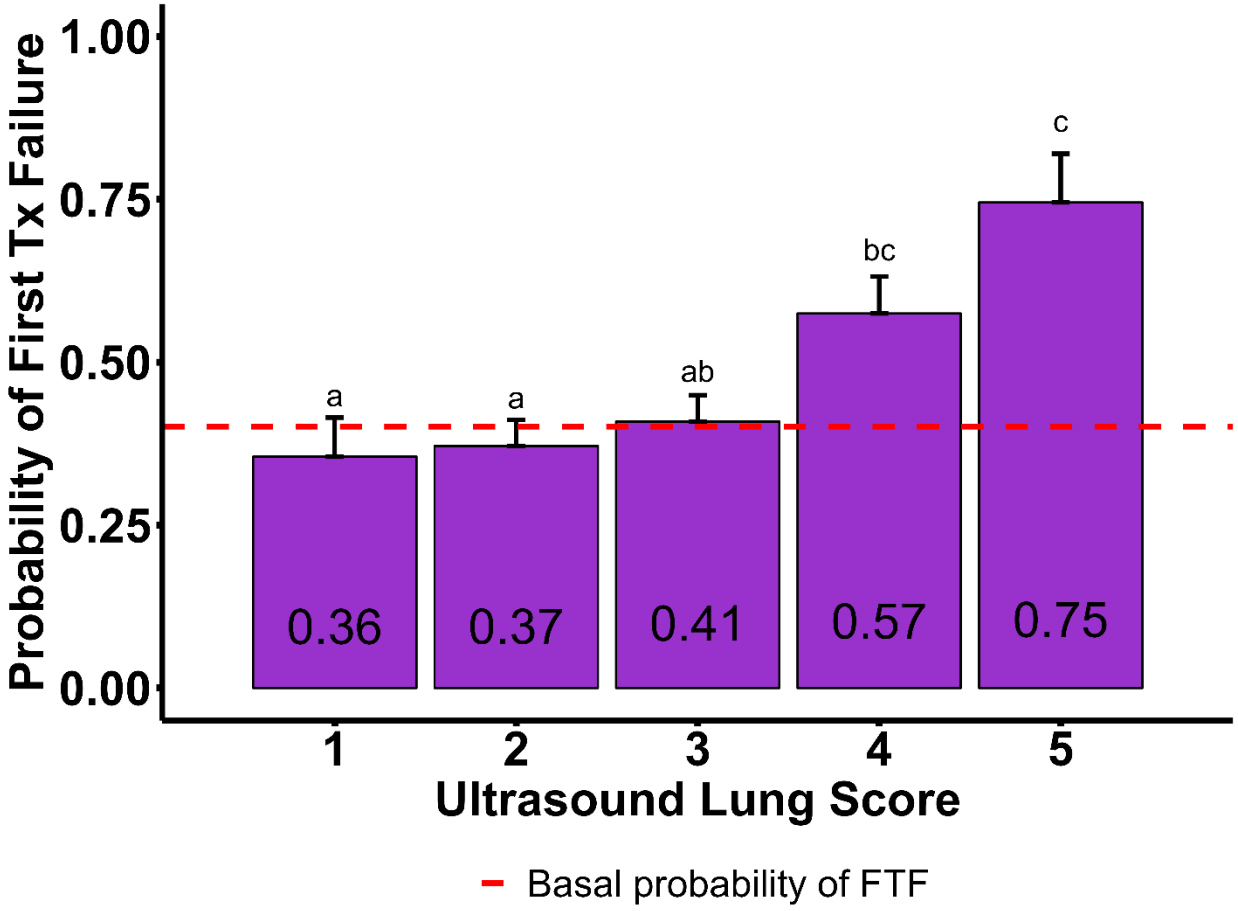
Figure 6.3 Model adjusted probability of did not finish (cull/death; DNF) by ultrasound lung score (ULS)* from a population of 819 cattle evaluated with targeted thoracic point of care ultrasound (TT-POCUS). Prevalence of DNF on this studied population was 23%.



*ULS scores defined as: 2 - presence of 3 or more thin B-lines; 3 - presence of merged B-lines; 4 - presence of several wide/merged B-lines, abnormal pleural (moth sign); 5 - consolidated lung, might be accompanied with moth sign and effusion based on description of lung injury.

[a,b] The ULS scores that do not share same superscript displays evidence of significant difference ($p < 0.05$).

Figure 6.4 Model adjusted probability of first treatment failure (re-treat/cull/death; FTF) by ultrasound lung score (ULS)* from a population of 819 cattle evaluated with targeted thoracic point of care ultrasound (TT-POCUS). Prevalence of FTF on this studied population was 40%.



*ULS scores defined as: Score 1 - presence of fewer than 3 thin B-lines (indicative of healthy lung); Score 2 – presence of 3 or more thin B-lines; Score 3 - merged B-lines; Score 4 - multiple wide/merged B-lines with abnormal pleural findings (moth sign); Score 5 - consolidated lung.

[a,b,c] The ULS scores that do not share same superscript displays evidence of significant difference ($p < 0.05$).

Chapter 7 - Conclusion and future research

Luis F. B. B. Feitoza[1]

[1]Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, 1800 Denison Ave., Manhattan KS 66506, USA

The objective of this dissertation was to explore innovative approaches to address three significant challenges within the cattle industry: fescue toxicosis,[1-3] liver abscesses in feedyard cattle,[4-8] and the development of chuteside evaluations for respiratory diseases.[9-14] By integrating diagnostic methods such as ultrasonography, this body of work sought to improve cattle health outcomes, management practices, and economic efficiency in feedyard operations.

Summary of findings

This dissertation presented three distinct but interrelated topics, each addressing a major challenge in the feedyard cattle industry. The first topic (Chapter 2 -) evaluated the use of vasoactive compounds in molasses-based block supplements to mitigate fescue toxicosis. Ultrasonography played a pivotal role in assessing vascular responses, where it was found that the molasses-based block supplements enhanced blood perfusion to the periphery, improving dry matter intake and average daily gain in cattle consuming ergot-infested tall fescue. These findings demonstrated that such supplementation could be a viable strategy to reduce the negative impact of fescue toxicosis on cattle performance.

The second topic evaluated the strategic use of tylosin phosphate as a feed additive for liver abscess mitigation in feedyard cattle (Chapter 3 -). While the overall prevalence of liver abscesses was not significantly reduced, a trend towards decreased severity was observed, along with increased body weight gain. This suggests that tylosin phosphate supplementation, particularly during dietary transitions when cattle are susceptible to ruminal acidosis, may have a role in improving health and performance, though further exploration into the optimal timing and dosage is warranted.

The third component of the research focused on chuteside evaluations for respiratory disease using different chute-side diagnostic tools (Chapter 1 - Chapter 4 - Chapter 5 - Chapter 6 -). Across one literature review and three cross-sectional observational studies, novel thoracic ultrasound parameters were evaluated in cattle clinically diagnosed with respiratory diseases. The results indicated that certain ultrasound features were associated with disease differentiation and prognosis, offering a promising approach to more precise disease management. The ability to differentiate interstitial pneumonia and predict disease outcomes using targeted thoracic ultrasonography represents a significant advancement in the point-of-care diagnostics for feedyard cattle.

Implications for the cattle industry

Collectively, the studies presented in this dissertation underscore the value of ultrasonography as a diagnostic and prognostic tool in managing health in feedyard cattle populations. The research provides evidence that targeted thoracic ultrasonography can be integrated into routine cattle management practices, aiding in accurate diagnosis, better prognosis, and informed treatment decisions. Furthermore, the use of molasses-bases block

supplements to enhance vascular perfusion and the strategic application of feed additives can contribute to improved animal health, welfare, and productivity.

These findings have practical implications for feedyard operators and veterinarians, who can apply these strategies to enhance cattle performance, reduce disease burden, and optimize health management protocols. Importantly, the economic implications extend beyond animal health by potentially improving feed efficiency, reducing mortality rates, and enhancing overall productivity in cattle operations.

Limitations and future research

While this dissertation's findings provide significant insights, certain limitations must be acknowledged. The studies on feed additives and their impact on vascular health and liver abscesses were conducted within specific environmental and management conditions, which may limit the external validity of the results. Moreover, while ultrasonography has shown promise in respiratory disease diagnostics, the standardization of ultrasound scoring and operator training are crucial for broader application.

Future research should aim to validate these findings across different conditions and management systems to establish external validity. Additionally, the development of standardized protocols for thoracic ultrasonography in cattle, as well as comprehensive operator training programs, will be essential to ensure consistent and reliable use of this diagnostic tool. Further investigation into the optimal use and dosage of feed additives for disease mitigation, as well as longitudinal studies on the impact of chuteside evaluations on long-term health outcomes, are warranted.

In conclusion, this dissertation advances the field of cattle health management by providing novel insights into the use of ultrasonography for diagnostic and prognostic purposes. The research emphasizes the importance of integrating targeted diagnostic tools into routine cattle management practices to enhance health outcomes, inform treatment decisions, and ultimately improve animal welfare and feedyard productivity. Future research building upon these findings will continue to refine cattle health management strategies, promoting both the health of the animals and the economic sustainability of the cattle industry.

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