

1 Performance and welfare-centered strategies in the beef industry: heat stress management, pain
2 mitigation, and diagnostics
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5 by
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Abstract

The objective of this dissertation is to explore both performance and welfare-based strategies within the beef industry to enhance cattle production and operational profitability by providing evidence-based management practices. The first chapter consists of a systematic literature review describing a diverse range of heat stress mitigation strategies employed within the feedyard sector and their effects on cattle performance, behavior, and physiological outcomes. A total of five studies were designed and conducted to describe two different heat stress mitigation strategies (Chapters 2 and 3), gross lesions at mortality (Chapter 4), prognostic abilities of biomarkers (Chapter 5), and effects of pain mitigation at castration (Chapter 6).

Two randomized controlled trials, consisting of approximately 8000 steers each, focused on strategies to mitigate heat stress within a commercial feedyard operation in the Pacific Northwest. One of these studies aimed to determine the effects of shade on steer performance, behavior, and carcass outcomes after a terminal sort event. The second study aimed to determine the effects of night-time feeding on steer performance, behavior, and carcass outcomes after a terminal sort event. Outcomes measured in both studies included daily feed delivery, water consumption, open mouth breathing prevalence, and disease events. Overall average daily gain and feed conversion were also calculated. Carcass traits recorded included final live weight, hot carcass weight, quality grade, yield grade, and dark cutter count. Overall, shade inclusion was seen to decrease water consumption, open mouth breathing prevalence, culling events, and dark cutter counts, while increasing the proportion of steers grading prime and choice vs select. No effects were seen between the night-fed group compared to the morning-fed group; however, the environmental heat load during the summer of this study was very mild, as measured and recorded by the Temperature-Humidity Index. The impact of shade in this dissertation, as well as

previously published literature, show benefits of providing shade to cattle on feed as a heat stress mitigation strategy. Further research is warranted on the effects of night-time feeding on heat stress mitigation, especially in the face of greater environmental heat burden.

One observational study was conducted over two summers in the Great Plains region, where approximately 800 mortalities within commercial feedyards were systematically necropsied. All gross lesions, or abnormalities, were recorded for each necropsy. Feedyard mortalities commonly exhibited multiple concurrent lesions, averaging 2.3 lesions per animal. The most common concurrent lesions seen consisted of pulmonary and gastrointestinal lesions, which agree with the most common diagnoses recorded in feedyard mortalities. Additionally, the probability of having more than one lesion or more than one body system affected increased as days on feed at death increased. Performing systematic necropsies and recording all lesions can help producers and veterinarians establish or modify evidence-based treatment protocols for morbidities, with the goal to improve overall performance.

Another observational study was conducted to determine potential associations between cardiac troponin (cTnI) serum concentration in cattle arriving at a commercial feedyard hospital for initial treatment of bovine respiratory disease, with the risk of retreatment, mortality, or culling within 60 days of first treatment. Blood was collected from over 300 steers and heifers, and 60-day outcomes were obtained. cTnI concentration was determined using an ELISA assay, and then further binomialized as LOW (< 0.156 ng/mg) or HIGH (≥ 0.156 ng/mg). The majority of samples had LOW cTnI concentrations; however, cattle with HIGH cTnI concentrations at first bovine respiratory disease treatment had a greater probability of not finishing the 60-day window due to either death or culling, compared to cattle with LOW cTnI concentrations.

Though cTnI concentrations may be valuable as an additional prognostic tool in a commercial feedyard, further research is needed to determine the optimal cTnI cutoff value.

Lastly, a single randomized controlled trial was performed to compare the use of lidocaine-infused castration bands to standard castration bands with no pain mitigation on 26 beef-dairy calves' performance and welfare. Calves were monitored for 7 weeks post-banding, and outcomes measured included body weight, average daily gain, feed conversion, clinical illness scores, banding site scores, physiological markers, as well as multiple different measures of animal behavior. Differences in behavior between these groups were observed close to time of scrotal sloughing, indicating the calves banded with the lidocaine-infused castration bands had less pain; however, no differences were seen in overall performance or physiological markers, indicating both groups experienced acute and chronic pain post castration. Further research could help researchers better understand the implications of pain mitigation around time of castration in calves on performance and welfare.

In conclusion, the studies presented in this dissertation evaluated strategies aimed at improving cattle performance and welfare. Collectively, these studies advance our understanding of management practices, necropsy evaluation, and welfare interventions that can improve cattle health, productivity, and decision making in commercial beef production.

Performance and welfare-centered strategies in the beef industry: heat stress management, pain mitigation, and diagnostics

by

Madeline Renee Rahikainen-Mancke

B.S., Kansas State University, 2022

A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Diagnostic Medicine and Pathobiology
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Abstract

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It takes a village, and I am incredibly fortunate for mine! Thank you for helping me become the person I am today.

Dedication

I dedicate this dissertation to my dad- known to many as Father Walter, but to me, simply my biggest fan.

Preface

This dissertation is presented in manuscript format and consists of one literature review and five research chapters. Chapter one is a literature review prepared by the author and has not been previously published. Chapters two through six have been published as manuscripts in peer-reviewed, scientific journals (MDPI Animals, MDPI Veterinary Sciences, Bovine Practitioner, and Translational Animal Sciences).

The research presented in this dissertation was conducted under the guidance of the supervisory committee. The author conducted the experimental design, data collection and analysis, interpretation of the results, and preparation of each manuscript. Coauthors contributed to all stages of each study.

The inclusion of published manuscripts in this dissertation has been approved by the respective publishers, where applicable. To ensure consistency with Kansas State University Graduate School dissertation requirements, minor modifications to formatting and organization have been made.

Chapter 1 - Heat Stress Mitigation Strategies in the Feedyard Sector of the Beef Industry; a systematic review

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Abstract

When the total heat load, including both environmental and metabolic heat production, surpasses an individual animal's ability to dissipate the heat, heat stress occurs. Heat stress can negatively affect cattle in the feedyard, resulting in detriments to cattle performance and welfare, and feedyard economics. Numerous heat stress mitigation strategies have been investigated to decrease the effects of hot environmental conditions, although their effectiveness varies. Differences in responses to these mitigation strategies are influenced by the heat tolerance of various cattle types, the frequency and duration of heat events, as well as the specific heat stress mitigation strategy employed. This systematic review describes the different heat stress mitigation strategies in greater detail, with specific attention to effects on cattle performance, behavior, and physiological outcomes. Overall, published articles fit into one of four categories of heat stress mitigation strategies; shade, water application, feedstuffs, and feeding management. A considerable amount of research has evaluated the effects of shade on cattle performance, however; the magnitude of effects varied, mostly due to shade type and area offered per animal. Water misting showed some benefits to cattle performance and behavior, however; acute (immediate, short term) effects were more commonly seen than long-term effects. A large variety of feedstuffs have been explored for their heat stress mitigation properties. Feeding management appears beneficial for mitigating heat stress, mostly through the

practice of restricting or limiting feed. This review highlights the importance of heat stress mitigation and concludes that future work should address a combination of specific mitigation strategies to continue to improve cattle performance and welfare under hot environmental conditions.

Keywords: feedlot, heat mitigation, heat stress, steers

Introduction

Heat stress occurs when the combination of environmental and metabolic heat load surpasses an animal's ability to adequately dissipate heat (Foroushani and Amon, 2022; Hahn and Becker, 1984). Signs of heat stress in cattle include increased respiration and heart rate, and decreased feed intake. Environmental conditions in the summer months that consist of above normal temperature, humidity, and solar radiation, coupled with low wind speeds, can have detrimental effects in the feedyard (Hubbard et al., 1999). When considering heat stress, groups of cattle at higher risk include dark hided cattle, cattle closer to slaughter, and animals that have previously been treated for pneumonia in the feedyard (Brown-Brandl et al., 2006)

Heat stress in finishing cattle negatively affects cattle performance, feedyard profitability, and animal welfare. The effects of heat stress on cattle performance include reductions in dry matter intake, growth, and feed conversion (Brown-Brandl et al., 2005; Brown-Brandl et al., 2006; Eigenberg et al., 2005), as well as an increase in water consumption (Arias and Mader, 2011). Physiological responses to heat stress include an increased sweating rate (Mader et al., 2010), respiration rate (Gaughan et al., 2000), panting score (Mader et al., 2006), and body temperature (Robertshaw, 1985). Behavioral changes are often seen in heat stress conditions, including increased time standing, increased time under shade or performing shade seeking behaviors, and body splashing at water tanks (Young and Hall, 1993). Biological markers in the

blood (such as cortisol) help measure animal stress (Collier et al., 2008). In extreme heat stress events, hyperthermia can result in mortality (Hubbard et al., 1999). In 2003, St-Pierre et al. (2003) estimated the annual economic burden on the beef industry attributable to heat stress to be \$370 million USD (St-Pierre et al., 2003).

The growing emphasis on heat stress management and mitigation in cattle production systems is driven by multiple factors, including climate variability, increased feedyard populations, greater demands for growth performance and carcass quality, and heightened public concern for animal welfare (Brown-Brandl et al., 2003). The goal of heat stress mitigation strategies is to decrease the heat load experienced by cattle, minimizing the likelihood and severity of heat stress. The National Cattlemen's Beef Association's Beef Quality Assurance program provides recommendations for reducing heat stress in high-risk cattle populations, primarily by modifying cattle handling and transportation practices during periods of extreme weather. Several beef cattle certification programs provide guidelines aimed at minimizing heat stress (Global Animal Partnership, 2022; Humane Farm Animal Care Welfare Standards: Beef Cattle, 2021). These guidelines commonly require access to shade and water-based cooling systems, such as sprinklers. The objectives of this review are to assess heat stress mitigation strategies on cattle performance and welfare, to identify gaps in current research regarding heat stress mitigation, and to provide a current resource for producers seeking ways to mitigate heat stress and the impacts on cattle performance and welfare.

Materials and Methods

The database used for this systematic review was PubMed. The initial search was conducted on July 7, 2025. The key search terms used were (((((((((((((((cattle) OR ("beef cattle")) OR (feedlot*)) OR (beef*)) OR (heifer)) OR (heifers)) OR (steer)) OR (steers)) AND

((((((((((("heat mitigation") OR ("heat elimination")) OR ("heat stress")) OR ("heat protection")) OR ("heat management")) OR ("heat mitigation strategy")) OR ("heat mitigation strategies")) OR ("thermal load")) OR ("high temperature")) OR ("hot temperature")) OR ("extreme heat")) OR ("heat exhaustion")) NOT (dairy) NOT (utero*)) NOT (reproduct*)) NOT (pasture) NOT (review). This search string included combinations of keywords related to cattle and heat stress mitigation, with exclusions applied for dairy, reproductive terms, pasture-based systems, in utero studies, and review articles. Papers considered for further review needed to be available in full text, published in the English language between 1985 and 2025, with species specified as “other animals” to exclude human studies.

After the initial search was conducted, titles were screened by one author. Abstracts were then evaluated by the same author to identify studies involving heat stress mitigation strategies in beef feedyard settings under natural environmental conditions (i.e., no artificial heat chambers). Uncertain inclusion decisions were resolved by a second author. This process resulted in the final set of records considered for full-text review.

Results and Discussion

The initial search yielded 2837 records. After title screening, 70 records were included in abstract reviews. The main reason for exclusion in the first round of screening was due to meat science studies evaluating temperature on meat composition and characteristics. Following abstract reviews, 28 records were considered for full review. Following full-text evaluation, two records were excluded due to artificial environmental conditions or absence of live animals. A total of 26 articles were included in the final dataset, with the selection diagram shown Figure 1.1.

Due to the heterogeneity of heat stress mitigation strategies explored in the 26 articles, results have been separated by type of mitigation strategy; shade, water application, feedstuffs, feeding management.

Shade

The effect of shade on cattle performance and welfare remains the most researched method of mitigating heat stress in feedyard setting, with this literature review consisting of 12 articles exploring the benefits of shade. Benefits to live animal performance, carcass quality, physiological measures (body temperature, heart rate, respiration or breathing rate), and behavior have been observed when comparing shaded cattle to unshaded cattle.

The inclusion of shade structures in feedyards has been shown to positively impact cattle performance and carcass quality. Some studies found increases in gain, dry matter intake, feed efficiency, body weight, hot carcass weight, quality grade, and yield grade (Blaine and Nsahlai, 2011; Boyd et al., 2015; Gaughan et al., 2010; Hagenmaier et al., 2016; Mitlöhner et al., 2001; Mitlöhner et al., 2002; Winders et al., 2023). Cattle provided shade maintain more consistent feed intake, even in the presence of rising heat load, whereas unshaded cattle have decreased feed intake during rising heat (Gaughan et al., 2010). Mitlöhner et al (2002) found that cattle provided shade were less likely to be dark cutters at harvest, indicating benefits of shade provided during the feeding period to both carcass quality as well as animal welfare (Mitlöhner et al., 2002). However, there are inconsistencies in literature, with some studies finding tendencies or differences in live cattle performance that benefit the unshaded cattle (Boyd et al., 2015; Hagenmaier et al., 2016). The impact of shade on water behavior or consumption varies, with some studies showing shaded cattle have more overall drinking events (Miller et al., 2024), while consuming less water (Gaughan et al., 2010). This behavior could be due to shaded cattle

coping with heat events better, which allow them to feel comfortable having more drinking events, and not having to consume significantly more water within a single event.

Physiological and behavioral benefits are commonly seen when cattle are provided shade compared to when they are not provided shade. The literature shows shaded cattle have lower body temperatures than unshaded cattle, measured in various ways (rectal, vaginal, tympanic, etc), indicating greater thermoregulation (Gaughan et al., 2010; Lees et al., 2018; Winders et al., 2023). However, Miller et al.(2024) found that shaded cattle had a minimum body temperature greater than the unshaded cattle, but lesser frequencies of increases in body temperature, indicating possibly less heat dissipation at night in the shaded group due to reduced heat accumulation during the hottest parts of the day (Miller et al., 2024). Cattle provided shade have also consistently shown decreased panting and respiration rates compared to cattle with no access to shade (Blaine and Nsahlai, 2011; Gaughan et al., 2010; Miller et al., 2024; Mitlöhner et al., 2002; Mitlöhner et al., 2002; Winders et al., 2023), indicating a lessened thermal load.

Changes in behavior have also been noted in shade literature. Winders et al (2023) found shaded cattle have more movement in the evening hours compared to unshaded cattle (Winders et al., 2023), suggesting unshaded cattle are having to dissipate more heat, therefore, conserving energy for thermoregulation by restricting movement during cooler parts of the day. The overall amount of time spent standing can be contradicting, with one study finding shaded cattle spend more time standing (Mitlöhner et al., 2001), while another found that shaded cattle spent less time standing (Mitlöhner et al., 2002). Due to the differences in behavior recording techniques (one study only monitored standing during day light hours over the entire duration, whereas the other study only compared behavior for one 24-hour period), these methods could explain standing behavior differently. Blaine and Nsahlai (2011) found shade increased the proportion of

cattle eating while taking fewer, larger feedings compared to unshaded cattle (Blaine and Nsahlai, 2011). Increased comfort in cattle under shade has been suggested due to decreases in agitation and anxious behavior (Miller et al., 2024), as well as agonist behavior and bullying activity (Mitlöhner et al., 2002). Overall, shade provision in the feedyard shows beneficial impacts to performance, behavior, and welfare, though the magnitude of effects varies, mostly due to shade type and the study duration.

Two studies conducted investigated the amount of shade on cattle performance and behavior. Collectively, the findings indicate that increasing shade area improves performance and behavior outcomes during heat stress events. Greater shade area was shown to improve weight gain, feed efficiency, and reduce panting behavior (Aguilar-Quñones et al., 2022; Sullivan et al., 2011). Sullivan et al. (2011) found that, during heat events, the number of total cattle under shade and number of cattle lying down under the shade increases as the amount of shade offered increases (Sullivan et al., 2011). Regardless of the amount of shade, dry matter intake increases and water consumption decreases compared to unshaded cattle (Sullivan et al., 2011). However, in extreme heat measured by the Heat Load Index, water intake was greater in the shaded pens compared to the unshaded pens, though more time spent at the waterer was seen in the unshaded group. This observation may indicate shaded cattle were able to maintain normal drinking behavior, seen in the quantity of water consumed, whereas unshaded cattle used the waterers as a means of behavioral thermoregulation, by seeking the cooler air near the water surface. Overall, the evidence suggests that providing cattle with a greater amount of shade benefits both performance and behavior.

Only one study was conducted comparing the type of shade on the physiological responses of heifers under heat stress conditions. Brown-Brandl et al. (2017) compared four

treatments: no shade, plastic snow fence, 60% solar block, and 100% solar block shade structures (Brown-Brandl et al., 2017). That study found that 100% solar block provided the greatest decrease in solar radiation exposure to shaded cattle; however, when the environmental conditions (including temperature, humidity, wind speed, and solar radiation) were most extreme, any type of shade was sufficient in reducing respiration rate. The lowest body temperature was seen in cattle utilizing 100% solar block shade structures, but the 60% solar block was deemed best for overall animal responses (e.g. respiration rate). This could possibly be due to increased airflow or less noise and movement in the wind compared to the 100% solar block shade. These findings indicate that although a greater impact on environmental heat load is seen with greater solar blockage, other design characteristics, such as structure material or slope of the roof, may influence the effectiveness of shade as a heat stress mitigation strategy.

Though the literature suggests shade is a useful heat stress mitigation strategy in the feedyard, the magnitude of differences seen can be contributed to multiple factors. The impact of shade varies based on breed differences, especially when comparing *Bos taurus* vs *Bos indicus* cattle. This can be attributed to the breed genetics, hide color, and ability to handle heat in native environments. Another reason for variability of results in shade literature can be attributed to the magnitude and severity of heat events seen during the duration of the study, which are unpredictable prior to the start of the study. The amount of shade and type of solar block contribute to differences seen in the effects of shade on cattle performance and behavior. Though benefits of shade on cattle performance and welfare are evident, there are several reasons why shade is not universally used in feedyards. The high installation cost and initial investment is the primary barrier, coupled with the uncertain economic return due to geographic region, length and severity of heat stress events, cattle type and weight, make producers hesitant to invest in shade

structures. Additionally, maintenance requirements of the shade structures and pen management, like manure accumulation and scraping the pen floor under the shade, adds additional challenges. Consequently, producers will have to weigh these factors against the expected heat stress frequency and duration to determine whether to install shade or not, though literature shows a consistent trend to benefitting animal welfare.

Water Application

Research evaluating water application, to either the pen floor or cattle, as a heat stress mitigation strategy in feedyard cattle remains limited; however, available literature overall indicates benefits of water application, when assessed by physiological and behavioral indicators of heat stress. Many studies have shown that, pens of cattle receiving water application had reduced body temperatures, respiration rates, and panting scores compared to cattle without water application (Davis et al., 2003; Mader et al., 2004; Mader et al., 2007; Mitlöhner et al., 2001). Indicative of thermal comfort, the application of water has been associated with changes in behavior, including reduced water-seeking behavior and increased use of wetted pen surfaces (Davis et al., 2003; Mader et al., 2007; Mitlöhner et al., 2001). Overall, these findings indicate water application to pens allows cattle to dissipate excess heat during periods of high environmental heat challenge.

Although physiological and behavioral responses to water application have been observed consistently, the effects on cattle performance have been less commonly observed. Most studies show that dry matter intake does not differ between groups of cattle with water applied versus cattle without water application. However, Mader and Davis (2004) found that water application may help maintain eating behavior, measured by bunk scores recorded at various times throughout the day, during heat stress events (Mader and Davis, 2004). Some

studies report tendencies for improved feed efficiency in cattle that had water applied, but carcass traits were inconsistent, with Mader and Davis (2004) showing that cattle fed in the morning that did not receive water application had greater marbling scores compared to cattle getting fed in the evening with or without water application (Mader and Davis, 2004), though direct effects of water application cannot be made due to the design of the study. These findings indicate that although water application can mitigate acute heat stress responses, it may not be enough to improve more long-term performance outcomes, such as weight gain, feed conversion, or carcass traits determined at the end of the feeding period.

The effectiveness of water application has also been shown to be influenced by the timing of the water application. Studies that have compared morning versus evening water application to the pen have reported differences in physiological responses, water consumption, and carcass characteristics (Mader et al., 2004; Mader et al., 2007; Davis et al., 2003). While both morning and evening water application showed reduced indicators of heat stress in the cattle, Mader et al. (2007) found that evening water application seems to be the most effective during heat stress events due to the lower panting scores during those specific periods (Mader et al., 2007). Water application also altered body temperature of the cattle throughout the day. Davis et al. (2003) showed that water application in the evening delayed the cattle's peak tympanic temperature and water application in the morning helped maintain a lower body temperature of cattle during peak environmental heat exposure (Davis et al., 2003). Water application timing also influenced behavioral responses. Regardless of timing of water application, Mader et al. (2007) found that cattle moved to the wetted surface following wetting events (Mader et al., 2007). These findings, together, indicate that the timing of water application can affect

physiological responses (body temperature), though either morning or evening water application can positively benefit cattle, both physiologically and behaviorally.

Overall, though the literature is somewhat limited, water application is an effective strategy for reducing physiological and behavioral responses due to heat stress. The application of water lowers body temperature, respiration rate, and panting scores, while encouraging cattle to utilize the cooler, wetted surfaces. However, benefits in performance and carcass characteristics have not been consistently reported. This could be due to water application only being applied for shorter periods of time, unlike shade, which is usually available for the entire day. Water application appears to be effective for improving welfare during heat stress events rather than improving animal performance.

Feedstuffs

Nutritional interventions are designed to improve resilience to heat stress effects seen in cattle. Many strategies have been used, such as changing energy density or sodium supplementation, to determine their effects on heat stress responses in feedyard cattle.

Several studies evaluated the inclusion of feed ingredients designed to alter nutrient density as a means of heat stress mitigation. Fat supplementation, as a way to maintain energy intake while reducing the heat increment of feeding, has been shown to have conflicting effects on cattle performance and physiology. Ceconi et al. (2022) found that cattle fed a ruminally-protected linseed oil-based product in the summer months increased dry matter intake and average daily gain, while not changing cortisol concentrations at slaughter compared to the other treatment groups; a negative control and a positive control (Ceconi et al., 2022). Two other studies conducted found no performance differences when supplementing fat into the diet (Gaughan and Mader, 2009; White et al., 1992). Conversely, Gaughan and Mader (2009) found

that cattle fed a fat supplementation in addition to sodium chloride during times of heat stress had a greater tympanic temperature than cattle without the fat supplementation (Gaughan and Mader, 2009).

Fish meal as a replacement for soybean meal, in means to alter nutrient density, was shown to be beneficial when fed during higher environmental temperatures compared to more moderate environmental temperatures, with increases in average daily gain and carcass weight reported (White et al, 1992). Wet distillers grains with solubles and zilpaterol hydrochloride were found to increase cattle performance and carcass traits, while having little to no effect on physiological indicators of heat stress (Boyd et al, .2015; Hales et al., 2014). These findings suggest strategies for nutrient partitioning improve productivity and thermoregulation but have no effect on behavioral indicators of heat stress.

Increases in water loss and impaired electrolyte balance often accompany heat stress. Limited studies have investigated the effects of sodium supplementation, through dietary inclusion, with little to no performance differences observed (Gaughan and Mader, 2009).

Functional feed additives have also been investigated to determine potential improvements in immune function and physiological stress responses. OmniGen-AF was found to decrease vaginal temperature in heifers (Colombo et al., 2019), while rumen-protected γ -aminobutyric acid (GABA) was seen to decrease physiological responses, measured by serum hormones, to heat stress while also increasing cattle performance while used in combination with an herbal supplement (Chen et al., 2020).

Plant-based and herbal supplements have been studied as potential heat stress mitigation strategies. Though the bioactive compounds may differ between products, the mechanisms involved in modulation of stress-related metabolic pathways and enhancement of antioxidant

capacity. The inclusion of *Allium Mongolicum* Regal powder was found to increase performance (Liu et al., 2024), while herbal tea residue showed benefits to performance as well as decreases in respiration rate and rectal temperature (Zhuang et al., 2021). Like mentioned above, Chinese herbal medicine prescriptions, concurrent with GABA, found to benefit performance, behavior, and physiological indicators of stress (Chen et al., 2020).

The effect of dietary composition has been explored as a means of mitigating heat stress by influencing fermentation processes and subsequent heat production. By altering forage-to-concentrate inclusion rates, energy density of the diet significantly influences the response to hot summer conditions, with a higher energy diet reducing the effects of heat stress on cattle performance, as well as the effects of saline water ingestion (Ray, 1989).

Unlike environmental modifications such as shade and water application, the diversity within the feedstuff-based approaches to mitigate heat stress lacks a clear consensus regarding the most effective mitigation strategy. Direct comparisons are difficult due to the variability in feedstuffs and the responses measured. Nonetheless, benefits to cattle performance, behavior, and physiological indicators show potential in coping with heat stress effects.

Feeding Management

Feeding management has been shown to be an additional strategy for mitigating heat stress in feedyard cattle, although it has received less attention compared to environmental modifications such as shade or application of water. Metabolic heat production is a major contributor to total heat load in cattle, which feeding strategies directly influence. Adjustments to feeding level, timing, and delivery method can alter the magnitude and temporal distribution of metabolic heat load, thereby affecting physiological responses, such as body temperature, respiration, and water intake. In times of thermal stress, understanding how feeding management

interacts with environmental heat load is necessary for developing heat stress mitigation approaches to improve cattle comfort and performance.

Restricted feeding reduced physiological effects of heat stress. Mader et al. (2002) found limit-fed steers had lower tympanic temperatures (reduced 0.2 to 0.4°C), coupled with decreased panting and bunching behavior compared to steers fed *ad libitum* (Mader et al., 2002). Restricted steers maintained the lowest body temperatures during the most severe heat load, with reductions in body temperature occurring 2 to 4 h earlier than the *ad libitum* or previously restricted cattle. Steers fed the restricted diet had a significantly lower dry matter intake following the restricted intake period compared to the steers fed *ad libitum* for the entire period, showing an incomplete compensatory intake after restriction. Davis et al. (2003) showed that the effectiveness of feeding management strategies to mitigate heat stress depends on heat stress severity (Davis et al., 2003). During mild heat stress conditions, the feeding program had minimal impact on tympanic temperature; however, under moderate heat stress conditions, lower body temperatures were consistently observed in the limit-fed steers compared to the *ad-libitum* steers. During severe heat stress conditions, advantages were seen in the limit-fed steers, with lower body temperatures recorded during the peak environmental heat hours compared to the traditional bunk-managed cattle. Importantly, previously limit-fed cattle maintained a reduced body temperature even after returning to an *ad libitum* feeding regimen, highlighting a potential carryover effect on thermal regulation.

In addition to feed intake level, time of feeding plays an important role in heat stress mitigation by influencing the time of peak metabolic heat production. In Experiment 3 conducted by Davis et al. (2003), dry matter intake did not differ between the AM- (0800 h) and PM-fed (1400 h) steers; however, under certain conditions, differences were observed in body

temperature (Davis et al., 2003). During severe heat stress conditions, there was a limited impact of feeding time, but the PM-fed cattle showed greater tympanic temperatures in the late afternoon hours, likely due to the peak environmental heat coinciding with the peak metabolic heat production. Additionally, AM-fed steers with no sprinkler cooling were noted to have the highest overall body temperatures (39.8°C), highlighting the importance of feeding time and environmental modification combination strategies.

Feeding management strategies have also been seen to influence feed intake behavior and resource use. Mader and Davis (2004) reported that cattle that had previously been limit-fed had a 7% compensatory increase in dry matter intake upon the return to ad libitum feeding (Mader and Davis, 2004). This resulted in improved gain and feed conversion compared to continuously ad libitum-fed cattle. Conversely, ad libitum-fed cattle exhibited greater variability, with reduced morning intake during moderate heat stress conditions and increased feed refusals during severe heat stress. To further support the role of feeding management on thermal regulation, differing water intake patterns observed in Mader and Davis (2004). Limit-fed cattle tended to consume 16 and 22% less water than ad libitum and bunk-managed cattle, respectively. This is likely reflected in reduced metabolic heat production and a lesser need for the cooling effects of water consumption. However, cattle fed in the AM with no sprinkler cooling were shown to have greater water intake, indicating an increased need of evaporative cooling. In a 2x2 factorial comparison with feeding time and sprinkling, AM-fed cattle without sprinkling consumed the most water, whereas the PM-fed cattle with the additional sprinkling consumed the least. Conversely, this study observed AM-fed cattle without water application exhibited greater carcass marbling scores, suggesting a potential influence of nutritional management on carcass traits.

Collectively, these studies suggest that feeding management can play a significant role in mitigating heat stress, although the effectiveness depends on other factors, such as environmental conditions and the specific strategy used. Limit feeding has been shown to consistently reduce metabolic heat load, resulting in lower body temperature and, in some cases, improved growth efficiency post-restriction of limit feeding. Altering feeding time can shift the timing of metabolic heat production but seems dependent on the metabolic heat production to coincide with the peak environmental heat. Managing bunks to where feed is only available in the cooler part of the day can keep intake behavior consistent during heat stress events. Overall, feeding management should be viewed as a supportive tool to complement other environmental heat mitigation strategies in feedyards during heat stress events.

Conclusions

This systematic review investigates heat stress mitigation strategies in the feedyard sector and their impact on cattle performance and welfare. As seen in the studies cited, 4 categories of heat stress mitigation strategies have been well studied in the literature; shade, water application, feedstuffs, and feeding management. The use of shade structures is the most consistent tool to improve cattle performance and welfare and is thus the most reliable means of limiting or mitigating environmental heat stress in feedyard cattle. Limited research has evaluated the impacts of water application on cattle performance and welfare; however, data shows acute physiological and behavioral benefits of water application to animals or their environment during heat stress events. Altering feedstuff compositions or specific feed additives has shown conflicting results on the impact those ingredients have on cattle with respect to mitigation of heat stress, and there is limited evidence to support adoption of any single nutritional intervention to mitigate heat related stress. Limit feeding and timing of feeding has been shown

to reduce metabolic heat load in cattle. Further research is warranted to study the impacts of heat stress mitigation strategies, alone or in combination, and how they may be utilized to improve cattle performance and welfare during heat stress events.

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Study Selection Flow Diagram

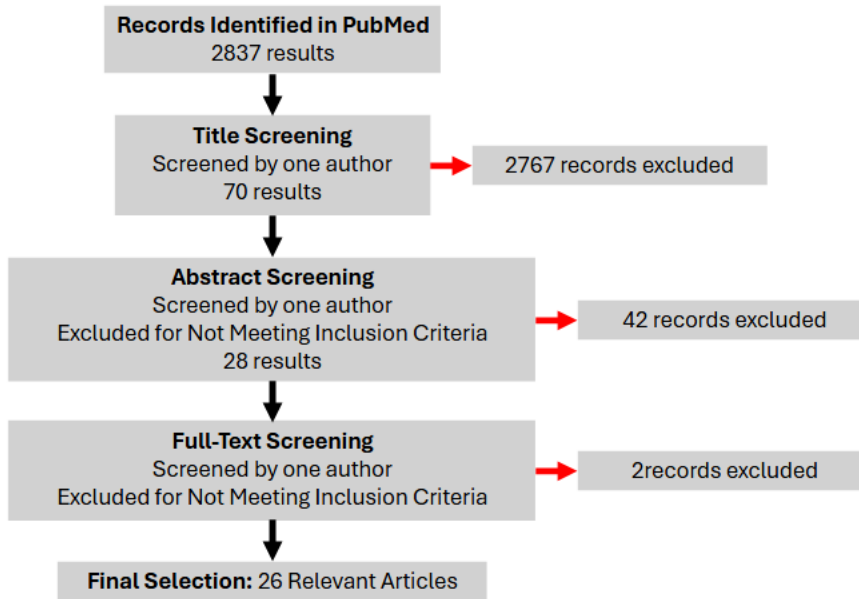


Figure 1.1. Study selection flow diagram

Study selection flow diagram of heat stress mitigation strategies in the feedyard sector of the beef industry, illustrating the process used to identify relevant articles for review. Of the 2,837 records identified, 2,767 were excluded at title screening, 42 at abstract screening, and 2 at full-text review, resulting in a final selection of 26 articles.

Chapter 2 - The effect of shade on steer performance after terminal sort

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Abstract

Heat stress negatively impacts performance and welfare and occurs when total environmental and metabolic thermal load exceeds an animal's ability to dissipate heat. Access to shade has been associated with reductions in body temperature and respiratory rate in cattle, but previous findings on cattle performance has been inconsistent. This pen-level randomized controlled trial evaluated the effects of shade (2.8 m²/head, 100% solar block; n= 12 pens) versus no shade (n= 12 pens) on steer performance following terminal sort at a commercial feedyard in the Pacific Northwest, where animals were housed on roller-compacted concrete floors. Data collected included water consumption, feed delivery, disease events, open mouth breathing (OMB) behavior, and carcass traits. Shade reduced water consumption by 15% (P<0.05), and pens without shade showed a 6% decrease in feed delivery when the previous day's temperature-humidity index (THI) was ≥ 80 (P<0.05). Additionally, shade reduced OMB behavior (P=0.09), the number of culled steers (P<0.05), and the incidence of dark cutters (P<0.05). A greater

proportion of steers in shaded pens graded choice and prime (93%), compared to those in unshaded pens (91%) ($P<0.05$). Inclusion of shade improved animal health, welfare, carcass quality, and environmental stewardship during the summer months in a commercial feedyard setting. Shade should be considered a valuable management tool for mitigating heat stress in finishing cattle.

Keywords: feedlot, heat mitigation, heat stress, shade, steers

Introduction

Heat stress occurs when total heat gain, both environmental and metabolic, exceeds the heat loss capabilities of the animal (Hahn and Becker, 1984). To maintain homeostasis in response to heat stress, cattle respond behaviorally and physiologically. A review focused on dairy cattle by Polsky and von Keyserlingk (2017) found that increased environmental heat load leads to increased hunger, thirst, frustration, aggression, and malaise, increased panting and decreased performance, as well as increased grouping and shade seeking behavior. An animal's difficulty or failure to cope with their environment can be an indicator of poor welfare, becoming detrimental to their well-being (Broom, 1986). Heat stress is a major concern in feedyards during the summer months due to increased environmental heat load. Physiologic responses in beef cattle due to increased heat load include increased body temperature (Robertshaw, 1985), panting scores and breaths per minute (Gaughan et al., 2000; Mader et al., 2006) and sweating (Mader et al., 2010). Behaviorally, changes can be seen with increased standing time, time spent under shade or seeking shade, and body splashing at the water trough (Young and Hall, 1993). Cattle under heat stress consume smaller meals and shift their feed intake to cooler parts of the day (Ray and Roubicek, 1971; Brown-Brandl et al., 2005). Overall dry matter intake, growth, and feed efficiency is affected by high heat load (Brown-Brandl et al., 2005; Eigenberg et al., 2005;

Brown-Brandl et al., 2006). Regarding finishing cattle, water intake has been seen to increase 87.3% in the summer compared to the winter (Arias and Mader., 2011). Decreased animal welfare and performance contribute to economic losses at the feedyard. In 2003, an economic assessment determined heat stress in the beef cattle industry is responsible for a US\$369 million loss (St-Pierre et al., 2003). Beef cattle at higher risk of heat stress include dark-hided cattle, cattle with higher body condition scores, and immunocompromised animals (Brown-Brandl et al., 2006).

Multiple methods exist to help mitigate heat stress in feedyards, such as nutritional management (Mader et al., 2002; Davis et al., 2003), and physical environmental modifications, such as water misting and shade (Edwards-Callaway et al., 2021; Azevedo et al., 2024). However, widespread adoption of shade in feedyards in the United States has been slow. Two surveys, conducted at feedyards in the High Plains region of the United States and of consulting cattle nutritionists, respectively, found only 17% of feedyards reported the use of shade structures in their pens (Samuelson et al., 2016; Simroth et al., 2017). This is much lower than the adaption of shade in other parts of the world. A recent report from Australia states that as of June 2024, there was an estimated 70.4% of feedlot cattle on feed that had access to shade, with a projected 83.5% of total capacity having access to shade by the end of 2026 (Meegan Agribusiness Solutions, 2026). Factors, such as high initial and maintenance cost of the shade, relatively short life span, and being an obstacle when cleaning pens, are common reasons for not providing shade to cattle.

Although substantial research has evaluated the effects of heat stress and shade in beef cattle, the dairy industry has generally received greater attention in this area, likely due to more immediate, detectable production responses to heat stress. Heat stress in dairy cattle is potentially

easier to recognize than heat stress in beef cattle, as milk production losses are observed rather quickly after a heat event (Collier et al., 1981). In feedyards, a response to heat, seen in dry matter intake, can be noticed within 3 to 5 days (Curtis et al., 2017), however, performance differences in carcass traits (beef yield and quality) are not observed until harvest, if observed at all. Currently, published literature has been inconsistent with determining the effects of shade on cattle performance and behavior, as well as carcass traits.

A meta-analysis performed by Edwards-Callaway et al. (2021), using fifteen shade vs. no shade published manuscripts, found cattle provided shade had a significantly higher final bodyweight, greater average daily gain (ADG), and gain efficiency compared to cattle without shade; however, there were not any differences seen in dry matter intake. The authors believe this has to do with the type or breed of study cattle, frequency, duration, and severity of heat events, as well as the type of shade provided (amount and solar block). There has been no published literature determining the effects of shade on steer performance in the Pacific Northwest region of the United States, or on cattle housed on roller-compacted concrete. Concrete has relatively high thermal conductivity and heat capacity compared with many natural ground surfaces, allowing it to absorb and retain heat for extended periods (Asadi et al., 2018). Roller compacted concrete has been implemented into feedyard pen construction recently, and one question posed is how the concrete pen floors will impact thermal load. The objective of this study was to compare performance and behavior outcomes of terminally sorted steers allowed shade to steers allowed no shade housed on roller compacted concrete in a commercial feedyard in the Pacific Northwest. The authors hypothesize that performance and behavior outcomes will be positively impacted in steers allowed shade.

Materials and Methods

All procedures described within this study were evaluated by the Animal Care and Use Committee at Kansas State University (IACUC-5013-DFT). Sample size was determined by logistical constraints of the feedyard and the number of shaded pens available.

Animals and study timeline

This study was performed at a commercial feedlot in the Pacific Northwest region of the United States. The feedyard location is in a dry, arid region, with an annual rainfall between 200-230 mm and an average temperature in the month of July being around 33 degrees Celsius (Climate-Charts.com, 2025). The Köppen climate classification describes this area as a cold, semi-arid climate (Peel et al., 2007). The cattle used in this study were predominantly crossbred *Bos taurus* beef steers with variable breed composition that were scheduled for terminal sort, approximately 60 days from their lots projected feedyard exit date. Steers were enrolled in this randomized controlled trial between June 11 - July 2, 2024. Terminal sorting events allow the feedyard to manage and market a more homogenous group, based on hip height and weight of the animal. Six pens of cattle were enrolled weekly, on a rolling basis, for a total of four weeks (blocks), totaling 24 pens. At terminal sort, each steer was reimplanted (200 mg trenbolone acetate and 28 mg estradiol benzoate, Synovex® Plus®, Zoetis, Parsippany, NJ). Body weight and hip measurements were used (PenPoint™ Sort, Elanco Animal Health, Greenfield, IN) to determine the respective terminal sort group for that individual animal; heavy (T3), medium (T2), or light (T1). The sorting system used was set to allow each terminal sort group to have similar head counts.

After each individual animal was given its respective sort group number, they were then randomly allocated to shade (S) or no shade (NS) treatment groups using a random number

generator (Microsoft Excel, version 2503, Microsoft Corp, Redmond, WA). Each week (block, $n=4$), six pens were enrolled: T3 shade, T3 no shade, T2 shade, T2 no shade, T1 shade, T1 no shade. After four weeks, 24 pens total had been enrolled in the trial (S; $n=12$, NS; $n=12$). A visual depiction of pen enrollment is shown in Fig. 2.1. Pen-location within block was not adjacent in order to keep cattle with the same lot visual identifiers separated in case of broken cable fencing and potentially mixed pens. Pen size allowed for approximately 350 animals, with 13.9 m² of pen space per head. All study pen floors were roller-compacted concrete (RCC). The shaded pens had permanent steel roofed shade structures (100% solar block) in the center of the pen, 15.2 m wide and 30.5 m long, with the height of the roof spanning from 4.3 m tall to 6.7 m tall (16% grade). This allowed for approximately 2.8 m² of shade per animal, and no heat trapped under the roof due to the slope. Due to the nature of the shade structure, blinding of treatment groups in this study was not possible. Pen served as the experimental and observational unit.

Steers were fed a finishing diet of flaked corn, high moisture corn, potato byproduct, alfalfa hay, wet distillers grain, liquid finisher, and micro ingredients (56.5% DM, 13.1% DM protein, 62.7 Mcal/cwt net energy for gain) for the duration of the study. Lubabegron (Exporior®, Elanco Animal Health, Greenfield, IN) was fed for the last 42 days before the pens projected ship date, with a wash out period of at least 5 days before harvest. All study animals were harvested at a commercial beef packing plant approximately 96.5 kilometers from the feedyard between August 4 and October 9, 2024. Paired pens (example: block 1 T1 S and block 1 T1 NS) were shipped to harvest on separate days of the same week. Carcass reports were collected from the packing plant to determine final live weight (FLW), hot carcass weight (HCW), quality grade, and yield grade. Pen level average daily gain (ADG) was calculated using the average FLW minus average enrollment weight, and average study days on feed (DOF). Pen

level feed conversion (F:G) was calculated using the average FLW minus average enrollment weight, divided by the average sum of feed delivered on a dry-matter basis (DM). These calculations followed standard beef cattle performance calculations (Nutrient Requirements of Beef Cattle, 2015). Any study pen with a head count discrepancy more than 15% from enrollment head count was removed from further statistical analysis (n= 4 S and 1 NS). This threshold was chosen to avoid potential bias from pens where a substantial number of carcass reports were missing due to incomplete data from the packing plant. To ensure that the carcass data represented a reliable sample of the original pen population, pens with greater than a 15% head count discrepancy were removed from further analysis.

Daily sampling

Daily data collections included water consumption, feed delivery, disease events, and open mouth breathing (OMB) behavior. Pens had fence line waterers, with both pens on each side of the water being from the same treatment group. Each waterer had a flow meter, allowing water flow to be measured in total liters, and extrapolated to water consumption. Water consumption was recorded starting when the first pen to use the waterer was enrolled until August 4. Each day, at 1400 h, water consumption was recorded for each waterer, as well as the waterers respective pens head count and approximate body weight. Any water leak visible at the 1400 h was noted. Daily liters consumed per 453.6 kg of body weight was used as the outcome. Head count and estimated daily body weight (determined through the feedyards management software system) were used to determine the approximate total kg of animal for each waterer. Temperature-humidity index (THI) was determined by an equation previously published ($THI = [0.8 \times \text{ambient temperature}] + [(\% \text{ relative humidity} \div 100) \times (\text{ambient temperature} - 14.4)] + 46.4$) (Mader et al., 2006) and recorded daily at 1600 h by a weather station located at the

feedyard. A THI threshold of greater than or equal to 80 was used to differentiate the potential for heat stress. This cutoff was determined based on industry guidance previously described (Ishmael, 2011; Texas A&M AgriLife Extension, 2022). For all statistical analyses, THI was binomialized to below 80 THI, or greater than or equal to 80.

Pen level feed delivery was collected, daily, through the feedyards data management system (Micro Technologies™, AmerisourceBergen, Amarillo, TX) on a DM per head basis for the entirety of the study. Disease event outcomes were also collected, daily, through the feedyards management system (Micro Technologies, AmerisourceBergen, Amarillo, TX) by recording the number of morbidities, mortalities, and culling for the entirety of the study. Culling was defined in this study as any animal that shipped to harvest or culled before the projected ship date for that pen. Feedyard personnel evaluated cattle daily for signs of illness and all treatment and culling decisions were based on existing protocols in place by the consulting veterinarian.

Open mouth breathing (OMB) behavior was determined by the proportion of OMB within study pens from day of enrollment to August 4 at 1600 h. Due to time constraints, each pen was randomly allocated to one of four days (six pens per day) for OMB assessment. Every four days, the same pen was reassessed. One picture was taken from the front of the pen, and one picture was taken from the back of the pen. A single, trained observer then counted the number of animals in each picture that were standing in that half (front or back) of the pen, with their faces and mouths fully visible. Due to assessment through still pictures, only OMB was able to be recorded. The case definition of OMB in this study included standing, mouth open, saliva present, and neck extended. This relates closest to a panting score of 4 in regards to Mader et al. (2006). This represents severe heat stress only, and does not account for total heat stress behavior.

Statistical analysis

Statistical models were built using R Studio (version 2024.12.0, PBC, Boston, MA). Pen served as the experimental and observational unit. Linear mixed effects models (“lme” function of R Studio) were utilized for continuous outcomes (identity link function): enrollment total pen weight, enrollment head count, enrollment average weight per head, water consumption, feed delivery, FLW, HCW, ADG and F:G. Generalized linear mixed effects models (logit link function), using events over trials (“glmer” function of R Studio) were used for disease event outcomes, OMB behavior, yield grades, quality grades, and dark cutter count. Treatment group (S or NS) was considered a fixed effect for all outcomes. Terminal sort group nested within week of enrollment (block) was considered a random effect to account for the lack of independence between groups; however, sort group was removed for the enrollment weight models because they were purposefully enrolled at different weights. For the water consumption and OMB models, THI category of that day (<80 or ≥ 80) was included as a fixed effect, and waterer or pen number were treated as a random effect to account for repeated measures. For the feed delivery model, the previous day THI was used in the statistical model because feed calls are based on previous day intake and pen number was treated as a random effect to account for repeated measures. For the OMB behavior model, location of picture taken (front or back) was considered a random effect as well as pen number as a random effect to account for repeated measures. For repeated measure outcomes (water consumption, feed delivery, OMB), a repeated covariance structure was used, including calendar date to account for serial dependence. Yield grade counts were categorized for statistical analysis as yield grade 1, 2, and 3 versus 4 and 5. Quality grade counts were categorized as prime and choice versus select. To stabilize variance and better meet the assumptions of normality for parametric analyses, a Box-Cox transformation was applied to

the dependent variable, determined by a Shapiro-Wilks test and visually examining histograms. The optimal lambda (λ) value was determined using maximum likelihood estimation. Box-Cox transformations were performed on water consumption, ADG, and F:G to obtain normality. For transformed data, the least square means is reported as the back-transformed value, with the standard error of the mean (SEM) reported as the transformed value. In each model, factors were considered significant if $P \leq 0.05$. For significant interaction effects, Tukey-adjusted pairwise comparisons were performed within levels of the interacting factors.

Economic analysis

An economic analysis was performed on a per head basis for each treatment group using a similar method previously published (Dennis et al., 2020). Net returns were calculated by using total revenue minus feeder cost, yardage cost, feeding cost, health cost, and interest cost. Initial cost and maintenance costs of the shade were not included in the economic analysis; therefore, this is based on returns based on production outcomes. Total revenue was calculated using the fed cattle price (\$/kg) for the week of September 2nd, 2024 (Cattle Markets, 2024), which was the middle of the shipping period, average finish weight of treatment group, a 5% shrink, and the average weight of the culled animals, with their return set at 75% of the fed cattle sale price. Feeder cost was calculated on a slide using average differences of weight groups of the feeder price (\$/kg) for the week of June 17th, 2024 (USDA National Feeder Cattle Summary, 2024), which was the middle of the enrollment period, the average enrollment weight, and the number of head enrolled in each treatment group. Yardage cost was determined by a national average of USD\$0.30/head day and the DOF for each treatment group. Feeding cost was calculated by using the average price of corn for the week of July 22nd (Agricultural Prices, 2024), which was halfway through the study period, calculated average feed conversion, finish weight, and percent

mortality and railers. Health costs were determined by the percent morbidity and cost, using USD\$23.60/treatment per the NAHMS Beef Feedlot (2011) survey of respiratory disease treatments in U.S. feedlots. Interest rate was calculated using the monthly prime rate for the month of July 2024 (FedPrimeRate.com).

Results

A total of 7,712 crossbred *Bos taurus* beef steers were enrolled between June 11 and July 2, 2024 at terminal sort and were harvested between August 4 and October 9, 2024. To confirm effective randomization between treatment groups at enrollment, there were no differences detected between treatment groups ($P>0.05$) regarding head counts, total pen weights, and average weight per head, as shown in Table 2.1.

From June 11 to October 8, 2024, the THI at 1600 hours was recorded. There were 19 days (16% of study days) that surpassed the threshold of 80 THI at 1600 h, occurring in the middle of July and again in the beginning of August. Figure 2.2 shows the THI over the duration of the study period.

No water leaks were recorded during the duration of the study. There were statistically significant ($P < 0.05$) differences observed in daily water consumption in liters per 453.6 kg of bodyweight between treatment groups and THI category, but no ($P>0.05$) interaction between treatment group and THI category was identified. Regardless of THI category, S pens had a 15% decrease in water consumption compared to NS pens (29.98 ± 0.04 SEM and 35.24 ± 0.05 SEM L per 453.6 kg bodyweight, respectively; $P<0.05$). When the THI was above the threshold of ≥ 80 , water consumption (35.28 ± 0.03 SEM L per 453.6 kg bodyweight) was 18% greater ($P<0.05$) compared to days with THI < 80 (29.98 ± 0.03 SEM), regardless of treatment group. A treatment by THI category interaction was detected in feed delivery on a DM per head (kg) basis,

with the NS pens having a 3% decrease in feed delivery following days with a THI ≥ 80 compared to days with a THI < 80 (Fig. 2.3; $P < 0.05$).

No significant differences ($P > 0.05$) were found between treatment groups in the proportion of morbidity, mortality, or did not finish (mortality and culls combined). However, a significant difference was found in the proportion of culls, with more culls in the NS pens compared to the S pens ($0.6\% \pm 0.002$ SEM and $0.3\% \pm 0.002$ SEM; $P < 0.05$). The reason for culling events was not recorded.

The probability of OMB resulted in a tendency ($P = 0.09$) found between treatment groups and a significant ($P < 0.05$) association between THI category, while no interaction between the two factors was identified. In NS pens, the model estimated probability of OMB was 0.9% (0.003 SEM), compared to 0.07% (0.005 SEM) in S pens ($P = 0.09$). Model estimated probability for OMB on days with THI < 80 (0.06%, 0.004 SEM) was lower ($P < 0.05$) compared to days with THI ≥ 80 (1.03%, 0.004 SEM).

Paired pens (example: block 1 T1 S and block 1 T1 NS) were shipped to harvest within two days of each other. Average DOF for T1's, T2's and T3's between terminal sort and harvest were 87, 76, and 57 DOF, respectively. After collecting carcass data, 4 S pens and 1 NS pen were removed from further carcass analysis due to head count discrepancy of more than 15% compared to enrollment head count, because of missing or incomplete carcass reports. Therefore, eight S pens and 11 NS pens were included in carcass analysis. There were no differences detected in FLW, HCW, ADG, and F:G between treatment groups ($P > 0.05$; Table 2.2). There were no detectable differences between S and NS in the probability of steers being yield grade 1,2, or 3 versus yield grade 4 or 5 (0.8 ± 0.02 SEM and 0.79 ± 0.02 SEM, respectively, $P > 0.05$). However, when considering quality grade, a higher ($P < 0.05$) proportion of S pens graded prime

and choice (0.93 ± 0.02 SEM) than NS pens (0.91 ± 0.02 SEM). A significant difference was also found when comparing dark cutter counts, with the probability of steers being dark cutters being lower ($P < 0.05$) in S pens (0.01 ± 0.002 SEM) compared to NS pens (0.02 ± 0.004 SEM).

The calculated net return on a per head basis for a steer after terminal sort for the S group was USD\$424.64 and USD\$413.73 for the NS group. This shows roughly a USD\$11.00 increase on return when steers are allowed shade in the last 70 DOF.

Discussion

The objective of the current study was to compare performance and behavior outcomes after terminal sort between steers allowed access to shade and steers without shade housed on roller compacted concrete in a commercial feedyard in the Pacific Northwest. As evidenced in Fig. 2.2, it was a moderate summer regarding heat, with no extreme heat events occurring. Results show beneficial impacts to carcass quality, environmental sustainability, and animal welfare and health.

In the current study, there was a statistically significant interaction between the treatment group (S or NS) and THI groups regarding feed delivery. Steers in the NS group were shown to have a 3% decrease in feed delivery when the THI of the previous day was above the threshold of 80, whereas no differences were observed in the S group. Because the cattle are limit fed, we are assuming that feed delivery and intake are similar. Previous studies show that beef cattle allowed shade had a higher dry matter intake compared to cattle not allowed shade (Mader et al., 1999; Mitlöhner et al., 2001; Mitlöhner et al., 2002; Barajas et al., 2013; Hagenmaier et al., 2016), however, other studies show no difference in dry matter intake (Mader et al., 1999; Boyd et al., 2015; Hayes et al., 2017). One study reported the interaction of feed intake and different categories of THI between a shade and no shade group and results show that the no shade group

had a higher intake in the “normal” THI category compared to the shaded group, however the reverse was true in the “emergency” THI category, with the shaded group having a higher intake than the no shade group (Brown-Brandl et al., 2005). Differences seen in literature, and described here, can be affected by cattle type, severity and duration of heat events or time of year, and type of shade (shade area and solar block).

Cattle daily response to thermal load was assessed by proportion of OMB and the NS group tended to have a greater probability of OMB compared to the S group. Though the probability was small in the NS group (approximately 0.9% OMB), this may be the result of multiple factors, such as logistical constraints of monitoring OMB, pictures were taken, and only extreme panting (OMB) would be noted. Therefore, this does not account for total heat stress behavior. Another factor leading to the low reported OMB may have been the low number of days during the study period where THI was not elevated enough to exceed the cattle comfort threshold, causing severe enough heat stress to exacerbate OMB in the pens. The current results are similar to previously published literature, with animals in the unshaded groups panting more or having an increased respiration rate compared to the shaded group (Brown-Brandl et al., 2005; Gaughan et al., 2010; Aengwanich et al., 2011; Blaine and Nsahlai, 2011; Sullivan et al., 2011; Van Laer et al., 2015; Hagenmaier et al., 2016; Hayes et al., 2017). The differences seen in the current study prove the benefits of shade on animal welfare, by seeing decreases in culling, dark cutters, and OMB behavior.

Behavioral and physiological processes involved in heat dissipation result in water loss, therefore water intake becomes crucial to prevent dehydration and aid in a small amount of thermal heat mitigation (Gebremedhin et al., 2008). The inclusion of shade in pens was shown to significantly impact water consumption. In the current study, the S group had a 15% decrease in

daily water consumption compared to NS group. This decrease was seen without subsequent changes identified in feed delivery or overall performance between the two treatment groups. Two previously published studies reported information concerning water consumption. One study noted a decrease in daily water consumption with the addition of shade compared to the non-shaded group (53.1 and 49.3 L/d, respectively) (Gaughan et al., 2010). Another study found no differences between shaded groups (square meters of shade per animal), but a significant decrease in water usage by cattle allowed shade versus not allowed shade (Sullivan et al., 2011). With increased concern regarding the environmental impacts of the feedyard and the limited water availability in certain areas of North America, shaded pens show a beneficial impact on water conservation and thus environmental sustainability. As thirst is a negative affective state in animals, a decrease in water consumption with the addition of shade proves beneficial to the animals' affective states.

In the current study, no differences were observed between the S and NS group in morbidity, mortality, or did not finish (mortalities and culls combined). However, there was a 50% decrease in the count of culls in the S group compared to the NS group. Though blinding was not possible in the current study, feedyard staff were making the culling decisions at the hospital, away from the study pens. One potential explanation for this finding is the lack of shade may have increased heat load and previously diseased or immuno-compromised animals may have decreased chances for recovery with increased thermal stress, as seen in Brown-Brandl et al. (2006) as a risk factor for heat stress. A decrease in the number of culls with the inclusion of shade benefits production and animal welfare. Cattle's capacity for thermoregulation is compromised when there is an active disease process (Lemal et al., 2023), in turn impacting the animals affective states. Therefore, culling becomes a humane option while considering the

prognosis of these animals. To the authors' knowledge, there are no other published manuscripts evaluating the effect of shade on culling rates.

Regarding steer performance, the current study only found statistically significant differences when comparing the proportion prime and choice versus select between the S and NS groups. Pens with the inclusion of shade were seen to have a higher proportion of steers grading prime and choice compared to non-shaded pens. Authors believe the higher proportion of quality grade prime and choice is due to the consistent feed intake seen in the S group compared to NS. Shade showing improvements to quality grade was also seen in another study comparing shade to no shade with beef cattle performance (Mitlöhner et al., 2002), though many studies did not find a difference in quality grade (Mitlöhner et al., 2001; Blaine and Nsahlai, 2011; Hagenmaier et al., 2016). The current study did not find any differences when comparing FLW, HCW, yield grade, ADG, and F:G. Previously published literature had inconsistent results on the effect of shade on steer performance with some studies illustrating no detectable differences in FLW (Mader et al., 1999; Sullivan et al., 2011; Rovira, 2014), HCW (Mader et al., 1999; Sullivan et al., 2011; DiGiacomo et al., 2014; Boyd et al., 2015; Hagenmaier et al., 2016), ADG (Rovira and Velazco, 2010; Brown-Brandl, 2013; Rovira, 2014; Hagenmaier et al., 2016; Hayes et al., 2017;), or feed conversion (Mader et al., 1999; Mitlöhner et al., 2001; Mitlöhner et al., 2002; Boyd et al., 2015). However, some studies identified differences in FLW (Mitlöhner et al., 2001; Mitlöhner et al., 2002; Blaine and Nsahlai, 2011; Boyd et al., 2015), HCW (Gaughan et al., 2010; Blaine and Nsahlai, 2011), ADG (McIlvain and Shoop, 1971; Mitlöhner et al., 2001; Mitlöhner et al., 2002; Gaughan et al., 2010; Blaine and Nsahlai, 2011; Barajas et al., 2013; Boyd et al., 2015), or feed conversion (Gaughan et al., 2010; Barajas et al., 2013), showing performance benefits in the shaded groups.

The probability of dark cutters was significantly different, with S pens having approximately 50% lower dark cutters compared to NS groups. This finding was also observed in a previous study, where the proportion of dark cutters in heat-stressed heifers allowed shade was half of the dark cutters from the heat-stressed heifers not allowed shade (Mitlöhner et al., 2002). Prolonged, or chronic, stress before harvest is considered to be the primary cause of dark cutters, with a variety of events, such as environmental stress, playing a role (Scanga et al., 1998; Ponnampalam et al., 2017). Chronic stress that leads to detrimental effects on muscle glycogen depletion, that lead to observing dark cutters, has been well documented in slaughter facilities (Edwards-Callaway et al., 2025). A study composed of nine commercial feedlots over a three-year period showed the incidence of dark cutters rises with increasing temperatures (Scanga et al., 1998), while a study conducted in Australia found that higher maximum ambient temperature, prior to feedlot exit, increased the incidence of dark cutters throughout a 3-, 7-, 14-, and 28-day lag period, though the differences were minimal (Steel et al., 2022). Decreasing the number of dark cutters not only improves quality grade and financial returns, but also shows an improvement in the welfare of these animals before harvest.

One major concern for producers is the cost and financial return from the shade structures. A study estimated the initial cost of investing in artificial shade structures to be USD\$90.00 per head (Maia et al., 2023). The economic analysis performed for this study showed beneficial to the S group, with a \$11.00 increase in net return per head for each steer enrolled at terminal sort. This shows potential for a large enough difference in the net returns to allow the increase in cattle performance to help offset the cost of the shade. However, these economic calculations were limited to the last 70 days on feed, using national averages for feed costs and

cattle prices during that period. Therefore, these results only provide a short-term economic perspective and do not include broader sensitivity analyses of feed or cattle price variation.

Limitations in this study include a specific study population, mild heat during the study period, and limited power to detect differences in treatment groups. Native beef steers, including both light and dark hided, were enrolled at terminal sort, approximately 60 days from harvest. There were only 19 days that passed the THI threshold of 80 (“danger” or “emergency” zone of the beef cattle temperature humidity chart) during the study period, proving the summer was milder compared to previous years. This study was a pen level study, with 12 S and 12 NS pens. The numbers per treatment group was determined based on feedyard logistics and how many pens had the permanent shade. After the exclusion of 4 S and 1 NS pen due to the lack of carcass reports obtained, power to detect differences was lessened. Pen exclusions occurred due to reasons unrelated to treatment effects, and therefore are unlikely to have introduced systematic bias. Data were analyzed using mixed models that accommodates unequal numbers of pens per treatment. Nonetheless, this potentially limited the ability to detect statistically significant differences in performance and carcass traits, if there truly was a difference between groups. A greater sample size may possibly be able to detect significant differences in more performance and carcass outcomes. Limitations regarding open mouth breathing behavior include the observation of only a subset of pens each day and the use of photographic assessment detecting more severe panting behavior. However, due to the large number of cattle in each pen (approximately 350 steers), panting behavior was evaluated using pen-level photographic scan sampling, which minimized animal disturbance and allowed observations to be collected consistently and within a similar environmental time frame across pens.

Further research is warranted on other heat stress mitigation strategies that could be used alone, or in combination, to help mitigate heat stress, such as misting cattle, fans, bedding type, or different feeding strategies. The implementation of shade in the US feedyard sector is slow, despite the multiple benefits on beef cattle performance and welfare. Common reasons for not providing shade include the initial cost and investment for the structures, as well as the questionable need for shade in some regions of the United States. The current study demonstrates the benefits of shade on carcass quality, animal welfare, and environmental sustainability under moderate heat stress conditions in a temperate region. Producers should treat shade as being another tool in the toolbox for mitigating heat stress, especially while prioritizing shade to the higher risk cattle for heat stress (e.g. dark-hided, later DOF).

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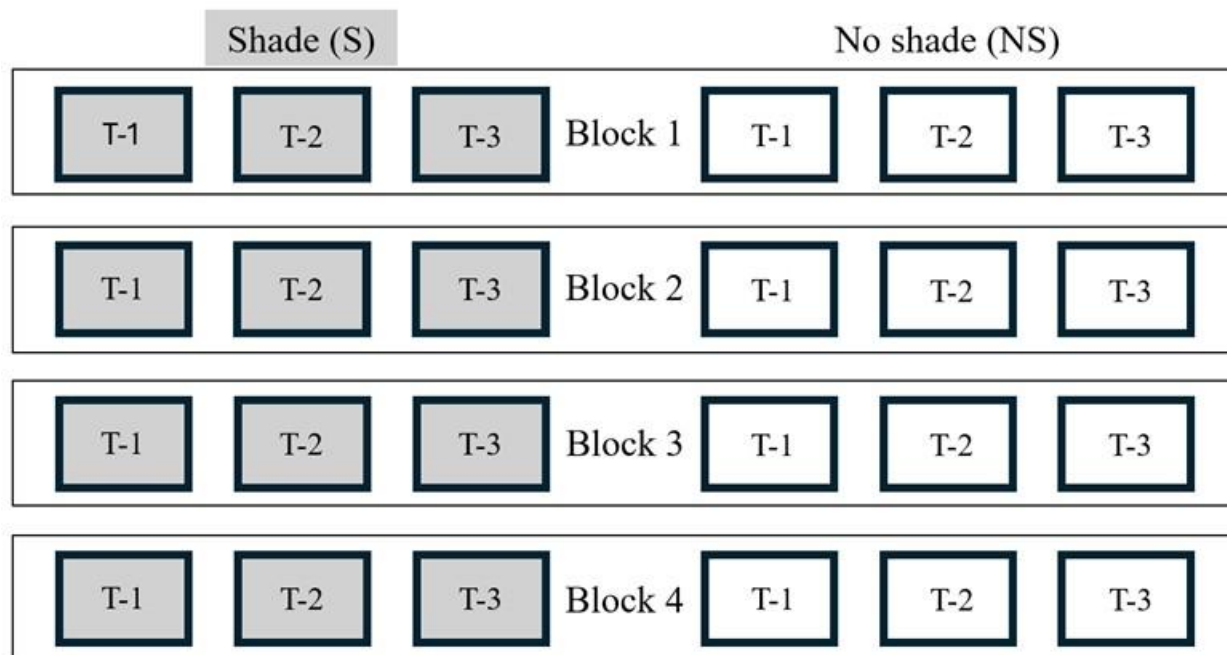


Figure 2.1. Pen enrollment visual

Pen enrollment visual including block, pen, and treatment group, depicting pens enrolled at terminal sort either into shade or no shade at a commercial feedyard in the Pacific Northwest in the summer of 2024.

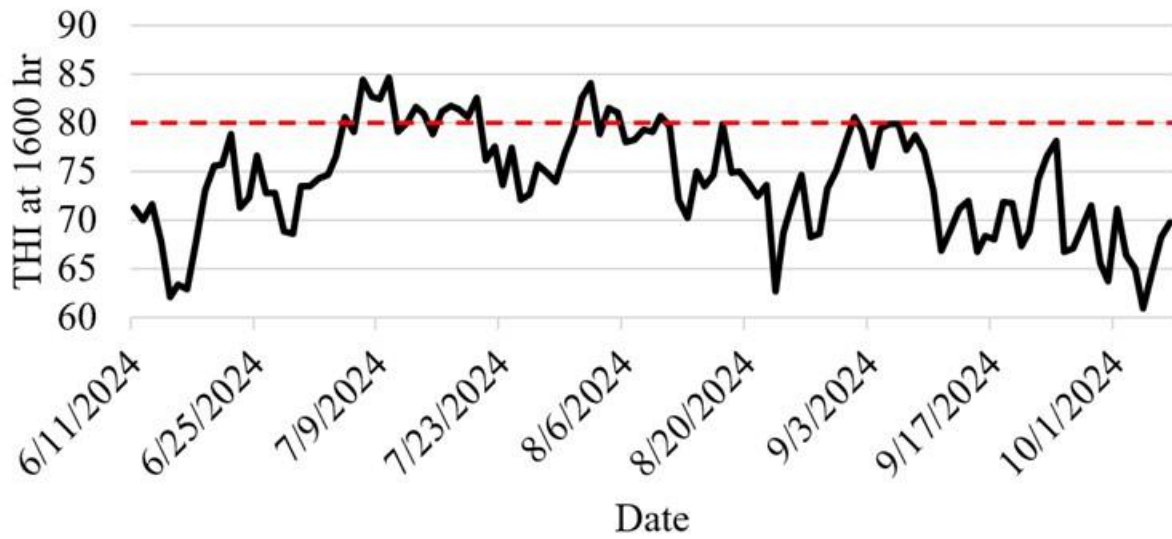


Figure 2.2. Temperature-humidity index at 1600 hr

Temperature-humidity index at 1600 hr over time in the Pacific Northwest region of the United States between June 12 and October 7, 2024. A horizontal dashed line at THI 80 depicts the THI cutoff to signify a potential heat stress event.

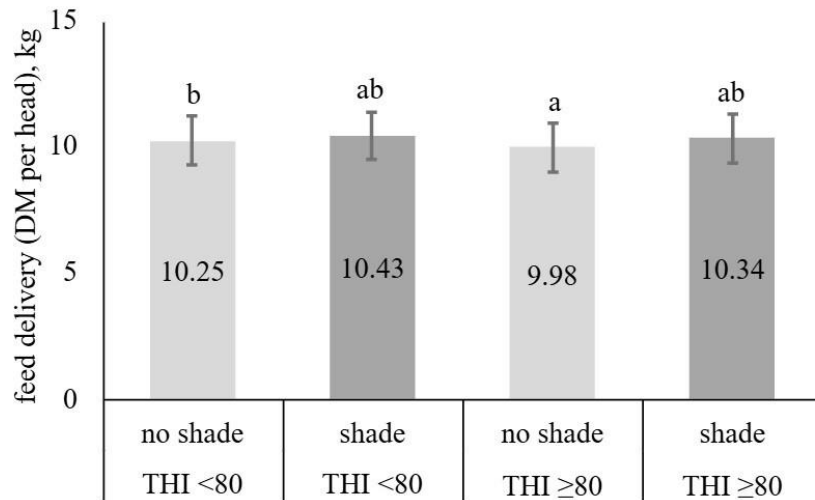


Figure 2.3. Mean feed delivery

Mean feed delivery, kg DM per head, and standard error (SE) between THI categories for steers enrolled at terminal sort into either shade or no shade pens at a commercial feedyard in the Pacific Northwest in the summer of 2024. A statistically significant difference was found between no shade THI <80 and no shade THI ≥80, depicted by a and b. No detectable differences found is depicted by ab.

Table 2.1. Mean and standard error of the mean (SEM) of head count, total pen weight (KG), and average weight per head (KG)

Mean and standard error of the mean (SEM) of head count, total pen weight (KG), and average weight per head (KG) of steers enrolled at terminal sort into either shade or no shade pens at a commercial feedyard in the Pacific Northwest in the summer of 2024. Averages are represented as raw values due to stratifying by terminal sort group. SEM and P-values are model outputs between the treatment groups.

Enrollment Outcome	Terminal Sort Group	Treatment				P-value
		Shade		No Shade		
		Mean	SEM	Mean	SEM	
Head count	Light (T1)	322	7.1	327	6.9	0.92
	Medium (T2)	315		329		
	Heavy (T3)	317		318		
Total pen weight, kg	Light (T1)	178,886	9932	182,244	9315	0.94
	Medium (T2)	190,462		199,242		
	Heavy (T3)	207,282		207,528		
Average weight per head, kg	Light (T1)	557	23	557	19	0.64

Table 2.2. Mean and standard error of the mean (SEM) of average final live weight, kg per head, average hot carcass weight per head, kg, average daily gain per head, kg, and average feed to gain conversion per head, kg

Mean and standard error of the mean (SEM) of average final live weight, kg per head, average hot carcass weight per head, kg, average daily gain per head, kg, and average feed to gain conversion per head, kg at harvest of steers enrolled at terminal sort into either shade or no shade pens at a commercial feedyard in the Pacific Northwest in the summer of 2024. 1Denotes outcomes that were transformed with a Box Cox transformation to obtain normality, with the mean being represented as back transformed and the SEM being represented as transformed.

Outcomes at harvest	Treatment				P-value
	Shade		No Shade		
	Mean	SEM	Mean	SEM	
Average FLW/head, kg	722.6	11.6	720.8	9.2	0.88
Average HCW/head, kg	459.5	7.2	454.0	5.7	0.53
ADG/head, kg*	1.6	0.9	1.5	0.8	0.75
Average F:G/head, kg1	6.12	0.003	5.98	0.003	0.54

Chapter 3 - The effect of night-time feeding on steer performance after terminal sort

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Abstract

Heat stress occurs when total environmental and metabolic heat production is greater than an animal's ability to dissipate that heat. Heat stress negatively impacts feeder cattle performance and welfare. Limited research has been conducted to determine if feeding cattle in the evening, thereby shifting their metabolic heat production to a cooler period of the day, can help mitigate heat stress. This pen-level randomized controlled trial evaluated the effects of evening feeding (PM; feedings at 2000, 2300, and 0200 h; $n = 24$ pens) versus morning feeding (AM; feedings at 0500, 0800, and 1200 h; $n = 24$ pens) on terminally sorted steer performance in a commercial feedyard in the Pacific Northwest. Data collection included feed delivery, water consumption, health events, open mouth breathing prevalence, and carcass traits. Linear and generalized linear mixed-effects models were used to determine potential differences between treatment group and temperature-humidity index (THI; <80 versus ≥ 80). Only 14% of the total study days had a THI

≥ 80 , indicating little to no heat stress impacts. There were no differences found between PM and AM for any outcome ($p < 0.05$). Regardless of treatment group, water consumption tended ($p = 0.07$) to increase, and open mouth breathing significantly ($p < 0.05$) increased on days with THI ≥ 80 . Further research is warranted to assess evening feeding as a heat stress mitigation strategy in a feedyard setting.

Keywords: feedyard, heat mitigation, heat stress, night-time feeding, steers

Introduction

Heat stress occurs when total heat accumulation (both environmental and metabolic) exceeds the animal's ability to dissipate heat (Hahn and Becker, 1984; Foroushani and Amon, 2022). Cattle respond to high environmental temperatures behaviorally, physiologically, and cellularly in an attempt to maintain homeostasis (Dos Santos et al., 2021; Mishra, 2021). In extreme situations, hyperthermia can result in a large number of deaths (Hubbard et al., 1999). In a review published by Lees et al. in 2019 (Lees et al., 2019), authors associated heat stress with reductions in dry matter intake, growth, and feed conversion efficiency in feedyard cattle. Additionally, an increase in respiration rate, panting score, and body temperature are commonly observed during times of heat stress (Idris et al., 2021; Idris et al., 2024; Islam et al., 2023). In 2003, St-Pierre estimated that there was a \$370 million (USD) economic burden on the beef industry attributable to heat stress (St-Pierre et al., 2003). Given that this estimate is over 20 years old, the current economic burden is likely higher today.

Today, cattle are grown to greater body weights and spend more time in the finishing phase, increasing their susceptibility to heat stress while simultaneously being exposed to challenging environmental conditions. As heat stress in feedyards becomes a greater concern for cattle performance, welfare, and economics, heat stress mitigation strategies have been explored.

The National Cattlemen's Beef Association Beef Quality Assurance program provides useful practices to help prevent heat stress in high-risk groups of cattle, mainly through altering cattle handling and transport in extreme weather (Beef Quality Assurance). Certain beef cattle certification programs have guidelines to follow to minimize heat stress, including Global Animal Partnership and Humane Farm Animal Care (Humane Farm Animal Care; Global Animal Partnership, 2022). These typically include access to shade and water-cooling systems (sprinklers). Cattle at greater risk for heat stress are generally considered first when prioritizing shade or sprinkler systems. Risk factors for heat stress include dark hide color, prior pneumonia treatment, greater fatness, and excitability (Brown-Brandl et al., 2006); therefore, shade and sprinklers are typically seen in receiving, shipping, and hospital pens. However, because of the cost of shade structures and increased water usage by sprinklers, alternative mitigation strategies warrant investigation.

Elevated body temperature typically leads to a protective lowering of metabolic activity and suppressed appetite (Baumgard and Rhoads, 2012; Oliveira et al., 2025; Renaudeau et al., 2012; Silanikove, 2000). Thermal heat load in cattle is determined by both environmental and metabolic heat production. Ray et al. (1971) (Ray and Roubicek, 1971) noted two major periods of feedlot cattle feed consumption, one at sunrise and one in the afternoon. However, hot summer conditions delayed and decreased time at the bunk in the afternoon, and increased feed consumption later in the evening. Conventionally, feedyard cattle are fed during the morning to early afternoon hours. Thus, peak metabolic heat production as a result of feed consumption occurs at a similar time as the peak environmental heat of the day. Feeding heifers in the afternoon was found to increase heat production in the cooler hours of the day when conduction and radiation were more efficient for dissipating heat (Brosh et al., 1998). One study found that

tympanic temperature decreased when steers were fed in the afternoon (1600 h), compared to the morning (0800 h), when the bunks were empty for several hours before feeding (Davis et al., 2003). Alternative ways to help mitigate heat stress include altering dietary components of the ration. Increased beef cattle performance in heat stress conditions have been found with dietary inclusion strategies consisting of linseed oil (Ceconi et al., 2022), *Allium mongolicum* Regel powder supplementation (Liu et al., 2024), wet distillers grains with solubles and zilpaterol hydrochloride (Boyd et al., 2015; Hales et al., 2014). Recently published reviews regarding microalgae inclusion into dairy goat diets (Boukrouh et al., 2025) and dairy cow diets (Boukrouh et al., 2026) found improved fatty acid profiles of milk produced by both species fed microalgae.

Limited, conflicting studies have been conducted to determine the effect of feeding time on cattle performance. Positive effects of feeding time were observed in Barajas et al. (2013), where they found that feeding 70% of the day's feed delivery to nonshaded crossbred steers during a period of high ambient temperatures at 1430 h compared to 0630 h increased average daily gain, gain efficiency, and dietary NE (Barajas et al., 2013); and a study conducted in 1994 found that feedlot limit-fed Holstein steers had greater gain and feed efficiency when fed at 2000 h compared to 0800 h (Brandt et al., 1994). A study conducted using Hereford steers found that steers with access to feed between 1600 and 0800 h the following day had increased feed intake during a hot period and a lower rectal temperature at 1800 h compared to steers fed ad libitum (Holt et al., 2004). Conversely, Mader and Davis did not find differences in Angus yearlings fed in the morning versus the afternoon on ADG during summer months (Mader and Davis, 2004). Regarding dairy cattle, a crossover study, using eight lactating cows, was used to determine if evening feeding (2030 h) would help alleviate associated production losses seen during heat stress events; however, no differences were seen in vaginal temperature, respiration rate, dry

matter intake, water intake, or milk production parameters (Ominski et al., 2002). The authors believe these inconsistent findings are due to the different feeding strategies (e.g., limit-fed/restricted), timing and number of feedings, and the variable heat load and duration within the studies. These inconsistent findings highlight the need for further research, particularly under large-scale commercial conditions.

The objective of this study was to compare performance and behavior outcomes of terminally sorted steers fed in the morning (AM) versus steers fed in the evening (PM) throughout the finishing period and through harvest. We hypothesized that PM-feeding will improve performance (average daily gain, feed conversion, carcass characteristics) and behavior (open mouth breathing prevalence) outcomes by shifting metabolic heat production to cooler periods of the day.

Materials and Methods

All procedures described within this study were approved by the Animal Care and Use Committee of Kansas State University (IACUC-5251-DFT). Sample size was determined based on an expected difference of 18 KG in final body weight (536 kg vs. 554 kg), derived from Experiment 1 reported by Knutsen et al. (Knutsen et al., 1994). Using an alpha of 0.05 and beta of 0.20 with standard deviation of 20 the number of pens to statistically identify this difference was calculated to be 21 per treatment group. We used 15% more pens ($n = 24$ per treatment group) in anticipation of potential data loss. Animal enrollment into the study began on 4 June 2025, and the final pens shipped to harvest on 25 September 2025.

Animals and Study Timeline

The location of the commercial feedyard was in the Pacific Northwest region of the United States. In this region, it is dry and arid, with approximately 8–9 inches of annual rainfall

and an average high temperature in the month of July around 33 degrees Celsius (Climate-Charts.com). The Köppen climate classification describes this area as a cold, semi-arid climate (Peel et al., 2007). This randomized controlled trial included native crossbred beef steers scheduled for terminal sort, approximately 60 days from their projected ship date. Terminal sorting allows for a large group of cattle to be sorted into smaller, more homogenous, marketing groups based on body weight and hip measurements. Allocation occurred between 4 June and 20 June 2025. Six pens were enrolled per day (blocks; $n = 8$), over a total of three weeks, totaling 48 pens. At terminal sort, each steer was reimplanted (Synovex Plus, Zoetis, Parsippany, NJ, USA). Using a sorting system (PenPoint Sort, Elanco Animal Health, Greenfield, IN, USA), body weight and hip measurements were collected to determine the respective terminal sort group for each individual animal; heavy (T3), medium (T2), or light (T1). Within the sorting system, a small variance set in between each sort group to allow for similar head counts. The average body weight and standard deviation for the T3, T2, and T1 groups were 645.01 ± 9.98 kg, 601.56 ± 9.53 kg, and 556.65 ± 8.16 kg, respectively.

After each animal was allocated to their respective sort group in the chute, they were then individually randomized to morning-time fed (AM) or night-time fed (PM) treatment groups using a random number generator (Microsoft Excel, Microsoft Corp, Redmond, WA, USA). Each terminal sort day (block; $n = 8$), six pens were enrolled: T3 AM ($n = 1$), T3 PM ($n = 1$), T2 AM ($n = 1$), T2 PM ($n = 1$), T1 AM ($n = 1$), T1 PM ($n = 1$), until 48 pens were enrolled (24 AM, 24 PM). Each dirt floor pen allowed approximately 150 animals, with 125 square feet of pen space per head. All pens were fed a finishing diet of flaked corn, high moisture corn, potato byproduct, alfalfa hay, wet distillers grain, liquid finisher, and micro ingredients (56.5% DM, 13.1% DM protein, 62.7 Mcal/cwt net energy for gain) for the duration of the study. Lubabegron

(Experior[®], Elanco Animal Health, Greenfield, IN, USA) was fed for the last 56 days before the pens' projected ship date, with a wash-out period of at least 3 days before harvest.

On day 0 (day of terminal sort), all AM and PM pens allocated were fed conventionally, with first feeding starting at 0500 h, second feeding at approximately 0800 h, and third feeding at approximately 1200 h. For the remainder of the study, AM pens were fed with this forementioned schedule. On the evening of day 0, PM pens were fed their next day's feed, with no feed call adjustment. Feeding times for the PM pens were 2000 h, 2300 h, and 0200 h the following morning. This feeding schedule continued for 2 evenings with no feed call adjustments. On the evening of day 3, feed calls for the PM pens were decreased to allow for cattle to consume the leftover feed in the bunks. Because of the nature of different feeding times and bunk conditions, blinding of treatment groups in this study was not possible.

Temperature-humidity index (THI) was recorded every day at 1600 h, using a previously published equation (Mader et al., 2006). The THI was then binomialized to <80 or ≥ 80 THI, signifying potential heat stress effects (Texas A&M University Animal Science, 2022).

Daily Collections

Water consumption was measured at 1500 h daily, from 6 June 2025 to 6 August 2025, by individual water meters attached to each waterer in liters. Water consumed in liters per 454 kg BW was calculated using the daily water meter reading and the average pen weight determined by Microbeef (Micro Technologies, AmerisourceBergen, Amarillo, TX, USA).

Feed delivery was measured daily for the duration of the study through Microbeef, as DM kgs per head delivered. Because of the differences in feed delivery for the PM pens during the transition, a 6-day acclimation period allowed for cattle to transition to the evening feeding before daily feed delivery was recorded.

Health events were recorded for the entire duration of the study through Microbeef, daily, including morbidity pulls, mortality, and culling events.

Open mouth breathing (OMB) behavior was determined daily from 6 June 2025 to 6 August 2025, at 1600 h, using a scan sample approach by taking a picture from the center of the front of the pen (feed bunk). Each pen was randomized to one of four days, allowing for OMB to be recorded every 4 days for each pen (12 pens/day). Pictures were then analyzed by one trained, blinded observer to determine the total number of heads seen in the pen, standing, with their faces clearly visible to determine open mouth breathing status. Then, the number of cattle that met the case definition of OMB (standing, mouth open, saliva present, and neck extended) were recorded.

Carcass Collections

Pens shipped to harvest between 5 August 2025 and 25 September 2025. Paired pens (AM and PM pen from same terminal sort group and block) were always shipped on the same week. Individual carcass reports were received from the packing plant and matched to the individual IDs and pen numbers at enrollment. Average pen hot carcass weight (HCW) was recorded using the carcass reports, and an assumed 62.5% dressing percentage was used to determine the approximate final live weight (FLW) (Cattly). The number of cattle that were graded in each quality grade category (prime, choice, select, other) and yield grade category (1, 2, 3, 4, 5) were recorded, as well as the total number of individual carcass reports received for each pen. ADG was calculated by dividing the difference between FLW and enrollment weight by DOF. Feed conversion (F:G) was calculated by dividing the total DM feed delivered by the total weight gain of the pen.

Statistical Design and Analysis

Pen served as the experimental and observational unit for all analyses except water consumption. A waterer was shared between two pens; therefore, the pair of pens served as the experimental and observational unit for water consumption. A significance level was set at $p \leq 0.05$, and tendencies at $0.05 < p \leq 0.10$. All statistical analyses were performed using R Studio (version 2026.01.0+392, PBC, Boston, MA, USA). Normality of continuous outcomes was assessed using the Shapiro–Wilk test and visualization of histograms.

Continuous outcomes (water consumption, feed delivery, FLW, HCW, ADG, F:G) were analyzed using linear mixed-effects models assuming normally distributed residuals and using an identity link function. Fixed effects included treatment group. Random effects included terminal sort group nested within block to account for lack of independence among observations. For repeated-measures outcomes (water consumption and feed delivery), THI and the potential interaction between treatment group and THI were included as additional fixed effects, and paired pen or pen number was included as an additional random effect. A repeated covariance structure was used, including calendar date to account for serial dependence. For the water consumption model, THI of the observed day was used and sort group nested within block was not included as a random effect as both pens sharing a water could come from different sort groups or blocks, as long as they were the same treatment. For the feed delivery model, THI recorded at 1600 h on the previous day was used because feed calls were based on the previous day's consumption.

Health, behavior, and carcass grade (quality and yield grade) outcomes were analyzed using generalized linear mixed-effects models, with events over trials as the outcome. Morbidity, mortality, culling, OMB prevalence, quality grade, and yield grade counts were analyzed

assuming a binomial distribution and a logit link function. Terminal sort group nested within block was included as a random effect, and pen was included as an additional random effect to account for repeated measures in OMB prevalence. A repeated covariance structure was used for the OMB model, including calendar date to account for serial dependence.

Results

A total of 7848 native crossbred beef steers were enrolled in this trial. At enrollment, steers were randomized, based on terminal sort group ($n = 3$), to either AM or PM pens. Table 3.1 shows central tendencies and variability between terminal sort groups and treatment groups based on number of heads per pen and average body weight per head at enrollment.

Figure 3.1 depicts the THI recorded at 1600 h over the entire study duration. A dotted red line differentiates the threshold of 80 used in statistical analyses. Sixteen days (14%) were recorded as having a $\text{THI} \geq 80$, with a maximum recorded THI of 84.

The PM-fed pens were successfully transitioned to night-time feeding using the methods described above. By day 6 after pen enrollment, the feed calls made to the PM pens matched those being made for the AM pens without any numerical increases in digestive morbidity or mortality.

Performance and behavioral variables were generally similar between treatments throughout the study period. Table 3.2 describes model estimated means of daily collections (water consumption, feed delivery, OMB); however, treatment group was not significant for any outcomes ($p > 0.05$). Overall health (morbidity, mortality, culling), performance (final live weight, daily gain, feed efficiency), and carcass (hot carcass weight, quality grade, yield grade) outcomes are shown in Table 3.3. No differences ($p > 0.05$) were seen between treatment groups for any outcome.

Temperature-humidity index (THI) was found to have a tendency and significant difference in regard to daily water consumption rate and OMB, respectively, regardless of treatment group (Table 3.2). When the THI at 1600 h was ≥ 80 , all cattle tended ($p = 0.07$) to consume more water and had a higher probability ($p < 0.01$) of OMB than on days with a THI < 80 , indicating marginal effects of heat stress occurring between both treatment groups.

Discussion

The aim of this study was to compare performance and behavior outcomes of terminally sorted steers fed in the morning (AM) versus steers fed in the evening (PM) throughout the finishing period and through harvest. Steers were transitioned to the PM-feeding schedule successfully; however, no detectable differences were seen between treatment groups. Regardless of AM- or PM-fed group, cattle seemed to respond to the effects of heat stress, and management practices should be put in place to mitigate these effects.

As a result of limited research published on transitioning fattening cattle from morning- to evening-fed, study personnel consulted multiple industry personnel (veterinarians, nutritionists, feedyard managers) to gauge opinions on how to successfully transition the study steers. In this study, steers were transitioned to PM-feeding by feeding the next day's ration the evening before and not changing the feed call for three days, allowing steers to consume their desired amount of feed at any time of the day. This prevented the bunk from becoming empty to avoid increasing the risk of acidotic events seen commonly with fluctuating feed consumption. On the fourth evening after enrollment, feed delivery was reduced to allow steers to consume the remaining feed in the bunk. By day 6, the PM pens' feed delivery was consistent with the AM pens within the same block. With this schedule, the PM steers' transition was achieved without observable digestive morbidity or mortality.

In the current study, no statistically significant differences were found between treatment groups for any outcome. Due to limited research on evening feeding, specifically regarding the capacity of a commercial feedyard with many cattle in each pen, authors compared the variation seen in the current study to other published studies. Ominski et al. (2002) had greater variation around dry matter intake and water consumption (Ominski et al., 2002). Brandt and Reinhardt (1994) found lesser variation compared to the current study regarding daily gain, but a greater variation in terms of feed efficiency (Brandt and Reinhardt, 1994). Mader and Davis (2004) had greater a variation compared to the current study regarding water intake, similar variation on overall daily dry matter intake, and lesser variation regarding feed efficiency compared to the current study, with no statistical differences found between treatment groups (Mader and Davis, 2004). Because of the variation between studies, we believe that the lack of differences detected in this study may have been due to having few days with high THI associated with heat stress. It has been shown that metabolic heat production produced by microbial fermentation accounts for 3 to 8% of the total heat production of cattle (Czerkawski, 1980). During increased environmental conditions, cattle compensate by eating smaller meals, more frequently, and shifting feed intake to cooler parts of the day (Arias and Mader, 2011; Hicks et al., 1989; Ray and Roubicek, 1971). The lack of differences seen in this study could be a result of the AM-fed cattle adjusting their feed consumption naturally. Another contributing factor may be that only 14% of the total study days had a $THI \geq 80$, with the maximum THI equaling 84. Due to the increased THI occurring in the middle of the summer, cattle may have had substantial time to acclimate to the increased THI. Other weather variables like windspeed, solar radiation, and night-time temperatures also play a role in thermal load, but were not recorded in the present study. The current study was conducted at a larger scale than many previous experiments,

involving approximately 7800 steers across 48 pens. This increase in scale more closely reflects commercial feedyard production conditions and may aid in explaining discrepancies between our findings and earlier studies conducted under smaller experimental settings. The present study was the first to feed three feedings throughout the night-time hours, rather than the late evening hours seen in previous studies. Differences in feeding strategies (timing and number of feedings) could result in the differences seen regarding outcomes.

Regardless of treatment groups, when the THI crossed the threshold of 80, water consumption and OMB prevalence increased, indicating heat stress in the cattle. The increase in water consumption and panting behavior is commonly seen under heat stress conditions (Idris et al., 2024; Lees et al., 2019). In just the summer months alone, Arias and Mader (2011) reported a 87.3% increase in water consumption compared to winter months in feedlot cattle (Arias and Mader, 2011). Idris et al. (2024) found that respiration rate and panting score nearly doubled in heat stress conditions compared to thermoneutral conditions (Idris et al., 2024). Heat stress mitigation strategies should continue to be explored and implemented by producers, including the use of shade or water-cooling systems. Further research is warranted on the effects of evening feeding on feedyard cattle performance, enabling producers to determine if this strategy can be effectively implemented either independently or in combination with other heat stress mitigation practices.

Limitations to this current study include the lack of extreme heat (days with $\text{THI} \geq 80$ at 1600 h) and the inability to blind to treatment group. Due to time constraints of capturing OMB prevalence daily, 12 pens per day were observed within a similar environmental time frame. Authors do recognize that short-term fluctuations in OMB within pens may not be captured with this data collection strategy; however, the same number of pens per treatment were recorded

daily. Possible confounding effects associated with different feed callers between the AM and PM group could have influenced feed delivery; however, all feed callers were trained using standardized company procedures.

Conclusions

Night-time feeding was successfully implemented in a commercial feedyard, demonstrating that this management strategy is feasible under practical conditions. However, no detectable differences were found between the morning and evening feeding schedules across all measured outcomes, possibly due to relatively mild environmental conditions during the study period. Despite the lack of treatment differences, cattle were shown to increase water consumption and open mouth breathing prevalence, indicating some effect of heat stress when the temperature-humidity index was greater than or equal to 80. This emphasizes the continued importance of heat stress mitigation strategies within the feedyard sector. The implementation of evening feeding is feasible, but not sufficient alone under mild heat stress conditions. Further research is warranted to evaluate the effects of evening feeding under more severe or prolonged environmental conditions, as well as in combination with other heat stress mitigation strategies, such as shade or cooling systems, to better determine its potential role in improving cattle productivity and welfare.

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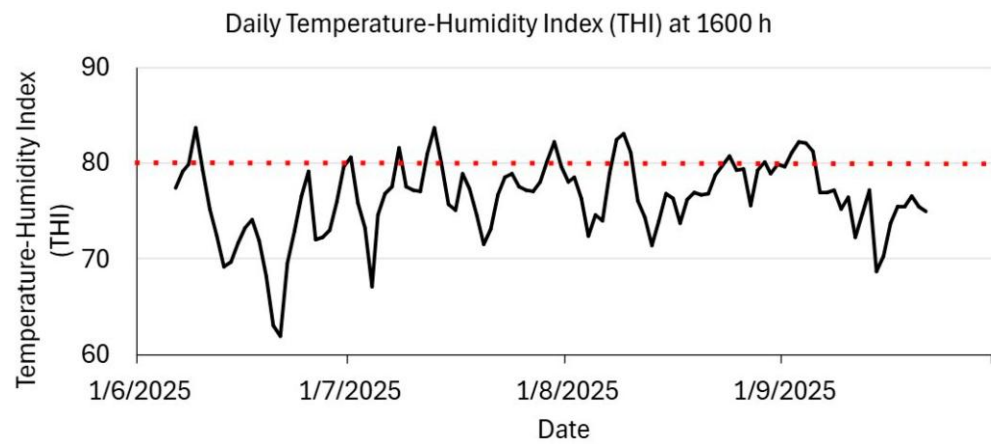


Figure 3.1. Temperature-humidity index (THI) recorded at 1600 h

Temperature-humidity index (THI) recorded at 1600 h from 6 June 2025 to 25 September 2025. A red dotted line depicts the threshold of 80 THI, the case definition for a potential heat stress event.

Table 3.1. Pen demographics at enrollment

Pen demographics at enrollment of steers allocated at terminal sort (T1: small; T2: medium; and T3: big) and randomized to either AM-fed (AM) or PM-fed (PM) treatment groups.

		Treatment Group			
		AM		PM	
	Terminal Sort Group	Mean	SD	Mean	SD
Total head per pen (n)	T1	163	10	164	11
	T2	165	9	164	11
	T3	162	10	163	11
Average bodyweight per head (kgs)	T1	555.52	8.64	556.11	8.31
	T2	601.20	10.55	599.94	9.34
	T3	645.53	9.06	644.57	11.87

Table 3.2. Treatment (trt) and temperature-humidity index (THI) model estimated daily outcomes

Treatment (trt) and temperature-humidity index (THI) model estimated daily outcomes of terminally sorted steers randomized to AM-feeding (AM) or PM-feeding (PM) during a heat stress trial in the summer of 2025 at a commercial feedyard in the Pacific Northwest.

	Model Estimated Means $\pm$ SE						
	Trt		THI		p-Value		
Daily Outcomes:	AM	PM	<80	$\geq$ 80	Trt	THI	Trt·THI
Water consumption (L/454 kgs BW)	31.38 $\pm$ 0.55	31.99 $\pm$ 0.60	31.15 $\pm$ 0.33	32.18 $\pm$ 0.63	0.14	0.07	0.40
Feed delivery (kgs DM/head)	10.8 $\pm$ 0.14	10.9 $\pm$ 0.14	10.8 $\pm$ 0.13	10.9 $\pm$ 0.13	0.17	0.26	0.71
Open mouth breathing (% pen)	0.6 $\pm$ 0.3	0.4 $\pm$ 0.2	0.2 $\pm$ 0.09	1.5 $\pm$ 0.78	0.66	<0.01	0.67

Table 3.3. Treatment model estimated overall outcomes

Treatment model estimated overall outcomes of terminally sorted steers randomized to AM-feeding (AM) or PM-feeding (PM) during a heat stress trial in the summer of 2025 at a commercial feedyard in the Pacific Northwest.

	Treatment Model Estimated Means $\pm$ SE		
Overall Health Outcomes:	AM	PM	p-Value
Morbidity (n treatment/hd count)	13 $\pm$ 1	14 $\pm$ 2	0.31
Mortality	1 $\pm$ 0.3	1 $\pm$ 0.3	0.77
Culling	0.6 $\pm$ 0.2	0.5 $\pm$ 0.1	0.50
Overall performance and carcass outcomes:			
Final live weight (kgs)	775 $\pm$ 5.17	774 $\pm$ 5.17	0.83
Hot carcass weight (kgs)	484 $\pm$ 3.23	484 $\pm$ 3.23	0.83
Daily gain (kgs)	2.1 $\pm$ 0.03	2.2 $\pm$ 0.03	0.38
Feed:gain (DM/kgs)	4.99 $\pm$ 0.08	4.92 $\pm$ 0.08	0.30
Quality grade prime and choice (% carcasses)	97 $\pm$ 0.3	97 $\pm$ 0.4	0.34
Yield grade 1, 2, 3 (% carcasses)	69 $\pm$ 1	70 $\pm$ 1	0.42

Chapter 4 - Determining frequency of multiple organ system involvement and concurrent lesions identified in feedyard mortalities and potential associations with cattle demographics

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Abstract

Necropsies are commonly used to diagnose the causes of death in feedyard cattle, but the documentation of multiple organ system involvement and concurrent lesions is limited. This observational study aimed to determine the frequency of such findings and their associations with animal demographics. Systemic necropsies were conducted for 889 cattle mortalities with minimal autolysis across six feedyards in the Central High Plains during the summers of 2022

and 2023. Lesions and abnormalities were recorded along with arrival weight, sex, days on feed (DOFs), and number of treatments. The results showed that 72% of mortalities had more than one gross lesion, averaging 2.3 lesions per animal. The most common organ systems affected together were digestive and pulmonary (19%), followed by cardiovascular, digestive, and pulmonary (6%), and cardiovascular and pulmonary (5%). Common concurrent lesions included bronchopneumonia with an interstitial pattern (BIP) and gastrointestinal lesions (GI) (8%), bronchopneumonia and GI (7%), and acute interstitial pneumonia (AIP) and GI (3%). A generalized linear mixed effects model revealed that the likelihood of multiple lesions increased with DOFs ($p = 0.02$). These findings highlight the value of thorough necropsy documentation to enhance our understanding of disease and guide improved feedyard management and treatment practices.

Keywords: feedyard; cattle; necropsy; lesions; concurrent

Introduction

Feedyard mortalities impact animal welfare, profitability, and overall production (Loneragan et al., 2001). Factors that may influence the health of feedyard cattle include but are not limited to the following: arrival weight, distance traveled and stressors associated with transportation, commingling, and extent of the feedyard personnel's experience in animal care (Galyean et al., 1995; Lechtenberg et al., 1998; Ribble et al., 1995). Studies in the previous literature focused on beef cattle found the incidence of overall morbidity rates to be 15–45% and overall mortality rates to be 1–5% (Irsik and Langemeier, 2003; Kelly and Janzen, 1986; Loneragan et al., 2001). Feedyard personnel often perform field necropsies on mortalities to evaluate and identify causes of death (Mason and Madden, 2007). The necropsy methodology varies among operations ranging from not performing a necropsy, performing a cursory

examination, or completing a thorough necropsy. Necropsy evaluations often result in a single final diagnosis for each animal, though necropsies should not be concluded upon finding a severe disease process.

Though mortalities are often attributed to a single disease process, multiple pathologic processes may contribute to diseases of feedyard cattle. Pulmonary disease continues to be the leading cause of feedyard mortality (Hay et al., 2014; Schneider et al., 2009; Smith, 1998). Gastrointestinal diseases, such as bloat, are also common causes of death (Belknap and Navarre, 2000). Cardiovascular disease is being reported with increased frequency in feedyard mortalities, and this could be due to increasing prevalence or increasing evaluations and awareness of the disease (Johnson et al., 2021). The frequency of singular disease processes has been established, but little information is available describing multiple organ system involvement or concurrent lesions of feedyard mortalities. Understanding the frequencies of concurrent lesions and potential associated risk factors could help identify diseased cattle ante mortem and facilitate the creation of more accurate treatment plans.

Due to limited research on multiple coexisting organ system involvement or concurrent lesions in feedyard cattle, producers are forced to focus on one disease in isolation from other diseases and adjust preventative and therapeutic plans accordingly. The primary study objective was to quantify the frequency of common concurrent pathologic lesions and organ system (pulmonary, gastrointestinal, cardiovascular, and other) involvement in feedyard mortalities. The second study objective was to determine potential associations between the probability of concurrent lesions or effects on organ systems with demographic factors (sex, days on feed, arrival weight, and number of treatments).

Materials And Methods

All procedures and observations were performed after the cattle's deaths. The Kansas State University Institutional Animal Care and Use Committee (IACUC) was contacted but deemed a protocol unnecessary due to no procedures being performed on live animals. This cross sectional, observational study was designed to determine the frequency of multiple organ system involvement and concurrent lesions in feedyard cattle mortalities throughout the feeding phase. In the months of June and July of 2022 and 2023, systemic necropsies were performed on feedyard cattle in six Central High Plains feedyards. Necropsies were performed by trained technicians through Kansas State University's Beef Cattle Institute. Enrollment into the study included cattle that were necropsied within 18 h of death with minimal to no autolysis. Following necropsy, treatment history and animal demographics were provided by the feedyard staff.

A systemic necropsy was performed on every mortality that qualified for study enrollment. Brain tissue was not examined due to logistical constraints concerning the rendering company disposing of the animals. Individual animal identification was recorded, and a case number was created for each mortality. A standardized necropsy evaluation form was used, in which all findings were recorded in a systematic fashion by recording designated abnormalities from a list for each area examined (Table 4.1). All necropsies were performed in a similar fashion, and the order of evaluation of organ systems was consistent among cases. To ease the removal of the animal, a necropsy technique was used to minimize the number of detached organs, similar to the previously published literature (Griffin, 2015).

An external examination was performed first, recording sex, extent of autolysis, and flesh category (degree of fat cover). The musculoskeletal system, including extremities, was then

examined. The tongue was removed, and the oral cavity was examined. The esophagus, trachea, and larynx were opened and examined.

After opening the chest cavity, the heart was completely removed and examined for the presence of pericardial effusion, determination of the heart shape, and observation of the external myocardial surface. Heart shape scores were recorded as 1–5 (1 = normal, 2 = mild changes, 3 = moderate changes, 4 = severe changes, and 5 = severe changes with flaccid heart), following a previously published scoring system (Heffernan et al., 2020). The heart was transected horizontally to examine papillary muscles and endocardium. All valves were examined for potential signs of endocarditis. The lungs were then removed from the thoracic cavity so that all eight lobes were visualized and percent involvement of pathology was estimated. Lung size, texture determined by palpation, and presence of pleural effusion, adhesions, abscesses, lungworms, or any acute or chronic disease processes were recorded.

The gastrointestinal tract was examined, including abomasum, rumen, small and large intestine serosa, mucosa, and content. Rumen scores were recorded as 1 through 5 (1 = normal—no evidence of scar tissue or active lesions; 2 = parakeratosis, abnormal papillae, and presence of clumps of papillae; 3 = one or more healed scars; 4 = mixture of one or more healed scars and active lesions; and 5 = one or more active lesions) based on an original scoring system that was created. In the abdomen, multiple areas were examined including mesenteric lymph nodes, liver, gallbladder, kidney, bladder, reproductive system, and spleen. Observations were made regarding the presence of peritoneal fluid, adhesions within the abdomen, and liver abscesses. Liver abscess scores were recorded as O (no abscesses), A (one or two small abscesses or up to four grouped abscesses that are usually less than an inch in diameter and surrounded by healthy liver), and A+ (one or more large abscesses greater than an inch in diameter, along with

inflammation of the liver tissues around the abscesses), as seen in the previously published literature (Brown et al., 1975).

During the full systemic necropsy, all lesions were recorded, and a final diagnosis was generated based on all findings. Lesions were described as any gross abnormality noted in the necropsy examination. All necropsies were performed by a trained research team, and all lesions and diagnoses were confirmed by a veterinarian through Kansas State University's Beef Cattle Institute. The final diagnosis was based on lesion severity and organ system affected to determine potential contribution to the fatality. A final diagnosis of multiple causes was assigned when two or more lesions that could have contributed to fatality (e.g., heart disease and acute interstitial pneumonia) were identified.

Each lesion or abnormality was classified to an organ system (SYST). The SYSTs included the following: pulmonary, gastrointestinal (GI), cardiovascular, and other (Figure 4.1). Within each SYST, lesions were categorized into lesion categories (LCATs). There were multiple LCATs for each SYST: four for the pulmonary SYST, three for GI SYST, three for cardiovascular SYST, and all miscellaneous LCATs going into other SYST. Pulmonary LCATs included acute interstitial pneumonia (AIP), bronchopneumonia (BP), bronchopneumonia with interstitial pneumonia (BIP), and other pulmonary lesions. The BIP lesions represent two separate disease processes (bronchopneumonia and interstitial pneumonia) (Haydock et al., 2023, Haydock et al., 2023), but their occurrence together was recorded as a single lesion. Other pulmonary lesions included all other respiratory diagnoses: embolic pneumonia, fibrinonecrotic larynx, granulomatous pneumonia, lung abscess, lung blood clot, lung nodules (small mass of dense tissue), pleuritis, and pulmonary hemorrhage (bleeding from the lungs). Gastrointestinal LCATs included bloat, liver abscesses, and other GI lesions (colon ulcers, congested liver,

diaphragmatic hernia, frothy rumen, hemoperitoneum, hemorrhagic/red abomasal mucosa, hemorrhagic/red rumen mucosa, intestinal strangulation, jaundiced liver, liver adhesions, liver flukes, liver laceration, liver nodules (small mass of dense tissue), liver scars, ruminal parakeratosis, and peritonitis). Cardiovascular lesions included heat stress, congestive heart failure (heart failure, right ventricular concentric hypertrophy; CHF), and other cardiovascular lesions (anemia, endocarditis, LV concentric hypertrophy, myocarditis, nutmeg liver, and pericarditis). Other lesions included dystocia/calving injury, jaundice, kidney stones, mummified fetus, musculoskeletal, neurological, pyometra, septicemia, splenomegaly, and ventral body wall abscess.

Cattle demographics were collected from individual animal health records. Arrival weights were categorized as less than 272 kg, 272–317 kg, 318–362 kg, 363–408 kg, and greater than 408 kg. Days on feed (DOFs) were categorized as 0–50 days, 51–100 days, 101–150 days, and greater than 150 days. The number of treatments prior to death were categorized as zero treatments, one treatment, two treatments, and three or more treatments.

The frequency of multiple affected SYSTs and concurrent LCATs was descriptively analyzed. Two separate models were created with the binomial outcomes being greater than one SYST or greater than one LCAT, respectively. Each case was included in each model. Using R Studio (version 2023.03.0, PBC, Boston, MA, USA), individual generalized linear mixed effect models (lme4 glmer function) were created to determine potential associations based on the probability of having greater than one SYST or LCAT with demographic factors. Fixed effects included demographic factors of arrival weight category, days on feed category, sex, and number of treatments. Univariate models were then assessed including the outcome of interest and each

individual fixed effect. Both models included feedyard and year identifiers as random effects to account for the lack of independence of mortalities within the same feedyard and year.

Results

Nine hundred and thirty-one (931) necropsies were performed between the two summers of 2022 and 2023. There were forty-two cases removed due to a lack of history data or the presence of extreme autolysis, resulting in 889 cases meeting the inclusion criteria for the final analysis. The classification of cases by SYST revealed that 58% (520/889) had multiple organ systems involved. The frequency of any single lesion recorded can be seen in Table 4.2. Figure 4.2, Figure 4.3, Figure 4.4, Figure 4.5, Figure 4.6, Figure 4.7 and Figure 4.8 provide example photographs illustrating common gross pathologies associated with each of the major lesion categories. The top five coexisting SYSTs were GI and pulmonary (19%, n = 170); cardiovascular, GI, and pulmonary (6.4%, n = 57); cardiovascular and pulmonary (5.4%, n = 48); GI1, GI2, and pulmonary (5.5%, n = 49); and GI, other, and pulmonary lesions (2.6%, n = 23). The cattle demographics of these top five SYSTs are shown in Table 4.3. The frequency of all organ systems affected for the study population can be seen in Appendix Table A.1.

Each mortality averaged 2.3 LCATs, and 72% (643/889) had more than one LCAT identified. The breakdown of individual lesion categories is shown in Table 4.3. The top five concurrent LCATs were BIP and other GI (8.8%, n = 72); BP and other GI (6.6%, n = 59); AIP and other GI (2.6%, n = 23); other GI and other (2.2%, n = 21); and BIP, CHF, and other GI lesions (1.8%, n = 16). The cattle demographics of these top five lesions are shown in Table 4.4. The frequency of all concurrent lesions in the study population can be seen in Appendix Figure A.2.

Univariate and multivariable analyses were performed to evaluate potential relationships between the outcomes of interest (more than one SYST or LCAT) and cattle demographic factors (arrival weight category, sex, DOFs at death, and number of previous treatments), though the significance of the fixed effects did not change between the multivariate and univariate analysis. The results presented are from the multivariate models, including all the fixed effects. The probability of a feedyard mortality having more than one SYST affected was only significantly associated ($p < 0.05$) with DOFs. Cattle that died between 0 and 50 DOFs had a lower probability of having more than one SYST affected than cattle that died between 101 and 150 DOFs (0.65 ± 0.13 SEM and 0.77 ± 0.10 SEM, respectively). Cattle in the 51–100 DOF category (0.65 ± 0.13 SEM) and >150 DOF category (0.76 ± 0.11 SEM) were not significantly different ($p > 0.05$) from any other DOF category. The probability of a feedyard mortality having greater than one LCAT was only significantly associated ($p < 0.05$) with DOF category at death. Cattle that died between 0 and 50 DOFs had a lower probability of having more than one LCAT than cattle that died between 101 and 150 DOFs (0.7 ± 0.11 SEM and 0.83 ± 0.08 SEM, respectively). Cattle in the 51–100 DOF category (0.75 ± 0.10 SEM) and >150 DOF category (0.8 ± 0.09 SEM) were not significantly different ($p > 0.05$) from any DOF category.

Discussion

Due to the negative impact that morbidities and mortalities have on feedyards, an improved understanding of the frequency of concurrent lesions could provide valuable information to decision makers for prevention and intervention. The objective of the current study was to quantify the frequency of multiple organ system involvement (pulmonary, gastrointestinal, cardiovascular, and other) and concurrent pathologic lesions in feedyard mortalities and to determine potential associations between the probability of multiple organ

system involvement or concurrent lesions with demographic factors (sex, days on feed, arrival weight, and number of treatments). In the current study, we found many mortalities (72%) had more than one lesion. Some combinations of SYSTs or LCATs were more common compared to others, and there were significant associations between DOFs and having more than one SYST or LCAT.

On average, each mortality had 2.3 lesions and, grossly, most lesions were found in the pulmonary system (93% of cases) and the GI system (85% of cases). The severity of the lesions was not recorded due to the subjectiveness of grossly identifying the progress of the lesions. In the current study, most concurrent lesions were identified in separate body systems (example: pulmonary and GI) compared to concurrent lesions seen within the same body system. To the authors' knowledge, there is no published literature focused solely on concurrently diseased body systems. Due to the nature of this cross-sectional study, there can be no inferences made on whether one lesion played a role in the development of another or if the concurrent lesions were simultaneous processes. Further research is warranted to determine possible associations or links between the involvement of multiple organ systems or concurrent lesions.

The percentage of AIP cases (9%) observed in the current study was similar to another study which also recorded the prevalence of BIP (Haydock et al., 2023). In this current study, BP was found in 39% of cases. Other studies show, of all morbidity and mortality events in feedyards, bovine respiratory disease accounts for 60–90% of cases (Baptista et al., 2017; Hay et al., 2014; Smith, 1998). Our study showed a smaller prevalence of BP than the other studies previously mentioned (Baptista et al., 2017; Hay et al., 2014; Smith, 1998), but the authors presume this is due to the BIP lesion category. BIP, which has not been reported commonly as a singular disease process, includes BP and interstitial pneumonia. BIP was another common

lesion found in the current study, with 32% of the cases having a BIP lesion. Another study showed that 11% of their mortalities were diagnosed as having BIP, which was the cause of death (Haydock et al., 2023). Pulmonary lesions were common in the current study population and seen often as a concurrent lesion, as they were present in four of the five top combinations of LCATs.

Concerning CHF, which is a significant cause of death in feedyard cattle in the Western Great Plains (Jensen et al., 1976; Neary et al., 2015), the mortality from CHF can be as high as 7.5% in severely affected pens (Heaton et al., 2019). However, the prevalence of CHF in mortalities can differ, with a retrospective study finding that less than 1% of deaths were attributed to CHF (Smith, 1998). In the current study, we found that 15% of cases had CHF lesions, but they were not necessarily severe enough to be the cause of death. The CHF lesions were commonly seen in concurrence with pulmonary lesions; however, to the authors' knowledge, there is no published literature describing the potential association between pulmonary and cardiovascular disease. More research is warranted on the relationship between pulmonary disease and CHF.

Gastrointestinal lesions were commonly seen in the current study (85% of cases had at least one GI lesion). A study from 1994 found that digestive disorders accounted for close to 26% of deaths in beef cattle, which was less than the number of deaths due to respiratory disorders and other causes of death (Vogel and Parrott, 1994). Another study shows that sudden deaths in feedyard cattle, often associated with digestive upsets, occur in cattle closer to market weight (Glock and DeGroot, 1998). This also agrees with a review of disease on feedlot performance showing digestive disorders tending to occur later in the feeding period (Smith, 1998). The current studies findings of a high prevalence of GI lesions, as well as a higher

probability of concurrent lesions in cattle with a greater number of DOFs can suggest that there is a possible association between GI lesions and DOFs (via spending more time eating a high-starch diet).

Another finding of the current study was that the top five concurrent SYST (Table 4.3) and LCAT (Table 4.4) combinations did not represent most of the cases: only 21% and 40%, respectively. This shows that there are many different SYST and LCAT combinations, with a small number of cases in each combination group. This is likely due to differences in disease processes and severity, allowing for a variety of SYST and LCAT combinations. The most common SYST combinations included pulmonary and GI lesions. Further research is warranted on the relationship between pulmonary and GI lesions.

The length of time at the feedyard (DOFs) was the only demographic factor found to be statistically significantly associated with having more than one SYST or LCAT affected. Cattle that died within 0–50 DOFs had a lower probability of having more than one SYST or LCAT than cattle that died between 101 and 150 DOFs. One factor that could impact this result is that the longer days on feed represents a greater amount of time at risk of contracting or developing an additional disease, as well as a greater amount of time on a high-starch diet. A study from 1996 reported the relationship between mortality and DOFs, with a similar mortality rate over the entire duration of the feeding period (Edwards, 1996). Even though that study recorded one singular cause of death, morbidity earlier in the feeding phase could play a role in co-morbidities at necropsy later in the feeding phase. Alternatively, more common concurrent lesions in cattle with a greater number of DOFs could be associated with impacts of the inciting disease, decreasing the animal's ability to prevent additional disease. The association between concurrent lesions and the timing of the feeding phase may also be indicative of potential challenges that

have been noted in treatment response late in the feeding phase. Previous research showed that cattle treated at later than 100 DOFs had a case fatality risk of 19.1% compared to cattle treated at 31–40 or 41–50 DOFs (8.5% and 8.7%, respectively) (Neal et al., 2024). The frequency of concurrent lesions observed later in the feeding period could decrease treatment success. For example, cattle with concurrent respiratory disease and CHF may only be treated for respiratory disease. Even if respiratory disease treatment is successful, CHF may persist. Concurrent lesions are more common in cattle with a greater number of DOFs, and further research is needed to determine the impact of concurrent lesions on treatment response.

The study's limitations include only performing necropsies on cattle from a limited population in the Central High Plains region. This study took place during the summer months, which is associated with increased temperature and humidity. The results might differ if the study took place in the winter months or year-round. Diagnosis was determined solely by gross evaluation; thus, the classification of conditions such as necrosis may not be as accurate as they would be if histopathology was conducted. Being able to match any lesion grossly to its histopathology would solidify these findings.

Recording more than one lesion at gross necropsy could allow producers, veterinarians, and researchers to study possible associations between two or more disease processes. And, over time, this could lead to the further identification of the order of disease onset.

Conclusions

This study showed that over 70% of the cases showed more than one lesion affecting deceased feedyard cattle. GI and pulmonary lesions were the top coexisting systems identified. Realizing that the morbidity of feedyard cattle can be the result of more than one pathological disease process can better allow producers and veterinarians to create appropriate therapeutic

interventions and health protocols. Even though the cause of death might be predominantly attributed to one organ system or lesion, recording all lesions at necropsy could be beneficial in the consideration of all disease processes and possible interactions. In the future, more refined diagnostic tools at necropsy could improve health protocols for morbidities.

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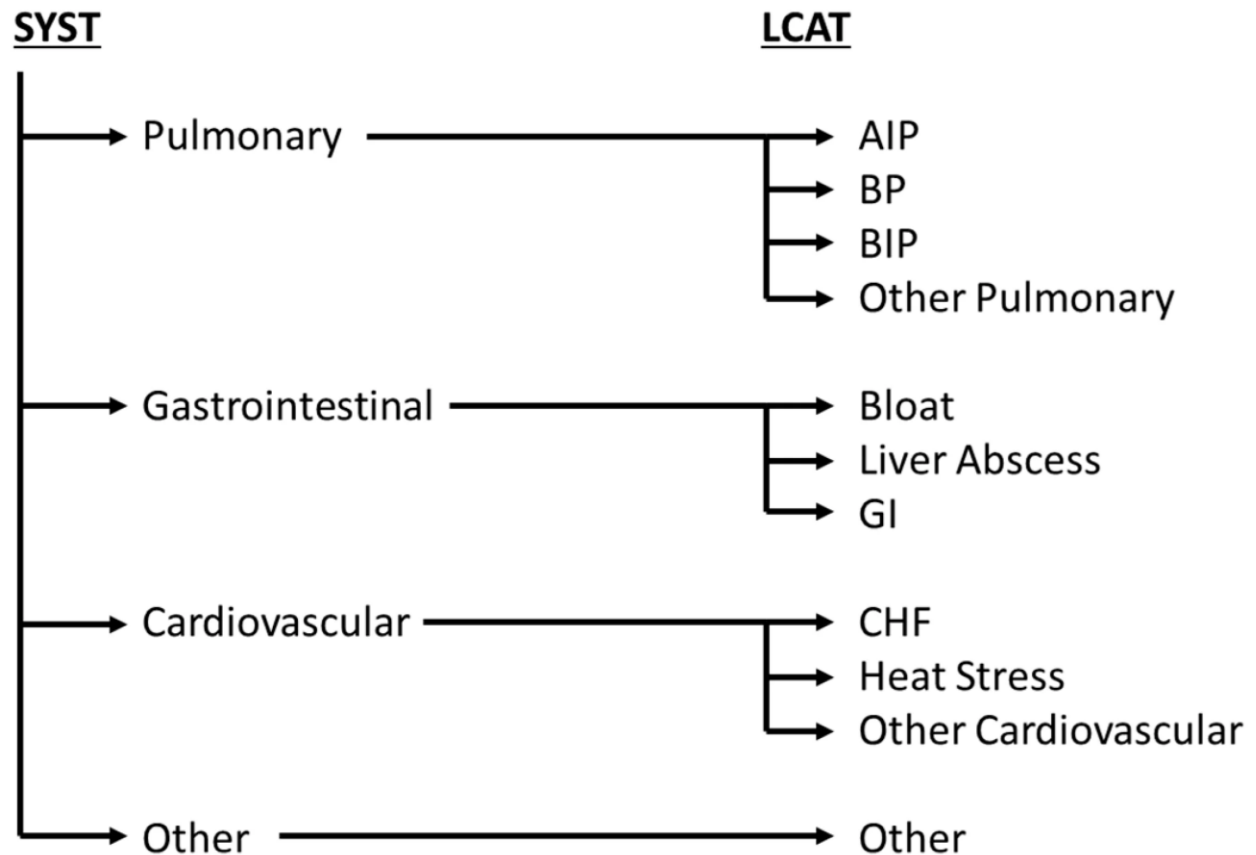


Figure 4.1. Organ systems affected (SYST) and lesion categories (LCATs)

Organ systems affected (SYST) and lesion categories (LCATs) on 889 Central High Plain feedyard mortalities at gross necropsy in the summers of 2022 and 2023. Abbreviations in the figure include AIP (acute interstitial pneumonia), BP (bronchopneumonia), BIP (bronchopneumonia with an interstitial pattern), GI (gastrointestinal), and CHF (congestive heart failure).

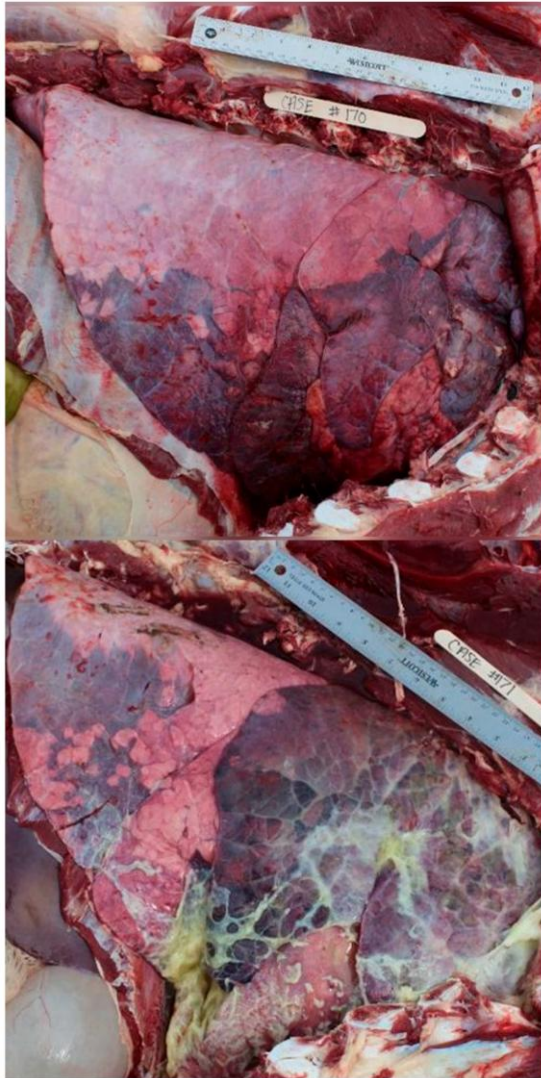


Figure 4.2. Examples of bronchopneumonia (BP) lesions.

Signs of BP lesions included but were not limited to gross lung consolidation, presence of purulent materials in airways, and presence of abscesses and/or adhesions. All pictures are right-sided thoracic pictures oriented with the dorsal aspect at the top of the picture and the cranial aspect on the right.

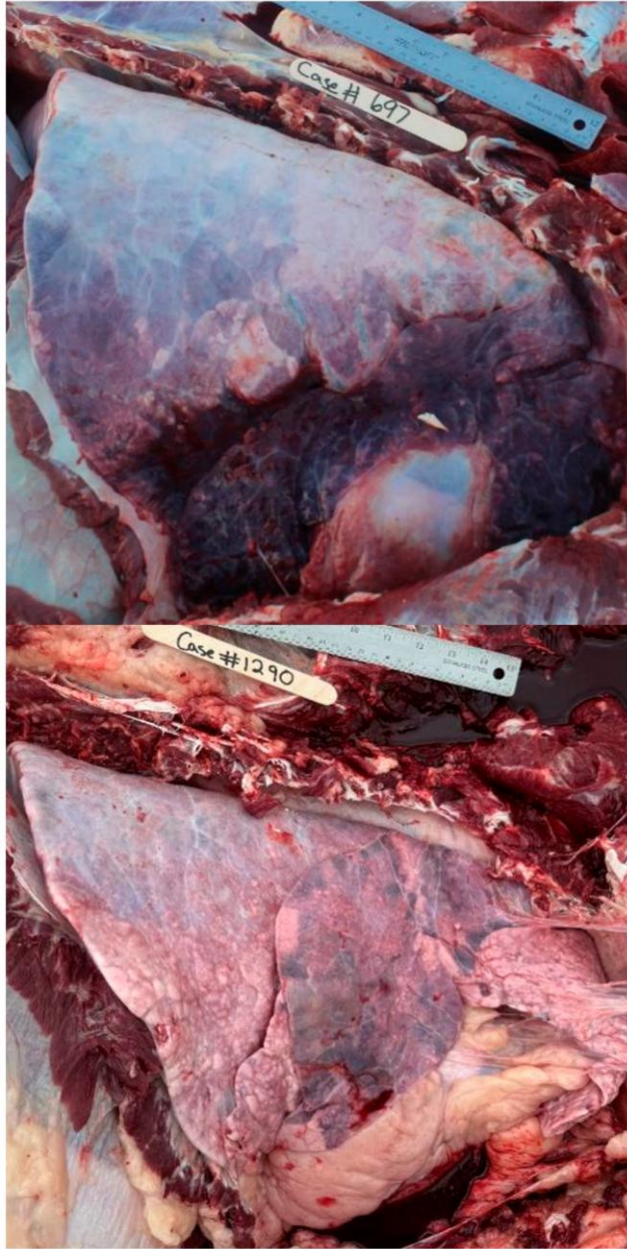


Figure 4.3. Examples of bronchopneumonia with interstitial pneumonia (BIP) lesions.

Cattle with BIP lesions represented pulmonary patterns consistent with both BP and AIP in the same animal. All pictures are right-sided thoracic pictures oriented with the dorsal aspect at the top of the picture and the cranial aspect on the right.

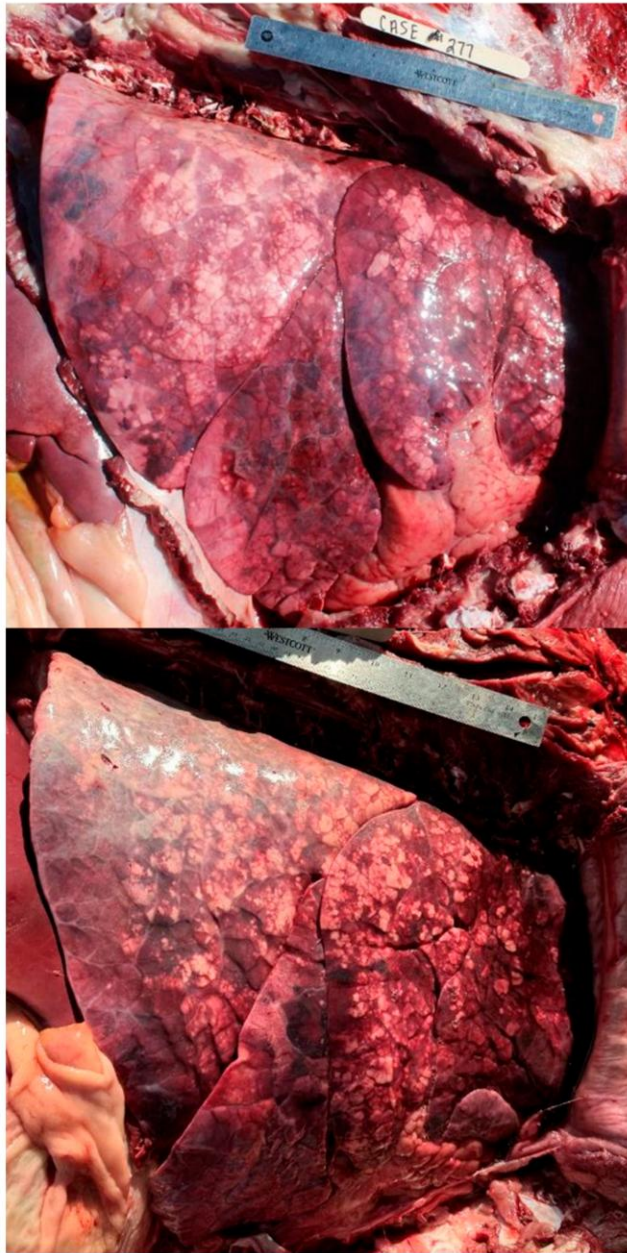


Figure 4.4. Examples of acute interstitial pneumonia (AIP) lesions.

Pulmonary lesions of AIP included overinflated, wet, and heavy lungs with typical differentiation in color patterns. All pictures are right-sided thoracic pictures oriented with the dorsal aspect at the top of the picture and the cranial aspect on the right.

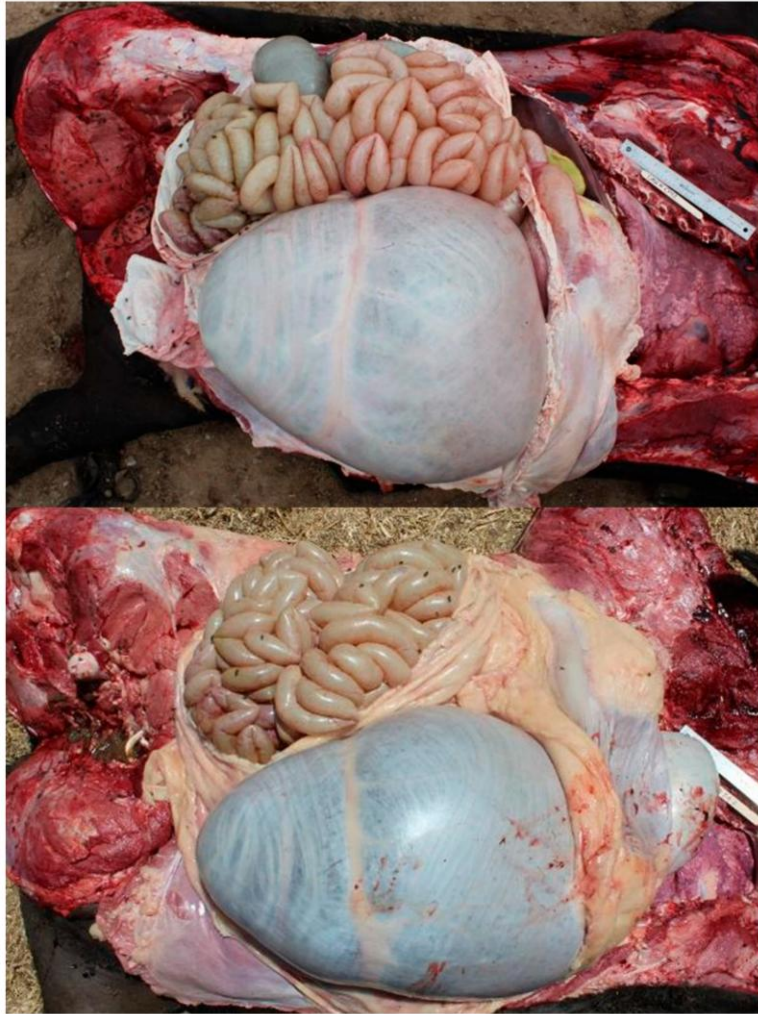


Figure 4.5. Examples of bloat lesions.

Typical signs of bloat include the following: enlarged, gas-filled rumen, pale hind-quarter musculature, and compressed, pale liver and/or lungs.

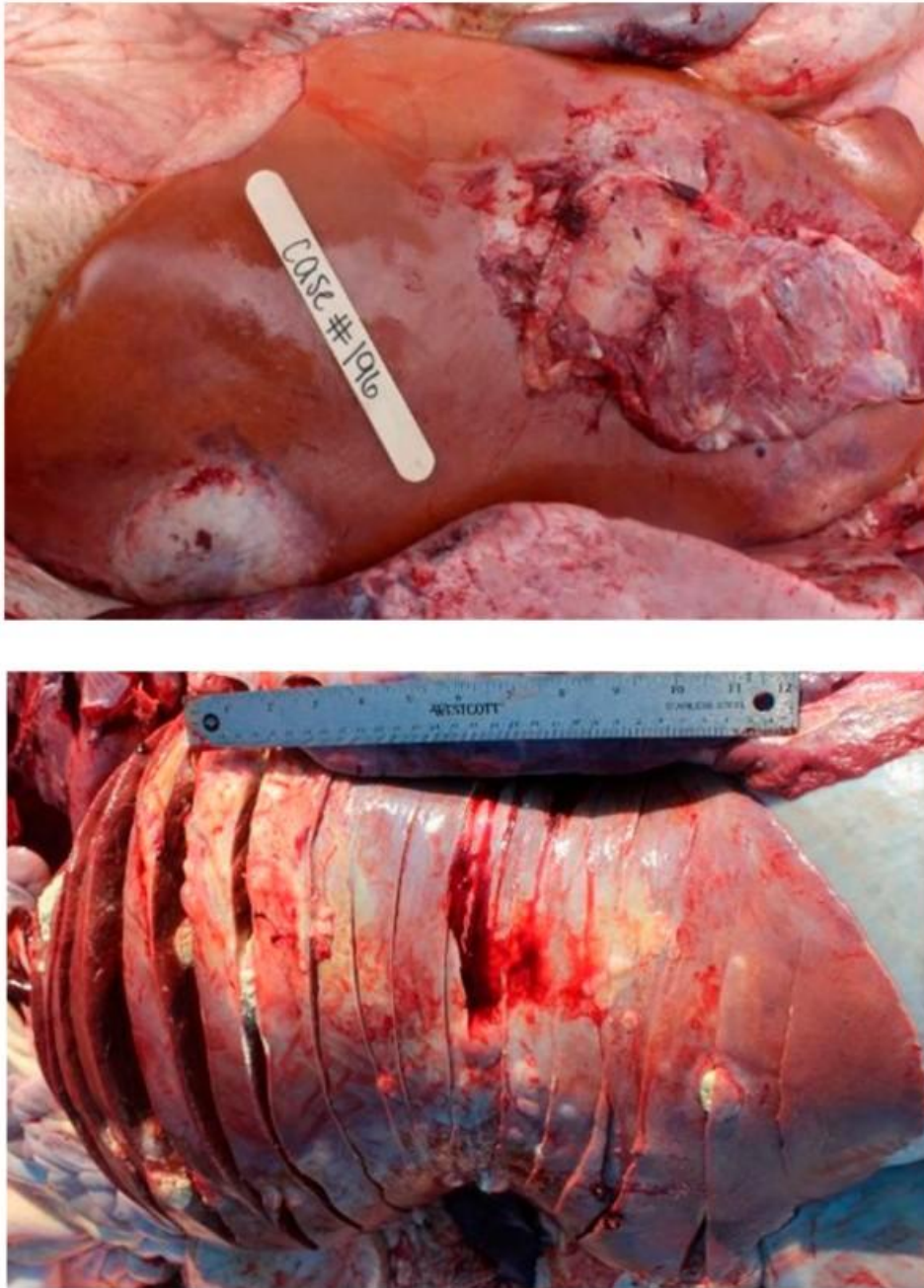


Figure 4.6. Examples of liver abscess lesions.



(a)



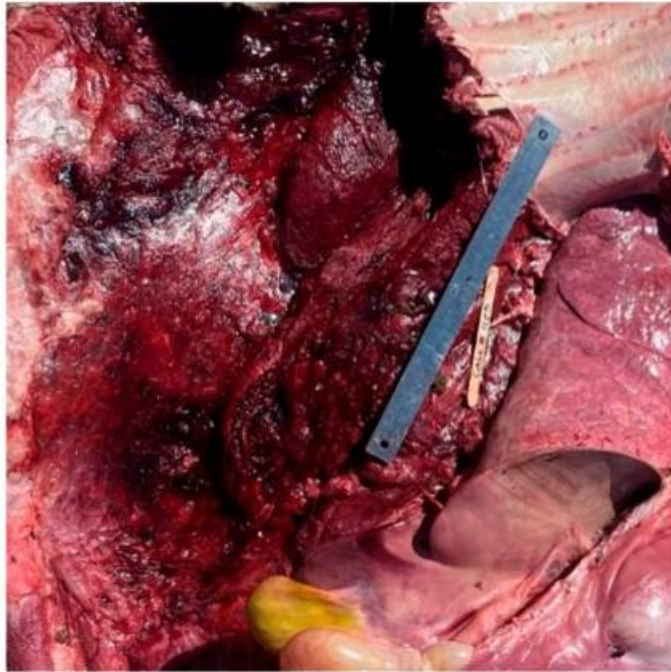
(b)



(c)

Figure 4.7. Examples of congestive heart failure (CHF) lesions.

Lesions consistent with diagnosis of CHF lesions included enlarged heart (heart score 3, 4, or 5), congested (nutmeg) liver, and effusion in peritoneal, pleural, or pericardial spaces. (a,b) illustrate hearts with heart scores of 5 while (c) illustrates congested liver.



(a)



(b)

Figure 4.8. Examples of heat stress lesions.

Heat stress lesions were associated with a variety of lesions including severe subcutaneous hemorrhage (a), cardiac petechia, and ecchymosis (b).

Table 4.1. Necropsy evaluation variables outside the normal limits

Necropsy evaluation variables outside the normal limits that were observed and recorded during 889 gross necropsies at Central High Plain of feedyard mortalities in the summers of 2022 and 2023.

Area of Examination:		Options, if Not Within Normal Limits:
External:	Sex	Steer, Heifer
	Breed	Native, Holstein, Mexican, Beef × Dairy
	Body condition	Thin, Moderate, Fat
	Lactation status	Lactating, Not Lactating
	Mastitis status	Mastitis, No Mastitis
Integumentary:	Oral cavity	Ulcers, Blunted Papillae, Petechia
Esophagus, Trachea, Heart:	Esophagus	Ulcers, Hemorrhage
	Larynx/Pharynx	Necrosis, Infection, Ulcers, Hemorrhage, Fibrinous
	Tracheal contents/Mucosa	Froth, Reflux, Hemorrhage, Fibrinonecrotic, Ulcers, Edema, Infection
	Pericardial effusion/Sac	Fibrinous, Muroid, Purulent, Sanguineous, Serous, Serosanguineous, Thickened, Adhesions
	Heart score	1, 2, 3, 4, 5
	Heart circumference (cm)	
	Heart weight (g)	
	Myocardium/Endocardium	Necrosis, Petechia, Ecchymosis, Dilated Ventricle, Myocarditis, Endocarditis

Area of Examination:		Options, if Not Within Normal Limits:
Lungs:	Pleural effusion	Fibrinous, Mucoid, Purulent, Sanguineous, Serous, Serosanguineous
	Pleural effusion volume	Mild, Moderate, Severe
	Pulmonary pathology	Bronchopneumonia, Fibrinous, Pleuritis, Acute Interstitial Pneumonia, Bronchopneumonia with Interstitial Pneumonia, Granulomatous, Embolic Pneumonia, Pulmonary Hemorrhage/Petechia, Pulmonary Edema
	Abscesses	Small, Medium, Large/Few, Moderate, Many
	Adhesions	Present, Absent
GI Tract:	Small intestine (SI) serosa	Red, Black
	SI mucosa	Ulcer, Hemorrhage, Thickened
	SI content	Gas, Fluid
	SI lesion	Obstructed, Inflamed, Parasites, Neoplasia
	Large intestine (LI) serosa	Red, Black
	LI mucosa	Ulcer, Hemorrhage, Thickened
	LI content	Gas, Fluid
	LI lesion	Obstructed, Inflamed, Parasites, Neoplasia
	Rumen score	1, 2, 3, 4, 5
	Rumen contents	Froth, Full, Empty, Bloat, Fluid
	Rumen mucosa	Ulcer, Hemorrhage, Thickened, Parakeratosis

Area of Examination:		Options, if Not Within Normal Limits:
	Abomasal mucosa	Ulcer, Hemorrhage, Thickened
Abdomen:	Mesenteric lymph nodes	Enlarged, Hemorrhage
	Peritoneal fluid	Fibrinous, Muroid, Purulent, Sanguineous, Serous, Serosanguineous
	Adhesions	Liver, SI, LI, Forestomach, Other
	Liver	Nutmeg, Congested, Jaundiced, Pale, Scars, Flukes, Enlarged
	Liver abscess grade	O, A, A+
	Kidney	Enlarged, Contracted, Infarcts, Petechia, Abscess, Infection
	Bladder/urine	Ecchymosis, Calculi, Cystitis, Pale, Dark Brown, Hemorrhage, Exudate, Ruptured, Stones
	Reproductive	Infected, Adhesions, Pregnant
	Spleen	Swollen, Contracted, Infarcted, Enlarged
Musculoskeletal:	Lesion	Swollen, Trauma, Muscle Necrosis, Ecchymosis
Final Diagnosis:		

Table 4.2. Frequency of organ systems affected (SYST) by lesion category (LCAT)

Frequency of organ systems affected (SYST) by lesion category (LCAT) from 889 Central High Plain feedyard mortalities at gross necropsy in the summers of 2022 and 2023. Abbreviations in the figure include AIP (acute interstitial pneumonia), BP (bronchopneumonia), BIP (bronchopneumonia with an interstitial pattern), GI (gastrointestinal), and CHF (congestive heart failure).

SYST:	LCAT:	Frequency of Lesion (n)	Percent of Cases ($n/889 \times 100$)
Pulmonary	BP	354	38.8%
	BIP	281	31.6%
	AIP	76	8.6%
	Other Pulmonary	122	13.7%
	Total Pulmonary	824	92.7%
GI	Bloat	83	9.3%
	Liver Abscess	47	5.3%
	Other GI	625	70.3%
	Total GI	755	84.9%
Cardiovascular	CHF	136	15.3%
	Heat Stress	40	4.5%
	Other Cardiovascular	141	15.9%
	Total Cardiovascular	317	35.7%
Other	Other	144	16.2%
	Total other	144	16.2%

SYST:	LCAT:	Frequency of Lesion (n)	Percent of Cases ($n/889 \times 100$)
Total lesions		2040	230%

Table 4.3. Top 5 concurrent organ systems (SYST) affected and cattle demographics

Top 5 concurrent organ systems (SYST) affected and cattle demographics from 889 Central High Plain feedyard mortalities at gross necropsy in the summers of 2022 and 2023. In this table, GI represents gastrointestinal lesions, GI1 represents the first gastrointestinal lesion, and GI2 represents the second gastrointestinal lesion.

	Top 5 Concurrent Systems (SYST)				
	GI and Pulmonary	Cardiovascular, GI, and Pulmonary	Cardiovascular and Pulmonary	GI1, GI2, and Pulmonary	GI, Other, and Pulmonary
	(n = 170)	(n = 57)	(n = 48)	(n = 49)	(n = 23)
Sex (%)					
Steer	31%	28%	33%	29%	26%
Heifer	69%	72%	67%	71%	74%
Arrival Weight (kg; avg)	324.6	331.4	324.4	323.1	353.8
Days on Feed (avg)	90	113	97	101	57
Number of Treatments (avg)	2	1	2	1	2

Table 4.4. Top 5 concurrent lesion categories (LCATs) and cattle demographics

Top 5 concurrent lesion categories (LCATs) and cattle demographics from 889 Central High Plain feedyard mortalities at gross necropsy in the summers of 2022 and 2023.

	Top 5 Concurrent Lesion Categories (LCATs)				
	BIP and Other GI	BP and Other GI	AIP and Other GI	Other GI and Other	BIP, CHF, and Other GI
	(n = 72)	(n = 59)	(n = 23)	(n = 21)	(n = 16)
Sex (%)					
Steer	25%	31%	35%	40%	13%
Heifer	75%	69%	65%	60%	87%
Arrival Weight (kg; avg)	326.2	324.5	321.3	329.5	325.3
Days on Feed (avg)	94	70	106	72	120
Number of Treatments (avg)	2	2	2	2	1

Chapter 5 - Evaluation of cardiac troponin I as a predictor of clinical outcomes in cattle treated for bovine respiratory disease (BRD) in commercial feedyards

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Abstract

Bovine respiratory disease (BRD) is a major cause of morbidity and mortality in feedlot cattle and prognostic tools would be valuable for managing individual BRD cases. Cardiac troponin I (cTnI) is a protein released into the circulatory system after myocardial damage that may serve as a prognostic biomarker. The study objective was to determine potential associations between cTnI serum concentration of cattle at initial BRD treatment with the risk of retreatment or mortality within 60 days. In this observational study, serum was collected from cattle in 2 central high plains feedyards at initial BRD treatment. Individual animal treatment outcomes were determined (treatment success, retreated, died, or culled due to BRD) at 60 days post enrollment.

Troponin concentrations were determined using a commercially available Bovine Cardiac Troponin ELISA assay and categorized as LOW (< 0.156 ng/mL) or HIGH (≥ 0.156 ng/mL). Of 318 tested serum samples, troponin concentrations were LOW for 310 and HIGH for 8 samples. Cattle with HIGH cTnI concentrations had a significantly ($P < 0.05$) greater probability of not finishing the 60-day post enrollment period (0.56 ± 0.22 SE) compared to cattle with LOW cTnI (0.11 ± 0.03 SE). Cattle with high cardiac troponin concentrations at initial BRD treatment were more likely to have negative clinical outcomes. However, a low number of cases were identified as having high cTnI. High troponin concentrations may be valuable to improve management decisions; however, further research is necessary to optimize cut-off values predictive of clinical outcome and evaluate prognostic value in select subpopulations of feedlot cattle.

Keywords: feedlot, cattle, cardiac troponin, bovine respiratory disease, prognostic

Introduction

Bovine respiratory disease (BRD) remains the major cause of morbidity and mortality in feedlot cattle, with many of these animals receiving antimicrobial therapy (USDA, 2013). Cattle with BRD may show clinical symptoms of depression, lethargy, increased respiratory rate, increased nasal discharge and inappetence (Apley, 2006). However, these non-specific clinical signs are often seen in animals with other disease syndromes, making accurate diagnosis and prognostication of suspected BRD cases challenging.

Cardiac troponins are proteins that are responsible for the regulation of contractile cardiac muscle tissue (Katrukha, 2013). Cardiac troponin I (cTnI) is expressed in the myocardium and is released into circulation when the cardiac myocytes sustain damage (Wells and Sleeper, 2008). A study in humans showed that increased cTnI concentrations can be induced by nonprimary

cardiac disease, such as acute or chronic systemic disorders (Giannitsis, 2013). A review paper, published in 2024, found that human cTnI concentrations can be elevated due to pulmonary, renal, neurologic, musculoskeletal, oncologic and gastrointestinal diseases, as well as acute illness and trauma (Maayah et al., 2024). Another study from the human medical literature demonstrated that detectable cTnI concentrations (1.0 gram per milliliter or higher) were associated with increased adverse events and mortality rate proportional to the amount of cTnI released (Ilva et al., 2008). Many studies have detected high cTnI concentrations in cattle with varying diseases, such as pericarditis, foot and mouth disease, LDA, dystocia and downer cow syndrome (Buczinski and Bélanger, 2010; Gunes et al., 2005; Gunes et al., 2008; Jesty et al., 2005; Mellanby et al., 2007; Peek et al., 2008; Serra et al., 2010; Suzuki et al., 2012; Tunca et al., 2008; Varga et al., 2009; Varga et al., 2013). In dairy cattle, elevated cTnI concentrations with non-cardiac diseases (metritis, mastitis, pneumonia, peritonitis, LDA, dystocia and cesarean section, and downer cow syndrome) were prognostic for negative outcomes (death or culling) (Varga et al., 2013).

Cardiac troponins may serve as a potential biomarker to determine the extent of cardiac damage in BRD cases (Fraser et al., 2014). A previous study demonstrated that cattle have a higher risk of congestive heart failure when they have been treated for BRD multiple times compared to cattle never treated (Johnson et al., 2022). The hypothesis of the current study is that feedyard cattle presenting with clinical signs of BRD, but with significant cardiac damage (either primary from congestive heart failure or secondary to severe BRD), will be at increased risk for culling or mortality. The objective of this study was to evaluate the association between cTnI serum concentration and risk of retreatment or BRD-specific mortality and culling in individual cattle in a 60-day post enrollment period following first BRD treatment. If cTnI

concentration at the time of first BRD treatment is associated with clinical outcome, this prognostic method could aid producers with early culling decisions, which would mitigate economic losses and reduce antimicrobial use in animals.

Materials and Methods

This study was approved by the Institutional Animal Care and Use Committee at Kansas State University (IACUC-4846-AS&I). This study was performed at commercial feedyards, and all appropriate animal care and housing were provided by the feedyards and personnel.

This study was conducted in 2 U.S. central High Plains commercial feedyards in June and July 2023. Study enrollments were conducted at the individual animal level with no restrictions based on sex or days on feed. Any animal that met the location-specific criteria for BRD treatment was eligible for study inclusion at the time of first BRD treatment only. At the time of enrollment, approximately 5 mL of blood was collected from the coccygeal vein into a 10 mL preservative free blood collection tube (BD Vacutainer, BD, Franklin Lakes, NJ, USA). All samples were kept on ice during the daily collection period. After daily collection was complete, all blood samples were centrifuged ($2000 \times g$ for 10 minutes) at the study sites, and serum was refrigerated during transport. Serum samples were frozen at -4°F until cTnI concentration determination. Animal identification and demographics (weight, sex, days on feed) were recorded at the time of sample collection.

After a period of 60 days from enrollment of the last animal in this clinical study, animal treatment records and outcomes were pulled from a database containing all events for the specific feedyards. A 60-day period was chosen relative to previous literature showing that most repulls and deaths happen within 60 days from first treatment (Smith et al., 2023). For an animal to be considered a first treatment success (FTS), there could be no additional BRD-associated

treatments, BRD-associated death, or BRD-associated culling within the 60 days follow-up period. For an animal to meet the definition of did not finish (DNF), the animal either died of BRD-related causes or was culled due to BRD within the 60-day period. Diagnosis, treatment and management decisions were made by personnel at each commercial feedyard, independent of study investigators. Both feedyards had similar protocols for identifying sick cattle (anorexia, depression, increased rectal temperature), however, treatment protocols were not obtained for the individual feedyards. All personnel influencing cattle management decisions were blinded to cTnI results until the entire data collection phase was completed (60 days post enrollment of last animal).

All feedyard data were moved to a statistical software program (R Studio, version 2022.02.3, Boston, MA, USA) for cleaning and analysis. Animal lot and identification numbers were matched to all animals enrolled in the study. After matching cases with 60 days post-enrollment records, cases that were shipped to harvest, died from a non-BRD cause, or were culled due to a non-BRD cause, were removed from study. The remaining cases met criteria for the FTS and DNF models.

A commercially available bovine cardiac troponin-I ELISA (Bovine Cardiac Troponin-I ELISA, CtnI-11-HS, Life Diagnostics, Inc., West Chester, PA, USA) kit was used according to the manufacturer's instructions to measure cTnI concentrations in the stored serum samples. Standards of 10, 5, 2.5, 1.25, 0.625, 0.313 and 0.156 ng/mL cTnI concentrations were included and run on each ELISA plate. All samples were randomized to plate and run in duplicate. Aliquots (100 ul) of the provided standards and serum samples were dispensed into a microtiter plate, followed by the addition of 100 ul of horseradish peroxidase (HRP)-conjugate into each well. The plates were incubated on a plate shaker (MB10-2A Thermo-Shaker, Allsheng, Xihu

District, Hangzhou, China) at 150 rpm and 25° C for one hour. Following incubation, the wells were emptied and washed 5 times with a 1x wash solution using a plate washer (405 TS, Agilent BioTek, Santa Clara, CA, USA). One hundred (100) ul of tetramethylbenzidine (TMB) was dispensed into each well and incubated on the plate shaker for an additional 20 minutes. Stop solution (100 ul) was added and the absorbance was measured at 450 nm using a plate reader (SpectraMax i3x, Molecular Devices, San Jose, CA, USA) within 5 minutes. The cTnI concentrations were determined using an online curve fitting software (MyAssays, Cambridge, United Kingdom).

The inter- and intra-assay coefficients of variation were 21 and 10%, respectively. Final results from the ELISA test were categorized as either LOW (< 0.156 ng/mL) or HIGH (≥ 0.156 ng/mL) cTnI concentrations, determined by the lower limit of quantification of the assay.

Randomly selected cases were removed from the study due to limited assay capacity. Of the remaining, each case was included in both FTS and DNF models. To determine prognostic ability, DNF cases were evaluated for sensitivity (the percent of cases that did not finish the 60-day period that had HIGH cTnI concentrations), specificity (the percent of cases that finished the 60-day period that had LOW cTnI concentrations), positive predictive value (the percent of cases that had HIGH cTnI concentrations that also did not finish the 60-day period), and negative predictive value (the percent of cases that had LOW cTnI concentrations that finished the 60-day period).

The binomial categories of cTnI concentration (HIGH; ≥ 0.156 ng/mL, or LOW; < 0.156 ng/mL) were used for statistical analysis. DOF at first BRD treatment was categorized into less than or equal to 60 days and greater than 60 days. Weight of the animal at first BRD treatment was categorized into ≤ 500 lb, 501 to 700 lb, 701 to 900 lb, 901 to 1100 lb, and > 1100 lb. Using

a statistical software program (R Studio, Version 2022.02.3, Boston, MA, USA), individual generalized linear mixed effect models (“glmer” function of the “lme4” package) were created to determine potential associations of fixed effects with the probability of DNF and FTS. The fixed effects included individual animal sex, DOF category, weight category, and cTnI category (HIGH/LOW) at the time of first BRD treatment. Animal lot number (given to a group of cattle with similar demographics for the entirety of the feeding period) was nested into feedyard as a random effect due to the lack of independence among samples. A statistically significant cutoff value was set at $P \leq 0.05$.

Results

Blood samples were collected from 343 steers and heifers at initial BRD treatment. Cattle were excluded ($n = 16$) from further analysis if during the 60-day post-treatment period the animal either died from a non-BRD cause, was removed for a non-BRD related cause, or was shipped to harvest. Due to limited ELISA plate capacity, 9 additional randomly selected animals were removed from further laboratory analysis. Three-hundred-and-eighteen cases were included in the final laboratory analysis. Of the 318 cases, there were 230 animals (72%) classified as FTS, and 88 that required additional BRD related therapy and/or died or were removed early for BRD causes. There were 39 cases (12%) that matched the case definition of DNF (died or were removed early for BRD causes), and 279 that finished the 60-day study period.

Of the 318 samples, 8 samples had HIGH cTnI concentrations with the remaining 310 samples classified as LOW cTnI concentrations. Of the 8 cases with HIGH cTnI, 2 were retreated for BRD, 2 died from BRD without additional treatment, and 4 additional animals were retreated and died of BRD within the 60 d post-treatment evaluation window. Table 5.1 depicts the number of cases that fit into each category of Days on Feed (DOF) and weight.

DOF category, weight category, and cTnI category at initial BRD treatment were not statistically associated with FTS ($P > 0.05$). Significant associations were identified between DNF and DOF category ($P < 0.05$), weight category ($P < 0.01$), and cTnI category ($P < 0.01$). Cattle greater than 60 DOF at the time of first BRD treatment had a greater probability of DNF (0.49 ± 0.16 SEM) than cattle less than 60 DOF (0.14 ± 0.07 SEM). Cattle within the 2 lowest bodyweight categories (≤ 500 lb and 501 to 700 lb) had a greater probability of DNF (0.72 ± 1.14 SEM and 0.66 ± 0.14 SEM, respectively) than cattle within all heavier weight categories (701 to 900 lb = 0.14 ± 0.09 SEM, 901 to 1100 lb = 0.16 ± 0.09 SEM, >1100 lb = 0.06 ± 0.06 SEM). Cattle with HIGH cTnI had a greater probability (0.56 ± 0.22 SEM) of DNF than cattle with LOW cTnI (0.11 ± 0.03 SEM).

Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of cTnI ELISA results at time of first treatment for BRD to determine DNF status were calculated (Table 5.2).

Discussion

The aim of the current study was to evaluate the association between cTnI and clinical outcome in BRD cases in feedyard cattle. Results of this study show that cattle with HIGH cTnI (≥ 0.156 ng/mL) at the time of first BRD treatment were significantly more likely to not finish the 60-day post-enrollment period than animals with LOW cTnI (< 0.156 ng/mL) at first BRD treatment. Although the current study considered both mortality and culling, these results are similar to previous research in stocker cattle with naturally occurring BRD, that reported a significant association between cTnI concentration at the time of first BRD treatment and subsequent mortality (Anderson et al., 2017). In that study, calves that died of BRD had cTnI concentrations of 0.1 ± 0.14 ng/mL (similar cutoff to the present study), while cTnI

concentrations in calves that did not die were 0.03 +/- 0.05 ng/mL. A previous report suggests a “normal” reference interval of cTnI in cattle serum of 0.00-0.05 ng/mL, determined by a point of care analyzer (Labonté et al., 2015).

Though the study results showed an association between cTnI concentrations and DNF, there was no significant association between cTnI concentrations and FTS. While previous research has shown the association between cTnI concentrations and negative outcomes like death or culling, we are unaware of research regarding the prognostic ability of cTnI to determine the probability of retreatment. Of the 88 cases that did not qualify as FTS in the 60-day post enrollment period, 33 (38%) of the cases were retreated for BRD, without subsequent death or culling. The subjectiveness of treating cattle for BRD not only impacts the initial case selection, but also the likelihood of being an FTS. If feedyard personnel are selecting BRD cases with mild clinical signs for treatment, these cases are likely to have a higher spontaneous cure rate; whereas animals with more severe BRD are more likely to be true BRD cases, but also may have a lower FTS. Because this research was conducted in 2 different feedlots, with multiple animal health personnel in each operation deciding to treat an animal or not, there could be observer variation. This inter-observer variation could have impacted the ability to find statistical significance relative to FTS because of the subjectiveness of treating cattle for BRD.

Results of the present study show that the majority of cTnI concentrations in cattle at the time of first BRD treatment are not detectable with the assay used in this study (LLOQ; lower limit of quantification, 0.156 ng/mL). Of 318 samples assessed in this study, only 8 animals had cTnI concentrations above the lower limit of quantification for this assay (0.156 ng/mL) at the time of first BRD treatment. One explanation for this finding is that cTnI is only released after damage to the cardiac muscle; therefore, only BRD cases with cardiac damage or primary heart

disease misdiagnosed as BRD would have measurable cTnI concentrations. Another explanation for the low number of animals with measurable cTnI could relate to timing of blood collection to disease progression. In the present study, a single blood sample was collected at the time of initial BRD diagnosis. Individual progression of BRD can be variable; therefore, the window between initial disease insult and sample collection is likely to be different for each animal sampled in the current study. In humans, cTnI concentrations increase within 2-3 hours after insult, peak within 12-48 hours, and then begin returning to normal baseline concentrations (Cardiovascular Education, 2023). It is possible that the time between cardiopulmonary insult and when an animal starts showing clinical signs is delayed and cTnI concentrations have already peaked and returned to the normal range. Another possibility is that the peak of cTnI concentration happened after initial BRD treatment, and cattle were treated before cardiopulmonary insult, therefore missing the peak concentration. Animals that were retreated in the 60-day post enrollment period could potentially have had higher cTnI concentrations at their retreatment date, due to a more severe disease process. Another population of cattle that could benefit from the prognostic ability of cTnI would be cattle getting retreated for a first or second time due to BRD. Another limitation of this study is the relatively high LLOQ compared to previously reported normal cTnI concentrations in cattle. It is likely that our assay was unable to detect milder cardiac insults.

This study shows that animals with HIGH cTnI concentrations were more likely to have negative clinical outcomes (DNF). Based on results shown in Table 5.2, the specificity of the cTnI ELISA was near perfect, demonstrating that most of the cattle that finished the 60-day period had LOW cTnI concentrations. The low sensitivity of the assay resulted from a number of animals that did not finish the 60-day period having LOW troponin concentration. This finding is

not completely unexpected as these animals could have died of BRD without substantial cardiac involvement or had cTnI concentrations below the LLOQ of the assay. The PPV indicates that 75% of cattle with HIGH cTnI did not finish the 60-day period. Though the NPV value shows 89% of cattle with LOW cTnI concentrations did finish the 60 day post enrollment period (there was a significantly higher number of cattle with LOW concentrations) the PPV is more useful in this scenario due to the negative outcome of death or culling associated with HIGH cTnI concentrations. These results show that cTnI could be a beneficial prognostic tool for producers to modify their treatment and management regimen or culling decisions. Since HIGH cTnI was associated with DNF, an individual animal with cTnI concentrations above 0.156 ng/ mL may warrant a culling decision, allowing the producer to receive a salvage value, rather than incurring the negative costs of treating, retreating, or mortality. Sensitivity, specificity, and predictive values were not calculated for FTS as cTnI concentration was not statistically associated with FTS.

One of the limitations of the present study relates to assay sensitivity. The lower limit of quantification for the ELISA was 0.156 ng/mL. Because there were cases that DNF and had LOW cTnI concentrations, using an assay with a lower LLOQ may improve the prognostic value for animals with cTnI concentrations below the 0.156 ng/mL cutoff used here, but still above the previously suggested reference range. Another limitation to the study is sample timing. Due to the short half-life of cTnI, it is possible that the peak cTnI concentration may have lapsed before clinical signs of disease were detected. Further research is warranted in optimizing sample timing and further identification of animal subpopulations that would benefit the most from the prognostic ability of cTnI.

Elevated cTnI concentrations at initial BRD diagnosis may be an effective prognosticator of negative clinical outcomes, like death and culling. Further research is warranted to optimize the use of cTnI for aiding BRD treatment decisions, which includes determining the population that could benefit the most from the prognostic ability of cTnI. Research evaluating the optimal case selection and alternative testing methodologies would improve the clinical utility of cTnI as a chute-side prognosticator. Additionally, using cTnI in combination with other predictive tools could potentially improve the prognostic utility for individual cases of BRD.

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Table 5.1. Cattle demographics and 60-day outcomes

Cattle demographics and 60-day outcomes from 318 feedyard cattle in U.S. central High Plains commercial operations at the time of initial BRD treatment by cTnI concentration categorized as HIGH (≥ 0.156 ng/mL) and LOW (< 0.156 ng/mL).

† Percentages in the 60-day outcomes do not add to 100% due to animals not classified as a FTS or DNF (e.g. animals that were retreated but did finish the 60-day post enrollment period).

Cattle demographics	All cattle (n=318)	cTnI concentration category	
		HIGH (n=8)	LOW (n=310)
DOF			
0-60	237 (75%)	7 (88%)	230 (74%)
> 60	81 (25%)	1 (12%)	80 (26%)
Weight, lb			
≤ 500	53 (17%)	3 (38%)	50 (16%)
501 to 700	64 (20%)	4 (50%)	60 (19%)
701 to 900	83 (26%)	1 (13%)	82 (26%)
901 to 1100	83 (26%)	0 (0%)	83 (27%)
> 1100	35 (11%)	0 (0%)	35 (11%)
Sex			
Steer	155 (49%)	4 (50%)	151 (49%)
Heifer	163 (51%)	4 (50%)	159 (51%)
60-day outcomes			
†First treatment success	230 (72%)	0 (0%)	230 (74%)
†Did not finish	39 (12%)	6 (75%)	33 (11%)

Table 5.2. 2x2 Table

Results of 318 feedyard cattle in U.S. central High Plains commercial operations at the time of initial BRD treatment by cTnI concentration categorized as HIGH (≥ 0.156 ng/mL) or LOW (< 0.156 ng/mL) that did or did not finish (DNF) the 60-day post-enrollment period. Calculated values for sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

		DNF		Total	Sensitivity	0.15
		Yes	No			
HIGH cTnI concentrations	Yes	6	2	8		
	No	33	277	310		
	Total	39	279	318		
					Specificity	0.99
					PPV	0.75
					NPV	0.89

Chapter 6 - The use of lidocaine-infused castration bands to castrate beef-dairy calves and its effect on animal welfare and performance

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Abstract

Castration is a common management practice among livestock producers, and animal welfare concerns exist following this procedure. The study's aim was to compare the behavior, performance, and blood parameters between calves castrated with a lidocaine-infused castration band (Lidoband™; LLB) to a standard band with no pain relief. In this blinded, randomized controlled trial, 26 male beef–dairy cross calves were followed for 7 weeks post banding. There were no significant differences between treatment groups in regard to overall body weight, average daily gain (ADG), feed conversion (G:F), clinical illness scores, banding site scores, and approach tests. However, there were treatment differences in the weekly ADG and G:F ($p < 0.05$). There was a significant treatment by time interaction between the number of lying bouts

and the average stand bout time ($p < 0.05$). There was also a significant treatment difference in the number of wound licks ($p < 0.05$). Overall, band castration presented acute and chronic pain, regardless of treatment. Performance, analyzed weekly, and behavioral patterns around the time of wound granulation (days 21–35 post castration) demonstrated positive outcomes for calves in the LLB group. Banding with an LLB proved to be beneficial in terms of both performance and behavioral aspects, implying greater animal welfare.

Keywords: banding, castration, cattle, lidocaine, welfare

Introduction

The practice of crossbreeding beef x dairy cattle has grown significantly in the U.S. The use of sexed beef semen in dairy herds is attributed to an effort to increase the value of surplus calves. In 2021, a survey conducted with 40 Wisconsin dairy farms indicated that on average, 454 beef–dairy cross calves were born per year per dairy farm; of those, we could consider 89% to be males (DeJarnette et al., 2009; Seidel, 2014; University of Wisconsin-Madison Division of Education, 2023). If we extrapolate this to all farms in the U.S. with milk cows in 2022 ($n = 36,024$) (2022 Census of Agriculture, 2024) starting to use beef semen, there is a possibility that 16,354,896 male beef x dairy calves will be born and supplied to the beef industry per year. Due to differences and a lack of research regarding beef–dairy cross calf management, as well as the implications for animal welfare compared to traditional beef calf management, more research on management strategies is warranted for these calves entering the beef supply chain.

A common management procedure that is performed in both the dairy and beef industries is castration. Castration is recommended to be performed early in life, between birth and 3 months of age (American Association of Bovine Practitioners Castration Guidelines, 2024). Male beef calves are castrated to reduce aggressive behavior, avoid pregnancies in cattle, and

enhance carcass quality traits (Stafford, 2007). In a 2010 survey, elastrator rubber rings were the most commonly used non-surgical castration method in calves less than 90 kg (44%) (Coetzee et al., 2010). Banding consists of placing a latex rubber band around the neck of the scrotum, entrapping the spermatic cords, and restricting blood supply to the testicles and scrotum, causing death and necrosis of these tissues and eventual “casting”, i.e., when necrotic tissues distal to the band fall off. Significant welfare concerns associated with this procedure are pain and distress due to the acute and chronic pain involved (Marti et al., 2017; Meléndez et al., 2017). Previous studies reported that calves castrated with bands presented reduced performance traits and increased chronic pain and discomfort-related behaviors compared to those treated by surgical castration (Nogues et al., 2021).

Lidocaine is a common analgesic, with many administrative routes being proven valuable in aiding in acute and chronic pain (Golzari et al., 2014). To aid in local pain mitigation, a lidocaine-infused castration band (LidoBand™, AVL/Solvat Ltd., Calgary, AB, Canada) was developed to slow-release therapeutic doses of lidocaine in the scrotum tissue under the band. It has been shown that calves banded with a lidocaine-infused castration band had tissue levels of lidocaine that approached 50% effective concentration (EC50) and exceeded 95% effective concentration (EC95) at 2 and 72 h, respectively, following castration, remaining above those levels for at least 28 days post castration (Ross et al., 2024). In lambs banded with a lidocaine-infused castration band, lidocaine concentration in tissue at castration and tail docking sites met or exceeded the EC50 for at least 21–28 days and showed local anesthesia at the tail site for at least 3 days compared to a control band, determined by the electrostimulation data (Ross et al., 2024). A study comparison between control bands, plus a local subcutaneous (SC) lidocaine injection and lidocaine-infused bands without a lidocaine injection used as castration methods,

reported that lidocaine-infused bands calves displayed less pain response upon in situ electrostimulation than the control-bands-plus-one-lidocaine-injection calves at 7 days post banding (Saville et al., 2020). However, no studies compared welfare outcomes based on basic health and function, affective states (pain and discomfort), and the natural living of calves castrated using lidocaine-infused bands for longer than 7 days.

The objectives of this study were to compare the measures of welfare (basic health and function and affective states (pain and discomfort)) of calves banded with Lidoband™ compared to calves banded with an identical standard band with no pain mitigation over a 49-day post castration period. We hypothesized that calves banded with Lidoband™ will show greater measures of basic health and function and decreased pain and discomfort, indicating the benefits of banding with a lidocaine-infused castration band.

Materials and Methods

All procedures described within this study were approved by the Animal Care and Use Committee of Kansas State University (IACUC-4923-AS&I). Due to the nature of a pilot study, the sample size was determined by previous studies with similar study designs (Nogues et al., 2021; Saville et al., 2020).

A total of 26 intact male Angus Holstein (beef–dairy) cross calves, less than 2 weeks old and weighing between 36 and 54 kg, were used to conduct this study at the Sheep and Meat Goat Unit at Kansas State University. This experiment was conducted as a blinded, randomized controlled trial, with the treatment groups being (1) lidocaine-infused castration band (Lidoband™; LLB; n = 13) and an identical standard castration band with no pain mitigation (CONT; n = 13). Calves were randomized to treatment group A or B (to ensure blinding) by their study identification number using a random number generator on Excel (version 3.12.1.,

Microsoft, Redmond, WA, USA). Individual calf was the experimental and observational unit. All castrations were performed by one unblinded veterinarian. Banding occurred on 11 October 2023, which notes Day 0 of the study, and calves were then followed for 49 days, with the intention being to observe scrotal sloughing for the majority of the calves.

All calves were transported approximately 300 miles on 9 October 2023 from a commercial calf-ranch in Southwest Kansas. Upon arrival at the study facility, all calves were weighed, given metaphylaxis (tulathromycin), a tetanus toxoid, and uniquely identified with an ear tag with their corresponding study identification number. Calves were given an oral coccidiostat (Corid (amprolium), Huvepharma, Peachtree City, GA, USA) once daily for 5 days after arrival. A two-day acclimation period was given to the calves prior to the start of the study. All calves had a health check performed by a veterinarian and were deemed clinically healthy prior to the study start date and at the conclusion of the study. During the experiment, calves were individually housed in raised pens (1.5×2.4 m) in an indoor facility, where the pens were cleaned twice daily. Sheep panels separated the individual pens, allowing calves to still see other calves, and neighboring calves were still able to touch and interact with each other. There was no roof over the pens. Each pen had an individual water bucket, feed bucket, and milk bottle holder. Calves were fed 2 L of milk replacer (Herd Maker® PB Milk Replacer, Land O'Lakes®, Arden Hills, MN, USA; 22% crude protein, 15% crude fat, 0.4% crude fiber) twice daily, and fresh water was always provided. A commercially available starter ration (Calf Grower B-68, Mid Kansas Coop, Manhattan, KS, USA; 14% crude protein, 2% crude fat, 7% crude fiber; 89% DM) was provided for the duration of the study. Health checks performed by experienced personnel were conducted on all calves twice daily at feeding, and band retention was also checked. All animal caretakers and analyzers were blinded to the treatment group. All outcome measures were

collected on calves in their individual pen, except body weights, which were measured by a stationary calibrated livestock scale located next to the pens. Data collection happened after morning feeding, at approximately hour 08:00, in the order of bodyweight, blood draws, banding site pictures, and video monitoring (15 min post human interaction).

Feed and milk intake was recorded once and twice daily, respectively, for 49 days by measuring the amount offered minus the amount refused (unconsumed). Body weight was measured at arrival and on days 0, 7, 14, 21, 28, 35, 42 and 49 to calculate the average daily gain (ADG). With daily feed and milk intake being measured on a dry matter basis, as were the weekly body weights, a gain to feed ratio (G:F) was calculated to determine feed conversion by dividing the weight gained by the feed consumed.

Banding site pictures were taken prior to banding, at the time of band castration, and on days 1, 2, 7, 14, 21, 28, 35, 42, and 49 following banding. Using a handheld camera (Canon EOS Rebel T3i, Ohta-ku, Tokyo, Japan), photographs were taken approximately 30 cm from the caudal end of the calves while they were standing, with the entirety of the scrotal tissue and band being visible. Banding site pictures were scored post-study by a single, blinded, trained observer, and scores were given to each banding site picture based on a published scored system from 1 to 5 (Marti et al., 2017). The present study's scoring rubric is shown in Figure 6.1. While checking for band retention, the date of scrotal sloughing was recorded, if applicable.

While calves were laying down and restrained by personnel within their individual pens, approximately 10 mL of blood was collected from the jugular vein using 10 mL syringes and a 12G $\times$ 1" needles at the following timepoints: days -1, 0 (band castration), hours 2 and 6, and days 1, 2, 7, 14, 21, 28, 35, and 42, at approximately hour 08:00, after body weights were calculated and before the banding site pictures were taken. Samples were immediately placed on

ice after collection. Five milliliters were added to a blood collection tube intended for cortisol analysis (6 mL Greiner Bio-One VACUETTE™ Lithium Heparin Blood Collection Tube, Greiner Bio-One North America Inc., Monroe, NC, USA) and 5 mL were added to a blood collection tube intended for Substance P analysis (6 mL Greiner Bio-One VACUETTE™ Lithium Heparin Blood Collection Tube, Greiner Bio-One North America Inc., Monroe, NC, USA). Forty-eight hours prior to the blood collection timepoints, benzamidine hydrochloride (final concentration 1 mM) was added to the EDTA blood tubes for Substance P (SP) analysis. The Benzamidine HCl solution was prepared in a clean lab wearing proper PPE including gloves and a lab coat. Briefly, 156.6 mg Benzamidine HCl was weighed out and added to 10 mL MilliQ ultrapure water in a sterile 15 mL conical. Solution was vortexed thoroughly and until dissolved. Blood tube caps were removed briefly to deliver the 60 μ L of Benzamidine HCL solution to each tube using an Eppendorf M4 Repeat pipet (Eppendorf North America, Enfield, CT, USA). Tubes were recapped immediately and stored at 4 °C until use within 48 h. Blood was centrifuged at 1000 $\times$ g for 15 min, and blood plasma was stored in a -80 °C freezer until analysis. Cortisol concentrations were determined through an enzyme-linked immunosorbent assay (ELISA; Caymen Chemical Cortisol ELISA kit item no. 500360, Ann Arbor, MI, USA). A total of 500 μ L of each sample was aliquoted into a 10 mL glass tube. The pH was adjusted to 1.5 to 2 by adding HCL to each sample. Ethyl acetate (2 mL) was added to each sample and then vortexed. After the layers had separated, the ethyl acetate was transferred to a clean glass tube. The vortex and extraction process were repeated three times more, yielding about 8 mL ethyl acetate extract per sample. The ethyl acetate was evaporated by drying samples in a CentriVap set on Program 9 (37 °C) for about 2 h (until the samples were barely dry). The extract was dissolved in 500 μ L of ELISA buffer (1X). The standard curve, ranging from 6.6 to 40,000 pg/mL, was created by

diluting synthetic cortisol with ELISA buffer. An amount of 50 μ L of each standard and sample was added to the appropriate wells in duplicate. A sample of 50 μ L of the AChE cortisol tracer and cortisol ELISA monoclonal antibody were added to each well. The plates were then incubated overnight in the dark at 4 °C. Plates were washed at 300 μ L for 5 cycles, and then 200 μ L of Ellman's Reagent was added to each well. Plates were then incubated in the dark for 90 min on a plate shaker. The plates were read at 405 nm. To determine the cortisol concentration of the samples, the raw data file was uploaded onto MyAssays Desktop software (version 7.0.211.1238 (200621)). Standard curves were plotted as a 4-parameter logistic curve. Samples were reanalyzed if the coefficient of variation (CV) was >16%.

A radioimmunoassay (RIA) was used to determine SP concentrations, ran in duplicate, using methods previously described (Van Engen et al., 2014). By diluting synthetic SP (Phoenix Pharmaceuticals cat. No. 061-05) with RIA buffer (50 mM sodium phosphate dibasic heptahydrate, 13 mM disodium EDTA, 150 mM sodium chloride, 1 mM benzamidine hydrochloride, 0.1% gelatin, 0.02% sodium azide; pH 7.4), a standard curve, ranging from 20 to 128- pg/mL was created. In plain 12 $\times$ 75 mm conical bottom tubes, 100 μ L of samples, standards, and quality controls (QCs) were aliquoted, followed by 100 μ L of rabbit anti-SP primary antibody (1:20,000; Phoenix Pharmaceuticals cat. no H-061-05). With an RIA buffer, iodine-125-SP tracer (custom iodination by Revvity, NEX083000MC) was diluted to 20,000 cpm. An amount of 100 μ L was then added to the samples, standards, and QCs. Samples were then covered and stored for 48 h at 4 °C. At the end of the incubation period, samples were placed on ice. A sample of 100 μ L of normal rabbit plasma (1:80) and goat anti-rabbit secondary antibody (1:40; Jackson ImmunoResearch cat no. 111-005-003) were added to each tube. For 10 min, samples were incubated at room temperature, then placed back on ice. A sample of 100 μ L

of blank bovine plasma was added to the blank, zero, standard, and QC tubes. An amount of 1 mL of 12% polypropylene glycol in 0.85% sodium chloride was added to all the tubes. The supernatant was aspirated by centrifuging the samples at 3000 rpm for 30 min at 4 °C. For 1 min, tubes were counted on a PerkinElmer Wizard2 gamma counter. To determine the SP concentration of the samples, the raw data file was uploaded onto MyAssays Desktop software (version 7.0.211.1238 (200621)). Standard curves were plotted as a 4-parameter logistic curve. Samples were reanalyzed if the coefficient of variation (CV) was >16%.

Every study day (days -1 to 42), at hour 12:00, a human approach test was performed by a single, blinded observer. This was conducted by approaching each calf individually and recording whether the calf made any approach (physical movement) towards the human, and it was recorded as a yes (approach) or no (did not approach).

Three-axis accelerometers (Hobo Pendant[®] G Data logger, Onset Computer Corp., Bourne, MA, USA) were fastened to the hind left leg (metatarsophalangeal joint) on arrival. To attach the accelerometer, a small dab of tag cement (Kamar Adhesive) was placed on the hair of a calf's leg and a four-inch cohesive bandage (AmerisourceBergen, Conshohocken, PA, USA) was loosely wrapped one time around the leg. The accelerometer was packed with a layer of foam and plastic film. Then, the accelerometer was fastened to the leg with a small dab of tag cement. A cohesive bandage was wrapped 6 more times around the leg to secure the accelerometer in place. Due to limited storage capacities, accelerometers were replaced twice over the duration of the study. Logger replacements happened on collection days while calves were laying down and restrained for blood collection. The time of removal and reposition were recorded, and, since loggers were launched for the day of replacement at midnight, it allowed the data to be stitched together without data loss. The axis position was recorded every 30 s for 24 h

for 42 days. Axis position results were converted into binomial data (standing/lying) using a modified version of a published protocol (UBC Animal Welfare Program: SOP – HOBO data Loggers, 2023). Data were then summarized into total minutes standing, total minutes lying, number of stand and lie bouts (>60 s), and the average bout time per day. A bout was defined as a movement from standing to lying, or vice versa, and staying in that new position for at least one minute.

Mounted video cameras (Camera Bullet 4 Megapixel Motorized 2.8–12 mm lens, Geovision, Taiwan) were used to continuously record video data. Cameras were mounted at a 45-degree angle on a rail that was 10 feet above the pens, with 2–3 calves being able to be monitored per camera (Cameras 1, 2, 3, 4, 5 and 6 capture 3 pens per camera; Cameras 7, 8, 9 and 10 captured 2 calves per pen). The differences in the number of animals per camera were due to the number of cameras we had available. Nevertheless, the bullet cameras allow for zoom and lens adjustment automatically using the Geovision program; that way, only the pens of calves assigned to that specific camera would appear in the video. Videos samples were rendered 30 min per day on days –1, 0, 1, 2, 7, 14, 21, 28, 35, and 42, relative to band castration (at approximately hour 08:30). Calves were allowed 15 min of rest after human interaction (body weights, blood collection, banding site pictures) before the 30 min videos were recorded. Behavior annotation and scoring utilized a behavior-specialized software (e.g., The Observer XT version 16, Noldus Information Technology, Wageningen, The Netherlands) using an adapted ethogram, shown in Table 6.1. One single, blinded to treatment and timepoint, trained observer analyzed all the videos. The outcomes measured included the percentage of time standing and eating, standing and not eating, and lying down. Counts of tail flicks, wound licks, looking at flank, and foot stamping were also recorded.

Treatment groups were assigned as A and B at randomization by a researcher not involved in data collection or analysis. Researchers did not become unblinded to the treatment group until all data analysis and results were completed. All statistical analyses were run on R Studio (Version 2022.02.3, Boston, MA, USA). Banding site scores were binomialized to the following categories: before wound granulation (scores 1 and 2), and after wound granulation (scores 3 and 4). Wound granulation in the current study was defined as the occurrence of new tissue, pink or red in color, formed on the surface of the scrotal wound during the healing process. Banding site scores of 5 were not included in the data analysis due to only 2 calves presenting this score at the end of the study. Regarding the daily human approach test, days 0, 1, 2, 7, 14, 21, 28, 35, and 42 were excluded from the data analysis due to calves being approached by humans for body weight, banding site pictures, and blood collection prior to the 12:00 pm approach test. All data were assessed for normality using a Shapiro–Wilk test. Transformations were performed for video behavior scoring to achieve normality prior to statistical analysis. The percentage of time spent standing while not eating, the percentage of time spent standing while eating, and the percentage of time spent lying were square root + 1 transformed to achieve normality. Counts of tail flicking, looking at flank, and wound licking were $\log_{10} + 1$ transformed to achieve normality. Behavior data were transformed for statistical analysis and are presented as the least squares means and standard error without transformation, with a *p*-value from transformed values. For models accounting for repeated measures, fixed effects included treatment, timepoint, and treatment by timepoint interaction. For the models accounting for overall performance (final bodyweight at day 49 post castration and overall ADG and G:F over the 49 days), fixed effects included the treatment group only. A calf identification number was used as a random effect for all models (except overall performance) to account for repeated

measures. An accelerometer number was used as a random effect for accelerometer data, as accelerometers were replaced twice during the study due to storage capabilities. Generalized linear mixed effects models (glmer function of lme4 package in R) were run to determine the possible differences between groups for banding site scores and approach tests. Linear mixed effects models (lme function of nlme package in R) were run to determine possible differences between the groups with all accelerometer data, video behavior scoring, performance data, and plasma biomarkers. Significance was established at $p \leq 0.05$, with a tendency at $0.05 < p < 0.10$, when biologically appropriate.

Results

Twenty-six single sourced beef-on-dairy calves were enrolled in this clinical trial. Thirteen calves were banded with a lidocaine-infused band (LLB) and 13 calves were banded with an identical standard band (CONT). The average initial weight of the LLB group was 43.8 kg, and the average initial weight of the CONT group was 46.5 kg (SEM 1.21, $p = 0.13$).

Body Weights, Average Daily Gain, Feed Conversion, and Banding Site Scores

The final body weight, overall ADG, and overall G:F were not significantly different between treatments ($p > 0.05$; Table 6.2). However, when data were analyzed weekly, a treatment by timepoint tendency was found in the ADG ($p = 0.06$) and a significant effect on G:F was found ($p < 0.01$). The LLB had a greater ADG than the CONT group in week 2 post castration ($p < 0.05$). A treatment effect was found in the G:F, with the LLB group having a greater feed conversion than the CONT group. No weekly treatment by time differences were found for bodyweights ($p > 0.05$). By day 49 post castration, 77% (10/13) of CONT calves and 62% (8/13) of LLB calves had cast their scrotal tissue. A timepoint effect for banding site scores was found, with the probability of the presence of granulating tissue increasing over time post

castration, independent of the treatment ($p < 0.05$). No effects of treatment or treatment by time interactions were found ($p > 0.05$).

Blood Parameters

The Substance P RIA intra- and inter-assay variation was 6.19% and 11.84%, respectively. Cortisol ELISA intra- and inter-assay variation was 12.24% and 8.41%, respectively. There were no significant treatments by timepoint interactions for plasma cortisol (5726 pg/mL and 5331 pg/mL, SEM = 0.84; LLB and CONT, respectively) or Substance P concentration (231 pg/mL and 221 pg/mL, SEM = 0.71; LLB and CONT, respectively; $p > 0.05$). Plasma cortisol concentrations peaked immediately after band castration (~2.3 min post band castration) and decreased overtime regardless of the treatment group (timepoint $p < 0.05$).

Animal Behavior

No effects of treatment, timepoint, or their interaction were found for the daily human approach test ($p > 0.05$). A treatment by time interaction was found for the number of lying bouts, with LLB calves performing a greater number of lying bouts on days 35 to 41 post castration compared to CONT calves ($p < 0.05$; Table 6.3). The number of lying bouts decreased for both treatments post castration starting on day 2. The average standing time within a bout had a significant treatment by timepoint interaction, with CONT calves spending more time standing within a bout between days 18 and 22, day 30, and day 41 post castration ($p < 0.05$; Figure 6.2). When behavior was annotated for 30 min per day using videos 15 min after human presence, treatment by the timepoint effect was found on days 21 and 35 for time spent lying (%), with the LLB calves lying down for longer than the CONT calves ($p < 0.05$; Table 6.3). A significant treatment and timepoint effect were found for the number of wound licks. The CONT group licked their wounds more often than the LLB group ($p < 0.05$; Figure 6.3). The number of wound

licks increased with time post castration for both the LLB and CONT groups. In addition, the number of times calves looked at their flank and the number of foot stomps performed also increased over time, regardless of treatment ($p < 0.05$; Figure 6.3).

Discussion

Castration pain has been demonstrated by several other studies looking at elements such as the acute pain in hours and days post procedure, as well as chronic pain weeks post procedure (Fisher et al., 1996; González et al., 2010; Marti et al., 2017; Meléndez et al., 2017; Molony et al., 1995; Nogues et al., 2021; Schwartzkopf-Genswein et al., 2005; Stafford, 2007; Thüer et al., 2007). Researchers were able to demonstrate evidence of decreased affective states by the presence of pain and distress when assessing basic health and function using plasma biomarkers and banding site scores, as well as natural living by changes in behavior. The present study evaluated calves castrated with a lidocaine-infused band (Lidoband™) compared to calves banded with an identical standard band with no pain mitigation over a 49-day post castration period. Outcomes selected for this study aimed to assess both acute and chronic pain.

In the present study, there were no observed differences in overall performance outcomes. Conflicting differences in overall body weight and ADG were noted in previous research that compared band castration, knife castration, and sham. In one study, band castration and sham presented greater overall weaning weights compared to knife castration (Marti et al., 2017). Another study reported lower weekly ADGs for band-castrated compared to knife-castrated calves (Nogues et al., 2021). However, each study had a different target population that included calves of similar ages but different breeds (extensive beef breeds, and individually housed dairy breeds, respectively). To the best of our knowledge, no studies tested differences in castration methods for young beef-on-dairy calves. Timepoint differences in weekly G:F, ADG,

and cortisol suggest that acute pain was present immediately and during the first week post castration regardless of the treatment group. The reduction in feed conversion and weight gain during the first couple of weeks seen in the present study has been previously attributed to pain decrease, as well as findings of the decrease in testosterone in a population older than the one of the present study (Pang et al., 2008; Purchas et al., 2002; Weary et al., 2006). The pain during this period is likely the result of constriction and ischemia promoted by the bands. Humans who submitted to the use of tourniquets during surgery described the pain of blood flow constriction as being dull, tight (with an aching sensation), and poorly localized (Kumar et al., 2016). However, the present study demonstrated differences and tendencies between treatments groups in G:F and ADG, respectively, when analyzed weekly. Calves in the LLB group had a greater weekly feed conversion and weekly ADG compared to CONT calves. The results indicate that LLB calves better coped with the acute pain caused by band-castration, which promoted better affective states. Nevertheless, no overall performance differences were noted. Cortisol and Substance P results found in the current study are similar to results from previous studies. The lack of differences in blood parameters (cortisol and Substance P) was previously described in rubber ring castration in young calves (one week old) compared to sham calves when assessing acute (Meléndez et al., 2017; Mellor et al., 1991) and chronic pain (Marti et al., 2017). Concerning cortisol, a similar lack of treatment differences was previously described in rubber-ring-castrated young calves (week old) compared to sham calves when assessing acute (Meléndez et al., 2017; Mellor et al., 1991) and chronic pain (Marti et al., 2017); however, cortisol peaked at castration time, indicating activation of the hypothalamic–pituitary–adrenal axis related to castration distress in both treatments. Although no treatment by time interactions were found, LLB calves seemed to have a greater G:F and ADG in the first two weeks post banding and lower cortisol

immediately after band castration. A study with a greater number of calves is necessary to confirm the interactions between treatment and time favoring the LLB treatment group.

Differences in behavior occurred in a similar temporal window as wound granulation, where the band started cutting through the scrotal tissue to begin the sloughing process (around 20–40 days post castration). We hypothesized that this is the time where chronic pain occurs for banded calves. Previous studies mentioned that chronic pain might be present at 7 days post band castration until the testicles slough off (Marti et al., 2017), while others, attributed chronic pain to an analogy with humans and behavioral changes (e.g., abnormal postures and walking, licking lesion sites) that would likely indicate to producers and veterinarians evidence of long lasting pain (Molony et al., 1995). Chronic pain or the long-term effects of castration were observed in the current study by timepoint effects observed for tail flicks, looking at flank, and foot stamping in the video analysis regardless of the treatment group. However, a treatment difference was found in the number of wound licks, with the LLB group licking their wounds less than the CONT group, indicating decreased pain in the scrotal area. Previous research demonstrated that wound-directed behaviors were associated with pain (Abbott et al., 1995; Fisher et al., 1996; Fisher et al., 2001; Mintline et al., 2014; Molony et al., 1995; Nogues et al., 2021). However, differences found between the treatment groups were very small, and previous research suggested that the size and less vascularized testicles in young calves could contribute to the lack of behavioral differences (Marti et al., 2017). The accelerometer data showed a treatment by timepoint interaction with the number of lying bouts and time spent standing in one bout. The LLB calves spent less time standing in one bout and had more lying bouts within a day compared to the CONT calves. The movement between lying to standing position in cattle is performed using the hind limbs first and then the front limbs, causing a stretch of the abdominal muscles

and skin. When a calf is castrated with a rubber band, the stretch of abdominal tissues is even more pronounced, which is believed to cause discomfort when lying to standing behavior is performed (Meléndez et al., 2017). The LLB calves moved up and down more often, indicating less discomfort or pain in the scrotal area and suggesting that lidocaine in the band promoted some local analgesia. The time of scrotum casting in the present study was similar to a previous study where they noticed that about 70% of the calves casted by day 56, with only one calf (1/11) showing complete healing (score of 5) (Nogues et al., 2021). A limitation to the current study is the fact that not all calves sloughed their scrotal tissue and had completely healed banding sites by day 49. Further research is warranted to follow all banded calves through the time of scrotal sloughing and complete banding site healing to determine possible differences in behaviors or performance that the chronic pain of scrotal sloughing could cause. Thüer et al. (2007) showed that calves who were banded had a significantly greater pain response to palpation than control calves or calves castrated with a burdizzo after day 10 and up to 8 weeks (Thüer et al., 2007), indicating the presence of chronic pain after banded castration.

Unfortunately, there are no set guidelines for times or intervals to score animal behavior. Other researchers assessing castration pain described their methodology of behavior scoring happening for 24 h once a week using a 5 min interval scan sampling (Nogues et al., 2021). Molony et al. (1995) behavior scored the first day for 3 h using 2 min intervals for the first 96 min and then 6 min intervals for the remaining 84 min (Molony et al., 1995). In addition, when Meléndez et al. (2017) scored the behaviors of calves submitted to castration, their strategy was to conduct continuous behavior sampling between 2 and 4 h post castration and then continuously scan samples in 2 min intervals every 10 min from 8:00 to 14:00 h on days 1, 2, 3, and 5 post castration using a subset of five animals per treatment (Meléndez et al., 2017). Later,

Marti et al. (2017) used similar continuous scan sampling from 08:00 to 17:00 h on days 5, 13, 20 and 27 post castration to evaluate chronic pain (Marti et al., 2017). Our strategy was to behavior score continuously for 30 min per day at the same time, following the exact same schedule on days -1, 0, 1, 2, 7, 14, 21, 28, 35, and 42 to maintain the homogeneity and consistency of the video data. Videos were rendered after morning feeding, bodyweight measures, blood collections, and banding site pictures were taken; then, animals were allowed a period of 15 min to return to natural behaviors without human interference. Our goal in allowing 15 min without human interaction was to diminish the masking of pain behaviors from human observation, since cattle can be stoic in the presence of humans. Nevertheless, the authors recognize that a 30 min behavior scoring may not represent behaviors occurring during the 24 h within a day. Behavior scoring present limitations due to amount of time necessary to score 24 h videos, which is clear not only from the present study but also from other research groups that have been previously mentioned (Marti et al., 2017; Meléndez et al., 2017; Molony et al., 1995; Nogues et al., 2021). New artificial intelligence behavior tools to decrease the amount of video hours needed to be watched by a researcher may provide a better tool to address the current challenge.

A limitation of this study is the small sample size. Our findings indicate that the use of lidocaine-infused castration bands may be a useful tool for producers to use for pain mitigation following banded castration. Collaboration with a calf ranch could lead to a commercial trial to assess performance metrics on a larger scale and increase external validity. Future work could include the use of Lidoband™ on different populations and sectors of the beef or dairy industry, giving valuable results to determine the population of cattle that would benefit the most from this product.

Results from the current study indicate that performance, behavior, and blood parameters are significantly influenced by banded castration. Performance differences observed early post castration and behavioral differences observed later post castration are indicative of Lidoband™ providing some alleviation from the acute and chronic pain that banding is known to cause. Regardless of treatment, acute and chronic pain are still present with banded castration and both short and long-term pain mitigation should be used to improve animal welfare.

Conclusions

This study demonstrates that the use of a lidocaine-infused castration band (LLB) offers significant advantages over standard banding for the welfare of young calves. While no major differences were observed in calf performance between the two treatment groups, the LLB-treated calves showed improved performance when the average daily gain (ADG) and feed efficiency (G:F) were analyzed weekly. Furthermore, behavioral indicators of pain, such as the number of wound licks and changes in standing and lying bouts, suggest that LLB treatment reduces pain and discomfort during the recovery period. These findings highlight the potential for LLB to mitigate the acute and chronic pain typically associated with band castration, making it a promising alternative that benefits both animal welfare and production outcomes.

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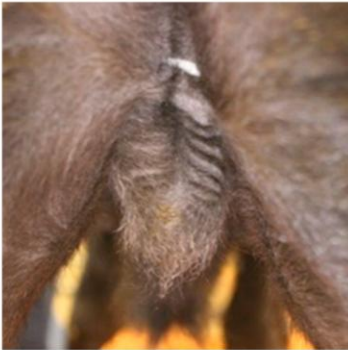
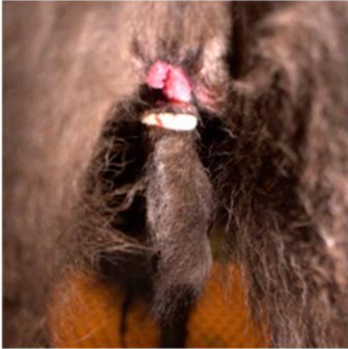
Score	Description	Picture
1	Band placed, restricting blood flow, but scrotum is not necrotic.	
2	Necrotic scrotum without purulent exudate.	
3	Necrotic scrotum with purulent exudate.	

Figure 6.1. Five-point banding site scoring system

Five-point banding site scoring system used to evaluate the wound healing associated with Lidoband™ castration (LLB; n = 13) and standard band castration (CONT; n = 13). The score system used is a published scoring system from 1 to 5 (Marti et al., 2017), with pictures from the current study.

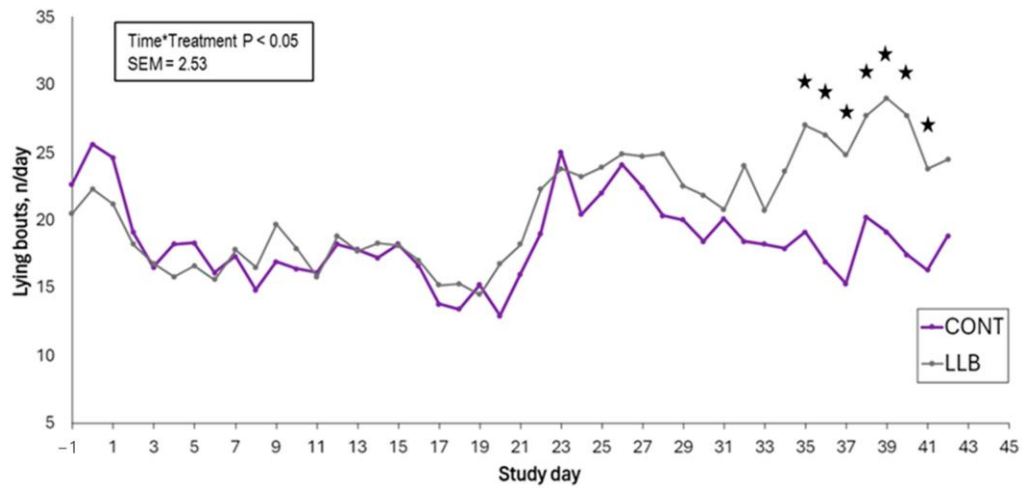


Figure 6.2. Average number of lying bouts per day

Average number of lying bouts per day of beef-on-dairy calves banded with a lidocaine-infused castration band (LLB; $n = 13$) and a standard band (CONT; $n = 13$), recorded by three-axis accelerometers. Stars in each study day denote differences among treatment groups ($p < 0.05$).

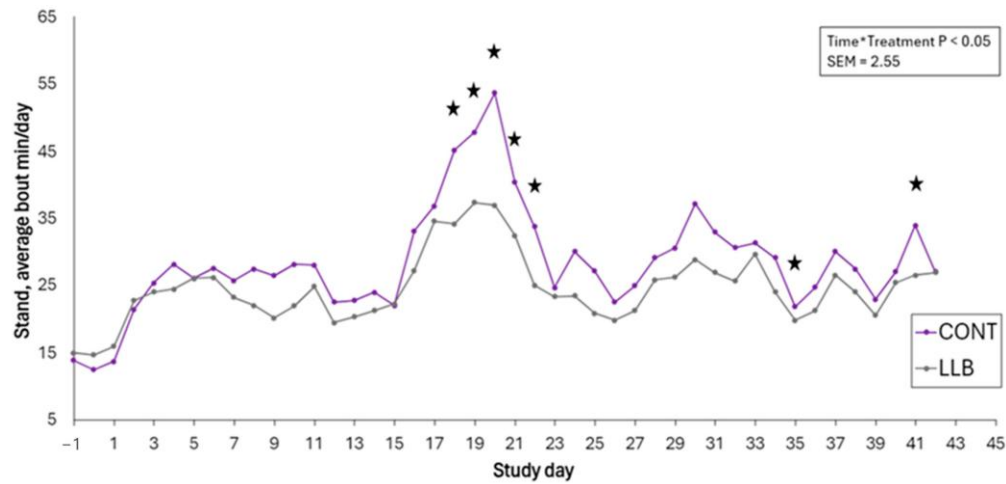


Figure 6.3. Average time (minutes) spent standing in a bout per day

Average time (minutes) spent standing in a bout per day of beef-on-dairy calves banded with a lidocaine-infused castration band (LLB; n = 13) and a standard band (CONT; n = 13), recorded by three-axis accelerometers. Stars in each study day denote differences among treatment groups ($p < 0.05$).

Table 6.1. Adapted ethogram used to evaluate behaviors

Adapted ethogram used to evaluate behaviors associated with Lidoband™ castration (LLB; n = 13) and standard band castration (CONT; n = 13) through video monitoring.

Behavior	Description
Duration, regardless of chewing status	
Stand eating, %	Percent of time standing with head on feed bucket
Stand not eating, %	Percent of time standing with head outside feed bucket
Lying down, %	Percent time in sternal or lateral recumbency
Count	
Tail flicks, n	Number of forceful tail movements beyond the widest part of rump while standing
Wound licks, n	Number of times their head turned to lick the castration wound site while standing
Looks at flank, n	Number of times their head turned to look at the flank area
Foot stamping, n	Number of times one of the hind legs was lifted and forcefully placed on the ground or kicked outward while standing

Table 6.2. Final body weight, overall average daily gain (ADG), and overall gain to feed (G:F)

Final body weight, overall average daily gain (ADG), and overall gain to feed (G:F) of beef-on-dairy calves banded with a lidocaine-infused castration band (LLB; n = 13) and a standard band (CONT; n = 13) and followed for 6 weeks post castration.

	Treatments			p-Value
	LLB	CONT	SEM	Treatment
Final body weight, kg	70.7	69.1	1.25	0.38
ADG, kg/d	0.51	0.49	0.02	0.49
G:F, kg	0.38	0.40	0.02	0.69

Table 6.3. The least square means and standard error of behavioral parameters

The least square means and standard error of behavioral parameters (duration and counts) obtained on day -1 (baseline), day 0 (immediately post castration), and days 1, 2, 7, 14, 21, 28, 35, and 42 post castration of beef-on-dairy cross calves band castrated with a standard band (CONTROL; n = 13) or with a lidocaine-loaded band (LLB; n = 13) scored in 30 min video samples. The data presented are the least squares means and standard error without transformation and the p-value from transformed values. 1 p-values correspond to the ANOVA analysis using square root + 1 transformed data. 2 p-values correspond to the ANOVA analysis using log 10 + 1 transformed data.

	Treatment			p-Value		
Behaviors	CONT	LLB	SEM	Treatment	Timepoint	Treatment X Timepoint
Stand not eating, %	22.0	16.3	2.55	0.20	<0.01	0.06
Stand eating, %	2.4	1.0	0.63	0.87	<0.05	0.36
Lying down, %	73.4	81.2	2.69	0.33	<0.01	<0.01
Tail flicks, n	4.2	3.6	1.31	0.13	<0.01	0.11
Wound licks, n	1.8	1.6	0.41	<0.05	<0.01	0.10
Looks at flank, n	1.1	1.4	0.20	0.13	<0.01	0.35
Foot stamping, n	2.4	2.7	1.16	0.30	<0.01	0.19

Appendix A - Frequency of total concurrent organ systems affected and lesions recorded

Appendix Table A.1. Frequency of SYST

Frequency of SYST from 889 central high plain feedyard mortalities at gross necropsy in the summers of 2022 and 2023, in descending order from most common to least common. An individual organ system can be counted multiple times to account for different lesions within the organ system.

Concurrent Organ Systems (SYST)	Count of cases (n)
GI, Pulmonary	170
Cardiovascular, GI, Pulmonary	57
Cardiovascular, Pulmonary	48
GI, GI, Pulmonary	49
GI, Other, Pulmonary	23
GI, Other	20
GI, Pulmonary, Pulmonary	18
Cardiovascular, GI, GI, Pulmonary	17
Cardiovascular, GI	15
Other, Pulmonary	14
GI, GI	13
GI, GI, GI, Pulmonary	12
Cardiovascular, Cardiovascular, GI, Pulmonary	9
Cardiovascular, GI, Pulmonary, Pulmonary	9
GI, GI, Other, Pulmonary	7
Cardiovascular, GI, GI, Pulmonary, Pulmonary	6
Cardiovascular, Cardiovascular, GI	5
Cardiovascular, Cardiovascular, GI, GI, Pulmonary	5
Cardiovascular, Cardiovascular, Pulmonary	5
Cardiovascular, GI, GI, GI, Pulmonary	5
GI, GI, Pulmonary, Pulmonary	5
Pulmonary, Pulmonary	5
Cardiovascular, Cardiovascular, GI, Pulmonary, Pulmonary	4

Other, Other, Pulmonary	4
Cardiovascular, Cardiovascular, Pulmonary, Pulmonary	3
Cardiovascular, GI, GI, Other, Pulmonary	3
Cardiovascular, GI, Other	3
Cardiovascular, Pulmonary, Pulmonary	3
GI, GI, Other	3
Other, Pulmonary, Pulmonary	3
Cardiovascular, Cardiovascular, Cardiovascular, Pulmonary	2
Cardiovascular, Cardiovascular, GI, Other, Pulmonary	2
GI, Other, Other	2
GI, Other, Other, Pulmonary	2
GI, Other, Pulmonary, Pulmonary	2
Cardiovascular, Cardiovascular, Cardiovascular, GI, Pulmonary	1
Cardiovascular, Cardiovascular, Cardiovascular, GI, Pulmonary, Pulmonary	1
Cardiovascular, Cardiovascular, Cardiovascular, Pulmonary, Pulmonary	1
Cardiovascular, Cardiovascular, GI, GI, GI, GI, Other, Pulmonary	1
Cardiovascular, Cardiovascular, GI, GI, GI, Pulmonary, Pulmonary	1
Cardiovascular, Cardiovascular, GI, Other, Pulmonary, Pulmonary	1
Cardiovascular, Cardiovascular, GI, Pulmonary, Pulmonary, Pulmonary	1
Cardiovascular, Cardiovascular, Other, Pulmonary	1
Cardiovascular, GI, GI	1
Cardiovascular, GI, GI, GI, GI, Pulmonary, Pulmonary	1
Cardiovascular, GI, GI, Other	1
Cardiovascular, GI, GI, Other, Pulmonary, Pulmonary	1
Cardiovascular, GI, Other, Other, Pulmonary, Pulmonary	1
Cardiovascular, GI, Other, Pulmonary	1
Cardiovascular, GI, Other, Pulmonary, Pulmonary, Pulmonary	1
Cardiovascular, GI, Pulmonary, Pulmonary, Pulmonary	1
Cardiovascular, Other	1
Cardiovascular, Other, Other, Pulmonary	1
Cardiovascular, Other, Pulmonary	1

Cardiovascular, Other, Pulmonary, Pulmonary	1
Cardiovascular, Pulmonary, GI	1
GI, Cardiovascular	1
GI, GI, GI	1
GI, GI, GI, GI, Pulmonary	1
GI, GI, GI, Other, Pulmonary	1
GI, GI, GI, Other, Pulmonary, Pulmonary	1
GI, GI, GI, Pulmonary, Pulmonary	1
GI, GI, Other, Other, Pulmonary	1
GI, Other, Other, Pulmonary, Pulmonary	1

Appendix Table A.2. Frequency of LCAT

Frequency of LCAT from 889 central high plain feedyard mortalities at gross necropsy in the summers of 2022 and 2023, in descending order from most common to least common.

Concurrent Lesion Categories (LCAT)	Count of cases (n)
BIP, GI	72
BP, GI	59
AIP, GI	23
GI, Other	21
BIP, CHF, GI	16
BIP, GI, GI	14
BIP, Other Cardiovascular	13
Bloat, GI	13
BP, GI, Other	13
BIP, CHF	12
Bloat, BP, GI	11
BP, GI, GI	11
BP, Other	11
Bloat, BP	10
BIP, GI, Other Pulmonary	9
BP, GI, Other Cardiovascular	9
BP, GI, Other Pulmonary	8
BP, Other Cardiovascular	8
GI, GI	8
BP, CHF, GI	7
AIP, Other Cardiovascular	6
BIP, GI, Other Cardiovascular	6
BIP, Other	6
AIP, CHF, GI	5
BP, CHF	5
BP, Liver Abscess	5
BP, Other Pulmonary	5

GI, Other, Other Pulmonary	5
GI, Other Cardiovascular	5
AIP, GI, Other Cardiovascular	4
BIP, GI, GI, GI	4
Bloat, CHF	4
Bloat, GI, GI	4
BP, CHF, GI, GI	4
BP, CHF, GI, GI, Other Cardiovascular	4
BP, CHF, GI, Other Pulmonary	4
BP, Heat Stress	4
CHF, GI	4
GI, GI, GI	4
Other, Other Pulmonary	4
AIP, CHF	3
AIP, GI, GI	3
BIP, CHF, GI, GI, Other Pulmonary	3
BIP, GI, Liver Abscess	3
BIP, GI, Other	3
BIP, Liver Abscess	3
Bloat, Other Cardiovascular	3
BP, CHF, Other Cardiovascular, Other Pulmonary	3
BP, GI, GI, Other	3
BP, GI, GI, Other Pulmonary	3
BP, Other, Other	3
BP, Other, Other Pulmonary	3
CHF, GI, Heat Stress	3
GI, Heat Stress	3
Heat Stress, Liver Abscess	3
AIP, CHF, GI, GI	2
AIP, CHF, GI, Other Cardiovascular	2
BIP, Bloat, GI	2

BIP, CHF, GI, GI	2
BIP, CHF, GI, GI, GI	2
BIP, CHF, GI, Other Cardiovascular	2
BIP, CHF, GI, Other Cardiovascular, Other Pulmonary	2
BIP, CHF, GI, Other, Other Cardiovascular	2
BIP, CHF, Liver Abscess	2
BIP, GI, GI, Other Cardiovascular	2
BIP, GI, GI, Other Pulmonary	2
BIP, GI, Liver Abscess, Other Cardiovascular	2
Bloat, BP, CHF, GI, Other	2
Bloat, BP, GI, GI	2
BP, GI, GI, GI	2
BP, GI, Heat Stress	2
BP, GI, Liver Abscess	2
BP, GI, Liver Abscess, Other Cardiovascular	2
BP, GI, Other Cardiovascular, Other Pulmonary	2
CHF, GI, Other Pulmonary	2
GI, GI, Other Cardiovascular	2
GI, GI, Other, Other Pulmonary	2
GI, Other Cardiovascular, Other Cardiovascular	2
GI, Other Cardiovascular, Other Pulmonary	2
GI, Other Pulmonary	2
GI, Other, Other Cardiovascular	2
Other, Other Cardiovascular, Other Pulmonary	2
AIP, Bloat	1
AIP, Bloat, CHF, GI, GI	1
AIP, CHF, Heat Stress	1
AIP, CHF, Other Cardiovascular	1
AIP, CHF, Other Cardiovascular, Other Cardiovascular, Other Pulmonary	1
AIP, GI, GI, Heat Stress, Other Pulmonary	1
AIP, GI, GI, Liver Abscess	1

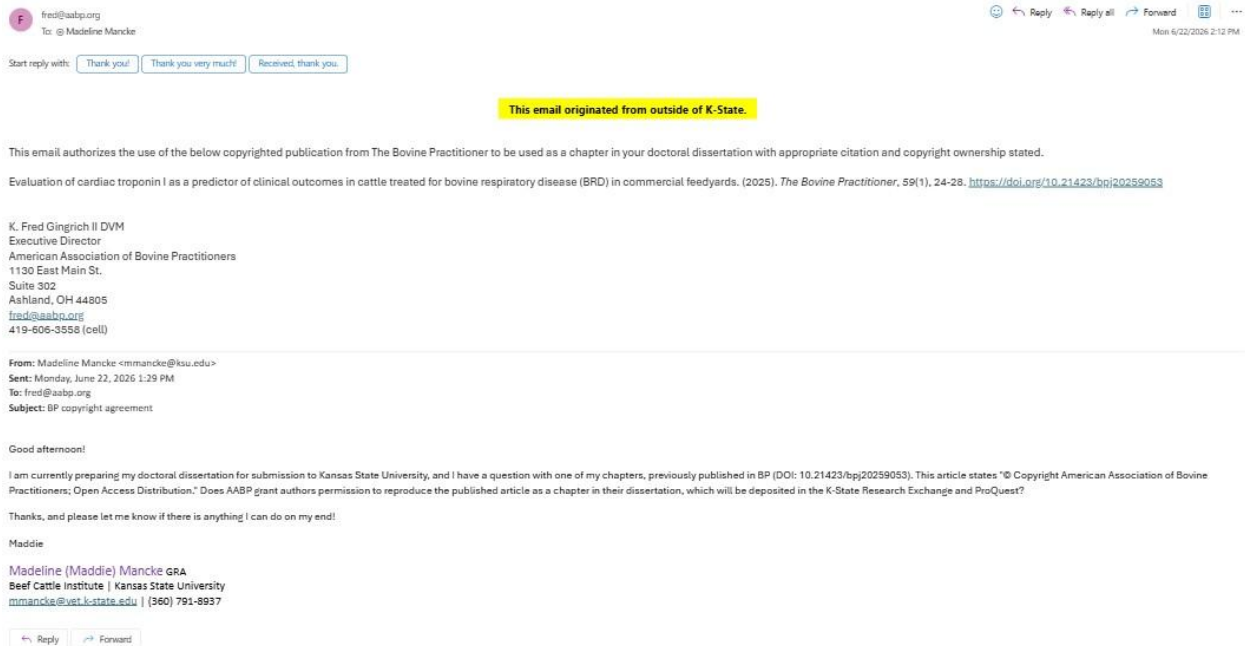
AIP, GI, GI, Other Cardiovascular, Other Pulmonary	1
AIP, GI, Heat Stress, Other Pulmonary	1
AIP, GI, Liver Abscess	1
AIP, GI, Other	1
AIP, GI, Other Cardiovascular, Other Pulmonary	1
AIP, Other Pulmonary	1
BIP, CHF, GI, GI, GI, GI, Other, Other Cardiovascular	1
BIP, CHF, GI, GI, Heat Stress	1
BIP, CHF, GI, Liver Abscess	1
BIP, CHF, GI, Liver Abscess, Other Pulmonary	1
BIP, CHF, GI, Other	1
BIP, CHF, GI, Other Pulmonary, Other Pulmonary	1
BIP, CHF, Other Cardiovascular	1
BIP, CHF, Other Cardiovascular, Other Cardiovascular	1
BIP, CHF, Other Pulmonary	1
BIP, GI, GI, GI, GI	1
BIP, GI, GI, GI, Other, Other Pulmonary	1
BIP, GI, GI, Heat Stress	1
BIP, GI, GI, Liver Abscess	1
BIP, GI, GI, Liver Abscess, Other	1
BIP, GI, GI, Other	1
BIP, GI, GI, Other Cardiovascular, Other Cardiovascular	1
BIP, GI, Heat Stress	1
BIP, GI, Other Cardiovascular, Other Cardiovascular	1
BIP, GI, Other, Other Pulmonary	1
BIP, Liver Abscess, Other, Other Cardiovascular, Other Pulmonary, Other Pulmonary	1
BIP, Other Pulmonary	1
BIP, Other, GI	1
Bloat, BP, CHF, GI, GI, Other Cardiovascular, Other Pulmonary	1
Bloat, BP, GI, Liver Abscess	1
Bloat, BP, Other	1

Bloat, BP, Other Cardiovascular	1
Bloat, CHF, Heat Stress	1
Bloat, CHF, Other	1
Bloat, GI, GI, Other Pulmonary	1
Bloat, GI, Liver Abscess	1
Bloat , GI, Other	1
Bloat, Liver Abscess	1
Bloat, Liver Abscess, Other Cardiovascular	1
Bloat, Other Pulmonary	1
BP, Bloat, GI, GI	1
BP, CHF, GI, GI, GI	1
BP, CHF, GI, GI, Other, Other Pulmonary	1
BP, CHF, GI, Heat Stress	1
BP, CHF, GI, Other Cardiovascular, Other Pulmonary	1
BP, CHF, GI, Other Cardiovascular, Other Pulmonary, Other Pulmonary	1
BP, CHF, Liver Abscess, Other Cardiovascular	1
BP, CHF, Liver Abscess, Other Pulmonary	1
BP, CHF, Other Cardiovascular	1
BP, CHF, Other Pulmonary	1
BP, CHF, Other, Other	1
BP, CHF, Other, Other Pulmonary	1
BP, GI, GI, GI, GI, Other Cardiovascular, Other Pulmonary	1
BP, GI, GI, GI, Other Pulmonary	1
BP, GI, GI, Heat Stress	1
BP, GI, GI, Heat Stress, Other	1
BP, GI, GI, Other, Other Pulmonary	1
BP, GI, Heat Stress, Other Cardiovascular, Other Pulmonary	1
BP, GI, Other Cardiovascular, Other Cardiovascular	1
BP, GI, Other, Other	1
BP, GI, Other, Other Cardiovascular	1
BP, GI, Other, Other Cardiovascular, Other Cardiovascular, Other Pulmonary	1

BP, Heat Stress, Other	1
BP, Heat Stress, Other Pulmonary	1
BP, Liver Abscess, Other	1
BP, Liver Abscess, Other, Other, Other Pulmonary	1
BP, Other Cardiovascular, GI	1
BP, Other, Other, Other Cardiovascular	1
CHF, GI, GI, Liver Abscess, Other Pulmonary	1
CHF, GI, Other Cardiovascular, Other Cardiovascular, Other Pulmonary	1
CHF, GI, Other, Other, Other Pulmonary, Other Pulmonary	1
CHF, Liver Abscess, Other Cardiovascular, Other Cardiovascular, Other Pulmonary, Other Pulmonary	1
CHF, Liver Abscess, Other Cardiovascular, Other Pulmonary	1
CHF, Liver Abscess, Other Pulmonary	1
CHF, Other	1
CHF, Other Cardiovascular, Other Cardiovascular, Other Pulmonary	1
CHF, Other Cardiovascular, Other Pulmonary	1
CHF, Other Pulmonary	1
GI, GI, GI, Liver Abscess, Other Pulmonary	1
GI, GI, Heat Stress, Other	1
GI, GI, Heat Stress, Other Pulmonary	1
GI, GI, Other	1
GI, GI, Other Pulmonary	1
GI, GI, Other, Other	1
GI, GI, Other, Other, Other Pulmonary	1
GI, Heat Stress, Other Cardiovascular	1
GI, Liver Abscess, Other	1
GI, Liver Abscess, Other, Other Cardiovascular	1
GI, Liver Abscess, Other, Other Pulmonary	1
GI, Other, Other, Other Pulmonary	1
Heat Stress, Other Cardiovascular	1
Heat Stress, Other Pulmonary	1
Liver Abscess, Other, Other	1

Liver Abscess, Other, Other Cardiovascular	1
Other Cardiovascular, Other Pulmonary	1
Other Pulmonary, Other Pulmonary, GI	1
Other, Other Cardiovascular, Other Cardiovascular, Other Pulmonary	1

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Appendix Figure B.1. Email from The Bovine Practitioner

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