

The health and welfare of fed cattle after transport to commercial slaughter facilities

by

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B.S., Kansas State University, 2008
M.S., Kansas State University, 2012
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Abstract

Cattle health and welfare are of utmost concern to producers, packers, processors, and consumers of beef. In addition, poor animal welfare can lead to decreases in economic return, in the form of production losses, product losses, or even live animal losses. Two major contributors to such losses include carcass bruising and cattle fatigue, or Fatigued Cattle Syndrome. Bruising in fed beef cattle costs the industry millions of dollars annually, and cattle fatigue leads to production losses and animal death during and after transport. Much research in cattle welfare is focused upon the more vulnerable classes of cattle in the industry, such as small calves, cull beef cows, and cull dairy cows. Limited research exists on the animal welfare concerns in fed beef cattle, likely because these animals are considered healthier and better fit for transport compared to other classes. The overall goal of this research was to assess the health and welfare of fed cattle after transport to commercial slaughter facilities by addressing two large concerns in the industry: 1) bruising in fed cattle and 2) Fatigued Cattle Syndrome and its prevalence and physiologic characteristics. The first objective of this research focused upon bruising, and was to determine whether a relationship exists between trauma incurred during unloading and prevalence of carcass bruising in finished beef cattle at commercial slaughter facilities. In addition, other risk factors which may contribute to carcass bruising in finished beef cattle are addressed. The second and third objectives focused upon Fatigued Cattle Syndrome in the fed cattle population. The second objective of this research was to determine the prevalence of abnormal mobility scores and the clinical signs associated with to abnormal mobility in finished cattle in six commercial slaughter facilities across the United States. The third objective was to determine if mobility score and clinical signs reflect concurrent changes in physiologic parameters such as blood concentrations of specific biochemical markers and biomechanical

integrity of hooves. This information is both valuable and novel in the fed beef cattle industry. Along with the implementation of practices that will promote better health and welfare of fed cattle presented to slaughter facilities, gathering such information will help improve animal welfare, increase economic returns, and strengthen consumer confidence in the industry.

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Table of Contents

List of Figures	xi
List of Tables	xiv
Acknowledgements	xvi
Dedication	xvii
Preface.....	xviii
Chapter 1 - Improving Cattle Welfare—An Evidence-Based Approach.....	1
Abstract.....	1
Introduction.....	1
Outcome-based Measures	3
Behavior.....	4
Morbidity and Mortality	8
Changes in Weight and Body Condition	16
Reproductive Efficiency	18
Physical Appearance.....	22
Handling Responses.....	24
Routine Procedure Management.....	30
Conclusion	34
References.....	37
Chapter 2 - Assessment of risk factors contributing to carcass bruising in fed cattle at commercial slaughter facilities	55
Abstract.....	55
Introduction.....	57
Materials & Methods	58
Results.....	60
Traumatic Events	61
Carcass Bruising	61
Discussion.....	62
Traumatic Events	62
Carcass Bruising	64

Conclusion	67
References.....	69
Chapter 3 - An Epidemiological Investigation to Determine the Prevalence and Clinical	
Manifestations of Slow-moving Finished Cattle at the Time of Slaughter	84
Abstract.....	84
Introduction.....	85
Materials & Methods	86
Results.....	90
Effect of Distance Traveled on Mobility Score Prevalence.....	91
Effect of Environmental Conditions on Mobility Score Prevalence	91
Effect of Time of Observation and Average Lairage Time on Prevalence of Mobility Score Prevalence	91
Effect of Sex and Breed on Mobility Score Prevalence.....	92
Effect of Distance Traveled on Prevalence of Clinical Diagnoses	92
Effects of Environmental Conditions on Prevalence of Clinical Diagnoses	93
Effect of Time of Observation and Average Lairage Time on Prevalence of Clinical Diagnoses	93
Effects of Sex on Prevalence of Clinical Diagnoses.....	94
Effects of Breed on Prevalence of Clinical Diagnoses	95
Mobility Score Prevalence	95
Prevalence of Clinical Diagnoses	97
Conclusion	101
References.....	103
Chapter 4 - Comparison of Physiologic Parameters of Normal Finished Cattle and Finished	
Cattle Diagnosed with Fatigued Cattle Syndrome at Commercial Slaughter Facilities	149
Abstract.....	149
Introduction.....	151
Materials & Methods	152
Results.....	156
Discussion.....	158
Conclusion	166

References..... 168

List of Figures

- Figure 2-1. Grid of sections used in the Harvest Audit Bruise Scoring System..... 81
- Figure 2-2. Prevalence of traumatic events for each combination of breed and trailer type. There was a significant interaction between trailer type and cattle breed, whereby Holstein cattle hauled in fat/feeder combination trailers experienced higher prevalence of traumatic events than their beef counterparts..... 82
- Figure 2-3. Relationship between average carcass weight and carcass bruising prevalence by lot for lots of Holstein and beef breed cattle ($P < 0.05$), results from multivariable linear regression model. Each point on the graph represents a lot of cattle observed. Triangles represent lots of Holstein cattle ($n=12$), while dots represent lots of beef breed cattle ($n=63$). 83
- Figure 3-1. Comparisons of point estimates for abnormal mobility score prevalence (scores 2, 3, and 4 as Figures A, B, and C, respectively) categorized by facility location in 65,600 finished cattle at 6 commercial slaughter facilities. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$). 113
- Figure 3-2. Comparisons of point estimates for abnormal mobility score prevalence (scores 2, 3, and 4 as Figures A, B, and C, respectively) categorized by distance traveled in 65,600 finished cattle at 6 commercial slaughter facilities. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$). 115
- Figure 3-3. Comparisons of point estimates for abnormal mobility score prevalence (scores 2, 3, and 4 as Figures A, B, and C, respectively) categorized by temperature ($^{\circ}\text{C}$) in 65,600 finished cattle at 6 commercial slaughter facilities. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$). 117
- Figure 3-4. Comparisons of point estimates for abnormal mobility score prevalence (scores 2, 3, and 4 as Figures A, B, and C, respectively) categorized by temperature-humidity index (THI) in 65,600 finished cattle at 6 commercial slaughter facilities. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$). 119
- Figure 3-5. Comparisons of point estimates for abnormal mobility score prevalence (scores 2, 3, and 4 as Figures A, B, and C, respectively) categorized by time observed in 65,600 finished

cattle at 6 commercial slaughter facilities. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$).....	121
Figure 3-6. Comparisons of point estimates for abnormal mobility score prevalence (scores 2, 3, and 4 as Figures A, B, and C, respectively) categorized by average lairage time (h) in 65,600 finished cattle at 6 commercial slaughter facilities. Lairage time is defined as {Lairage Time = (time of observation - average time the trucks carrying the lots passed over the slaughter facility scale)}. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$).....	123
Figure 3-7. Comparisons of point estimates for abnormal mobility score prevalence (scores 2 and 3, Figures A and B, respectively) categorized by sex in 65,600 finished cattle at 6 commercial slaughter facilities. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$).....	125
Figure 3-8. Comparisons of point estimates for abnormal mobility score prevalence (scores 2, 3, and 4 as Figures A, B, and C, respectively) categorized by breed in 65,600 finished cattle at 6 commercial slaughter facilities. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$).....	126
Figure 3-9. Comparisons of point estimates for clinical diagnoses (Figures A, B, C, D, and E) categorized by distance traveled (mi) in 65,600 finished cattle at 6 commercial slaughter facilities. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$).....	128
Figure 3-10. Comparisons of point estimates for clinical diagnoses (Figures A, B, C, D, and E) categorized by temperature ($^{\circ}$ F) in 65,600 finished cattle at 6 commercial slaughter facilities. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$).....	131
Figure 3-11. Comparisons of point estimates for clinical diagnoses (Figures A, B, C, D, and E) categorized by time of observation in 65,600 finished cattle at 6 commercial slaughter facilities. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$).....	134
Figure 3-12. Comparisons of point estimates for clinical diagnoses (Figures A, B, C, D, and E) categorized by time of observation in 65,600 finished cattle at 6 commercial slaughter	

facilities. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$). 137

Figure 3-13. Comparisons of point estimates for clinical diagnoses (Figures A, B, C, D, and E) categorized by average lairage time in 65,600 finished cattle at 6 commercial slaughter facilities. Lairage time is defined as {Lairage Time = (time of observation - average time the trucks carrying the lots passed over the slaughter facility scale)}. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$). 140

Figure 3-14. Comparisons of point estimates for clinical diagnoses (Figures A, B, C, D, and E) categorized by sex in 65,600 finished cattle at 6 commercial slaughter facilities. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$). 143

Figure 3-15. Comparisons of point estimates for clinical diagnoses (Figures A, B, C, D, and E) categorized by breed in 65,600 finished cattle at 6 commercial slaughter facilities. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$). 146

List of Tables

Table 1-1. A list of outcome-based measures for specific incidences in management of cattle welfare.....	36
Table 2-1. Description of cattle hauled in each trailer type (fat vs. combination trailers).	75
Table 2-2. Description of lots observed for both traumatic events and carcass bruising.	75
Table 2-3. Description of lots, including carcass characteristics, prevalence of traumatic events experienced, and prevalence of carcass bruising.	76
Table 2-4. <i>P</i> -values generated from univariable and multivariable analyses for the outcome traumatic events. Only 2-way interactions were evaluated in the multivariable analysis. Interaction effects are listed in the order by which they were removed from the model using backward selection at a threshold of $P>0.05$	77
Table 2-5. Percent of carcass bruising on the left side, the dorsal midline, and the right side of beef carcasses. Superscripts indicate a significant difference between the observed values and the expected values of the bruising in each region ($P<0.05$). Equal distribution between all regions was expected.	77
Table 2-6. Percent of carcass bruising on the front, middle, and rear thirds of beef carcasses. Superscripts indicate a significant difference between the observed values and the expected values of the bruising in each region ($P<0.05$). Equal distribution between all regions was expected.	78
Table 2-7. Percent of carcass bruising categorized as small, medium, or large bruises. Superscripts indicate a significant difference between the observed values and the expected values of the bruise sizes. Equal distribution between all sizes was expected.	78
Table 2-8. Estimates of parameters for the fixed effects of average carcass weight and breed of cattle assessed with multiple linear regression.	78
Table 2-9. Estimate of mean carcass bruise prevalence per lot by breed (cattle were categorized as either Holstein or beef breeds). Estimates with different superscripts differ significantly ($P<0.05$).	79
Table 2-10. <i>P</i> -values generated from univariable and multivariable analyses for the outcome carcass bruising. Only 2-way interactions were evaluated in the multivariable analysis.	

Interaction effects are listed in the order by which they were removed from the model using backward selection at a threshold of $P > 0.05$	80
Table 3-1. Description of cattle observed by facility, breed, and gender (n=65,600).	108
Table 3-2. Description of environmental conditions and average mobility scores in each facility where observations were made.	108
Table 3-3. Prevalence of mobility scores 1, 2, 3, and 4 (n=65,600) observed.....	109
Table 3-4. Mobility score counts and percent prevalence observed in each facility.	109
Table 3-5. Mobility scores by gender and breed (n=65,600).....	110
Table 3-6. Prevalence of each clinical diagnosis (n=65,600).	110
Table 3-7. Prevalence of abnormalities within each abnormal mobility score (MS 2, 3, and 4).	111
Table 3-8. Clinical signs observed in animals assigned to each clinical diagnosis category. ...	112
Table 4-1. Clinical signs displayed by normal cattle (MS = 1) and cattle displaying signs of Fatigued Cattle Syndrome (FCS) with MS > 3.....	173
Table 4-2. Comparisons of biochemical markers in normal cattle (MS = 1) and cattle displaying signs of Fatigued Cattle Syndrome (FCS) with MS > 3.	174
Table 4-3. Descriptions and comparisons of biomechanical integrity of dorsal hoof walls in normal cattle (MS = 1) and cattle displaying signs of Fatigued Cattle Syndrome (FCS) with MS > 3.....	175
Table 4-4. Sensitivity and specificity results for diagnostic test of mobility score as an indicator of FCS, compared to the Gold Standard of diagnosis using clinical presentation. Serum concentrations of lactate and creatine kinase (CK) were used as tests, while the Gold Standard in determining true disease status was clinical diagnosis by a trained veterinarian. Serum lactate was above the normal reference range (>5mmol/L), therefore different cutoff values were explored to determine the cutoff which yielded the highest sensitivity and specificity results.	175
Table 4-5. Benchmarks for determining strength of agreement (not due to chance) between two diagnostic tests using Cohen's Kappa coefficient. ¹⁰	176

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Dedication

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Preface

Chapter 1 in this dissertation entitled “Improving cattle welfare—an evidence-based approach was prepared as an invited review in *CAB Reviews* and was accepted for publication on May 22, 2015. Chapter 2, entitled “Assessment of risk factors contributing to carcass bruising in fed cattle at commercial slaughter facilities” was prepared for publication in *Journal of Animal Science*. Chapters 3 and 4, entitled “An epidemiological investigation to determine the prevalence and clinical manifestations of slow-moving finished cattle at the time of slaughter” and “Comparison of physiologic parameters of normal finished cattle and finished cattle diagnosed with Fatigued Cattle Syndrome at commercial slaughter facilities” were prepared for publication in *Journal of the American Veterinary Medical Association*. The text and figures within these chapters are formatted according to the guidelines specified by the journal in which they are published or will be submitted for publication.

Chapter 1 - Improving Cattle Welfare—An Evidence-Based

Approach

Abstract

Animal welfare is a scientific field that is constantly changing and improving. The importance of this field is becoming more evident in all production agriculture settings, including cattle and dairy production units. In 2007, Fraser defined animal welfare as a “mandatory science”, one in which science has been used to guide actions, decisions, and policy. While values-based ideas are an integral part of the idea of animal welfare, science-based ideas and the scientific method must be used to assess and improve animal welfare in our production units. Animal welfare practices must advance from the laboratory to the field. In this light, animal welfare outcome measures must be defined to allow people to measure and assess animal welfare in beef and dairy production units daily. This review focuses on defining the outcome-based measures that can be used in the cattle industry to increase our understanding of animal health and well-being. This will provide a framework to help farmers, ranchers, and veterinarians determine if changes in management or other husbandry practices improve or compromise cattle health or welfare.

Introduction

Animal welfare, including cattle welfare, is a complex and often highly debated topic. Animal welfare is a term that has arisen in society to express ethical concerns about the quality of life experienced by animals, particularly animals that are used by human beings in production agriculture (1, 2).

Animal welfare has become an established scientific field. However, as an industry, we must understand that this science evolved from society's concern about the quality of life experienced by the animals we raise. Therefore, it must be clear that there are multiple groups which approach animal welfare from different viewpoints, and put emphasis on different aspects of the science (1). The definition of animal welfare as reported by the OIE in 2008: "Animal welfare means how an animal is coping with the conditions in which it lives. An animal is in a good state of welfare if (*as indicated by scientific evidence*) it is healthy, comfortable, well-nourished, safe, able to express innate behavior, and if it is not suffering from unpleasant states such as pain, fear, and distress. Good animal welfare requires disease prevention and veterinary treatment, appropriate shelter, management, nutrition, humane handling and humane slaughter/killing. Animal welfare refers to the state of the animal; the treatment that an animal receives is covered by other terms such as animal care, animal husbandry, and humane treatment (3, emphasis added). Therefore, while multiple groups approach animal welfare from different viewpoints, it is generally accepted that scientific evidence must be used to indicate the welfare situation. The science must be emphasized when making assessments of animal welfare.

Fraser (2008) stated that our understanding of animal welfare is both values-based and science-based. It is a "mandated" science, in which the tools of science are used within a framework of values (2). When different groups discuss and debate animal welfare, most disagreements arise from a difference in values, and not from differences in factual evidence. There is now a growing acceptance among animal welfare scientists of the importance of feelings in determining welfare (4), however, an evidence-based approach to cattle welfare is necessary to provide such factual evidence. Like all other scientific fields, knowledge should be obtained and tested through the scientific method. And like all practices, the study of animal

welfare must be translated to the field where producers and veterinarians can calibrate their assessment tools to determine normal and abnormal animal welfare on the farm at any time. There are a number of assessments and audits available to first and third parties for the recording of animal welfare conditions, however, animal welfare should be measured on a day-to-day basis, by those who work directly with the animals themselves. It is for this reason that outcome-based measures are essential in the measurement and management of animal welfare in cattle.

Outcome-based Measures

Broom (1991) and Curtis (1986) emphasized that the concept of welfare varies over a range, and it should be measurable by using a variety of indicators (5-6). Decisions should be made using these indicators to determine if the situation is tolerable for the animals in question.

Outcome-based measures, specifically animal-based measures, are useful indicators of animal welfare. The use of these indicators and the appropriate thresholds should be adapted to the different situations where cattle are managed. However, certain measurements will likely be similar across all production systems, and these should be focused upon first, as the industry evolves toward a more specific measurement system for animal welfare. Animal-based measures are important because they require the observer to focus on the animals themselves, while assessments and audits tend to focus the observer's attention on environmental concerns. Producers and veterinarians use animal-based measures to make production decisions daily. Animal-based measures for welfare can easily be integrated into these production decisions as well.

According to the OIE definition such criteria include cattle behavior, morbidity rates, mortality rates, changes in weight and body condition, reproductive efficiency, physical appearance, handling responses, and complications due to routine procedure management (7). These measures are tracked by both producers and veterinarians on a regular basis for the routine management of cattle operations. It is important for typically-measured animal health and production outcome measures to be used to assess animal welfare so farmers, ranchers, and veterinarians can provide useful information about health and welfare status of the herd as a whole, on a day-to-day basis. With the help of these animal-based measures, recommendations can be made to improve cattle welfare on production facilities (Table 1-1). Management practices should consider productivity, but also health and welfare of the animals.

Behavior

Many management practices considering welfare of animals are based on cattle behavior. Certain behaviors can indicate an animal welfare problem (7). It is important to have a clear understanding of an animal's behavior under various environmental conditions for consideration of research results on physiology, nutrition, breeding, and management (8).

Feeding behavior is generally consistent and repeatable. Feeding behavior can be related to health (9), performance, and feed conversion (10-11). Feed intake is measured daily in some, but not all, cattle operations, but should be monitored regularly to recognize any changes (increases or decreases). Sowell *et al.* in 1999 showed that decreased feed intake can be seen in the first four days on feed in morbid steers versus healthy steers, but there was no significant difference in water intake between healthy and morbid cattle in the study (9). In dairy operations, Sepulveda-Varas *et al.* (2014) showed that cows with clinical mastitis showed a

decrease in feed intake in the days before treatment, and a rapid improvement in the days after treatment (12). Such information about decreased feed intake in morbid animals can be of use when determining animal health, which is an indicator of animal welfare. Feed intake can also be affected by social relationships between cattle. It has been shown that dominance can affect feed intake in cattle by potentiating feeding displacement (13-15) and indicating the possible need for a decreased stocking density or increased bunk space. Val-Laillet *et al.* (2008) also concluded that there may be different motivations for different behaviors, so dominance is not necessarily the only variable that can affect feeding behavior—motivation such as hunger or access to fresh versus older feed, can also affect the feeding behavior of cattle (13). However, it must be remembered that decreased feed intake due to any circumstance can indicate a welfare problem, and must be addressed when observed.

Producers regularly monitor feeding behavior in intensive production systems. For example, daily intake is measured by bunk readers in feedlots. Intake in dairy cattle can be measured on a daily basis as well. Feed intake in extensive production systems may not be monitored every day, but producers make efforts to track forage quality and quantity in pastures, and ensure that all cows maintain adequate body condition. Written records and documentation are encouraged to ensure that changes in feed intake are monitored and addressed, if needed.

In periods of hot weather, other behaviors can be used to address animal welfare, such as respiratory rate or panting (7). A panting score system was developed by Gaughn and Mader (2014) to evaluate heat stress in cattle (16). The risk of heat stress for cattle is influenced by environmental factors including air temperature, relative humidity and wind speed, and animal factors including breed, age, body condition, metabolic rate, and coat color/density (17-18). During hot weather, cattle welfare can be improved by assessing the animals' response to heat

stress using body temperature, respiratory rates, and panting scores and implementing management plans to alleviate the negative impact of increased heat stress on cattle.

Social behaviors can be used as tools in the assessment of animal welfare as well. Dominance behaviors, such as bullying, can be indicators of animal welfare (19). For example, less dominant animals may suffer from reduced access to resources such as food, resting places, shade, and other general activities (13, 19). Such behaviors are usually demonstrated when animal welfare has been compromised, as in situations with high stocking rates, insufficient bunk space, or mixing of animals of different age, size or gender (20-21). Water tanks in the summer may also have an effect on displays of social dominance in a herd. Taylor *et al.* in 1997 discussed data that supported that the use of different management strategies can help to alleviate some of the negative consequences of buller steer syndrome, such as permanent removal of animals from home pens (22). Buhman *et al.* (2000) stated that measurement of eating and drinking behaviors might allow development of applications to improve cattle productivity, however the results of their study showed large variations in eating and drinking duration and frequency, and led the authors to question the use of eating and drinking behaviors to predict social rank or health status (23). Miranda *et al.* (2013) studied the cortisol levels of bulls that ranked low, medium, and high in the social dominance structure, and determined that animals in the “medium” group experienced lower levels of cortisol than those in the groups ranked low and high (19). Such results indicate that social structure has an effect on not only the lower-ranked animals, but on the stress indices of higher-ranked animals as well. This indicates that the stressful situations perceived by researchers may not only have an effect on only a few of the animals observed, but on a number of the animals in a herd. A small number of animals in the herd are usually directly involved in the stressful activities, however, a large number of animals

observe such behavior, and may be affected by it. Studies of social dominance are relevant for the management of herds in feedlots and dairies as they give information on individual susceptibility to social stress under certain environmental conditions.

Behavioral changes in cattle can also indicate illness or pain, very important factors in the assessment of animal welfare. Cattle can experience pain from routine management procedures, including the processing procedure, in which injections of vaccines and implants occur, and surgical procedures such as castration and dehorning (24-25). It is widely accepted that the benefits of routine cattle processing procedures such as vaccination and the administration of anabolic implants greatly outweigh the risks to animal welfare in both animal health and production measures. Behaviors observed in cattle due to routine surgical procedures such as castration and dehorning practices indicate a stronger pain response than responses caused by administration of vaccines and implants (24-25). Stamping, kicking, abnormal gait, vocalization, decreased movement, and decreased feed intake have been observed in cattle after the castration procedure (24, 26). Increased lying, head shaking, rubbing, ear-flicking, and vocalization have been observed in cattle after dehorning (25). Such behaviors are important indicators of pain, and therefore, animal welfare status. Care should be taken to alleviate pain associated with routine surgical procedures when at all possible, whether it be with the use of a local anesthetic before or during the procedure, the use of sedation prior to the procedure, or the use of post-procedural pain management, which will be discussed in further detail in this review.

Producers spend hours observing cattle in their environment, and make production changes based on many of the observations discussed here. Observing cattle behavior is one of the most important acts producers perform to ensure good animal health and welfare. In the future, recording these observations of feed intake, social behaviors such as bullying, and

behaviors indicative of injury and morbidity (such as lameness and panting scores) must be recorded for benchmarking purposes, so that changes can be observed and addressed accordingly. Keeping records of changes in cattle behavior will add to a growing database of welfare indicators, and will contribute to the improvement of animal welfare for both the producers involved and the industry as a whole.

Morbidity and Mortality

Morbidity rates, including disease, lameness, post-procedural complication and injury rates above recognized thresholds may be direct or indirect indicators of the animal welfare status of whole herds. Benchmarking data on morbidity, mortality, and case fatality rates can be accessed for both beef and dairy cattle in the USDA's National Animal Health Monitoring System to help determine the proper thresholds for particular types of operations, such as feedlot, cow/calf, and dairy operations (27-29). However, other peer-reviewed research on morbidity and mortality rates in cattle should be utilized as well, as such research can provide valuable insight into region- or class-specific data that may not be reflected when data from a general population is provided. For example, Martin *et al.* (1980) reported morbidity and mortality rates in beef cattle in Bruce County, western Ontario. This report was used to improve morbidity and mortality rates in the feedlot cattle in that region by addressing issues such as diet, prophylactic practices, and other management strategies (30). McGuirk (2008) presented a systematic approach to enteric and respiratory diseases in dairy calves and heifers (31). By using the benchmarking data available, veterinarians, nutritionists, and producers can determine a level of measure that is appropriate for individual operations.

Cattle health is an important aspect of animal welfare and can serve as an important outcome measure in assessment. There are a number of diseases and syndromes that can affect cattle in intensive and extensive production systems. Cattle raised in intensive beef production systems such as feedlots are subject to a number of diseases, including bovine respiratory disease (BRD), lameness, and acidosis. Lameness and acidosis are common diseases in intensive dairy production systems, along with mastitis and a number of transition cow diseases, such as ketosis and milk fever. Diseases causing lameness also affect cattle in extensive production systems, along with parasitic diseases. Morbidity and mortality are also indicators of calf health and welfare in both intensive and extensive systems. Disease prevalence rates are important for detecting potential animal welfare problems or fundamental changes in animal husbandry practices. Many farm management practices decrease stress in cattle through providing shelter, preventative medicine, and good nutrition, which all lead to improved immune status, thus decreasing diseases in the herd.

When considering animal welfare on any production unit, calf morbidity and mortality are of utmost importance. Calf morbidity and mortality can be caused by a number of diseases and conditions, including occurrence of dystocia, hypothermia, undernutrition, and infection by enteric and respiratory diseases (32). Mortality rates in neonatal calves have been reported to be as high as 50% (32). These rates vary by age, passive transfer status, type of operation, housing, season, management, country, region, and origin of the data, however the most common reasons for disease-related deaths in dairy calves are enteric and respiratory disease (31). In beef calves, the leading cause of mortality in calves less than three weeks of age is birth-related or weather-related causes (33). In beef calves over three weeks of age, leading causes of mortality are digestive (including bloat, scours, and enterotoxemia) and respiratory problems (33). McGuirk

(2008) and Larson *et al.* (2004) provide extensive literature on management decisions that impact dairy and beef calf morbidity and mortality (31, 34). Preventive measures include detailed record-keeping, prevention of dystocia, colostrum management, and proper environmental management (housing, bedding, etc.), all of which are outcome-based measures that producers can track and use to improve animal welfare on their operations.

In older cattle, bovine respiratory disease is the most common disease in the United States. The 2011 USDA National Animal Health Monitoring System report states that the average morbidity rate for cattle housed in all U.S. feedlots reporting is 16.2% due to respiratory disease (28). Every production unit should determine a normal rate for the occurrence of BRD, specific to the unit's management style and the type of cattle that are housed in the production unit, and maintain levels below such a threshold to insure animal welfare is not compromised. Proper diagnosis and timely treatment should also be implemented. Morbidity rates, retreatment rates, mortality rates, and case fatality rates can be useful to determine animal welfare status, as good health is necessary for good welfare (35). Necropsy can be a diagnostic tool that can provide useful information on treatment and care of diseased animals, and the use of post-harvest measurement of lung lesions can aid in the quantification of both clinical sub-clinical disease (36). Roeber *et al.* (2001) reported that preweaning health management programs are usually important to cattle buyers, as they play a significant role in determining profitability and economic risk (37), however the same can be said for the role that such programs play in animal welfare. Preweaning and preconditioning programs can be critical in the prevention of disease later in life, and implementation of such programs is greatly encouraged.

The presence of parasites can indicate an animal health and welfare problem, and contribute to increased morbidity and mortality rates. Parasites can affect productivity of cattle,

as well as economics of production (38). Parasitic infestations often lead to decreased appetite, and therefore decreased intake. Byford *et al.* (1992) suggested that this reduced amount of feed ingested by parasitized animals may also be digested less efficiently than by nonparasitized animals (38). The group also suggested that the presence of parasites may increase metabolic rate, reducing the amount of metabolizable energy available for production. Decreased intake, less efficient digestion, and decreased amounts of energy available for production all have detrimental effects on animal welfare. However, good management practices, including the use of a parasite prevention program, can decrease the parasitic load on cattle, improving both animal health and production.

Lameness in cattle is a significant animal welfare problem and causes significant economic losses (39-41). While lameness is most often termed a behavioral response, in most cases, it is a response to an underlying pathology, such as infection or inflammation. In dairy cows, an annual incidence of lameness of up to 54% has been reported (41). There are a number of lameness scoring systems that have been assessed (42-47). Most of these scoring systems use a five-point scale. A scoring system should be used to evaluate lameness in cattle within a herd, and a pre-determined threshold should be used to assess whether the scores are within limits acceptable for good animal welfare. A number of studies have reported prevalence of lameness in different regions of the United States and the world, and in different production systems, and the data from such studies should be used as a benchmark.

Average prevalence of lameness has been found to range from 7.0%-36.8% (44-52), but individual farm prevalence has ranged from 3.3 to 79.2% (48, 52). It must be noted that prevalence of lameness is not the same as prevalence of disease or lesions. Under most circumstances, the prevalence of lameness is dependent upon the prevalence of pathology,

however, one must always distinguish between the two. There has been much data put forth on the prevalence of pathology in the feet/legs of dairy cattle, which is the primary cause of lameness. Clinical lesions such as digital dermatitis, interdigital hyperplasia, sole hemorrhages, sole ulcers, white-line disease, and others have been included in studies reporting prevalence of disease causing lameness (50, 53-54), but when considering such information, one must consider the different production systems, housing systems, and other factors that also contribute to prevalence.

The amount of information on lameness in beef cattle is much more limited than information from the dairy industry. However, in a survey of 147 feedlot industry members, Terrell *et al.* (2014) reported that the median estimated lameness was 2.0% (55). The majority of the participants also estimated that 0-9% of feedlot mortality was associated with lameness (55). When asked to identify the diagnosis that most commonly caused lameness in feedlot cattle, 42.2% of participants selected footrot, 35.4% selected injury, 4.1% selected sole bruises/ulcers, 0.7% selected hairy heel wart, and 0.7% selected other causes (55). The most common contributing factors to lameness of infectious origin were reported as pen conditions, pen surface, weather patterns, and handling of cattle before arrival (55). The four most common contributing factors for non-infectious lameness were reported as cattle handling after arrival, cattle temperament, cattle handling before arrival, and pen conditions (55). Such risk factors are of great importance for the monitoring and measuring of animal welfare, as they can potentially contribute not only to lameness, but to other indicators of animal welfare, such as cattle comfort and animal handling.

Other risk factors of lameness have been discussed extensively by Chesterson *et al.* (2011), who found 24 potential risk factors that were statistically significantly associated with

lameness prevalence (56). The authors found that the two risk factors most highly associated with lameness prevalence were the average maintenance state of the main track on the farm, and the patience of the farmer handling the cows on that track (56). Again, the information provided about such risk factors is valuable, as the factors have implications in other areas of animal welfare, such as animal handling, which will be discussed further.

The incidence of ruminal acidosis, both acute and subacute, can also be an important indicator of the welfare of both beef and dairy cattle. In cattle, acidosis occurs when a high-energy diet (usually very palatable) is introduced to animals that do not have a rumen that is fully adapted to such digestible substrate (57). Nagaraja and Lechtenberg (2007) stated that morbidity and mortality associated with digestive diseases in feedlot cattle is second only to that of respiratory disease (58). In 2014, Terrell *et al.* reported that participants in a survey of feedlot industry members indicated that approximately 35% of respondents agreed that digestive disorders contributed to 10-19% of mortality in feedlot cattle (55). In dairy cattle, a high concentrate diet tends to increase milk production, but possibly at the expense of cow health in the long term (59). Incidence of acute ruminal acidosis was reported as 0.3% in dairy cows during lactation (60). It is important for producers, nutritionists, and veterinarians to understand that morbidity and mortality due to digestive disorders can be monitored and used to determine welfare status and to implement prevention plans, such as changes in step-up diets and utilization of emergency diets in periods of inclement weather. Changes in intake can be a way to indirectly measure the potential for acidosis and other digestive disorders that can be detrimental to animal welfare.

Acidosis in cattle has further implications in the area of animal health and welfare as well. Liver abscesses can be caused by bacteria invading the liver through a number of

pathways, however the most common route that bacteria take to the liver is through the acidosis-rumenitis-liver abscess complex (61-62). Lesions in the rumen caused by acidosis allow bacteria to access the liver via the portal vein. Such bacteria then establish themselves in the liver and produce hepatic abscesses (62). Prevalence of rumen lesions has been reported as 24.1% (36). The reported prevalence of liver abscesses in feedlot cattle averages between 12% and 32% (36, 63). Jensen *et al* (1954) showed a high statistical correlation between the occurrence of liver abscesses and rumen pathology (61). Animals that show clinical signs often display non-specific signs, such as anorexia, decreased production parameters (decreased intake, or decreased milk yield), bloat, and diarrhea, all of which are abnormal, and contribute to morbidity (64). However, the majority of animals affected by rumen lesions and liver abscesses do not display severe clinical signs (65). Regardless of the presence of clinical signs, the occurrence of such lesions still pose a risk to animal welfare. The lack of clinical signs, along with the reported presence of underlying disease indicates the need for measurement of post-harvest parameters, such as those found in the Harvest Audit ProgramTM, which can aid in the implementation of prevention plans in the future (36).

As with acidosis, digestive and metabolic disorders can pose as animal welfare issues in the transition cow in dairy operations. A majority of the health problems in dairy cows occur during the periparturient period, due to the body's inadequate adaptation to the metabolic demands of lactation (66-67). Dairy cows which are in transition from dry to lactating periods experience changes in metabolic, endocrine, and immune status, which can make them more susceptible to diseases such as ketosis, hypocalcemia, and displacement of the abomasum. In addition, retained placenta and/or metritis can follow parturition. Incidence of hypocalcemia can range from 0.03% to 44%, while incidence of ketosis and displaced abomasum range from 0% to

20% and 0% to 14%, respectively, however, there is a wide variation in incidences reported, likely due to feeding regimens, housing, milk yield, and management decisions (66, 68). The same can be said for the variation in incidence of retained placenta and metritis: 0% to 22.6% and 0% to 66%, respectively (66). Regardless of the incidence of these diseases on the operation, it is essential that incidence and prevalence of these diseases be recorded and monitored. The causes of these diseases are multifactorial, including milk yield, feeding regimen, and genetics. Mulligan, et al. (2006) proposed an integrated approach to the monitoring and prevention of such diseases based on farm management and environmental factors, clinical data, milk production records, dietary analysis, and assessment of blood and liver concentrations of various metabolites and trace elements (67). Many of these parameters are already measured by producers, and such an approach gives both producers and veterinarians another tool by which both production and welfare can be assessed. Continual monitoring of the parameters listed here, along with morbidity and mortality due to transition diseases is critical for future management decisions and improvement of both production and animal welfare on dairy operations.

The same can be said for the incidence and prevalence of mastitis in dairy operations. Mastitis is the most prevalent disease in dairy herds, and can have significant economic and welfare effects on production units and animals within them (69). As with transition diseases, the causes of mastitis are multifactorial, including but not limited to housing, milking machines or technique, or other environmental influences. Mastitis can occur in dairy cattle of any age, with prevalence ranging from 0% to 97% in dairy heifers to 1.7% to 54.6% in cows (66, 70). Both clinical and subclinical mastitis can be monitored with evaluation of animals by producers and veterinarians and with somatic cell counts from frequent milk samples. Use of diagnostic

tools, as well as monitoring of morbidity and mortality rates due to mastitis are critical in the evaluation of animal health and welfare on dairy operations.

As discussed here, mortality rates and morbidity rates may be direct or indirect indicators of the animal welfare status. They are useful in assessing poor welfare associated with disease and lack of care (35). Accurate records are not always available, so producers should work with extension, veterinarians, and others to continually improve record maintenance and be knowledgeable about what forms of data are valuable in the assessment of animal welfare. Depending on the production system, estimates of mortality rates can be obtained by analyzing the causes of death and the rate and pattern of mortality. Post-mortem examination is useful to establish causes of death, and to aid in the quantification of subclinical disease. Finally, both clinical and post-mortem pathology could be utilized as an indicator of disease, injury, or other problems that may compromise animal welfare.

Changes in Weight and Body Condition

In growing and adult animals, weight and body condition may be an indicator of animal health and animal welfare. Poor body condition score and significant weight loss may be an indicator of compromised welfare (7). Body condition scoring has been widely accepted as the most practical method for assessing changes in energy reserves (71). Management of body condition on cattle production units has implications not only for animal welfare, but for yield (meat or milk), reproductive performance, and herd health (72-73). In both beef and dairy cattle, body condition can influence subsequent performance, whether it be from a reproductive or a feeding standpoint (71, 73-74). For example, Selk *et al.* (1988) showed that body condition

scores precalving and at the start of the subsequent breeding season is a major factor that influences pregnancy rate of beef cows (75).

As stated in the definition of animal welfare, an animal must be well-nourished, among other things, to experience good animal welfare (3). If one considers other definitions of animal welfare, such as the Five Freedoms, the same trend will be found (76). The first of the Five Freedoms states that “Freedom from hunger and thirst” is a guideline that should be used to determine if good animal welfare exists in the production system (76).

While the body condition score of animal is used as an indicator of animal welfare, it is not the *cause* of welfare problems. Body condition scores, particularly low scores, can be indicative of a number of conditions that compromise animal welfare. Agenas *et al.* (2006) stated that adequate nutrition is a fundamental requirement for the welfare of all livestock (77). Inadequate nutrition is likely the most severe animal welfare issue that can be measured with body condition scoring. The most obvious cause of inadequate nutrition is undernutrition, which can result from a lack of foodstuffs in general.

Malnutrition is an improper balance of nutrients in the diet (77). Malnutrition can result from provision of inadequate diets, or from improper ration formulation. Nutrient requirements of cattle depend upon the physiological status, maturity, and plane of growth, and indicate a specific set of nutrients be provided to the cattle. If such nutrients are not provided in the proper amounts, welfare can be compromised.

Other causes of inadequate nutrition and subsequent welfare issues include disease states, and malabsorption. This can be due to the specific pathology of the disease, such as decreased absorption of nutrients in the case of Johne’s disease. Metabolic disease is also of concern in cattle when body condition is considered. The increased incidence of metabolic diseases in high

producing dairy cows indicates a need for concern with each stage of the lactation cycle and effects on subsequent lactation cycles (71). Diseases such as acidosis and Salmonella infections can cause malabsorption due to high rates of passage. High parasite load can cause decreased absorption of nutrients in the GI tract, which can cause a decrease in the animal's body condition score (78). Disease can also cause an animal to change its behavior, particularly feeding behavior. If an animal experiences lack of motivation to acquire foodstuffs due to decreased mobility caused by foot lesions, or decreased appetite caused by metabolic disease, intake can be compromised, and will be reflected in a subsequent decrease in body condition score. As Broom (1986) indicates, this ability (or lack thereof) of the animal to cope with the disease or the state in which it is living is a welfare concern, and should be addressed (79).

Because changes in body weight are influenced by changes in internal protein and water, gastrointestinal content, changing organ weights, and frame size, body condition scoring is used most frequently (80-84) when determining nutrient status of cattle. By using a standard body condition scoring system, the amount of fat and muscle can be evaluated, and can contribute to the assessment of the health and welfare of live animals. A body condition scoring system using a 1 to 5 scale was developed for dairy cows. This scoring system can be used at any time during the lactation cycle. In beef cattle, a scoring system from 1 to 9 is typically used (75), with one score representing approximately 75-100 pounds of live weight (73, 75).

Reproductive Efficiency

Reproductive efficiency is the most economically important aspect in beef production (85). In dairy production, reproductive efficiency is also key, as the calving interval has a large effect on milk yield for each cow. Reproductive efficiency can be an indicator of animal health

and animal welfare status, as poor reproductive performance can indicate underlying disease or management problems. Poor reproductive efficiency, including anestrus (the absence of an estrus cycle) or increased time between estrus cycles, can be caused by a number of factors, including disease, decreased nutritional status, or problems with management programs.

Infectious disease can cause a number of reproductive efficiency problems, including decreased ovulation rates, fertilization rates, embryonic survival rates, perinatal survival rates (86). Non-infectious causes such as metabolic disorders, toxins, and environmental stress may contribute as well. In 1990, Oltenacu *et al.* reported that retained placenta, metritis, and cystic ovaries were the diseases which caused the largest effects on reproductive performance in dairy cows (87). In beef cows, a number of infectious diseases are discussed by Givens, including *Leptospira sp.*, *Trichomonas foetus*, and *Neospora caninum* (86). As discussed previously, morbidity rates can contribute to the compromise of animal welfare, and should be monitored to see that disease rates, including those causing reproductive inefficiency, are kept at levels acceptable for good animal welfare. Good management, which includes proper vaccination protocols, is key to maintain the reproductive efficiency of herds when disease states are considered.

Nutrition status and its effect on fertility has also been investigated. Relationships between body condition scores and reproductive measures are discussed in a number of publications (72, 74, 88-89). A strong relationship between the two was found by Pryce *et al.* in 2001 (72). The impact of nutrient status on formation of spermatozoa and oocytes, ovulation, fertilization, and embryo and fetal development was discussed extensively by Robinson *et al.* in 2006 (89). As an example, after calving and during early lactation, dairy cows are usually in a negative energy balance, as the amount of energy needed to produce milk is greater than the

energy in a ration can provide. At this point, the body mobilizes tissue to make up for the energy needed (72). Komaragiri *et al.* (1998) showed that under conditions in which an adequate diet is provided, the amount of dietary fat and protein did not have a significant effect on the amount of tissue mobilization in post-partum dairy cows (90). They suggested that under such conditions, hormonal status of the cow may exert more pressure on tissue mobilization than diet (90). The key to such a conclusion as it relates to animal welfare is that an adequate diet was provided. If such conditions of tissue mobilization exist without adequate nutrition, decreases in body condition would likely be seen, and animal welfare could then be compromised. Such nutritional inadequacies should always be avoided, but especially when cattle are in a periparturient period.

The occurrence of dystocia in cattle herds is a situation in which animal welfare can also potentially be compromised. Cows and heifers that experience dystocia experience a great deal of pain, and are at greater risk for mortality due to various complications (91). In addition, calves which survive dystocia experience lower passive immunity transfer, higher mortality, and higher indicators of physiological stress (92). This is particularly important in beef production systems where cattle may not be observed as frequently as other systems, such as dairies (91). However, dystocia can also have negative effects on dairy production units, as calf survival to adulthood and subsequent milk production can be decreased with increased calving difficulty (92, 93). The incidence of dystocia can vary widely between farms (94). A survey conducted to obtain information about the incidence of assistance in beef heifers and to identify factors contributing to farmers' decisions about breeding heifers reported that half of the farmers surveyed viewed dystocia as "not a problem" (94). However, because calves that are born through dystocia are more likely to die than those born naturally (95), and cows and heifers that

experience dystocia can experience many complications, including death (91), the occurrence of dystocia should not be taken lightly, and incidence should be noted in welfare assessments.

Genetic selection for fertility has been looked at extensively, particularly in dairy operations (96-99). Increased genetic capability for milk production, along with changes in nutritional management and larger herd size have been associated with a decline in fertility of lactating cows (100). Some have suggested that genetic selection for increased milk yield is viewed as increasing profit at the expense of reducing animal welfare through decreased reproductive efficiency, increased metabolic disorders, and decreased longevity in dairy cows (96). In 2005, Oltenacu and Algers commented on such compromise, again using the definition of animal welfare provided by Broom in 1986, “The welfare of an individual is its state as regards its attempts to cope with its environment” (79, 96). Such comments could facilitate the argument that the focus of genetic selection for production traits cause a compromise of animal welfare because the animals, as a herd, are unable to cope with the heavy selection for milk yields, by not being able to adapt to such selection bias. On the other hand, Simm *et al.* (1996) stated that a better scientific understanding of adaptation to extensive systems is needed, and particularly of the genetic variation in components of that adaptation (101). It may be that selection for milk yield is important, but as a part of a whole of selection parameters, including the ability to adapt to intensive management practices (which may include selection based on morbidity and mortality parameters, udder conformation parameters, etc.). Longevity in the herd must be considered in both genetic selection and in animal welfare considerations. As in most areas of animal welfare, more research, such as collection of out-come based measures, is always the key to understanding such complex issues involving biological processes and subsequent effects on animal welfare.

Physical Appearance

Physical appearance may be an indicator of animal health and welfare, as well as the conditions of management. Grandin (2001) stated that many problems that occur during the transport of livestock are caused by animals that are not fit for transport (102). While this statement is undeniably true, unfit animals can experience welfare problems throughout the production system, and not just in periods of transport. Animals can be deemed unfit after a number of diagnostic tests, however, physical appearance can be just as instrumental in determining the fitness of cattle.

As stated previously, adequate nutrition is a fundamental requirement for the welfare of all livestock (77). Emaciation can develop if inadequate nutrition is provided. Physical appearance can be indicative of nutrient status, and is evident when body condition score is determined (71). Loss of flesh over the tail head, the hips, and the ribs can indicate low body condition and states of emaciation in cattle. Such signs are evident upon physical examination, even to an untrained eye. Evidence of undernutrition in farm animals is currently a matter of subjective assessment, however, as discussed further in this review, body condition can be recorded and used as a measure of animal welfare. People manage the things that they measure (102), therefore even subjective measurement with a widely-accepted scoring system is useful for the management of welfare issues such as body condition and states of emaciation.

An animal's physical appearance can be used to evaluate hydration status as well. Water is the most important nutrient to cattle in any situation. It is vital to nutrition (103), and required for all of life's processes (104). While early dehydration can be observed in plasma and serum total protein measurements (105) and serum osmolality (106), animals must be in a state of

dehydration for a longer period to display clinical signs of dehydration. Severely dehydrated animals commonly show clinical signs, such as depression, sunken eyes, and skin-tenting (107). A reliable quantitative measure for hydration status for young calves was developed by Constable *et al.* (1998), which provides a guideline for estimating the percent dehydration in cattle (108). Some of the principles of assessing dehydration in mature cattle are similar to those used in younger animals, but some exceptions must be considered. Body weight and rumen fill should be considered in the assessment, and care should be taken to determine that clinical signs are actually due to dehydration, rather than emaciation (107). Observation of such signs are indicative of such dehydration as to cause a significant welfare issue, and management practices should be implemented to assure adequate rehydration and maintenance of hydration status.

Another indicator of animal welfare can be the presence of excessive mud or feces on and in the coat. Under winter conditions, if an animal's coat is wet and muddy, energy requirements for maintenance for that animal can easily double (109). Production parameters in beef cattle are also affected by mud, as average daily gains were shown to decrease substantially in the presence of mud over 6 inches in depth (110). Sant'Anna and de Costa (2011) recommended the establishment of management procedures to control hygiene in cows in order to reduce production losses due to high somatic cell counts and to improve the welfare of dairy cows (111). Honeyman *et al.* (2008) used a mud scoring system to evaluate live cattle in bedded hoop barns (112). The scoring system ranked cattle from 1 to 5, with 1 = no visible mud and 5 = heavy mud on the animal. Jordan *et al.* (1999) also suggested the use of mud scoring systems to improve the cleanliness of animals presented for slaughter (113). This could not only improve the welfare of the animals, but also increase performance parameters, and help control contamination of udders to prevent mastitis. Other abnormalities can be observed in the coat of

cattle as well. Abnormal coat color and texture can indicate change in nutrient status, such as copper deficiency (114). Such deficiencies can indicate the presence of undernutrition or malnutrition, and the need for management practices to solve the problems.

The presence of ectoparasites can be observed upon physical examination. As stated previously, such infestations can contribute to increased morbidity and mortality rates and changes in body condition, which can both indicate a welfare issue. Production parameters such as weight loss/gain, meat value, and milk production can be used to determine the losses caused by parasite load (115). Steelman (1976) emphasizes the need for the establishment of adequate control measures using quantification of losses caused by ectoparasites, the rate of response of animals to control measures, and the interactions of these factors over time (115). Such measures can also be used in consideration of animal welfare, as production losses due to the presence of parasites can indicate an animal's inability to cope with such a heavy parasite load, and therefore compromised welfare (3, 79).

Physical appearance is likely the easiest parameter to observe when assessing animal welfare, but many times may be overlooked because of the simplicity of the subject. It is important that a thorough assessment be made in initial observations of animals before further welfare assessment is continued.

Handling Responses

Improper handling can result in fear and distress in cattle (116). The proper handling of cattle requires the knowledge of cattle behavior and the presence of adequate handling facilities (117). In a dairy, proper handling of cattle should be assessed when cows are on the way to the milking parlor, and on the way back. In a feedlot, handling procedures can be

evaluated during processing, when cattle are moved to a home pen, and when cattle are moved from the home pen for transport to a slaughter facility. In all cattle production systems, the handling of sick cattle should be observed during movement to the hospital, through treatment, and when returning to hospital or home pens.

Handlers of cattle have a large effect on the responses exhibited, but both previous experiences *and* genetic factors have a part in the behavior of cattle during handling (118). Grandin (1993) reported that in certain animals, there was a tendency to become behaviorally agitated, and the behavior was consistent over time (118). Such behavior is dangerous to handlers and other cattle, and handlers should be cautious when moving highly temperamental cattle. Cattle that are flighty and temperamental should be removed from a production unit (118).

With exception to temperamental animals (those which are flighty and over-responsive to external stimuli), cattle respond to the manner in which they are handled (117). Hemsworth *et al.* (2002) demonstrated that a handler's beliefs about how animals should be treated was associated with the use of either positive or negative behavior when handling cattle (119). It was stated that when handlers thought more positively about the animals and the effort required to handle them, those handlers used more positive and less negative interactions with cattle (119). It is important to remember that attitude of handlers must be addressed when assessing animal welfare, as attitudes are often indicative of behaviors.

The human-animal interaction has also been demonstrated not only in the responses of cattle to actual handling procedures, but in their responses to individual humans (120), and in production responses (121). Munksgaard *et al.* (1997) demonstrated that animals learned, very readily, in fact, to discriminate between people who treated them gently during handling, and

people who handled them aversively (120). In the tests performed, animals maintained a greater distance between themselves and handlers who had handled them poorly than they did with gentle handlers. The study also demonstrated that the learned aversion may be demonstrated not only in the area where the cattle are handled initially, but in other areas of the farm, indicating the possibility of the cattle being able to generalize their experience with good or bad handlers to other locations on the farm (120).

Poor handling may also have a negative effect on production in cattle. While further research is warranted, evidence suggests that poor handling techniques and the behavioral responses by cattle to such techniques may have negative effects on milk let-down and milk yield in dairy cattle (119, 122-123). In beef cattle, it has also been shown that the fear-related behavioral responses of beef cattle to human handling impacts productive, reproductive, and health characteristics (124), along with meat quality characteristics (125).

Behavioral responses of cattle to human interaction are important to observe, however, responses to other stimuli encountered during handling are equally important, and objective measures of responses to both human interaction and other stimuli can be used in the assessment of animal welfare. As noted previously, there are many situations in which cattle are handled on various production units, but some of the major areas of cattle handling include processing through an alley and chute, loading and unloading, transport, driving cattle, and movement of sick animals.

When processing cattle, objective measures including number of injuries, vocalization, slipping and falling in the alley, jumping and running out of the chute, electric prod use, chute miscatches, and subjective measures such as crush scores can be used to determine whether handling practices are consistent with good welfare of the animals. The percentage of animals

injured during handling (i.e. broken horns, broken legs, lacerations, etc.) can be an indicator of welfare issues that should be addressed immediately. Vocalization can be used as a simple method for detecting welfare problems (126). Research shows that different methods of restraint can influence vocalization in cattle (127). Vocalization is a reliable measure of animal welfare, as it is very objective, easy to tabulate, and no sophisticated equipment is required (126). The Beef Quality Assurance Program has set the industry standard of vocalization while processing cattle at <5% (128). Percentages of animals slipping or falling in alleys, chutes, and chute exit areas can indicate welfare problems such as improper handling or improper facility design. Industry standard for cattle slipping and falling during processing has been set at <2% (128). Also, the percentage of animals moved with an electric prod can be assessed, and a “minimum use goal” should be set (the industry standard has been set at 10% (116)). Chute miscatches are defined as the animal being in any position other than with its head fully outside of the chute and the balance of the body within the chute, or if an animal is caught in the tail/back gate and not released (128). There should be a 0% miscatch rate during processing (118). Chute exit speed and chute behavior can be measured objectively. Flight speeds can be obtained with infrared sensors (129). Strain gauges and load cells, as well as movement-measuring devices have been used in assessing behavior of cattle in the chute (130-132). Many production units do not have such technologies, so other, more subjective methods have been used to evaluate cattle behavior in and around the chute. Turner *et al.* (2011) used a race score to determine the speed of movement of the animal along the alley and the extent of encouragement needed from handlers (125). A crush score was also used in their assessment to measure the behavior of cattle in the chute. While subjective measures are not as ideal as objective, if scorers are consistent, they can help in the assessment of animal handling with regards to welfare.

When loading and unloading, the handling of cattle is very important. Responses to aggressive versus low stress handling can be used in the measurement of animal welfare during handling. Recent research shows that blood indicators of stress, such as lactate and creatine kinase, are greatly elevated in finished beef cattle that have been run in an alley to load out, rather than walked (133). Vocalization and electric prod use are measures of welfare used during loading and unloading as well. Again, industry standards have been set for electric prod use in processing barns, but a standard has not been set for loading, unloading, and transport of animals. Likely, the standard would be similar to the 10% in processing barns, as cattle being loaded and unloaded have similar facilities to walk through, but may balk at the prospect of entering the trailer. When cattle are unloaded, care should be taken to observe groups for cattle that are fatigued, those who show obvious lameness or short-stepping, or cattle that move more slowly than the group, and tend to be left behind. Finally, the percent of injured animals is a very important welfare measure. Broken legs, lacerations, and other serious injuries should not be tolerated, and measures should be taken to solve and prevent such problems.

Measures of welfare for the transport of animals could include percent of injured animals, electric prod use, bruising observed after slaughter, and the number of fatigued cattle that come off the truck, both in the production unit and at slaughter facilities. Injured animals that come off the truck should be managed with care, as they can present a risk for injury to employees, other cattle, and further injury to themselves. If an injured animal comes off a truck, management should take care to inspect the truck and loading/unloading facilities to insure that if the injury was caused by facilities, the problem is solved. Bruising due to rough handling or rough transport can be observed in animals after slaughter (102). Finally, cattle that come off of trucks, especially heavy, finished cattle and cows that have been removed from the herd for udder

problems, chronic lameness, or other disease/abnormality should be monitored for fatigue, and for any other abnormality that could cause compromised welfare. These cattle could be at a higher risk for becoming non-ambulatory, and should be monitored closely.

The same can be said for driving or moving cattle to a truck, pen, or pasture. Injury rates, electric prod use, cattle speed, and the number of fatigued cattle are all important areas to measure, and can all be measured objectively, if desired. The same industry standards can be used in driving and moving cattle as in situations discussed previously.

Finally, the handling of sick and/or injured animals can be a situation in which welfare can be demonstrated very readily as excellent or severely compromised. Sick animals can be ambulatory or non-ambulatory. They can also become non-ambulatory if proper handling techniques are not utilized. Electric prods should be used at a minimum (less than industry standards of 10%). Vocalization can also be used as a measure of welfare for handling sick/injured animals. The number of animals that go down as a result of handling should be monitored and recorded, and if reported, immediate action should be taken to prevent further injury, as such situations should not be tolerated. In animals experiencing symptoms due to respiratory disease or heat stress, welfare measures such as panting scores can be used. Cattle in situations discussed above should always be handled with care, and at the pace that the animal sets. It is essential to measure such welfare outcomes in order to improve handling of not only sick and injured animals, but all cattle.

The information provided in handling studies and the recommendations made are essential material to consider when assessing animal welfare. While industry standards for some of these measures have not been set, the research described here can create a spring-board for further research which can help to determine normal parameters for the indicators noted. Using

such information, cattle handlers are better equipped to understand that their attitudes affect not only their own behavior, but the animals' behavior as well. Understanding the way that cattle react to different stimuli will help handlers improve their skills in working with cattle, and subsequently positively influence the welfare of the animals.

Routine Procedure Management

As discussed previously, surgical and non-surgical procedures are commonly performed in cattle for improving animal performance, facilitating management, and improving human safety and animal welfare. Such procedures include castration, dehorning, ovariectomy, tail-docking, and various methods of identification, among others. However, if these procedures are not performed properly, animal welfare can be compromised.

Pain response to routine management procedures can be measured in a number of ways, including the use of performance data (dry matter intake, average daily gain) and behavioral assessment (26). Physiological responses, such as plasma cortisol concentrations, have also been used to assess pain during procedures such as castration (134). The assessment of pain is useful, especially when looking at methods to relieve pain, such as the use of local anesthetic or drugs for systemic pain relief.

Cattle owners routinely use castration to facilitate safe and convenient management of livestock. Each year, millions of cattle are castrated by physical methods which cause pain (24). Different castration methods are used by different production systems, but the most common methods of castration are surgical and banding (26). Surgical castration causes an acute pain response, and cattle typically demonstrate behavior associated with pain, such as vocalization, immediately after the procedure is done. Cattle which are banded usually do not display as

severe a reaction to acute pain, but may experience more chronic pain due to the necrotizing effects of decreased and finally no blood flow to the scrotal sac. Regardless of the method of castration used, a reduction in gain and/or intake has been demonstrated in cattle which are castrated, at least for the first 1-4 weeks after castration (26, 134-136). However, Rust *et al.* (2007) and Fisher *et al.* (2001) demonstrated that cattle which are surgically castrated gained more than cattle which were banded (26, 137).

The use of anesthesia and analgesia has been researched in castrated versus intact animals as well. The most common method of anesthesia is local anesthesia, through either an epidural or more local injection of an anesthetic in the spermatic cord, scrotum, or the testicle itself. There has been conflicting evidence that the administration of local anesthesia is useful for pain mitigation in surgically castrated animals. Rust *et al.* (2007) demonstrated no differences in production parameters between cattle castrated with and without the use of local anesthesia (26). Fisher *et al.* (1996) demonstrated a decrease in plasma cortisol levels in animals castrated after administration of local anesthesia versus cattle castrated without it (134). Some have questioned the use of local anesthesia, saying that the administration of anesthetic may actually cause added unwarranted stress on the animal (138).

The use of analgesia for the relief of pain in cattle after castration has also been studied. Ting *et al.* (2003) demonstrated that the systemic analgesia provided by the NSAID ketoprofen decreased the inflammatory and stress responses associated with two different methods of castration (139). Pang *et al.* (2006) demonstrated a decrease in plasma cortisol response in castrated calves with the use of carprofen (140). The effects of oral meloxicam have been studied as well, particularly in association with castration and animal health status in feedlot cattle (141). Studies such as these will be useful in the future to help determine drugs that should

be approved and used for pain mitigation for routine management procedures to aid in the improved welfare of cattle.

The same can be said for dehorning of cattle. There are a number of ways that horns can be removed, including the use of cautery for disbudding, application of a chemical paste to horn buds, and surgical amputation of horns. All procedures cause cattle to show behaviors indicative of pain at one point or another. Again, performance data, behavioral assessment, and physiological responses can indicate pain in these animals (25).

Similar to research done with local anesthetic administered to calves being castrated, local anesthetic showed mixed results when calves were dehorned. Some may argue that the method of dehorning may contribute to the presence of pain relief provided by local anesthetics, however studies comparing different methods of dehorning with the use of local anesthesia have still yielded conflicting results. For example, Vickers *et al.* (2005) demonstrated that the use of local anesthesia when calves were dehorned using a caustic paste did not provide a positive effect on the behavior of the calves in the first four hours after the procedure (142). Sylvester *et al.* in 2004 concluded that the use of a local anesthetic does alleviate pain during the dehorning procedure of scooping (143). However, Petrie *et al.* (1996) concluded that the use of local anesthesia for scoop disbudding provided little benefit in reducing distress caused by the procedure, while its use during cautery disbudding provided some benefits for pain mitigation (144). Similar to the castration procedure again, the use of analgesics has been studied for pain relief for the dehorning procedure. In 2000, Faulkner and Weary showed a significant difference in the behaviors associated with dehorning in cattle given oral ketoprofen versus those who were not (145). Other studies have shown that the administration of both systemic analgesia and local anesthesia may be a more sufficient way to mitigate the pain experience in cattle during and after

the dehorning process (146-147). While further research on the use of local anesthesia to alleviate the pain that occurs due to dehorning is warranted, one thing that all of these studies have concluded is that dehorning is a painful process, and the mitigation of pain should be considered, regardless of the method of dehorning used.

Tail docking has been common in the United States, Europe, Australia, and New Zealand, but has recently lost favor in many production units, and is not recommended in dairies at this time. This is because the disadvantages to the cow, including both acute and chronic pain and reduced ability to repel flies, very clearly outweigh any advantages that the procedure might provide to the producer (148).

Regardless of the procedure performed, animal welfare can be compromised if it is not performed properly or if cleanliness is neglected. Indicators of such problems could include post-procedure swelling and/or infection, hemorrhage, myiasis, or even mortality (149-151). If such problems occur, they are clearly evident, and can be assessed easily.

Measurement of outcomes for animal welfare can be utilized in nearly every routine surgical procedure performed on production units. For example, performance data can be used to measure the effects of castration on cattle. As discussed previously, intake, average daily gain, and overall weight gain/loss can help to quantify an animal's response to the pain of different castration procedures (26). Neely (2013) showed that appetite scores of animals were different among groups of cattle that experienced different dehorning procedures (150). The number of cattle with an abnormal gait, the number of cattle that lie down after procedures, and the number of cattle that display an abnormal posture or that vocalize during or after a procedure can all be quantified by either veterinarians or producers, and used to determine whether the procedure was or was not painful for the animals, or whether methods of anesthesia and

analgesia were effective or not. Again, the research described here has provided some information on the effects of castration and dehorning, and some of the ways to mitigate the painful effects, however, implementing the measures in a production unit will be the best way to determine normal rates of pain indicators in animals. If veterinarians and producers make an effort to measure and record such parameters, and continually find ways to improve welfare during routine procedures (such as administration of anesthesia and analgesia), that improved welfare will show in continued measurement and record-keeping. Many industry standards for normal have not been set, however, use of these measures in production units, and the recording and reporting of them, will help to develop such standards for use in the improvement of animal welfare, first in individual production units, and then in the cattle industry as a whole.

Conclusion

Scientists are taught that science is “value free”. However, the science of animal welfare, among others, is an exception to this rule. Values are clearly involved in this science, and we must take great care to remember that when studying the concept scientifically. Society’s and consumers’ concern for the quality of life experienced by production animals will continue to influence the study of animal welfare. It is our job as scientists to carry out such studies using the scientific method. Welfare decisions should be based on a sound scientific understanding of animals and how they are affected by production management practices.

Outcome-based measures such as those discussed here are an integral part of the scientific study and implementation of good animal welfare practices in cattle operations. Outcome-based measures give the industry a scientific basis for assessment of animal welfare, and provide straightforward methods by which producers and veterinarians are able to measure

and record animal welfare. They demonstrate producers' dedication to the animals they care for, while simultaneously providing a scientifically-based measurement system for animal welfare in the cattle industry.

Table 1-1. A list of outcome-based measures for specific incidences in management of cattle welfare.

Outcome-based Measure	Procedure/incidence
Cattle behavior	Castration, dehorning, spaying, feeding, heat stress, weaning, transportation, cattle handling, down animal management, euthanasia, biosecurity, parasite control, nutrition, cattle comfort, stocking density, protection from predators, employee training
Morbidity rate	Dystocia, calving management, orphan calf care, colostrum management, castration, dehorning, spaying, branding, weaning, transportation, cattle handling, down animal management, biosecurity, parasite control, nutrition, cattle comfort, stocking density, protection from predators, employee training
Mortality rate	Dystocia, calving, management, orphan calf care, colostrum management, castration, dehorning, spaying, branding, weaning, transportation, cattle handling, down animal management, biosecurity, parasite control, nutrition, cattle comfort, stocking density, protection from predators, employee training
Changes in weight, body condition	Calving management, orphan calf care, colostrum management, castration, dehorning, spaying, branding, weaning, cattle handling, down animal management, biosecurity, parasite control, nutrition, cattle comfort, stocking density, employee training
Reproductive efficiency	Dystocia, weaning, cattle handling, biosecurity, parasite control, nutrition, cattle comfort, employee training
Physical appearance	Calving management, orphan calf care, castration, dehorning, branding, down animal management, euthanasia, parasite control, nutrition, cattle comfort, stocking density, protection from predators, employee training
Handling responses	Transportation, cattle handling, down animal management, euthanasia, employee training
Routine procedure management	Castration, dehorning, spaying, branding, employee training

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Chapter 2 - Assessment of risk factors contributing to carcass bruising in fed cattle at commercial slaughter facilities

Abstract

Cattle injuries can occur during transportation due to vehicle design, transport conditions, and loading or unloading procedures and lead to carcass bruising and economic loss due to decreased carcass value. The objectives of this study were to determine whether a relationship exists between trauma incurred during unloading and prevalence of carcass bruising in finished beef cattle at commercial slaughter facilities and determine related risk factors which contribute to both trauma and carcass bruising. Breed (classified as either Holstein cattle or beef breeds), sex, distance traveled, and trailer type (“fat/feeder combination” vs. “fat” trailer) were considered risk factors which may contribute to traumatic event prevalence. When carcass bruise prevalence within each lot was used as the dependent variable, breed, sex, distance traveled, traumatic event prevalence, ribeye area, fat thickness, yield grade, and average carcass weight were considered potential risk factors. Carcass bruises were categorized by location and size, according to the Harvest Audit Program™ Carcass Bruise Scoring System. Traumatic events were observed as cattle exited trailers onto the unloading docks, and were categorized by location. Average traumatic event prevalence per lot was 20.4% (\pm 1.11%). Average carcass bruise prevalence by lot was 68.2% (\pm 1.15%). There was a significant interaction between breed and trailer type when multiple linear regression was used to explore variables contributing to traumatic events observed at unloading ($P \leq 0.05$). Traumatic events did not contribute to carcass bruising, while average carcass weight and breed affected carcass bruising prevalence significantly. Carcass bruising was more prevalent in Holstein cattle than in cattle which were

predominantly beef breeds ($P \leq 0.01$). Average carcass weight was negatively associated with carcass bruise prevalence ($P \leq 0.05$). The association between traumatic events at unloading and carcass bruising is not significant when multiple variables are considered, indicating that bruising may occur at numerous other points prior to and during the transportation process, including loading and transport, and that other variables can contribute to carcass bruise prevalence. These areas should be explored to determine all potential causes of bruising in beef carcasses, and to help implement prevention practices.

Introduction

Bruising in fed beef cattle costs the industry millions of dollars annually (Garcia et al., 2008). Bruised tissue must be discarded because it provides an ideal environment for bacterial proliferation, which poses a significant food safety concern (Marshall, 1977). In addition, bruising is an indicator of poor animal welfare during the pre-slaughter period (Broom, 2003). Hoffmann et al. (1998) defined a bruise as “a tissue injury without laceration usually produced by a blunt object impacting an animal with sufficient force to cause rupture of the vascular supply and accumulation of blood and serum in tissues.” This definition indicates that a bruise follows after the animal experiences some sort of trauma. Many potential sources of bruising have been suggested in the literature, including vehicle design, transport conditions, and loading and unloading procedures, however none of these have been explored extensively, and the trauma associated with these areas of the transport process is not addressed in fed beef cattle in the United States (Strappini et al., 2009; Strappini et al., 2013). Grandin (1980) and Broom (2003) reported that much of the bruising observed in livestock results from rough handling during loading, transport, and unloading, but clear supportive data is lacking. It’s been reported that 43% of carcass bruising observed occurs at the slaughter facility, however handling practices have improved immensely since the publication of such research (McCausland & Millar, 1982). Therefore, the primary objective of this study was to determine whether a relationship exists between trauma incurred during unloading and prevalence of carcass bruising in finished beef cattle at commercial slaughter facilities. In addition, other risk factors which may contribute to carcass bruising in finished beef cattle are addressed, including breed, sex, distance traveled, carcass characteristics, and the trailer type used during transport.

Materials & Methods

Permission to observe live animals was approved by the Kansas State University Institutional Care and Use Committee, IACUC #3598. Permission to observe animals unloading and carcasses on the line was obtained from corporate and management personnel for each slaughter facility prior to observation days. Permission to record trailer design was also obtained from the transporters and the slaughter facilities.

No treatments were assigned for this observational study. Fed beef cattle were observed at 3 commercial slaughter facilities during July and August of 2015. Lots of finished beef cattle were selected from the slaughter facility's daily slaughter order sheet. Whole lots were observed, even if the lot arrived in multiple trailers. Individual animal identification was not recorded.

To record traumatic events at unloading, a trained observer watched the cattle coming off the trailers, and counted the cattle that hit any part of the trailer during unloading. Multiple events were recorded for the individual animal if the animal experienced more than one traumatic event. Each traumatic event was classified by its location. Locations were specified as shoulder, back, rib, or hip areas. Some cattle experienced multiple traumatic events. Prevalence of traumatic event occurrence was calculated using the number of traumatic events observed at unloading over the total number of cattle in the trailer.

The same lots of cattle were observed by a second trained observer for carcass bruising prevalence using the Harvest Audit Program™ Carcass Bruise Scoring System, developed at Kansas State University (Rezac, 2013). The scoring system allows the observer to record the presence of all bruises on a carcass, their location, and the size category in which they fall.

Location was determined by dividing the carcass into a grid of 9 sections (Figure 2-1), and recording the presence or absence of a bruise in each section. Size of the bruises was categorized as small (<5cm in diameter), medium (5-15cm in diameter), or large (>15cm in diameter). Bruise severity was not addressed, as the severity of a bruise depends on the density of the affected tissue, and the vascularity of said tissue, making such a measurement impossible in the fast-paced environment of a commercial slaughter facility in the United States (Strappini et al., 2009).

Multiple linear regression (PROC GLIMMIX in SAS v. 9.4) with backward variable selection was used to develop a statistical model exploring risk factors which may contribute to traumatic events and/or carcass bruising. The experimental unit for evaluation of traumatic events was trailer load. Breed (classified as either Holstein cattle or beef breeds), sex, distance traveled, and trailer type (“fat/feeder combination” vs. “fat” trailer) were used as independent variables, or fixed effects, when developing a model to investigate factors contributing to traumatic event prevalence. The experimental unit for evaluation of carcass bruising was lot. When carcass bruise prevalence within each lot was used as the dependent variable, breed, sex, distance traveled, traumatic event prevalence, ribeye area, fat thickness, yield grade, and average carcass weight were considered independent variables. In both models, slaughter facility and feedyard nested within slaughter facility were considered random effects.

Each analysis started with exploration of frequency distributions, raw means, and other patterns in the data. The GLIMMIX procedure in SAS 9.4 (Cary, North Carolina) was used to develop univariable linear regression models for each independent variable to explore linear relationships between the dependent and independent variables. Then, using the GLIMMIX procedure, a full multivariable linear model containing all predictor variables was used to

estimate effects on the outcome of interest (traumatic event prevalence or carcass bruising prevalence). Using backward selection, independent variables and their 2-way interactions were eliminated from the model one by one, using a *P*-value of ≥ 0.05 as exclusion criteria, starting with interactions displaying the highest *P*-value, then moving to individual variables displaying *P*-values over 0.05. Forward selection was used to confirm the results of models developed from the backward selection process.

Linear regression was used rather than logistic regression as the data were normally distributed, with seemingly equal variance among the residual errors. In addition, such data must be easily interpreted by industry personnel such as slaughter facility employees, truck drivers, and other personnel involved in the movement of animals from feedyards to slaughter facilities.

Chi-square goodness of fit tests were used to determine differences of observed versus expected values of carcass bruising by location on the carcass and bruise size. Expected values consisted of equal distribution of bruising on the left side, the right side, and the dorsal midline of the carcass; the cranial, middle, and caudal thirds of the carcass; and small, medium, and large bruises.

Results

A total of 9,860 animals in 75 lots were observed at 3 different slaughter facilities in the United States. Two hundred seventy-five trailer loads were observed. Combination trailers were more frequently observed than fat trailers. The average number of animals hauled in combination trailers was 37 head, and the average number of animals hauled in fat trailers was 33 head (Table 2-1). The average number of cattle per lot was 131. More lots comprised of beef

breeds were observed than Holstein, and there were more lots made up of steers than lots of heifers and/or mixed sex (Table 2-2). Along with traumatic event and carcass bruise prevalence, a description of carcass data, including average hot carcass weight (kg), average ribeye area (REA), average fat thickness (in), and yield grade by lot is presented in Table 2-3.

Traumatic Events

Average traumatic event prevalence in finished cattle by lot was 20.4% ($\pm 1.11\%$, Table 2-3). When the multiple linear regression model was developed for the outcome of prevalence of traumatic events, a significant interaction between breed and trailer type (Figure 2-2, $P \leq 0.05$) was observed with traumatic event prevalence being highest in Holstein cattle hauled in fat/feeder combination trailers. No other risk factors measured were found to contribute to traumatic event prevalence in cattle during unloading at the slaughter facilities (Table 2-4).

Carcass Bruising

Average carcass bruise prevalence in finished cattle by lot was 68.2% ($\pm 1.15\%$, Table 3). Prevalence of carcass bruising in beef breed cattle was 66.6%, compared to a prevalence of 76.6% in Holstein cattle (Table 3, $P \leq 0.05$). Over half the bruises on the beef carcasses observed occurred along the dorsal midline ($53.5 \pm 1.12\%$, Table 2-5, $P \leq 0.05$), which is in agreement with previous research using the HAP Bruise Scoring system and the 2011 National Beef Quality Audit (Youngers et al., 2016, McKeith et al., 2012). Carcass bruising was highest in the middle third of the carcass, followed by the cranial third, then the caudal third, which is also in agreement with Youngers et al. (2016, Table 2-6, $P \leq 0.05$). More medium-sized bruises were observed on the carcasses than small or large bruises (Table 2-7, $P \leq 0.05$).

When carcass bruising was considered the dependent variable, no statistically significant interactions were observed. However, breed and average carcass weight were predictive of bruising of cattle carcasses (Table 2-8). Holstein cattle displayed significantly higher carcass bruising than did beef breeds (Table 2-7, $P \leq 0.05$). In both beef cattle and Holsteins, as average carcass weight increased, the prevalence of carcass bruising decreased linearly (Figure 2-3, $P \leq 0.05$). *P*-values for all univariable and multivariable analyses for the outcome of carcass bruising are listed in Table 2-10.

Discussion

Traumatic Events

A significant interaction was observed between breed and trailer type when traumatic events were used as the dependent variable. In the United States, trailer types are usually observed as “fat/feeder combination (combo)” trailers, and “fat” trailers. In other countries, such as Colombia, studies have been conducted exploring the effect of transport vehicle on carcass bruising (Romero et al., 2013). However, the trucks and trailers used in other countries differ greatly from those used in the United States. In most cases, they are smaller, holding only 14-16 animals, with open sides and canvas roofing—vastly different from the large aluminum trailers used to haul 30-40 animals at a time in the United States. In the current study, trailer type was defined by the truck drivers hauling the cattle enrolled. Fat/feeder combo trailers are those which are used to haul both feeder calves and finished beef cattle. Fat trailers are usually used to haul finished cattle only. The differences between these types of trailers include the presence or absence of a “jail” or “doghouse” in the upper rear compartment of the trailer, used to contain

very small calves (present in fat/feeder combo trailers, Beef Quality Assurance, 2006), the presence of a small compartment in the nose of the trailer, used as a counter-balance (also present in fat/feeder combo trailers), and the clearance height of the entrance into the “belly”, or lower compartment of the trailer (approximately 2-3 inches shorter in fat/feeder combo trailers). Either type of trailer can have a slide-in or fold-up ramp leading into the upper deck compartment—ramp type was not part of the data collected in this study.

Holsteins experienced more traumatic events compared to beef breeds when hauled in fat/feeder combination trailers than when hauled in trailers for fat cattle only. Dairy breeds, particularly Holsteins, often display larger frame sizes than their beef breed counterparts (Long et al, 1979; Tatum et al., 1986). Therefore, this difference could be due to the decreased space allowance and clearance in the different trailer types and larger frame size of Holstein cattle. Data on frame size would help to make more solid conclusions about the effect height of cattle on traumatic events experienced. Hip height would be a measure which could influence the trauma experienced in different types of trailers, as taller cattle may be more likely to experience trauma and subsequent bruising. In addition, heavier cattle may move slower than lighter ones, decreasing the pressure at which traumatic events would occur.

Grandin (1997) indicated that more temperamental or excitable cattle will move faster and are more prone to injury, which could have an effect on traumatic event prevalence. Cattle temperament and speed at both loading and unloading are measures that could help to determine contributing factors to trauma incurred, and should be measured in subsequent studies, especially those exploring the trauma experienced by cattle during the transport process. A method to measure flight speed was proposed by Veters et al. (2013) to determine speed of cattle at processing, and could potentially be used to determine if speed at loading or unloading has an

effect on traumatic events or carcass bruising in fed cattle. Temperament scores, handling techniques, and speed at which cattle are moved were not assessed in the current study, but could contribute to the bruising observed in this and other studies.

Carcass Bruising

It is generally accepted that animals which experience traumatic events will subsequently display bruising, however the contribution of each traumatic event to the actual bruising displayed is not well documented (Stedman, 2006; Strappini et al., 2013). The correlation between traumatic events and bruising was not found to be related in this study. This could possibly be explained due to the fact that traumatic events were only observed at unloading at the slaughter facility. No observations were made at other points where trauma could occur, such as at loading or during the transport process itself. Jarvis et al. (1995) explored the relationship between the same variables, but found no significant correlation between potentially traumatic events at unloading and the number of bruises per animal. Traumatic events and bruising relationships due to trailer type could not be directly observed in this study, as cattle in the same lot usually arrived in multiple truckloads. After unloading, these loads were combined back into their original lots and penned together in the slaughter facility holding pens, making it impossible to measure the effect of trailer type on actual carcass bruising in the animals observed.

There was no observed effect of distance traveled on the prevalence of carcass bruising or traumatic events observed in finished cattle. Jarvis et al. (1995) also found that there was no effect of distance travelled on the bruising scores observed in finished cattle at slaughter. Hoffman et al. (1998) observed that cattle hauled longer distances to slaughter had more bruising

on their carcasses than cattle hauled shorter distances. However, that study included mature beef cows, which usually display different physical characteristics than fed cattle, such as less fat cover, and more pronounced bony prominences. The environment in which these studies were conducted must be considered, as the current study focused on fed cattle coming into slaughter facilities which are built relatively close to cattle sources. Jarvis et al. (1995) included cattle which traveled up to and over 80 miles, but Hoffman et al. (1998) included cattle which had traveled over 580 miles. In the current study, no cattle observed had traveled over 300 miles, and it could be that cattle traveling well over the distances observed here could display higher carcass bruising. In addition, the sources of the cows were different than the sources of the fed cattle observed here, in that the cows used by Hoffman et al. came from ranches and livestock auctions, where the cattle observed here came directly from the feedlot. Movement through livestock auctions could have contributed to carcass bruising in the cows.

In this study, there was no statistical difference between bruising observed in animals of different sexes. Previous research has found sex to be a significant contributor to the carcass bruising observed at slaughter (Romero et al., 2013; Leach, 1982). Research from Romero et al. (2013) indicated that carcass bruising was significantly different between males and females, with males displaying more carcass bruising than females. Another study found that male cattle are more likely to display higher serum creatine kinase (CK) levels, which the authors link to stress and bruising (Mpakama et al., 2014). This difference in CK levels has been documented in humans as well, and is attributed to larger body mass in males (Brancaccio et al., 2007). However, Leach (1982) reported that the occurrence of bruised tissue from cull cows was significantly higher than that of steers. Again, animal type and origin must be considered when comparing results of such studies, as many bruising studies involve a mixture of fed steers and

heifers, cull cows, and cull bulls. Such variation in animal type and source was not observed here, as all cattle were sourced from feedyards with the sole intent of being slaughtered as fed beef.

Results show that average carcass weight was significantly correlated with carcass bruise prevalence. Intuitively, one may think that bruising would increase as carcass weight increased, as there may be increased risk of trauma, however the opposite effect was observed. As average carcass weight of the lots increased, carcass bruise prevalence decreased. Some researchers hypothesized that a decrease in fat cover will lead to increased bruising, as the fatty tissue offers some protection from the effects of outside trauma however did not explore the idea extensively (Knowles et al., 1982). Strappini et al. (2010) did explore this relationship, and confirmed that as fat cover increased, carcass bruising decreased. Due to the decreased vascularity of fat, it could be that animals experienced similar events which may cause bruising, but the fatty tissue did not hemorrhage as much as the highly vascular muscle tissue in lighter-weight animals.

It may be that heavier cattle may move slower than lighter ones, decreasing the pressure at which potentially traumatic events would occur, which may in turn decrease the potential for carcass bruising. As stated previously, speed of cattle exiting the trucks was not measured in this study. Grandin (1997) indicated that more temperamental or excitable cattle will move faster and are more prone to injury, however bruising was not assessed in that review. Fordyce et al. (1985) reported that temperament had no effect on carcass bruising, but the cattle used in the study were reported to be “relatively quiet.” A method to measure flight speed was proposed by Veters et al. (2013) to determine speed of cattle at processing, and could potentially be used to determine if speed at loading or unloading has an effect on traumatic events or carcass bruising in fed cattle. To better understand how differences in temperament can affect carcass bruising,

temperament scores, handling techniques, and speed at which cattle are moved were not recorded in the current study, but could contribute to carcass bruising, and should be assessed when considering trauma and carcass bruising outcomes.

Holsteins displayed more carcass bruising than beef breeds. Dairy breeds, particularly Holsteins, often display larger frame sizes than their beef breed counterparts (Tatum et al., 1986). Research shows that in feeder cattle, frame size has a significant effect on carcass weight, where larger frame size leads to higher carcass weight (Dolezal et al., 1993). An interaction between breed and average carcass weight would better support such a hypothesis. Since frame size or hip height were not measured in this study, it is impossible to conclude the effect of frame size on carcass bruise prevalence. Mpkama et al. (2014) reported on the association of breed with creatine kinase levels, but did not report on the relationship between breed and carcass bruising, and did not assess the breeds represented in the current study. In addition, while mature body size is genetically determined, research shows that it can be altered by nutritional or hormonal factors, including malnutrition and hormonal growth implant status (Owens et al., 1993). In this study, the number of Holstein animals observed compared to the number of beef animals could contribute to the lack of a statistically significant interaction between breed and average carcass weight. More data should be collected to determine how frame size, as measured by hip height or a frame score, affects bruising in both beef and Holstein cattle.

Conclusion

While there are limitations to this and other observational studies, the information gleaned here can contribute to an existing knowledge base. Here, Holstein cattle hauled in trailers with smaller dimensions experienced more traumatic events than when hauled on larger

trailers. Holstein cattle also displayed a higher prevalence of carcass bruising than cattle of beef breeds, and bruising decreased in both breeds as hot carcass weight increased. More research is needed to better understand how the entire transportation process, including animal handling at loading and unloading, trailer type, and animal risk factors contribute to carcass bruising in fed cattle. Risk factors such as breed, sex, cattle temperament, and carcass traits should not be overlooked. In addition, the type of cattle being observed must be considered, and comparisons between groups should be made with caution, always remembering that risk factors can differ between the groups. However, no matter what cattle group or type is included in subsequent research, carcass bruising in cattle is a significant economic and animal welfare issue, and only more research can help decrease the number of animals which experience trauma during the transport process and carcass bruising at slaughter.

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Table 2-1. Description of cattle hauled in each trailer type (fat vs. combination trailers).

		Fat Trailers¹	Combination Trailers¹
Number of trailers observed		129	146
Average #head/trailer		33	37
Breed			
	Beef	99	132
	Holstein	30	14
Sex			
	Steers	104	108
	Heifers	18	26
	Mixed²	7	11
	Not specified		1

¹Fat/feeder combo trailers are those which are used to haul both feeder calves and finished beef cattle. Fat trailers are usually used to haul finished cattle only. The differences between these types of trailers include the presence or absence of a “jail” or “doghouse” in the upper rear compartment of the trailer, used to contain very small calves (present in fat/feeder combo trailers, Beef Quality Assurance, 2006), the presence of a small compartment in the nose of the trailer, used as a counter-balance (also present in fat/feeder combo trailers), and the clearance height of the entrance into the “belly”, or lower compartment of the trailer (approximately 2-3 inches shorter in fat/feeder combo trailers). Either type of trailer can have a slide-in or fold-up ramp leading into the upper deck compartment.

²Mixed lot refers to a lot comprised of both heifers and steers.

Table 2-2. Description of lots observed for both traumatic events and carcass bruising.

Total Number of lots		75
Average #head/lot		131
Breed		
	Beef	63
	Holstein	12
Sex		
	Steer	54
	Heifer	13
	Mixed¹	8

¹Mixed lot refers to a lot comprised of both heifers and steers.

Table 2-3. Description of lots, including carcass characteristics, prevalence of traumatic events experienced, and prevalence of carcass bruising.

	Number of lots (n)	Average Carcass Weight (kg, SEM)	Average REA ¹ (in, SEM)	Average Fat Thickness (in, SEM)	Average YG ² (SEM)	Prevalence of Traumatic Events ³ (SEM)	Prevalence of Carcass Bruising ⁴ (SEM)
Beef							
Heifer	13	371.01 (+ 6.5)	14.09 (+ 0.28)	0.51 (+ 0.02)	2.62 (+ 0.08)	17.2% (+ 3.0%)	67.1% (+ 2.8%)
Mixed ⁵	8	375.0 (+ 5.7)	14.01 (+ 0.23)	0.56 (+ 0.02)	2.73 (+ 0.10)	18.4% (+ 2.9%)	64.9% (+ 3.5%)
Steer	42	419.2 (+ 4.1)	14.12 (+ 0.17)	0.56 (+ 0.02)	2.65 (+ 0.07)	19.5% (+ 1.4%)	66.7% (+ 1.4%)
Total	63	403.7 (+ 3.1)	14.1 (+ 0.13)	0.55 (+ 0.03)	2.66 (+ 0.08)	18.9% (+ 1.1%)	66.6% (+ 2.5%)
Holstein							
Steer	12	394.6 (+ 4.2)	13.85 (+ 0.32)	0.57 (+ 0.01)	2.81 (+ 0.05)	28.6% (+ 2.5%)	76.6% (+ 1.2%)
Total	75	402.2 (+ 3.6)	14.05 (+ 0.12)	0.55 (+ 0.01)	2.68 (+ 0.04)	20.4% (+ 1.1%)	68.2% (+ 1.2%)

¹REA = Ribeye area

²YG = Yield grade

³Prevalence of traumatic event occurrence was calculated dividing the number of traumatic events observed at unloading by the total number of cattle in the trailer.

⁴Prevalence of carcass bruising was calculated by dividing the number of carcasses with a bruise present over the total number of animals in the lot.

⁵Mixed lot refers to a lot comprised of both heifers and steers.

Table 2-4. *P*-values generated from univariable and multivariable analyses for the outcome traumatic events. Only 2-way interactions were evaluated in the multivariable analysis. Interaction effects are listed in the order by which they were removed from the model using backward selection at a threshold of $P>0.05$.

Independent variable	Univariable <i>P</i>-values	Multivariable <i>P</i>-values	Final model <i>P</i>-values
Distance	0.7026	0.4542	N/A
Sex¹	0.0091	0.1159	N/A
Breed²	0.0001	0.0042	0.0042
Trailer Type³	0.0591	0.0507	0.0507
Sex*Trailer	N/A	0.8501	N/A
Distance*Trailer	N/A	0.6945	N/A
Distance*Sex	N/A	0.2727	N/A
Distance*Breed	N/A	0.0713	N/A
Breed*Trailer	N/A	0.0111	0.0111

¹Sex was categorized as “Steer,” “Heifer,” or “Mixed.”

²Breed was categorized as “Beef” or “Holstein.”

³Fat/feeder combo trailers are those which are used to haul both feeder calves and finished beef cattle. Fat trailers are usually used to haul finished cattle only. The differences between these types of trailers include the presence or absence of a “jail” or “doghouse” in the upper rear compartment of the trailer, used to contain very small calves (present in fat/feeder combo trailers, Beef Quality Assurance, 2006), the presence of a small compartment in the nose of the trailer, used as a counter-balance (also present in fat/feeder combo trailers), and the clearance height of the entrance into the “belly”, or lower compartment of the trailer (approximately 2-3 inches shorter in fat/feeder combo trailers). Either type of trailer can have a slide-in or fold-up ramp leading into the upper deck compartment.

Table 2-5. Percent of carcass bruising on the left side, the dorsal midline, and the right side of beef carcasses. Superscripts indicate a significant difference between the observed values and the expected values of the bruising in each region ($P\leq 0.05$). Equal distribution between all regions was expected.

Bruise location	Mean, %	SEM, %
Left¹	26.46 ^a	1.10
Midline²	53.52 ^b	1.12
Right³	19.98 ^c	1.04

¹Bruises along the left side of the carcass were those which occurred in areas 3, 6, and 9 (see Figure 1).

²Bruises along the left side of the carcass were those which occurred in areas 2, 5, and 8 (see Figure 1).

³Bruises along the left side of the carcass were those which occurred in areas 1, 4, and 7 (see Figure 1).

Table 2-6. Percent of carcass bruising on the front, middle, and rear thirds of beef carcasses. Superscripts indicate a significant difference between the observed values and the expected values of the bruising in each region ($P \leq 0.05$). Equal distribution between all regions was expected.

Bruise location	Mean, %	SEM, %
Front ¹	31.30 ^a	1.05
Middle ²	56.13 ^b	1.02
Rear ³	12.57 ^c	0.71

¹Bruises along the front third of the carcass were those which occurred in areas 7, 8, and 9 (see Figure 1).

²Bruises along the middle third of the carcass were those which occurred in areas 4, 5, and 6 (see Figure 1).

³Bruises along the rear third of the carcass were those which occurred in areas 1, 2, and 3 (see Figure 1).

Table 2-7. Percent of carcass bruising categorized as small, medium, or large bruises. Superscripts indicate a significant difference between the observed values and the expected values of the bruise sizes. Equal distribution between all sizes was expected.

Bruise Size	Mean, %	SEM, %
Small (<5cm)	28.64 ^a	1.32
Medium (5-15cm)	41.77 ^b	0.97
Large (>15cm)	29.58 ^c	1.81

Table 2-8. Estimates of parameters for the fixed effects of average carcass weight and breed of cattle assessed with multiple linear regression.

Effect	Class ¹	Estimate ²	SEM	P-value
Intercept		1.0952	0.1447	<0.001
Average Carcass Weight		-0.00082	0.00035	0.022
Breed	Beef	-0.9515	0.03519	0.009
	Holstein	Ref.3	.	.

¹Refers to breed of cattle

²Parameter estimates

³Ref. stands for reference category

Table 2-9. Estimate of mean carcass bruise prevalence per lot by breed (cattle were categorized as either Holstein or beef breeds). Estimates with different superscripts differ significantly ($P \leq 0.05$).

Class¹	Estimate, %	SEM, %
Beef	67.20 ^a	3.0
Holstein	76.70 ^b	4.3

¹Refers to breed of cattle

Table 2-10. *P*-values generated from univariable and multivariable analyses for the outcome carcass bruising. Only 2-way interactions were evaluated in the multivariable analysis. Interaction effects are listed in the order by which they were removed from the model using backward selection at a threshold of $P > 0.05$.

	Univariable	Multivariable	Final model
	<i>P</i>-value	<i>P</i>-value	<i>P</i>-value
Traumatic Events	0.1158	0.3155	N/A
Average Carcass Weight	0.0195	0.0222	0.0222
Distance	0.2169	0.4166	N/A
Sex¹	0.747	0.5208	N/A
Breed²	0.0078	0.0087	0.0087
Ribeye Area³	0.2375	0.1019	N/A
Average Yield Grade⁴	0.0786	0.4627	N/A
Fat Thickness	0.3968	0.5064	N/A
Traumatic Events*REA	N/A	0.9543	N/A
Traumatic Events*Fat Thickness	N/A	0.8967	N/A
REA*Distance	N/A	0.8023	N/A
Average Carcass Weight*Distance	N/A	0.8797	N/A
Traumatic Events*Distance	N/A	0.8359	N/A
Distance*Breed	N/A	0.6229	N/A
Fat Thickness*Average YG	N/A	0.5394	N/A
Average YG*Distance	N/A	0.3544	N/A
Fat Thickness*Distance	N/A	0.7798	N/A
Average Carcass Weight*Breed	N/A	0.4482	N/A
Traumatic Events*Average Carcass Weight	N/A	0.3222	N/A
REA*Average YG	N/A	0.3068	N/A
Average YG*Breed	N/A	0.1105	N/A
Average Carcass Weight*REA	N/A	0.1875	N/A
Traumatic Events*Breed	N/A	0.2778	N/A
REA*Breed	N/A	0.8703	N/A
Average Carcass Weight*Average YG	N/A	0.1413	N/A
Average Carcass Weight*Fat Thickness	N/A	0.3681	N/A
Fat Thickness*Breed	N/A	0.1259	N/A
Traumatic Events*Average YG	N/A	0.1139	N/A
REA*Fat Thickness	N/A	0.0745	N/A

¹Sex was categorized as “Steer,” “Heifer,” or “Mixed.”

²Breed was categorized as “Beef” or “Holstein.”

³Ribeye Area = REA

⁴Yield Grade = YG

Figure 2-1. Grid of sections used in the Harvest Audit Bruise Scoring System.

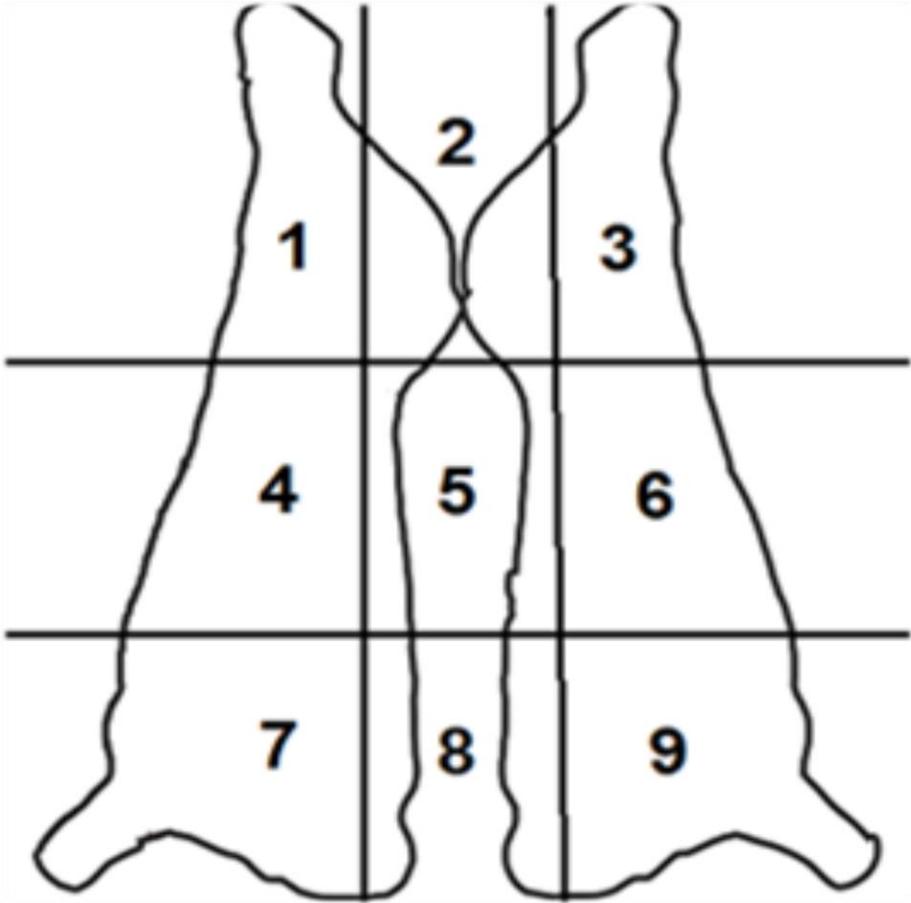
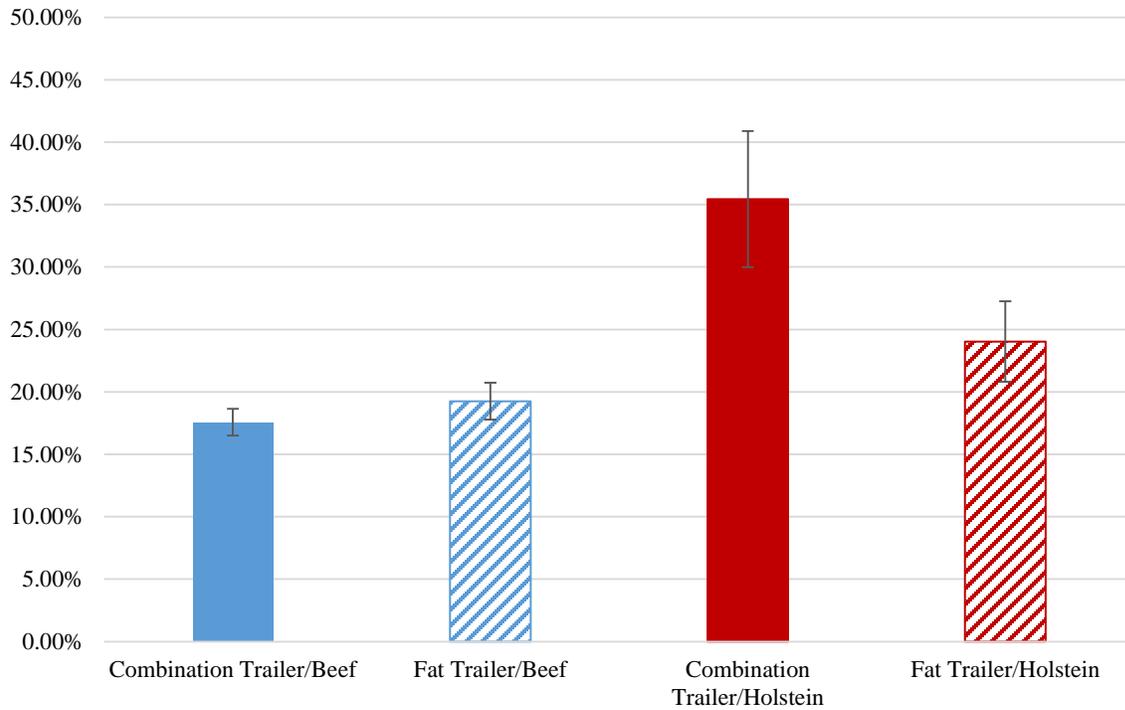
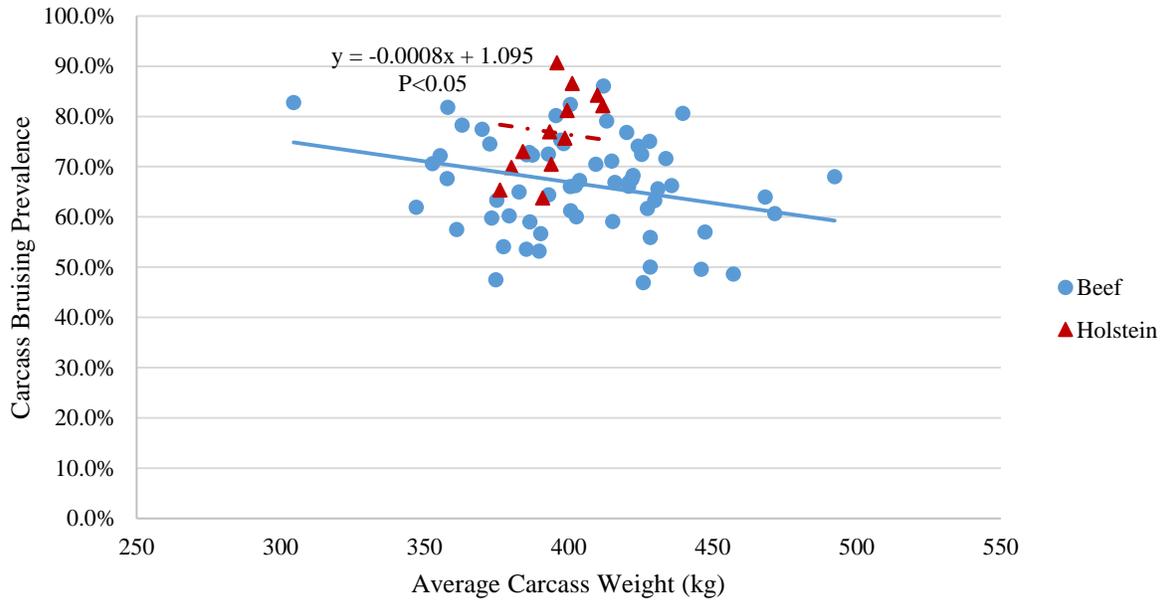


Figure 2-2. Prevalence of traumatic events for each combination of breed and trailer type. There was a significant interaction between trailer type and cattle breed, whereby Holstein cattle hauled in fat/feeder combination trailers experienced higher prevalence of traumatic events than their beef counterparts.



¹Fat/feeder combo trailers are those which are used to haul both feeder calves and finished beef cattle. Fat trailers are usually used to haul finished cattle only. The differences between these types of trailers include the presence or absence of a “jail” or “doghouse” in the upper rear compartment of the trailer, used to contain very small calves (present in fat/feeder combo trailers, Beef Quality Assurance, 2006), the presence of a small compartment in the nose of the trailer, used as a counter-balance (also present in fat/feeder combo trailers), and the clearance height of the entrance into the “belly”, or lower compartment of the trailer (approximately 2-3 inches shorter in fat/feeder combo trailers). Either type of trailer can have a slide-in or fold-up ramp leading into the upper deck compartment.

Figure 2-3. Relationship between average carcass weight and carcass bruising prevalence by lot for lots of Holstein and beef breed cattle ($P \leq 0.05$), results from multivariable linear regression model. Each point on the graph represents a lot of cattle observed. Triangles represent lots of Holstein cattle ($n=12$), while dots represent lots of beef breed cattle ($n=63$).



Chapter 3 - An Epidemiological Investigation to Determine the Prevalence and Clinical Manifestations of Slow-moving Finished Cattle at the Time of Slaughter

Abstract

Cattle mobility is routinely measured at commercial slaughter facilities. However, the clinical signs and causes of decreased mobility in cattle at slaughter are poorly defined. The objective of this study was to determine the prevalence of abnormal mobility scores (MS) and clinical diagnoses for decreased mobility in finished cattle. Finished beef cattle (n = 65,600) were observed at 6 slaughter facilities in the United States. All cattle were observed during the morning shift by a veterinarian who assigned mobility scores (MS) and clinical diagnoses for cattle displaying abnormal mobility. Cattle displaying abnormal mobility were categorized into one of five clinical diagnosis categories: 1) lameness, 2) poor conformation, 3) laminitis, 4) Fatigued Cattle Syndrome (FCS), and 5) general soreness/stiffness. Prevalence of MS 1, 2, 3 and 4 in cattle observed were 97.02%, 2.69%, 0.27%, and 0.01%, respectively. Of all cattle observed, 0.23% were categorized as lame, 0.20%, as poor conformation, 0.72%, as laminitis, 0.14% as FCS, and 1.68% as general soreness/stiffness. Longer lairage time was associated with increasing prevalence of MS 2 and 3 up to 10 hours ($P \leq 0.01$). Prevalence of lameness and general soreness/stiffness was higher in steers than heifers, but prevalence of laminitis was higher in heifers than steers ($P \leq 0.05$). Fatigued Cattle Syndrome prevalence was higher in dairy cattle than in beef cattle ($P \leq 0.05$). These data indicate the prevalence of cattle displaying abnormal mobility at slaughter is low and causes of abnormal mobility are multifactorial.

Introduction

Cattle health and welfare, including mobility status, is a significant concern for producers, packers, retailers, and consumers of beef. The mobility of finished cattle at commercial slaughter facilities gained attention after an adverse animal welfare event was reported in 2013, resulting in increased awareness of severe cattle fatigue and its effects, now defined as “Fatigued Cattle Syndrome”.^{1,2} A similar condition has been described in swine, where a portion of hogs exposed to stress at the time of transport may display decreased mobility and become non-ambulatory as the result of metabolic acidosis and muscle fatigue.³ Fatigued Cattle Syndrome, or FCS, is manifested in clinical signs such as tachypnea with an abdominal component, muscle tremors, stiff gait with shortened strides, and reluctance to move.^{2,4} Previous reports show abnormalities in biochemical variables of cattle diagnosed with FCS include markedly increased lactate, creatine kinase (CK), and aspartate aminotransferase (AST) compared to normal reference ranges.²

A 4-point mobility scoring system is commonly used to assess mobility of finished cattle at commercial slaughter facilities.⁵ Mobility scoring of cattle at commercial slaughter facilities is now common, however, the specific diagnosis of the cause of abnormal mobility in finished cattle at slaughter has not been investigated. The etiology of abnormal mobility in cattle at abattoirs has been poorly defined, and it is unlikely that all cattle with abnormal mobility scores are necessarily afflicted with the aforementioned FCS. Rather, some cattle may experience pain due to acute or chronic lameness, which can be amplified during the transport process. Such discrepancies in the system warrant further investigation to determine the true prevalence and causes of abnormal mobility. Therefore, the objectives of this observational study were to determine the prevalence of abnormal mobility using the 4-point mobility scoring system, and to

determine the clinical signs contributing to abnormal mobility in finished cattle in six commercial slaughter facilities across the United States.

Materials & Methods

All procedures were approved by the Institutional Animal Care and Use Committee of Kansas State University (IACUC # 3708).

Slaughter facilities selected for observation were chosen based on data from a cattle mobility scoring program and logistical considerations.⁶ The 6 facilities selected for the study were in different locations in the U.S., and their operations reflect the current population of finished cattle slaughtered in the U.S., each facility slaughtering over 1,500 animals in a single 8-hour shift. Observations were made each week in the months of April-August, 2016. Each facility was visited on 5 different days throughout the summer, so that scoring could be completed on each day of the business week (Monday through Friday) in an effort to eliminate potential confounding introduced by observing cattle on the same day every week.

Data pertaining to facility design and environmental conditions were collected, including flooring type and weather parameters. For each lot, pertinent information was recorded, including feedlot of origin, slaughter facility lot number, number of head in the lot, breed of cattle (categorized as beef or dairy), sex of cattle (categorized as steer, heifer, or mixed), and distance traveled. Weather measurements included temperature (°C) at the start and end of each shift, percent humidity at the start and end of each shift, and wind speed (mph) at the start and end of each shift. Cut points for ambient temperature, temperature-humidity index (THI), and distance traveled exists, categories were selected based on common numeric cut points such as every 5 or 10 degrees, and every 100 miles.

Temperature and humidity recorded at the end of each shift were used to determine the day's temperature-humidity index, using the equation from Mader, et al. (2010) where: $\{THI = (0.8 \times \text{ambient temperature in } ^\circ\text{C}) + [(\% \text{ relative humidity} / 100) \times (\text{ambient temperature in } ^\circ\text{C} - 14.4)] + 46.4\}$.^{7,8} Time of observation was recorded. Average lairage time for each lot was recorded and expressed as: $\{\text{Lairage Time} = (\text{time of observation} - \text{average of the times all trucks carrying the lots passed over the slaughter facility scale})\}$. This measurement therefore included the time cattle spent on the trucks waiting to be unloaded, time spent on the unloading dock, and time spent in lairage pens.

Cattle were monitored between the hours of 0600 and 1600 (the entirety of Shift 1) at each slaughter facility on each day of observation. Cattle were observed after being moved from lairage pens, on their way to the serpentine alley leading to the restrainer. Observation locations were not the same at each facility due to logistical considerations, however all observations were made at the same point of the animals' drive from lairage pens to the restrainer.

Mobility score (MS) was assigned to each animal observed using the scoring system adopted by the North American Meat Institute (NAMI, 2015), where: 1 = Normal, walks easily with no apparent lameness or change in gait; 2 = Keeps up with normal cattle when the group is walking, exhibits one or more of the following: stiffness, shortened stride, or slight limp; 3 = Lags behind normal cattle when the group is walking, exhibits one or more of the following: obvious stiffness, difficulty taking steps, obvious limp or discomfort; 4 = Extremely reluctant to move, even when encouraged by handlers.⁵

Mobility scores were observed and recorded by the same trained veterinarian (T.L. Lee). Any animal deemed to have impaired mobility ($MS \geq 2$) was observed further for other clinical signs of injury or stress and a clinical diagnosis of the cause of abnormal mobility was

determined and recorded by the observer. Cattle with normal mobility ($MS = 1$) were not uniquely identified.

For cattle displaying abnormal mobility, all observed clinical signs were recorded. Obvious disease or injury was noted, including lameness due to broken legs, toes, or any other obvious injury (the leg(s) on which the animal was lame were recorded as well). Evidence of acute or chronic laminitis (founder) was documented, including the presence of abnormally long hoof walls, walking on heels, and/or sloughed hoof walls. Reluctance to move without obvious disease or injury, including failure to keep up with contemporaries without extra handling pressure or use of electric prods, paddles, or sticks was noted. Evidence of shortened strides, stiffened gait or difficulty walking was recorded. In addition, the presence of nervous system abnormalities such as muscle tremors or ataxia, or other signs of distress such as an increased respiratory rate or vocalization were documented. Other signs not listed were recorded as comments during data collection.

Based on the observed clinical signs, cattle receiving a $MS \geq 2$ were categorized into one of five categories: 1) Lameness/injury = lameness on one or more limbs caused by broken toes or legs, or any shoulder or rear leg injuries; 2) Poor conformation = abnormalities in shape or structure of legs and feet, which contribute significantly to the animal's decreased mobility.⁹; 3) Laminitis = founder/laminitis, including animals with abnormally long hooves, animals walking on their heels, presence of cracked hooves, or possibly sloughed hoof walls; 4) Fatigued Cattle Syndrome (FCS) = animals displaying abnormal mobility, with clinical signs not due to injury or founder, including but not limited to nervous system abnormalities such as muscle tremors, increased respiratory rate, increased vocalization, obvious stiffness, and shortened strides; and 5) General soreness/stiffness = Cattle with abnormal mobility ($MS \geq 2$) not due to any obvious

disease, injury, or syndrome. Sore and stiff cattle displayed normal behavior with the exception of abnormal mobility or range of movement.

All data were entered and tabulated in a Microsoft Excel spreadsheet.^a Means, standard deviations, frequency distributions, and minimum and maximum values were calculated using spreadsheet formulas.

Data were further analyzed using the proc PROC GLIMMIX procedure in SAS v. 9.4^b to perform univariable analyses. Because this is an exploratory prevalence study, and the primary objective was to determine the prevalence of and clinical signs associated with abnormal mobility, multivariable analyses were not performed, therefore no interactions or specific random effects are included. The univariable analyses conducted were to identify specific risk factors that may warrant further investigation.

Mobility score and clinical diagnosis category (lameness, poor conformation, laminitis, FCS, and general soreness/stiffness) were considered dependent variables. Facility location, distance traveled, ambient temperature, THI, breed, gender, time of observation, and average lairage time were treated as independent variables. Comparisons of least square mean estimates of the dependent variables were made between categories of each independent variable using the LSMEANS procedure, with a Tukey-Kramer adjustment for multiple comparisons. Statistical significance was determined at $P \leq 0.05$. The count of the observations per group were assumed to follow a Poisson distribution, and the natural logarithm of the cattle in the group was treated as the offset (denominator) variable. An overdispersion term was included in the model to account for within-group dependency of each outcome and to inflate variance associated with the model estimates.

Results

A total of 65,600 head of finished cattle were observed at 6 slaughter facilities over 30 days of observation. Over all cattle evaluated, steers (n = 39,690) were observed more frequently than heifers (n = 19,734) or animals in mixed lots (n = 6,176). There were a greater number of beef breed cattle observed than cattle from dairy breeds (n = 58,124 v. 7,476, respectively). The description of cattle number observed within slaughter facility, breed type, and sex is displayed in Table 3-1.

Low temperatures on observation days ranged from 3.8°C to 22.2°C. High temperatures ranged from 18.9°C to 37.2°C. Distance traveled by cattle coming into the slaughter facilities ranged from 5 miles to 1191 miles. Average lairage time ranged from 30 minutes to over 12 hours (Table 3-2). In all but one facility, which had both grooved concrete and rebar, grooved concrete was the flooring surface in the lairage pens and alley ways. Table 3-2 shows a description of distance traveled, average lairage time, and average mobility score for cattle at each slaughter facility.

Cattle exhibiting a MS 1 were most prevalent (97.02%), and cattle exhibiting a MS = 4 were the least prevalent (0.01%, Table 3-3). In all facilities, MS = 1 was most prevalent, followed by MS = 2, then MS = 3, and finally MS = 4 (Table 3-4). Abnormal mobility was observed more frequently in steers versus heifers or cattle in mixed lots (1.92% v. 0.79% and 0.27%, respectively; Table 3-5), but more steers were observed overall compared to heifers or cattle in mixed lots. Prevalence each clinical diagnosis is shown in Table 3-6. General soreness/stiffness was observed to be the biggest contributor to prevalence of animals displaying a MS = 2 (Table 3-7). Animals displaying FCS comprised 50% of the animals displaying MS = 3 and MS = 4. Of all clinical signs observed in cattle displaying abnormal MS, shortened strides

and stiffness were most commonly reported (Table 3-8). Comparisons of point estimates of abnormal MS prevalence ($MS \geq 2$) categorized by slaughter facility are displayed in Figure 3-1 (A, B, and C, respectively).

Effect of Distance Traveled on Mobility Score Prevalence

There was no observed effect of distance traveled to the slaughter facility on the prevalence of MS 2 or 4 across all plants (Figure 3-2A and C; $P > 0.05$). Prevalence of MS 3 was lower at 0-100 miles traveled compared to 101-200 and 201-300 miles traveled (Figure 3-2B; $P = 0.0114$ and 0.003 , respectively) but no difference was observed in prevalence of MS = 3 in cattle which had traveled over 300 miles ($P > 0.05$).

Effect of Environmental Conditions on Mobility Score Prevalence

Ambient temperature and temperature-humidity index were not observed to have an effect on the prevalence of abnormal mobility (Figures 3-3 and 3-4; $P > 0.05$).

Effect of Time of Observation and Average Lairage Time on Prevalence of Mobility Score Prevalence

There were no differences detected in any abnormal MS at different times of the day. (Figure 3-5; $P > 0.05$). Prevalence of MS 2 and 3 increased as average lairage time increased up to 8 hours, then became more variable up to and over 14 hours (Figures 3-6A and 3-6B; $P < 0.0001$ and $P = 0.001$, respectively). There were no differences detected in MS = 4 at different average lairage times ($P = 0.613$).

Effect of Sex and Breed on Mobility Score Prevalence

Numerically, more steers exhibited abnormal MS than heifers and animals from mixed lots (1.92% v. 0.79% and 0.27%, respectively), however no differences were detected between animals of different sexes within each abnormal mobility score category (Figure 3-7; $P > 0.05$). There were no significant differences detected in the prevalence of abnormal mobility scores between breeds (Figure 3-8; $P > 0.05$). All animals displaying MS = 4 were steers, with 5 being of beef breeds and 1 of dairy.

Effect of Distance Traveled on Prevalence of Clinical Diagnoses

No differences were detected in prevalence of the clinical diagnoses lameness and poor conformation due to distance traveled ($P > 0.05$). There were significant differences between distance traveled with regards to cattle diagnosed with laminitis, FCS, and general soreness/stiffness (Figure 3-9, A, B, C, D, and E, respectively; $P \leq 0.05$). Prevalence of laminitis was greater in animals which had traveled 301-400 miles compared to those traveling 101-200 miles ($P = 0.002$), but no differences in prevalence of laminitis were detected when these animals were compared to animals traveling any other distance ($P = 0.451$). With the exception of animals which traveled 300 to 500 miles (where prevalence of FCS was 0%), prevalence of FCS was numerically lowest in cattle traveling 0-100 miles, and was lower than prevalence of FCS in animals which had traveled 201-300 miles ($P = 0.0016$). No other differences were observed with regards to distance traveled and its effect on prevalence of FCS ($P > 0.05$). General soreness/stiffness increased as distance increased up to 300 miles ($P = 0.0002$), after which no differences were observed.

Effects of Environmental Conditions on Prevalence of Clinical Diagnoses

Prevalence of lameness was greater at 15 – 21°C when compared to ambient temperatures between 27 – 32°C (Figure 3-10A; $P = 0.007$), but was not different from ambient temperatures between 21 – 27°C and 32 – 38°C ($P > 0.05$). Prevalence of poor conformation was greater at ambient temperatures below 21°C than above 21°C (Figure 3-10B; $P \leq 0.0001$). There were no differences detected between the prevalence of laminitis, FCS, or general soreness/stiffness across different ambient temperatures (Figures 3-10C, D, and E; $P > 0.05$). Regarding THI, the prevalence of lameness was higher at THIs of 54-57 and 62-65 compared to THIs of 66 to 69 (Figure 3-11A; $P = 0.0013$). Poor conformation was higher at THI of 50-53 compared to THI between 62 and 69 (Figure 3-11B; $P = 0.0107$). No differences between the prevalence of laminitis were detected across THI levels (Figure 3-11C; $P = 0.2033$). There was an overall effect of THI on the prevalence of FCS ($P = 0.026$), but no differences were detected between different levels of THI when a Tukey-Kramer adjustment for multiple comparisons was implemented. General soreness and stiffness prevalence was lowest at THI of 54-57 compared to THI of 66-69 (Figure 3-11E; $P = 0.0015$).

Effect of Time of Observation and Average Lairage Time on Prevalence of Clinical Diagnoses

Prevalence of lameness and poor conformation were higher from 6am-8am than from 8am-10am (Figures 3-12A and 3-12B; $P = 0.0449$ and 0.0174 , respectively), but no other differences in clinical diagnoses were detected at different times of observation. No differences were detected in the prevalence of lameness and poor conformation with regards to lairage time (Figure 3-13A and 3-13B; $P > 0.05$). Prevalence of laminitis was greater in animals

experiencing a lairage time of 8-10h compared to those spending 0-2 h in lairage (Figure 3-13C; $P = 0.0019$), but no other differences were detected across other lairage times. Animals experiencing lairage times of 2-4 h displayed a lower prevalence of FCS than those in lairage for 4-6 h (Figure 3-13D; $P = 0.0009$), but prevalence of FCS did not appear to differ when these groups were compared to other lairage times. Prevalence of general soreness/stiffness was greater at 4-6 h lairage than < 4 h (Figure 3-13E; $P < 0.001$), and animals experiencing lairage times >10 h displayed greater prevalence than those in lairage for < 2 h ($P < 0.0094$). Prevalence of general soreness/stiffness was not observed to differ between other lairage time categories ($P > 0.05$).

Effects of Sex on Prevalence of Clinical Diagnoses

Steers displayed higher prevalence of lameness than heifers at the time of slaughter (Figure 3-13A, $P = 0.0034$). No differences in the prevalence of lameness was detected in animals of mixed lots when compared to heifers or steers ($P = 0.8125$ and 0.2865 , respectively). Laminitis was observed to be more prevalent in heifers than in steers (Figure 3-14C; $P = 0.0103$), but no differences were detected between the prevalence of laminitis in animals of mixed sex lots when compared to heifers or steers ($P = 0.9406$ and 0.2703 , respectively). Steers displayed a higher prevalence of general soreness/stiffness compared to heifers (Figure 3-14E; $P = 0.0032$), but no difference was detected when animals in mixed lots were compared to steers or heifers ($P = 0.4719$ and 0.3565 , respectively). There were no observed differences in the prevalence of poor conformation or FCS detected across sex groups (Figure 3-14B and D; $P > 0.05$).

Effects of Breed on Prevalence of Clinical Diagnoses

Prevalence of FCS was higher in dairy breeds compared to beef breeds (0.28% v. 0.13%, respectively; Figure 3-15D; P = 0.0195). However, no effects of breed type on any of the other 4 clinical diagnosis categories were observed.

DISCUSSION:

This study is the first to explore the prevalence of MS and causes of abnormal mobility in the population of fed cattle entering slaughter facilities in the U.S.. Prevalence of cattle with abnormal mobility scores was found to be low compared to cattle with normal mobility, and prevalence of abnormal scores decreased with increasing severity. General soreness/stiffness was the most frequently observed clinical diagnosis. This study identifies potential factors that could contribute to abnormal mobility, and provides information upon which future research studies can be designed.

Mobility Score Prevalence

Prevalence of MS = 3 increased with increasing distance traveled to the slaughter facility, up to approximately 300mi (Figure 2B). In swine, losses due to deceased or nonambulatory pigs increase as distance moved during loading and distance traveled increase.^{10,11} Data collected in this study indicate prevalence of abnormal mobility increases to a certain distance traveled and/or lairage time, then stays the same or decreases. This could suggest that animals traveling longer distances may have the ability to rest and recover during the journey. Gonzalez et al. (2012) indicated that distance should be considered along with ambient temperature to properly

address their effects on cattle stress, however temperature was not recorded during transport for each truckload of cattle observed in the present study.¹²

While some suggest that increased ambient temperatures or seasonal changes cause a decrease in cattle mobility at slaughter facilities, there was no effect of temperature or THI on prevalence of different abnormal MS found in this study.^{13,14} However, all observations were made during the first slaughter shift, which typically runs from 0600 to 1400 or 1500, therefore cattle were not observed in the hottest hours of the day. In addition, all observations were made in the spring or summer months (late April to mid-August), which did not allow for observations to be made during times of large temperature changes. Month of slaughter has been associated with increased mortality in fed cattle, but the contribution of abnormal mobility to such mortality has not been explored.¹³ Because observations were made only in the late spring and summer in this study, seasonal effects on the prevalence of abnormal MS could not be assessed, therefore further research in the area is warranted.

Mader et al. (2010) suggested that solar radiation and wind speed should be incorporated into THI calculations, however such information was used in assessments of environmental stress in animals in feedyards, not in slaughter facilities.⁸ Solar radiation may contribute to heat load in animals contained in slaughter facility lairage pens, however wind may not be a major contributing factor in the ability of animals to cool themselves in such an environment. Lairage pens in slaughter facilities are small areas, where stocking density is greatly increased in comparison to feedyard pens, likely eliminating most of the positive impact wind may have on the cooling abilities of cattle. Shade has been shown to decrease heat stress in cattle in feedyards, but the use of shade in slaughter facility lairage pens may in fact be detrimental to the animals' ability to dissipate heat, as shade structures may contribute to a further decrease in air

flow throughout slaughter facility pens.¹⁵ In addition, there are reports of mixed results on the effect of implant status and its effect on animals' ability to deal with heat stress. In the current study, prevalence of abnormal mobility observed does not seem to be affected by temperature, and implant status was not recorded, therefore no conclusion can be made upon the relationship between MS, ambient temperature, and implant status of the cattle^{7,16} Hahn remarked that cattle are well-equipped to cope with environmental stressors, and such adaptability may be reflected in the non-significant results seen here.¹⁷

Differences in abnormal MS prevalence between breeds and sexes was not observed here. Given the nature of the fed cattle industry, however, it is possible that the higher prevalence of beef animals and steers in the population observed could contribute to the lack of difference. In the summer of 2016, approximately twice as many steers were commercially slaughtered compared to heifers.¹⁸ More data may be required to determine a true difference in the prevalence of abnormal MS between breeds and sexes.

Prevalence of Clinical Diagnoses

Increases in distance traveled and average lairage time was associated with increased prevalence of FCS and general soreness/stiffness to a point (400km distance, 8h lairage), after which prevalence of each diagnosis decreased or stabilized. As with MS, this could indicate that increased length of journey or increased amount of time in lairage pens could give animals a chance to recover from the fatigue induced by the stress of transport. A substantial amount of research exists on the effects of distance traveled on stress and bruising in cattle, but such research does not include the assessment of cattle mobility or the presence of any abnormalities other than biomarkers of stress, including blood cortisol, lactate, catecholamines, creatine kinase,

and others.¹⁹⁻²² In two separate studies, Frese and Hagenmaeir showed that rough handling can increase stress and these stress markers in cattle, and also showed that such stress can result in abnormal mobility.^{4,23} If blood metabolites were measured in all cattle displaying abnormal mobility status due to any of the clinical diagnoses defined here, it is likely that these blood biomarkers of stress would be increased in such compromised animals.

Laminitis was more prevalent in heifers versus steers, while lameness and general soreness/stiffness was more prevalent in steers. Laminitis is commonly observed in operations where high-concentrate diets are fed, such as those fed to feedlot cattle.²⁴ High or inconsistent feed intake could contribute to differences in the prevalence of laminitis, especially across sexes. According to the Nutrient Requirements for Cattle, predicted DMI for medium-framed heifers should be decreased by 10% as compared to steers, which does not support an effect of increased DMI on the prevalence of laminitis.²⁵ It could be that because heifers are not as efficient as steers, they require longer days on feed, increasing chances laminitis in heifers compared to steers.²⁶ In fact, research shows that cattle fed a high concentrate diet had a net sole horn growth of 2.5 times that of cattle fed a high concentrate diet for 30 days less.²⁷ However, this hypothesis is not supported when the prevalence of laminitis in beef cattle is compared to that in dairy cattle, which typically require longer days on feed than either steers or heifers of beef breeds.²⁸

With regards to lameness and general soreness/stiffness, it could be hypothesized that steers are more prone to fighting and are more temperamental than their female counterparts, which could cause an increase in prevalence of these diagnoses seen in steers, but Voisinet et al. (1997) showed that heifers displayed higher temperament scores than steers.²⁹ No data on temperament were collected in the current study, therefore increased lameness and general

soreness/stiffness in steers cannot be attributed to temperament here. Differences in prevalence of FCS or poor conformation between sexes were not detected in this study.

Dairy animals displayed a higher prevalence of FCS than beef breeds, possibly indicating that dairy animals are more susceptible to the rigors of the transport and lairage process. Dairy animals typically take more time to mature than beef breeds due to larger frame sizes, resulting in longer days on feed.²⁸ In addition, cattle from dairy breeds are typically fed higher concentrate diets earlier in life than their beef counterparts. This could increase the risk of subclinical acidosis, which could lead to unobservable subclinical laminitis.²⁷ It is possible that such pathology could be mistaken for fatigue. In addition, differences in the way in which cattle are raised and their level of exposure to humans contribute to differences in docility between dairy and beef breeds.³⁰ Feedlot cattle from dairy breeds are typically exposed to humans more than cattle of beef breeds. This could have implications at shipping and slaughter, as feedyard personnel may feel the need to handle dairy animals roughly to get them to move, increasing chances of stress and subsequent fatigue. Finally, it has been proposed that responses to stress are the result of a complex interaction between genetics and previous experience of the animals.³¹ It is possible that animals of dairy origin with different genetics or experiencing different handling experiences are more predisposed to FCS, but more research is warranted to provide conclusive evidence of such relationships.

Of the animals displaying $MS \geq 3$, 50% were diagnosed as having FCS (Table 7). In hogs, approximately 50% of the nonambulatory animals are considered fatigued.³ It should be noted that of the 65,600 animals observed in the current study, only one animal was observed to be non-ambulatory. In addition, compared to earlier observational reports of increased

prevalence of cattle displaying signs of FCS, it does not seem to be as prevalent as previously reported, based on the results of this study.^{32,33}

Inherently, the use of subjective measurements (MS and clinical diagnoses) creates some room for differences in opinion as to what constitutes the different levels of mobility. However, the use of the same trained observer on each day and the use of a mobility scoring system and specific case definitions to define the clinical diagnosis categories helps to eliminate some of the subjectivity. In addition, the categories used for clinical diagnosis of the abnormalities reported here are quite broad, and animals entering slaughter facility could have displayed more specific problems than the ones reported here (for example, if a tumor affecting mobility was present on an animal's leg, the abnormality was classified as "poor conformation" and a comment was made to note the presence of the tumor). Finally, depending on the observer's location in the slaughter facility yards, which was largely determined by facility employees and the necessity of eliminating any chance of the animals noticing the observer, observations were more easily made in some facilities than others.

Since 2013 when FCS became a well-known problem within the industry, some slaughter facilities have implemented mitigation strategies to decrease the prevalence of cattle displaying such signs, including communicating with feedyards about incoming cattle condition, making truck drivers and facility employees aware of the clinical signs of FCS, and contracting with feedyards which employ mitigation strategies as well (Siemens and Alexander, personal communication). Such strategies not only help to decrease the incidence of FCS, but likely serve to promote better health and well-being in all fed cattle transported to slaughter facilities, and the industry should be commended for implementing such practices.

Conclusion

Abnormal mobility scores ($MS \geq 2$) were found to be relatively low compared to animals with an $MS = 1$, and the prevalence of these scores decreased with severity. The most common abnormal MS observed was 2. Only 6 animals of the 65,600 animals observed were reported to have a MS of 4, and only 1 animal was non-ambulatory. Mobility scores did not appear to be affected by ambient temperature, THI, breed, or sex. However, differences in the distance traveled and lairage time at the slaughter facility contributed to differences in the prevalence of abnormal MS .

General soreness/stiffness was the most frequently reported clinical diagnosis, followed by laminitis, lameness, poor conformation, and finally FCS. The most commonly observed clinical signs with regards to decreased mobility were stiffness and shortened strides, followed by long toes, lameness, and muscle tremors. Compared to abnormal mobility, differences in the prevalence of lameness, poor conformation, laminitis, FCS, and general soreness/stiffness seem to be more vulnerable to the effects of the independent variables reported. Differences in the prevalence of laminitis, FCS, and general soreness/stiffness were detected at different distances traveled to the slaughter facilities, but no consistent trend was observed. Lameness and poor conformation differed at different temperatures and THI, while differences in general soreness/stiffness were only observed at different levels of THI. Prevalence of FCS and general soreness/stiffness increased with increasing lairage times, but only up to approximately 4-6h. Dairy cattle were more susceptible to FCS compared to beef breeds. Of the animals displaying mobility score 3 or higher, 50% were diagnosed as having FCS.

The information reported here is both valuable and novel in the fed beef cattle industry. A sample of such magnitude is difficult to find in the literature, and much information can be

gleaned from the observations made in this study, including the contributions of different clinical abnormalities to the mobility of cattle entering commercial slaughter facilities. Along with the implementation of practices that will promote better health and welfare of fed cattle presented to slaughter facilities, measuring MS and identifying the etiologies causing abnormal mobility may help improve animal welfare and strengthen consumer confidence in the industry. Further research is needed to more fully explore the risk factors contributing to decreased mobility in commercial slaughter facilities, but observational studies such as the one described here are important steps in the process of determining the environmental and animal factors upon which that research should be focused.

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Table 3-1. Description of cattle by slaughter facility location in 65,600 finished cattle observed for mobility scores and clinical signs.

	Beef			Dairy¹		
	Heifer	Mixed²		Heifer	Mixed	
Lot		Steer	Lot		Steer	
Facility 1	3827	180	9524	98	0	910
Facility 2	2884	3558	3499	0	0	769
Facility 3	3620	527	6343	0	0	1009
Facility 4	4077	360	4926	0	0	1006
Facility 5	1719	691	5577	0	25	1001
Facility 6	3244	335	3233	265	500	1893

¹ “Dairy” refers to Holstein animals.

² “Mixed” refers to lots of animals which were comprised of both steers and heifers.

Table 3-2. Description of environmental conditions by commercial slaughter facility location in 65,600 finished cattle observed for mobility scores and clinical signs.

	Average distance traveled (mi)¹	Average lairage time (min)²	Flooring type	Average mobility score³
Facility 1	103	223	Grooved concrete	1.03
Facility 2	220	337	Grooved concrete	1.03
Facility 3	75	258	Grooved concrete	1.04
Facility 4	90	392	Grooved concrete	1.03
Facility 5	269	354	Grooved concrete	1.04
Facility 6	134	503	Grooved concrete + rebar	1.03

¹ “Average distance traveled” was defined as miles traveled from the feedyard to the slaughter facility.

² “Average lairage time” is expressed as: {Lairage Time = (time of observation - average time the trucks carrying the lots passed over the slaughter facility scale)}. The measurement includes the time that animals spent on the trucks waiting to be unloaded, time spent on the unloading dock, and time spent in lairage pens.

³ Mobility score (MS) was assigned to each animal observed using the scoring system adopted by the North American Meat Institute, where: 1 = Normal, walks easily with no apparent lameness or change in gait; 2 = Keeps up with normal cattle when the group is walking, exhibits one or more of the following: stiffness, shortened stride, or slight limp; 3 = Lags behind normal cattle when the group is walking, exhibits one or more of the following: obvious stiffness, difficulty taking steps, obvious limp or discomfort; 4 = Extremely reluctant to move, even when encouraged by handlers.

Table 3-3. Prevalence of mobility scores 1, 2, 3, and 4 in 65,600 finished cattle at 6 commercial slaughter facilities.

	Count	Percent
Mobility Score¹ 1	63647	97.02%
Mobility Score 2	1767	2.69%
Mobility Score 3	180	0.27%
Mobility Score 4	6	0.01%

¹ Mobility score (MS) was assigned to each animal observed using the scoring system adopted by the North American Meat Institute, where: 1 = Normal, walks easily with no apparent lameness or change in gait; 2 = Keeps up with normal cattle when the group is walking, exhibits one or more of the following: stiffness, shortened stride, or slight limp; 3 = Lags behind normal cattle when the group is walking, exhibits one or more of the following: obvious stiffness, difficulty taking steps, obvious limp or discomfort; 4 = Extremely reluctant to move, even when encouraged by handlers.

Table 3-4. Prevalence of mobility scores 1, 2, 3, and 4 by commercial slaughter facility in 65,600 finished cattle at 6 commercial slaughter facilities.

	Mobility Score¹ 1		Mobility Score 2		Mobility Score 3		Mobility Score 4		Total	
	Count	Percent	Count	Percent	Count	Percent	Count	Percent	Count	Percent
Facility 1	14098	96.97%	389	2.68%	51	0.35%	1	0.01%	14539	100.00%
Facility 2	10403	97.13%	282	2.63%	25	0.23%	0	0.00%	10710	100.00%
Facility 3	11119	96.70%	342	2.97%	37	0.32%	1	0.01%	11499	100.00%
Facility 4	10093	97.34%	265	2.56%	10	0.10%	1	0.01%	10369	100.00%
Facility 5	8713	96.67%	255	2.83%	43	0.48%	2	0.02%	9013	100.00%
Facility 6	9221	97.37%	234	2.47%	14	0.15%	1	0.01%	9470	100.00%
Total	63647	97.02%	1767	2.69%	180	0.27%	6	0.01%	65600	100.00%

¹ Mobility score (MS) was assigned to each animal observed using the scoring system adopted by the North American Meat Institute, where: 1 = Normal, walks easily with no apparent lameness or change in gait; 2 = Keeps up with normal cattle when the group is walking, exhibits one or more of the following: stiffness, shortened stride, or slight limp; 3 = Lags behind normal cattle when the group is walking, exhibits one or more of the following: obvious stiffness, difficulty taking steps, obvious limp or discomfort; 4 = Extremely reluctant to move, even when encouraged by handlers.

Table 3-5. Description of gender and breed by mobility score in 65,600 finished cattle at 6 commercial slaughter facilities.

	Beef			Dairy ¹		
	Heifer	Mixed lot ²	Steer	Heifer	Mixed lot	Steer
Mobility Score³ 1	18862	5485	32069	354	513	6364
Mobility Score 2	476	154	924	7	10	196
Mobility Score 3	33	12	104	2	2	27
Mobility Score 4	0	0	5	0	0	1

¹ “Dairy” refers to Holstein animals.

² “Mixed lot” refers to animals which came in lots comprised of both heifers and steers.

³ Mobility score (MS) was assigned to each animal observed using the scoring system adopted by the North American Meat Institute, where: 1 = Normal, walks easily with no apparent lameness or change in gait; 2 = Keeps up with normal cattle when the group is walking, exhibits one or more of the following: stiffness, shortened stride, or slight limp; 3 = Lags behind normal cattle when the group is walking, exhibits one or more of the following: obvious stiffness, difficulty taking steps, obvious limp or discomfort; 4 = Extremely reluctant to move, even when encouraged by handlers.

Table 3-6. Prevalence of animals displaying abnormal mobility scores (MS \geq 2) categorized by clinical diagnosis in 65,600 finished cattle at 6 commercial slaughter facilities.

Clinical Diagnosis	Count	Percent of Total Observations
Lameness¹	153	0.23%
Poor Conformation²	130	0.20%
Laminitis³	471	0.72%
FCS⁴	94	0.14%
General Soreness/Stiffness⁵	1105	1.68%

¹ Lameness/injury was defined as obvious lameness on one or more limbs caused by broken toes or legs, or any shoulder or rear leg injuries.

² Poor conformation was defined as abnormalities in shape or structure of legs and feet which contribute significantly to the animal’s decreased mobility.

³ Laminitis was defined as founder/laminitis including animals with abnormally long hooves, animals walking on their heels, presence of cracked hooves, or possibly sloughed hoof walls.

⁴ Fatigued Cattle Syndrome was defined as animals with abnormal mobility, with clinical signs not due to injury or founder, including but not limited to nervous system abnormalities such as muscle tremors, increased respiratory rate, increased vocalization, obvious stiffness, and shortened strides.

⁵ General soreness/stiffness was recorded when animals presented with decreased mobility not due to any obvious disease, injury, or syndrome. Sore and stiff cattle displayed normal behavior with the exception of decreased mobility or range of movement.

Table 3-7. Prevalence of animals displaying abnormal mobility scores (MS \geq 2) categorized by clinical diagnosis in 65,600 finished cattle at 6 commercial slaughter facilities.

	Mobility Score ¹		
	2 (n=1767)	3 (n=180)	4 (n=6)
Lameness²	132	19	2
Poor Conformation³	121	9	0
Laminitis⁴	423	47	1
FCS⁵	1	90	3
General Soreness/stiffness⁶	1090	15	1

¹ Mobility score (MS) was assigned to each animal observed using the scoring system adopted by the North American Meat Institute, where: 1 = Normal, walks easily with no apparent lameness or change in gait; 2 = Keeps up with normal cattle when the group is walking, exhibits one or more of the following: stiffness, shortened stride, or slight limp; 3 = Lags behind normal cattle when the group is walking, exhibits one or more of the following: obvious stiffness, difficulty taking steps, obvious limp or discomfort; 4 = Extremely reluctant to move, even when encouraged by handlers.

² Lameness/injury was defined as obvious lameness on one or more limbs caused by broken toes or legs, or any shoulder or rear leg injuries.

³ Poor conformation was defined as abnormalities in shape or structure of legs and feet which contribute significantly to the animal's decreased mobility.

⁴ Laminitis was defined as founder/laminitis including animals with abnormally long hooves, animals walking on their heels, presence of cracked hooves, or possibly sloughed hoof walls.

⁵ Fatigued Cattle Syndrome was defined as animals with abnormal mobility, with clinical signs not due to injury or founder, including but not limited to nervous system abnormalities such as muscle tremors, increased respiratory rate, increased vocalization, obvious stiffness, and shortened strides.

⁶ General soreness/stiffness was recorded when animals presented with decreased mobility not due to any obvious disease, injury, or syndrome. Sore and stiff cattle displayed normal behavior with the exception of decreased mobility or range of movement.

Table 3-8. Number of animals displaying clinical signs within each clinical diagnosis category in 65,600 finished cattle at 6 commercial slaughter facilities.

	Lameness ¹ (n=153)	Poor conformation ² (n=130)	Laminitis ³ (n=471)	FCS ⁴ (n=94)	General soreness/stiffness ⁵ (n=1106)
Lame	151	0	0	0	2
Broken leg	1	0	0	0	0
Broken toe	3	0	2	0	0
Sloughed hoof	0	0	0	0	0
Long toes	2	2	466	7	5
Shortened strides	10	105	420	92	1004
Walking on heels	0	1	59	0	0
Increased respiratory rate	1	0	1	41	13
Muscle tremors	3	1	1	82	9
Stiffness	4	41	41	91	1042
Vocalization	0	0	0	0	0
Nonambulatory	0	0	0	1	0

¹Lameness/injury was defined as obvious lameness on one or more limbs caused by broken toes or legs, or any shoulder or rear leg injuries.

²Poor conformation was defined as abnormalities in shape or structure of legs and feet which contribute significantly to the animal's decreased mobility.

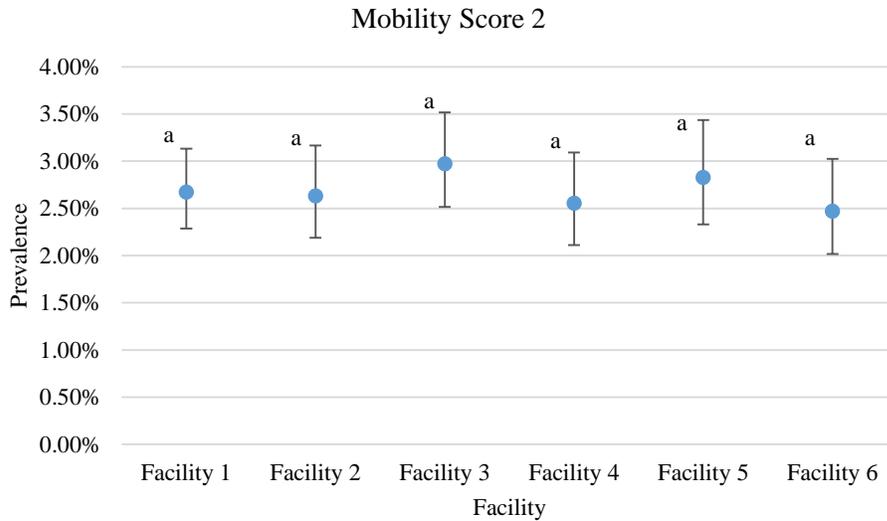
³Laminitis was defined as founder/laminitis including animals with abnormally long hooves, animals walking on their heels, presence of cracked hooves, or possibly sloughed hoof walls.

⁴Fatigued Cattle Syndrome was defined as animals with abnormal mobility, with clinical signs not due to injury or founder, including but not limited to nervous system abnormalities such as muscle tremors, increased respiratory rate, increased vocalization, obvious stiffness, and shortened strides.

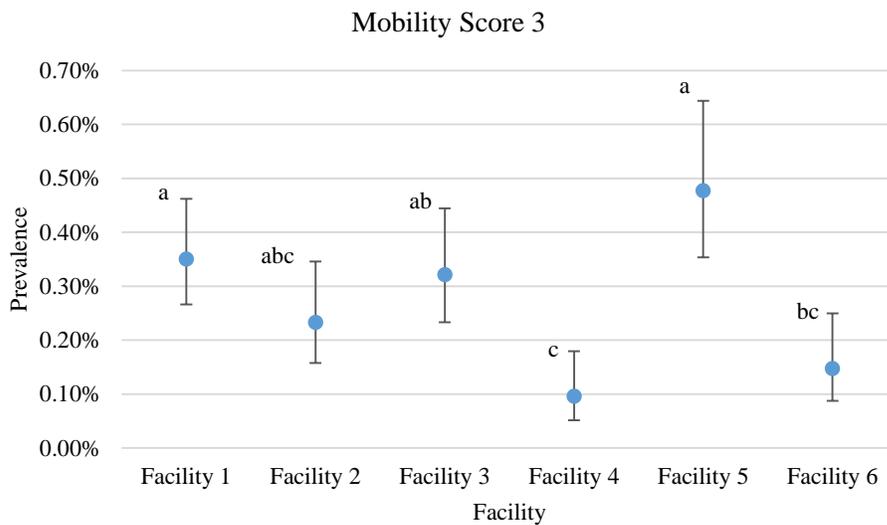
⁵General soreness/stiffness was recorded when animals presented with decreased mobility not due to any obvious disease, injury, or syndrome. Sore and stiff cattle displayed normal behavior with the exception of decreased mobility or range of movement.

Figure 3-1. Comparisons of point estimates for abnormal mobility score prevalence (scores 2, 3, and 4 as Figures A, B, and C, respectively) categorized by facility location in 65,600 finished cattle at 6 commercial slaughter facilities. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$).

A.



B.



C.

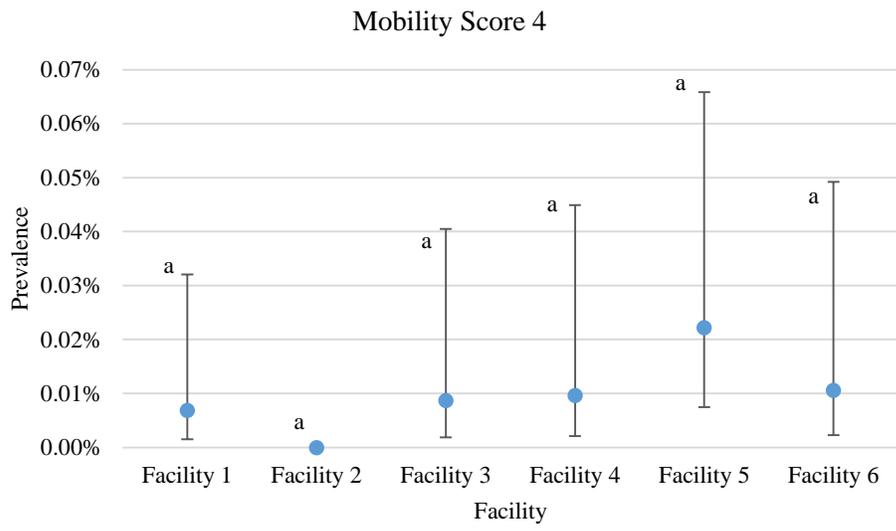
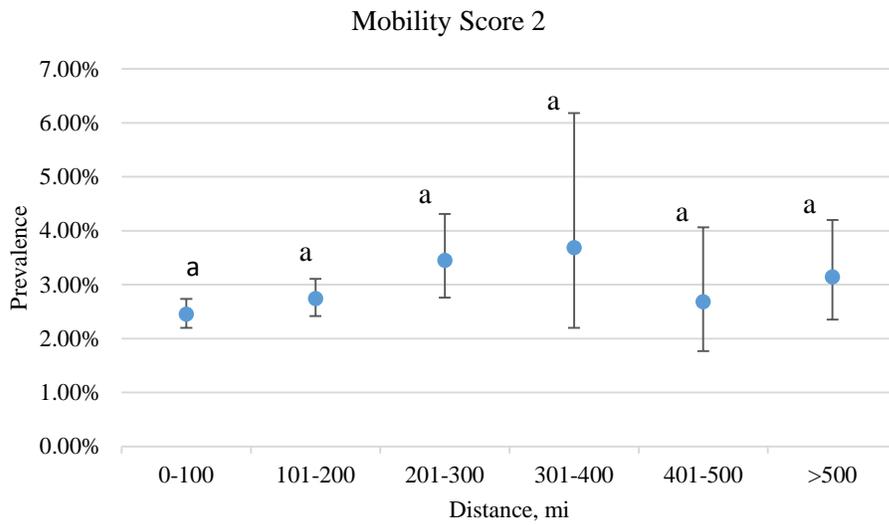
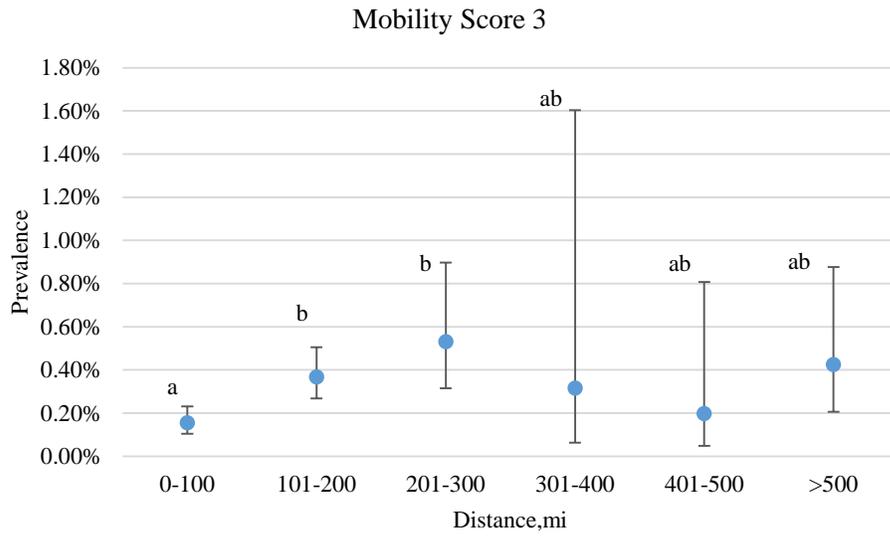


Figure 3-2. Comparisons of point estimates for abnormal mobility score prevalence (scores 2, 3, and 4 as Figures A, B, and C, respectively) categorized by distance traveled in 65,600 finished cattle at 6 commercial slaughter facilities. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$).

A.



B.



C.

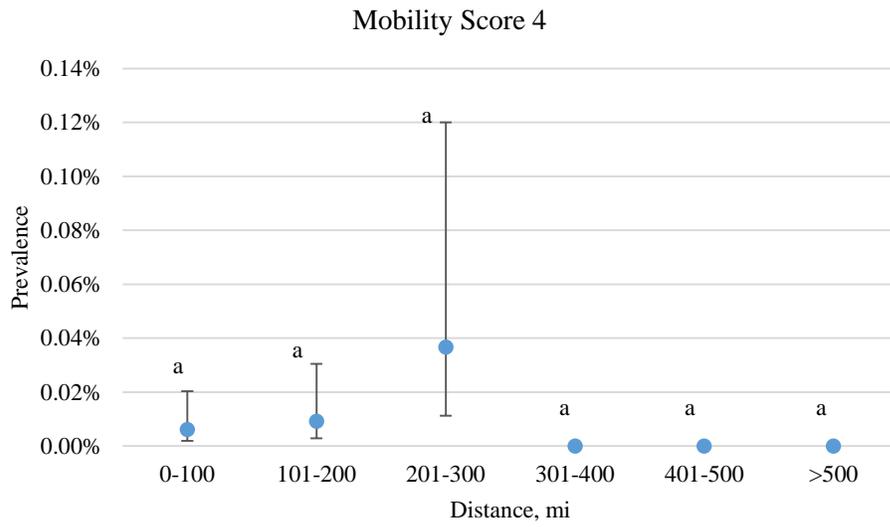
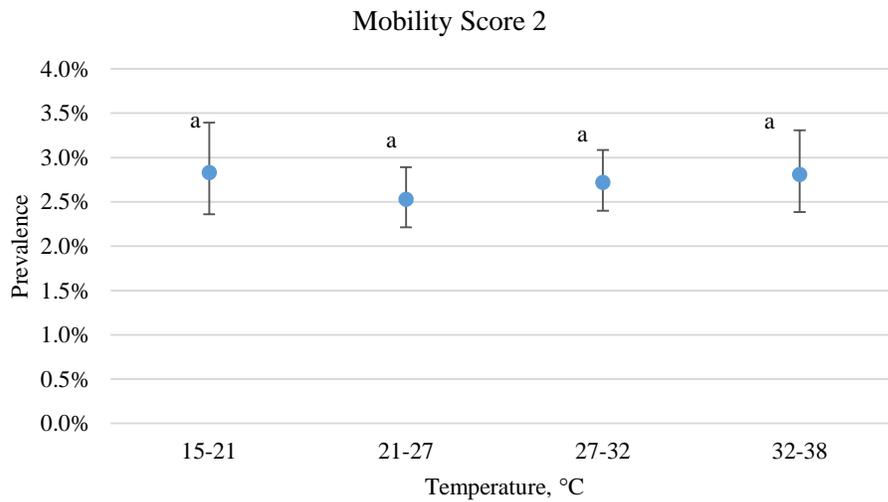
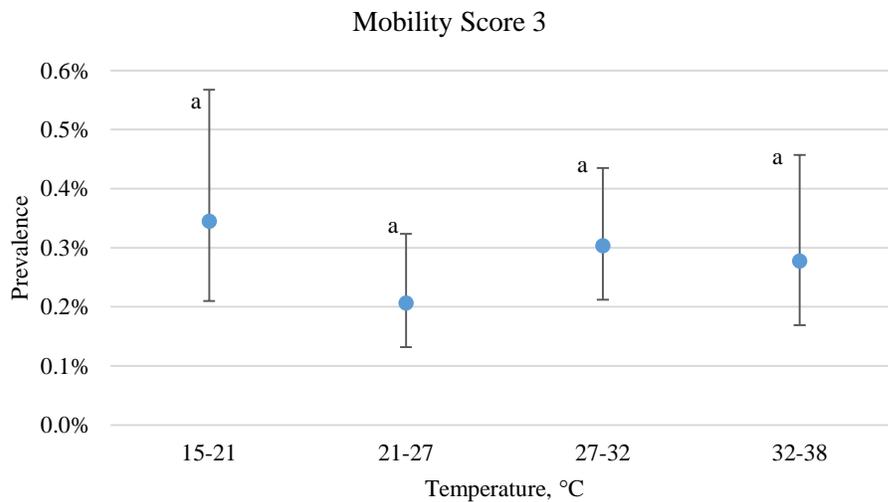


Figure 3-3. Comparisons of point estimates for abnormal mobility score prevalence (scores 2, 3, and 4 as Figures A, B, and C, respectively) categorized by temperature ($^{\circ}\text{C}$) in 65,600 finished cattle at 6 commercial slaughter facilities. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$).

A.



B.



C.

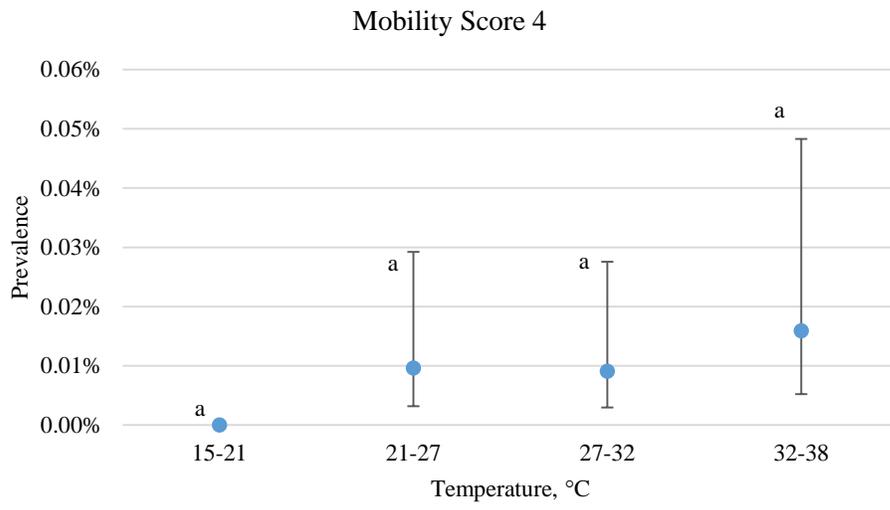
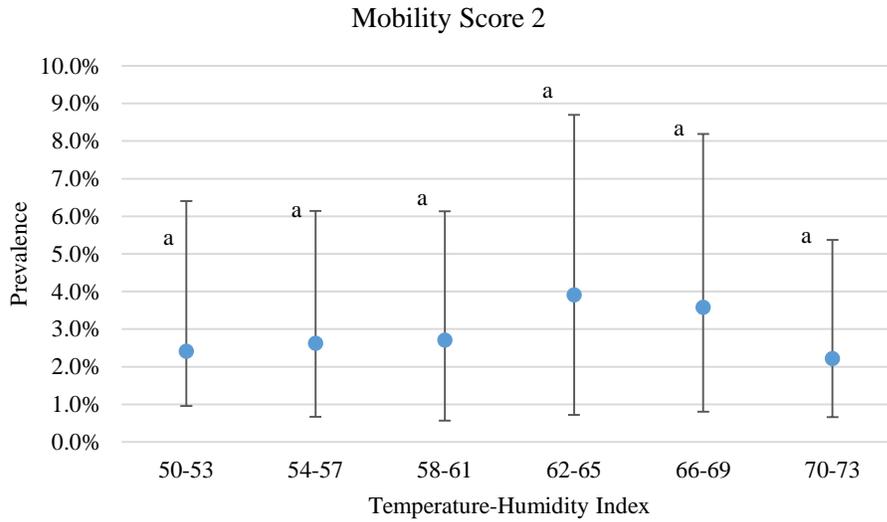
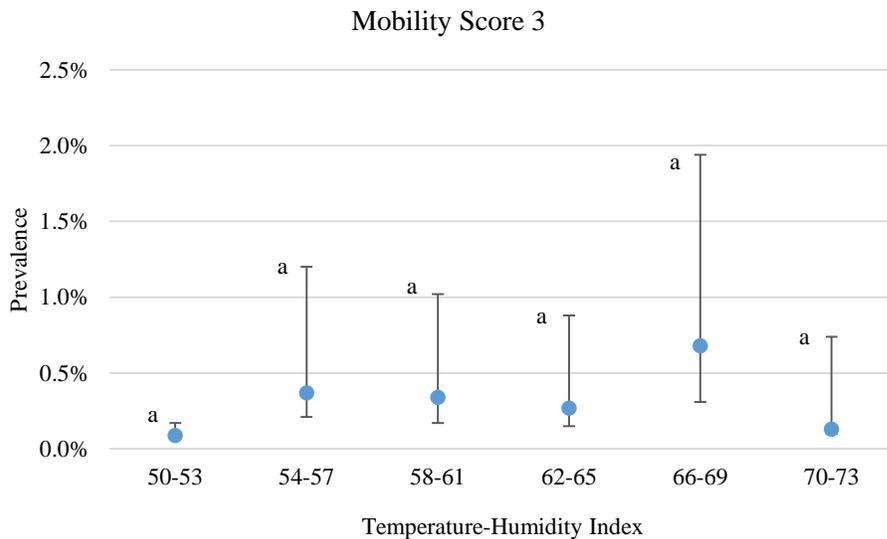


Figure 3-4. Comparisons of point estimates for abnormal mobility score prevalence (scores 2, 3, and 4 as Figures A, B, and C, respectively) categorized by temperature-humidity index (THI) in 65,600 finished cattle at 6 commercial slaughter facilities. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$).

A.



B.



C.

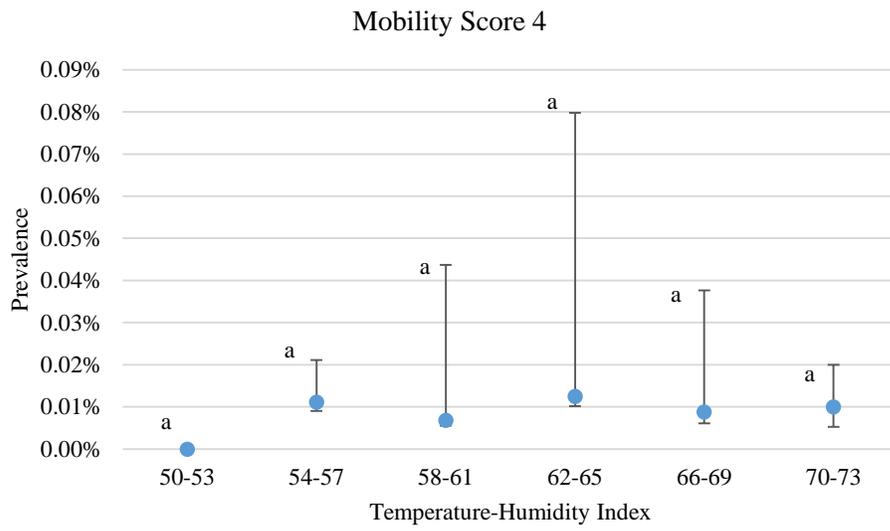
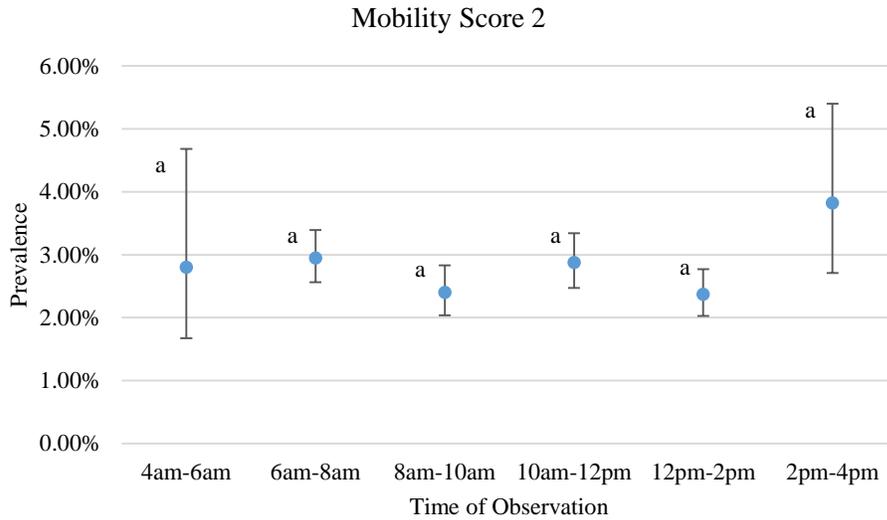
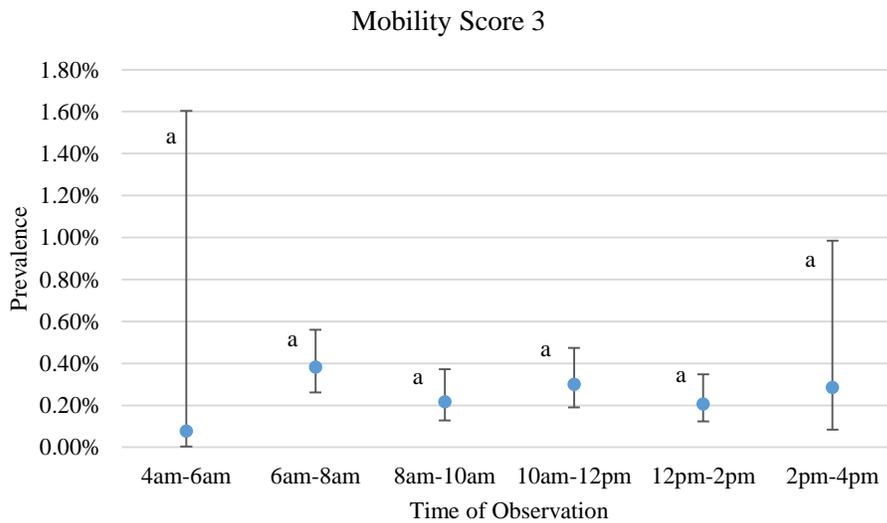


Figure 3-5. Comparisons of point estimates for abnormal mobility score prevalence (scores 2, 3, and 4 as Figures A, B, and C, respectively) categorized by time observed in 65,600 finished cattle at 6 commercial slaughter facilities. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$).

A.



B.



C.

Mobility Score 4

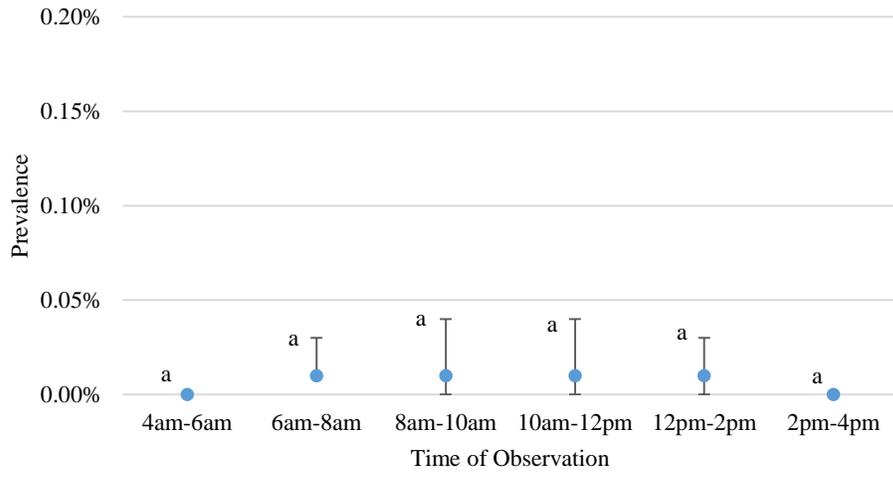
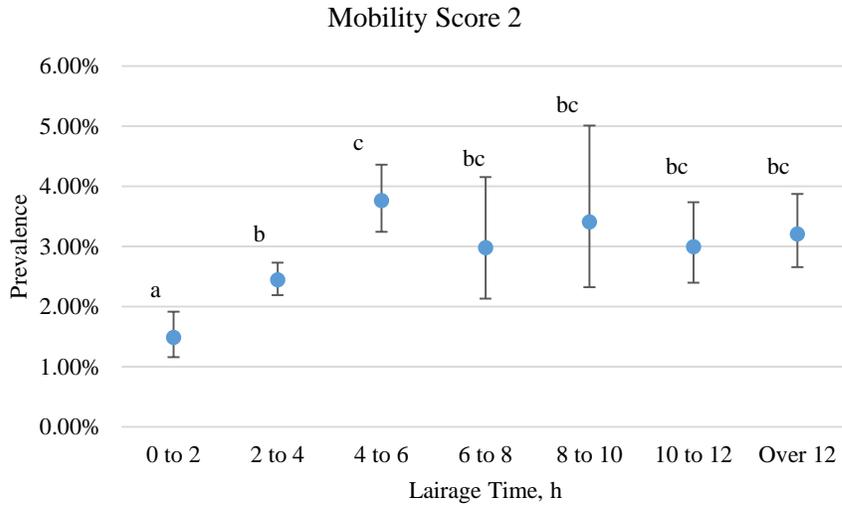
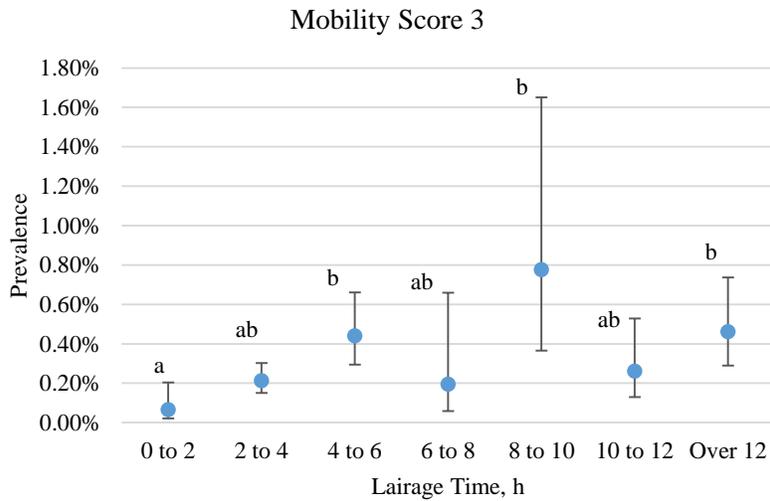


Figure 3-6. Comparisons of point estimates for abnormal mobility score prevalence (scores 2, 3, and 4 as Figures A, B, and C, respectively) categorized by average lairage time (h) in 65,600 finished cattle at 6 commercial slaughter facilities. Lairage time is defined as {Lairage Time = (time of observation - average time the trucks carrying the lots passed over the slaughter facility scale)}. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$).

A.



B.



C.

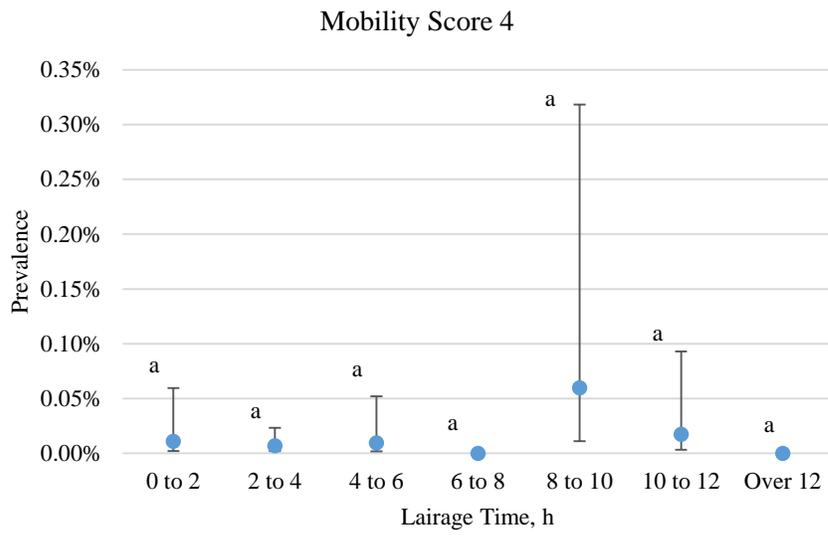
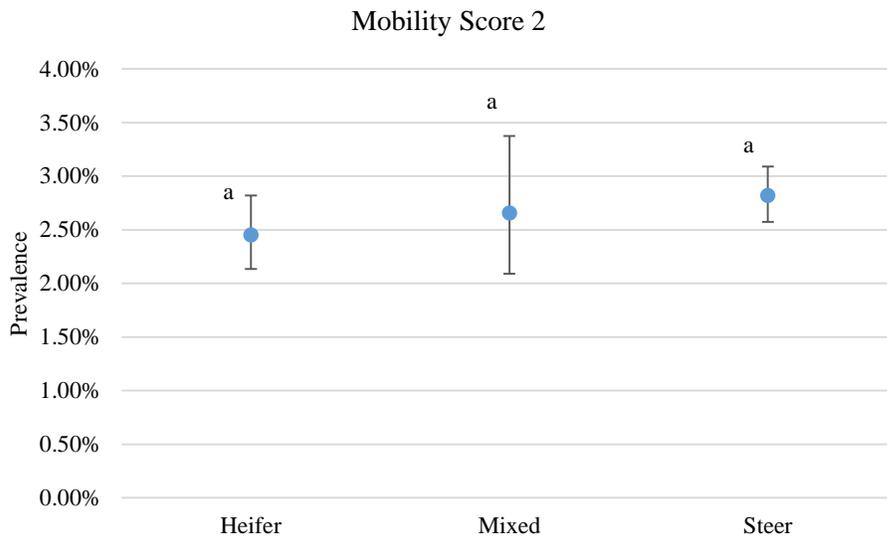


Figure 3-7. Comparisons of point estimates for abnormal mobility score prevalence (scores 2 and 3, Figures A and B, respectively) categorized by sex in 65,600 finished cattle at 6 commercial slaughter facilities. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$).

A.



B.

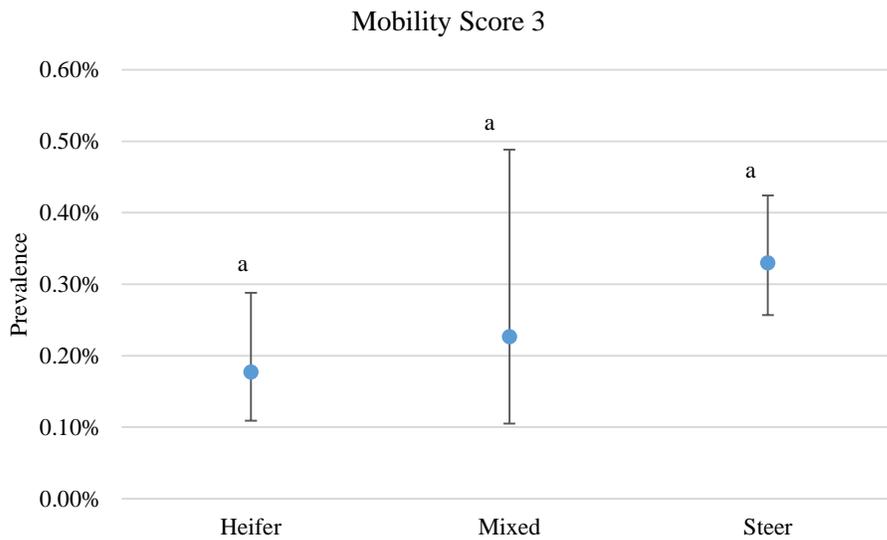
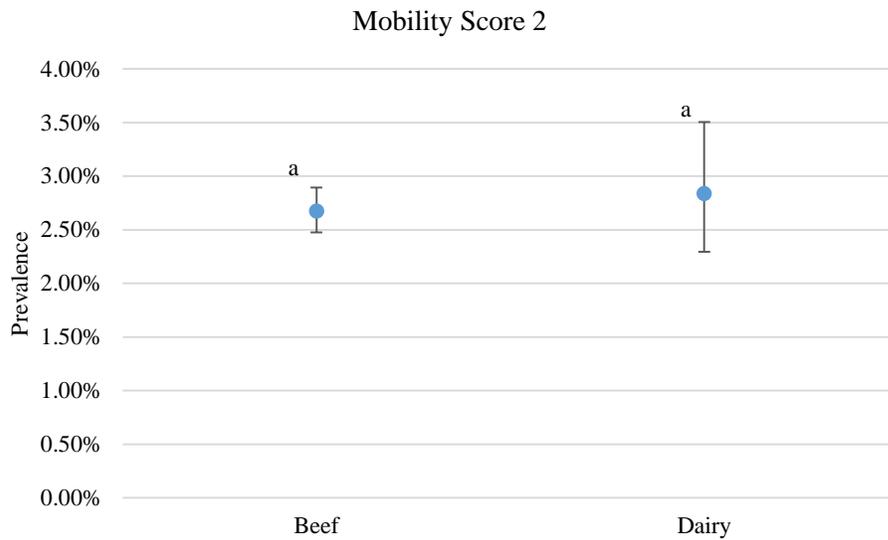
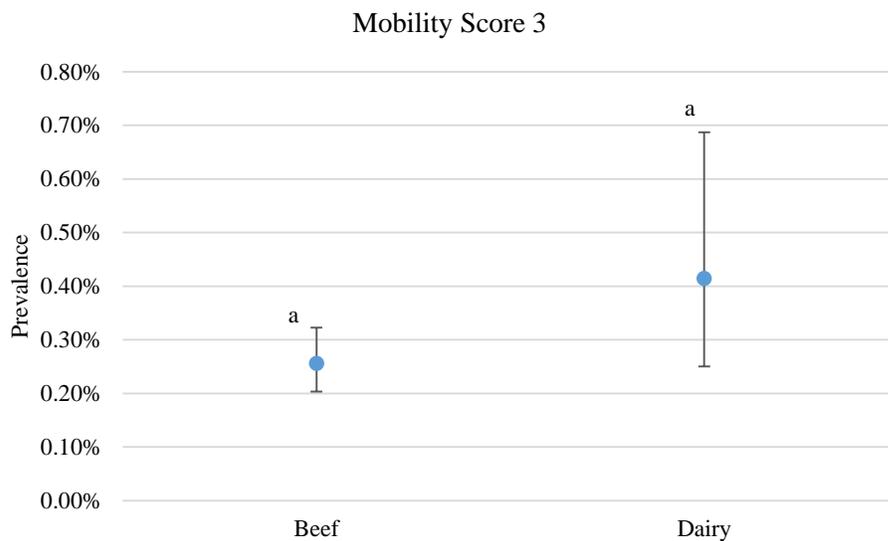


Figure 3-8. Comparisons of point estimates for abnormal mobility score prevalence (scores 2, 3, and 4 as Figures A, B, and C, respectively) categorized by breed in 65,600 finished cattle at 6 commercial slaughter facilities. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$).

A.



B.



C.

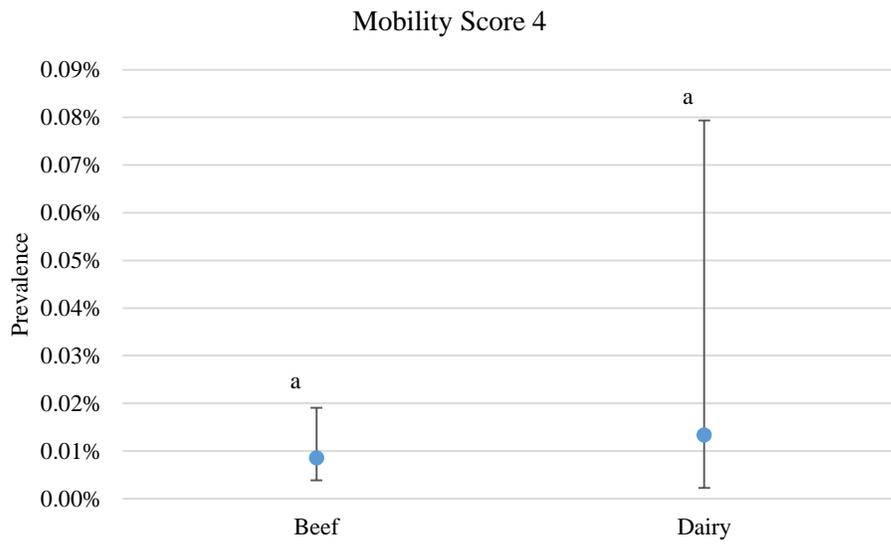
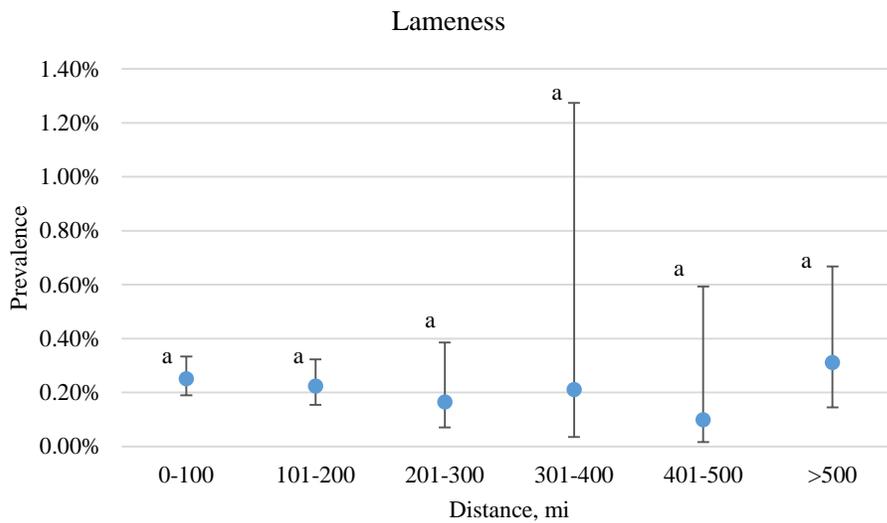
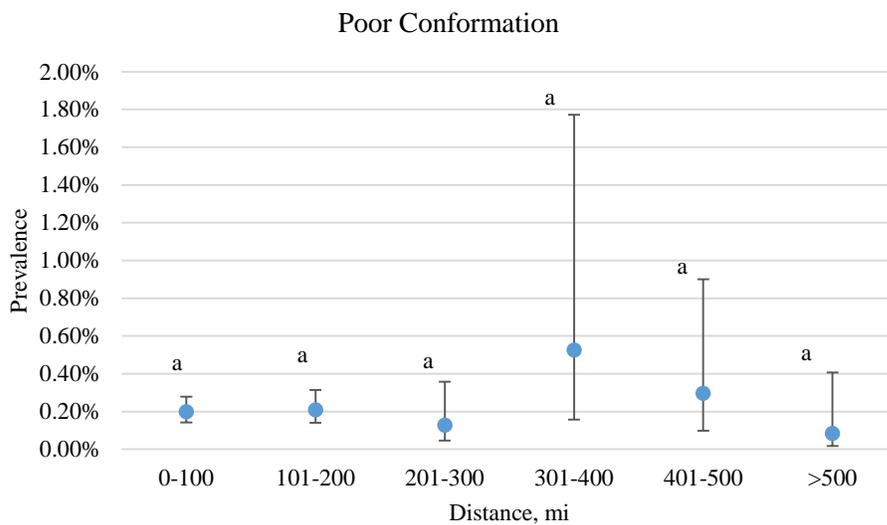


Figure 3-9. Comparisons of point estimates for clinical diagnoses (Figures A, B, C, D, and E) categorized by distance traveled (mi) in 65,600 finished cattle at 6 commercial slaughter facilities. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$).

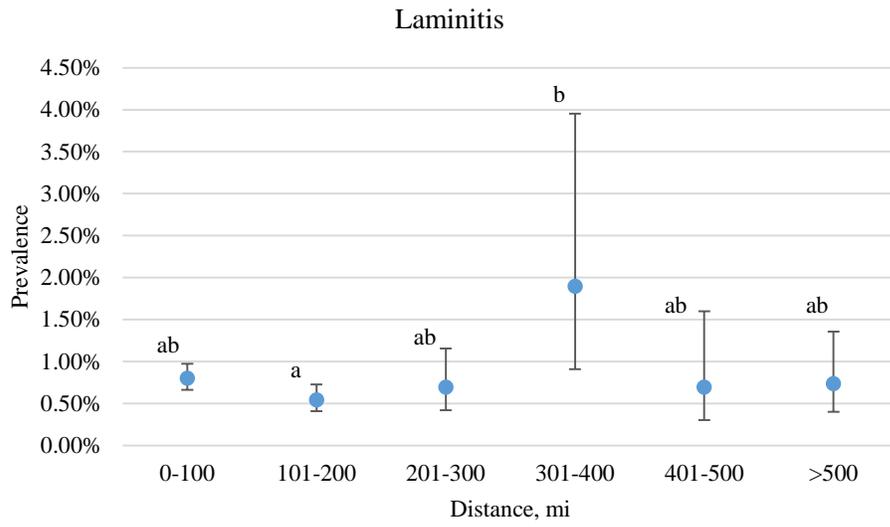
A.



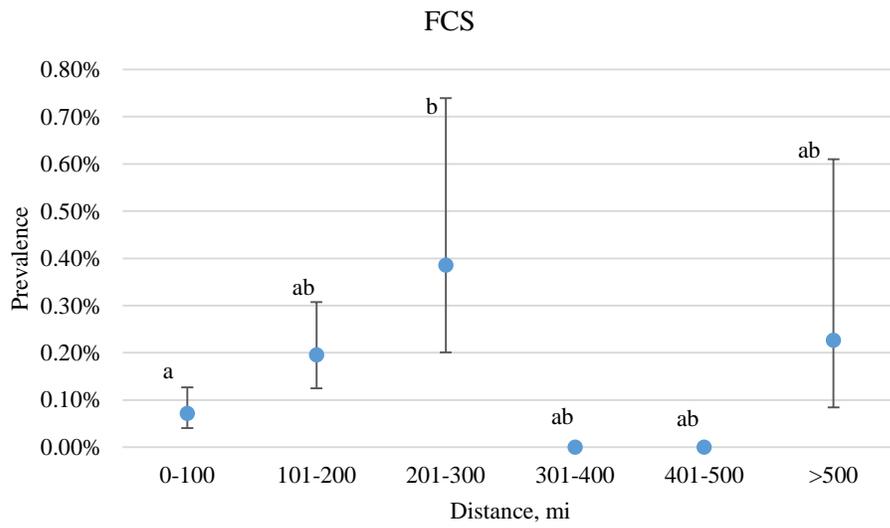
B.



C.



D.



E.

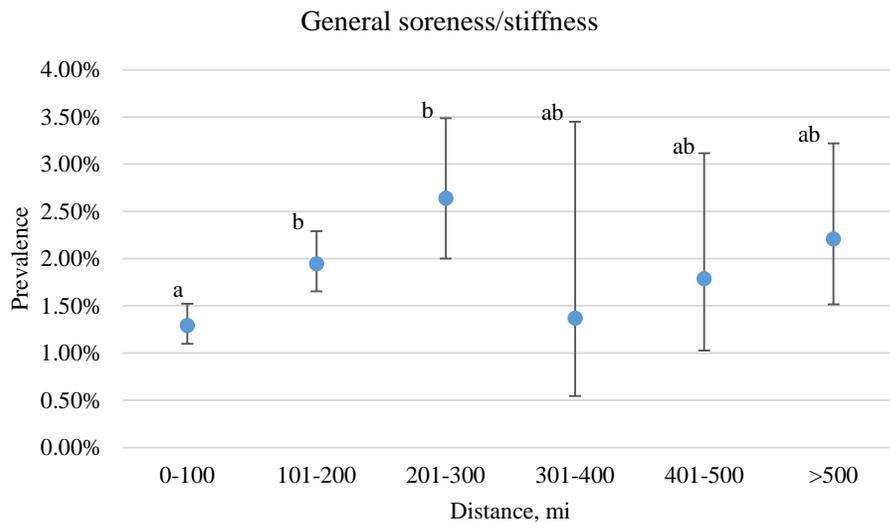
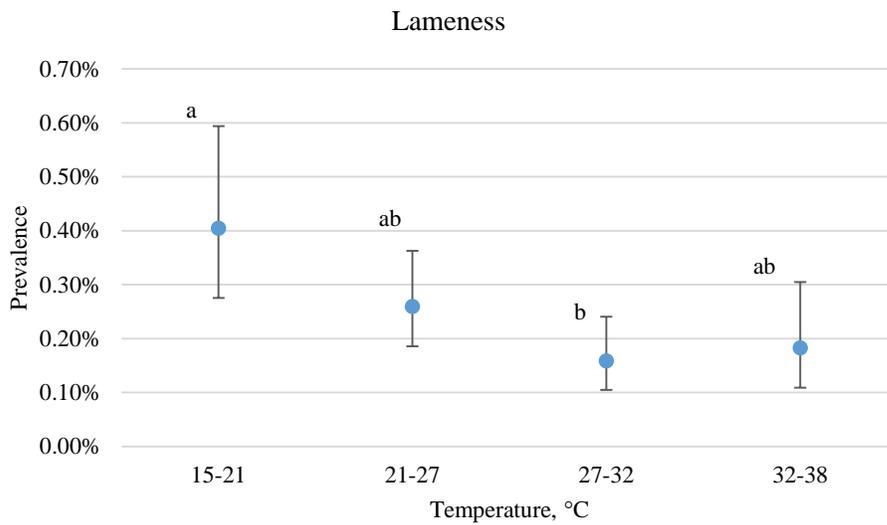
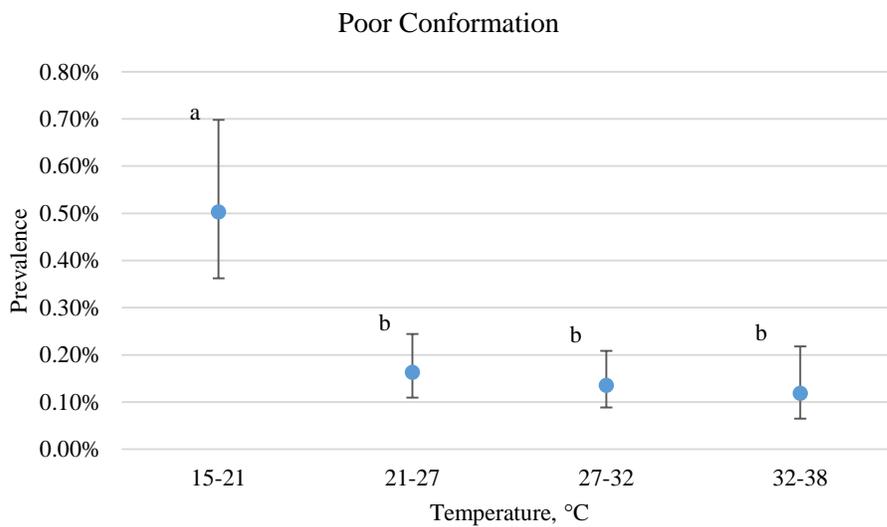


Figure 3-10. Comparisons of point estimates for clinical diagnoses (Figures A, B, C, D, and E) categorized by temperature (°F) in 65,600 finished cattle at 6 commercial slaughter facilities. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$).

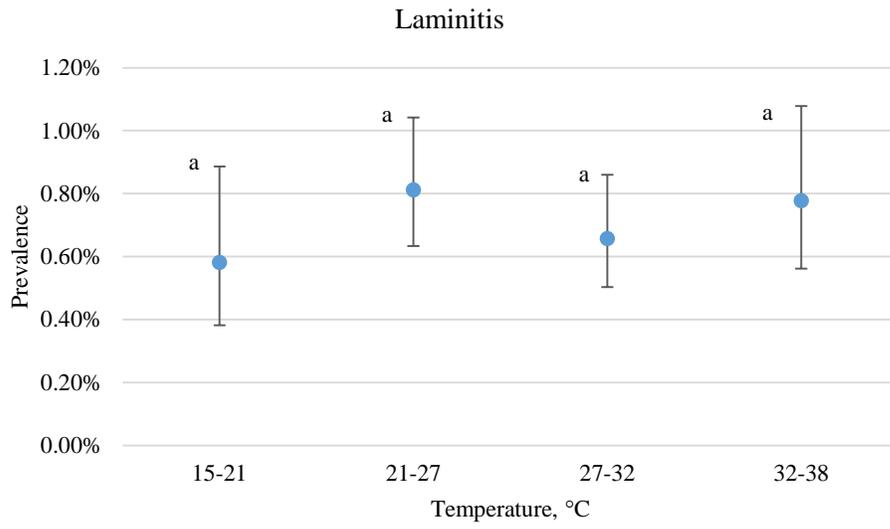
A.



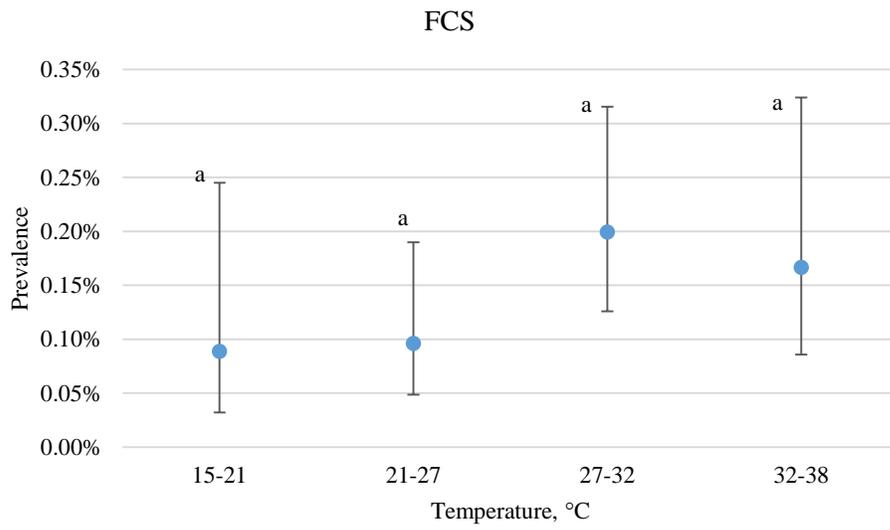
B.



C.



D.



E.

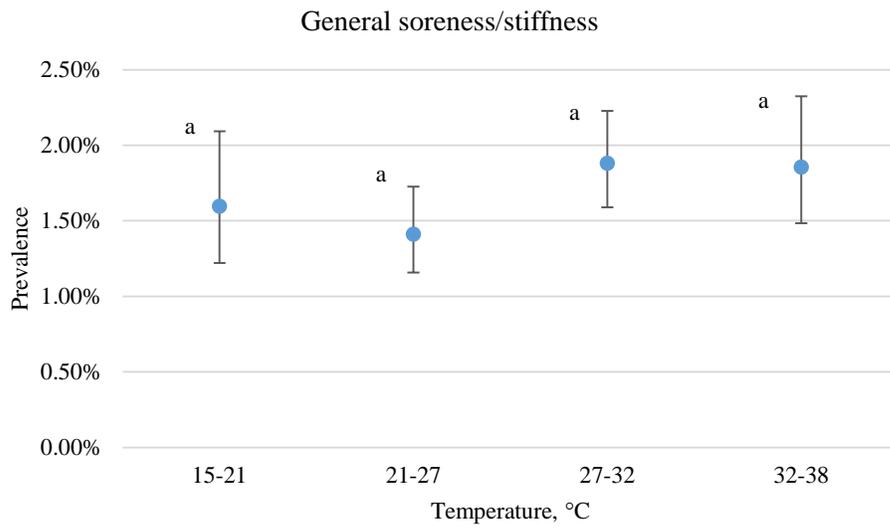
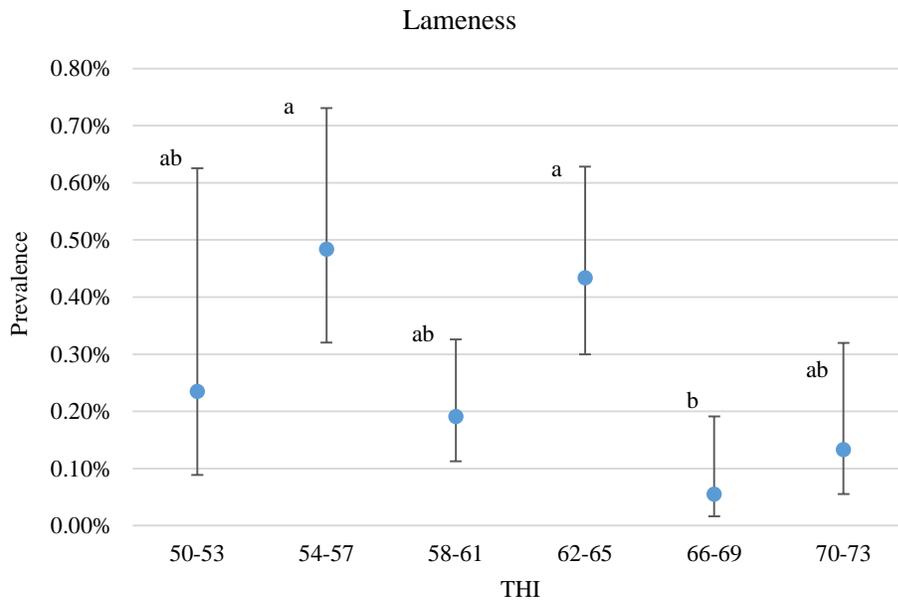
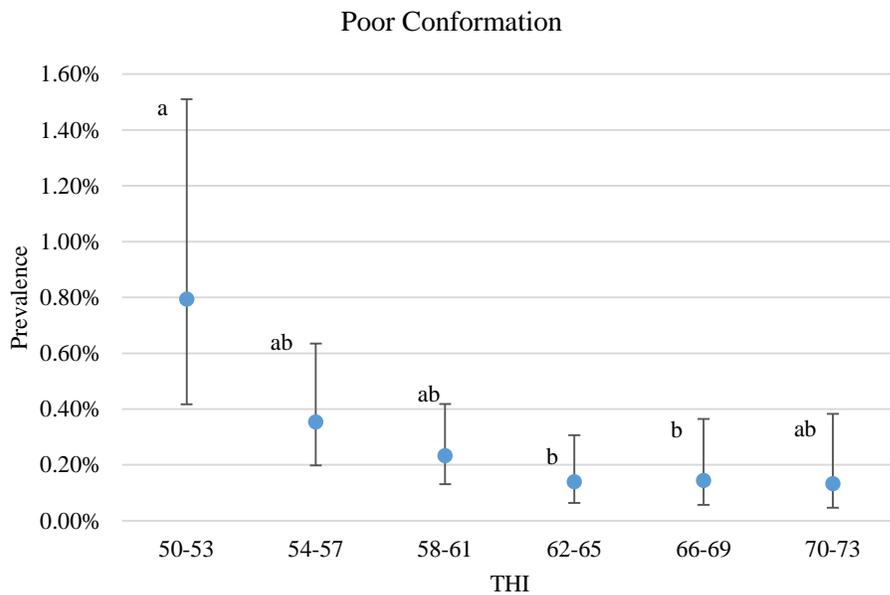


Figure 3-11. Comparisons of point estimates for clinical diagnoses (Figures A, B, C, D, and E) categorized by time of observation in 65,600 finished cattle at 6 commercial slaughter facilities. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$).

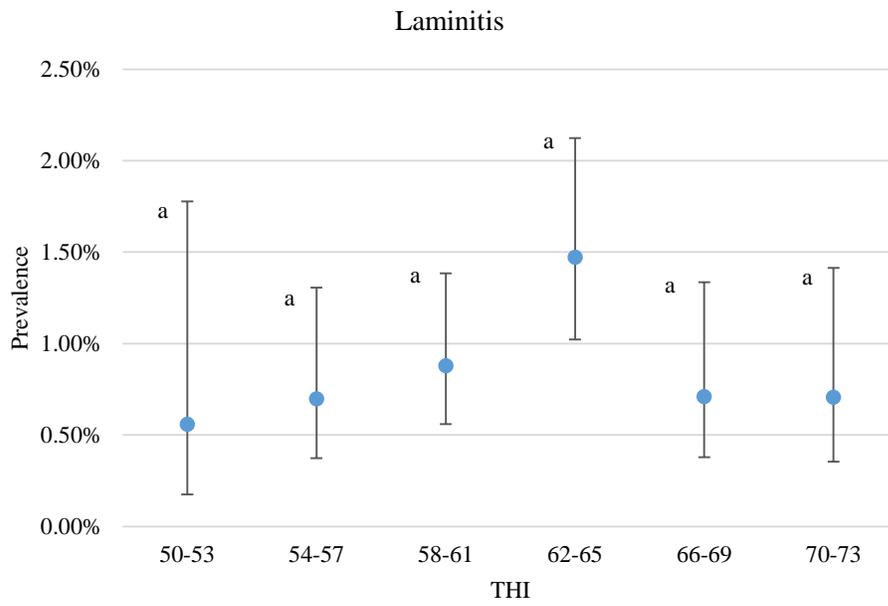
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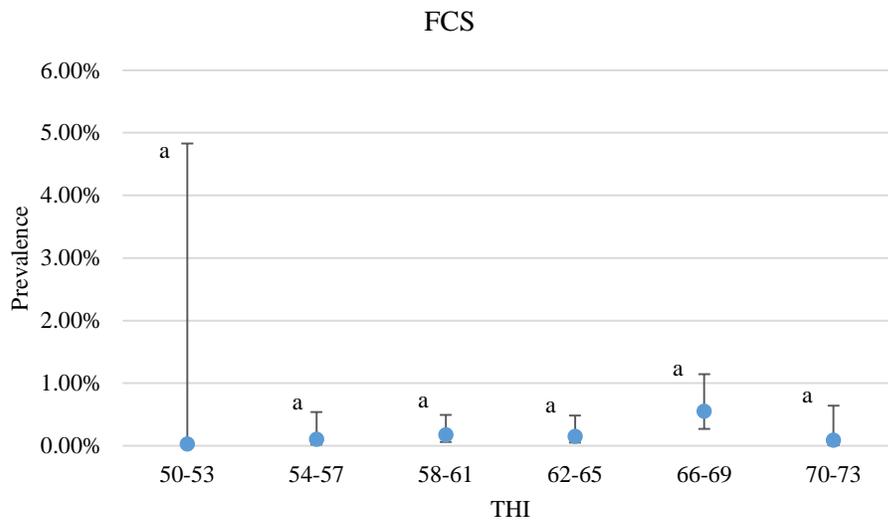
B.



C.



D.



E.

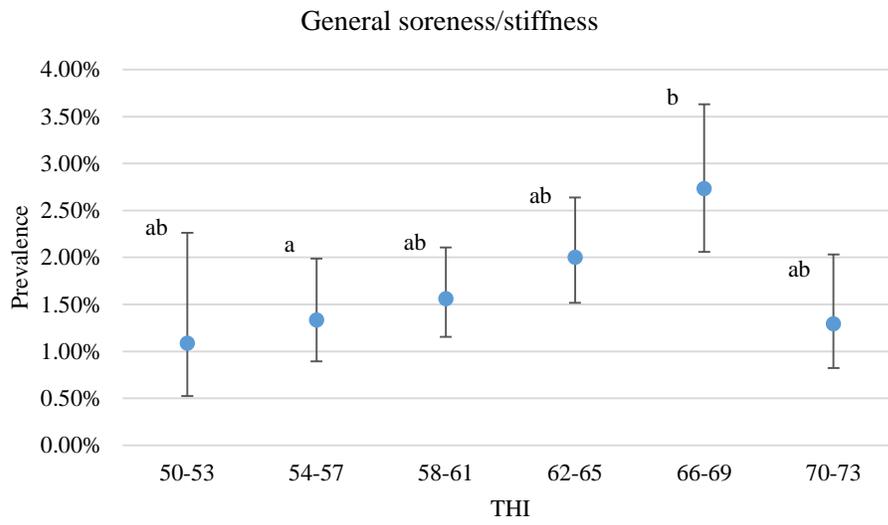
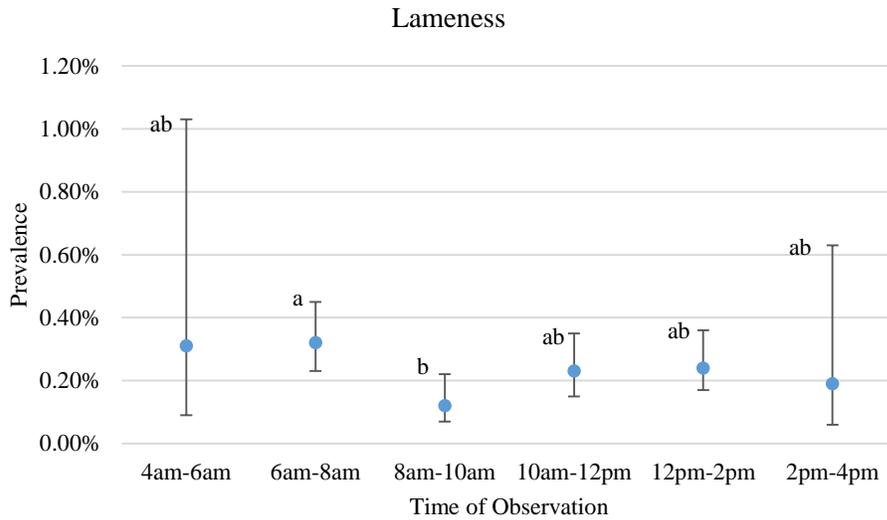
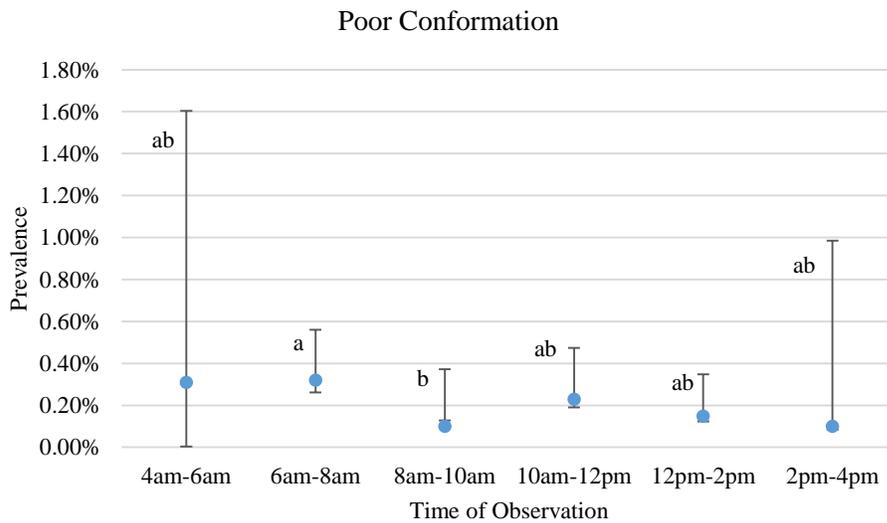


Figure 3-12. Comparisons of point estimates for clinical diagnoses (Figures A, B, C, D, and E) categorized by time of observation in 65,600 finished cattle at 6 commercial slaughter facilities. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$).

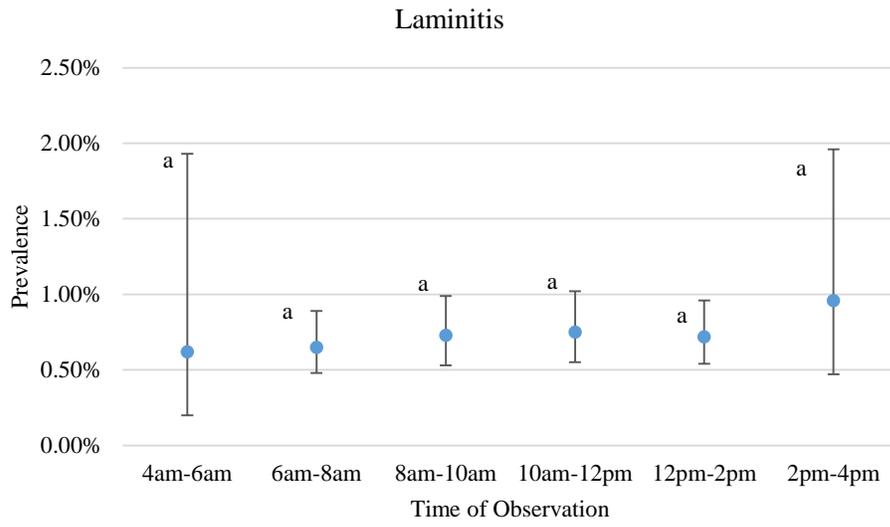
A.



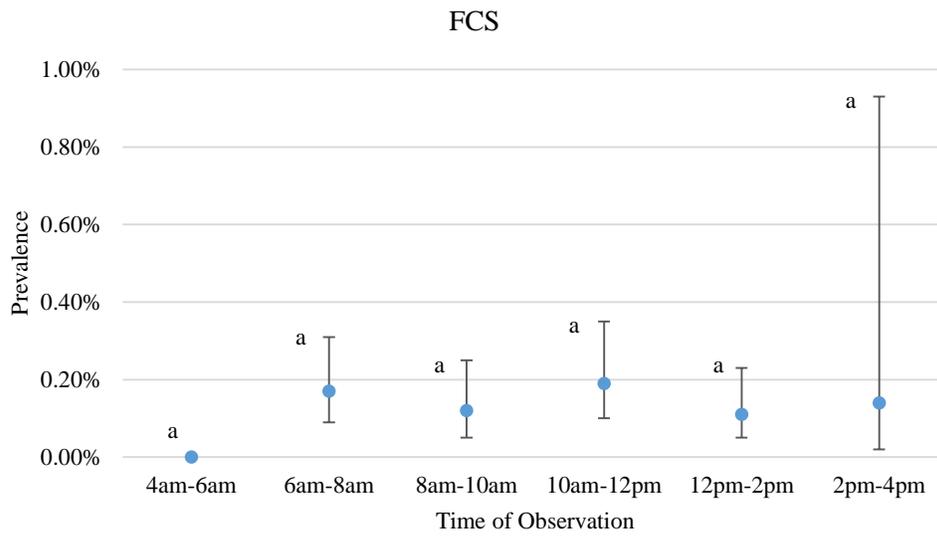
B.



C.



D.



E.

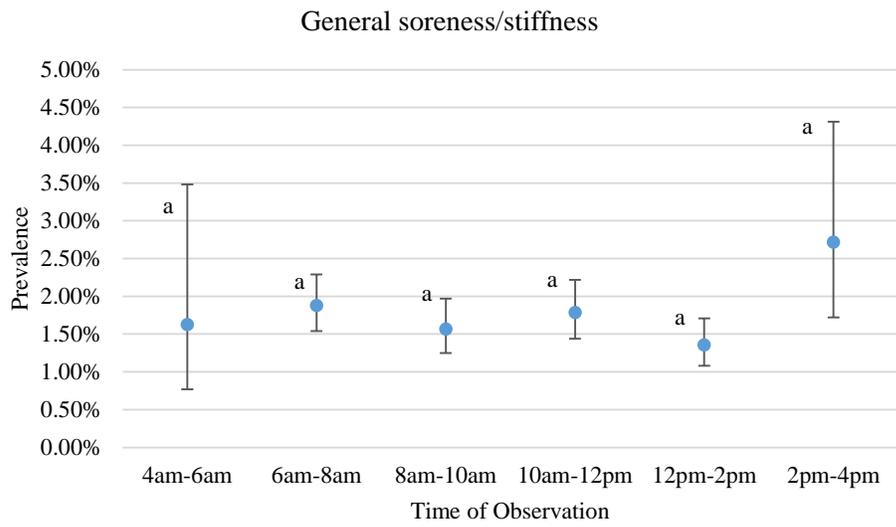
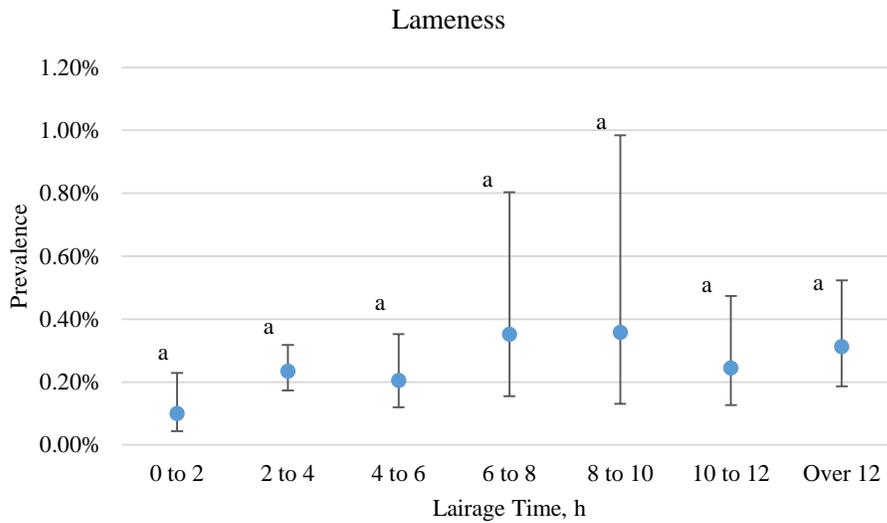
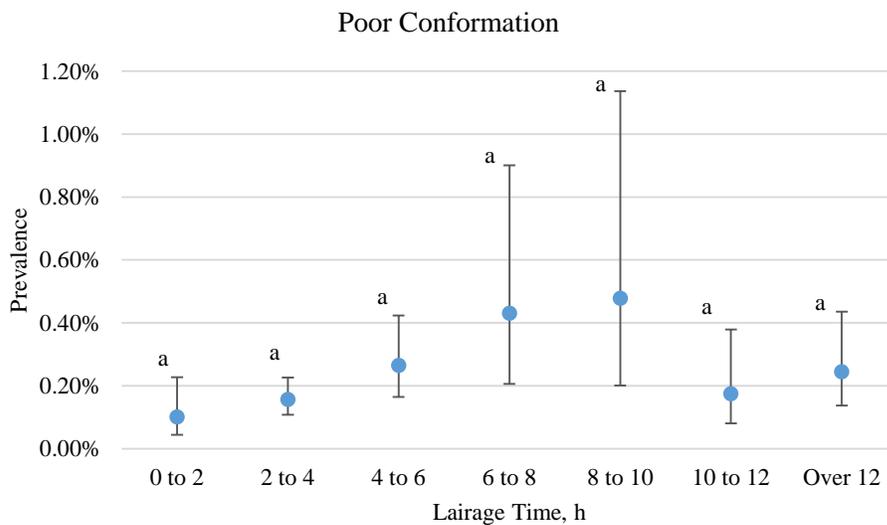


Figure 3-13. Comparisons of point estimates for clinical diagnoses (Figures A, B, C, D, and E) categorized by average lairage time in 65,600 finished cattle at 6 commercial slaughter facilities. Lairage time is defined as {Lairage Time = (time of observation - average time the trucks carrying the lots passed over the slaughter facility scale)}. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$).

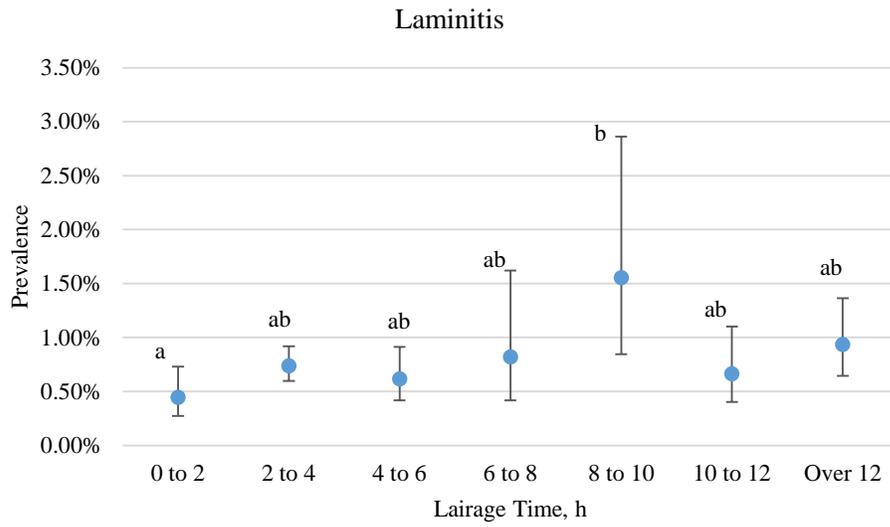
A.



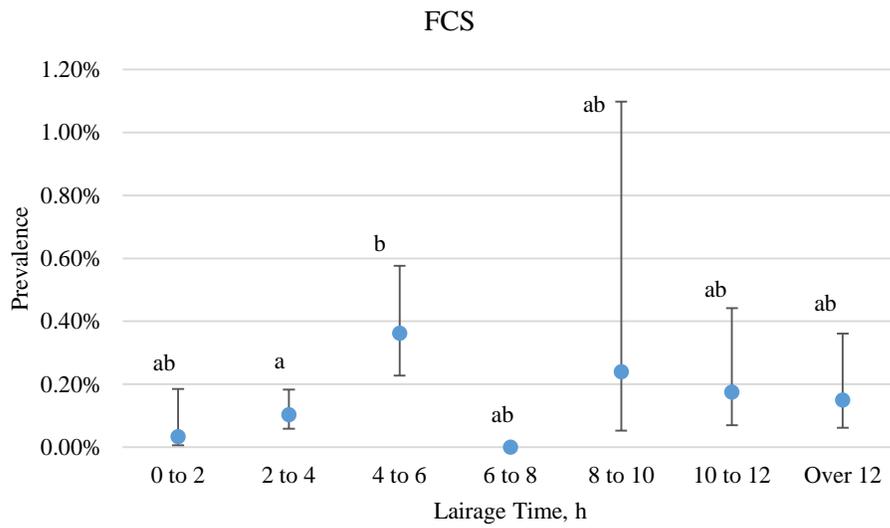
B.



C.



D.



E.

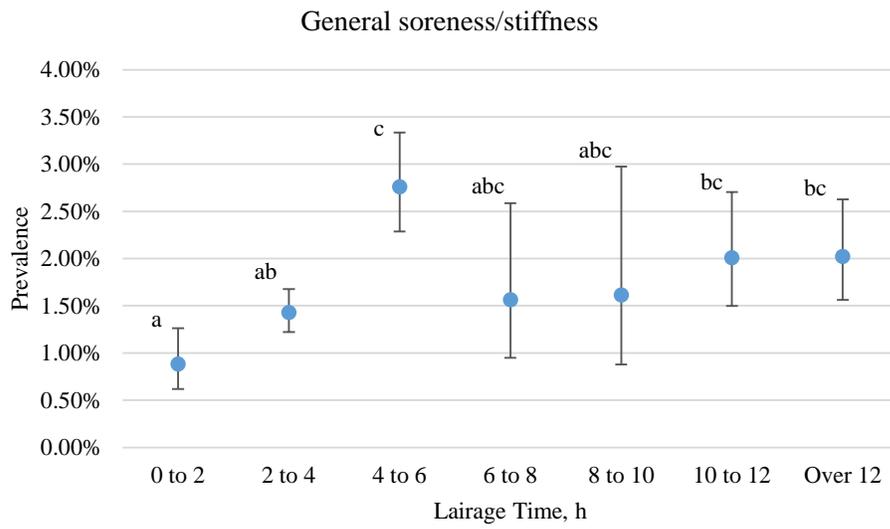
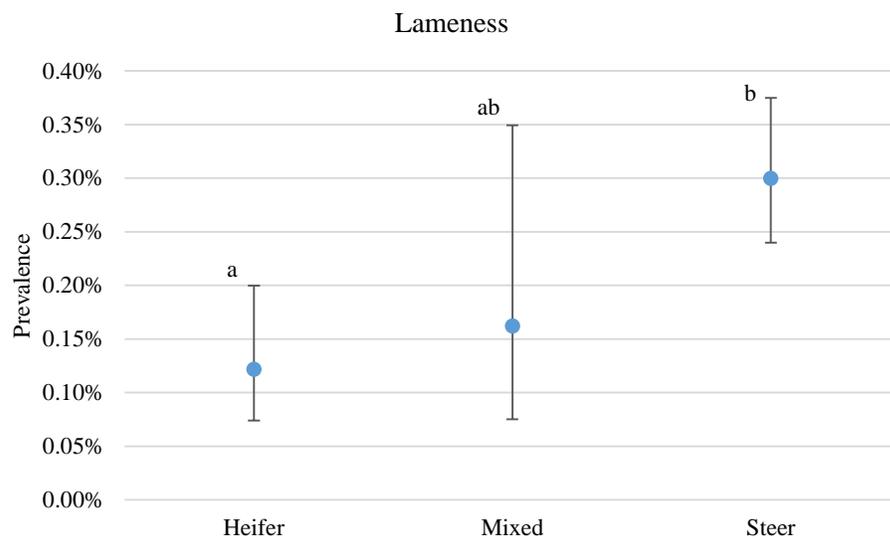
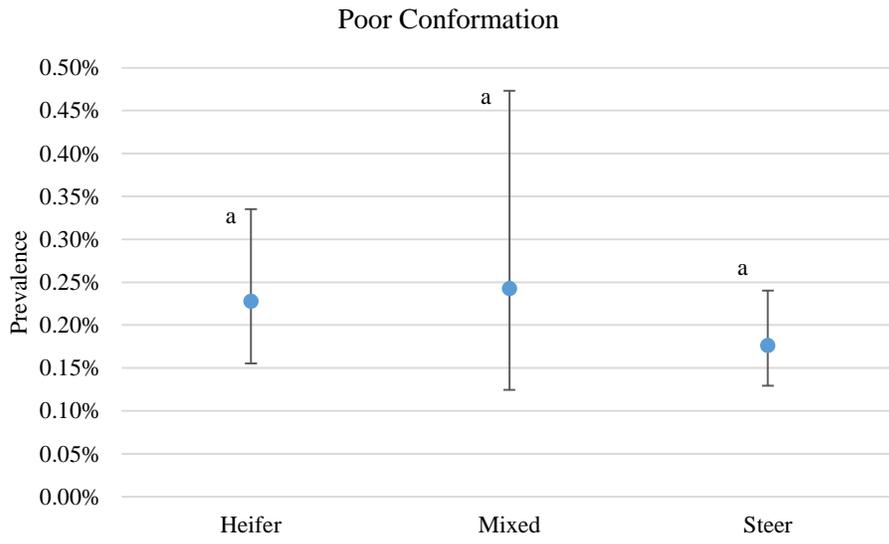


Figure 3-14. Comparisons of point estimates for clinical diagnoses (Figures A, B, C, D, and E) categorized by sex in 65,600 finished cattle at 6 commercial slaughter facilities. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$).

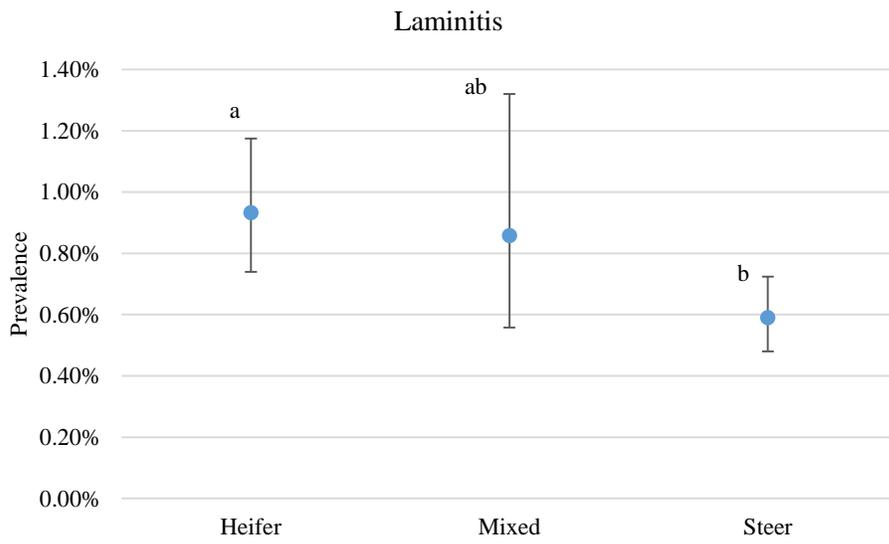
A.



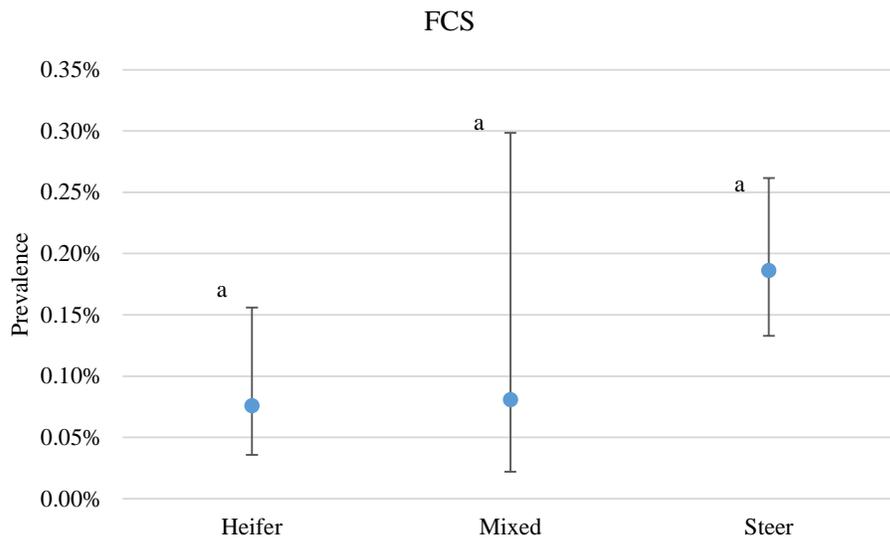
B.



C.



D.



E.

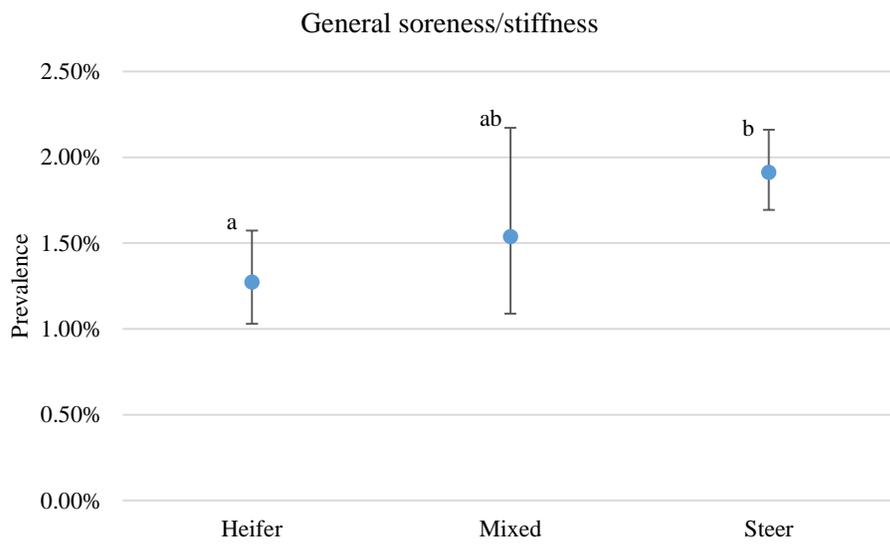
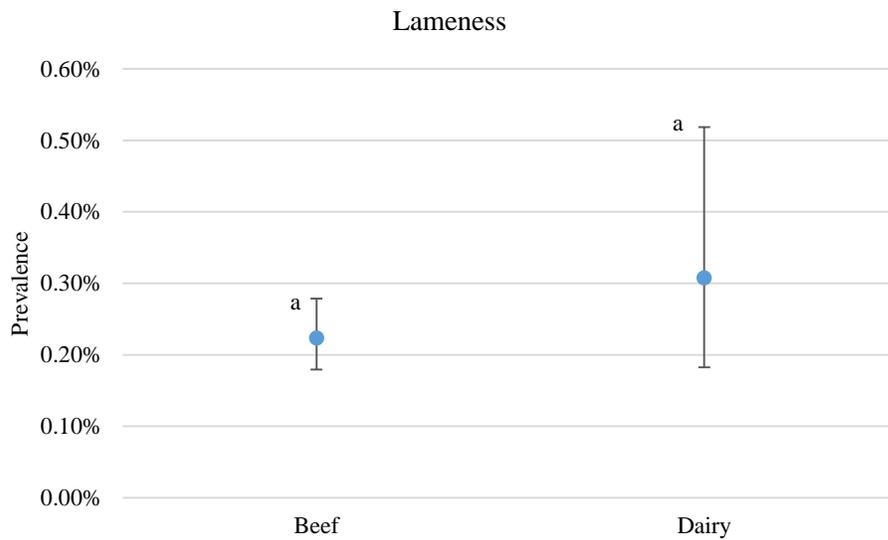
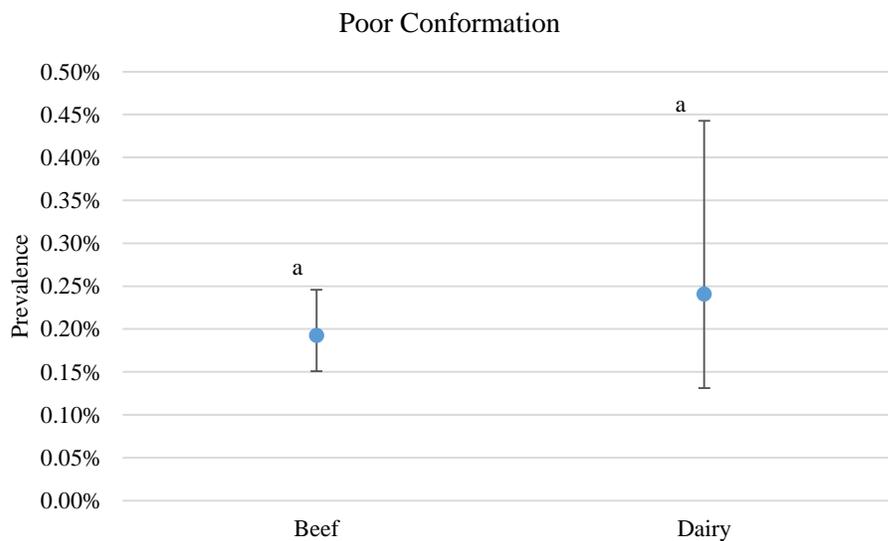


Figure 3-15. Comparisons of point estimates for clinical diagnoses (Figures A, B, C, D, and E) categorized by breed in 65,600 finished cattle at 6 commercial slaughter facilities. Variation is expressed as 95% confidence intervals, and estimates without common superscripts differ ($P \leq 0.05$).

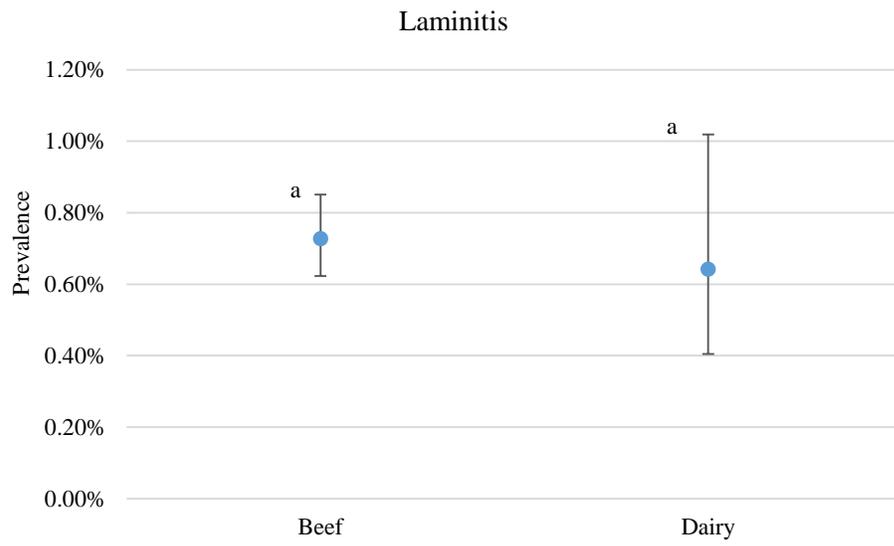
A.



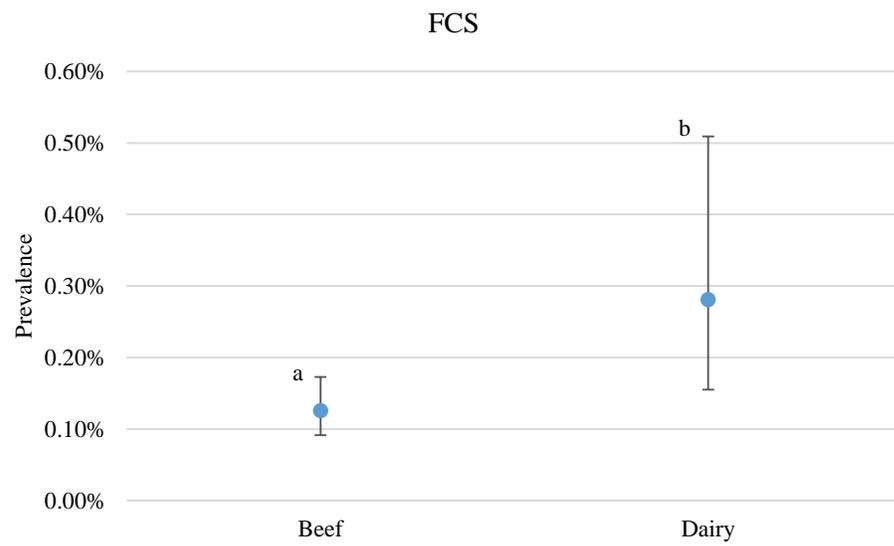
B.



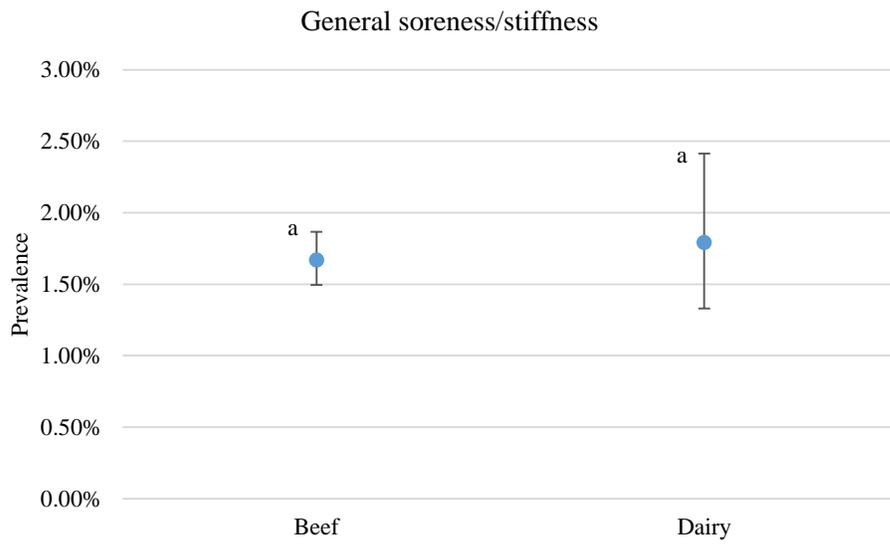
C.



D.



E.



Chapter 4 - Comparison of Physiologic Parameters of Normal Finished Cattle and Finished Cattle Diagnosed with Fatigued Cattle Syndrome at Commercial Slaughter Facilities

Abstract

Fatigued Cattle Syndrome (FCS) led to the adoption of a mobility scoring system for cattle entering commercial slaughter facilities. The objective of this study was to determine the correlation of FCS clinical signs, including abnormal mobility, with changes in physiologic parameters of cattle at slaughter. Cattle displaying clinical signs of FCS and mobility scores >2 ($n=20$) were enrolled. A normal cohort of the same breed type (color) and sex was selected from the same lot as a paired control. Blood and feet samples were collected from each animal at slaughter. Lactate concentrations were greater in cattle with FCS compared to normal cattle (12.88 v. 10.76 mmol/L; $P = 0.0037$). Creatine kinase concentrations did not appear to differ between FCS and normal cattle (561.30 v. 449.88 U/L, $P = 0.5066$). Glucose concentrations were greater in normal cattle (179.05 v. 138.80 mg/dL, $P = 0.0179$). No difference in epinephrine levels was observed (629.00 v 657.00 pg/mL, $P = 0.2503$). There was a significant interaction between sex and diagnosis of FCS in norepinephrine levels, where heifers showing signs of FCS displayed lower levels of norepinephrine than their normal cohorts, but steers did not ($P = 0.0314$). These data indicate cattle diagnosed with FCS display higher serum lactate concentrations than normal cattle. Perhaps utilizing pen-mate cohorts as controls, subjected the same heat, handling, and transportation stress, may diminish the difference in concentrations of

certain physiological markers. More research is needed to develop definitive biological markers to confirm FCS in cattle.

Introduction

Recently, adverse events involving abnormal mobility of cattle in commercial slaughter facilities resulted in the detection of a novel syndrome experienced by finished cattle, termed Fatigued Cattle Syndrome (FCS) which was reported by Thomson *et al.* in 2015.^{1,2} The clinical signs and diagnostic findings in finished cattle are similar to those reported in finished hogs at the time of slaughter with a similar syndrome, Fatigued Pig Syndrome (FPS).³ Both syndromes impact animal health that lead to economic issues in the pork and beef industries in the form of losses in production and even animal death during and after transport.⁴

The clinical signs of FCS include tachypnea with an abdominal component, muscle tremors, stiff gait with shortened stride, and reluctance to move, possibly to the point of nonambulatory status.^{1,5,6} Suggested biochemical markers include markedly increased lactate and creatine kinase (CK) concentrations in serum or plasma.¹ Recent research by Frese *et al.* (2016) and Hagenmaier *et al.* (2016) shows that FCS can be induced in cattle by aggressive handling.^{5,6} However, evidence shows that the prevalence of FCS is very low in the general population of fed cattle, and detection via analysis of physiologic blood parameters can be time-consuming, labor-intensive, and exceedingly expensive (Lee *et al.*, unpublished data). The use of a mobility scoring system based upon evident clinical signs may contribute to the diagnosis of FCS in the field. Therefore, the objective of this study was to determine if mobility score and clinical signs reflect concurrent changes in bovine physiologic parameters such as blood concentrations of lactate, glucose, creatine kinase (CK), epinephrine and norepinephrine, and biomechanical integrity of hooves, represented by peak stress and strain on the dorsal hoof wall.

Materials & Methods

All procedures were approved by the institutional animal care and use committee of Kansas State University (IACUC # 3708).

Slaughter facilities selected for observation were selected based on data from a cattle mobility scoring program and logistical considerations.⁷ The three facilities selected for the study were in different locations in the United States, and their operations reflect the current population of finished cattle slaughtered in the United States. Observations were made during June and July, 2016. Animals were observed by the same trained veterinarian from Kansas State University.

Animals displaying a $MS \geq 2$ and displaying clinical signs of FCS (including increased respiratory rate, muscle tremors, and/or vocalization with no other signs of contributing disease) were eligible for enrollment in the study (n=20). After an animal displaying signs of FCS was detected, a normal cohort (n=20) of the same sex and breed type/color was selected from the same lot for comparison.

Mobility score was assigned to each animal enrolled using the scoring system adopted by the North American Meat Institute (NAMI, 2015), where: 1 = Normal, walks easily with no apparent lameness or change in gait; 2 = Keeps up with normal cattle when the group is walking, exhibits one or more of the following: stiffness, shortened stride, or slight limp; 3 = Lags behind normal cattle when the group is walking, exhibits one or more of the following: obvious stiffness, difficulty taking steps, obvious limp or discomfort; 4 = Extremely reluctant to move, even when encouraged by handlers; statue-like.⁸

Clinical signs recorded regarding the animal's clinical presentation included, but were not limited to, reluctance to move without obvious disease or injury; failure to keep up with

contemporaries without extra handling pressure, or use of electric prods, paddles, or sticks; shortened strides, stiffened gait or difficulty walking; nervous system abnormalities such as muscle tremors; or other signs of distress such as an increased respiratory rate or vocalization. Other signs not listed were recorded as comments in the data set. Additional information collected included the slaughter facility lot number, time of observation, sex of each animal, and breed type/color of each animal. Animals were followed through euthanasia via captive bolt according to the slaughter facility's euthanasia SOP, through exsanguination, where blood samples were collected, and after exsanguination, where the left front foot was collected.

Blood samples were collected following exsanguination using a 60-mL syringe with needle, and then immediately transferred to two clotting (10 mL serum separator, no anticoagulant) tubes and one tube containing potassium EDTA.^a Plasma was centrifuged immediately following collection at 3000G for 15 minutes, harvested and placed in 2mL cryovials, and immediately placed on dry ice. Plasma was kept on dry ice for transport to permanent storage at -80°C until analysis. Blood samples intended for serum were allowed to clot for 30 minutes and then centrifuged at 3000G for 15 minutes. Following centrifugation, serum was harvested, placed in 2mL cryovials, and placed on dry ice. Serum samples were also kept on dry ice for transport to permanent storage at -80°C until analysis. Times at which blood samples were collected, centrifuged, serum harvested, placed on ice, and placed in permanent freezer storage were recorded.

Plasma samples were assayed for catecholamines Michigan State University's Veterinary Diagnostic Center and serum samples were assayed at for lactate, cortisol and full chemistry panels at Kansas State University's Veterinary Diagnostic Lab. Plasma samples were assayed for epinephrine and norepinephrine using a commercially available radioimmunoassay (RIA) kit.^b

Serum samples were assayed for lactate using a Nova CCX analyzer^c and full serum chemistry panels including analysis of CK, glucose, urea nitrogen, creatinine, total protein (TP), albumin, globulin, total calcium, phosphorous, sodium, potassium, chloride, bicarbonate, anion gap, sodium: potassium ratio, aspartate transaminase (AST), alkaline phosphatase (ALP), gamma glutamyltransferase (GGT), and sorbitol dehydrogenase (SDH) were assayed using a Cobas c501 analyzer.^d Cortisol concentrations were assayed using serum with a solid-phase competitive chemiluminescent immunoassay and an automated analyzer system.^e

The left front hoof of each animal was collected after exsanguination. Each sample was double-bagged in plastic resealable bags, and placed on dry ice for transport to permanent storage at -20°C until testing.⁹ Specimens were collected using a 14mm hole saw and a drill press while the foot was frozen. A full thickness, 10mm core was obtained and kept frozen until testing. To assure consistency, each core was obtained from the point midway down the dorsal hoof wall as measured from the coronary band to the solar surface.

Biomechanical testing of the hoof wall was performed at Iowa State University's Veterinary Medical Center, where specimens were allowed to thaw for 30 minutes until they reached room temperature. The temperature of each specimen was obtained prior to testing. An Instron Model 4402^f was then used to determine the elongation to rupture as well as the peak stress. Specimens were placed in a custom grip designed specifically for hoof core specimens and elevated using standard stress and strain methodology.⁹ Diameter and soft tissue thickness were measured and entered into TestWorks4^g to determine peak stress and strain at rupture.

Data were analyzed using the PROC GLIMMIX procedure in SAS Version 9.4,^h in which individual animals were the experimental unit and pairs of animals with decreased mobility and their normal cohorts served as a block. The statistical model was used to estimate the outcome

of each blood marker and the peak stress and strain of the dorsal hoof wall. The model included the fixed effects of mobility status, sex, and the interaction between mobility status and sex. Pair (block) was considered a random effect. Degrees of freedom were calculated using the Kenward-Roger method. Transformation of glucose, CK, AST, and TP data were performed by computing the natural logarithm of the data. Differences in average levels of physiologic parameters between the groups (normal and FCS) were reported as significant when P-values were ≤ 0.05 .

Sensitivity and specificity was calculated using the clinical diagnosis of FCS as the gold standard or “true” disease state, and the hematological profile as the “test” disease state. True FCS status was determined by the observing veterinarian. Based on previous research, a positive hematological test for FCS was defined as an animal with CK levels above normal reference ranges for cattle, reported by the Kansas State University Veterinary Diagnostic Laboratory.^{1,2} Sensitivity and specificity were calculated using different cutpoints for lactate concentration, as all animals enrolled in the study displayed serum lactate concentrations above the normal reference range. Sensitivity was calculated as {Sensitivity = #true positives / (#true positives + #false negatives)} and specificity was calculated as {Specificity = # true negatives / (#true negatives + #false positives)}. Cohen’s Kappa (Kappa) was used to measure the amount of agreement between the diagnostic test of mobility score and the use of serum biochemical markers using the equation {K = (observed agreement – chance agreement / maximum agreement beyond chance)}, where {maximum agreement beyond chance = 1 – chance agreement}.¹⁰

Results

Twenty finished cattle displaying clinical signs of FCS were enrolled in the study, along with 20 normal cohorts from the same lot. All animals displaying signs of FCS exhibited a MS = 3. All animals displaying normal mobility were scored as MS = 1. Nine of the 20 pairs of normal and abnormal animals were heifers, and 11 were steers. All animals displaying signs of FCS showed reluctance to move and shortened strides. Fifteen of the 20 animals had increased respiratory rates. Seventeen animals were unable to keep up with their contemporaries. Nineteen of the 20 animals displaying signs of FCS had muscle tremors, and 1 animal displayed neurologic symptoms (Table 4-1).

Serum lactate concentrations were greater in cattle diagnosed with FCS (abnormal) compared to normal animals and were outside the normal reference range in animals in both groups (12.88 mmol/L v. 10.76 mmol/L \pm 0.59; ref. <5mmol/L; $P=0.0020$). No differences in CK concentration in abnormal versus normal animals were observed, but average CK levels for both normal and abnormal groups were again higher than normal reference ranges (561.30 U/L v. 449.88 U/L, \pm 77.1; ref. 171-357 U/L; $P=0.1147$). Aspartate transaminase (123.50 v. 105.20 U/L; $P = 0.0127$) concentrations were greater in cattle diagnosed with FCS compared to normal cattle. No difference was detected between the groups with regards to cortisol concentrations (160.28 v. 131.20 nmol/L \pm 12.34; $P = 0.0630$). Normal cattle displayed greater glucose concentrations compared to cattle diagnosed with FCS (179.05 v. 138.80 \pm 14.30 mg/dL; $P = 0.0179$), and both groups had glucose levels above normal reference ranges (ref. range = 29-73 mg/dL). There was no difference in total protein in cattle diagnosed with FCS compared to their normal cohorts (8.56 v. 8.30 g/dL, \pm 0.14; $P = 0.0615$), but remained within the normal reference range (6.0 – 9.0 g/dL). There was a sex \times mobility score interaction ($P = 0.0314$), with

regards to norepinephrine levels, where slow-moving heifers displayed reduced levels of norepinephrine compared to their normal cohorts but steers did not ($P = 0.0314$). No differences were detected in concentrations of epinephrine between the two groups. Blood concentrations of each marker for each group are presented in Table 4-2.

During collection of hooves for biomechanical analysis, five samples from the 40 cattle selected could not be collected due to the inability to identify animals after hide washing. Strain was not detected in two samples due to structural abnormalities. Peak stress was measured as the force per unit area that was required to cause specimen failure (measured in pascal; pascal = Newton/m²). Seventeen feet from cattle diagnosed with FCS and 18 feet from normal cattle were used in the analysis. No difference in peak stress was detected in normal cattle compared to cattle diagnosed with FCS (5.67 ± 0.797 v. 5.09 ± 0.806 mPa, respectively; $P = 0.5295$; Table 3). Strain at break was defined as the amount of deformation an object experiences compared to its original size and shape. Sixteen feet from normal cattle and 17 feet from cattle diagnosed with FCS were used. No difference in strain was detected in normal cattle compared to cattle diagnosed with FCS ($50.1\% \pm 5.713\%$ v. $53.3\% \pm 5.615\%$, respectively; $P = 0.4487$; Table 3).

The sensitivity and specificity of the current test for FCS was computed comparing the Gold Standard of clinical diagnosis to blood lactate at different cutoff levels (10 - 14 mmol/L). The greatest sensitivity of the test using 14 mmol/L lactate was 83.3%, however specificity of the test at this level of lactate was only 25%. The greatest sensitivity and specificity combination was obtained using a cutoff level of 12 mmol/L lactate, where sensitivity was 65% and specificity was also 65%. Positive and negative predictive values were 65% as well (Table 4). The Kappa coefficient for the comparison of the Gold Standard and blood levels of lactate at 12 mmol/L was 0.3, indicating “fair” agreement (Table 5).¹⁰

The sensitivity of the test using CK compared to the Gold Standard was 90%. Specificity was 70%. Positive and negative predictive values of the test using CK to detect FCS were 75% and 88%, respectively (Table 4-4). The agreement not due to chance, as indicated by the Kappa value (0.6) was “substantial,” indicating that the use of blood levels of CK to determine FCS status is quite accurate (Table 4-5).¹⁰

Discussion

Fatigued Cattle Syndrome is the clinical manifestation of a number of physiologic processes in the body, which result from an abnormal amount of stress and fatigue experienced by an animal. Many factors may contribute to the expression of this syndrome, including genetics, exercise status, handling techniques, nutritional status, and environmental and transport conditions.^{1,5,6,11,12} However, while the factors which contribute to FCS can be numerous, the physiologic processes remain relatively constant. The syndrome is characterized by an increase in blood lactate, CK, AST, and other markers which indicate that the animal has undergone some inciting stress event.^{1,5}

Fatigued Cattle Syndrome results in increased levels of lactate, CK, and other biochemical markers due to the depletion of muscle energy supplies and subsequent breakdown of muscle tissue. Lactate is produced as a byproduct in the muscle tissue when glucose is broken down in skeletal muscle cells. In normal skeletal muscle, conversion of glucose to two molecules of pyruvate yields 2 molecules of ATP through the breakdown of glucose itself, and 30 molecules of ATP when pyruvate enters the Krebs cycle, which occurs in the mitochondria.¹³ This energy, in the form of ATP, is used in multiple cellular functions, including maintenance

activities such as maintenance of cell membranes. However, the Krebs cycle can produce ATP only in the presence of oxygen. When muscle cells are deprived of oxygen, pyruvate does not enter the Krebs cycle. Rather, it is further broken down to lactic acid by lactate dehydrogenase. This yields just 2 molecules of NAD⁺, which is used in the same cycle to break down more molecules of glucose for the production of only 2 ATP molecules.¹⁴ Because such a small amount of ATP is produced in the absence of oxygen, maintenance of regular cellular activities is compromised, and cell membranes begin to lose their integrity. This causes the membranes to “leak” intracellular materials such as the lactic acid being produced, as well as other enzymes and molecules, including CK and AST. If cells are compromised entirely, they will break down completely, releasing all intracellular contents into the surrounding tissue. This causes an increase of the biochemical markers lactate, CK, and AST in the blood, hallmarks of FCS.¹

The use of diagnostic tests such as serum chemistry or histopathology to detect these hallmarks of FCS would require a large amount of time and labor, and would impose an extraordinary expense upon feedyards, processing companies, and ultimately, the consumer. The use of mobility scoring and observation of clinical signs to help diagnose FCS in commercial slaughter facilities is ideal, as such observation is not labor-intensive, and does not incur a large expense upon any entity in the production system, relative to diagnostic testing. However, since mobility scores are a subjective measure, validation of the system should be performed not just once, but periodically throughout the duration of the system’s implementation. The data collected here provides information for development of future validation strategies.

While the physiologic processes for increased lactate is relatively constant between animals, reference ranges for lactate concentration in normal animals are not reported consistently. Some reports indicate that lactate should be below 2.2 mmol/L, and others indicate

that normal lactate values can reach up to 5 mmol/L.^{1,15,16} In this study, the serum lactate concentrations seen in both normal cattle and cattle diagnosed with FCS are higher than reference ranges for normal, unstressed animals.¹ However, a difference of 2 mmol/L was still detected between normal animals and cattle diagnosed with FCS.

Parker et al. (2003) found that acid-base values remained normal in cattle subjected to normal transport conditions, and attributed the normalcy to compensation mechanisms stimulated by the respiratory or renal systems.¹⁶ The values reported in the study by Parker et al. were quite low compared to values reported in other studies, and the current study (0.59 mmol/L, 0.75mmol/L, and 0.62mmol/L).^{1,5,15,16} This could be due to the differences in collection method, as the use of a glycolytic agent was reported by Parker et al. which was not used in the current study. Intuitively, however, the relative differences in lactate values of cattle subjected to transport stress vs. unstressed animals would be reflected in proportion, and they are not. In addition, if respiratory or renal compensation mechanisms are compromised, for example, if cattle had decreased lung capacity due to previous respiratory disease or pneumonia, it is possible that physiologic mechanisms for maintenance of homeostasis could be disrupted and result in decreased ability to blow off carbon dioxide and decreased oxygen perfusion to tissues, including skeletal muscle, leading to increased anaerobic respiration, increased lactate production, and a decreased ability to maintain acid-base balance. This was demonstrated in high lactate values found some animals with acute respiratory disease, particularly in animals near death.¹⁵

Additionally, reports in humans indicate that decreased lactate elimination by the liver can result in high concentrations of blood lactate.¹⁷⁻¹⁹ However, it has not been concluded if decreased elimination is due primarily to parenchymal disease or to decreased portal blood flow.

In cattle, it could be hypothesized that a decrease in liver function (in the form of liver abscesses) could lead to a decrease in lactate elimination and subsequent disruptions in acid-base status.

In addition to high lactate levels, serum creatine kinase (CK) was proposed as a potential biomarker for the diagnosis of FCS, as high levels of the enzyme indicate the breakdown of skeletal muscle.²⁰ Increased levels of CK depend on muscle damage from either strenuous exercise or muscular pathology.²¹ It is unlikely that most animals entering commercial slaughter facilities experience a specific muscular pathology, but in severe cases of FCS and other cases of decreased mobility, muscular pathology in the individual animal should not be ruled out. In the current study, no difference was detected in CK levels between normal and abnormal animals, however both groups displayed higher average CK concentrations than the normal reference range. In addition, while a statistically significant difference between the two groups observed here may not exist, it is possible that a biological difference may. As stated previously, cell membranes become compromised and release their contents after experiencing stress, but this cascade occurs along a continuum, and it could be that CK levels in FCS cattle did not accumulate fast enough to detect a statistically significant difference between the two groups.

In humans, CK levels are related to body mass, where people of higher body mass display increased concentrations of CK.^{21,22} Higher levels of CK in large cattle could reflect such a relationship as well, which would account for the higher-than-normal concentrations of CK in cattle presenting with both normal and decreased mobility. In addition, Buckham Sporer et al. (2008) reported that in young beef bulls transported 9 hours by road, CK levels changed over time, with lower concentrations observed immediately after the journey, and higher concentrations approximately 15 hours after the trip.²³ In contrast, Frese et al. reported CK values of approximately double those found in the current study two hours after cattle had been

handled aggressively.⁵ Other previous research shows even higher CK values at exsanguination after low-stress and high-stress handling was performed at the feedyard (7,810 v. 8,502 U/L, respectively).⁶ It must be noted that the samples collected in the current study were collected upon exsanguination, and were collected from cattle with unknown history. Such discrepancies indicate that there may be a time or stress component which affects CK in animals experiencing the stressors of handling and transportation, and more time or stress may be required to see comparable CK levels in non-experimental cattle.

Serum AST is a muscle- and liver-specific enzyme in cattle.²⁴ Increases in AST were seen in cattle presenting with FCS in the summer of 2013, however the use of AST as a biomarker for the diagnosis of FCS, or any other disease in beef cattle, has not been explored extensively.¹ In the current study, cattle presenting with signs of FCS had higher blood AST concentrations than normal animals ($P \leq 0.05$). In dairy cattle, the use of CK and AST as diagnostic indicators of muscle and liver damage due to displaced abomasum and endometritis has been documented, indicating that AST may be a helpful tool for use in detecting muscle damage in cattle.²⁴ However, since AST is also an indicator of disruption of liver function, and results should be interpreted with such information in mind.²⁵

Elevated cortisol is a hallmark of stress due to transport and many other stressors.²³ Cortisol has been used as a measure of stress in animals for a number of years.^{3,5,23,25,26} In the current study, there is marginal evidence to conclude that cortisol levels in cattle with signs of FCS were higher than their normal cohorts ($P = 0.0528$). However, as a biomarker for the diagnosis of FCS, cortisol may not be ideal. Cortisol levels seem to decrease after the stress of transport, indicating that the hormone may be more indicative of acute stress, rather than the stress of overall fatigue.^{23,25,27} It may be that lactate status and muscle enzyme activity are more

important biomarkers of FCS, due to the physiologic processes they represent (i.e. acid-base status, respiration status, and muscle degradation), rather than the acute stress of which cortisol is usually indicative.

The same may be said about glucose levels in cattle experiencing FCS. In the current study, glucose levels were higher in normal cattle compared to cattle presenting with FCS, but both groups displayed blood glucose concentrations higher than the normal reference range. This is in agreement with Mitchell *et al.* (1988), where glucose and lactate concentrations were higher in cattle at exsanguination than after transport or handling.²⁸ The use of glucose as a biochemical marker may be confounded by a number of factors, including the fasting of animals immediately prior to and during transport and acute stress of handling during transport and in the slaughter facilities.²⁸⁻³⁰ However, it could be argued that lower glucose levels are seen in animals with compromised mobility or FCS due to the use of any available glucose for the purposes of anaerobic respiration. Indeed, the cases reported during the adverse welfare event in 2013 displayed highly variable blood glucose concentrations (26 to 296 mg/dL).¹ Additionally, levels of total protein were significantly different between the two groups observed here, however the TP of neither group was outside the reference range of normal animals. More information about the role of glucose and TP in FCS is needed to determine if they are useful biochemical markers.

Blood levels of the catecholamines epinephrine and norepinephrine were not different between the animals displaying normal vs. abnormal mobility. This is likely due to the method of blood collection used. Blood was collected from animals at exsanguination, which in these commercial slaughter facilities, occurs after stunning via captive bolt. It has been reported that all stunning methods trigger a massive release of catecholamines.³¹⁻³⁴ This is demonstrated in

the work of Hagenmaier *et al.* (2017), where concentrations of epinephrine and norepinephrine increased 2 to 3-fold in cattle after slaughter compared to levels in the same animals before slaughter.⁶ This massive secretion likely masked any differences which may have been detected between the groups. In addition, high blood cortisol levels seen here could be due to increased catecholamine release, which increases the amount of adrenocorticotrophic hormone circulation, which subsequently increases the amount of cortisol released into the blood. Such a confounding effect increases evidence that cortisol may not be an ideal biochemical marker for the diagnosis of FCS. Finally, Stott *et al.* (1978) suggested that because no reference ranges for catecholamines exist, and their release is variable, a series of measurements should be collected to accurately describe animals' reaction to stress using hormone levels.³⁵ Because samples were collected at only one time point in the current study, differences in catecholamine levels within animals could not be measured. As blood collection at commercial slaughter facilities is difficult even at exsanguination and next to impossible in lairage pens, the use of catecholamines as diagnostic indicators of FCS in cattle is less than ideal.

The use of biomechanical data describing foot wall integrity, was not statistically significant in this study. Peak stress represents the force required to cause breakage of the dorsal hoof wall. This value was slightly higher in normal cattle compared to cattle diagnosed with FCS. Greater force may be required to break the hoof wall of normal cattle compared to hooves of compromised cattle. On the other hand, strain, which is the percent change in linear tension (stretch) compared to normal soft tissue thickness, was slightly higher in cattle diagnosed with FCS. Weaker connective tissue would likely stretch more, and one could hypothesize that the increased stress in cattle experiencing FCS could contribute to this physiologic mechanism, however these differences were small, and not statistically significant. Research shows that

dairy cattle undergoing the stress of parturition experience loss of integrity of the connective tissue in the support structures of the foot, and such losses seem to increase as time to parturition nears.^{9,36} After parturition, evidence of recovery was seen in histopathological analysis of connective tissue from the hoof wall.⁹ However, parturition is not the only stressor which contributes to these changes. Differences in housing can cause biomechanical and histopathological changes in hoof wall connective tissue as well.³⁶ More research in this area is needed, including effects of stress, management, and other factors, to assess the true effects of FCS on the biomechanical integrity of the hoof wall, or vice versa, the effects of changes in connective tissue on the clinical presentation of FCS in beef cattle.

Finally, based on the information discussed here, the use of blood biochemical markers lactate and CK as tests to diagnose FCS was compared to the clinical diagnosis of FCS by a trained veterinarian. It must be noted that the prevalence of FCS in the population of fed cattle is very low (approximately 0.16%, Lee et al., unpublished data), therefore the true prevalence of diseased vs. non-diseased animals in the population is not represented here. However, measurements of agreement via a Kappa coefficient should be reflective of the agreement of the two diagnostic tests (clinical signs vs. biochemical markers) due to chance and true agreement. The greatest sensitivity of the test using 14 mmol/L lactate was 85%, however specificity of the test at this level of lactate was only 25%. The greatest sensitivity and specificity combination was obtained using a cutoff level of 12 mmol/L lactate, where sensitivity was 65% and specificity was also 65%. Based on the results reported here, the use of a cutoff of 12 mmol/L indicates “fair” agreement between the two tests. The highest agreement observed (0.4) was at a lactate concentration of 14 mmol/L, indicating “moderate” agreement. Table 4 shows the strength of agreement at different Kappa values, adapted from Landis and Koch, 1977.¹⁰ The

sensitivity of the test using CK compared to the Gold Standard was 90%. Specificity was 70%. Positive and negative predictive values of the test using CK to detect FCS were 75% and 88%, respectively. The agreement not due to chance, as indicated by the Kappa value (0.6) was “substantial,” indicating that the use of blood levels of CK to determine FCS status is quite accurate, and is likely a very good biomarker to help diagnose FCS.

Validation of the use of clinical signs and mobility status as diagnostic tools for FCS should be performed periodically to assess the ability of scorers to diagnose FCS in slaughter facilities. However, it is extremely important to note that while certain biomarkers are likely indicative of FCS, the syndrome is clearly manifested as decreased mobility. Many times, especially with regards to interpretation of good animal welfare, visual assessment may be the most important indicator of fatigued cattle entering commercial slaughter facilities.

Conclusion

Cattle with impaired mobility following lairage at commercial slaughter facilities had altered levels of numerous blood biochemical markers, specifically lactate, AST, glucose, and norepinephrine. The absolute values of the biomarkers obtained in the current study are lower than other reported research evaluating these parameters in FCS cattle, however still indicate an altered physiologic state.^{1,5,6} This could be due to the observational nature of the study, in that experimentally-induced FCS could cause more dramatic changes in blood biomarkers than the changes observed here. In addition, these data indicate that the severity of the clinical manifestation of FCS could occur along a continuum, and perhaps animals more severely affected may display greater concentrations of these biochemical markers. The abnormalities in

blood biochemical markers, along with the clinical signs of impaired mobility, muscle tremors, increased respiratory rate, and others are indicative of decreased animal welfare, and should be addressed.

The use of mobility score and other visual clinical signs to diagnose FCS is important because measurement of animal welfare is not only a matter of normal vs. abnormal physiological status. Just like a physical examination is part of all health assessments conducted by veterinarians and producers, visual assessment of animals must be used in order to completely assess the health and welfare of animals in all areas of the production process.

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Table 4-1. Clinical signs displayed by normal cattle (MS = 1) and cattle displaying signs of Fatigued Cattle Syndrome (FCS) with MS \geq 3 in finished cattle at slaughter.

	Normal cattle¹		FCS² cattle	
	(n=20)		(n=20)	
	Count	Percent	Count	Percent
No disease	20	100%	0	0%
Reluctance to move	0	0%	20	100%
Vocalization	0	0%	0	0%
Shortened strides	0	0%	20	100%
Recumbent	0	0%	0	0%
Foundered³	0	0%	0	0%
Increased RR	0	0%	15	75%
Open-mouthed breathing	0	0%	1	5%
Unable to keep up	0	0%	17	85%
Muscle tremors	0	0%	19	95%
Nervous system signs	0	0%	1	5%

¹Normal cattle were cattle presenting as MS =1 and displaying no clinical signs of Fatigued Cattle Syndrome.

²Fatigued Cattle Syndrome

³Foundered was defined as signs of laminitis, including but not limited to abnormally long hoof walls, elongated toes, and walking on heels.

Table 4-2. Comparisons of biochemical markers in normal cattle (MS = 1) and cattle displaying signs of Fatigued Cattle Syndrome (FCS) with MS \geq 3 in finished cattle at slaughter.

	Animals displaying signs of FCS¹ (n=20)	Normal animals (n=20)	SEM	Reference Range	P-value² for Pairs³	P-value for Sex*Pair Interaction
Epinephrine (pg/mL)	629.00	657.00	0.028	N/A	0.2503	0.2458
Norepinephrine (pg/mL)	1940.00	2280.00	0.270	N/A	0.0314	0.0314
Lactate (mmol/L)	12.88	10.76	0.590	< 5	0.0020	0.9486
Cortisol (nmol/L)	160.28	131.20	12.336	N/A	0.0630	0.8278
Glucose (mg/dL)¹¹	138.80	179.05	14.300	29-73	0.0179	0.167
Creatine Kinase (U/L)¹¹	561.30	449.88	77.100	171-357	0.1147	0.6223
AST (U/L)^{4,11}	123.50	105.20	9.090	53-156	0.0127	0.5591
TP (g/dL)^{5,11}	8.56	8.30	0.140	6.0-9.0	0.0615	0.779
BUN (mg/dL)^{6,11}	13.40	13.60	0.628	9-24	0.5338	0.1184
Creatinine (mg/dL)	1.64	1.58	0.058	0.5-1.6	0.5066	0.5792
Albumin (g/dL)	5.58	3.91	1.590	3.1-4.3	0.3501	0.3859
Globulin (g/dL)	4.62	4.43	0.170	N/A	0.1612	0.571
Total Ca (mg/dL)	10.69	10.58	0.100	8.1-10.3	0.4646	0.8409
Phosphorous (mg/dL)¹¹	8.34	8.43	0.310	4.9-9.0	0.5237	0.3689
Sodium (mmol/L)	148.15	146.55	0.720	138-155	0.0546	0.5689
Potassium (mmol/L)¹¹	7.25	7.370	0.210	4.2-6.3	0.602	0.374
Chloride (mmol/L)	99.45	98.85	0.590	92-117	0.3446	0.803
Bicarb (mmol/L)	20.66	20.41	0.498	21-31	0.5589	0.7212
Anion Gap (mmol/L)	36.30	35.60	0.780	N/A	0.4035	0.8387
Na/K Ratio⁷	21.10	20.60	1.130	N/A	0.4815	0.4815
ALP (U/L)⁸	121.04	124.38	11.110	20-76	0.6954	0.7753
GGT (U/L)^{9,11}	37.90	30.95	4.490	10-39	0.3506	0.7588
SDH (U/L)^{10,11}	21.55	16.44	4.520	171-357	0.3919	0.5398

¹Fatigued Cattle Syndrome

²Statistical significance was declared when $P \leq 0.05$.

³Creatine kinase

⁴Aspartate transaminase

⁵Total protein

⁶Sodium/potassium ratio

⁷Alkaline phosphatase

⁸Gamma glutamyltransferase

⁹Sorbitol dehydrogenase

¹⁰Statistical analysis was conducted on log transformed values and treatment estimates were back-transformed for reporting purposes.

Table 4-3. Descriptions and comparisons of biomechanical integrity of dorsal hoof walls in normal cattle (MS = 1) and cattle displaying signs of Fatigued Cattle Syndrome (FCS) with MS ≥ 3 in finished cattle at slaughter.

	Animals displaying signs of FCS¹ (SEM)	Number of feet from FCS cattle	Normal animals (SEM)	Number of feet from normal cattle	P-value²
Diameter (mm)	9.15 (\pm 0.086)	18	9.73 (\pm 0.086)	17	N/A ³
Soft Tissue Thickness (mm)	4.08 (\pm 0.155)	18	4.3 (\pm 0.155)	17	N/A ³
Peak Stress (mPa)	5.09 (\pm 0.797)	18	5.67 (\pm 0.806)	17	0.5295
Strain at Break (%)	53.25 (\pm 5.615)	17	50.05 (\pm 5.713)	16	0.4487

¹Fatigued Cattle Syndrome

²Statistical significance was declared when $P \leq 0.05$.

³Statistical analysis not performed.

Table 4-4. Sensitivity and specificity results for diagnostic test of mobility score as an indicator of FCS, compared to the Gold Standard of diagnosis using clinical presentation.

Biomarker¹

Lactate, mmol/L²	Sensitivity	Specificity	PPV⁴	NPV⁵	Kappa
10	85%	25%	53%	63%	0.15
11	70%	45%	56%	60%	0.15
12	65%	65%	65%	65%	0.3
13	55%	80%	73%	64%	0.35
14	50%	90%	83%	64%	0.4
Creatine Kinase (CK; U/L)³	90%	70%	75%	88%	0.6

¹Serum lactate and creatine kinase concentrations were used as tests for determining disease status, measured against the Gold Standard of clinical diagnosis of FCS by a trained veterinarian. Serum concentrations of lactate and creatine kinase (CK) were used as tests, while the Gold Standard in determining true disease status was clinical diagnosis by a trained veterinarian. Serum lactate was above the normal reference range (>5mmol/L), therefore different cutoff values were explored to determine the cutoff which yielded the highest sensitivity and specificity results.

²Reference value = <5mmol/L

³Reference value = 171-357 U/L

⁴Positive predictive value

⁵Negative predictive value

Table 4-5. Benchmarks for determining strength of agreement (not due to chance) between two diagnostic tests using Cohen's Kappa coefficient.¹⁰

Kappa	0	0.01-0.2	0.21-0.4	0.41-0.6	0.61-0.8	0.81-1.0
Strength of Agreement	None	Slight	Fair	Moderate	Substantial	Near Perfect